ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2021 September 15; 12(9): 1363-1586

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 12 Number 9 September 15, 2021

REVIEW

MINIREVIEWS

World Journal of Diabetes

Monthly Volume 12 Number 9 September 15, 2021

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Jayaprakash Sahoo, MBBS, MD, Associate Professor, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605006, India. jppgi@yahoo.com

AIMS AND SCOPE

The primary aim of *World Journal of Diabetes* (*WJD*, *World J Diabetes*) is to provide scholars and readers from various fields of diabetes with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJD mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

INDEXING/ABSTRACTING

The *WJD* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports/Science Edition, PubMed, and PubMed Central. The 2021 Edition of Journal Citation Reports® cites the 2020 impact factor (IF) for *WJD* as 3.763; IF without journal self cites: 3.684; 5-year IF: 7.348; Journal Citation Indicator: 0.64□Ranking: 80 among 145 journals in endocrinology and metabolism; and Quartile category: Q3.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Lin-YuTong Wang*; **Production Department Director:** *Yu-Jie Ma*; **Editorial Office Director:** *Jia-Ping Yan*.

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

PUBLICATION DATE
September 15, 2021

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1363-1385

DOI: 10.4239/wjd.v12.i9.1363

ISSN 1948-9358 (online)

REVIEW

Conundrum of vitamin D on glucose and fuel homeostasis

Maria Mercedes Chang Villacreses, Rudruidee Karnchanasorn, Panadeekarn Panjawatanan, Horng-Yih Ou, Ken C Chiu

ORCID number: Maria Mercedes Chang Villacreses 0000-0001-9713-1450; Rudruidee Karnchanasorn 0000-0001-7134-1097; Panadeekarn Panjawatanan 0000-0003-3665-7904; Horng-Yih Ou 0000-0002-3350-6548; Ken C Chiu 0000-0002-2226-1039.

Author contributions: Chang Villacreses MM, Chiu KC, Karnchanasorn R, and Ou HY developed the central theme and concepts of this manuscript; Panjawatanan P collected the data and participated in data analyses with Chang Villacreses MM, Chiu KC, Karnchanasorn R, and Ou HY; Chang Villacreses MM and Chiu KC prepared the first draft of manuscript; Chang Villacreses MM, Chiu KC, Karnchanasorn R, and Ou HY took part in critical review and revision of manuscript.

Conflict-of-interest statement: Authors declare no conflict-of-interest.

Maria Mercedes Chang Villacreses, Panadeekarn Panjawatanan, Ken C Chiu, Department of Clinical Diabetes, Endocrinology, and Metabolism, City of Hope National Medical Center, Duarte, CA 91010, United States

Maria Mercedes Chang Villacreses, Ken C Chiu, Division of Endocrinology, Metabolism and Nutrition, Department of Internal Medicine, Harbor-UCLA Medical Center, Torrance, CA 90509, United States

Rudruidee Karnchanasorn, Division of Endocrinology, Department of Medicine, University of Kansas Medical Center, Kansas City, KS 66160, United States

Panadeekarn Panjawatanan, Department of Internal Medicine, Bassett Medical Center, Cooperstown, NY 13326, United States

Horng-Yih Ou, Department of Internal Medicine, National Cheng Kung University Hospital, National Cheng Kung University, Tainan 700, Taiwan

Corresponding author: Ken C Chiu, FACE, FACP, MD, Professor, Department of Clinical Diabetes, Endocrinology, and Metabolism, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010, United States. kenchiumd@gmail.com

Abstract

As an endocrine hormone, vitamin D plays an important role in bone health and calcium homeostasis. Over the past two decades, the non-calcemic effects of vitamin D were extensively examined. Although the effect of vitamin D on beta cell function were known for some time, the effect of vitamin D on glucose and fuel homeostasis has attracted new interest among researchers. Yet, to date, studies remain inconclusive and controversial, in part, due to a lack of understanding of the threshold effects of vitamin D. In this review, a critical examination of interventional trials of vitamin D in prevention of diabetes is provided. Like use of vitamin D for bone loss, the benefits of vitamin D supplementation in diabetes prevention were observed in vitamin D-deficient subjects with serum 25-hydroxyvitamin D < 50 nmol/L (20 ng/mL). The beneficial effect from vitamin D supplementation was not apparent in subjects with serum 25-hydroxyvitamin D > 75 nmol/L (30 ng/mL). Furthermore, no benefit was noted in subjects that achieved serum 25-hydroxyvitamin D > 100 nmol/L (40 ng/mL). Further studies are required to confirm these observations.

Key Words: Vitamin D; Glucose metabolism; Diabetes mellitus; Insulin sensitivity; Beta

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: United States

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: May 3, 2021
Revised: May 10, 2021
Accepted: August 5, 2021
Article in press: August 5, 2021
Published online: September 15, 2021

P-Reviewer: Yang L
S-Editor: Liu M
L-Editor: A
P-Editor: Guo X

cell function

Core Tip: Vitamin D deficiency is a well-recognized health issue and contributes to bone loss and calcium dysregulation. Evidence suggests that excess vitamin D is not in and of itself of therapeutic benefit. Available clinical data suggests that vitamin D supplementation appears to limit the development of diabetes in vitamin D deficient subjects. However, no benefit was observed in non-vitamin D deficient subjects. Furthermore, overreplacement of vitamin D is of no beneficial effect and could possibly be harmful.

Citation: Chang Villacreses MM, Karnchanasorn R, Panjawatanan P, Ou HY, Chiu KC. Conundrum of vitamin D on glucose and fuel homeostasis. *World J Diabetes* 2021; 12(9): 1363-1385
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1363.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1363

INTRODUCTION

The potential role of vitamin D deficiency induced by migration of human beings has been suggested to be involved in human evolution and various modern health conditions[1]. The history prospective of vitamin D evaluation will enhance our understanding of the development in this field. The role of dietary deficiency in the pathogenesis of rickets was established by Platt[2] in 1919. Although it was thought to be caused by vitamin A deficiency initially, McCollum *et al*[3] identified a vitamin deficiency other than vitamin A that caused rickets in 1922. Since vitamin A, B, and C were already identified, the new molecule was named as vitamin D[4].

Beginning with its discovery in 1922, scientific publications focusing upon vitamin D numbered no more than some 10 per year but this increased to 35 per year by 1945 (Figure 1). As knowledge of the structure, molecular biology and function of vitamin D increased[5,6], there was a concurrent increase in vitamin D-specific publications. With the observations of the non-calcemic effects of vitamin D[7], vitamin D-focused publications peaked at 5152 in 2017. Recently, the role of vitamin D deficiency in relation to coronavirus disease 2019 (COVID-19) infection attracted attention[8].

Vitamin D on bone health

The role of vitamin D on calcium and bone metabolism was well-summarized[9]. There is no doubt about the association between rickets and vitamin D deficiency and the reversal and prevention of rickets with vitamin D supplementation. However, controversy still surrounds the efficacy of vitamin D supplementation upon bone mineral density and fracture prevention. Multiple studies failed to demonstrate any benefit from vitamin D supplementation[10-12] and a systematic review and meta-analysis also failed to confirm any beneficial effect on bone density or fracture prevention from vitamin D supplement[13]. Nevertheless, placebo-control randomized clinical trials revealed a threshold effect of vitamin D[14,15] with no benefit observed on the subjects with baseline 25-hydroxyvitamin D level ≥ 75 nmol/L (30 ng/mL). Furthermore, possible detrimental effects on bone mineral density were observed in subjects who received a higher dose of vitamin D (250 μg or 10000 IU daily) with a mean 25-hydroxyvitamin D of 200 nmol/L or 80 ng/mL[12]. While not conclusive, these data suggest that the optimal effects of vitamin D are found at a 25-hydroxyvitamin D level of 75 nmol/L (30 ng/mL).

Vitamin D as a hormone

Vitamins are defined as micronutrients that cannot be self-synthesized and that necessary for the proper function of key enzymatic processes. Consequently, vitamins must be obtained through the diet. Vitamin D is synthesized from cholesterol to 7-dehydrocholesterol, also known as pro-vitamin D_3, in the skin through the action of ultraviolet radiation[16]. In addition, the liver forms 25-hydroxyvitamin D_3, also

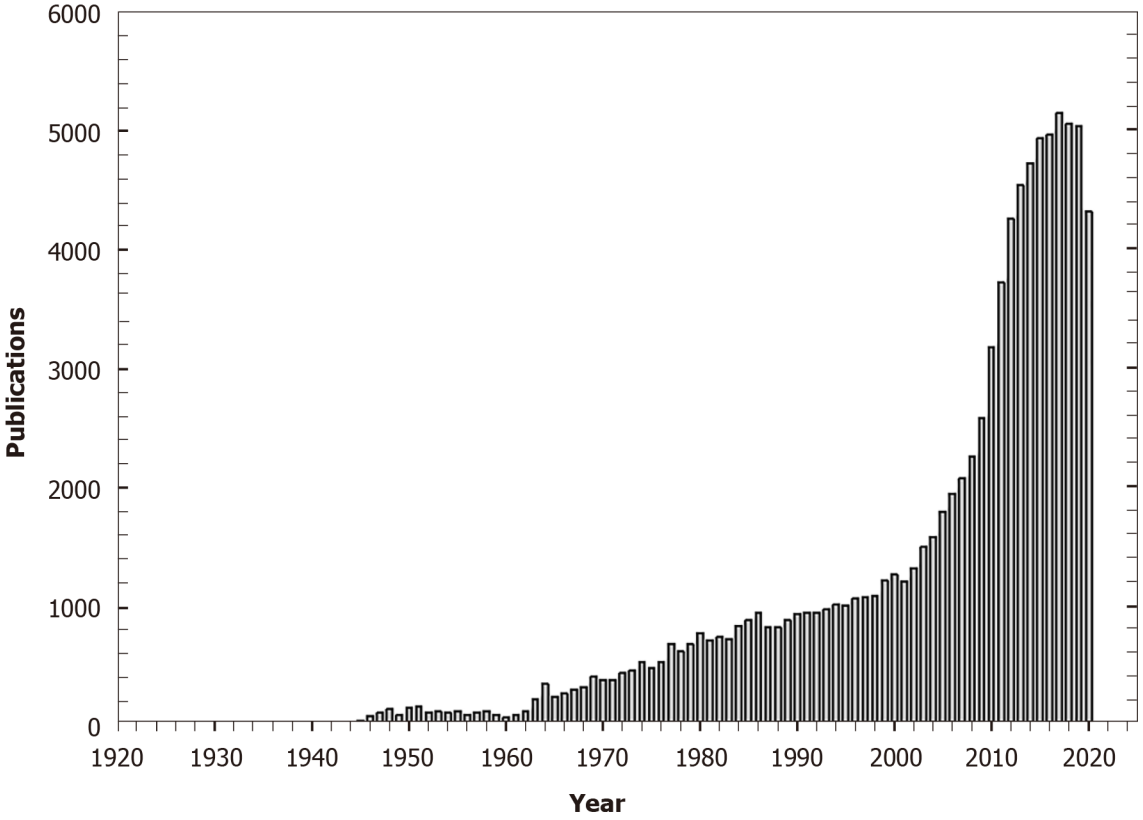

Figure 1 Vitamin D publications from 1922 to 2020. Data were obtained from PubMed (https://pubmed.ncbi.nlm.nih.gov/) accessed on October 20, 2020.

known as pre-vitamin D_3. To become an active compound, further hydroxylation in the kidney is required to form 1,25-dihydroxyvitaomin D_3, which is a biologically active vitamin D. Then, 1,25-dihydroxyvitamin D is released into circulation to exert its effects on the target cells and promote calcium and bone homeostasis. Thus, vitamin D is a hormone and, like the pituitary-thyroid axis, has a complex natural history in the body (Table 1).

The half-life of thyroid hormone depends upon thyroid status[17]. The half-life for levothyroxine (T4) is 6-7 d in euthyroid subjects, 9-10 d in subjects with hypothyroidism, and 3-4 d in subjects with hyperthyroidism. The half-life of liothyronine (T3) is 18-24 h in euthyroid subjects, 12-16 h in hyperthyroid subjects, and 26-32 h in hypothyroid subjects. The half-life of vitamin D averages 15 h but depends upon of the type of vitamin D (cholecalciferol or vitamin D_3 *vs* ergocalciferol or vitamin D_2) and vitamin D binding protein concentration[18]. The half-life of 1,25-dihydroxyvitamin D is 10-20 h[19], while there is no information regarding the half-life of 1,25-dihydroxyvitamin D_3 *vs* D_2. Since 1,25-dihydroxyvitamin D is released into the blood and exerts its effects upon osteocytes to promote mineralization and on the gastrointestinal epithelium to increase calcium and phosphorus absorption, it is appropriate to classify vitamin D as a hormone.

EVIDENCE OF NON-CALCEMIC EFFECTS

In addition to the target organs, both the vitamin D receptor and 1alpha-hydroxylase (CYP27B1) are expressed in various other tissues[20], suggesting additional functions of vitamin D beyond bone metabolism and calcium homeostasis. Interestingly, the vitamin D receptor is expressed in the pancreatic islets[21], liver[22], muscle[23], and adipose tissue[24]. 1alpha-hydroxylase (CYP27B1) is expressed in pancreatic islets[25], liver[26], muscle[27], and adipose tissue[28]. Thus, it is possible that vitamin D could take part in glucose and fuel homeostasis.

In contrast to calcemic effects of vitamin D which is primary mediated by circulating 1,25-dihydroxyvitamin D produced in the kidney, the non-calcemic effects of vitamin D are mediated by circulating 25-hydroxyvitamin D through a paracrine or autocrine function[29]. Within the target cells or its vicinity, circulatory 25-hydroxyvitamin D

Table 1 Vitamin D as a hormone: Comparison of the pituitary-thyroid and parathyroid hormone-vitamin D axes

	Pituitary-thyroid axis	Parathyroid-vitamin D axis
Organ(s)	Thyroid glands	Skin/liver/kidney
Source compound	Iodine, tyrosine	Cholecalciferol (cholesterol), ergocalciferol
Prehormone	Levothyroxine, $T_{1/2}$ = 6-7 d	25-hydoxyvitamin D_2/D_3, $T_{1/2}$ = 13-17 d
Active hormone	Triiodothyronine, $T_{1/2}$ = 14-24 h	1,25-dihydroxyvitamin D_2/D_3, $T_{1/2}$ = 10-20 h
Transportation	Thyroxine binding globulin	Vitamin D binding protein
Receptor	Thyroid hormone receptor	Vitamin D receptor
Stimulating factor	Thyroid stimulating hormone	Parathyroid hormone
Effect	Energy homeostasis	Calcium homeostasis

enters cells and is converted to 1,25-dihydroxyvitamin D by the locally existing 1alpha-hydroxylase (CYP27B1). Hence, 25-hydroxyvitamin D is the key circulatory element for the non-calcemic effects of vitamin D whereas 1,25-dihydroxyvitamin D the promotes the calcemic effects.

EFFECTS UPON CELL DIFFERENTIATION AND CELL PROLIFERATION

Colon, prostate, breast, and ovarian cancer

A role for vitamin D in the pathogenesis of cancer was proposed in 1980[30] after it was observed that colon cancer rates were higher in the northern rather than the southern United States. The association of vitamin D deficiency with cancer, including breast[31], prostate[32], and colon cancer[33] was attributed to the ability of vitamin D to differentiation cells[34] and to suppress cell proliferative[35] along with other effects [36,37].

Immunity, autoimmunity, and inflammation

The risk of type 1 diabetes was reduced by vitamin D supplement in a birth-cohort study from Finland[38]. Furthermore, a polymorphism in the vitamin D receptor was associated with increased risk of type 1 diabetes[39]. Not unexpectedly, a role of vitamin D deficiency in the pathogenesis of type 1 diabetes was proposed[40]. In addition, the association of vitamin D deficiency with multiple sclerosis[41], systemic lupus erythematosus[42], and other autoimmune diseases[43] was attributed to the immunomodulatory and anti-inflammatory effects of vitamin D[44]. Furthermore, vitamin D plays an important role in the maintenance of B cell homeostasis[45], and vitamin D replacement may reduce B cell-mediated autoimmune disorders.

The role of vitamin D in the treatment of tuberculosis was appreciated with the observation that sun exposure altered the clinical presentation of tuberculosis[46]. Subsequently, vitamin D was administered as part of the treatment of tuberculosis [47]. Vitamin D deficiency was frequently observed in patient with untreated tuberculosis[48]. It is now known that Toll-like receptors up-regulate expression of the vitamin D receptor and the vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular Mycobacterium tuberculosis[49]. Thus, the role of vitamin D in fighting infection is established[50]. Further, vitamin D deficiency is associated with acute respiratory tract infection[51], bacterial vaginosis[52], pneumonia[53], foot infection in diabetics[54], chronic hepatitis C infection[55], and human immunodeficiency virus infection[56]. Recently, vitamin D deficiency was recognized as a risk factors for COVID-19 infection[57-61]. Thus, vitamin D could play a role in fighting infection.

An association between vitamin D receptor polymorphism and the severity of coronary artery disease was reported[62]. Deficiency was also noted to associate with an increased risk of myocardial infraction[63], hypertension[64], and stroke[65]. The mechanism proposed to account for these associations included activation of the renin-angiotensin system[66], coronary calcification[67], platelet activation and aggregation [68], increased proinflammatory cytokines[69], and vascular endothelial dysfunction [65].

Fuel metabolism

In patients with vitamin D deficiency and diabetes, vitamin D supplementation improved beta cell function and glucose tolerance[70]. An association between vitamin D deficiency and glucose intolerance and beta cell dysfunction was observed in east London Asians[71]. Similarly, alternations in vitamin D metabolism in obese subjects manifesting as low 25-hydroxyvitaimin D is well-recognized[72]. This topic will be reviewed in this article.

Neuropsychiatric disorders

Vitamin D deficiency was reported to be associated with depression[73], schizophrenia [74], autism[75], and Parkinson's disease[76]. Various mechanisms have been reported to support a role of vitamin D in neuropsychiatric disorders. Vitamin D has a protective effect on dopaminergic neurons[77]. Vitamin D deficiency could result in altered synaptic plasticity through its effect on perineuronal nets leading to cognitive deficits[78]. Vitamin D deficiency alters brain protein expression in rats[79]. Furthermore, immunohistochemical study revealed the expression of vitamin D receptor and 1alpha-hydroxylase (CYP27B1) in various regions of human brain with the strong expression in the hypothalamus and in the large (presumably dopaminergic) neurons within the substantia nigra[80]. Thus, vitamin D deficiency could play a role in the pathogenesis of various neuropsychiatric disorders.

VITAMIN D REPLACEMENT THERAPY

Source of vitamin D

Vitamin D is available in two forms: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Ergocalciferol comes from plants in the form of ergosterol (provitamin D_2). Ergosterol is an important component of mushrooms. Through ultraviolet b (UVB) irradiation, which can occur within mushroom or artificially, it becomes ergocalciferol [81]. Cholecalciferol comes from animals and people through the biosynthesis of cholesterol to 7-dehydrocholesterol (Provitamin D_3). Again, through UVB irradiation, this intermediate becomes cholecalciferol. Thus, dietary intake and sun exposure are the major determinants of serum 25-hydroxyvitamin D levels.

Sun, mainly UVB irradiation, plays an important role in biosynthesis of vitamin D. Since 7-dehydrocholesterol can be synthesized from cholesterol, theoretically vitamin D supplementation is not required once sun exposure is adequate. Skin color is a key determinant of vitamin D synthesis[82]. Vitamin D has been proposed to play a role in human evolution and migration away from equator by affecting skin color through the development of depigmented and tannable skin *via* genetic pathways under positive selection[1,83]. Sun exposure is highly effective in raising serum 25-hydroxyvitamin D concentration, while its effects diminish significantly on donning clothing and using sun screen[84]. In this regard, more body surface area exposure is more effective than longer exposure time[85]. However, the efficacy of sun exposure to increase serum 25-hydroxyvotamin D concentrations diminishes with the degree of skin tanning[86]. Thus, minimized sun exposure time for 5 min to 30 min (depending on time of day, season, latitude, and skin pigmentation) with maximize body surface exposure is recommended[9]. However, increased risk of sun-mediated skin cancer makes this approach to prevent vitamin D deficiency less optimum[87].

Vitamin D can be obtained through dietary intake. However, except for cod liver oil, vitamin D content in naturally occurring food is relatively low, even in mushrooms (Table 2). Although ergosterol is highly abundant in the membrane of mushrooms, mushroom are cultivated under shadow without UVB irradiation[81]. Thus, dietary intake of vitamin D is inadequate and vitamin D supplement is often needed to avoid deficiency.

Comparison of metabolism of vitamin D_2 vs vitamin D_3

It is estimated that 65% of vitamin D is present as vitamin D while 35% is in the form of 25-hydroxyvitaomn D. As well, almost 75% of vitamin D is in adipose tissue, while 25-hydroxyvitamin D is distributed 20% in muscle, 30% in serum, 35% in fat, and 15% in other tissues[88]. The metabolism of vitamin D_3 and vitamin D_2 is summarized in Table 3. Vitamin D binding protein transports the various forms of vitamin D in circulation, including vitamin D, 25-hydroxyvtamin D, and 1,25-dihydroxyvitamin D [89]. Each vitamin D binding protein molecule has one binding site for vitamin D and/or its metabolites. The relative affinity of vitamin D binding protein to vitamin D_3

Table 2 Vitamin D content of selected foods

Food	Per serving		Percent DV
	IU	μg	
Cod liver oil, 1 tablespoon	1360	34.00	170
Trout (rainbow), farmed, cooked, 3 ounces	645	16.13	81
Salmon (sockeye), cooked, 3 ounces	570	14.25	71
Mushrooms, white, raw, sliced, exposed to UV light, 1/2 cup	366	9.15	46
Milk, 2% milkfat, vitamin D fortified, 1 cup	120	3.00	15
Soy, almond, and oat milks, vitamin D fortified, various brands, 1 cup	100-144	2.50-3.60	13-18
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 1 serving	80	2.00	10
Sardines (Atlantic), canned in oil, drained, 2 sardines	46	1.15	6
Egg, 1 large, scrambled (Vitamin D is in the yolk)	44	1.10	6
Liver, beef, braised, 3 ounces	42	1.05	5
Tuna fish (light), canned in water, drained, 3 ounces	40	1.00	5
Cheese, cheddar, 1 ounce	12	0.30	2
Mushrooms, portabella, raw, diced, ½ cup	4	0.10	1
Chicken breast, roasted, 3 ounces	4	0.10	1
Beef, ground, 90% lean, broiled, 3 ounces	1.7	0.04	0

Adapted from: https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/#en25. The Food and Drug Administration developed daily values (DVs) to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. The DV for vitamin D on the new Nutrition Facts and Supplement Facts labels used for the values in Table 2 is 20 μg (800 IU) for adults and children aged 4 years and older. Foods providing 20% or more of the DV are considered to be high sources of a nutrient, but foods providing lower percentages of the DV also contribute to a healthful diet. DV: Daily value.

Table 3 Comparison of transportation and metabolism of vitamin D_3 *vs* D_2

Ref.	Symbol	Name (chromosome location)	Function	D3/D2
Haddad *et al*[90], 1993	VBP	Vitamin D binding protein (4q12-q13)	Vitamin D transportation	1.14
Holmberg *et al*[91], 1986	*CYP2R1*	25-hydroxylase (11p15.2)	Conversion of vitamin D to 25-hydroxy vitamin D	5.0
Zarei *et al*[93], 2016	*CYP27B1*	1alpha-hydroxylase (12q13.1-q13.3)	Conversion of 25(OH)D to 1,25(OH)2D	2.4
Jones *et al*[94], 1980	VDR	Vitamin D receptor (7q36)	Receptor for vitamin D	1.3

is 1.14 times stronger than to vitamin D_2[90]. 25-hydroxylase (CYP2R1) catalyzes 25-hydroxylation of vitamin D_3 5 times more efficiently than vitamin D_2[91]. Thus, after administration of a single oral dose of vitamin D_3 and vitamin D_2, a more sustainable and prolonged increase in serum 25-hydroxybitamin D_3 concentration is observed compared to serum 25-hydroxybitamin D_2 concentration[92]. 1alpha-hydroxylase (CYP27B1) coverts 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 2.4-time more efficiently than 25-hydroxyvitamin D_2[93]. In receptor binding assays, 1,25-dihydroxyviramin D_3 has 1.3 times more receptor affinity than 1,25-dihdroxyvitamin D_3[94]. These data indicate that vitamin D_3 is more biologically potent than vitamin D_2.

Comparison of biological potency of vitamin D_2 vs vitamin D_3

Vitamin D_2 and vitamin D_3 were reported to have similar efficacy in raising serum 25-hydroxyviramin D concentration[95]. However, other studies demonstrated that vitamin D_3 was more efficacious at raising serum 25(OH)D concentrations than vitamin D_2[96-100]. This finding was confirmed by a meta-analysis of the randomized

control trials[101]. Furthermore, 25-hydroxyvitamin D_3 has a longer half-life compared to 25-hydroxyvitamin D_2 (15.1 ± 3.1 d *vs* 13.9 ± 2.6 d, P = 0.001, mean ± STD)[18]. In comparison to oral vitamin D_2, oral vitamin D_3 achieves a higher serum concentration of 1,25-dihydroxyvitamin D[100,102] and a more effective suppression of serum parathyroid hormone concentration[97]. Physicians preferring use of vitamin D_2 should be aware of its markedly lower potency and shorter duration of action when compared to vitamin D_3. Thus, vitamin D_3 is the preferred form of vitamin D for replacement therapy.

OPTIMAL SERUM 25-HYDROXYVITAMIN D CONCENTRATION

Minimal serum 25-hydroxyvitamin D concentration

The primary function of vitamin D is to maintain calcium homeostasis. The minimal serum 25-hydroxyvitamin D concentration for health was defined based on the serum parathyroid hormone response to replacement therapy with ergocalciferol[103]. A serum 25-hydroxyvitamin D concentration of 50 nmol/L (20 ng/mL) was recommended since no further changes in serum parathyroid hormone levels were found in subjects with a serum 25-hydroxyvitamin D level of 50 nmol/L (≥ 20 ng/mL). In 2010, the United Sates Institute of Medicine adapted this value as a target for ensuring good bone health[104]. However, based on a larger observational study with 1569 subjects in France, serum parathyroid hormone concentration were noted to still decrease when the serum 25-hydroxyvitamin D rose to 78 mmol/L (31 ng/mL)[105]. Furthermore, a serum 25-hydroxyvitamin level of 75 nmol/L (30 ng/mL) is a recognized threshold for intestinal calcium absorption[106]. As shown in Table 4, many professional organizations and agencies have since adapted 75 nmol/L (30 ng/mL) as the minimal acceptable serum 25-hydroxyvitamin D concentration recognizing this may have beneficial effects beyond bone health, targeting beyond bone health while the Institute of Medicine define the minimal 25-hydroxyvitamin D concentration 50 nmol/L (20 ng/mL) on bone health with a public health interest.

Maximal serum 25-hydroxyvitamin D concentration

The maximal allowed serum 25-hydroxyvitamin D concentration is defined by the appearance of adverse effects. Although the Institute of Medicine dose not define maximal serum 25-hydroxyvitamin D concentration[104], a warning against elevated serum 25-hydroxyvitamin D concentrations is stated. This warning is based upon the observed association of increasing mortality with serum 25-hydroxyvitamin D concentration > 125 nmol/L (50 ng/mL)[107] by limiting the maximal daily vitamin D allowance (Table 4). This notion was further supported by the finding of increased cardiovascular mortality with serum 25-hydroxyvitaminD > 125 nmol/L (50 ng/mL)[108]. In addition, a progressive decline in bone mineral density with serum 25-hydroxyvitamin D greater than 125 nmol/L (50 ng/mL) was observed in a United States population[109]. Conversely, bone mineral density improved after discontinuation of vitamin D supplementation in patients with a serum 25-hydroxyvitamin D concentration greater than 50 ng/mL[110]. Although vitamin D supplementation increased calcium absorption without a threshold effect[111], reanalysis of the data revealed a diminished response (per 1000 IU of vitamin D in Table 5) with increasing dose of vitamin D supplement suggesting a threshold effect of vitamin D on calcium absorption[112], something noted by others[106]. We reported lack of improvement in insulin sensitivity in individuals with a serum 25-hydroxyvitamin D concentration > 125 nmol/L (50 ng/mL)[113]. Although hypercalcemia from vitamin D intoxication occurs mainly when the serum 25-hydroxyvitamin D concentration is > 374 nmol/L (150 ng/mL)[114], serum 25-hydroxyvitamin D concentrations > 75 nmol/L (50 ng/mL) could be either harmful or lack beneficial effect.

Comparison of daily replacement vs intermittent replacement of vitamin D

The observation that a single oral dose of vitamin D_3 2.5 mg (100000 IU) can maintain serum 25-hydroxyvitamin D above the target goal[115] provides a unique dosing strategy of vitamin D replacement therapy with greater adherence. It could even ensure 100% compliance if given by or under the direct supervision of a health care provider. Weekly[103], monthly[116], biyearly[117], and even yearly[118] schedules were reported in various trials leading to initiation of more convenient dosing schedule at less frequent intervals in clinical practice. To reduce the dosing frequency, a much higher dose of vitamin D is required which is predicted to cause a short-term spike (> 75 nmol/L or 50 ng/mL) in serum 25-hydroxyvitamin D concentration shortly

Table 4 Recommended daily vitamin D intake as promulgated by selected organizations and agencies

Organization	Daily intake		Goal	
	IU	μg	ng/mL	nmol/L
Institute of Medicine	600-800	15-20	> 20 (20-50)	> 50 (50-125)
Agency of Healthcare Research and Quality, Department of Health and Human Services	> 1000	> 25	> 30	> 75
Office of Dietary Supplements, NIH	600-800	15-20	20-50	50-125
National Osteoporosis Foundation	800-1000	20-25	> 30	> 75
American Association of Clinical Endocrinologists	1000-2000	25-50	30-60	75-150
Endocrine Society	1500-2000	37.5-50	30-100	75-250

Table 5 Diminished response of intestinal calcium absorption in response to increasing vitamin D supplementation

Daily vitamin D supplementation		Observed increase in calcium absorption	Estimated increase in calcium absorption per 1000 IU (25 μg)
IU	μg		
800	20	3.90%	4.88%
2000	50	5.00%	2.50%
4000	100	6.70%	1.68%

after oral administration. In addition to the adverse effects as described in the above section 4.2, increased falls and fracture are observed with annual vitamin D replacement therapy. These mainly occur within the first 3 mo after oral administration of 12.5 mg vitamin D_3[118]. Furthermore, the associations of high-dose vitamin D treatment with gastrointestinal complaints[119], increased bone turnover markers [120], hypercalcemia[121], hypercalciuria[122], and increased urinary magnesium loss [123] have been reported. Similar levels of serum 25-hydroxyvitamin D concentration were achieved at the end of a 56-d trial from daily (1500 IU/d), weekly (10500 IU/wk), and monthly (45000 IU/4 wk) replacement therapy. Excessive serum 25-hydroxyvitamin D concentration was not observed in those on the daily regimen but was observed in individuals on the weekly regimen and was still more common in those on monthly regimen[124]. Thus, high-dose vitamin D replacement therapy results in excessive serum 25-hydroxyvitamin D concentration.

A Lysine (K) amino acid polymorphism, in replacement of Threonine (T), at position 436 of vitamin D binding protein is associated with increased affinity of vitamin D and is associated a 416% elevation in serum 25-hydroxyvitamin D concentration if high-dose (4000 IU) vitamin D_3 replacement therapy if given as opposed to low-dose (600 IU) vitamin D_3 replacement therapy. Individuals carrying the TT SNP showed only a 136% increase in circulating vitamin[125]. Since the K allele is a minor allele and KK genotype accounts for less than few percent of population, the KK subjects may account for the excessive serum 25-hydroxyvitamin D-associated complications noted in certain studies. Given the above, daily vitamin D supplementation would seem to be most physiological and safest way to correct vitamin D deficiency and avoid the possible adverse effects associated with the excessive serum 25-hydroxyvitamin D concentration.

Factors affecting serum 25-hydroxyvitamin D concentration

Various genetic loci are associated with serum 25-hydroxyvitamin D concentration [126] with 4 major loci identified (Table 6). These are all key proteins involved in the transportation and metabolism of vitamin D. Race and ethnicity were noted to have significant impact on serum 25-dihyrdroxyvitamin D concentration[127], again implicating a genetic influence[126] including skin color[128].

Seasonable variations in serum 25-hydroxyvitanim D concentrations related to sun exposure are well described[126]. Consistent with this, latitude has a significant impact on serum 25-dihydrocyvitamin D concentration[129]. Living closer to the equator and increasing sun exposure can improve vitamin D levels. However, the increased risk of skin cancer from sun exposure should be balanced employing maximum skin

Table 6 Major loci associated with changes in serum 25-hydroxyvitamin D concentration

Chromosome	SNP	Gene symbol	Protein	*P* value
4p12	rs2282679	GC	Vitamin D binding protein	1.9×10^{-109}
11q12	rs12785878	DHCR7	7-dehydrocholsterol reductase	2.1×10^{-27}
11p15	rs10741657	CYP2R1	1-alpha-hydroxylase	3.3×10^{-20}
20q13	rs6013897	CYP24A1	1,25-dihydroxyvitamin D3 24-hydroxylase	6.0×10^{-10}

Adapted from Wang *et al*[126]. SNP: Single nucleotide polymorphism.

exposure area with decreased exposure time[85]. Dietary supplementation also corrects deficiency. Obesity is associated with a lower serum 25-hydroxyvitamin D concentration[72] while weight reduction with loss of adipose tissue is associated with improvement in serum 25-hydroxyvitamin D concentration[130]. These findings indicate that vitamin D status may be improved through modification of lifestyle.

Practical recommendations for vitamin D replacement therapy

As showed in Table 4, the recommended vitamin D supplement varies between organizations and agencies. The reasons for this relate to the purpose of vitamin D supplementation, visive calcemic *vs* non-calcemic effects. For calcemic effects, bone health is the goal of supplementation and is maximized through using a conservative daily vitamin D to achieve the minimal serum 25-hydroxyvitamin D concentration while avoiding possible adverse effects associated with overreplacement. A public health approach to this is displayed in Table 7. In contrast, a more personized approach is rationale when the target is to promote the non-calcemic effects of vitamin D.

We recommend using vitamin D_3, instead of vitamin D_2, for the rationale as discussed in the sections 3.2 and 3.3. We are in favor of daily replacement therapy and against intermittent mega dose replacement. This is supported by the recommendations of the Endocrine Society for indefinitely intermittent mega dose replacement [131]. It has been estimated that supplement with cholecalciferol 1000 IU (50 μg) daily will increase serum 25-hydroxyvitamin D concentration by 10 ng/mL[132]. Since vitamin D is a fat soluble, replacement therapy can be further enhanced by taking it with the largest meal of day[133]. We recommend vitamin D_3 1000 IU daily for achievement of an initial serum 25-hydroxyvitamin D concentration between 51 nmol/L (21) ng/mL and 75 nmol/L (30 ng/mL); 2000 IU daily for between 26 nmol/L (11 ng/mL) and 50 nmol/L (20 ng/mL); and 5000 IU for equal or less than 25 nmol/L (10 ng/mL). Serum 25-hydroxyvitamin concentration should be measured within 3 mo for assessment and, if indicated, dose adjustment. We are targeting serum 25-hydroxyvitamin D concentration between 75 nmol/L (30 ng/mL) and 125 nmol/L (50 ng/mL).

VITAMIN D AND DIABETES PREVENTION

Vitamin D diabetes prevention trials

To date, eight clinical trials employed vitamin D to reduce prediabetes progression to overt diabetes (Table 8). Only two studies[134,135] demonstrated positive results. Although these two studies had small sample size, they recruited true vitamin D deficient (25-hydroxyvitamin D < 50 nmol/L or 20 ng/mL) subjects and achieved final 25-hydroxyvitamin D concentration at 89-90 nmol/L, after intervention for 1 year and 6 mo, respectively. Of note, the study in India[134] was a randomized open label study demonstrating an odds ratio of 0.31 [95% confidence intervals (CI): 0.11-0.90]. The study in Iran was a randomized placebo control study[135] revealing an odds ratio of 0.06 (95%CI: 0.01-0.51). Because of relatively small sample sizes of both studies, the CI were very wide. Additional studies with similar initial and final 25-hydroxyvitamin D concentration (< 50 nmol/L and 90-100 nmol/L, respectively) and much larger sample sizes are required to confirm these data.

Two negative studies[136,137] were noted to have similar initial 25-hydroxyvitamin D concentrations (25-42 nmol/L). The negative results could be due to the relatively short interventions (8-16 wk) and small sample sizes. The study in Holland only

Table 7 Vitamin D supplementation versus vitamin D replacement therapy

	Vitamin D supplement	Vitamin D replacement therapy
Target goal	Bone health	Beyond bone health
Target 25-hydroxyvitamin D level	> 20 ng/mL (50 nmol/L)	> 30 ng/mL (75 nmol/L)
Initial testing for 25-hydroxyvitamin D level	No	Yes
Concern of over-replacement	Yes	Yes
Follow-up testing for 25-hydroxyvitamin D level	No	Yes
Dose adjustment	No	Yes
Approach	Public health	Individualized

Table 8 Preventive trials of vitamin D supplementation to prevent the development of type 2 diabetes

Ref.	Country Race/ethnicity	Placebo control			Intervention			Dose	Frequency	Duration	Diabetes prevention
		n	25(OH)D nmol/L		*n*	25(OH)D nmol/L					
			Initial	Final		Initial	Final				
Dutta *et al*[134], 2014[1]	IndiaAsian Indian	49	45	44	55	43	89	1500 μg	Weekly X 8, monthly	1 yr	Positive[2]
Niroomand *et al* [135], 2019	IranIranian	83	32	40	83	31	90	1250 μg	Weekly for 3 mo, monthly	6 mo	Positive[3]
Wagner *et al* [136], 2016[4]	Sweden	22	47	46	21	42	83	750 μg	weekly	8 wk	Negative
Oosterwerff *et al* [137], 2014	HollandNon-Western	65	22	23	65	25	60	30 μg	daily	16 wk	Negative
Barengolts *et al* [141], 2015[5]	United States African American	86	35	50	87	37	120	1250 μg	weekly	12 m	Negative
Davidson *et al* [139], 2013[6]	United States Latino and African American	53	55	60	56	55	167	2222 μg	weekly	12 mo	Negative
Jorde *et al*[140], 2016	Norway	255	61	64	256	60	110	500 μg	weekly	5 yr	Negative
Pittas *et al*[138], 2019	United States mixed	1212	70	72	1211	69	136	100 μg	daily	24 mo	Negative

[1]This study was an open label randomized design, instead of randomized placebo-control design as other studies.
[2]Intervention is associated with significantly lower progression to diabetes (11% *vs* 27%; $P = 0.04$) and higher reversal to normoglycemia (43% *vs* 20%; $P = 0.02$).
[3]The rate of progression toward diabetes was significantly lower in the intervention group (3% *vs* 28%; $P = 0.002$).
[4]Meadian 25-hydroxyvitamin was provided, rather than mean 25-hydroxyvitamin D as in other studies.
[5]Ergocalciferol was used, rather than cholecalciferol in other studies.
[6]Weekly dose of cholecalciferol was adjusted to titrate serum 25-hydroxyvitamin D between 162 nmol/L and 200 nmol/L.

achieved a final suboptimal 25-hydroxyvitamin D concentration of 60 nmol/L.

The other four studies[138-141] had a final 25-hydroxyvitamin D concentration > 100 nmol/L which might not be optimal for glucose metabolism. Among them, the study in African American[141] was the only study that recruited true vitamin D deficient subjects (initial 25-hydroxyvitamin D 37 nmol/L). Of note, ergocalciferol was used which could be less effective biologically as discussed above in 3.2 and 3.3. Enrollment of non-vitamin D deficient (25-hydroxyvitamin D < 50 nmol/L) subjects [138-140] could further reduce the chance of finding any effect. Furthermore, the study in Norway had a significant dropout rate in the interventional group with only 45% of participants completing the planned 5-year visit. The largest intervention trial[138] included more than 1000 subjects in each group. To be able to apply to the general population in the United States, this study did not target vitamin D deficient subjects and allowed the participants to take additional vitamin D up to 25 μg daily. Therefore, it had the highest initial 25-hydroxyvitamin D among these studies, 70 nmol/L in the

control group and 69 nmol/L in the interventional group, which might diminish the power of this study to detect the beneficial effect of vitamin D. Regardless of the negative results in most studies, the beneficial effect of vitamin D supplementation cannot be completely excluded, especially in subjects with vitamin D deficiency (25-hydroxyvitamin D < 50 nmol/L).

The effects of vitamin D supplement on parameters of glucose metabolism

Various parameters of glucose metabolism were reported in most of above-mentioned studies, except one[138]. After vitamin D intervention for 1 year, the study from India [134] observed improvement in fasting and 2-hr post-challenge glucose concentrations, insulin sensitivity by Homeostasis Model (HOMA) insulin resistance index, QUICKI, and 1/fasting insulin concentration while no impact on HbA1c and beta cell function by HOMA. Following vitamin D supplement for 6 mo, the study from Iran[135] reported the improvement in the HOMA insulin resistance index and marginal improvement in fasting insulin concentration (P = 0.05) and 2-hour post-challenge blood glucose concentration (P = 0.07) with no impact on fasting blood glucose concentration.

After an 8-wk intervention, the study from Sweden[136] assessed insulin sensitivity and beta cell function using the hyperglycemic clamp. They observed a significant improvement in deposition index based on the first phase insulin response (P = 0.005) and marginal improvement in first phase insulin response (P = 0.06), insulin sensitive index (P = 0.09), deposition index based on the second phase insulin response (P = 0.06), and A1c (P = 0.06) but no impact on the second phase insulin response and fasting and 2-hr post-challenge blood glucose concentration.

In contrast, the study from Holland[137] evaluated glucose metabolism parameters based on the 75-g glucose tolerance test following intervention for 16 wk. They reported negative results, finding no effects upon insulin area under curve, glucose area under curve, insulin sensitivity by composite insulin sensitivity index, Stumvoll index, insulin resistance index by HOMA, and beta cell function by insulinogenic index. Of note, the final 25-hydroxyvitamin D concentration was only 60 nmol/L which could be suboptimal for glucose metabolism. Similarly, after the vitamin D supplementation for 5 years, the study from Norway[140] observed no impact on fasting and 2-hr post-challenge serum glucose concentration, fasting and post challenge serum insulin concentration, fasting serum C-peptide concentration, HbA1c, and insulin sensitivity by HOMA insulin resistance index and QUICKI.

Following a 12-mo intervention, the study involving Latino and African Americans [139] observed a significant improvement in HbA1c but no effects on fasting and 2-hr post-challenge blood glucose concentration, beta cell function by the ratio of insulin and glucose area under curve, Stumvoll first and second insulin response, insulinogenic index, insulin sensitivity index by HOMA insulin resistance index and composite insulin sensitivity index, and oral disposition index. However, a significant improvement in composite insulin sensitivity index but not Matsuda index, insulinogenic index, C-peptidogenic index, and HbA1c was noted.

Excepting two studies[137,140] with negative results, favorable outcomes on parameters of glucose metabolism were reported in five studies[134-136,139,141] suggesting some benefits to supplementation under these conditions.

Summary of vitamin D and diabetes prevention

In vitamin D deficient (25-hydroxyvitamin D < 50 nmol/L) prediabetic subjects, vitamin D supplement appears to be effective in reduction of the development of overt diabetes. However, there appears to be no benefit in vitamin D sufficient subjects, which was noted in a study from Norway[142]. Based on pooled data from four intervention trials, in subjects without vitamin D deficiency there is no improvement in glucose metabolism with high dose vitamin D supplementation and if anything, the effect is negative[143]. This notion is consistent with the observed threshold effect of vitamin D on bone health and lack of benefit in subjects with baseline 25-hydroxyvitamin D level ≥ 75 nmol/L (30 ng/mL)[14,15].

LABORATORY EVIDENCE SUPPORTING THE EFFECT OF VITAMIN D ON GLUCOSE AND FUEL HOMEOSTASIS

Beta cell function

Functional beta cell studies: The important role of vitamin D on insulin secretion has

been noted in laboratory animals since 1980. Insulin secretion was reduced by about 50% in isolated perfused islets from vitamin D-deficient rats compared to controls [144]. Interestingly, 1,25-dihydroxyvitamin D_3 was noted in cell nuclei in the islets of langerhans[145]. Furthermore, administration of 1,25-dihydroxyvitamin D3 to vitamin D-deficient rats improved insulin secretion significantly when compared to controls [146]. Vitamin D deficiency impaired both phases of insulin release in rats while correction of hypocalcemia failed to reverse the defect in insulin release[147]. Vitamin D, but not calcium, was essential for normal insulin secretion from the perfused rat pancreas[148]. The positive effect of single dose of 1,25-dihydroxyvitamin D_3 on insulin secretion was apparent at 8 h in perfused rat pancreata, peaked at 14 h, and then decreased to pretreatment baseline values by 36 h[149]. Dietary vitamin D3 supplementation improved impaired glucose tolerance and insulin secretion in the vitamin D-deficient rats[150]. A dose-dependent effect from parenteral 1,25-dihydroxyvitamin D on insulin secretion and glucose metabolism was observed within 3 h and remained effective up to 20 h in the vitamin D-deficient rats[151]. The role of vitamin D on insulin synthesis and secretion was supported by studies in vitamin D receptor knockout mice. Insulin secretory capacity was reduced by 60% in vitamin D receptor knockout mice[152] with increased post-challenged blood glucose but normal fasting blood glucose concentration and reduced insulin mRNA levels in pancreatic islets but normal pancreatic beta cell mass, islet architecture, and islet neogenesis when compared to wild type mice. Thus, vitamin D plays an important role in pancreatic insulin synthesis and secretion in vivo.

Mechanistic studies of beta cell function: Although the essential role of vitamin D on insulin secretion has been established in vitamin D depleted laboratory animal, details of the underlying molecular mechanism remain to be defined. Employing a proteomic approach, treatment with 1,25-dihydroxyvitamin D_3 resulted in 31 differentially expressed proteins in INS-1 beta-like cells[153] with 29 upregulated, some of which were implicated in insulin granule motility and insulin exocytosis as well as regulation of ions. Pretreatment of INS1E cells with 1,25-dihydroxyvitamin D or 25-hydroxyvitamin D and glucose resulted in 526 and 181 differentially expressed genes, respectively[154].

Several molecular mechanisms were proposed to account for the effects of vitamin D on beta cells, including changes in the local pancreatic islet renin-angiotensin system [155], restoration of GLUT2 expression[156], enhancement of IP3 and AMPA receptor expression[157], vitamin D-binding protein-induced beta cell dedifferentiation[158], reduction of oxidative damage[159], reduced cholinergic pancreatic effects[160], enhanced transcriptional regulation of voltage-gated calcium channels[161], and elevation of PPAR-γ expression[162]. However, further studies are required to confirm the proposed mechanisms.

Insulin sensitivity

Functional studies of insulin sensitivity: In contrast to beta cell function, there are fewer studies of insulin sensitivity. Dietary supplementation of vitamin D improved insulin sensitivity, hepatic steatosis, and myocardial fibrosis in Western diet fed rats [163]. In dietary-induced obese mice, vitamin D receptor activation in liver macrophages improved insulin sensitivity with reduction of hepatic inflammation and steatosis[164]. Vitamin D treatment improved insulin resistance index in a nongenetic model of type 2 diabetes[165]. However, vitamin D status were not reported in these studies.

Mechanistic studies of insulin sensitivity: Chronic central administration of 1,25-dihydroxyvitamin D_3 dramatically reduced body weight, putatively by lowering food intake, in obese rodents[166]. Treatment with vitamin D increased mitochondrial function and insulin sensitivity, in part, through upregulation of perilipin 2, a perilipin protein upregulated with 1,25-dihydroxyvitamin D treatment[167]. In skeletal myocytes, vitamin D reduced insulin resistance by altering lipid partitioning and lipid droplet packaging in favor of lipid turnover[168]. FGF-23 knockout mice are hypoglycemic with profoundly increased peripheral insulin sensitivity and improved subcutaneous glucose tolerance. Ablation of vitamin D signaling in these mice normalized subcutaneous glucose tolerance tests and insulin sensitivity[169]. Caveolin-1 protein, which is necessary for vitamin D signaling, could play a role in vitamin D-induced insulin sensitivity in skeletal muscle[170]. In cultured rat osteoblasts, 1,25-dihydroxyvitamin D_3 treatment increased expression of the insulin and vitamin D receptors, and elevated osteocalcin levels under high glucose exposure [171], which may in turn improve insulin sensitivity.

However, the results of vitamin D receptor knockout mice were less uniform. Skeletal muscle-specific vitamin D receptor knockout mice developed insulin resistance and glucose intolerance accompanied by increased expression and activity of FOXO1[172]. Deletion of macrophage vitamin D receptor promoted insulin resistance and monocyte cholesterol transport and accelerated atherosclerosis[173]. In contrast, deletion of the vitamin D receptor gene in endothelial cells improved glucose tolerance and insulin sensitivity in skeletal muscle and reduced expression and secretion of insulin in pancreatic islets[174]. Together these data indicate that vitamin D has positive and negative effects on insulin sensitivity that are cell and organ specific.

CONCERNS ARISING WITH REPORTED STUDIES

Lack of true vitamin D deficient subjects

Due to publicity and potential non-calcemic benefits of vitamin D supplementation, the sale of vitamin D supplements increased significantly and taking vitamin D supplements is common. Thus, there are less true vitamin D deficient subjects available for inclusion in clinical trials. As well, a general lack of funding support for large trials impedes addressing the ability of researchers to address the gaps in knowledge surrounding vitamin D and its beneficial effects.

Lack of beneficial effects from suboptimal replacement and detrimental effects of over-replacement

To obtain the maximal effect of vitamin D, serum 25-hydroxyvitamin D concentration should be maintained in an optimal range, namely between 75 nmol/L (30 ng/mL) and 125 nmol/L (50 ng/mL). Inadequate vitamin D replacement therapy will reduce the chance to observe the expected beneficial effect of vitamin D while adverse effects associated with excessive serum 25-hydroxyvtamin D concentration will also cloud data interpretation. Although mega doses of vitamin D given intermittently could improve compliance in a study protocol, the predicted wide swings in serum 25-hydroxyvitamin D concentrations will confound outcomes. It is important in clinical studies to use a proper daily dose to avoid these pitfalls.

Inadequate sample size

The Diabetes Prevention Program demonstrated a 58% (95%CI: 48%-66%) reduction in the incidence of diabetes in the lifestyle intervention group (cumulative incidence of diabetes 14.4% in 1079 participants) and a 31% reduction in diabetes (95%CI: 17%-43%) in the metformin treated group (cumulative incidence of diabetes 21.7% in 1073 participants) when compared to the placebo (cumulative incidence of diabetes 28.9% in 1082 participants)[175]. Insulin sensitivity improved by 61.8% in the lifestyle intervention group and 28.3% in the metformin group[176]. This study can be employed to calculate a sample size sufficient for assessing the effects of vitamin D intervention.

Based on the non-linear relationship of serum 25-hydroxyvotamin D concentration and insulin sensitivity index as we reported[113], we constructed Table 9. Assuming a linear relationship between improvement in insulin sensitivity and reduction of diabetes from the Diabetes Prevention Program[175,176], we calculated the required sample size to detect the reduction of diabetes incidence after vitamin D replacement therapy in a population similar to that of the Diabetes Prevention Program[175] with a power of 0.80 to detect the proposed difference and a type I error rate, alpha, of 0.05 in a clinical trial of 3 years. Starting with a baseline serum 25-hydroxyviyamin D of 25 ng/mL (10 ng/mL), 170 subjects would be needed. Such a study cohort size is not excessive. However, if the baseline serum 25-hydroxyvitamin D is equal or greater than 50 nmol/L (20 ng/mL) the cohort size needed increases markedly. These calculations suggest that all studies to date are flawed secondary to inadequate sample size.

It has been frustrating to confound the published negative reports while ample evidence supports the benefit of vitamin D. Accordingly, we propose these guidelines [177]. Future studies into the effects of vitamin D supplementation need to ensure the proper selection of study subjects, adequate vitamin D replacement to achieve an optimal serum 25-hydroxyvitamin D concentrations, avoidance over-placement to eliminate detrimental effects, and adequate sample size to detect the proposed effects.

Table 9 Calculated sample size requirement to detect an improvement in insulin sensitivity based on a baseline serum 25-hydroxyvitamin D concentration of 40 ng/mL (100 nmol/L) and a power of 0.80 and alpha of 0.05

Initial serum 25-hydroxy-vitamin D concentration		Estimated insulin sensitivity index(μM/min/m²/pM)	Improvement in insulin sensitivity index with postintervention Serum 25-hydroxyvitamin D concentration 40 ng/mL (100 nmol/L)	Diabetes reduction based on the Diabetes Prevention Program	Sample size
ng/mL	nmol/L				
10	25	4.1326	0.8664	0.4361	340
15	37	5.4144	0.4246	0.2118	1602
20	50	6.2812	0.2280	0.1121	5934
25	62	6.8674	0.1232	0.0589	21878
30	75	7.2638	0.0619	0.0278	99260
35	87	7.5319	0.0241	0.0086	1041162

THE ISSUES THAT NEED TO BE ADDRESSED BY THE FUTURE STUDIES

Optimal serum 25-hydroxyvitamin D concentration for glucose metabolism

Table 4 summarizes the recommended serum vitamin D concentrations from several institutions and agencies. As appreciated, studies on bone health[14,15] showed no additional benefit in the subjects with serum 25-hydroxyvitmanin D > 75 nmol/L (30 ng/mL) and this agrees with the effects upon diabetes prevention. However, increased all-cause mortality[107] and cardiovascular mortality[108] occurred prior to the 125 nmol/L (50 ng/mL) threshold, implying a much lower maximum dose for optimal serum 25-hydroxyvitamin D concentration. The question remains whether the same relationship applies to glucose homeostasis.

Detrimental effects on glucose metabolism for serum 25-hydroxyvitamin D concentrations above a maximum threshold

The detrimental effects noted in individuals with serum 25-hydroxyvitamin D concentration above a maximum threshold was observed in a cross-sectional study[109]. Further, improvement in bone density after discontinuation of vitamin D supplementation in osteoporotic patients with elevated serum 25-hydroxyvitamin D concentration was reported[110]. Elevated serum 25-hydroxyvitamin D concentrations were also associated with increased falls and fracture[118]. These reports suggest that assessment of negative effects from elevated serum 25-hydroxyvitamin D concentration may be uncovered with additional study.

Diabetes prevention in vitamin D deficit subjects

Although various evidence suggests the benefit of vitamin D on glucose metabolism, published diabetes prevention trails are not convincing and suffer from improper designed and execution. To address this issue, a well-designed and well-conducted randomized, placebo-control trial to test the effects of vitamin D to limit development of diabetes is warranted, by selecting true vitamin D deficient subjects, achieving optimal but not excessive serum 25-hydroxyvitamin concentration, and enrolling adequate number of subjects. Properly monitoring serum 25-hydroxyvitamin D concentrations is required during the study.

CONCLUSION

The role of vitamin D in glucose metabolism and fuel homeostasis is supported by a number of observational studies. We reported that serum 25-hydroxyviatmin D concentration accounted for 21.2% of the variation in insulin sensitivity index in univariate analysis and 6.1% by itself among 42% with other covariates in multivariate analysis[178]. We also reported that serum 25-hydroxyviatmin D concentration accounted for 8.2% of the variation in beta cell function in univariate analysis and 4.5% by itself among 25.5% with other covariates in multivariate analysis[179]. Although the intervention studies have failed to provide concordant data for multiple reasons, laboratory studies revealed a number of molecular mechanisms that underlie the effect

of vitamin D supporting the important role of the vitamin in glucose metabolism and fuel homeostasis. Since the independent contributions of vitamin D to insulin sensitivity[178] and beta cell function[179] are relatively small, vitamin D deficiency could be the last straw that breaks camel's back in polygenetic and multifactorial diseases, such as diabetes, obesity, and hyperlipidemia.

ACKNOWLEDGEMENTS

A special acknowledgement is due to Chiu-Tien Chiu, MD, PhD for his unconditional support to KCC. We are in debt to Jeffrey Isenberg MD, MPH for critical reading and editing of the manuscript.

REFERENCES

1 **Diamond J**. Evolutionary biology: geography and skin colour. *Nature* 2005; **435**: 283-284 [PMID: 15902239 DOI: 10.1038/435283a]
2 **Platt BS**. Sir Edward Mellanby, G.B.E., K.C.B., F.R.S. *Nature* 1955; **175**: 530-532 [PMID: 14370159 DOI: 10.1038/175530a0]
3 **McCollum EV**, Pitz W, Simmonds N, Becker JE, Shipley PG, Bunting RW. The effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. 1922. The effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. *J Biol Chem* 2002; **277**: E8 [PMID: 11991957]
4 **Deluca HF**. History of the discovery of vitamin D and its active metabolites. *Bonekey Rep* 2014; **3**: 479 [PMID: 24466410 DOI: 10.1038/bonekey.2013.213]
5 **Askew FA**, Bourdillon RB, Webster TA. The production of vitamin D in a glow discharge. *Biochem J* 1932; **26**: 814 [PMID: 16744889 DOI: 10.1042/bj0260814]
6 **DeLuca HF**. Current concepts. Vitamin D. *N Engl J Med* 1969; **281**: 1103-1104 [PMID: 4309963 DOI: 10.1056/NEJM196911132812006]
7 **Holick MF**. Noncalcemic actions of 1,25-dihydroxyvitamin D3 and clinical applications. *Bone* 1995; **17**: 107S-111S [PMID: 8579891 DOI: 10.1016/8756-3282(95)00195-j]
8 **Mitchell F**. Vitamin-D and COVID-19: do deficient risk a poorer outcome? *Lancet Diabetes Endocrinol* 2020; **8**: 570 [PMID: 32445630 DOI: 10.1016/S2213-8587(20)30183-2]
9 **Holick MF**. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266-281 [PMID: 17634462 DOI: 10.1056/NEJMra070553]
10 **Aloia J**, Fazzari M, Islam S, Mikhail M, Shieh A, Katumuluwa S, Dhaliwal R, Stolberg A, Usera G, Ragolia L. Vitamin D Supplementation in Elderly Black Women Does Not Prevent Bone Loss: A Randomized Controlled Trial. *J Bone Miner Res* 2018; **33**: 1916-1922 [PMID: 29905969 DOI: 10.1002/jbmr.3521]
11 **Aspray TJ**, Chadwick T, Francis RM, McColl E, Stamp E, Prentice A, von Wilamowitz-Moellendorff A, Schoenmakers I. Randomized controlled trial of vitamin D supplementation in older people to optimize bone health. *Am J Clin Nutr* 2019; **109**: 207-217 [PMID: 30624670 DOI: 10.1093/ajcn/nqy280]
12 **Burt LA**, Billington EO, Rose MS, Raymond DA, Hanley DA, Boyd SK. Effect of High-Dose Vitamin D Supplementation on Volumetric Bone Density and Bone Strength: A Randomized Clinical Trial. *JAMA* 2019; **322**: 736-745 [PMID: 31454046 DOI: 10.1001/jama.2019.11889]
13 **Reid IR**, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet* 2014; **383**: 146-155 [PMID: 24119980 DOI: 10.1016/S0140-6736(13)61647-5]
14 **Reid IR**, Horne AM, Mihov B, Gamble GD, Al-Abuwsi F, Singh M, Taylor L, Fenwick S, Camargo CA, Stewart AW, Scragg R. Effect of monthly high-dose vitamin D on bone density in community-dwelling older adults substudy of a randomized controlled trial. *J Intern Med* 2017; **282**: 452-460 [PMID: 28692172 DOI: 10.1111/joim.12651]
15 **Macdonald HM**, Reid IR, Gamble GD, Fraser WD, Tang JC, Wood AD. 25-Hydroxyvitamin D Threshold for the Effects of Vitamin D Supplements on Bone Density: Secondary Analysis of a Randomized Controlled Trial. *J Bone Miner Res* 2018; **33**: 1464-1469 [PMID: 29665087 DOI: 10.1002/jbmr.3442]
16 **Glossmann HH**. Origin of 7-dehydrocholesterol (provitamin D) in the skin. *J Invest Dermatol* 2010; **130**: 2139-2141 [PMID: 20445550 DOI: 10.1038/jid.2010.118]
17 **Nicoloff JT**, Low JC, Dussault JH, Fisher DA. Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. *J Clin Invest* 1972; **51**: 473-483 [PMID: 4110897 DOI: 10.1172/jci106835]
18 **Jones KS**, Assar S, Harnpanich D, Bouillon R, Lambrechts D, Prentice A, Schoenmakers I. 25(OH)D2 half-life is shorter than 25(OH)D3 half-life and is influenced by DBP concentration and genotype. *J Clin Endocrinol Metab* 2014; **99**: 3373-3381 [PMID: 24885631 DOI:

10.1210/jc.2014-1714]
19 **Berry D**, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. *Curr Opin Nephrol Hypertens* 2011; **20**: 331-336 [PMID: 21654390 DOI: 10.1097/MNH.0b013e328346d6ba]
20 **Fagerberg L**, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlén M. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics* 2014; **13**: 397-406 [PMID: 24309898 DOI: 10.1074/mcp.M113.035600]
21 **Lee S**, Clark SA, Gill RK, Christakos S. 1,25-Dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology* 1994; **134**: 1602-1610 [PMID: 8137721 DOI: 10.1210/endo.134.4.8137721]
22 **Zhang H**, Shen Z, Lin Y, Zhang J, Zhang Y, Liu P, Zeng H, Yu M, Chen X, Ning L, Mao X, Cen L, Yu C, Xu C. Vitamin D receptor targets hepatocyte nuclear factor 4α and mediates protective effects of vitamin D in nonalcoholic fatty liver disease. *J Biol Chem* 2020; **295**: 3891-3905 [PMID: 32051143 DOI: 10.1074/jbc.RA119.011487]
23 **Bischoff-Ferrari HA**, Borchers M, Gudat F, Dürmüller U, Stähelin HB, Dick W. Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res* 2004; **19**: 265-269 [PMID: 14969396 DOI: 10.1359/jbmr.2004.19.2.265]
24 **Yuzbashian E**, Asghari G, Hedayati M, Zarkesh M, Mirmiran P, Khalaj A. Determinants of vitamin D receptor gene expression in visceral and subcutaneous adipose tissue in non-obese, obese, and morbidly obese subjects. *J Steroid Biochem Mol Biol* 2019; **187**: 82-87 [PMID: 30412764 DOI: 10.1016/j.jsbmb.2018.11.004]
25 **Bland R**, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, Hewison M. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol* 2004; **89-90**: 121-125 [PMID: 15225758 DOI: 10.1016/j.jsbmb.2004.03.115]
26 **Vuica A**, Ferhatović Hamzić L, Vukojević K, Jerić M, Puljak L, Grković I, Filipović N. Aging and a long-term diabetes mellitus increase expression of 1 α-hydroxylase and vitamin D receptors in the rat liver. *Exp Gerontol* 2015; **72**: 167-176 [PMID: 26471398 DOI: 10.1016/j.exger.2015.10.005]
27 **Srikuea R**, Zhang X, Park-Sarge OK, Esser KA. VDR and CYP27B1 are expressed in C2C12 cells and regenerating skeletal muscle: potential role in suppression of myoblast proliferation. *Am J Physiol Cell Physiol* 2012; **303**: C396-C405 [PMID: 22648952 DOI: 10.1152/ajpcell.00014.2012]
28 **Wamberg L**, Christiansen T, Paulsen SK, Fisker S, Rask P, Rejnmark L, Richelsen B, Pedersen SB. Expression of vitamin D-metabolizing enzymes in human adipose tissue -- the effect of obesity and diet-induced weight loss. *Int J Obes (Lond)* 2013; **37**: 651-657 [PMID: 22828938 DOI: 10.1038/ijo.2012.112]
29 **Hewison M**, Zehnder D, Bland R, Stewart PM. 1alpha-Hydroxylase and the action of vitamin D. *J Mol Endocrinol* 2000; **25**: 141-148 [PMID: 11013342 DOI: 10.1677/jme.0.0250141]
30 **Garland CF**, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 1980; **9**: 227-231 [PMID: 7440046 DOI: 10.1093/ije/9.3.227]
31 **Garland FC**, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. *Prev Med* 1990; **19**: 614-622 [PMID: 2263572 DOI: 10.1016/0091-7435(90)90058-r]
32 **Ahonen MH**, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* 2000; **11**: 847-852 [PMID: 11075874 DOI: 10.1023/a:1008923802001]
33 **Garland CF**, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1989; **2**: 1176-1178 [PMID: 2572900 DOI: 10.1016/s0140-6736(89)91789-3]
34 **Abe E**, Miyaura C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Suda T. Differentiation of mouse myeloid leukemia cells induced by 1 alpha,25-dihydroxyvitamin D3. *Proc Natl Acad Sci U S A* 1981; **78**: 4990-4994 [PMID: 6946446 DOI: 10.1073/pnas.78.8.4990]
35 **Peehl DM**, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. *Cancer Res* 1994; **54**: 805-810 [PMID: 7508338]
36 **Masuda S**, Jones G. Promise of vitamin D analogues in the treatment of hyperproliferative conditions. *Mol Cancer Ther* 2006; **5**: 797-808 [PMID: 16648549 DOI: 10.1158/1535-7163.MCT-05-0539]
37 **Samuel S**, Sitrin MD. Vitamin D's role in cell proliferation and differentiation. *Nutr Rev* 2008; **66**: S116-S124 [PMID: 18844838 DOI: 10.1111/j.1753-4887.2008.00094.x]
38 **Hyppönen E**, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001; **358**: 1500-1503 [PMID: 11705562 DOI: 10.1016/S0140-6736(01)06580-1]
39 **Chang TJ**, Lei HH, Yeh JI, Chiu KC, Lee KC, Chen MC, Tai TY, Chuang LM. Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. *Clin Endocrinol (Oxf)* 2000; **52**: 575-580 [PMID: 10792336 DOI: 10.1046/j.1365-2265.2000.00985.x]
40 **Zella JB**, DeLuca HF. Vitamin D and autoimmune diabetes. *J Cell Biochem* 2003; **88**: 216-222

[PMID: 12520517 DOI: 10.1002/jcb.10347]
41 **Munger KL**, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, Ascherio A. Vitamin D intake and incidence of multiple sclerosis. *Neurology* 2004; **62**: 60-65 [PMID: 14718698 DOI: 10.1212/01.wnl.0000101723.79681.38]
42 **Kamen DL**, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS. Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev* 2006; **5**: 114-117 [PMID: 16431339 DOI: 10.1016/j.autrev.2005.05.009]
43 **Maruotti N**, Cantatore FP. Vitamin D and the immune system. *J Rheumatol* 2010; **37**: 491-495 [PMID: 20080911 DOI: 10.3899/jrheum.090797]
44 **Murdaca G**, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, Gangemi S. Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications. *Autoimmun Rev* 2019; **18**: 102350 [PMID: 31323357 DOI: 10.1016/j.autrev.2019.102350]
45 **Chen S**, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* 2007; **179**: 1634-1647 [PMID: 17641030 DOI: 10.4049/jimmunol.179.3.1634]
46 The sun cure for surgical tuberculosis. *Br Med J* 1923; **2**: 111-112 [PMID: 20771236]
47 **Klip W**. The tuberculostatic action of vitamin D2. *Antonie Van Leeuwenhoek* 1952; **18**: 217-226 [PMID: 14944180 DOI: 10.1007/BF02538610]
48 **Davies PD**, Brown RC, Woodhead JS. Serum concentrations of vitamin D metabolites in untreated tuberculosis. *Thorax* 1985; **40**: 187-190 [PMID: 3872485 DOI: 10.1136/thx.40.3.187]
49 **Liu PT**, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006; **311**: 1770-1773 [PMID: 16497887 DOI: 10.1126/science.1123933]
50 **Zasloff M**. Fighting infections with vitamin D. *Nat Med* 2006; **12**: 388-390 [PMID: 16598282 DOI: 10.1038/nm0406-388]
51 **Laaksi I**, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamäki H, Ylikomi T. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. *Am J Clin Nutr* 2007; **86**: 714-717 [PMID: 17823437 DOI: 10.1093/ajcn/86.3.714]
52 **Bodnar LM**, Krohn MA, Simhan HN. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. *J Nutr* 2009; **139**: 1157-1161 [PMID: 19357214 DOI: 10.3945/jn.108.103168]
53 **Inamo Y**, Hasegawa M, Saito K, Hayashi R, Ishikawa T, Yoshino Y, Hashimoto K, Fuchigami T. Serum vitamin D concentrations and associated severity of acute lower respiratory tract infections in Japanese hospitalized children. *Pediatr Int* 2011; **53**: 199-201 [PMID: 21648117 DOI: 10.1111/j.1442-200x.2010.03224.x]
54 **Tiwari S**, Pratyush DD, Gupta B, Dwivedi A, Chaudhary S, Rayicherla RK, Gupta SK, Singh SK. Prevalence and severity of vitamin D deficiency in patients with diabetic foot infection. *Br J Nutr* 2013; **109**: 99-102 [PMID: 22715859 DOI: 10.1017/S0007114512000578]
55 **Terrier B**, Jehan F, Munteanu M, Geri G, Saadoun D, Sène D, Poynard T, Souberbielle JC, Cacoub P. Low 25-hydroxyvitamin D serum levels correlate with the presence of extra-hepatic manifestations in chronic hepatitis C virus infection. *Rheumatology (Oxford)* 2012; **51**: 2083-2090 [PMID: 22908327 DOI: 10.1093/rheumatology/kes209]
56 **Pinzone MR**, Di Rosa M, Malaguarnera M, Madeddu G, Focà E, Ceccarelli G, d'Ettorre G, Vullo V, Fisichella R, Cacopardo B, Nunnari G. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. *Eur Rev Med Pharmacol Sci* 2013; **17**: 1218-1232 [PMID: 23690192]
57 **Abrishami A**, Dalili N, Mohammadi Torbati P, Asgari R, Arab-Ahmadi M, Behnam B, Sanei-Taheri M. Possible association of vitamin D status with lung involvement and outcome in patients with COVID-19: a retrospective study. *Eur J Nutr* 2021; **60**: 2249-2257 [PMID: 33123774 DOI: 10.1007/s00394-020-02411-0]
58 **Panagiotou G**, Tee SA, Ihsan Y, Athar W, Marchitelli G, Kelly D, Boot CS, Stock N, Macfarlane J, Martineau AR, Burns G, Quinton R. Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity. *Clin Endocrinol (Oxf)* 2020; **93**: 508-511 [PMID: 32621392 DOI: 10.1111/cen.14276]
59 **Merzon E**, Tworowski D, Gorohovski A, Vinker S, Golan Cohen A, Green I, Frenkel-Morgenstern M. Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *FEBS J* 2020; **287**: 3693-3702 [PMID: 32700398 DOI: 10.1111/febs.15495]
60 **Carpagnano GE**, Di Lecce V, Quaranta VN, Zito A, Buonamico E, Capozza E, Palumbo A, Di Gioia G, Valerio VN, Resta O. Vitamin D deficiency as a predictor of poor prognosis in patients with acute respiratory failure due to COVID-19. *J Endocrinol Invest* 2021; **44**: 765-771 [PMID: 32772324 DOI: 10.1007/s40618-020-01370-x]
61 **Meltzer DO**, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. *JAMA Netw Open* 2020; **3**: e2019722 [PMID: 32880651 DOI: 10.1001/jamanetworkopen.2020.19722]
62 **Van Schooten FJ**, Hirvonen A, Maas LM, De Mol BA, Kleinjans JC, Bell DA, Durrer JD. Putative susceptibility markers of coronary artery disease: association between VDR genotype, smoking, and

aromatic DNA adduct levels in human right atrial tissue. *FASEB J* 1998; **12**: 1409-1417 [PMID: 9761785 DOI: 10.1096/fasebj.12.13.1409]

63 **Giovannucci E**, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008; **168**: 1174-1180 [PMID: 18541825 DOI: 10.1001/archinte.168.11.1174]

64 **Scragg R**, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertens* 2007; **20**: 713-719 [PMID: 17586404 DOI: 10.1016/j.amjhyper.2007.01.017]

65 **Pilz S**, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, März W. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008; **39**: 2611-2613 [PMID: 18635847 DOI: 10.1161/STROKEAHA.107.513655]

66 **Xiang W**, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* 2005; **288**: E125-E132 [PMID: 15367398 DOI: 10.1152/ajpendo.00224.2004]

67 **Watson KE**, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R, Demer LL. Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 1997; **96**: 1755-1760 [PMID: 9323058 DOI: 10.1161/01.cir.96.6.1755]

68 **Sultan M**, Twito O, Tohami T, Ramati E, Neumark E, Rashid G. Vitamin D diminishes the high platelet aggregation of type 2 diabetes mellitus patients. *Platelets* 2019; **30**: 120-125 [PMID: 29313404 DOI: 10.1080/09537104.2017.1386298]

69 **Schleithoff SS**, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 2006; **83**: 754-759 [PMID: 16600924 DOI: 10.1093/ajcn/83.4.754]

70 **Kumar S**, Davies M, Zakaria Y, Mawer EB, Gordon C, Olukoga AO, Boulton AJ. Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D. *Postgrad Med J* 1994; **70**: 440-443 [PMID: 8029165 DOI: 10.1136/pgmj.70.824.440]

71 **Boucher BJ**, Mannan N, Noonan K, Hales CN, Evans SJ. Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. *Diabetologia* 1995; **38**: 1239-1245 [PMID: 8690178 DOI: 10.1007/BF00422375]

72 **Bell NH**, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* 1985; **76**: 370-373 [PMID: 2991340 DOI: 10.1172/JCI111971]

73 **Ganji V**, Milone C, Cody MM, McCarty F, Wang YT. Serum vitamin D concentrations are related to depression in young adult US population: the Third National Health and Nutrition Examination Survey. *Int Arch Med* 2010; **3**: 29 [PMID: 21067618 DOI: 10.1186/1755-7682-3-29]

74 **Zhu JL**, Luo WW, Cheng X, Li Y, Zhang QZ, Peng WX. Vitamin D deficiency and Schizophrenia in Adults: A Systematic Review and Meta-analysis of Observational Studies. *Psychiatry Res* 2020; **288**: 112959 [PMID: 32335466 DOI: 10.1016/j.psychres.2020.112959]

75 **Wang T**, Shan L, Du L, Feng J, Xu Z, Staal WG, Jia F. Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. *Eur Child Adolesc Psychiatry* 2016; **25**: 341-350 [PMID: 26514973 DOI: 10.1007/s00787-015-0786-1]

76 **Lv Z**, Qi H, Wang L, Fan X, Han F, Wang H, Bi S. Vitamin D status and Parkinson's disease: a systematic review and meta-analysis. *Neurol Sci* 2014; **35**: 1723-1730 [PMID: 24847960 DOI: 10.1007/s10072-014-1821-6]

77 **Smith MP**, Fletcher-Turner A, Yurek DM, Cass WA. Calcitriol protection against dopamine loss induced by intracerebroventricular administration of 6-hydroxydopamine. *Neurochem Res* 2006; **31**: 533-539 [PMID: 16758362 DOI: 10.1007/s11064-006-9048-4]

78 **Mayne PE**, Burne THJ. Vitamin D in Synaptic Plasticity, Cognitive Function, and Neuropsychiatric Illness. *Trends Neurosci* 2019; **42**: 293-306 [PMID: 30795846 DOI: 10.1016/j.tins.2019.01.003]

79 **Almeras L**, Eyles D, Benech P, Laffite D, Villard C, Patatian A, Boucraut J, Mackay-Sim A, Mgrath J, Féron F. Developmental vitamin D deficiency alters brain protein expression in the adult rat: implications for neuropsychiatric disorders. *Proteomics* 2007; **7**: 769-780 [PMID: 17295352 DOI: 10.1002/pmic.200600392]

80 **Eyles DW**, Smith S, Kinobe R, Hewison M, Mgrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J Chem Neuroanat* 2005; **29**: 21-30 [PMID: 15589699 DOI: 10.1016/j.jchemneu.2004.08.006]

81 **Phillips KM**, Ruggio DM, Horst RL, Minor B, Simon RR, Feeney MJ, Byrdwell WC, Haytowitz DB. Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. *J Agric Food Chem* 2011; **59**: 7841-7853 [PMID: 21663327 DOI: 10.1021/jf104246z]

82 **Libon F**, Cavalier E, Nikkels AF. Skin color is relevant to vitamin D synthesis. *Dermatology* 2013; **227**: 250-254 [PMID: 24134867 DOI: 10.1159/000354750]

83 **Jablonski NG**, Chaplin G. Colloquium paper: human skin pigmentation as an adaptation to UV radiation. *Proc Natl Acad Sci U S A* 2010; **107** Suppl 2: 8962-8968 [PMID: 20445093 DOI: 10.1073/pnas.0914628107]

84 **Holick MF**. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes* 2002; **9**: 87-98

85 **Barger-Lux MJ**, Heaney RP. Effects of above average summer sun exposure on serum 25-

hydroxyvitamin D and calcium absorption. *J Clin Endocrinol Metab* 2002; **87**: 4952-4956 [PMID: 12414856 DOI: 10.1210/jc.2002-020636]

86 **Rockell JE**, Skeaff CM, Williams SM, Green TJ. Association between quantitative measures of skin color and plasma 25-hydroxyvitamin D. *Osteoporos Int* 2008; **19**: 1639-1642 [PMID: 18408879 DOI: 10.1007/s00198-008-0620-4]

87 **Epstein JH**. Photocarcinogenesis, skin cancer, and aging. *J Am Acad Dermatol* 1983; **9**: 487-502 [PMID: 6355213 DOI: 10.1016/s0190-9622(83)70160-x]

88 **Heaney RP**, Horst RL, Cullen DM, Armas LA. Vitamin D3 distribution and status in the body. *J Am Coll Nutr* 2009; **28**: 252-256 [PMID: 20150598 DOI: 10.1080/07315724.2009.10719779]

89 **Bouillon R**, Schuit F, Antonio L, Rastinejad F. Vitamin D Binding Protein: A Historic Overview. *Front Endocrinol (Lausanne)* 2019; **10**: 910 [PMID: 31998239 DOI: 10.3389/fendo.2019.00910]

90 **Haddad JG**, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J. Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* 1993; **91**: 2552-2555 [PMID: 8390483 DOI: 10.1172/JCI116492]

91 **Holmberg I**, Berlin T, Ewerth S, Björkhem I. 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D2 and D3. *Scand J Clin Lab Invest* 1986; **46**: 785-790 [PMID: 3026027 DOI: 10.3109/00365518609084051]

92 **Armas LA**, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab* 2004; **89**: 5387-5391 [PMID: 15531486 DOI: 10.1210/jc.2004-0360]

93 **Zarei A**, Hulley PA, Sabokbar A, Javaid MK, Morovat A. 25-Hydroxy- and 1α,25-Dihydroxycholecalciferol Have Greater Potencies than 25-Hydroxy- and 1α,25-Dihydroxyergocalciferol in Modulating Cultured Human and Mouse Osteoblast Activities. *PLoS One* 2016; **11**: e0165462 [PMID: 27893751 DOI: 10.1371/journal.pone.0165462]

94 **Jones G**, Byrnes B, Palma F, Segev D, Mazur Y. Displacement potency of vitamin D2 analogs in competitive protein-binding assays for 25-hydroxyvitamin D3, 24,25-dihydroxyvitamin D3, and 1,25-dihydroxyvitamin D3. *J Clin Endocrinol Metab* 1980; **50**: 773-775 [PMID: 6965943 DOI: 10.1210/jcem-50-4-773]

95 **Holick MF**, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W, Ameri A, Tannenbaum AD. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab* 2008; **93**: 677-681 [PMID: 18089691 DOI: 10.1210/jc.2007-2308]

96 **Trang HM**, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am J Clin Nutr* 1998; **68**: 854-858 [PMID: 9771862 DOI: 10.1093/ajcn/68.4.854]

97 **Romagnoli E**, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmo E, Carnevale V, Scillitani A, Minisola S. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *J Clin Endocrinol Metab* 2008; **93**: 3015-3020 [PMID: 18492750 DOI: 10.1210/jc.2008-0350]

98 **Heaney RP**, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab* 2011; **96**: E447-E452 [PMID: 21177785 DOI: 10.1210/jc.2010-2230]

99 **Glendenning P**, Chew GT, Seymour HM, Gillett MJ, Goldswain PR, Inderjeeth CA, Vasikaran SD, Taranto M, Musk AA, Fraser WD. Serum 25-hydroxyvitamin D levels in vitamin D-insufficient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. *Bone* 2009; **45**: 870-875 [PMID: 19631774 DOI: 10.1016/j.bone.2009.07.015]

100 **Shieh A**, Chun RF, Ma C, Witzel S, Meyer B, Rafison B, Swinkels L, Huijs T, Pepkowitz S, Holmquist B, Hewison M, Adams JS. Effects of High-Dose Vitamin D2 Versus D3 on Total and Free 25-Hydroxyvitamin D and Markers of Calcium Balance. *J Clin Endocrinol Metab* 2016; **101**: 3070-3078 [PMID: 27192696 DOI: 10.1210/jc.2016-1871]

101 **Tripkovic L**, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hyppönen E, Berry J, Vieth R, Lanham-New S. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr* 2012; **95**: 1357-1364 [PMID: 22552031 DOI: 10.3945/ajcn.111.031070]

102 **Cipriani C**, Romagnoli E, Pepe J, Russo S, Carlucci L, Piemonte S, Nieddu L, McMahon DJ, Singh R, Minisola S. Long-term bioavailability after a single oral or intramuscular administration of 600,000 IU of ergocalciferol or cholecalciferol: implications for treatment and prophylaxis. *J Clin Endocrinol Metab* 2013; **98**: 2709-2715 [PMID: 23766519 DOI: 10.1210/jc.2013-1586]

103 **Malabanan A**, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998; **351**: 805-806 [PMID: 9519960 DOI: 10.1016/s0140-6736(05)78933-9]

104 **Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D, Calcium**. Dietary Reference Intakes for Calcium and Vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington (DC): National Academies Press (US), 2011

105 **Chapuy MC**, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997; **7**: 439-443 [PMID: 9425501 DOI: 10.1007/s001980050030]

106 **Heaney RP**. Functional indices of vitamin D status and ramifications of vitamin D deficiency. *Am J Clin Nutr* 2004; **80**: 1706S-1709S [PMID: 15585791 DOI: 10.1093/ajcn/80.6.1706S]

107 **Melamed ML**, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008; **168**: 1629-1637 [PMID: 18695076 DOI:

10.1001/archinte.168.15.1629]

108 **Durup D**, Jørgensen HL, Christensen J, Tjønneland A, Olsen A, Halkjær J, Lind B, Heegaard AM, Schwarz P. A Reverse J-Shaped Association Between Serum 25-Hydroxyvitamin D and Cardiovascular Disease Mortality: The CopD Study. *J Clin Endocrinol Metab* 2015; **100**: 2339-2346 [PMID: 25710567 DOI: 10.1210/jc.2014-4551]

109 **Bischoff-Ferrari HA**, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006; **84**: 18-28 [PMID: 16825677 DOI: 10.1093/ajcn/84.1.18]

110 **Adams JS**, Lee G. Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Intern Med* 1997; **127**: 203-206 [PMID: 9245225 DOI: 10.7326/0003-4819-127-3-199708010-00004]

111 **Aloia JF**, Dhaliwal R, Shieh A, Mikhail M, Fazzari M, Ragolia L, Abrams SA. Vitamin D supplementation increases calcium absorption without a threshold effect. *Am J Clin Nutr* 2014; **99**: 624-631 [PMID: 24335055 DOI: 10.3945/ajcn.113.067199]

112 **Huang J**, Ou HY, Karnchanasorn R, Chiu KC. Clinical implication of vitamin D threshold. *Am J Clin Nutr* 2014; **100**: 295-296 [PMID: 24951577 DOI: 10.3945/ajcn.114.087171]

113 **Ou HY**, Karnchanasorn R, Lee LZ, Chiu KC. Interaction of BMI with vitamin D and insulin sensitivity. *Eur J Clin Invest* 2011; **41**: 1195-1201 [PMID: 21434896 DOI: 10.1111/j.1365-2362.2011.02525.x]

114 **Heaney RP**. Nutrition and chronic disease. *Mayo Clin Proc* 2006; **81**: 297-299 [PMID: 16529131 DOI: 10.4065/81.3.297]

115 **Ilahi M**, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. *Am J Clin Nutr* 2008; **87**: 688-691 [PMID: 18326608 DOI: 10.1093/ajcn/87.3.688]

116 **Ghazi AA**, Hosseinpanah F, M Ardakani E, Ghazi S, Hedayati M, Azizi F. Effects of different doses of oral cholecalciferol on serum 25(OH)D, PTH, calcium and bone markers during fall and winter in schoolchildren. *Eur J Clin Nutr* 2010; **64**: 1415-1422 [PMID: 20823895 DOI: 10.1038/ejcn.2010.169]

117 **Carnes J**, Quinn S, Nelson M, Jones G, Winzenberg T. Intermittent high-dose vitamin D corrects vitamin D deficiency in adolescents: a pilot study. *Eur J Clin Nutr* 2012; **66**: 530-532 [PMID: 22190133 DOI: 10.1038/ejcn.2011.204]

118 **Sanders KM**, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA* 2010; **303**: 1815-1822 [PMID: 20460620 DOI: 10.1001/jama.2010.594]

119 **Leventis P**, Kiely PD. The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. *Scand J Rheumatol* 2009; **38**: 149-153 [PMID: 18991184 DOI: 10.1080/03009740802419081]

120 **Rossini M**, Gatti D, Viapiana O, Fracassi E, Idolazzi L, Zanoni S, Adami S. Short-term effects on bone turnover markers of a single high dose of oral vitamin D_3. *J Clin Endocrinol Metab* 2012; **97**: E622-E626 [PMID: 22298802 DOI: 10.1210/jc.2011-2448]

121 **von Restorff C**, Bischoff-Ferrari HA, Theiler R. High-dose oral vitamin D3 supplementation in rheumatology patients with severe vitamin D3 deficiency. *Bone* 2009; **45**: 747-749 [PMID: 19539796 DOI: 10.1016/j.bone.2009.06.012]

122 **Tellioglu A**, Basaran S, Guzel R, Seydaoglu G. Efficacy and safety of high dose intramuscular or oral cholecalciferol in vitamin D deficient/insufficient elderly. *Maturitas* 2012; **72**: 332-338 [PMID: 22613271 DOI: 10.1016/j.maturitas.2012.04.011]

123 **Witham MD**, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. *Nutr Metab Cardiovasc Dis* 2012; **22**: 864-870 [PMID: 21194910 DOI: 10.1016/j.numecd.2010.11.001]

124 **Ish-Shalom S**, Segal E, Salganik T, Raz B, Bromberg IL, Vieth R. Comparison of daily, weekly, and monthly vitamin D3 in ethanol dosing protocols for two months in elderly hip fracture patients. *J Clin Endocrinol Metab* 2008; **93**: 3430-3435 [PMID: 18544622 DOI: 10.1210/jc.2008-0241]

125 **Fu L**, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. *Clin Biochem* 2009; **42**: 1174-1177 [PMID: 19302999 DOI: 10.1016/j.clinbiochem.2009.03.008]

126 **Wang TJ**, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 2010; **376**: 180-188 [PMID: 20541252 DOI: 10.1016/S0140-6736(10)60588-0]

127 **Hsu S**, Hoofnagle AN, Gupta DK, Gutierrez OM, Peralta CA, Shea S, Allen NB, Burke G, Michos ED, Ix JH, Siscovick D, Psaty BM, Watson KE, Kestenbaum B, de Boer IH, Robinson-Cohen C.

Race, Ancestry, and Vitamin D Metabolism: The Multi-Ethnic Study of Atherosclerosis. *J Clin Endocrinol Metab* 2020; **105**: e4337-e4350 [PMID: 32869845 DOI: 10.1210/clinem/dgaa612]

128 **Clemens TL**, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* 1982; **1**: 74-76 [PMID: 6119494 DOI: 10.1016/s0140-6736(82)90214-8]

129 **Webb AR**, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988; **67**: 373-378 [PMID: 2839537 DOI: 10.1210/jcem-67-2-373]

130 **Gangloff A**, Bergeron J, Lemieux I, Després JP. Changes in circulating vitamin D levels with loss of adipose tissue. *Curr Opin Clin Nutr Metab Care* 2016; **19**: 464-470 [PMID: 27537278 DOI: 10.1097/MCO.0000000000000315]

131 **Holick MF**, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; **96**: 1911-1930 [PMID: 21646368 DOI: 10.1210/jc.2011-0385]

132 **Vieth R**. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999; **69**: 842-856 [PMID: 10232622 DOI: 10.1093/ajcn/69.5.842]

133 **Mulligan GB**, Licata A. Taking vitamin D with the largest meal improves absorption and results in higher serum levels of 25-hydroxyvitamin D. *J Bone Miner Res* 2010; **25**: 928-930 [PMID: 20200983 DOI: 10.1002/jbmr.67]

134 **Dutta D**, Mondal SA, Choudhuri S, Maisnam I, Hasanoor Reza AH, Bhattacharya B, Chowdhury S, Mukhopadhyay S. Vitamin-D supplementation in prediabetes reduced progression to type 2 diabetes and was associated with decreased insulin resistance and systemic inflammation: an open label randomized prospective study from Eastern India. *Diabetes Res Clin Pract* 2014; **103**: e18-e23 [PMID: 24456991 DOI: 10.1016/j.diabres.2013.12.044]

135 **Niroomand M**, Fotouhi A, Irannejad N, Hosseinpanah F. Does high-dose vitamin D supplementation impact insulin resistance and risk of development of diabetes in patients with pre-diabetes? *Diabetes Res Clin Pract* 2019; **148**: 1-9 [PMID: 30583032 DOI: 10.1016/j.diabres.2018.12.008]

136 **Wagner H**, Alvarsson M, Mannheimer B, Degerblad M, Östenson CG. No Effect of High-Dose Vitamin D Treatment on β-Cell Function, Insulin Sensitivity, or Glucose Homeostasis in Subjects With Abnormal Glucose Tolerance: A Randomized Clinical Trial. *Diabetes Care* 2016; **39**: 345-352 [PMID: 26786573 DOI: 10.2337/dc15-1057]

137 **Oosterwerff MM**, Eekhoff EM, Van Schoor NM, Boeke AJ, Nanayakkara P, Meijnen R, Knol DL, Kramer MH, Lips P. Effect of moderate-dose vitamin D supplementation on insulin sensitivity in vitamin D-deficient non-Western immigrants in the Netherlands: a randomized placebo-controlled trial. *Am J Clin Nutr* 2014; **100**: 152-160 [PMID: 24898240 DOI: 10.3945/ajcn.113.069260]

138 **Pittas AG**, Dawson-Hughes B, Sheehan P, Ware JH, Knowler WC, Aroda VR, Brodsky I, Ceglia L, Chadha C, Chatterjee R, Desouza C, Dolor R, Foreyt J, Fuss P, Ghazi A, Hsia DS, Johnson KC, Kashyap SR, Kim S, LeBlanc ES, Lewis MR, Liao E, Neff LM, Nelson J, O'Neil P, Park J, Peters A, Phillips LS, Pratley R, Raskin P, Rasouli N, Robbins D, Rosen C, Vickery EM, Staten M; D2d Research Group. Vitamin D Supplementation and Prevention of Type 2 Diabetes. *N Engl J Med* 2019; **381**: 520-530 [PMID: 31173679 DOI: 10.1056/NEJMoa1900906]

139 **Davidson MB**, Duran P, Lee ML, Friedman TC. High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. *Diabetes Care* 2013; **36**: 260-266 [PMID: 23033239 DOI: 10.2337/dc12-1204]

140 **Jorde R**, Sollid ST, Svartberg J, Schirmer H, Joakimsen RM, Njølstad I, Fuskevåg OM, Figenschau Y, Hutchinson MY. Vitamin D 20,000 IU per Week for Five Years Does Not Prevent Progression From Prediabetes to Diabetes. *J Clin Endocrinol Metab* 2016; **101**: 1647-1655 [PMID: 26829443 DOI: 10.1210/jc.2015-4013]

141 **Barengolts E**, Manickam B, Eisenberg Y, Akbar A, Kukreja S, Ciubotaru I. Effect of high-dose vitamin D repletion on glycemic control in african-american males with prediabetes and hypovitaminosis d. *Endocr Pract* 2015; **21**: 604-612 [PMID: 25716637 DOI: 10.4158/EP14548.OR]

142 **Jorde R**, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr* 2009; **48**: 349-354 [PMID: 19370371 DOI: 10.1007/s00394-009-0020-3]

143 **Jorde R**, Strand Hutchinson M, Kjærgaard M, Sneve M, Grimnes G. Supplementation with High Doses of Vitamin D to Subjects without Vitamin D Deficiency May Have Negative Effects: Pooled Data from Four Intervention Trials in Tromsø. *ISRN Endocrinol* 2013; **2013**: 348705 [PMID: 23577264 DOI: 10.1155/2013/348705]

144 **Norman AW**, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 1980; **209**: 823-825 [PMID: 6250216 DOI: 10.1126/science.6250216]

145 **Clark SA**, Stumpf WE, Sar M, DeLuca HF, Tanaka Y. Target cells for 1,25 dihydroxyvitamin D3 in the pancreas. *Cell Tissue Res* 1980; **209**: 515-520 [PMID: 6996826 DOI: 10.1007/BF00234764]

146 **Clark SA**, Stumpf WE, Sar M. Effect of 1,25 dihydroxyvitamin D3 on insulin secretion. *Diabetes* 1981; **30**: 382-386 [PMID: 7014306 DOI: 10.2337/diab.30.5.382]

147 **Chertow BS**, Sivitz WI, Baranetsky NG, Clark SA, Waite A, Deluca HF. Cellular mechanisms of insulin release: the effects of vitamin D deficiency and repletion on rat insulin secretion.

Endocrinology 1983; **113**: 1511-1518 [PMID: 6352248 DOI: 10.1210/endo-113-4-1511]

148 **Kadowaki S**, Norman AW. Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. *J Clin Invest* 1984; **73**: 759-766 [PMID: 6323527 DOI: 10.1172/JCI111269]

149 **Kadowaki S**, Norman AW. Time course study of insulin secretion after 1,25-dihydroxyvitamin D3 administration. *Endocrinology* 1985; **117**: 1765-1771 [PMID: 3899614 DOI: 10.1210/endo-117-5-1765]

150 **Cade C**, Norman AW. Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. *Endocrinology* 1986; **119**: 84-90 [PMID: 3013599 DOI: 10.1210/endo-119-1-84]

151 **Cade C**, Norman AW. Rapid normalization/stimulation by 1,25-dihydroxyvitamin D3 of insulin secretion and glucose tolerance in the vitamin D-deficient rat. *Endocrinology* 1987; **120**: 1490-1497 [PMID: 3549262 DOI: 10.1210/endo-120-4-1490]

152 **Zeitz U**, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J* 2003; **17**: 509-511 [PMID: 12551842 DOI: 10.1096/fj.02-0424fje]

153 **Pepaj M**, Bredahl MK, Gjerlaugsen N, Bornstedt ME, Thorsby PM. Discovery of novel vitamin D-regulated proteins in INS-1 cells: a proteomic approach. *Diabetes Metab Res Rev* 2015; **31**: 481-491 [PMID: 25449168 DOI: 10.1002/dmrr.2629]

154 **Bornstedt ME**, Gjerlaugsen N, Olstad OK, Berg JP, Bredahl MK, Thorsby PM. Vitamin D metabolites influence expression of genes concerning cellular viability and function in insulin producing β-cells (INS1E). *Gene* 2020; **746**: 144649 [PMID: 32251702 DOI: 10.1016/j.gene.2020.144649]

155 **Cheng Q**, Li YC, Boucher BJ, Leung PS. A novel role for vitamin D: modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. *Diabetologia* 2011; **54**: 2077-2081 [PMID: 21424540 DOI: 10.1007/s00125-011-2100-1]

156 **Lahbib A**, Ghodbane S, Louchami K, Sener A, Sakly M, Abdelmelek H. Effects of vitamin D on insulin secretion and glucose transporter GLUT2 under static magnetic field in rat. *Environ Sci Pollut Res Int* 2015; **22**: 18011-18016 [PMID: 26169817 DOI: 10.1007/s11356-015-4844-5]

157 **Jayanarayanan S**, Anju TR, Smijin S, Paulose CS. Vitamin D3 supplementation increases insulin level by regulating altered IP3 and AMPA receptor expression in the pancreatic islets of streptozotocin-induced diabetic rat. *J Nutr Biochem* 2015; **26**: 1041-1049 [PMID: 26054778 DOI: 10.1016/j.jnutbio.2015.04.011]

158 **Kuo T**, Damle M, González BJ, Egli D, Lazar MA, Accili D. Induction of α cell-restricted Gc in dedifferentiating β cells contributes to stress-induced β-cell dysfunction. *JCI Insight* 2019; **5**: e128351 [PMID: 31120862 DOI: 10.1172/jci.insight.128351]

159 **He D**, Wang Y, Liu R, He A, Li S, Fu X, Zhou Z. 1,25$(OH)_2D_3$ Activates Autophagy to Protect against Oxidative Damage of INS-1 Pancreatic Beta Cells. *Biol Pharm Bull* 2019; **42**: 561-567 [PMID: 30930416 DOI: 10.1248/bpb.b18-00395]

160 **Guareschi ZM**, Valcanaia AC, Ceglarek VM, Hotz P, Amaral BK, de Souza DW, de Souza TA, Nardelli T, Ferreira TR, Leite NC, Lubackzeuski C, de O Emilio HR, Grassiolli S. The effect of chronic oral vitamin D supplementation on adiposity and insulin secretion in hypothalamic obese rats. *Br J Nutr* 2019; **121**: 1334-1344 [PMID: 30924427 DOI: 10.1017/S0007114519000667]

161 **Kjalarsdottir L**, Tersey SA, Vishwanath M, Chuang JC, Posner BA, Mirmira RG, Repa JJ. 1,25-Dihydroxyvitamin D_3 enhances glucose-stimulated insulin secretion in mouse and human islets: a role for transcriptional regulation of voltage-gated calcium channels by the vitamin D receptor. *J Steroid Biochem Mol Biol* 2019; **185**: 17-26 [PMID: 30071248 DOI: 10.1016/j.jsbmb.2018.07.004]

162 **Park S**, Kim DS, Kang S. Vitamin D deficiency impairs glucose-stimulated insulin secretion and increases insulin resistance by reducing PPAR-γ expression in nonobese Type 2 diabetic rats. *J Nutr Biochem* 2016; **27**: 257-265 [PMID: 26522682 DOI: 10.1016/j.jnutbio.2015.09.013]

163 **Mazzone G**, Morisco C, Lembo V, D'Argenio G, D'Armiento M, Rossi A, Giudice CD, Trimarco B, Caporaso N, Morisco F. Dietary supplementation of vitamin D prevents the development of western diet-induced metabolic, hepatic and cardiovascular abnormalities in rats. *United European Gastroenterol J* 2018; **6**: 1056-1064 [PMID: 30228894 DOI: 10.1177/2050640618774140]

164 **Dong B**, Zhou Y, Wang W, Scott J, Kim K, Sun Z, Guo Q, Lu Y, Gonzales NM, Wu H, Hartig SM, York RB, Yang F, Moore DD. Vitamin D Receptor Activation in Liver Macrophages Ameliorates Hepatic Inflammation, Steatosis, and Insulin Resistance in Mice. *Hepatology* 2020; **71**: 1559-1574 [PMID: 31506976 DOI: 10.1002/hep.30937]

165 **Sadek KM**, Shaheen H. Biochemical efficacy of vitamin D in ameliorating endocrine and metabolic disorders in diabetic rats. *Pharm Biol* 2014; **52**: 591-596 [PMID: 24251869 DOI: 10.3109/13880209.2013.854812]

166 **Sisley SR**, Arble DM, Chambers AP, Gutierrez-Aguilar R, He Y, Xu Y, Gardner D, Moore DD, Seeley RJ, Sandoval DA. Hypothalamic Vitamin D Improves Glucose Homeostasis and Reduces Weight. *Diabetes* 2016; **65**: 2732-2741 [PMID: 27217488 DOI: 10.2337/db16-0309]

167 **Schnell DM**, Walton RG, Vekaria HJ, Sullivan PG, Bollinger LM, Peterson CA, Thomas DT. Vitamin D produces a perilipin 2-dependent increase in mitochondrial function in C2C12 myotubes. *J Nutr Biochem* 2019; **65**: 83-92 [PMID: 30658160 DOI: 10.1016/j.jnutbio.2018.11.002]

168 **Jefferson GE**, Schnell DM, Thomas DT, Bollinger LM. Calcitriol concomitantly enhances insulin sensitivity and alters myocellular lipid partitioning in high fat-treated skeletal muscle cells. *J Physiol Biochem* 2017; **73**: 613-621 [PMID: 28980208 DOI: 10.1007/s13105-017-0595-8]

169 **Hesse M**, Fröhlich LF, Zeitz U, Lanske B, Erben RG. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in Fgf-23 deficient mice. *Matrix Biol* 2007; **26**: 75-84 [PMID: 17123805 DOI: 10.1016/j.matbio.2006.10.003]

170 **Boucher BJ**. Does vitamin D status contribute to caveolin-1-mediated insulin sensitivity in skeletal muscle? *Diabetologia* 2009; **52**: 2240 [PMID: 19672573 DOI: 10.1007/s00125-009-1478-5]

171 **Wu YY**, Yu T, Zhang XH, Liu YS, Li F, Wang YY, Gong P. 1,25(OH)2D3 inhibits the deleterious effects induced by high glucose on osteoblasts through undercarboxylated osteocalcin and insulin signaling. *J Steroid Biochem Mol Biol* 2012; **132**: 112-119 [PMID: 22595150 DOI: 10.1016/j.jsbmb.2012.05.002]

172 **Chen S**, Villalta SA, Agrawal DK. FOXO1 Mediates Vitamin D Deficiency-Induced Insulin Resistance in Skeletal Muscle. *J Bone Miner Res* 2016; **31**: 585-595 [PMID: 26462119 DOI: 10.1002/jbmr.2729]

173 **Oh J**, Riek AE, Darwech I, Funai K, Shao J, Chin K, Sierra OL, Carmeliet G, Ostlund RE Jr, Bernal-Mizrachi C. Deletion of macrophage Vitamin D receptor promotes insulin resistance and monocyte cholesterol transport to accelerate atherosclerosis in mice. *Cell Rep* 2015; **10**: 1872-1886 [PMID: 25801026 DOI: 10.1016/j.celrep.2015.02.043]

174 **Ni W**, Glenn DJ, Gardner DG. Tie-2Cre mediated deletion of the vitamin D receptor gene leads to improved skeletal muscle insulin sensitivity and glucose tolerance. *J Steroid Biochem Mol Biol* 2016; **164**: 281-286 [PMID: 26369613 DOI: 10.1016/j.jsbmb.2015.09.017]

175 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403 [PMID: 11832527 DOI: 10.1056/NEJMoa012512]

176 **Knowler WC**, Hamman RF, Edelstein SL, Barrett-Connor E, Ehrmann DA, Walker EA, Fowler SE, Nathan DM, Kahn SE; Diabetes Prevention Program Research Group. Prevention of type 2 diabetes with troglitazone in the Diabetes Prevention Program. *Diabetes* 2005; **54**: 1150-1156 [PMID: 15793255 DOI: 10.2337/diabetes.54.4.1150]

177 **Karnchanasorn R**, Ou HY, Chiu KC. Proposed Guidelines for Future Vitamin D Studies. *JAMA Intern Med* 2016; **176**: 280-281 [PMID: 26830241 DOI: 10.1001/jamainternmed.2015.7974]

178 **Chiu KC**, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**: 820-825 [PMID: 15113720 DOI: 10.1093/ajcn/79.5.820]

179 **Karnchanasorn R**, Ou HY, Chiu KC. Plasma 25-hydroxyvitamin D levels are favorably associated with β-cell function. *Pancreas* 2012; **41**: 863-868 [PMID: 22258069 DOI: 10.1097/MPA.0b013e31823c947c]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1386-1400

DOI: 10.4239/wjd.v12.i9.1386

ISSN 1948-9358 (online)

REVIEW

Review of the management of sight-threatening diabetic retinopathy during pregnancy

Priscilla Peixi Choo, Norshamsiah Md Din, Nooraniah Azmi, Mae-Lynn Catherine Bastion

ORCID number: Priscilla Peixi Choo 0000-0002-1518-3685; Norshamsiah Md Din 0000-0002-5137-1632; Nooraniah Azmi 0000-0002-2446-258X; Mae-Lynn Catherine Bastion 0000-0002-6856-8052.

Author contributions: Choo PP conducted the literature review, wrote the first draft, and performed plagiarism and grammar checks; Bastion MLC revised the drafts, formatted the article, and directed the writing; Azmi N and Md Din N critically evaluated the writing and made further amendments to it.

Supported by National University of Malaysia, Bangi, Selangor, Malaysia for English Editing, No. GP-K009894 (2015).

Conflict-of-interest statement: Mae-Lynn Catherine Bastion has received fees for serving as a speaker and/or an advisory board member for Novartis, Alcon, and Santen. She received fees for serving as a speaker for Bayer and Allergan. She has received research funding from Alcon, Novartis, Santen, and Universiti Kebangsaan Malaysia. She is an employee of National University of Malaysia. She holds a patent draft (PT/7449/UKM/20) pending for topical insulin eyedrops. Norshamsiah Md Din has received fees for serving as a speaker

Priscilla Peixi Choo, Norshamsiah Md Din, Nooraniah Azmi, Mae-Lynn Catherine Bastion, Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur 56000, Wilayah Persekutuan, Malaysia

Nooraniah Azmi, Department of Ophthalmology, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Corresponding author: Mae-Lynn Catherine Bastion, FRCS, MBBS, Doctor, Professor, Surgeon, Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre, Faculty of Medicine Universiti Kebangsaan Malaysia Jalan Yaacob Latif, Kuala Lumpur 56000, Wilayah Persekutuan, Malaysia. mae-lynn@ppukm.ukm.edu.my

Abstract

Diabetes mellitus (DM) is a noncommunicable disease reaching epidemic proportions around the world. It affects younger individuals, including women of childbearing age. Diabetes can cause diabetic retinopathy (DR), which is potentially sight threatening when severe nonproliferative DR (NPDR), proliferative DR (PDR), or sight-threatening diabetic macular oedema (STDME) develops. Pregnancy is an independent risk factor for the progression of DR. Baseline DR at the onset of pregnancy is an important indicator of progression, with up to 10% of women with baseline NPDR progressing to PDR. Progression to sight-threatening DR (STDR) during pregnancy causes distress to the patient and often necessitates ocular treatment, which may have a systemic effect. Management includes prepregnancy counselling and, when possible, conventional treatment prior to pregnancy. During pregnancy, closer follow-up is required for those with a long duration of DM, poor baseline control of blood sugar and blood pressure, and worse DR, as these are risk factors for progression to STDR. Conventional treatment with anti-vascular endothelial growth factor agents for STDME can potentially lead to foetal loss. Treatment with laser photocoagulation may be preferred, and surgery under general anaesthesia should be avoided. This review provides a management plan for STDR from the perspective of practising ophthalmologists. A review of strategies for maintaining the eyesight of diabetic women with STDR with emphasis on prepregnancy counselling and planning, monitoring and safe treatment during pregnancy, and management of complications is presented.

Key Words: Sight-threatening diabetic retinopathy; Severe nonproliferative diabetic retinopathy; Proliferative diabetic retinopathy; Diabetic macula oedema; Pregnancy;

and/or an advisory board member for Novartis. She receives fees for serving as a speaker for Santen, DKSH, and Novartis. She has received research funding from Allergan. She is an employee of Universiti Kebangsaan Malaysia. She holds a patent draft (file no: UKM. IKB. 800–4/1/1960) for the synthetic eye model for cataract surgery simulation. Choo Peixi and Nooraniah Azmi have no conflicts of interest to disclose.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Malaysia

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 25, 2021
Peer-review started: January 25, 2021
First decision: June 16, 2021
Revised: June 25, 2021
Accepted: August 12, 2021
Article in press: August 12, 2021
Published online: September 15, 2021

P-Reviewer: Tolunay HE
S-Editor: Fan JR
L-Editor: A

Panretinal photocoagulation

Core Tip: Progression of diabetic retinopathy (DR) to the sight-threatening DR (STDR) is rare during pregnancy but can cause significant ocular morbidity and distress to the mother. Good prepregnancy and intrapartum control of systemic risk factors, especially blood sugar and blood pressure, and adequate prepregnancy treatment of STDR will reduce complications during pregnancy. When STDR develops, conventional therapy for nonpregnant individuals may not be applied. This includes avoidance of anti-vascular endothelial growth factor agents conventionally for diabetic macular oedema and proliferative DR (PDR), especially during early trimesters. Panretinal photocoagulation is a safe option for PDR. Surgical treatments should be performed under local anaesthesia or preferentially deferred until postpartum.

Citation: Choo PP, Md Din N, Azmi N, Bastion MLC. Review of the management of sight-threatening diabetic retinopathy during pregnancy. *World J Diabetes* 2021; 12(9): 1386-1400
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1386.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1386

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disease that involves multiple organs and may cause severe visual impairment. DM is known to affect several ocular structures, including the extraocular muscles, the intraocular lens, the optic nerve, and the retina. However, diabetic retinopathy (DR) is the most common and leading cause of blindness among working-age adults in developing countries[1]. Of 285 million people worldwide with diabetes in 2010[2], approximately one-third have signs of DR, and one-third of these patients may have vision-threatening retinopathy, defined as severe nonproliferative DR (NPDR), proliferative DR (PDR), or diabetic macular oedema (DME)[3]. In Southeast Asia alone, the total number of people with diabetes is expected to reach more than 140 million by 2040. Over 20 years, the prevalence has more than doubled among Malaysians aged 30 or more years, with a prevalence of 22.6% in 2013[4,5].

CLINICAL FEATURES OF DR

DR can be classified into several stages: (1) Mild NPDR characterized by increased vascular permeability; (2) Moderate NPDR depicted by vascular closure with less than 20 microaneurysms; (3) Severe NPDR, which is identified as any of the following clinical features: Microaneurysms in all 4 quadrants, venous beading in 2 or more quadrants, and intraretinal microvascular abnormalities in 1 or more quadrant; (4) Very severe NPDR if they have 2 or more of the criteria for severe NPDR; and (5) PDR which is characterized by the growth of new blood vessels (neovascularization) on the optic disc, retina, or on the posterior surface of the vitreous. DME is characterized by retinal thickening from leaky blood vessels that can develop at any stage of DR.

The progression of DR during pregnancy increases the frequency of perinatal follow-ups and may necessitate stressful treatments[6]. Sight-threatening DR (STDR) can cause ocular morbidity, which can lead to psychological distress in new mothers [7]. Poor vision may also lead to adverse effects on newborns through neglect and postnatal depression in the mother[7].

RISK FACTORS FOR DR PROGRESSION

The incidence of DR is highly dependent on the duration and control of diabetes, and risk factors such as hyperglycaemia[8,9], hypertension[10], dyslipidaemia[11], and

P-Editor: Ma YJ

nephropathy[12] may accelerate DR progression in both pregnant and nonpregnant individuals.

In women with pre-existing DM, pregnancy is also known to be associated with worsening DR[13]. As the prevalence of type 1 DM (T1DM)[14] and type 2 DM (T2DM)[15] increases globally, recent studies have found that the incidence of DR in early pregnancy is approximately 63% in T1DM[16] and 14% in T2DM[13]. The adverse effects of pregnancy on retinal status occur by the end of the second trimester and regress after delivery, but some severe cases may persist into the first year postpartum[13,17-19]. Risk factors such as poor glycaemic control during pregnancy [13], longer duration of diabetes before conception[20], rapid normalization of glycated haemoglobin (HbA1c) at the beginning of pregnancy[20], hypertension[21], and preeclampsia[22] may influence the development and progression of DR during pregnancy.

The severity of DR at conception also has an impact on DR progression during pregnancy, as progression was more significant in pregnant women with moderate and severe forms of DR than in those with mild or no DR[16]. According to the Diabetes in Early Pregnancy Study, approximately 55% of pregnant women with moderate-to-severe NPDR and 21% with mild NPDR showed deterioration of DR[20]. A review by Morrison *et al*[23] found that when NPDR was present at baseline, 30.2% worsened, and 9.8% progressed to proliferative disease[23]. Macular oedema typically occurs alongside proteinuria or hypertension and may progress throughout pregnancy and resolve during the postpartum period; however, some cases may persist and cause long-term vision loss[24].

PREPARING FOR DR PROGRESSION IN PREGNANT DIABETICS

Screening and pre-pregnancy counselling and treatment

Screening for DR is an important aspect of diabetes management, as it aims to detect DR as early as possible to enable timely treatment and prevent vision loss[25]. Diabetic women should have a preconception retinal screening and counselling on the risk of development and progression of DR, as well as comprehensive care by a multidisciplinary team consisting of an endocrinologist, an ophthalmologist, and a perinatologist [26]. Comprehensive eye assessment, tight glycaemic control, and other assessments will be performed throughout the pregnancy period[27]. The duration of the follow-up is dependent on the stage of DR; the more severe the DR is at diagnosis during the initial check-up, the more frequent the follow-up schedule will be. Maximal control of both glucose levels and blood pressure is essential in the treatment of DR during pregnancy[16].

Laser photocoagulation for DR

Currently, scatter or panretinal photocoagulation (PRP) is a preferred treatment modality for all patients, including pregnant women with DR, which involves applying laser burns on the retina while sparing the central macular area to reduce the ischaemic drive and the risk of vision loss[28]. In an unfortunate event of DR progression, pregnant women with severe NPDR and PDR at the preproliferative stage may consider either scatter or PRP, as both are effective and safe treatments with minimal side effects to the foetus[29,30]. Although the results from protocol S of DRCR.net found that both anti-vascular endothelial growth factor agents (anti-VEGF) and PRP are effective for PDR, anti-VEGF in pregnancy should be avoided whenever possible to minimize the placental transfer of drugs and risk to the foetus[30,31]. However, PRP treatment is associated with potential side effects, including worsening of macular oedema that may lead to transient or permanent vision loss, peripheral visual field defects, night vision loss, loss of contrast sensitivity, potential complications from misdirected or excessive burns, and progression of visual loss[32].

Anti-VEGF agents for PDR and DME in pregnancy

VEGF, an endothelial-cell-specific angiogenic factor[33], was suggested to be the primary mediator of diabetic retinal neovascularization, as its concentration in ocular fluid samples from patients with PDR was found to be significantly increased compared to samples from patients with NPDR[34]. Since then, clinical studies have suggested that anti-VEGF therapy is effective for PDR[30], and various anti-VEGF drugs, such as pegaptanib, ranibizumab, bevacizumab, and aflibercept, have been used. Pegaptanib (Macugen®; Pfizer Inc.) is a 28-base ribonucleic acid aptamer that specifically binds to and blocks the activity of the 165 amino acid isoform of VEGF

($VEGF_{165}$)[35] and was approved by the United States Federal Drug Administration (FDA) for the treatment of neovascular age-related macular degeneration in 2004[36]; administration of a 0.3 mg (0.9 mL) dose is recommended once every six weeks by intravitreal injection. The use of pegaptanib has been shown to reduce retinal thickness and improve vision in PDR[37] and macular oedema[38]. However, its use worldwide and in Malaysia for DME and PDR in nonpregnancy diabetic patients has been largely superseded by the other 3 anti-VEGF agents.

Ranibizumab (Lucentis®; Genentech Inc.) is a humanized monoclonal antibody fragment directed at all isoforms of VEGF-A and contains only the Fab fragment of the parental anti-VEGF antibody with a weight of 48 kDa[39]. The DR Clinical Research Network's (DRCR.net) Protocol S study found that eyes treated with ranibizumab were less likely to have vitreous haemorrhage (VH) and progress from severe NPDR to PDR than those treated with PRP[30]**.** The use of ranibizumab 0.3 to 0.5 mg (0.05 mL) as a monthly intravitreal injection attained FDA approval for the treatment of all forms of DR in 2017.

Bevacizumab (Avastin®; Genentech Inc.), a full-length recombinant humanized monoclonal immunoglobulin G1κ antibody weighing 149 kDa, which inactivates all VEGF isoforms[39], was FDA-approved as a treatment for colorectal carcinoma in 2004. It is used as an off-label therapy by many ophthalmologists, as trials found its side-effect profile with doses of either 1.25 mg or 2.5 mg (0.05 mL) to be similar to ranibizumab[40]. A 2-year randomized controlled trial also provided evidence supporting the use of bevacizumab for persistent centre-involving macular oedema [41].

Aflibercept (Eylea®; Regeneron Inc.) is a 115 kDa recombinant fusion protein that consists of VEGF-binding domains for human VEGF receptors 1 and 2 fused to the Fc domain of human immunoglobulin-G1 and binds to all isomers of the VEGF-A family [38]. In 2014, the FDA approved aflibercept for the treatment of macular oedema after significant improvements in the primary endpoint of mean change in best-corrected visual acuity were achieved for the aflibercept-treated group in completed phase III VIVID and VISTA[42] trials, and the 52-wk visual and anatomic superiority of the intravitreal aflibercept injection group was sustained through week 100[43]. The Panorama trial[44] was then conducted to investigate aflibercept for the improvement of moderate-severe to severe NPDR without macular oedema, and the safety data were consistent with the results of phase III VIVID and VISTA trials, and the outcome was sustained through week 100[45]; thus, it obtained FDA approval for the treatment of DR in 2019. The recommended dosage of aflibercept injection for the treatment of macular oedema and DR is 2 mg (0.05 mL) every 8 wk after five initial monthly injections.

VEGF also plays a role in the maintenance of foetal and placental vasculature[46]; thus, a reduction in VEGF expression has been linked with defective embryogenesis and foetal loss in humans[47]. Studies also found that the inhibition of VEGF activity and signalling pathways may lead to hypertension[48-50]**.** Despite this, the relationship between VEGF, hypertension, and preeclampsia is poorly understood. The teratogenicity of anti-VEGF drugs have been explored, categorized, and detailed by the FDA as follows[31]: Pegaptanib has been assigned to Pregnancy Category B, where no teratogenicity was found in mice when given an intravenous dose of up to 40 mg/kg/d (approximately 7000 times the recommended human dose of 0.3 mg *per* eye), while human studies are not yet available[51]; ranibizumab is designated Pregnancy Category C, where an embryo-foetal developmental toxicity study was performed on pregnant cynomolgus monkeys, and skeletal abnormalities were found in foetuses from monkeys treated with a dose of 1 mg/eye (approximately 13 times higher than predicted mean-steady stage C_{max} levels with single eye treatment in humans); no skeletal abnormalities were observed at the lower dose of 0.125 mg/eye (equivalent to C_{max} levels with single eye treatment in humans), and no adequate and well-controlled studies of the administration have been conducted in pregnant women [52]; bevacizumab has been assigned to Pregnancy Category C, as pregnant rabbits dosed with 10 mg/kg to 100 mg/kg (approximately 1 to 10 times the clinical dose of 10 mg/kg) every three days during day 6–18 of gestation showed decrease in maternal and foetal body weights, increased number of foetal resorptions, skeletal deformities, and corneal opacity in all doses, while controlled data are not yet available in human pregnancy[53]; and aflibercept is designated Pregnancy Category C, where embryo-foetal development studies on rabbits with intravenous doses of ≥ 3 mg/kg have revealed evidence of embryo-foetal toxicity such as post-implantation loss and foetal malformations including skeletal abnormalities in all doses, while no controlled data are yet available in pregnant women[54].

The pharmacokinetics of these anti-VEGF drugs have been tested in animals and humans, but not all pharmacokinetic values in humans have been obtained. Nevertheless, the pharmacokinetic characteristics of these 4 drugs appear to be similar. Following intravitreal injections, these anti-VEGF drugs leave the eye by crossing the retina and retinal pigment epithelium to the choroidal circulation, passing through the ciliary body and iris, or moving into the anterior chamber by diffusion and bulk flow before exiting through the trabecular meshwork, and none of the drugs degrades within the eye[55]. Systemic half-lives vary from hours to weeks before drug elimination *via* glomerular filtration or pinocytotic elimination occurs.

Pegaptanib was found to have an intravitreal half-life of 3.9 d in monkeys[56] and an estimated half-life of 7 d in humans. After entering the systemic circulation in humans, the maximum serum concentration is reached in 1–4 d, and the serum half-life is 10 d. It is metabolized by endonucleases and exonucleases, which are then excreted primarily in the urine. On the other hand, after intravitreal injection into rabbits, ranibizumab has a half-life of 2.6–2.88 d[57-59] with a maximum aqueous concentration after 3 d. Ranibizumab fully penetrates the retina one day after injection, and the concentrations in the serum are either very low (1/10000 that of the vitreous) [58] or undetectable[59]. The half-life of ranibizumab in monkeys is 3 d, and serum concentrations are 1000-fold lower than those in the vitreous[60]. Intravitreal ranibizumab is found to distribute rapidly to the monkeys' retina within 6–24 h[60]. The half-life of intravitreal ranibizumab in humans is estimated to be 4.8–9 d, with serum concentrations approximately 90000-fold lower than intraocular concentrations [55]. The intravitreal and serum half-lives of bevacizumab in rabbits are 4.32 and 6.8 d, respectively[61,62], with a maximum serum concentration reached in 8 d. After intravitreal injections in rabbits, bevacizumab appeared in the subretinal space within 2 h [63], the inner retina and choroid within the first day, and the outer layers and choroid in subsequent days, but no drugs were found at 4 wk[64]. The half-life of intravitreal bevacizumab in a human was estimated to be 6.7–10 d depending on the use of either a one-compartment model or two-compartment model[65-68], while the half-life of bevacizumab in human serum is 17–21 d, similar to that of other full-length antibodies. Intravitreal aflibercept has a half-life of 4.7 d in rabbits[69] and an estimated 9 d in humans based on the intermediate size of the molecule (between ranibizumab and bevacizumab), while bound aflibercept in human serum has a half-life of 18 d[70]. Table 1 summarizes the structural and pharmacokinetic characteristics of the four anti-VEGF drugs. No study has been found to determine whether these drugs cross the placenta in pregnant women.

Several studies on the use of ranibizumab and bevacizumab in pregnant women have been reported of which some have been summarized by Polizzi and Mahajan [31]. Most of the studies in pregnant women are case reports, and initial intravitreal ranibizumab was given either 8–17 wk post last menstrual period (LMP)[70] or in the third trimester[71,72]; all reported no complications.

However, intravitreal anti-VEGF injections given as early as 5 wk postconception were associated with miscarriage within a week[73]. A total of 8 papers comprising 16 pregnancies in 15 women using intravitreal bevacizumab have been published since 2009[74-83]. The injection was given between a few days before or after the LMP and during the third trimester. There were 5 cases of abortion[76,79,82,83] and one case of pre-eclampsia[80] after the use of intravitreal bevacizumab. Petrou *et al*[76] described 2 women who received intravitreal bevacizumab at approximately 4 and 3 wk of gestation, respectively, followed by spontaneous miscarriage 7 and 10 d, respectively, after administration of the drug[76]. Gómez Ledesma *et al*[79] also reported a 41-year-old woman who received intravitreal bevacizumab a few days before or after the LMP and suffered a miscarriage approximately 7 wk after the injection[79]. Kianersi *et al*[82, 83] reported pregnancy loss within 18 to 24 h in two patients who received intravitreal bevacizumab injection while they were between 10 and 12 wk pregnant[82,83]. Intravitreal bevacizumab given preconception and continued after 29 wk of gestation was associated with preeclampsia requiring urgent caesarean section[80].

Despite these reports of spontaneous miscarriages and preeclampsia occurring after intravitreal anti-VEGF injections given within 13 wk of gestation, other reports did not find adverse events with injections given within the same time frame[70,74,75,77,78,80, 81]; thus, it is uncertain whether anti-VEGF therapy played a role in these pregnancy losses, as the rate of spontaneous miscarriage is between 15% and 20%[84] and may increase to as high as 41% if maternal age is over 35 years[85]. There were no reports on pegaptanib and aflibercept being administered in pregnant women. Hence, the use of anti-VEGF should be weighed against the possible risk of foetal developmental abnormalities or pregnancy loss and should only be administered following a thorough discussion with the patient and consultation with an obstetrician, and the

Table 1 Structural and pharmacokinetic characteristics of the four anti-vascular endothelial growth factor drugs[55]

	Pegaptanib	Ranibizumab	Bevacizumab	Aflibercept
Structure	Pegylated aptamer	Recombinant monoclonal antibody fragment (Fab)	Recombinant monoclonal antibody (Mab)	Fusion protein
Molecular weight (kDa)	50	48	149	115
Recommended dose (volume)	0.3 mg (0.9 mL)	0.5 mg (0.05 mL)	1.25 mg (0.05 mL)	2 mg (0.05 mL)
Intravitreal half-life (d)	3.9 (monkeys)	2.6-2.88 (rabbits)	4.32-6.61 (rabbits)	4.5-4.7 (rabbits)
		3-3.2 (monkeys)	3.1 (monkeys)	
		7.1 (humans)	6.7-10 (humans)	
Serum half-life humans (d)	10	0.25	21	18

potential benefit outweighs the potential risk to the foetus. Indeed, DM patients of child-bearing age should have PDR and DME treated before conceiving. This even means the need for contraception during anti-VEGF treatment.

Topical nonsteroidal anti-inflammatory agents in pregnancy

Apart from VEGF, elevated inflammatory markers have been found in patients with DR, which suggests that inflammation may play a role in the pathogenesis of DR[86] and macular oedema[87,88]. Both animal and human studies have found increased levels of inflammatory mediators and prostaglandins (PGs) in DR in the vitreous cavity[89-91], and prostaglandin E_2 levels correlate with vitreous levels of VEGF[92]. As topical nonsteroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of cyclooxygenase enzymes and reduce the synthesis of proinflammatory PGs with few documented risks, they have recently become readily available in the form of topical ophthalmic formulations[93]. New topical NSAIDs such as nepafenac (Nevanac®; Alcon Inc.) were formulated to be able to reach the posterior segment of the eye[94,95]. It rapidly penetrates the cornea and is deaminated by intraocular hydrolases in uveal tissue and retina to form the active metabolite amfenac[96].

Several small randomized case studies on the use of topical nepafenac 0.1% for the treatment of DME have been published[97-100] and revealed the effectiveness of the drug and improvement in visual acuity and retinal/foveal/macular thickness. However, a phase II, multicentre, double-masked randomized clinical trial conducted by DRCR.net found that topical nepafenac 0.1% three times a day for a year on eyes with noncentral DME does not show a beneficial effect on OCT-measured retinal thickness or visual acuity outcomes[101], which is in contrast to the results of other smaller, randomized published case reports. Small quantifiable plasma concentrations of nepafenac and amfenac have been found in subjects 2–3 h after topical administration, and the C_{max} of nepafenac and amfenac in serum was approximately 0.31 and 0.42 ng/mL, respectively[102]. The elimination of orally administered nepafenac in rats was shown to be in the urine (57%) and faeces (40%) over 7 d[103]. The FDA has also categorized nepafenac under pregnancy category C, as reproduction studies performed in rabbits and rats at oral doses of up to 10 mg/kg/d have revealed maternal toxicity and no teratogenicity[104]. Animal exposure to nepafenac and amfenac was approximately 260- to 2400-fold human plasma exposure at the recommended human topical ophthalmic dose for rats and approximately 80- and 680-fold human plasma exposure for rabbits, respectively, at this dose. Dystocia increased post-implantation loss, reduced foetal weight and growth, and reduced foetal survival in maternal rats when given doses of ≥ 10 mg/kg. Although nepafenac could cross the placental barrier in rats, no adequate and well-controlled studies in pregnant women have been conducted; therefore, nepafenac should be used in pregnancy only if the potential benefit outweighs the potential risk to the foetus and should be avoided in the third trimester due to the known effects of prostaglandin biosynthesis inhibition on the foetal cardiovascular system (closure of ductus arteriosus)[105].

Vitrectomy for complications of STDR in pregnancy

VH secondary to PDR is one of the most common vision-threatening complications of DR other than DME. In mild to moderate cases of VH, PRP is performed when possible to prevent further episodes of VH, and it may eventually resolve spontaneously[106]. However, approximately 5% of PDR cases develop VH even after PRP is

initiated, which often requires pars plana vitrectomy (PPV)[107], a technique introduced in the 1970s[108]. Despite vision improvement reported in approximately 75% of PDR patients after PPV, major complications associated with PPV include cataract formation, elevated intraocular pressure, recurrent vitreous cavity haemorrhage (early, delayed, or persistent), iatrogenic retinal breaks, tractional and rhegmatogenous retinal detachment, and neovascular glaucoma[109]. Several studies have been conducted on the use of anti-VEGF drugs as a treatment for VH due to PDR and found that intravitreal ranibizumab[110], bevacizumab[111,112], and aflibercept [113] had good short-term safety and efficacy for new or recurrent VH in PDR eyes with and without a previously lasered approach, reducing the need for PPV. As the use of anti-VEGF drugs is associated with pregnancy loss and foetal abnormalities, PRP and PPV remain the treatment of choice for VH in pregnant patients with PDR. Surgery should be conducted under the assistance of an experienced anaesthetist to anticipate pregnancy-related anaesthetic complications[114].

Advances in PPV instrumentation have led to small-gauge vitrectomy increasing in popularity, improving the surgical experience, and allowing PPV to be performed under local anaesthesia. Nevertheless, surgical treatment of any kind is a form of stress during pregnancy. The supine position required for PPV may even prove challenging for pregnant patients due to the gravid uterus. Hence, this reiterates the need to stabilize PDR before pregnancy with a PRP laser and, if needed, PPV in diabetic patients. Although anti-VEGF has advantages, it cannot be used as a prepregnancy therapy for diabetic women with active PDR who are intending to conceive. This is due to the risk of conception loss when they subsequently conceive while treatment has to continue during pregnancy. If PDR progression occurs, surgical treatment should be delayed after delivery if this option is available.

Anaesthesia in the pregnant diabetic

Management of DR in pregnancy is essential, and preventing the development and progression of DR should be at all costs, as well as ensuring maternal and foetal safety. However, ophthalmic surgery during pregnancy poses additional challenges, which include the timing of the surgery, the posture during surgery, and the type of anaesthesia. Elective surgery is recommended to be postponed until 6 wk postpartum, while essential surgery should be performed in the second trimester if possible when preterm contractions and spontaneous abortions are least likely[115]. Pregnant women are susceptible to hypoxia, hypercapnia, and systemic hypotension due to altered maternal physiology, which exposes both the mother and the foetus to the risk of surgical anaesthesia, particularly general anaesthesia. Moreover, the supine position in the second and third trimesters can induce profound hypotension due to aortic and vena cava compression by the uterus. Pregnant patients should therefore be positioned with their hips, abdomen, and thighs on their left side while maintaining a normal head position for ophthalmic surgery[116].

Current anaesthetic medications, including general anaesthetics (nitrous oxide excluded), benzodiazepines, and opioids, have not been shown to have any teratogenic effects in humans when using standard concentrations at any gestational age[117,118] and have not been associated with increased rates of stillbirths or adverse pregnancy outcomes[119]. However, reports have shown an increased incidence of low birth weight and neural tube defects with exposure to general anaesthesia in the first trimester[120]; thus, general anaesthesia should be avoided whenever possible.

Local anaesthetics work by blocking sodium channels in nerve membranes, leading to absent nerve impulses and anaesthesia[121]. An extensive study on local anaesthetic use in 60000 pregnant females included benzocaine, procaine, tetracaine, and lidocaine and revealed no increased incidence of foetal complications[122] or foetal birth defects [123].

Under circumstances where general anaesthesia was necessary, an appropriate understanding of additional pregnancy-related risks should be considered, including intubation difficulties, aspiration risks, thromboprophylaxis, and foetal well-being [124]. General anaesthetics work at the level of the spinal cord and in different areas of the brain, which results in relaxation of the muscles and central nervous system depression, although the exact mechanism of action has not been ascertained[125]. Thiopentone in late pregnancy showed no significant effect on intrauterine pressure, while ketamine was found to cause a uterine contraction in early pregnancy and no effect in late pregnancy[126]. Volatile anaesthetics such as halothane, sevoflurane, desflurane, and isoflurane have been shown to inhibit uterine contractility; thus, they may be beneficial in preventing preterm contractions[127]. Nonetheless, the choice of anaesthetic technique and the selection of appropriate anaesthetic drugs should be carefully considered to preserve maternal safety, maintain the pregnancy state, and

achieve the best possible foetal outcome.

Screening for DR during pregnancy

According to Malaysia's Clinical Practice Guidelines: Screening of DR, individuals with pre-existing DM who are planning for pregnancy should have their eyes examined before conception and counselled on the risk of DR development and progression[128]. Subsequent follow-up is dependent on the stage of DR found on the initial examinations: Every 3 mo for mild to no DR and referral to an ophthalmologist is necessary for moderate to severe DR. Women with gestational DM (GDM) do not require DR screening, as it carries no risk of DR unless GDM is diagnosed in the first trimester of pregnancy. GDM is a glucose intolerance state induced by pregnancy that may resolve or persist after the pregnancy period[129,130], and the prevalence of GDM in Malaysia was reported to be approximately 8.8%[131]. Women with GDM have a sevenfold increased relative risk of progressing to T2DM[132-134], and they are usually asymptomatic until macular oedema or PDR has developed.

Bastion *et al*[135] reported a case of a 36-year-old pregnant woman who had GDM at her previous pregnancy with an elevated post-delivery maternal glucose tolerance test. Her first-trimester fundoscopy found no DR. By the second trimester, she had developed PDR, and PRP was performed on both eyes during her pregnancy. This was followed by PPV with membrane peeling in the right eye at five months postpartum, as the right VH did not resolve spontaneously, leaving her with counting-finger vision [135]. On the other hand, Raman and Livingstone reported a case study of a 31-year-old pregnant woman with underlying T2DM who had diffuse VH on both eyes at her 22nd week of gestation, which required urgent PRP. However, she developed recurrent VH in her third trimester, and PPV was then performed at 2 wk postpartum for her right eye, as it then developed inferior combined rhegmatogenous and tractional retinal detachment (TRD). Her left eye had a nonclearing VH requiring PPV a month later[136]. Both cases reported safe delivery of the baby and good postoperative visual acuity[135,136], highlighting the rapid progression of DR and the importance of follow-up and timely surgical intervention for a good final vision outcome.

However, Helen *et al*[137] reported four T1DM women with PDR who had adverse maternal outcomes, including abortion in one patient, preeclampsia, and preterm delivery in one patient, renal failure requiring dialysis in one patient, neonatal death occurring in one case, and premature delivery occurring in another case. All except one woman had stable or improved visual acuity. One woman progressed to develop neovascular glaucoma[137]. Hence, prepregnancy counselling and close follow-up during pregnancy and the postpartum period are essential for diabetic women.

The recommended ophthalmic management of DR during pregnancy at each stage [23,26] is summarized in the following flowchart (Figure 1).

Despite the best efforts to monitor and manage DR during pregnancy, the literature suggests that compliance with treatment and follow-up is still a struggle for pregnant women with diabetes. Hampshire *et al*[138] looked at attendance at a prepregnancy care program for adequate retinal assessment in the subsequent pregnancy and found that 70% of women with pregestational diabetes had incomplete follow-up[138], suggesting a lack of awareness on sight-threatening complications of diabetes[139].

CONCLUSION

There is limited evidence for the management of STDR in pregnancy, with evidence mainly from case reports and series. Management of STDR in pregnancy requires prepregnancy counselling, treatment, and stabilization of DM and STDR. It involves appropriate control of systemic risk factors for DR progression, monitoring of DR with fundus imaging at least every trimester, and prompt referral to the ophthalmologist when there is DR progression during pregnancy. Treatments that are conventional for DME, such as anti-VEGF, should not be given during pregnancy in diabetic patients, particularly in the early trimester, as there have been several reports of foetal loss. PRP can be given for severe NPDR and PDR; however, surgical management for VH or TRD in pregnancy should be deferred. If at all required, surgery should be performed under local anaesthesia, at an earlier trimester, or deferred until after delivery.

PRE-CONCEPTION

Pre-conception counselling addressing clinical modifiers (*e.g.* glycaemic control, hypertension and DR requiring treatment)

Pre-conception fundus ophthalmic examination to determine DR stage prior to conception preferably a dilated fundus examination/at the very least a fundus photographic screening

If STDR is detected, **referral should be made to an ophthalmologist** to ensure that treatment required is applied prior to pregnancy

Contraception may be required if patient needs anti-VEGF

Optimal glycaemic control

FIRST TRIMESTER

Comprehensive eye examination includes photographic screening or dilated exam

If severe NPDR or PDR progresses, consider PRP laser or if DME is detected consider grid or focal laser (Anti-VEGF not advised)

Consider more frequent follow-up, even if vision is normal

Control of risk factors for progression such as blood pressure, blood sugar level

If blood sugar not controlled, initiate insulin therapy

Consider more frequent eye follow-up if control is poor

Review of risk factors for progression during poor glycaemic control, longer duration of diabetes and hypertension

Consider more frequent follow-up

SECOND AND THIRD TRIMESTER

Comprehensive eye examination includes photographic screening or dilated exam

If severe NPDR or PDR progresses, consider PRP laser or if DME is detected, consider grid or focal laser

Consider more frequent eye follow-up, even if vision is normal

Control of risk factors for progression such as blood pressure, blood sugar level

If blood sugar not controlled, initiate insulin therapy

Consider more frequent eye follow-up if control is poor

Review of risk factors for progression during poor glycaemic control, longer duration of diabetes and hypertension

Consider more frequent follow-up if control is poor

Anti-VEGF and surgery permissible if patient understands the risks (Deferment if possible)

POST-PARTUM

Ophthalmic follow-up for DR/DME progression and complications

Majority regressed post-partum

Some may require PRP or PPV after delivery

Figure 1 Flow chart showing the suggested management of sight-threatening diabetic retinopathy during pregnancy. DR: Diabetic retinopathy; STDR: Sight-threatening diabetic retinopathy; DME: Diabetic macular oedema; VEGF: Vascular endothelial growth factor; PRP: Panretinal photocoagulation; PDR: Proliferative DR; NPDR: Nonproliferative diabetic retinopathy.

REFERENCES

1 **Semeraro F**, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic Retinopathy: Vascular and Inflammatory Disease. *J Diabetes Res* 2015; **2015**: 582060 [PMID: 26137497 DOI: 10.1155/2015/582060]

2 **Shaw JE**, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]

3 **Yau JW**, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; **35**: 556-564 [PMID: 22301125 DOI: 10.2337/dc11-1909]

4 **Wan Nazaimoon WM**, Md Isa SH, Wan Mohamad WB, Khir AS, Kamaruddin NA, Kamarul IM, Mustafa N, Ismail IS, Ali O, Khalid BA. Prevalence of diabetes in Malaysia and usefulness of HbA1c as a diagnostic criterion. *Diabet Med* 2013; **30**: 825-828 [PMID: 23413941 DOI: 10.1111/dme.12161]

5 **Hussein Z**, Taher SW, Gilcharan Singh HK, Chee Siew Swee W. Diabetes Care in Malaysia:

Problems, New Models, and Solutions. *Ann Glob Health* 2015; **81**: 851-862 [PMID: 27108152 DOI: 10.1016/j.aogh.2015.12.016]

6 **Fenwick E**, Rees G, Pesudovs K, Dirani M, Kawasaki R, Wong TY, Lamoureux E. Social and emotional impact of diabetic retinopathy: a review. *Clin Exp Ophthalmol* 2012; **40**: 27-38 [PMID: 21575125 DOI: 10.1111/j.1442-9071.2011.02599.x]

7 **Rees G**, Xie J, Fenwick EK, Sturrock BA, Finger R, Rogers SL, Lim L, Lamoureux EL. Association Between Diabetes-Related Eye Complications and Symptoms of Anxiety and Depression. *JAMA Ophthalmol* 2016; **134**: 1007-1014 [PMID: 27387297 DOI: 10.1001/jamaophthalmol.2016.2213]

8 **Harris Nwanyanwu K**, Talwar N, Gardner TW, Wrobel JS, Herman WH, Stein JD. Predicting development of proliferative diabetic retinopathy. *Diabetes Care* 2013; **36**: 1562-1568 [PMID: 23275374 DOI: 10.2337/dc12-0790]

9 **Klein R**, Lee KE, Gangnon RE, Klein BE. The 25-year incidence of visual impairment in type 1 diabetes mellitus the wisconsin epidemiologic study of diabetic retinopathy. *Ophthalmology* 2010; **117**: 63-70 [PMID: 19880184 DOI: 10.1016/j.ophtha.2009.06.051]

10 **Leske MC**, Wu SY, Hennis A, Hyman L, Nemesure B, Yang L, Schachat AP; Barbados Eye Study Group. Hyperglycemia, blood pressure, and the 9-year incidence of diabetic retinopathy: the Barbados Eye Studies. *Ophthalmology* 2005; **112**: 799-805 [PMID: 15878059 DOI: 10.1016/j.ophtha.2004.11.054]

11 **Chew EY**, Davis MD, Danis RP, Lovato JF, Perdue LH, Greven C, Genuth S, Goff DC, Leiter LA, Ismail-Beigi F, Ambrosius WT; Action to Control Cardiovascular Risk in Diabetes Eye Study Research Group. The effects of medical management on the progression of diabetic retinopathy in persons with type 2 diabetes: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Eye Study. *Ophthalmology* 2014; **121**: 2443-2451 [PMID: 25172198 DOI: 10.1016/j.ophtha.2014.07.019]

12 **Estacio RO**, McFarling E, Biggerstaff S, Jeffers BW, Johnson D, Schrier RW. Overt albuminuria predicts diabetic retinopathy in Hispanics with NIDDM. *Am J Kidney Dis* 1998; **31**: 947-953 [PMID: 9631838 DOI: 10.1053/ajkd.1998.v31.pm9631838]

13 **Diabetes Control and Complications Trial Research Group**. Effect of pregnancy on microvascular complications in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. *Diabetes Care* 2000; **23**: 1084-1091 [PMID: 10937502 DOI: 10.2337/diacare.23.8.1084]

14 **You WP**, Henneberg M. Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. *BMJ Open Diabetes Res Care* 2016; **4**: e000161 [PMID: 26977306 DOI: 10.1136/bmjdrc-2015-000161]

15 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]

16 **Vestgaard M**, Ringholm L, Laugesen CS, Rasmussen KL, Damm P, Mathiesen ER. Pregnancy-induced sight-threatening diabetic retinopathy in women with Type 1 diabetes. *Diabet Med* 2010; **27**: 431-435 [PMID: 20536515 DOI: 10.1111/j.1464-5491.2010.02958.x]

17 **Moloney JB**, Drury MI. The effect of pregnancy on the natural course of diabetic retinopathy. *Am J Ophthalmol* 1982; **93**: 745-756 [PMID: 6178293 DOI: 10.1016/0002-9394(82)90471-8]

18 **Temple RC**, Aldridge VA, Sampson MJ, Greenwood RH, Heyburn PJ, Glenn A. Impact of pregnancy on the progression of diabetic retinopathy in Type 1 diabetes. *Diabet Med* 2001; **18**: 573-577 [PMID: 11553188 DOI: 10.1046/j.1464-5491.2001.00535.x]

19 **Schultz KL**, Birnbaum AD, Goldstein DA. Ocular disease in pregnancy. *Curr Opin Ophthalmol* 2005; **16**: 308-314 [PMID: 16175045 DOI: 10.1097/01.icu.0000179803.42218.cc]

20 **Chew EY**, Mills JL, Metzger BE, Remaley NA, Jovanovic-Peterson L, Knopp RH, Conley M, Rand L, Simpson JL, Holmes LB. Metabolic control and progression of retinopathy. The Diabetes in Early Pregnancy Study. National Institute of Child Health and Human Development Diabetes in Early Pregnancy Study. *Diabetes Care* 1995; **18**: 631-637 [PMID: 8586000 DOI: 10.2337/diacare.18.5.631]

21 **Rosenn B**, Miodovnik M, Kranias G, Khoury J, Combs CA, Mimouni F, Siddiqi TA, Lipman MJ. Progression of diabetic retinopathy in pregnancy: association with hypertension in pregnancy. *Am J Obstet Gynecol* 1992; **166**: 1214-1218 [PMID: 1566772 DOI: 10.1016/s0002-9378(11)90608-5]

22 **Lövestam-Adrian M**, Agardh CD, Aberg A, Agardh E. Pre-eclampsia is a potent risk factor for deterioration of retinopathy during pregnancy in Type 1 diabetic patients. *Diabet Med* 1997; **14**: 1059-1065 [PMID: 9455934 DOI: 10.1002/(SICI)1096-9136(199712)14:12<1059::AID-DIA505>3.0.CO;2-8]

23 **Morrison JL**, Hodgson LA, Lim LL, Al-Qureshi S. Diabetic retinopathy in pregnancy: a review. *Clin Exp Ophthalmol* 2016; **44**: 321-334 [PMID: 27062093 DOI: 10.1111/ceo.12760]

24 **Omoti AE**, Waziri-Erameh JM, Okeigbemen VW. A review of the changes in the ophthalmic and visual system in pregnancy. *Afr J Reprod Health* 2008; **12**: 185-196 [PMID: 19435022]

25 **Wang LZ**, Cheung CY, Tapp RJ, Hamzah H, Tan G, Ting D, Lamoureux E, Wong TY. Availability and variability in guidelines on diabetic retinopathy screening in Asian countries. *Br J Ophthalmol* 2017; **101**: 1352-1360 [PMID: 28292772 DOI: 10.1136/bjophthalmol-2016-310002]

26 **Mallika P**, Tan A, S A, T A, Alwi SS, Intan G. Diabetic retinopathy and the effect of pregnancy. *Malays Fam Physician* 2010; **5**: 2-5 [PMID: 25606177]

27 **Klein BE**, Moss SE, Klein R. Effect of pregnancy on progression of diabetic retinopathy. *Diabetes*

Care 1990; **13**: 34-40 [PMID: 2404715 DOI: 10.2337/diacare.13.1.34]
28 **Canadian Diabetes Association Clinical Practice Guidelines Expert Committee**, Cheng AY. Canadian Diabetes Association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. Introduction. *Can J Diabetes* 2013; **37** Suppl 1: S1-S3 [PMID: 24070926 DOI: 10.1016/j.jcjd.2013.01.009]
29 **American Academy of Ophthalmology Retina/Vitreous Panel**. Preferred Practice Pattern® Guidelines. Diabetic Retinopathy. San Francisco, CA: American Academy of Ophthalmology, 2014
30 **Writing Committee for the Diabetic Retinopathy Clinical Research Network**. Gross JG, Glassman AR, Jampol LM, Inusah S, Aiello LP, Antoszyk AN, Baker CW, Berger BB, Bressler NM, Browning D, Elman MJ, Ferris FL 3rd, Friedman SM, Marcus DM, Melia M, Stockdale CR, Sun JK, Beck RW. Panretinal Photocoagulation *vs* Intravitreous Ranibizumab for Proliferative Diabetic Retinopathy: A Randomized Clinical Trial. *JAMA* 2015; **314**: 2137-2146 [PMID: 26565927 DOI: 10.1001/jama.2015.15217]
31 **Polizzi S**, Mahajan VB. Intravitreal Anti-VEGF Injections in Pregnancy: Case Series and Review of Literature. *J Ocul Pharmacol Ther* 2015; **31**: 605-610 [PMID: 26302032 DOI: 10.1089/jop.2015.0056]
32 **American Academy of Ophthalmology**. Preferred practice pattern diabetic retinopathy. San Francisco, CA: American Academy of Ophthalmology, 1998
33 **Ferrara N**, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 1992; **13**: 18-32 [PMID: 1372863 DOI: 10.1210/edrv-13-1-18]
34 **Aiello LP**, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994; **331**: 1480-1487 [PMID: 7526212 DOI: 10.1056/NEJM199412013312203]
35 **Ng EW**, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, Adamis AP. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov* 2006; **5**: 123-132 [PMID: 16518379 DOI: 10.1038/nrd1955]
36 **Gragoudas ES**, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* 2004; **351**: 2805-2816 [PMID: 15625332 DOI: 10.1056/NEJMoa042760]
37 **González VH**, Giuliari GP, Banda RM, Guel DA. Intravitreal injection of pegaptanib sodium for proliferative diabetic retinopathy. *Br J Ophthalmol* 2009; **93**: 1474-1478 [PMID: 19692371 DOI: 10.1136/bjo.2008.155663]
38 **Sultan MB**, Zhou D, Loftus J, Dombi T, Ice KS; Macugen 1013 Study Group. A phase 2/3, multicenter, randomized, double-masked, 2-year trial of pegaptanib sodium for the treatment of diabetic macular edema. *Ophthalmology* 2011; **118**: 1107-1118 [PMID: 21529957 DOI: 10.1016/j.ophtha.2011.02.045]
39 **Kubota T**, Kiuchi Y, Sheridan C. Anti-vascular endothelial growth factor agents for ocular angiogenesis and vascular permeability. *J Ophthalmol* 2012; **2012**: 898207 [PMID: 22523655 DOI: 10.1155/2012/898207]
40 **Comparison of Age-related Macular Degeneration Treatments Trials(CATT) Research Group**, Martin DF. Maguire MG, Fine SL, Ying GS, Jaffe GJ, Grunwald JE, Toth C, Redford M, Ferris FL 3rd. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012; **119**: 1388-1398 [PMID: 22555112 DOI: 10.1016/j.ophtha.2012.03.053]
41 **Rajendram R**, Fraser-Bell S, Kaines A, Michaelides M, Hamilton RD, Esposti SD, Peto T, Egan C, Bunce C, Leslie RD, Hykin PG. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Arch Ophthalmol* 2012; **130**: 972-979 [PMID: 22491395 DOI: 10.1001/archophthalmol.2012.393]
42 **Korobelnik JF**, Do DV, Schmidt-Erfurth U, Boyer DS, Holz FG, Heier JS, Midena E, Kaiser PK, Terasaki H, Marcus DM, Nguyen QD, Jaffe GJ, Slakter JS, Simader C, Soo Y, Schmelter T, Yancopoulos GD, Stahl N, Vitti R, Berliner AJ, Zeitz O, Metzig C, Brown DM. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology* 2014; **121**: 2247-2254 [PMID: 25012934 DOI: 10.1016/j.ophtha.2014.05.006]
43 **Brown DM**, Schmidt-Erfurth U, Do DV, Holz FG, Boyer DS, Midena E, Heier JS, Terasaki H, Kaiser PK, Marcus DM, Nguyen QD, Jaffe GJ, Slakter JS, Simader C, Soo Y, Schmelter T, Yancopoulos GD, Stahl N, Vitti R, Berliner AJ, Zeitz O, Metzig C, Korobelnik JF. Intravitreal Aflibercept for Diabetic Macular Edema: 100-Week Results From the VISTA and VIVID Studies. *Ophthalmology* 2015; **122**: 2044-2052 [PMID: 26198808 DOI: 10.1016/j.ophtha.2015.06.017]
44 **Brown DM**. Intravitreal aflibercept injection (IAI) for moderately severe to severe nonproliferative diabetic retinopathy (NPDR): the phase 3 PANORAMA study. *Invest Ophthalmol Vis Sci* 2018; **59**: 1889
45 **Lim JI**. Intravitreal aflibercept injection for nonproliferative diabetic retinopathy: year 2 results from the PANORAMA study. *Invest Ophthalmol Vis Sci* 2020; **61**: 1381
46 **Almawi WY**, Saldanha FL, Mahmood NA, Al-Zaman I, Sater MS, Mustafa FE. Relationship between VEGFA polymorphisms and serum VEGF protein levels and recurrent spontaneous

miscarriage. *Hum Reprod* 2013; **28**: 2628-2635 [PMID: 23900206 DOI: 10.1093/humrep/det308]
47 **Galazios G**, Papazoglou D, Tsikouras P, Kolios G. Vascular endothelial growth factor gene polymorphisms and pregnancy. *J Matern Fetal Neonatal Med* 2009; **22**: 371-378 [PMID: 19529993 DOI: 10.1080/14767050802645035]
48 **Granger JP**. Vascular endothelial growth factor inhibitors and hypertension: a central role for the kidney and endothelial factors? *Hypertension* 2009; **54**: 465-467 [PMID: 19652083 DOI: 10.1161/HYPERTENSIONAHA.109.132274]
49 **Sane DC**, Anton L, Brosnihan KB. Angiogenic growth factors and hypertension. *Angiogenesis* 2004; **7**: 193-201 [PMID: 15609074 DOI: 10.1007/s10456-004-2699-3]
50 **Robinson ES**, Khankin EV, Karumanchi SA, Humphreys BD. Hypertension induced by vascular endothelial growth factor signaling pathway inhibition: mechanisms and potential use as a biomarker. *Semin Nephrol* 2010; **30**: 591-601 [PMID: 21146124 DOI: 10.1016/j.semnephrol.2010.09.007]
51 **U.S. Food and Drug Administration**. Food and Drug Administration. Highlights of prescribing information: MACUGEN® (pegaptanib sodium injection) intravitreal injection, revised: 07/2011. [cited 10 January 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2011/021756s018Lbl.pdf
52 **U.S. Food and Drug Administration**. Food and Drug Administration. Highlights of prescribing information: LUCENTIS® (ranibizumab injection) for intravitreal injection, revised: 04/2017. [cited 10 January 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2017/125156s114Lbl.pdf
53 **U.S. Food and Drug Administration**. Food and Drug Administration. Highlights of prescribing information: AVASTIN® (bevacizumab) injection for intravenous use, revised: 10/2020. [cited 10 January 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2020/125085s336Lbl.pdf
54 **U.S. Food and Drug Administration**. S. Food and Drug Administration. Highlights of prescribing information: EYLEA® (aflibercept) injection, for intravitreal use, revised: 5/2019. [cited 10 January 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2019/125387s061Lbl.pdf
55 **Stewart MW**. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind VEGF. *Expert Rev Clin Pharmacol* 2014; **7**: 167-180 [PMID: 24483136 DOI: 10.1586/17512433.2014.884458]
56 **Veronese FM**, Mero A. The impact of PEGylation on biological therapies. *BioDrugs* 2008; **22**: 315-329 [PMID: 18778113 DOI: 10.2165/00063030-200822050-00004]
57 **Christoforidis JB**, Williams MM, Wang J, Jiang A, Pratt C, Abdel-Rasoul M, Hinkle GH, Knopp MV. Anatomic and pharmacokinetic properties of intravitreal bevacizumab and ranibizumab after vitrectomy and lensectomy. *Retina* 2013; **33**: 946-952 [PMID: 23407351 DOI: 10.1097/IAE.0b013e3182753b12]
58 **Gaudreault J**, Fei D, Beyer JC, Ryan A, Rangell L, Shiu V, Damico LA. Pharmacokinetics and retinal distribution of ranibizumab, a humanized antibody fragment directed against VEGF-A, following intravitreal administration in rabbits. *Retina* 2007; **27**: 1260-1266 [PMID: 18046235 DOI: 10.1097/IAE.0b013e318134eecd]
59 **Bakri SJ**, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ. Pharmacokinetics of intravitreal ranibizumab (Lucentis). *Ophthalmology* 2007; **114**: 2179-2182 [PMID: 18054637 DOI: 10.1016/j.ophtha.2007.09.012]
60 **Gaudreault J**, Fei D, Rusit J, Suboc P, Shiu V. Preclinical pharmacokinetics of Ranibizumab (rhuFabV2) after a single intravitreal administration. *Invest Ophthalmol Vis Sci* 2005; **46**: 726-733 [PMID: 15671306 DOI: 10.1167/iovs.04-0601]
61 **Bakri SJ**, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). *Ophthalmology* 2007; **114**: 855-859 [PMID: 17467524 DOI: 10.1016/j.ophtha.2007.01.017]
62 **Christoforidis JB**, Carlton MM, Knopp MV, Hinkle GH. PET/CT imaging of I-124-radiolabeled bevacizumab and ranibizumab after intravitreal injection in a rabbit model. *Invest Ophthalmol Vis Sci* 2011; **52**: 5899-5903 [PMID: 21685343 DOI: 10.1167/iovs.10-6862]
63 **Dib E**, Maia M, Longo-Maugeri IM, Martins MC, Mussalem JS, Squaiella CC, Penha FM, Magalhães O Jr, Rodrigues EB, Farah ME. Subretinal bevacizumab detection after intravitreous injection in rabbits. *Invest Ophthalmol Vis Sci* 2008; **49**: 1097-1100 [PMID: 18326736 DOI: 10.1167/iovs.07-1225]
64 **Shahar J**, Avery RL, Heilweil G, Barak A, Zemel E, Lewis GP, Johnson PT, Fisher SK, Perlman I, Loewenstein A. Electrophysiologic and retinal penetration studies following intravitreal injection of bevacizumab (Avastin). *Retina* 2006; **26**: 262-269 [PMID: 16508424 DOI: 10.1097/00006982-200603000-00002]
65 **Zhu Q**, Ziemssen F, Henke-Fahle S, Tatar O, Szurman P, Aisenbrey S, Schneiderhan-Marra N, Xu X; Tübingen Bevacizumab Study Group, Grisanti S. Vitreous levels of bevacizumab and vascular endothelial growth factor-A in patients with choroidal neovascularization. *Ophthalmology* 2008; **115**: 1750-1755, 1755.e1 [PMID: 18708261 DOI: 10.1016/j.ophtha.2008.04.023]
66 **Krohne TU**, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. *Am J Ophthalmol* 2008; **146**: 508-512 [PMID: 18635152 DOI: 10.1016/j.ajo.2008.05.036]

67 **Csaky KG**, Gordiyenko N, Rabena MG, Avery RL. Pharmacokinetics of intravitreal bevacizumab in humans. *Invest Ophthalmol Vis Sci* 2007; **48**: 4936

68 **Meyer CH**, Krohne TU, Holz FG. Intraocular pharmacokinetics after a single intravitreal injection of 1.5 mg *vs* 3.0 mg of bevacizumab in humans. *Retina* 2011; **31**: 1877-1884 [PMID: 21738089 DOI: 10.1097/IAE.0b013e318217373c]

69 **Furfine E**, Coppi A, Koehler-Stec E, Zimmer E, Tu W, Struble C. Pharmacokinetics and ocular tissue penetration of VEGF Trap after intravitreal injections in rabbits. *Invest Ophthalmol Vis Sci* 2006; **47**: 1430

70 **Fossum P**, Couret C, Briend B, Weber M, Lagarce L. Safety of intravitreal injection of ranibizumab in early pregnancy: a series of three cases. *Eye (Lond)* 2018; **32**: 830-832 [PMID: 29350689 DOI: 10.1038/eye.2017.305]

71 **Sarhianaki A**, Katsimpris A, Petropoulos IK, Livieratou A, Theoulakis PE, Katsimpris JM. Intravitreal administration of ranibizumab for idiopathic choroidal neovascularization in a pregnant woman. *Klin Monbl Augenheilkd* 2012; **229**: 451-453 [PMID: 22496030 DOI: 10.1055/s-0031-1299207]

72 **Jouve L**, Akesbi J, Nordmann JP. Safety and efficacy of ranibizumab for pregnant women in idiopathic choroidal neovascularization. *Acta Ophthalmol* 2015; **93**: e597-e598 [PMID: 25483229 DOI: 10.1111/aos.12611]

73 **Akkaya S**. Early Miscarriage Occurring Six Days After Intravitreal Ranibizumab Injection. *Med Hypothesis Discov Innov Ophthalmol* 2019; **8**: 69-72 [PMID: 31263715]

74 **Rosen E**, Rubowitz A, Ferencz JR. Exposure to verteporfin and bevacizumab therapy for choroidal neovascularization secondary to punctate inner choroidopathy during pregnancy. *Eye (Lond)* 2009; **23**: 1479 [PMID: 18617903 DOI: 10.1038/eye.2008.218]

75 **Wu Z**, Huang J, Sadda S. Inadvertent use of bevacizumab to treat choroidal neovascularisation during pregnancy: a case report. *Ann Acad Med Singap* 2010; **39**: 143-145 [PMID: 20237737]

76 **Petrou P**, Georgalas I, Giavaras G, Anastasiou E, Ntana Z, Petrou C. Early loss of pregnancy after intravitreal bevacizumab injection. *Acta Ophthalmol* 2010; **88**: e136 [PMID: 19740128 DOI: 10.1111/j.1755-3768.2009.01572.x]

77 **Tarantola RM**, Folk JC, Boldt HC, Mahajan VB. Intravitreal bevacizumab during pregnancy. *Retina* 2010; **30**: 1405-1411 [PMID: 20924262 DOI: 10.1097/IAE.0b013e3181f57d58]

78 **Introini U**, Casalino G, Cardani A, Scotti F, Finardi A, Candiani M, Bandello F. Intravitreal bevacizumab for a subfoveal myopic choroidal neovascularization in the first trimester of pregnancy. *J Ocul Pharmacol Ther* 2012; **28**: 553-555 [PMID: 22662749 DOI: 10.1089/jop.2012.0067]

79 **Gómez Ledesma I**, de Santiago Rodríguez MÁ, Follana Neira I, León Garrigosa F. [Neovascular membrane and pregnancy. Treatment with bevacizumab]. *Arch Soc Esp Oftalmol* 2012; **87**: 297-300 [PMID: 22824650 DOI: 10.1016/j.oftal.2011.09.011]

80 **Sullivan L**, Kelly SP, Glenn A, Williams CP, McKibbin M. Intravitreal bevacizumab injection in unrecognised early pregnancy. *Eye (Lond)* 2014; **28**: 492-494 [PMID: 24434664 DOI: 10.1038/eye.2013.311]

81 **Polizzi S**, Ferrara G, Restaino S, Rinaldi S, Tognetto D. Inadvertent use of bevacizumab in pregnant women with diabetes mellitus type 1. *J Basic Clin Physiol Pharmacol* 2015; **26**: 161-163 [PMID: 25153234 DOI: 10.1515/jbcpp-2014-0058]

82 **Kianersi F**, Ghanbari H, Naderi Beni Z, Naderi Beni A. Intravitreal vascular endothelial growth factor (VEGF) inhibitor injection in unrecognised early pregnancy. *Invest New Drugs* 2016; **34**: 650-653 [PMID: 27251054 DOI: 10.1007/s10637-016-0361-8]

83 **Kianersi F**, Ghanbari H, Naderi Beni Z, Naderi Beni A. Intravitreal vascular endothelial growth factor (VEGF) inhibitor injection in patient during pregnancy. *J Drug Assess* 2021; **10**: 7-9 [PMID: 33796345 DOI: 10.1080/21556660.2020.1847926]

84 **Robinson GE**. Pregnancy loss. *Best Pract Res Clin Obstet Gynaecol* 2014; **28**: 169-178 [PMID: 24047642 DOI: 10.1016/j.bpobgyn.2013.08.012]

85 **Knudsen UB**, Hansen V, Juul S, Secher NJ. Prognosis of a new pregnancy following previous spontaneous abortions. *Eur J Obstet Gynecol Reprod Biol* 1991; **39**: 31-36 [PMID: 2029953 DOI: 10.1016/0028-2243(91)90138-b]

86 **Adamis AP**, Berman AJ. Immunological mechanisms in the pathogenesis of diabetic retinopathy. *Semin Immunopathol* 2008; **30**: 65-84 [PMID: 18340447 DOI: 10.1007/s00281-008-0111-x]

87 **Funatsu H**, Yamashita H, Ikeda T, Mimura T, Eguchi S, Hori S. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. *Ophthalmology* 2003; **110**: 1690-1696 [PMID: 13129863 DOI: 10.1016/S0161-6420(03)00568-2]

88 **Funatsu H**, Noma H, Mimura T, Eguchi S, Hori S. Association of vitreous inflammatory factors with diabetic macular edema. *Ophthalmology* 2009; **116**: 73-79 [PMID: 19118698 DOI: 10.1016/j.ophtha.2008.09.037]

89 **Johnson EI**, Dunlop ME, Larkins RG. Increased vasodilatory prostaglandin production in the diabetic rat retinal vasculature. *Curr Eye Res* 1999; **18**: 79-82 [PMID: 10223650 DOI: 10.1076/ceyr.18.2.79.5386]

90 **Lane LS**, Jansen PD, Lahav M, Rudy C. Circulating prostacyclin and thromboxane levels in patients with diabetic retinopathy. *Ophthalmology* 1982; **89**: 763-766 [PMID: 6750495 DOI: 10.1016/s0161-6420(82)34729-6]

91 **Zhou J**, Wang S, Xia X. Role of intravitreal inflammatory cytokines and angiogenic factors in proliferative diabetic retinopathy. *Curr Eye Res* 2012; **37**: 416-420 [PMID: 22409294 DOI:

10.3109/02713683.2012.661114]
92 **Schoenberger SD**, Kim SJ, Sheng J, Rezaei KA, Lalezary M, Cherney E. Increased prostaglandin E2 (PGE2) levels in proliferative diabetic retinopathy, and correlation with VEGF and inflammatory cytokines. *Invest Ophthalmol Vis Sci* 2012; **53**: 5906-5911 [PMID: 22871833 DOI: 10.1167/iovs.12-10410]
93 **Schoenberger SD**, Kim SJ. Nonsteroidal anti-inflammatory drugs for retinal disease. *Int J Inflam* 2013; **2013**: 281981 [PMID: 23365785 DOI: 10.1155/2013/281981]
94 **Kapin MA**, Yanni JM, Brady MT, McDonough TJ, Flanagan JG, Rawji MH, Dahlin DC, Sanders ME, Gamache DA. Inflammation-mediated retinal edema in the rabbit is inhibited by topical nepafenac. *Inflammation* 2003; **27**: 281-291 [PMID: 14635785 DOI: 10.1023/a:1026024409826]
95 **Kern TS**, Miller CM, Du Y, Zheng L, Mohr S, Ball SL, Kim M, Jamison JA, Bingaman DP. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. *Diabetes* 2007; **56**: 373-379 [PMID: 17259381 DOI: 10.2337/db05-1621]
96 **Ke TL**, Graff G, Spellman JM, Yanni JM. Nepafenac, a unique nonsteroidal prodrug with potential utility in the treatment of trauma-induced ocular inflammation: II. In vitro bioactivation and permeation of external ocular barriers. *Inflammation* 2000; **24**: 371-384 [PMID: 10850858 DOI: 10.1023/a:1007001131987]
97 **Hariprasad SM**, Callanan D, Gainey S, He YG, Warren K. Cystoid and diabetic macular edema treated with nepafenac 0.1%. *J Ocul Pharmacol Ther* 2007; **23**: 585-590 [PMID: 18001248 DOI: 10.1089/jop.2007.0062]
98 **Callanan D**, Williams P. Topical nepafenac in the treatment of diabetic macular edema. *Clin Ophthalmol* 2008; **2**: 689-692 [PMID: 19668417 DOI: 10.2147/opth.s3965]
99 **Garcia-Gonzalez JM**, Emanuelli A, Berrocal MH. Topical nepafenac 0.1% for the treatment of macular edema secondary to diabetic retinopathy and retinal vascular occlusions. *Invest Ophthalmol Vis Sci* 2009; **50**: 1349
100 **Vignesh TP**. Topical nepafenac in the treatment of center involving diabetic macular edema. *TNOA J Ophthalmic Sci Res* 2019; **57**: 109-112 [DOI: 10.4103/tjosr.tjosr_12_19]
101 **Friedman SM**, Almukhtar TH, Baker CW, Glassman AR, Elman MJ, Bressler NM, Maker MP, Jampol LM, Melia M; Diabetic Retinopathy Clinical Research Network. Topical nepafenec in eyes with noncentral diabetic macular edema. *Retina* 2015; **35**: 944-956 [PMID: 25602634 DOI: 10.1097/IAE.0000000000000403]
102 **Gamache DA**, Graff G, Brady MT, Spellman JM, Yanni JM. Nepafenac, a unique nonsteroidal prodrug with potential utility in the treatment of trauma-induced ocular inflammation: I. Assessment of anti-inflammatory efficacy. *Inflammation* 2000; **24**: 357-370 [PMID: 10850857 DOI: 10.1023/a:1007049015148]
103 **Bucci FA Jr**, Waterbury LD, Amico LM. Prostaglandin E2 inhibition and aqueous concentration of ketorolac 0.4% (acular LS) and nepafenac 0.1% (nevanac) in patients undergoing phacoemulsification. *Am J Ophthalmol* 2007; **144**: 146-147 [PMID: 17601444 DOI: 10.1016/j.ajo.2007.02.034]
104 **U.S. Food and Drug Administration**. Food and Drug Administration. Highlights of prescribing information: NEVANAC® (nepafenac ophthalmic suspension) 0.1%, for topical ophthalmic use, revised: 11/2020. [cited 10 January 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2020/021862s017 Lbl.pdf
105 **Coceani F**, Olley PM. Involvement of prostaglandins in the fetal and neonatal circulation. In: Berti F, Folco G, Velo GP (eds) Leukotrienes and Prostacyclin. NATO Advanced Science Institutes Series (Series A: Life Sciences). Boston: Springer, 1983
106 **El Annan J**, Carvounis PE. Current management of vitreous hemorrhage due to proliferative diabetic retinopathy. *Int Ophthalmol Clin* 2014; **54**: 141-153 [PMID: 24613890 DOI: 10.1097/IIO.0000000000000027]
107 **Flynn HW Jr**, Chew EY, Simons BD, Barton FB, Remaley NA, Ferris FL 3rd. Pars plana vitrectomy in the Early Treatment Diabetic Retinopathy Study. ETDRS report number 17. The Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology* 1992; **99**: 1351-1357 [PMID: 1407968 DOI: 10.1016/s0161-6420(92)31779-8]
108 **Machemer R**, Buettner H, Norton EW, Parel JM. Vitrectomy: a pars plana approach. *Trans Am Acad Ophthalmol Otolaryngol* 1971; **75**: 813-820 [PMID: 5566980]
109 **Yorston D**, Wickham L, Benson S, Bunce C, Sheard R, Charteris D. Predictive clinical features and outcomes of vitrectomy for proliferative diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 365-368 [PMID: 18303158 DOI: 10.1136/bjo.2007.124495]
110 **Diabetic Retinopathy Clinical Research Network***. Randomized clinical trial evaluating intravitreal ranibizumab or saline for vitreous hemorrhage from proliferative diabetic retinopathy. *JAMA Ophthalmol* 2013; **131**: 283-293 [PMID: 23370902 DOI: 10.1001/jamaophthalmol.2013.2015]
111 **Wirkkala J**, Bloigu R, Hautala NM. Intravitreal bevacizumab improves the clearance of vitreous haemorrhage and visual outcomes in patients with proliferative diabetic retinopathy. *BMJ Open Ophthalmol* 2019; **4**: e000390 [PMID: 31909195 DOI: 10.1136/bmjophth-2019-000390]
112 **Taskintuna I**, Elsayed MEAA, Taskintuna K, Ahmad K, Khandekar R, Schatz P, Kozak I. Comparison of outcomes of four different treatment modalities for diabetic vitreous haemorrhage. *Sci Rep* 2020; **10**: 3674 [PMID: 32111892 DOI: 10.1038/s41598-020-60378-8]

113 **Mansour AM**, Ashraf M, El Jawhari KM, Farah M, Souka A, Sarvaiya C, Singh SR, Banker A, Chhablani J. Intravitreal ziv-aflibercept in diabetic vitreous hemorrhage. *Int J Retina Vitreous* 2020; **6**: 2 [PMID: 31956432 DOI: 10.1186/s40942-019-0204-9]

114 **Abdelaal AM**, Alqahtani AS. Mode of Delivery in the Setting of Repeated Vitreous Hemorrhages in Proliferative Diabetic Retinopathy: A Case Report and Review of the Literature. *Cureus* 2020; **12**: e11239 [PMID: 33269167 DOI: 10.7759/cureus.11239]

115 **Jacobson MS**. Ophthalmology surgery during pregnancy. In: Nezhat C, Kavic M, Lanzafame R, Lindsay M, Polk T (eds) Non-obstetric surgery during pregnancy. Boston: Springer, 2019

116 **Kuczkowski KM**. Nonobstetric surgery in the parturient: anesthetic considerations. *J Clin Anesth* 2006; **18**: 5-7 [PMID: 16517324 DOI: 10.1016/j.jclinane.2005.11.003]

117 **Samples JR**, Meyer SM. Use of ophthalmic medications in pregnant and nursing women. *Am J Ophthalmol* 1988; **106**: 616-623 [PMID: 2903673 DOI: 10.1016/0002-9394(88)90597-1]

118 **Schaefer C**, Peters PW, Miller RK. Drugs during pregnancy and lactation: treatment options and risk assessment. London: Academic Press, 2014

119 **Reitman E**, Flood P. Anaesthetic considerations for non-obstetric surgery during pregnancy. *Br J Anaesth* 2011; **107** Suppl 1: i72-i78 [PMID: 22156272 DOI: 10.1093/bja/aer343]

120 **Mazze RI**, Källén B. Reproductive outcome after anesthesia and operation during pregnancy: a registry study of 5405 cases. *Am J Obstet Gynecol* 1989; **161**: 1178-1185 [PMID: 2589435 DOI: 10.1016/0002-9378(89)90659-5]

121 **Hemmings HC Jr**, Greengard P. Positively active: how local anesthetics work. *Anesthesiology* 2010; **113**: 250-252 [PMID: 20526177 DOI: 10.1097/ALN.0b013e3181e32e84]

122 **Turner MD**, Singh F, Glickman RS. Dental management of the gravid patient. *N Y State Dent J* 2006; **72**: 22-27 [PMID: 17203851]

123 **Hagai A**, Diav-Citrin O, Shechtman S, Ornoy A. Pregnancy outcome after in utero exposure to local anesthetics as part of dental treatment: A prospective comparative cohort study. *J Am Dent Assoc* 2015; **146**: 572-580 [PMID: 26227642 DOI: 10.1016/j.adaj.2015.04.002]

124 **Upadya M**, Saneesh PJ. Anaesthesia for non-obstetric surgery during pregnancy. *Indian J Anaesth* 2016; **60**: 234-241 [PMID: 27141105 DOI: 10.4103/0019-5049.179445]

125 **Kopp Lugli A**, Yost CS, Kindler CH. Anaesthetic mechanisms: update on the challenge of unravelling the mystery of anaesthesia. *Eur J Anaesthesiol* 2009; **26**: 807-820 [PMID: 19494779 DOI: 10.1097/EJA.0b013e32832d6b0f]

126 **Oats JN**, Vasey DP, Waldron BA. Effects of ketamine on the pregnant uterus. *Br J Anaesth* 1979; **51**: 1163-1166 [PMID: 526384 DOI: 10.1093/bja/51.12.1163]

127 **Yoo KY**, Lee JC, Yoon MH, Shin MH, Kim SJ, Kim YH, Song TB, Lee J. The effects of volatile anesthetics on spontaneous contractility of isolated human pregnant uterine muscle: a comparison among sevoflurane, desflurane, isoflurane, and halothane. *Anesth Analg* 2006; **103**: 443-447, table of contents [PMID: 16861431 DOI: 10.1213/01.ane.0000236785.17606.58]

128 **Ministry of Health**, Malaysia. Clinical Practice Guidelines: Screening of Diabetic Retinopathy. Putrajaya, Malaysia: Ministry of Health, 2011

129 **Chiefari E**, Arcidiacono B, Foti D, Brunetti A. Gestational diabetes mellitus: an updated overview. *J Endocrinol Invest* 2017; **40**: 899-909 [PMID: 28283913 DOI: 10.1007/s40618-016-0607-5]

130 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** Suppl 1: S81-S90 [PMID: 24357215 DOI: 10.2337/dc14-S081]

131 **Jeganathan R**, Karalasingam SD. 4th report of national obstetric registry. Ministry of Health, Malaysia, 2013-2015. [cited 10 January 2021]. Available from: http://www.acrm.org.my/nor/reports.php.

132 **Zhu Y**, Zhang C. Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. *Curr Diab Rep* 2016; **16**: 7 [PMID: 26742932 DOI: 10.1007/s11892-015-0699-x]

133 **Sandsæter HL**, Horn J, Rich-Edwards JW, Haugdahl HS. Preeclampsia, gestational diabetes and later risk of cardiovascular disease: Women's experiences and motivation for lifestyle changes explored in focus group interviews. *BMC Pregnancy Childbirth* 2019; **19**: 448 [PMID: 31775681 DOI: 10.1186/s12884-019-2591-1]

134 **Nguyen CL**, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of Gestational Diabetes Mellitus in Eastern and Southeastern Asia: A Systematic Review and Meta-Analysis. *J Diabetes Res* 2018; **2018**: 6536974 [PMID: 29675432 DOI: 10.1155/2018/6536974]

135 **Bastion ML**, Barkeh HJ, Muhaya M. Accelerated diabetic retinopathy in pregnancy--a real and present danger. *Med J Malaysia* 2005; **60**: 502-504 [PMID: 16570717]

136 **Raman P**, Livingstone BI. Advanced diabetic eye disease in pregnancy. *J Clin Gynecol Obstet* 2018; **7**: 72-75 [DOI: 10.14740/jcgo487w]

137 **Helen CC**, Tajunisah I, Reddy SC. Adverse outcomes in Type I diabetic pregnant women with proliferative diabetic retinopathy. *Int J Ophthalmol* 2011; **4**: 443-446 [PMID: 22553697 DOI: 10.3980/j.issn.2222-3959.2011.04.23]

138 **Hampshire R**, Wharton H, Leigh R, Wright A, Dodson P. Screening for diabetic retinopathy in pregnancy using photographic review clinics. *Diabet Med* 2013; **30**: 475-477 [PMID: 23252726 DOI: 10.1111/dme.12077]

139 **Buari NH**, Dian NI. The Association of Awareness and Knowledge of Diabetic Retinopathy with Age and Residential Area In Selangor. *Environ Proc J* 2017; **2**: 125 [DOI: 10.21834/e-bpj.v2i6.942]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1401-1425

DOI: 10.4239/wjd.v12.i9.1401

ISSN 1948-9358 (online)

REVIEW

Epidemiology of type 2 diabetes in the Middle East and North Africa: Challenges and call for action

Imad M El-Kebbi, Nayda H Bidikian, Layal Hneiny, Mona Philippe Nasrallah

ORCID number: Imad M El-Kebbi 0000-0003-4177-5438; Nayda H Bidikian 0000-0001-9717-8849; Layal Hneiny 0000-0003-1073-4879; Mona Philippe Nasrallah 0000-0001-7382-8835.

Author contributions: El-Kebbi IM conceptualized the outline, wrote major section of the manuscript, generated some of the tables and figures, and provided final review and edits of the manuscript; Bidikian NH contributed to the literature search, wrote a large portion of the manuscript, and generated some of the tables and graphs; Hneiny L performed the literature search, and wrote one small section of the manuscript; Nasrallah MP accepted the editor invitation, conceptualized the paper, wrote one section of the manuscript, generated one table, and proofread and edited the whole manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: All authors declare no relationships/conditions/circumstances that present a potential conflict of interest.

Imad M El-Kebbi, Mona Philippe Nasrallah, Department of Internal Medicine, Division of Endocrinology, American University of Beirut Medical Center, Faculty of Medicine, Beirut 11072020, Lebanon

Imad M El-Kebbi, Department of Internal Medicine, Sheikh Shakhbout Medical City, Abou Dhabi 11001, United Arab Emirates

Nayda H Bidikian, School of Medicine, American University of Beirut, Faculty of Medicine, Beirut 11072020, Lebanon

Layal Hneiny, University Libraries, Saab Medical Library, American University of Beirut, Beirut 11072020, Lebanon

Corresponding author: Mona Philippe Nasrallah, MD, Associate Professor, Department of Internal Medicine, Division of Endocrinology, American University of Beirut Medical Center, Faculty of Medicine, Riad El-Solh, Beirut 11072020, Lebanon. mn36@aub.edu.lb

Abstract

Type 2 diabetes continues to be a serious and highly prevalent public health problem worldwide. In 2019, the highest prevalence of diabetes in the world at 12.2%, with its associated morbidity and mortality, was found in the Middle East and North Africa region. In addition to a genetic predisposition in its population, evidence suggests that obesity, physical inactivity, urbanization, and poor nutritional habits have contributed to the high prevalence of diabetes and prediabetes in the region. These risk factors have also led to an earlier onset of type 2 diabetes among children and adolescents, negatively affecting the productive years of the youth and their quality of life. Furthermore, efforts to control the rising prevalence of diabetes and its complications have been challenged and complicated by the political instability and armed conflict in some countries of the region and the recent coronavirus disease 2019. Broad strategies, coupled with targeted interventions at the regional, national, and community levels are needed to address and curb the spread of this public health crisis.

Key Words: Type 2 diabetes; Middle East and North Africa; Epidemiology; Prevalence; Prediabetes; Complications

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Lebanon

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 29, 2021
Peer-review started: January 29, 2021
First decision: June 5, 2021
Revised: June 21, 2021
Accepted: August 2, 2021
Article in press: August 2, 2021
Published online: September 15, 2021

P-Reviewer: Cojocariu C, Tavan H
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Wang LYT

Core Tip: The Middle East and North Africa region has the world's highest diabetes prevalence, the second highest rate of rise, the highest adjusted mortality from noncommunicable disease, and the highest diabetes-related disability adjusted life years. This review provides an up-to-date review of the diabetes status in this dynamic region of the world and touches on new elements that affect diabetes such as the high number of refugees and the coronavirus disease 2019 pandemic. This review identifies gaps and weaknesses in type 2 diabetes in the Middle East and North Africa region and highlights areas where planning and action are highly needed.

Citation: El-Kebbi IM, Bidikian NH, Hneiny L, Nasrallah MP. Epidemiology of type 2 diabetes in the Middle East and North Africa: Challenges and call for action. *World J Diabetes* 2021; 12(9): 1401-1425
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1401.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1401

INTRODUCTION

The worldwide prevalence of diabetes mellitus continues to grow with no sign of reversal. Data from the World Health Organization (WHO) shows that diabetes rose 80% in prevalence between 1980 and 2014[1], and the International Diabetes Federation (IDF) estimates that in 2019, 9.3% of the global adult population age 20-79 years suffered from diabetes[2]. Compared to high income countries (HIC), this increase disproportionately affects low- and middle-income countries and adds a burden of excess morbidity, mortality, and health care costs[3]. Specifically, the Middle East and North Africa region (MENA) carried the highest prevalence of diabetes in 2019 at 12.2% and is expected to witness a 96% increase in diabetes prevalence between 2019 and 2045, second only to the African region with a 143% projected rise [4]. To compare, over the same time period, the prevalence in Europe and North America/Caribbean regions is expected to increase by 15% and 33%, respectively. Moreover, 44.7% of people with type 2 diabetes (T2D) in the MENA region are unaware of their condition[4].

Despite the heterogeneity within the MENA countries in terms of culture, income, population size, and sociopolitical stability[5,6], multiple common predisposing factors for diabetes have been implicated, including aging of the population, the change in lifestyle with reduction in physical activity, and increased consumption of calories and unhealthy food items, which have led to a rise in the prevalence of overweightness and obesity[2]. Genetic and epigenetic factors may also be contributing elements[7]; in a region that has a high rate of consanguinity[8], multiple gene loci that predispose to diabetes have been identified in the Eastern Mediterranean Region (EMR) population[9]. In addition to diabetes, prediabetes has been identified in a sizable proportion of the MENA population[2], out of whom a majority is expected to progress to diabetes over time[10].

This unrelenting diabetes epidemic was an important driving factor that spurred the 2011 declaration of the United Nations general assembly, in which countries committed to work on national plans for preventing and controlling noncommunicable diseases (NCD)[11]. In addition to the risk factors behind the worldwide rise in diabetes prevalence, other factors, specific to the MENA region, are contributing to the epidemic[12]. Measures to address the high numbers in the MENA area have been ineffective partly due to inadequate funding, insufficient commitment, political instability, and armed conflict in multiple countries[13,14].

This review aims at describing the current prevalence of diabetes and prediabetes in the MENA region, the contributing risk factors, common diabetes complications, and strategies that can help curb its spread and complications.

METHODOLOGY

We searched Medline (OVID electronic database Ovid MEDLINE(R) and Epub Ahead of Print, In-Process and Other Non-Indexed Citations and Daily 1946 to December 07,

2020) for studies reporting in 20 countries of the MENA region not only diabetes mellitus and cardiovascular diseases (CVDs), but also diabetic foot or amputation, diabetic nephropathies, diabetic retinopathy (DR), the prevalence of diabetes, and the prevalence of diabetes for the pediatric and adolescent age group. Each of the latter searches was exported into Endnote X9 Software, and the library was screened for relevant literature. The search did include the Medical Subject headings (MESH) for all the concepts except for countries in which keywords were added along to MESH to ensure a wider range of results. Only articles in the English language and with studies where specific complication prevalence was evaluated were selected for our paper. We limited the prevalence studies to publications within the last decade (2010-2020). For diabetes prevalence studies, we excluded hospital and clinic-based studies.

There has been no consensus on which countries define the MENA region. For our review, we have included the following 20 countries: Afghanistan, Algeria, Bahrain, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Palestine, Qatar, Saudi Arabia, Syria, Tunisia, United Arab Emirates (UAE), and Yemen. These countries are commonly included in the definition of EMR (EMRO). Therefore, we included data from the WHO, which reports on EMRO, and IDF, which reports on MENA.

PREVALENCE OF T2D

Diagnostic criteria for diabetes

The diagnostic criteria for diabetes by the American Diabetes Association (ADA) include the following: hemoglobin A1c 6.5%, fasting plasma glucose 126 mg/dL (7.0 mmol/L), oral glucose tolerance test with 2 h plasma glucose 200 mg/dL (11.1 mmol/L), or casual plasma glucose 200 mg/dL (11.1 mmol/L) in the presence of hyperglycemic symptoms[15]. It is worth noting that the IDF follows the ADA criteria in the diagnosis of T2D[16]. On the other hand, the WHO defines diabetes as raised fasting glucose 126 mg/dL (7.0 mmol/L), history of diabetes, or using antidiabetic medication[17]. Unless stated otherwise, the studies included in the prevalence paragraph follow either ADA or WHO diagnostic criteria.

Prevalence

In 2019 and as previously stated, 9.3% of adults were living with diabetes worldwide, with a predicted rise to 10.2% and 10.9% by years 2030 and 2045, respectively. The highest age adjusted prevalence was reported in the MENA reaching 12.2%, where 1 in 8 adults was living with diabetes[2]. The WHO provides periodic country-specific rates of diabetes compiled from various studies[17]. We used WHO data to rank MENA countries by diabetes prevalence between 2000 and 2014[17], as shown in Figure 1.

The highest age standardized diabetes prevalence in the MENA region in the year 2000 was in Kuwait with a prevalence of 15.4%, and the lowest prevalence was 6.8% in Yemen. Between 2000 and 2014, all 20 countries of the MENA region discussed in this paper experienced an increase in prevalence while Kuwait kept its first ranking among these countries, for having the highest prevalence among them at 19.6% (Figure 1). Kuwait has already exceeded its projected prevalence of diabetes for 2030, which was anticipated to be 16.9%[18]. Another high-income country, Qatar, ranked second in prevalence among MENA countries, following Kuwait, both in 2000 and 2014 (Figure 1). In Saudi Arabia, a cross-sectional study was conducted from 2007 to 2009 and included 18034 individuals older than 30 years. It found the prevalence of diabetes was 25.4% (out of which 10.2% were previously undiagnosed), and it was significantly higher in urban compared to rural areas[19].

Moving to Pakistan, in 2017, a large-scale national study including 18856 adults (above 20 years of age), found that T2D prevalence was 16.9% (n = 3201), and diabetes was significantly associated with age[20]. In 2019, the age-adjusted comparative prevalence in Pakistan rose to 19.9%, and that country was ranked first among the MENA countries for having the highest number of people (19.4 million) living with diabetes[2]. To understand the trend in diabetes prevalence in Jordan, four surveys using the same diagnostic criteria (the WHO criteria) were conducted in the years 1994, 2004, 2009, and 2017. Over the years, the age-adjusted prevalence increased from 17.1% in 2004 to 23.7% in 2017. This steep increase was attributed not only to increased incidence, but also to other factors like aging of the population and better survival of individuals with diabetes. In addition, the percentage of previously diagnosed diabetes increased as well, accounting for 82.6% of all diabetes cases in 2017, indicating

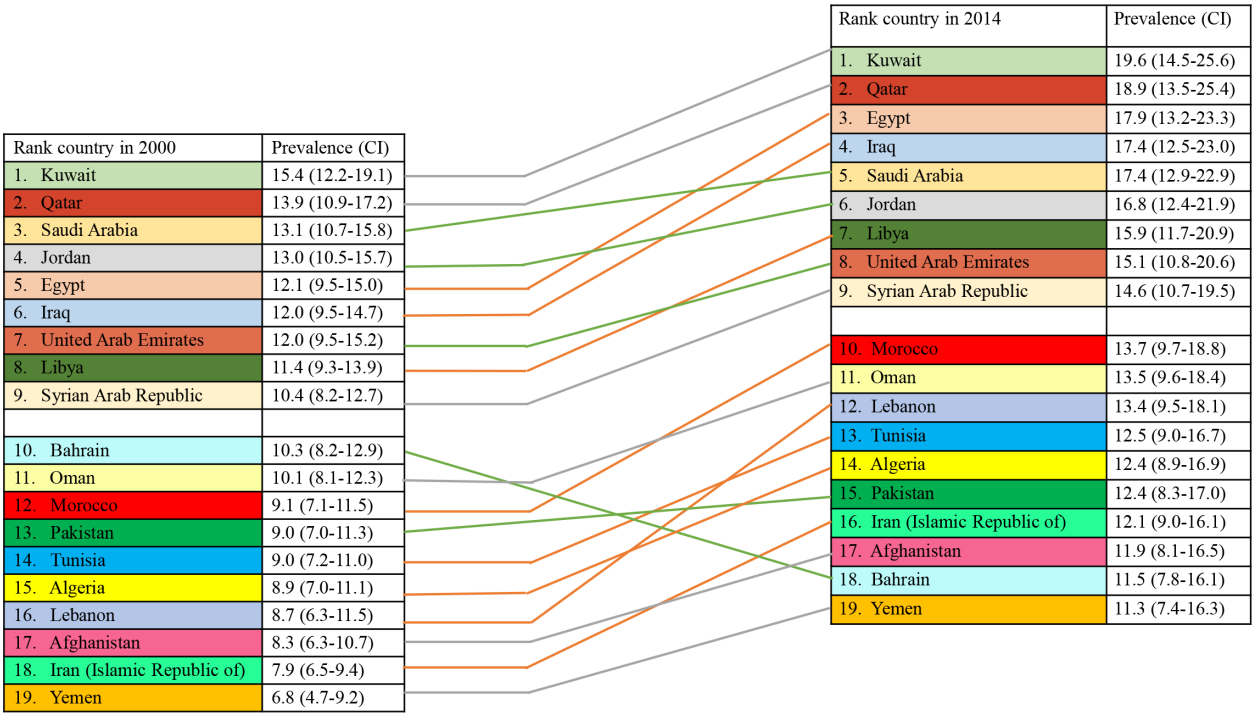

Figure 1 Middle East and North Africa countries ranked by prevalence of type 2 diabetes in 2000 and 2014 (prevalence and confidence intervals are in %)[1]. CI: Confidence interval.

improved screening and diabetes awareness[21].

Moving to North Africa, and according to the WHO, the prevalence of diabetes in Egypt in 2014 was 17.9%, the third highest prevalence in the MENA region. In 2019, Egypt occupied the second highest rank among MENA countries with respect to number of adults living with diabetes, with almost 9 million cases[2]. However, within Egypt, the prevalence was much lower in rural areas compared to the national average. As an example, a community-based cross-sectional study conducted in Qena Governorate over 2 years (2013-2015) found that 811 out of 9303 had T2D (8.7%). It is worth noting that the majority of participants (n = 7701) were younger than 40 years of age, which likely contributed to the relatively low prevalence in this governorate[22].

In a neighboring country to Egypt, Tunisia, the most recent national cross-sectional survey we could identify was conducted in 2005 and involved 7700 adults, age 35 to 70 years. It found that the prevalence of T2D, based on WHO criteria, was 15.5%. Again, within that study, the urban prevalence was twice as high as in rural areas (17.7% *vs* 9.7%, respectively)[23].

As for Iran, a national survey conducted in 2011 found that the prevalence of diabetes (T1D and T2D combined) was 11.4%, and the annual incidence was estimated to be 1%[24]. Two large community-based cross-sectional studies yielded the following: in the Yazd area of central Iran, out of 2269 adults above 20 years, the crude prevalence of self-reported diabetes in 2014-2015 was 14.1%, and 1 out of every 5 people over 40 years of age was living with diabetes[25]. In contrast, the Pars Cohort Study in Southern Iran conducted on 9264 adults aged 40-75 years, found a slightly lower prevalence of diabetes of 9.9% using self-report and fasting plasma glucose[26, 27]. In either case, the prevalence is increasing over time, as was shown in a 5 year study in the city of Ahvaz. Out of 593 participants above 20 years of age, the prevalence of diabetes was 15.2% in 2009 and increased to 20.9% in 2014[27]. In both Southern and Southwest areas of Iran, diabetes prevalence was positively correlated with low education level, body mass index (BMI), and age.

Moving to Lebanon, a cross-sectional national survey including adults above 25 years of age found a prevalence of self-reported diabetes of 8.5%; the prevalence was higher among older age, obese, and less physically active groups[28]. A more recent community-based survey that was conducted in Beirut in 2014, found that the prevalence of diabetes, based on self-report, fasting glucose, or hemoglobin A1c, was 18.0%. Similarly, increasing age and BMI were risk factors[29]. The higher prevalence reported in this study, compared to the previously mentioned one, can be attributed to the different diagnostic criteria of diabetes and the characteristics of the participants,

where they had a higher obesity rate and were residing in Beirut, the capital of Lebanon, unlike the first survey that included both an urban and a rural population. In 2019, the IDF estimated the age adjusted comparative diabetes prevalence in Lebanon at 11.2%[2]. Other countries in the MENA region did not have recent or large community-based studies to estimate the prevalence of diabetes in the population. These countries (except for Morocco and Algeria) are more likely to have political instability and/or conflict (Libya, Iraq, Yemen, Afghanistan, Syria, Palestine, and Bahrain). The most recent community-based survey from Syria was conducted in 2006 in the city of Aleppo and reported the total prevalence of T2D to be 15.6%, out of whom 5% were newly diagnosed, while the rest were self-reported cases. Like other studies, the prevalence was correlated with obesity and a positive family history[30]. In 2019, based on extrapolation from similar countries, the IDF stated that more than 1 million adults were living with diabetes in Syria[2]. Unfortunately, Palestine, like Syria, lacks recent surveys and studies on a national level; however, the IDF estimated that the prevalence of diabetes in Palestine was 7% in 2017[31] and 9.5% in 2019[2].

Prevalence of diabetes by gender

The prevalence of diabetes was higher in women than men in several of the MENA countries (Table 1). Yet, some countries showed a higher prevalence in males compared to females, as is the case for Lebanon[28]. Gender differences may vary even within regions of the same country; for example, women in Central Iran[25] and the rural area of Kurdistan province[32] had a higher prevalence than men, whereas no significant differences in prevalence by genders was observed in the Southwest of Iran [27].

Given that other global studies do not show a higher predisposition to diabetes among females, it is likely that it is the gender factor, and not the physiologic sex factor, that contributes to the risk. This is supported by the higher prevalence of obesity and sedentary lifestyle among women, as described in the risk factors section. The prevalence by gender is shown in Table 1 and Figure 2.

Urban vs rural

Studies mentioned previously for Egypt[21], Tunisia[22], and Lebanon[28,29] have shown a higher T2D prevalence in urban compared to rural areas. Similarly, a study including 9149 participants aged 7-80 years from Riyadh, the capital of Saudi Arabia, found that the prevalence of diabetes, based on the WHO definition, was 31.6%[33], which is higher than the overall reported national prevalence[17].

The higher urban compared to rural prevalence is not consistent across or within studies. For example, a national diabetes survey in Pakistan conducted in the years 2016-2017 found a significantly higher prevalence in urban *vs* rural areas among males above 60 years of age and among females; however, younger males showed a higher prevalence in rural areas[34].

Similarly, the prevalence of diabetes in Iran was higher in rural compared to urban areas; it was noted that in the rural population of Kurdistan province, the prevalence of T2D in 2011-2017 was 19.6%. The prevalence was significantly associated with age and lower level of education. In this specific population, genetic polymorphisms were more common among rural populations, predisposing them to a higher risk of diabetes[32].

It is likely that the difference in prevalence in urban *vs* rural areas is attributed to different nutritional habits, activity level, and possibly a more health-promoting environment.

Unknown vs known diabetes

The rate of unknown or undiagnosed diabetes can be detected by population-based studies that collect blood samples and measure hemoglobin A1c or glucose levels. The proportion of undiagnosed diabetes in the MENA region was 44.7% in 2019[16]. In Kuwait, a cross-sectional survey in 2007 found that 23 subjects out of 120 diabetic adults were previously undiagnosed (19%)[35]. Similarly, in Pakistan, the rate of unknown diabetes was 27%[34].

In central Iran, undiagnosed diabetes was found to be more common in men (4%) than in women (3.7%), and it was significantly associated with older age; the prevalence of undiagnosed diabetes was 4.8 times higher in the age group 60-69 years compared to the youngest age group 20-29 years, indicating a higher level of diabetes unawareness in the older population[25]. In Beirut (Lebanon), 26 subjects out of 90 were unaware that they had diabetes (29%)[29]. In Jordan, the percentage of newly diagnosed cases compared to all diabetic cases was 25.5% in 2004, and it dropped to 17.4% in 2017[21]. In Qena, Egypt, around 35% of all the diabetes cases (both types)

Table 1 Gender specific prevalence of type 2 diabetes in % (CI) in 20 Middle East and North Africa countries in 2000 and 2014 as reported by the World Health Organization[1] and for Palestine[144]

Country	2000		2014	
	Men	Women	Men	Women
Afghanistan	8.1 (5.3-11.5)	8.5 (5.7-11.9)	11.6 (6.4-18.2)	12.2 (6.8-18.8)
Algeria	8.6 (6.0-11.8)	9.2 (6.5-12.3)	12.3 (7.4-18.8)	12.6 (7.7-18.9)
Bahrain	10.6 (7.6-14.2)	9.9 (7.1-13.5)	12.0 (7.0-18.5)	10.6 (6.1-16.7)
Egypt	10.8 (7.5-14.7)	13.3 (9.6-17.5)	16.0 (10.0-23.6)	19.8 (12.9-28.2)
Iran	7.4 (5.5-9.4)	8.5 (6.4-10.7)	11.4 (7.2-17.2)	12.9 (8.4-18.8)
Iraq	11.5 (8.2-15.4)	12.4 (9.1-16.2)	17.2 (10.7-25.3)	17.5 (11.1-25.4)
Jordan	12.0 (8.8-15.9)	14.0 (10.5-18.0)	16.5 (10.5-24.0)	17.2 (11.3-24.6)
Kuwait	15.3 (11.2-20.3)	15.6 (11.4-20.4)	19.7 (12.8-28.1)	19.6 (12.9-27.7)
Lebanon	9.0 (5.7-13.1)	8.4 (5.3-12.2)	14.5 (8.7-21.8)	12.2 (7.4-18.5)
Libya	10.7 (7.9-14.1)	12.2 (9.1-15.8)	15.2 (9.5-22.5)	16.6 (10.7-23.8)
Morocco	9.0 (6.2-12.4)	9.2 (6.4-12.6}	14.0 (8.4-21.5}	13.4 (8.1-20.5}
Oman	10.2 (7.6-13.6)	9.9 (7.3-13.1)	14.3 (8.6-21.7)	12.3 (7.4-18.4)
Pakistan	9.1 (6.3-12.2)	9.0 (6.3-12.2)	12.6 (7.0-19.5)	12.1 (7.0-18.6)
Palestine	10.6 (7.8-14.0)	11.8 (8.9-15.2)	16.5 (10.3-24.3)	17.5 (11.4-24.9)
Qatar	13.7 (9.9-18.1)	14.2 (10.4-18.5)	18.9 (12.0-27.0)	18.8 (12.2-26.8)
Saudi Arabia	13.1 (9.8-17.1)	13.1 (9.8-17.0)	17.6 (11.5-25.4)	17.0 (11.1-24.4)
Syria	9.8 (7.0-13.2)	10.9 (8.1-14.2)	14.0 (8.5-21.0)	15.3 (9.6-22.4)
Tunisia	8.3 (6.0-11.1)	9.7 (7.2-12.7)	12.1 (7.4-18.3)	12.9 (7.9-19.0)
United Arab Emirates	11.8 (8.6-16.0)	12.4 (9.1-16.5)	15.0 (9.2-22.5)	15.4 (9.7-22.6)
Yemen	7.4 (4.5-11.3)	6.2 (3.6-9.4)	12.6 (6.7-20.6)	10.1 (5.3-17.0)

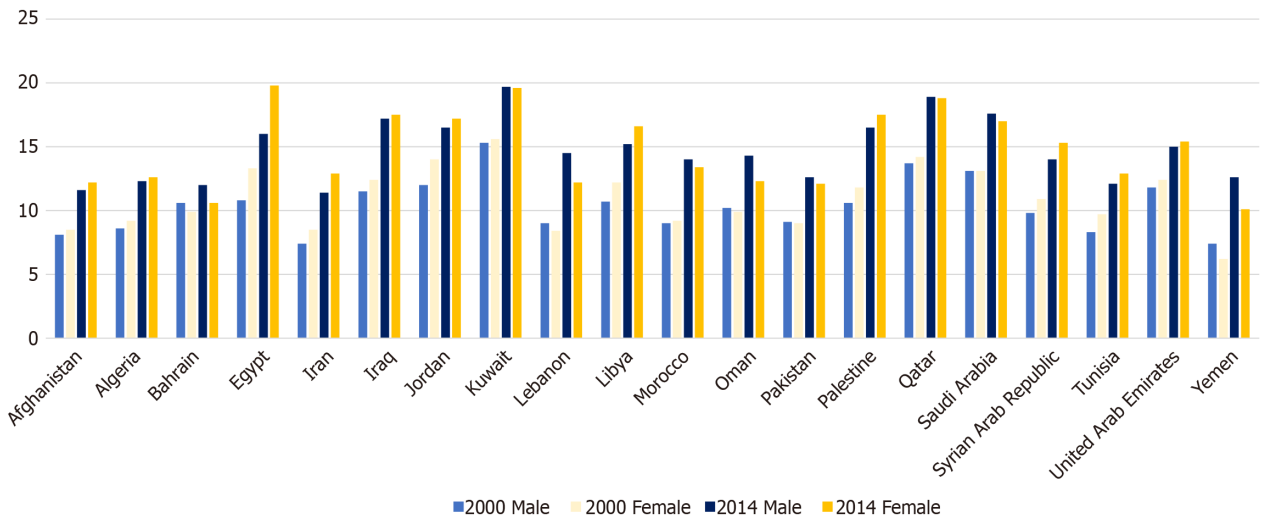

Figure 2 Prevalence of type 2 diabetes by gender in years 2000 and 2014 from the World Health Organization[1] and for Palestine[144].

were newly diagnosed[22], and Tunisia showed one of the highest rates of undiagnosed diabetes at 51.1%[23].

PREDIABETES

The development of diabetes tends to be a gradual process with rising glucose levels from the normoglycemic range to the diabetic range. This process is driven by a combination of metabolic disorders that include both insulin resistance and a progressive decline in insulin secretion[10]; the term prediabetes has been used to characterize the intermediate state between normoglycemia and the glucose levels that define diabetes[2,15]. Patients with prediabetes comprise those with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)[15]. Like diabetes, patients with prediabetes are at an increased risk of cardiovascular events but to a lesser extent. Previous studies have suggested that up to 70% of patients with prediabetes will eventually progress to diabetes[10], however implementation of lifestyle changes or pharmacologic therapy may prevent or delay that progression[36]. It is estimated that 25%-50% of patients with prediabetes may progress to diabetes within 5 years of diagnosis[37].

In addition to the high prevalence of diabetes, countries in the MENA region also suffer from a high prevalence of prediabetes. The IDF estimates that in 2019, the worldwide age adjusted comparative prevalence of IGT in adults aged 20-79 years was 8.6%, with the European region being least affected at a prevalence of 4.4%, and the Western Pacific region having the highest prevalence at 10.4%[2]. The estimated MENA prevalence of IGT was intermediate at 9.2% and is projected to increase to 9.9% by 2045. Even though the projected prevalence goes up only by 0.7%, it translates into near doubling of the number from 35.5 million to 64.5 million adults with IGT mostly due to population growth.

Within the past 20 years, a relatively small number of studies have reported on the prevalence of prediabetes in the MENA countries, however there was a wide variability among them in the definition of prediabetes, the sample size and sampling technique, and the age group sampled (Table 2). Overall, there did not appear to be a major difference between gender subgroups. A relatively high prevalence of prediabetes was reported in Iraq[38], Saudi Arabia[19], the UAE[39], and Kuwait[40], ranging between 19.3% and 28.6%. An intermediate prevalence was found in the countries of Iran[41], Pakistan[34], and Qatar[42] with a prevalence ranging from 13.8% to 14.6%. The countries of Yemen[43], Syria[30], Oman[44], and Tunisia[45] boasted a relatively low prevalence with a range of 4.6%-9.0%. A Lebanese study limited to the greater Beirut area reported an unusually high prevalence of prediabetes of 40.3%[29]; the way prediabetes was defined could have been a factor affecting the results, since either an elevated fasting glucose or a high A1c were acceptable parameters, while most of the other studies limited the prediabetes definition to IFG.

The values estimated by IDF for the prevalence of IGT in 2019 are overall lower compared to those reported for individual countries in the table, possibly because IDF limited their data collection to studies of IGT and excluded those that had also included isolated IFG in the definition of prediabetes. In addition, for the IDF data, only Algeria, Jordan, Oman, Pakistan, Saudi Arabia, Palestine, and the UAE had estimates based on oral glucose tolerance test. Diabetes prevalence for the remaining countries were extrapolated using values from countries deemed to be similar (geographic location, World Bank income group, ethnicity, language, and IDF region) and may be under-estimated[4].

RISK FACTORS

Multiple risk factors have been implicated in the increase in T2D prevalence. The change to a more sedentary lifestyle and the westernization of dietary habits with a shift to fast food and items rich in refined sugar and animal fat play a major role. Additional factors may also contribute to the rising prevalence, including cigarette and waterpipe smoking[46], pollution of the environment[47], and a high prevalence of hepatitis C in some countries (mainly Egypt and Pakistan)[48,49]. Moreover, there is evidence that people in lower socioeconomic groups are at increased risk of T2D[50].

Genetics

In addition to the well-known contributing effect of aging, sedentary lifestyle, unhealthy diets, and obesity in the development of diabetes, genetics also appear to play a role. A family history of diabetes in first degree relatives has long been known to increase the diabetes risk by up to 3-fold[51].

Table 2 Prevalence of prediabetes (%) in some Middle East and North Africa countries

Ref.	Country, yr	Age group (*n*)	Definition of prediabetes	Sampling technique	Prevalence of prediabetes % (95%CI)		
					Male	Female	Total
Nasrallah *et al* [29]	Lebanon (Beirut area), 2014	≥ 18 yr (501)	IFG: FPG 5.6-6.9 mmol/L or A1c 5.8%-6.49%	Probability multistage random sampling	48.0 (40.6-55.4)	36.0 (30.7-41.3)	40.3 (36.0-44.6)
Mansour *et al*[38]	Iraq, 2011-2012	19-94 yr (5445)	IFG: FPG 5.7-6.9 mmol/L or A1c: 5.7%-6.4%	Population-based random sample	28.6	29.5	29.1
Al-Rubeaan *et al* [19]	Saudi Arabia, 2007-2009	≥ 30 yr (18034)	IFG: FPG 5.6-6.9 mmol/L	Random household national sample	26.4	24.7	25.5
Saadi *et al*[39]	United Arab Emirates (Al-Ain), 2005-2006	> 18 yr (2455)	IFG: FPG 5.6-6.9 mmol/l or IGT	Simple random sample	19.7	22.8	22.8
Alkandari *et al* [40]	Kuwait, 2014	18-69 yr (2561)	IFG: FPG 6.1-6.9 mmol/L	Random sample	19.3 (16.9-22.0)	19.5 (17.6-21.5)	19.4 (17.9-21.0)
Esteghamati *et al* [41]	Iran, 2011	25-70 yr (11867)	IFG: FPG 5.6-6.9 mmol/L	Randomized multistage cluster sample	15.45 (12.71-18.18)	13.74 (11.55-15.94)	14.6 (12.41-16.78)
Basit *et al*[34]	Pakistan, 2016-2017	≥ 20 yr (10834)	IFG: FPG 6.1-6.9 mmol/L orIGT	Multistage clustering technique	NA	NA	14.4
Bener *et al*[42]	Qatar, 2007-2008	> 20 yr (1117)	IFG: FPG 5.6-6.9 mmol/L or IGT	Multistage stratified cluster sampling	NA	NA	13.8
Gunaid *et al*[43]	Yemen, 2000	≥ 35 yr (250)	IFG: FPG 5.6-6.1 mmol/L or IGT	Multistage random sampling	5.7 (2.8-8.6)	10.9 (7.1-14.7)	9.0 (6.0-12.0)
Albache *et al*[30]	Syria (Aleppo), 2006	≥ 25 yr (806)	IFG: FPG 6.1-6.9 mmol/L	Random sampling	10.4 (4.7-21.0)	6.8 (2.9-15.1)	8.6 (3.8-18.1)
Al-Lawati *et al* [44]	Oman, 2000	≥ 20 yr (5838)	IFG: FPG 6.1-6.9 mmol/L	Multistage stratified probability sampling	7.1 (6.2-8.1)	5.1 (4.4-6.0)	6.1 (5.5-6.8)
Bouguerra *et al* [45]	Tunisia, 1996-1997	≥ 19 yr (7860)	IFG: FPG 6.1-6.9 mmol/L	National cross-sectional sample	4.58	4.91	NA

IFG: Impaired fasting glucose according to specified glucose range; FPG: Fasting plasma glucose; IGT: Impaired glucose tolerance (using World Health Organization definition, glucose ≥ 7.8 but < 11.1 mmol/L, 2 h after 75 gm oral glucose load); CI: Confidence interval; NA: Not available; A1c: Hemoglobin A1c.

Reports from Palestine, Iran, and Lebanon showed that a positive family history of T2D raised the risk by 1.6, 1.8, and 3.4 times, respectively[28,52,53]. Moreover, despite a higher prevalence of obesity in North America and Europe, MENA has a comparatively higher prevalence of diabetes, suggesting the presence of a genetic predisposition to glucose intolerance.

Genome wide association studies have yielded several single nucleotide polymorphisms that appear to be associated with the development of diabetes. A recent meta-analysis reported that, for people in the MENA area, 71 single nucleotide polymorphisms in 32 genes increased the risk of T2D by 24%-69%[7]. There was a strong association with single nucleotide polymorphisms in the *TCF7L2* (in 9 countries) and *CDKAL1* genes (in 4 countries), in addition to a variety of other loci, including *ADIPOQ*, *FTO*, *MC4R*. *COL8A1*, *KCNQ1*, *ALX4*, and *HNF1*. *TCF7L2* was the most widely reported gene in the region, in countries that include Palestine[54], Lebanon[55], UAE[56], Egypt[57], Qatar[58], and Tunisia[59]. In the Lebanese population, associations with T2D have been found with variants of the *COL8A1*, *KCNQ1*, *ALX4*, and *HNF1* genes[60]. The high rate of consanguinity reported for the MENA region, varying from 30% and up to 60%[8], likely further enhances the genetic susceptibility observed.

Transition in nutrition

In addition to the genetic predisposition, a worldwide transition to unhealthy diets and reduction in physical activity[61] plays a role in the development of obesity and diabetes. Diets have shifted to a higher consumption of calories, processed food, and animal fat, and a lower intake of fiber, fruits, and vegetables. In particular, the MENA area has experienced a rapid rate of modernization and urbanization over the past decades. An analysis of food availability and consumption by Mehio Sibai *et al*[62]

showed a gradual and significant rise in daily caloric, protein, and fat intake between 1969-1971 and 2002-2004. It is estimated that the energy supply during that period rose from 2200 up to 2930 kilocalorie per day. In parallel, there was an increase in sugar intake and a reduction in the intake of fruits and vegetables. In Saudi Arabia, a recent study of people aged 35-70 years showed that 34% of participants reported an unhealthy diet, with a higher rate in younger individuals and those living in urban areas[63]. Similarly, a review paper from Lebanon found a rising trend of increased energy consumption from fat and animal product in the population, with a reduction in carbohydrate and cereal intake[64]. Another study from Lebanon found that consuming minimally processed food such as fruits, vegetables, legumes, breads, and cheeses was less likely to be associated with the metabolic syndrome (odds ratio = 0.18, 95%CI: 0.04-0.77) and hyperglycemia (odds ratio = 0.25, 95%CI: 0.07-0.98) compared to the consumption of highly processed food such as fast foods, snacks, meat, nuts, sweets, and liquor[65].

Physical inactivity

With the rapid worldwide modernization and advancement in technology, a reduction in the rate of physical activity has occurred. In the MENA region, all countries have demonstrated an increase in physical inactivity, with a higher prevalence among females compared to males. Figure 3 shows the prevalence of physical inactivity in MENA countries subdivided by gender. Insufficient physical activity was defined as the percentage of the population aged above 18 years who are not performing at least 150 min/wk of moderate-intensity physical activity or its equivalent[66]. There was a very high prevalence of insufficient physical activity in the high income Gulf countries with a prevalence ranging from 33%-67%, possibly because of a shift from manual labor/high physical activity jobs to occupations that are more sedentary in the services sector[67]. A study in Saudi Arabia found that more than 90% of surveyed individuals had an inadequate level of physical activity[68]. In contrast, that prevalence was under 30% for Jordan, Morocco, and Tunisia. Females were consistently less active compared to males, possibly due to prevailing local customs in conservative countries where women may not spend as much time outside the home or in public places and may not frequent exercise facilities.

Obesity

Because of the adoption of unhealthy dietary habits and food choices, and the significant reduction in physical activity, the prevalence of obesity has been steadily increasing worldwide including in the MENA region. It is well-known that obesity is a significant risk factor for diabetes, with many studies showing a correlation between BMI and the incidence of diabetes[69].

Data from the WHO risk surveillance program has shown a significant rise in the rate of obesity across all countries. The countries most affected are the high-income Gulf countries, in addition to Egypt, Libya, Lebanon, and Iraq with rates exceeding 30%. In contrast, Afghanistan, Pakistan, and Yemen boasted a relatively low prevalence, ranging from 5% to 17%. However, all countries suffered a rise in obesity prevalence between 2000 and 2016, ranging from 30%-100% (Figure 4). Interestingly, females were disproportionately more affected than males, with a prevalence that is comparatively 1.5-fold to 2.0-fold higher. Specifically, the prevalence of female obesity exceeded 40% in some Gulf countries (Saudi Arabia, Kuwait, Qatar, and UAE), in addition to Egypt and Jordan[70] (Figure 5).

COMPLICATIONS OF DIABETES

Mortality

NCDs account for around 70% of deaths worldwide[70]. In 2019, diabetes was the ninth leading cause of death with around 1.8 million directly attributed to hyperglycemia[71]. In addition, CVD, which is the leading cause of death and commonly a chronic complication of diabetes[72], claimed during the same year around 9 million lives[71]. Since 2019 and up until January 2021, the coronavirus disease 2019 (COVID-19) pandemic had already surpassed world diabetes mortality with over 2 million deaths; however, diabetes was again an important risk factor for mortality or the development of severe COVID-19 infection[73].

Regionally, the MENA region scores the worst in terms of hyperglycemia-related mortality with an age-standardized mortality rate per 100000 (ASM) of 139.6 in 2016, followed by Southeast Asia, which has an ASM of 115.3 and in contrast to Europe with

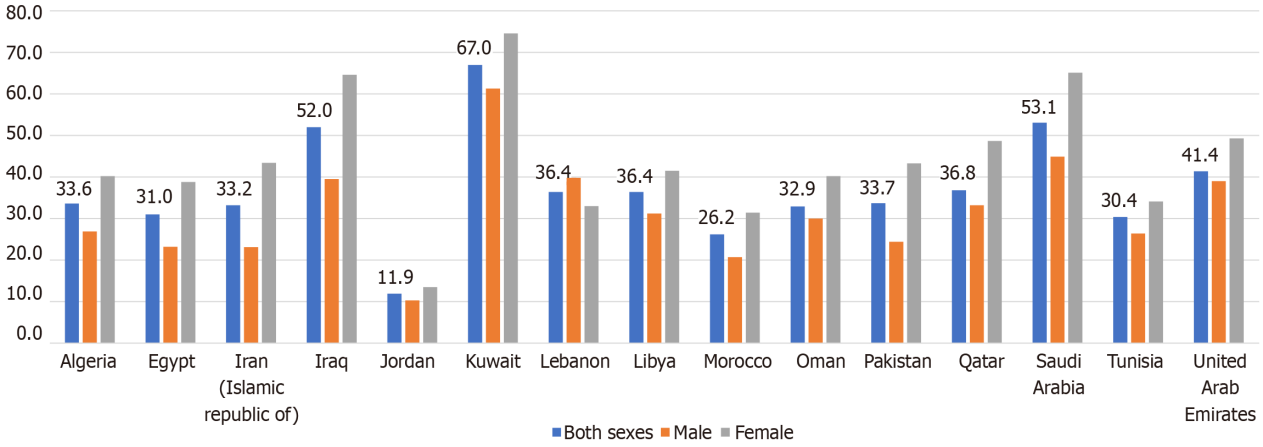

Figure 3 Prevalence (%) of insufficient physical activity among adults aged 18+ year in 2016 (age-standardized estimate)[1].

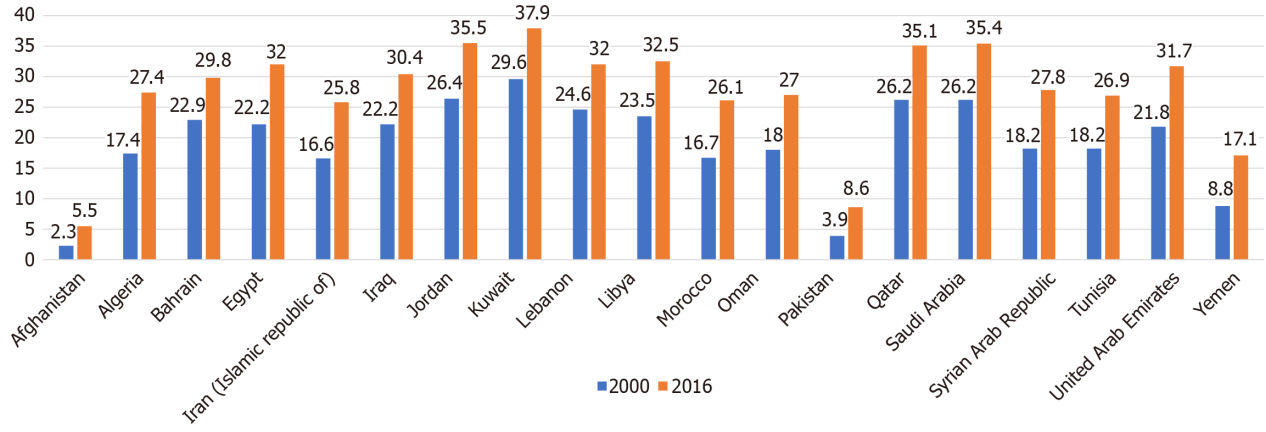

Figure 4 Prevalence (%) of obesity by country among adults aged 18 years or older, defined as body mass index ≥ 30 kg/m² (age-standardized estimate) in 2000 and 2016[70].

an ASM of 55.7 and the Americas with an ASM of 72.6[74].

Focusing on the MENA region, there seems to be a pattern of lower ASM mortality from NCDs (diabetes, CVD, cancer, and chronic obstructive pulmonary disease) for some HIC of the Gulf and for the North African countries. Thus, in 2016, the ASMs for Oman, Qatar, Bahrain, and UAE were 404.6, 425.5, 430.1, and 460, respectively; similarly, for the North African countries of Algeria, Tunisia, and Morocco, they were 430.7, 460.6, and 483.9, respectively. In contrast, in low/middle low-income countries, ASM was 681.0 for Pakistan, 805.3 for Afghanistan, and 819.7 for Yemen, per 100000 [75]. The ASM from NCDs for the MENA countries as well as the absolute number of deaths from diabetes or from CVD are presented Table 3. In addition to the apparently higher mortality in middle-income countries and low-income countries, the largest proportion of diabetes-related deaths occur among individuals under 60 years of age, with loss of productive years, adding to the socioeconomic burden of diabetes[2]. The finding of lower mortality for the HIC is consistent with recent world data[76,77].

Even if not leading to premature death, diabetes causes significant morbidity with disability, lower productivity, and quality of life. The MENA region again has the highest rate of disability-adjusted life years caused by diabetes[77].

Macrovascular complications

Having diabetes essentially increases the risk of having a major adverse vascular event defined as nonfatal myocardial infarction, stroke, heart failure, and/or cardiovascular death by 2-3-fold after adjusting for age, sex, and smoking status[72,76,78]. We could not find any large cohort studies nor national data on the above hard outcomes even from countries with a high prevalence of T2D who have a national diabetes registry

Table 3 Mortality from high glucose and cardiovascular disease extracted from the World Health Organization country profile data site for 2016[75]

2016	Total population (million)	Age-standardized mortality rate for NCD per 100000	Diabetes deaths (*n*)	Diabetes mortality per 10000	CVD deaths (*n*)	CVD mortality per 10000
Afghanistan (LIC)	32527000	805.3	7056	2.17	51244	15.75
Algeria (MIC)	39667000	430.7	8390	2.12	69173	17.44
Bahrain (HIC)	1377000	430.1	404	2.93	775	5.63
Egypt (MIC)	91508000	711.8	17851	1.95	245904	26.87
Iran (MIC)	79109000	532.5	14842	1.88	160823	20.33
Iraq (MIC)	36423000	604.5	7279	2.00	51593	14.16
Jordan (MIC)	7595000	542.4	2347	3.09	13384	17.62
Kuwait (HIC)	3892000	541.4	326	0.84	4552	17.62
Lebanon (MIC)	5851000	516.4	1886	3.22	17814	30.45
Libya (MIC)	6278000	567.0	1292	2.06	11638	18.54
Morocco (MIC)	34378000	483.9	10645	3.10	69457	20.20
Oman (HIC)	4491000	404.6	903	2.01	4047	9.01
Pakistan (MIC)	189000000	681	44666	2.36	411569	21.78
Qatar (HIC)	2235000	425.5	359	1.60	1054	4.72
Saudi Arabia (HIC)	31540000	508.5	3737	1.18	42440	13.46
Syria (MIC)	18502000	594.7	1322	0.71	37885	20.48
Tunisia (MIC)	11254000	460.6	3523	3.13	31987	28.42
UAE (HIC)	9157000	460	707	0.77	5970	6.52
Yemen (MIC)	26832000	819.7	3854	1.44	56793	21.17

NCD: Noncommunicable diseases; CVD: Cardiovascular disease; LIC: Low income countries; MIC: Middle income countries; HIC: High income countries.

and/or have a relatively well-funded health care system[79]. The heterogeneity of the MENA population, the presence or absence of risk factors, and the inconsistent definition of outcomes makes comparisons between individual regions challenging. However, a recent study of 143567 adults with diabetes aged 35-70 years from 21 countries around the world, including 5 countries from the MENA (Saudi Arabia, UAE, Iran, Palestine, and Pakistan) and followed for 9 years found an absolute incidence of major CVD among people with diabetes of 8.3; 9.2; and 10.3 per 1000 person-years in HIC, middle-income countries, and low-income countries, respectively, as compared to 3.4; 4.9; and 5.3 per 1000 person-years in people without diabetes, respectively[76].

Specific to the MENA, the Tehran Lipid Study sampled 1198 adults aged ≥ 30 years with T2D and followed them for a median of 10 years. It reported a 23.4% and 14.3% cardiovascular and all-cause mortality, respectively. More than half of the mortality was due to cardiovascular events. Risk factors for death were male gender, smoking, and hypertension[80]. In a sample of 1308 adults with T2D recruited from primary care centers in Palestine, the prevalence of self-reported CVD was 12.2%[81]. The prevalence for CVD for Iran[4,80] and Palestine[82] is shown in Table 4.

On the other hand, risk factors for CVD, such as hypertension, dyslipidemia, smoking, physical inactivity, and obesity, are well documented through the implementation of the STEPS program by the WHO for most countries[17]. As an example, the prevalence of raised blood pressure (systolic blood pressure ≥ 140 and diastolic blood pressure ≥ 90 mmHg) was 26.3% for the EMR, which is the second highest in the world, with no gender predilection. It ranged from a low of 16.2% in Oman to as high as 25.0% in Pakistan. Similarly, the habit of smoking was high in this population, with a large gender difference, with 36.3% of men being active smokers (third highest in the world) *vs* 2.9% of women[17]. Of more concern is that a

Table 4 Select studies reporting on diabetic foot ulcer and macrovascular complications of diabetes for the Middle East and North Africa region

Complication	Ref.	Country	Sample size (% male)	Setting	Duration of diabetes (yr)	Method of assessment	Prevalence %
Diabetic foot ulcer	Assaad-Khalil *et al* [88], 2015	Egypt (Alexandria)	2000 (50.0)	Diabetes Foot Clinic	11.7 ± 8.3	Physical exam	8.7
	Al-Rubeaan *et al* [89], 2015	Saudi Arabia	62681 (52.4)	Saudi National Diabetes Registry	13.3 ± 8.1	Chart review	2.1
	Yazdanpanah *et al* [92], 2018	Iran (Ahfaz)	605 (42.8)	Diabetes Clinic	9.2 ± 7.1	Physical exam	6.4
	AlAyed *et al*[93], 2017	Jordan	1000 (48.2)	Diabetes Clinic	57.1% ≥ 5	Physical exam	5.3
Peripheral vascular disease	Akram *et al*[90], 2011	Pakistan	830 (49.0)	Outpatient Clinic	8.1 ± 6.2 low ABI; .4 ± 6.4 normal ABI	ABI below 0.9	31.6
Coronary artery disease	Saeedi *et al*[4], 2020	Iran (Kurdistan)	400 (18.0)	Diabetes Clinic	14.6 ± 4.1	Angiography or physician	21.7 (5.75 CABG, 3.75 angioplasty)
	Abu Al-Halaweh *et al*[81], 2017	Palestine	1308 (35.9)	Primary Care Centers	7.1 ± 6.3	Questionnaire	12.2 (myocardial infarction)
	Afsharian *et al*[80], 2016	Iran (Tehran)	1198 (42.1)	Community-based	NA	Physician assessment	23.4

ABI: Ankle brachial index; NA: Not available; CABG: Coronary artery bypass grafting.

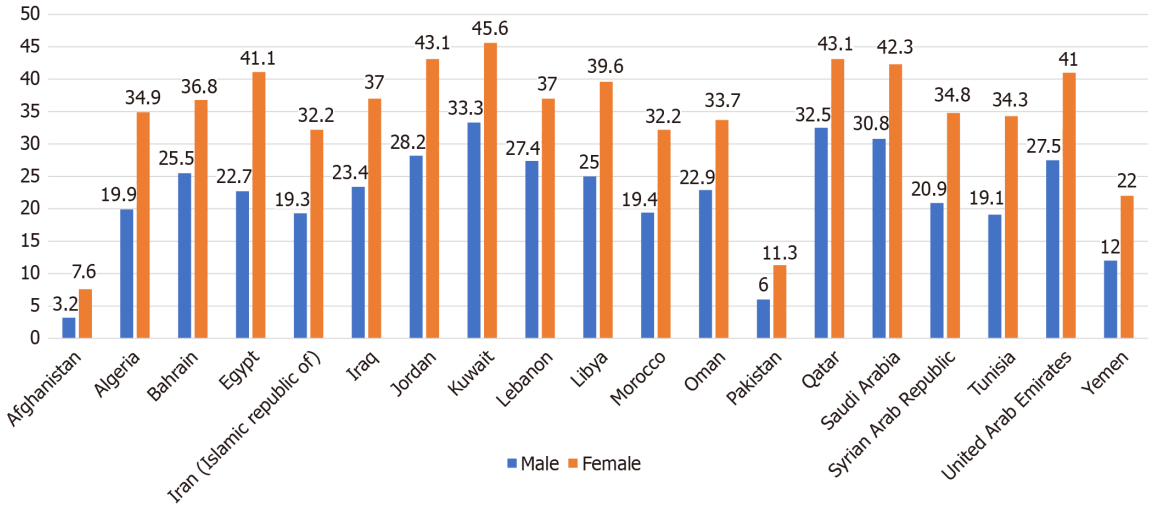

Figure 5 Prevalence (%) of obesity by country among male and female adults aged 18 years or older. Obesity defined as body mass index ≥ 30 kg/m^2 (age-standardized estimate) in 2000 and 2016[70].

substantial proportion of youth (aged 13-15 years) smoke cigarettes, reaching 10% in Qatar and 35% in the West Bank (Palestine)[82]. Additionally, the habit of waterpipe smoking is highly prevalent among youth in predominantly Arabic-speaking countries of the MENA region, with waterpipe exceeding cigarette smoking[82]. The prevalence of hypercholesterolemia (total cholesterol level ≥ 5.2 mmol/L) was 36.8% in the EMR [70], which is comparable to the world prevalence. However, there is a higher predisposition to the atherogenic dyslipidemias in this region, with an elevated prevalence of low high-density lipoprotein-cholesterol and familial hypercholesterolemia[83,84].

Given that the prevalence of CVD risk factors in the MENA region is high, it would be desirable to develop a predictive model adapted to its population. One such CVD predictive tool used 1314 Omani adults to develop it and another 405 individuals for validating it; that population sample was followed prospectively for 6 years and confirmed a 9% incidence of CV events[85].

Diabetic foot ulcers

Diabetic foot ulcers (DFU) are a consequence of neuropathy and/or peripheral vascular disease. The lifetime risk for a person with diabetes to develop a DFU is 15%-25%, and the global prevalence is 3%-8%[86]. In a systematic review of DFU involving Arabic speaking countries, a total of 9 studies were reviewed, representing 16512 participants recruited from outpatient clinics. The prevalence of DFU was available for the following countries: Saudi Arabia (8.5%), Egypt (3.6%), Jordan (4.6%), Bahrain (5.9%), and Iraq (2.7%)[87]. A clinic-based study of 2000 adults with T2D in Alexandria (Egypt) found that 8.7% had DFU[88]. In a retrospective review of 62675 patients in Saudi Arabia, the prevalence of DFU was 2.05%, with an additional 1.06% suffering amputations[89].

Risk factors for DFU were a longer diabetes duration, male gender, higher BMI, the presence of an abnormal ankle-brachial index[90], and sensory neuropathy[88,91,92]. Prevalence of amputations varied from 1% to 2%[91,93]. Among 840 patients with diabetes in the Saudi National Diabetes Registry, the risk of mortality rose with DFU and increased further in the presence of lower extremity amputation, with a standardized mortality ratio of 4.39 and 7.21, respectively[94].

Microvascular complications

Most studies reporting on microvascular complications from the MENA region are either clinic or hospital based. However, some countries, like Saudi Arabia, possess a national diabetes registry, and others such as Iran have large cohorts followed longitudinally, like the Tehran Lipid Cohort. Among the three microvascular complications of diabetes namely nephropathy, neuropathy, and retinopathy, the latter is the most documented and studied.

Retinopathy

The overall global prevalence of DR is 34.6%, including 7.0% proliferative DR and 6.8% clinically significant macular edema[95]. It is the most common cause of blindness in adults worldwide including the MENA region. The overall prevalence of DR in the MENA countries ranges from 12.6% to 37.8%, with proliferative DR ranging from 2.3% to 10.6%[96-99].

In a nationwide study of 50464 Saudi adults with T2D from the Saudi National Diabetes Registry, the prevalence of DR was 19.7%, with 10.6% proliferative and 5.7% clinically significant macular edema[100]. In a hospital-based study of 1325 adults with T2D from Egypt, the prevalence of DR was 20.5%[101]. In a clinic-based survey of 1308 Palestinian adults with T2D, the prevalence of DR using nonmydriatic images was 21.8%[81]. In a cross-sectional Tunisian clinic-based study of 2320 adults, the prevalence of DR was 26.3%, out of whom 3.4% had proliferative DR and 4.2% had clinically significant macular edema[102]. In a systematic review in Iran which included 17079 individuals, the overall prevalence of DR was 37.8%, with wide variability among regions[103]. The prevalence of DR for the various countries is shown in Table 5.

Importantly, retinopathy was found even among newly diagnosed adults with T2D. In Pakistan, DR was present at diagnosis in 15.9% of 958 adults with T2D[99]. Similarly, in Jordan, DR was documented in 7.9% of 127 adults with T2D within 6 mo of diagnosis[104]. Finally, in a retrospective chart review from Lebanon of 484 adults with T2D, DR was present in 26.6% at first ophthalmologic examination[105].

The elevated prevalence of DR at diagnosis is in line with the high proportion (44.7%) of undiagnosed diabetes for the MENA region[2]. It is likely that there is a latency period from onset of diabetes to time of its diagnosis, during which damage to the retina is taking place. In support of this theory is that duration of diabetes has been consistently reported as a risk factor for DR across most studies[96,100,101]. Even after being diagnosed, regular ophthalmologic check-ups were uncommon. This was evidenced in a community-based screening campaign of 2205 adults with mostly T2D, for whom only one third had regular retinal exams by an ophthalmologist[96]. Similarly, in Egypt, out of 1325 adults with long-standing diabetes, 82% were not aware of the need to do retinal checks[101]. Other risk factors for DR were poor glycemic control, older age, higher BMI, hypertension, smoking, the use of insulin, and the presence of other microvascular complications[97,98,100].

Nephropathy

Studies assessing nephropathy are more heterogeneous, with some reporting on albuminuria, others on glomerular filtration rate, and very few on end-stage renal disease. A global study evaluating the impact of diabetes on disability-adjusted life

Table 5 Select studies reporting on microvascular complications of diabetes in the Middle East and North Africa region

Complication	Ref.	Country	Sample size (% male)	Setting	Duration of diabetes (yr)	Method of assessment	Prevalence %
Retinopathy	Al-Rubeaan *et al* [89], 2015	Saudi Arabia	50464 (56.0)	Saudi National Diabetes Registry	13.4 ± 8.2	Chart review	19.7 (10.6 PDR)
	Macky *et al*[101], 2011	Egypt	1325 (28.5)	Hospital-based	48% for 5-15	Slit lamp	20.5 (2.3 PDR)
	Jammal *et al* [104], 2013	Jordan	127 (63.8)	Clinic-based	Newly diagnosed	Slit lamp	7.9
	Uddin *et al*[99], 2018	Pakistan	958 (56.0)	Multi-Clinics	Newly diagnosed	Slit lamp	15.9
	Abu Al-Halaweh *et al*[81], 2017	Palestine	1308 (35.9)	Primary Care Centers	7.1 ± 6.3	Digital retinal photo	21.8
	Elshafei *et al*[97], 2011	Qatar	540 (360/540)	Community-based	12.9 ± 9.1	Slit lamp	23.5
	Heydari *et al* [98], 2012	Iran	1022 (40.2)	Clinic-based	11.2 ± 8.2 DR; 5.8 ± 5.4 no DR	Slit lamp	23.6
	Arej *et al*[96], 2019	Lebanon	2205	Community-based	9.1 ± 7.1	Digital retinal photo	12.6
	Kahloun *et al* [102], 2014	Tunis	2320 (39.8)	Hospital-based	7.6	Slit lamp	26.3 (3.4 PDR)
Nephropathy	Al-Rubeaan *et al* [107], 2018	Saudi Arabia	54670 (51.2)	Saudi National Diabetes Registry	13.6 ± 8.1	ACR and GFR	10.8 (1.2 micro; 8.1 macro; 1.5 ESRD)
	Uddin *et al*[99], 2018	Pakistan	958 (56.0)	Multi-Clinics	Newly diagnosed	ACR	24.0
	Zakkerkish *et al* [110], 2013	Iran	350 (32.0)	Diabetes Clinic	4.6 ± 5.5	ACR	20.6 (5.1 macro)
	Shahwan *et al* [108], 2019	Palestine (Ramallah)	550 (54.7); Age above 35 yr	Diabetes Clinic	8.9 ± 6.8	ACR	34.6 (5.8 macro)
	Ali and Al Lami [109], 2016	Iraq	224 (58.9)	Diabetes Clinic	23.2 % ≥ 9	ACR (2 out of 3)	16.1
Neuropathy	Khedr *et al*[22], 2016	Egypt (Qena)	9303 (51.1); 837 with diabetes	Community	NR	MNSI, then ENG	18.5
	Ghandour *et al* [114], 2018	Palestine (Ramallah)	517 (32.0)	Primary Health Clinic	9.0 ± 7.5	Monofilament test	38.2
	Chahbi *et al* [115], 2018	Morocco	300 (50.7)	Diabetes Clinic	10.6 ± 7.4	Diabetic Neuropathy Score	15.4 (DN4 painful)
	Garoushi *et al* [112], 2019	Libya	450 (50.2)	Diabetes Clinic	15.1± 7.1	Diabetic Neuropathy Score	42.2 (s-LANSS ≥ 12 pain)
	Kiani *et al*[113], 2013	Iran	521 (NR)	Diabetes Clinic	9.2 ± 7.4	NSS and NDS	49.3

NR: Not recorded; MNSI: Michigan neuropathy screening instrument, ENG: Electroneurogram; PDR: Proliferative diabetic retinopathy; ACR: Albumin to creatinine ratio; S-LANSS: Leeds assessment of neuropathic symptoms and signs; NSS: Neuropathy symptom score; NDS: Neuropathy disability score; DR: Diabetic retinopathy; GFR: Glomerular filtration rate; ESRD: End-stage renal disease.

years in the EMR reported more than doubling of diabetes-related chronic kidney disease between 1990 and 2005[77]. This doubling was not only due to the increased prevalence of diabetes or aging but also due to more obesity, salt intake, and uncontrolled blood pressure[77]. In a national study from Libya, the estimated prevalence of end-stage renal disease was 624 per million. The major cause was diabetes, followed by hypertension[106].

The prevalence of microalbuminuria ranged from 10.8% in Saudi Arabia[107] to 34.6% in Palestine[108], with Iraq[109], Iran[110], and Pakistan[99] at 16.1%, 20.6%, and 24.0%, respectively. Macroalbuminuria constitutes about 15% of the reported albuminuria. The prevalence of nephropathy from selected studies is shown in Table 5.

Risk factors for albuminuria were elevated blood pressure, high BMI, duration of disease, hyperglycemia, and the presence of diabetes complications[107,109,110].

Neuropathy

Diabetic peripheral neuropathy is the most common complication of diabetes; at least 50% of individuals with diabetes will develop it to some extent[111]. Neuropathy negatively affects quality of life and constitutes a major risk for DFUs. Despite its high prevalence, studies on neuropathy are heterogeneous in terms of the populations studied, the setting (clinic or community-based), and the testing procedure used (questionnaire, monofilament test, or electroneurogram). Similarly, the prevalence in the MENA region varies from 18.5% (Egypt)[22] to 42.2% (Libya)[112] and 49% (Iran)[113], using a standardized scoring system. Using a simple monofilament test, the prevalence was 38% in an outpatient clinic in Palestine[114]. Painful neuropathy is less common and was 15.4% in a cross-sectional study of 300 adults in Morocco[115]. Increasing age, duration of disease, high BMI, poor glycemic control, smoking, and low educational level were predisposing factors[112,113]. Peripheral neuropathy is overall more common in men, whereas painful neuropathy was more common in women[115].

DIABETES IN CHILDREN AND ADOLESCENTS

Whereas diabetes in children and adolescents used to be mostly limited to T1D, there has been, over the last two decades, an increasing proportion developing T2D globally[15]. Moreover, in some countries, such as Japan, T2D in children and adolescents has become more prevalent than T1D[2]. Because of the earlier onset of the disease, affected children are expected to have a higher prevalence of complications in adulthood compared to others with adult-onset T2D within the same age group[17]. Furthermore, T2D starting at an early age would adversely affect productivity at its peak, raise the healthcare costs, and increase morbidity and mortality[15].

We could not find recent data about the overall prevalence of T2D in children and adolescents in the MENA region; however, data from specific countries reflect an increasing incidence and prevalence of T2D in this age group. In Kuwait, for instance, the incidence rate of T2D between 2011 and 2013 among children and adolescents, aged ≤ 14 years, was 2.56 per 100000 per year[116]. In Qatar, there were no registered cases of T2D among children and adolescents before 2008. Afterwards, the incidence of T2D increased from 1.16 per 100000 in 2012 to 2.72 per 100000 in 2016[117]. In Saudi Arabia, a nationwide survey conducted over years 2007-2009 found that 0.45% of the adolescents aged less than 18 years were known to have diabetes; out of which, 0.07% was T2D (n = 17). Moreover, the prevalence of newly diagnosed IFG and diabetes (both types) were 6.1% and 4.3%, respectively[118]. In Riyadh, T2D in the age group 7-17 years was found to be more common than T1D with a prevalence of 4.5%[33].

Moving to Iran, from 2001 to 2011, 1% of adolescents developed prediabetes and T2D each year[119]. The study found that males were 1.28 times more likely to develop prediabetes and T2D than females, which differs from an American study for diabetes in youth, the SEARCH study, that found the prevalence to be higher among females. This lower prevalence among Iranian females could be attributed to the ethnic differences and to how prediabetes was defined. This study included IFG only in its definition of prediabetes, which is, in contrast to IGT, a more common phenotype in males than females, thus contributing to the higher prediabetes prevalence among Iranian males[119]. As for Lebanon, a cross-sectional study was conducted in 2007 among students aged 11-18 years at three private schools in an urban area. It found that 10.5% of the students had IFG, and 3.5% had diabetes. Among overweight and obese individuals, the risk of developing prediabetes or diabetes was 4.93 and 2.85 times higher, respectively. The diagnostic criteria used did not differentiate between T1D and T2D; however, since the majority of those diagnosed with prediabetes and diabetes were overweight or obese, they were likely insulin resistant. Therefore, T2D is on the differential and should be confirmed for such a patient profile[120].

Modifiable risk factors

Obesity: One of the most important risk factors for T2D in adolescents is obesity. In the UAE, the prevalence of prediabetes and T2D among overweight and obese public school students, aged 11-17 years, were 5.4% and 0.87%, respectively[121]. Furthermore, 100% of all the children and adolescents with T2D in Qatar were either overweight or obese[117], and 23.35% of Saudi adolescents with diagnosed diabetes

were obese[118].

Obesity presents a challenging health problem in the MENA region, where the rates of obesity have been increasing rapidly among children and adolescents at a faster rate than adults[122]. According to the WHO, the crude prevalence of obesity among children and adolescents in the region ranged from 2.4% in Afghanistan to 22.8% in Kuwait in 2016[123].

In Lebanon, two cross-sectional studies were conducted in 1997 and 2009 using the WHO definition for obesity in children and adolescents as age and sex specific scores + 2. The prevalence of obesity in the age group 6-19 years increased from 7.3% in 1997 to 10.9% in 2009[124]. Moreover, obesity was more common in boys (10.1%) than in girls (4.2%) in private schools, whereas there was no gender difference in public schools (6.7% and 6.0% for boys and girls, respectively)[120]. In Pakistan, recent data from Hazara city showed that the prevalence of obesity among school students was 4.78%, and similarly to Lebanon the prevalence was higher in private compared to public schools[125].

Physical inactivity: The Global School-based Student Health Surveys found that physical inactivity, defined as less than 60 min activity per day on 5 or more days in the past 7 d, was very high among children and adolescents in MENA countries and was higher in girls compared to boys. The prevalence was 65.4% in Lebanon, 72.5% in the UAE, 80% in Iraq, and more than 80% in many MENA countries, reaching as high as 90.6% in Egypt. The high sedentary lifestyle rates can be partly due to lack of encouragement of physical activities from parents, teachers, and friends, while favoring spending more time on other tasks such as their education[126].

Non modifiable risk factors

Family history: Along with obesity and physical inactivity, the genetic predisposition and family history present significant risk factors for T2D in adolescents[127]. The risk of abnormal glycemic status among Emirati school students was 1.9 times higher among those who have first degree relatives with diabetes; moreover, it was found that more than half of Emirati students with prediabetes and all those with T2D had a positive family history of diabetes[121]; the same applies to Qatari adolescents with T2D[117].

Other non-modifiable risk factors are low birth weight, maternal diabetes during pregnancy, and the postmenarchal phase in females[126]. During puberty, there is a physiological 30% increase in insulin resistance that, in the presence of other risk factors, can precipitate T2D; thus, the onset of T2D in youth is earlier in girls than boys and manifests itself usually during the second decade of life.

In summary, the youth of the MENA region are particularly vulnerable to developing T2D because of a culmination of risk factors. Multilevel interventions are needed to be able to effectively reverse this wave.

The management of T2D in children and adolescents presents a real challenge due to the poor adherence to the management recommendations of this age group and the difficulty in reversing obesity[128]; moreover, many of the young T2D patients are asymptomatic. Thus, it is important to screen those at risk. The ADA guidelines recommend screening children starting age 10 years or earlier (in case of an earlier onset of puberty) for all obese or overweight children if they have one additional risk factor[128]. The management for T2D in children and adolescents should include reduction of 7%-10% of the excess weight, physical activity for at least 60 min/d, and a healthy balanced diet rich in nutrients. Moreover, optimal blood pressure control and annual screening for neuropathy and retinopathy are crucial to prevent and manage complications[128]. All of these should be accompanied by educating patients and their parents about self-management including self-monitoring of blood glucose. We could not identify any studies from the MENA on the management of T2D in this age group.

CHALLENGES

In brief, the MENA region has the highest prevalence of T2D, and the rates continue to rise steeply. Furthermore, it has one of the highest diabetes related disability-adjusted life years and mortality rates from NCDs. Modifiable risk factors for both diabetes and its complications are highly prevalent in this population and extend to the pediatric and adolescent age group.

To address and reverse this situation is a daunting yet unavoidable task. Emerging factors add to the challenge, such as the increasing pollution level and environmental degradation, the high level of geopolitical instability, and most recently the COVID-19 pandemic. The latter two have had a direct impact on diabetes care.

Refugees and displaced people

In 2021, the United Nations High Commissioner Report indicated that there are 17.4 million refugees, internally displaced or physically constrained people in the MENA region. These are predominantly from Syria, Iraq, Yemen, and Libya[129]. They constitute about 3% of the MENA population, and 18% of the global population of concern. During the year 2000, 24 million Yemeni people depended on assistance. In parallel, the rates of extreme poverty have doubled in the region, from 3.8% to 7.2% between 2015 and 2018, and are likely to rise further due to the financial strain of the COVID-19 pandemic[130]. Furthermore, in 2020 the MENA region was deemed the 'least peaceful' area in the world with its many armed conflicts and/or political instability[131].

The above factors negatively influence NCDs in general and diabetes in particular. They also weaken the public healthcare system. The optimal management of diabetes requires a patient-centered approach with continuity of care[132]. Instead, in an unstable setting, the care is typically fragmented and interrupted, and the health of refugees and of displaced civilians is very likely to suffer. Unfortunately, very few national studies address the health status of refugees, so the prevalence of diabetes complications may be underestimated.

One such study was conducted in Lebanon on both the Lebanese population and Syrian refugees, as part of the STEPwise approach[133]. Out of 1899 Lebanese and 2134 Syrian adults aged 18-69 years, 51.2% of Lebanese had three or more cardiovascular risk factors *vs* 59.8% of Syrians. A 2014 survey by the United Nations High Commissioner Report found that close to 15% of adult Syrian refugees had at least one chronic condition, with 16% of those having diabetes, half of whom reported difficulties accessing health services[13]. With social instability and lack of security in parts of other MENA countries, it is expected that similar conditions may be prevalent [14]. The situation is likely to be complicated by poverty, food insecurity, poor nutrition, and inability to access health care. A concerted effort by the international community and governmental health agencies is needed to assist vulnerable people and provide them with access to medical care.

COVID-19 and diabetes

The COVID-19 pandemic has stressed the healthcare system and highlighted the need for a stronger infrastructure. Resources of all countries, even those that are relatively affluent, have been strained. National health systems should invest in interventions that aim at improving diabetes care. This is of paramount importance, in view of the data that shows a worse prognosis and an increased risk of complications and mortality for patients with diabetes hospitalized with COVID-19 infections[134]. A recent report from Qatar suggested that patients with diabetes hospitalized with COVID-19 infection had more severe clinical manifestations of pneumonia and acute respiratory distress syndrome and longer duration of hospitalization, intensive care stay, and mechanical ventilation[135]. Another study from Saudi Arabia reported that during the COVID-19 lockdown, patients with diabetes had reduced compliance with their medications and with healthy lifestyle habits[136]. Moreover, it is likely that many patients with diabetes have had a disrupted outpatient follow-up care due to visit cancellations during the pandemic, a situation that may adversely impact their overall diabetes control.

CONCLUSION

Call for action

There is ample evidence that diabetes can be prevented or delayed and its complications significantly reduced[137]. A recent editorial emphasized that worldwide implementation of diabetes prevention measures is an urgent matter[138]. Our review of T2D in the MENA region provides an opportunity to identify gaps and potential remedies for diabetes-related public health problems.

More accurate and complete data collection is needed for individual countries because available data is frequently scarce, outdated, relies on historical estimates, or is extrapolated from other similar countries. Without reliable data, the magnitude of

the problem can be under/overestimated, and areas of need cannot be properly identified.

In addition, the socioeconomic disparities in the care of diabetes need to be addressed because a lower socioeconomic background frequently emerged as a risk factor for diabetes and for complications. Strategies need to be put in place to address this issue, with interventions that are evidence-based, cost effective, and designed to improve access to care.

Another worrisome finding is the gender difference manifesting as a higher prevalence of obesity and physical inactivity among women compared to men. It calls for an investigation of potential reasons and for implementation of remedial solutions, especially that gestational diabetes carries the risk of generational transmission of metabolic syndrome[139].

Furthermore, there is an urgent need to introduce programs that educate about healthy nutrition choices and promote healthy habits. Proper monitoring of implementation of planned interventions followed by evaluation of their impact is mandatory. Strategies to promote and encourage physical activity are needed, whether through urban planning with introduction of sidewalks and walking trails or through building exercise facilities that are accessible and affordable.

Policies are needed to label food items with their nutritional and caloric content. Educational programs need to be introduced at the school, community, and workplace levels, with emphasis on adapting them to the local cultures and norms. To be effective, educational programs need to be culturally sensitive and tailored to the functional health literacy of the local population[140]. The national media and advertising industry need to be involved in educating the public, in partnership with the national health system. Telehealth may play an important role in terms of raising awareness and providing better access to care[141], especially in view of the geopolitical instability and the COVID-19 pandemic.

In addition, there is a need to promote a public health awareness at the level of primary health care providers for screening and managing diabetes and associated metabolic comorbidities. There is evidence to suggest that quality of care is not optimal[142]. Educational activities need not only address risk factors for diabetes such as obesity and unhealthy lifestyles but also how to manage diabetes and its potential complications and how to implement preventive measures to avoid complications, such as regular eye and foot exams and kidney function tests.

The prevention and management of diabetes should be the perfect prototype of 'health into all policies.' In 2013, the WHO announced the Global Action Plan for the Prevention and Control of Non-Communicable Diseases 2013-2020 aimed at curbing the rise in obesity and diabetes. However, it is evident that not enough has been done so far to achieve this goal, and efforts have fallen short of the magnitude of the problem. Therefore, national strategies need to be put in place to address noncommunicable diseases in general and diabetes in particular, with an emphasis not only on planning but also on implementation. In 2021, the WHO is launching the WHO Global Diabetes Compact, which would enable countries (especially low and middle income) to prevent diabetes by addressing risk factors and help these countries develop their capabilities in identifying and treating people with diabetes[143]. Such programs are urgently needed in the MENA region.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Amy Zenger for her kind and valuable assistance in English revisions.

REFERENCES

1 **World Health Organization**. Diabetes: Key Facts 2020. [cited 27 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/diabetes

2 **International Diabetes Federation**. IDF Diabetes Atlas. 9th ed. [cited 27 Jan 2021]. In: International Diabetes Federation [Internet]. Available from: https://www.diabetesatlas.org

3 **Moradi-Lakeh M**, Forouzanfar MH, El Bcheraoui C, Daoud F, Afshin A, Hanson SW, Vos T, Naghavi M, Murray CJ, Mokdad AH; Global Burden of Disease Collaborators on Eastern Mediterranean Region and Diabetes. High Fasting Plasma Glucose, Diabetes, and Its Risk Factors in the Eastern Mediterranean Region, 1990-2013: Findings From the Global Burden of Disease Study 2013. *Diabetes Care* 2017; **40**: 22-29 [PMID: 27797926 DOI: 10.2337/dc16-1075]

4 **Saeedi P**, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, Unwin N, Wild SH, Williams R. Mortality attributable to diabetes in 20-79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2020; **162**: 108086 [PMID: 32068099 DOI: 10.1016/j.diabres.2020.108086]

5 **Azizi F**, Hadaegh F, Hosseinpanah F, Mirmiran P, Amouzegar A, Abdi H, Asghari G, Parizadeh D, Montazeri SA, Lotfaliany M, Takyar F, Khalili D. Metabolic health in the Middle East and north Africa. *Lancet Diabetes Endocrinol* 2019; **7**: 866-879 [PMID: 31422063 DOI: 10.1016/S2213-8587(19)30179-2]

6 **MENARA Project**. European policy brief. [cited 27 Jan 2021]. In: Istituto Affari Internazionali [Internet]. Available from: https://www.iai.it/sites/default/files/menara_pb_2.pdf

7 **Abuhendi N**, Qush A, Naji F, Abunada H, Al Buainain R, Shi Z, Zayed H. Genetic polymorphisms associated with type 2 diabetes in the Arab world: A systematic review and meta-analysis. *Diabetes Res Clin Pract* 2019; **151**: 198-208 [PMID: 30954515 DOI: 10.1016/j.diabres.2019.03.037]

8 **Bener A**, Mohammad RR. Global distribution of consanguinity and their impact on complex diseases: Genetic disorders from an endogamous population. *Egypt J Med Human Genet* 2017; **18**: 315-320 [DOI: 10.1016/j.ejmhg.2017.01.002]

9 **Musambil M**, Siddiqui K. Genetics and genomics studies in type 2 diabetes: A brief review of the current scenario in the Arab region. *Diabetes Metab Syndr* 2019; **13**: 1629-1632 [PMID: 31336532 DOI: 10.1016/j.dsx.2019.03.017]

10 **Nathan DM**, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, Zinman B; American Diabetes Association. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes Care* 2007; **30**: 753-759 [PMID: 17327355 DOI: 10.2337/dc07-9920]

11 **United Nations**. Leaders Gather at UN Headquarters for a High-Level Meeting on Non-communicable Diseases (NCDs). [cited 26 Jan 2021]. In: United Nations [Internet]. Available from: https://www.un.org/en/ga/ncdmeeting2011

12 **World Health Organization**. Diabetes Programme: Diabetes Action Now 2021. [cited 26 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/diabetes/actionnow/en/

13 **Khan Y**, Albache N, Almasri I, Gabbay RA. The Management of Diabetes in Conflict Settings: Focus on the Syrian Crisis. *Diabetes Spectr* 2019; **32**: 264-269 [PMID: 31462883 DOI: 10.2337/ds18-0070]

14 **Qirbi N**, Ismail SA. Health system functionality in a low-income country in the midst of conflict: the case of Yemen. *Health Policy Plan* 2017; **32**: 911-922 [PMID: 28402469 DOI: 10.1093/heapol/czx031]

15 **American Diabetes Association**. Understanding A1C. [cited 27 Jan 2021]. In: American Diabetes Association [Internet]. Available from: https://www.diabetes.org/a1c/diagnosis

16 **International Diabetes Federation**. Insulin at 100. [cited 27 Jan 2021]. In: International Diabetes Federation [Internet]. Available from: https://idf.org/

17 **World Health Organization**. Raised fasting blood glucose (>=7.0 mmol/L or on medication)(age-standardized estimate). [cited 27 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/data/gho/data/indicators/indicator-details/GHO/raised-fasting-blood-glucose-(-=7-0-mmol-l-or-on-medication)(age-standardized-estimate)

18 **Shaw JE**, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]

19 **Al-Rubeaan K**, Al-Manaa HA, Khoja TA, Ahmad NA, Al-Sharqawi AH, Siddiqui K, Alnaqeb D, Aburisheh KH, Youssef AM, Al-Batel A, Alotaibi MS, Al-Gamdi AA. Epidemiology of abnormal glucose metabolism in a country facing its epidemic: SAUDI-DM study. *J Diabetes* 2015; **7**: 622-632 [PMID: 25266306 DOI: 10.1111/1753-0407.12224]

20 **Aamir AH**, Ul-Haq Z, Mahar SA, Qureshi FM, Ahmad I, Jawa A, Sheikh A, Raza A, Fazid S, Jadoon Z, Ishtiaq O, Safdar N, Afridi H, Heald AH. Diabetes Prevalence Survey of Pakistan (DPS-PAK): prevalence of type 2 diabetes mellitus and prediabetes using HbA1c: a population-based survey from Pakistan. *BMJ Open* 2019; **9**: e025300 [PMID: 30796126 DOI: 10.1136/bmjopen-2018-025300]

21 **Ajlouni K**, Batieha A, Jaddou H, Khader Y, Abdo N, El-Khateeb M, Hyassat D, Al-Louzi D. Time trends in diabetes mellitus in Jordan between 1994 and 2017. *Diabet Med* 2019; **36**: 1176-1182 [PMID: 30614070 DOI: 10.1111/dme.13894]

22 **Khedr EM**, Fawi G, Allah Abbas MA, El-Fetoh NA, Al Attar G, Zaki AF, Gamea A. Prevalence of Diabetes and Diabetic Neuropathy in Qena Governorate: Population-Based Survey. *Neuroepidemiology* 2016; **46**: 173-181 [PMID: 26849453 DOI: 10.1159/000444056]

23 **Ben Romdhane H**, Ben Ali S, Aissi W, Traissac P, Aounallah-Skhiri H, Bougatef S, Maire B, Delpeuch F, Achour N. Prevalence of diabetes in Northern African countries: the case of Tunisia. *BMC Public Health* 2014; **14**: 86 [PMID: 24472619 DOI: 10.1186/1471-2458-14-86]

24 **Esteghamati A**, Larijani B, Aghajani MH, Ghaemi F, Kermanchi J, Shahrami A, Saadat M, Esfahani EN, Ganji M, Noshad S, Khajeh E, Ghajar A, Heidari B, Afarideh M, Mechanick JI, Ismail-Beigi F. Diabetes in Iran: Prospective Analysis from First Nationwide Diabetes Report of National Program for Prevention and Control of Diabetes (NPPCD-2016). *Sci Rep* 2017; **7**: 13461 [PMID: 29044139 DOI: 10.1038/s41598-017-13379-z]

25 **Mirzaei M**, Rahmaninan M, Mirzaei M, Nadjarzadeh A, Dehghani Tafti AA. Epidemiology of diabetes mellitus, pre-diabetes, undiagnosed and uncontrolled diabetes in Central Iran: results from

Yazd health study. *BMC Public Health* 2020; **20**: 166 [PMID: 32013917 DOI: 10.1186/s12889-020-8267-y]

26 **Akbarzadeh A**, Salehi A, Molavi Vardanjani H, Poustchi H, Gandomkar A, Fattahi MR, Malekzadeh R. Epidemiology of Adult Diabetes Mellitus and its Correlates in Pars Cohort Study in Southern Iran. *Arch Iran Med* 2019; **22**: 633-639 [PMID: 31823628]

27 **Latifi SM**, Karandish M, Shahbazian H, Hardani Pasand L. Incidence of Prediabetes and Type 2 Diabetes among People Aged over 20 Years in Ahvaz: A 5-Year Perspective Study (2009-2014). *J Diabetes Res* 2016; **2016**: 4908647 [PMID: 28004008 DOI: 10.1155/2016/4908647]

28 **Costanian C**, Bennett K, Hwalla N, Assaad S, Sibai AM. Prevalence, correlates and management of type 2 diabetes mellitus in Lebanon: findings from a national population-based study. *Diabetes Res Clin Pract* 2014; **105**: 408-415 [PMID: 25005850 DOI: 10.1016/j.diabres.2014.06.005]

29 **Nasrallah MP**, Nakhoul NF, Nasreddine L, Mouneimne Y, Abiad MG, Ismaeel H, Tamim H. PREVALENCE OF DIABETES IN GREATER BEIRUT AREA: WORSENING OVER TIME. *Endocr Pract* 2017; **23**: 1091-1100 [PMID: 28683240 DOI: 10.4158/EP171876.OR]

30 **Albache N**, Al Ali R, Rastam S, Fouad FM, Mzayek F, Maziak W. Epidemiology of Type 2 diabetes mellitus in Aleppo, Syria. *J Diabetes* 2010; **2**: 85-91 [PMID: 20923489 DOI: 10.1111/j.1753-0407.2009.00063.x]

31 **International Diabetes Federation**. IDF Diabetes Atlas 8th ed. [cited 27 Jan 2021]. In: International Diabetes Federation [Internet]. Available from: https://www.diabetesatlas.org/upload/resources/previous/files/8/IDF_DA_8e-EN-final.pdf

32 **Ghafuri S**, Ghaderi E, Fahami Y, Rajabnia M, Naleini SN. Epidemiologic study of type 2 diabetes mellitus and metabolic syndrome in rural population of kurdistan province, Iran, in 2011-2017. *Diabetes Metab Syndr* 2019; **13**: 1689-1697 [PMID: 31235080 DOI: 10.1016/j.dsx.2019.03.037]

33 **Al-Daghri NM**, Al-Attas OS, Alokail MS, Alkharfy KM, Yousef M, Sabico SL, Chrousos GP. Diabetes mellitus type 2 and other chronic non-communicable diseases in the central region, Saudi Arabia (Riyadh cohort 2): a decade of an epidemic. *BMC Med* 2011; **9**: 76 [PMID: 21689399 DOI: 10.1186/1741-7015-9-76]

34 **Basit A**, Fawwad A, Qureshi H, Shera AS; NDSP Members. Prevalence of diabetes, pre-diabetes and associated risk factors: second National Diabetes Survey of Pakistan (NDSP), 2016-2017. *BMJ Open* 2018; **8**: e020961 [PMID: 30082350 DOI: 10.1136/bmjopen-2017-020961]

35 **Al Khalaf MM**, Eid MM, Najjar HA, Alhajry KM, Doi SA, Thalib L. Screening for diabetes in Kuwait and evaluation of risk scores. *East Mediterr Health J* 2010; **16**: 725-731 [PMID: 20799528]

36 **Tuomilehto J**, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/nejm200105033441801]

37 **Richter B**, Hemmingsen B, Metzendorf MI, Takwoingi Y. Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. *Cochrane Database Syst Rev* 2018; **10**: CD012661 [PMID: 30371961 DOI: 10.1002/14651858.CD012661.pub2]

38 **Mansour AA**, Al-Maliky AA, Kasem B, Jabar A, Mosbeh KA. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. *Diabetes Metab Syndr Obes* 2014; **7**: 139-144 [PMID: 24833912 DOI: 10.2147/DMSO.S59652]

39 **Saadi H**, Carruthers SG, Nagelkerke N, Al-Maskari F, Afandi B, Reed R, Lukic M, Nicholls MG, Kazam E, Algawi K, Al-Kaabi J, Leduc C, Sabri S, El-Sadig M, Elkhumaidi S, Agarwal M, Benedict S. Prevalence of diabetes mellitus and its complications in a population-based sample in Al Ain, United Arab Emirates. *Diabetes Res Clin Pract* 2007; **78**: 369-377 [PMID: 17532085 DOI: 10.1016/j.diabres.2007.04.008]

40 **Alkandari A**, Longenecker JC, Barengo NC, Alkhatib A, Weiderpass E, Al-Wotayan R, Al Duwairi Q, Tuomilehto J. The prevalence of pre-diabetes and diabetes in the Kuwaiti adult population in 2014. *Diabetes Res Clin Pract* 2018; **144**: 213-223 [PMID: 30179683 DOI: 10.1016/j.diabres.2018.08.016]

41 **Esteghamati A**, Etemad K, Koohpayehzadeh J, Abbasi M, Meysamie A, Noshad S, Asgari F, Mousavizadeh M, Rafei A, Khajeh E, Neishaboury M, Sheikhbahaei S, Nakhjavani M. Trends in the prevalence of diabetes and impaired fasting glucose in association with obesity in Iran: 2005-2011. *Diabetes Res Clin Pract* 2014; **103**: 319-327 [PMID: 24447808 DOI: 10.1016/j.diabres.2013.12.034]

42 **Bener A**, Zirie M, Janahi IM, Al-Hamaq AO, Musallam M, Wareham NJ. Prevalence of diagnosed and undiagnosed diabetes mellitus and its risk factors in a population-based study of Qatar. *Diabetes Res Clin Pract* 2009; **84**: 99-106 [PMID: 19261345 DOI: 10.1016/j.diabres.2009.02.003]

43 **Gunaid AA**, Assabri AM. Prevalence of type 2 diabetes and other cardiovascular risk factors in a semirural area in Yemen. *East Mediterr Health J* 2008; **14**: 42-56 [PMID: 18557451]

44 **Al-Lawati JA**, Al Riyami AM, Mohammed AJ, Jousilahti P. Increasing prevalence of diabetes mellitus in Oman. *Diabet Med* 2002; **19**: 954-957 [PMID: 12421434 DOI: 10.1046/j.1464-5491.2002.00818.x]

45 **Bouguerra R**, Alberti H, Salem LB, Rayana CB, Atti JE, Gaigi S, Slama CB, Zouari B, Alberti K. The global diabetes pandemic: the Tunisian experience. *Eur J Clin Nutr* 2007; **61**: 160-165 [PMID: 16900086 DOI: 10.1038/sj.ejcn.1602478]

46 **Khattab A**, Javaid A, Iraqi G, Alzaabi A, Ben Kheder A, Koniski ML, Shahrour N, Tarigh S, Idrees M, Polatli M, Rashid N, El Hasnaoui A; BREATHE Study Group. Smoking habits in the Middle

East and North Africa: results of the BREATHE study. *Respir Med* 2012; **106** Suppl 2: S16-S24 [PMID: 23290700 DOI: 10.1016/S0954-6111(12)70011-2]

47 **Meo SA**, Memon AN, Sheikh SA, Rouq FA, Usmani AM, Hassan A, Arian SA. Effect of environmental air pollution on type 2 diabetes mellitus. *Eur Rev Med Pharmacol Sci* 2015; **19**: 123-128 [PMID: 25635985]

48 **Lonardo A**, Adinolfi LE, Petta S, Craxì A, Loria P. Hepatitis C and diabetes: the inevitable coincidence? *Expert Rev Anti Infect Ther* 2009; **7**: 293-308 [PMID: 19344243 DOI: 10.1586/eri.09.3]

49 **Gomaa A**, Allam N, Elsharkawy A, El Kassas M, Waked I. Hepatitis C infection in Egypt: prevalence, impact and management strategies. *Hepat Med* 2017; **9**: 17-25 [PMID: 28553150 DOI: 10.2147/HMER.S113681]

50 **Agardh E**, Allebeck P, Hallqvist J, Moradi T, Sidorchuk A. Type 2 diabetes incidence and socio-economic position: a systematic review and meta-analysis. *Int J Epidemiol* 2011; **40**: 804-818 [PMID: 21335614 DOI: 10.1093/ije/dyr029]

51 **Florez JC**, Hirschhorn J, Altshuler D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. *Annu Rev Genomics Hum Genet* 2003; **4**: 257-291 [PMID: 14527304 DOI: 10.1146/annurev.genom.4.070802.110436]

52 **Mousa HS**, Yousef S, Riccardo F, Zeidan W, Sabatinelli G. Hyperglycaemia, hypertension and their risk factors among Palestine refugees served by UNRWA. *East Mediterr Health J* 2010; **16**: 609-614 [PMID: 20799587]

53 **Harati H**, Hadaegh F, Saadat N, Azizi F. Population-based incidence of Type 2 diabetes and its associated risk factors: results from a six-year cohort study in Iran. *BMC Public Health* 2009; **9**: 186 [PMID: 19531260 DOI: 10.1186/1471-2458-9-186]

54 **Ereqat S**, Nasereddin A, Cauchi S, Azmi K, Abdeen Z, Amin R. Association of a common variant in TCF7L2 gene with type 2 diabetes mellitus in the Palestinian population. *Acta Diabetol* 2010; **47** Suppl 1: 195-198 [PMID: 19885641 DOI: 10.1007/s00592-009-0161-0]

55 **Ghassibe-Sabbagh M**, Haber M, Salloum AK, Al-Sarraj Y, Akle Y, Hirbli K, Romanos J, Mouzaya F, Gauguier D, Platt DE, El-Shanti H, Zalloua PA. T2DM GWAS in the Lebanese population confirms the role of TCF7L2 and CDKAL1 in disease susceptibility. *Sci Rep* 2014; **4**: 7351 [PMID: 25483131 DOI: 10.1038/srep07351]

56 **Osman W**, Tay GK, Alsafar H. Multiple genetic variations confer risks for obesity and type 2 diabetes mellitus in arab descendants from UAE. *Int J Obes (Lond)* 2018; **42**: 1345-1353 [PMID: 29717269 DOI: 10.1038/s41366-018-0057-6]

57 **El-Lebedy D**, Ashmawy I. Common variants in TCF7L2 and CDKAL1 genes and risk of type 2 diabetes mellitus in Egyptians. *J Genet Eng Biotechnol* 2016; **14**: 247-251 [PMID: 30647622 DOI: 10.1016/j.jgeb.2016.10.004]

58 **O'Beirne SL**, Salit J, Rodriguez-Flores JL, Staudt MR, Abi Khalil C, Fakhro KA, Robay A, Ramstetter MD, Al-Azwani IK, Malek JA, Zirie M, Jayyousi A, Badii R, Al-Nabet Al-Marri A, Chiuchiolo MJ, Al-Shakaki A, Chidiac O, Gharbiah M, Bener A, Stadler D, Hackett NR, Mezey JG, Crystal RG. Type 2 Diabetes Risk Allele Loci in the Qatari Population. *PLoS One* 2016; **11**: e0156834 [PMID: 27383215 DOI: 10.1371/journal.pone.0156834]

59 **Mtiraoui N**, Turki A, Nemr R, Echtay A, Izzidi I, Al-Zaben GS, Irani-Hakime N, Keleshian SH, Mahjoub T, Almawi WY. Contribution of common variants of ENPP1, IGF2BP2, KCNJ11, MLXIPL, PPARγ, SLC30A8 and TCF7L2 to the risk of type 2 diabetes in Lebanese and Tunisian Arabs. *Diabetes Metab* 2012; **38**: 444-449 [PMID: 22749234 DOI: 10.1016/j.diabet.2012.05.002]

60 **Almawi WY**, Nemr R, Keleshian SH, Echtay A, Saldanha FL, AlDoseri FA, Racoubian E. A replication study of 19 GWAS-validated type 2 diabetes at-risk variants in the Lebanese population. *Diabetes Res Clin Pract* 2013; **102**: 117-122 [PMID: 24145053 DOI: 10.1016/j.diabres.2013.09.001]

61 **Popkin BM**. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. *Am J Clin Nutr* 2006; **84**: 289-298 [PMID: 16895874 DOI: 10.1093/ajcn/84.1.289]

62 **Mehio Sibai A**, Nasreddine L, Mokdad AH, Adra N, Tabet M, Hwalla N. Nutrition transition and cardiovascular disease risk factors in Middle East and North Africa countries: reviewing the evidence. *Ann Nutr Metab* 2010; **57**: 193-203 [PMID: 21088386 DOI: 10.1159/000321527]

63 **Alhabib KF**, Batais MA, Almigbal TH, Alshamiri MQ, Altaradi H, Rangarajan S, Yusuf S. Demographic, behavioral, and cardiovascular disease risk factors in the Saudi population: results from the Prospective Urban Rural Epidemiology study (PURE-Saudi). *BMC Public Health* 2020; **20**: 1213 [PMID: 32770968 DOI: 10.1186/s12889-020-09298-w]

64 **Nasreddine L**, Naja FA, Sibai AM, Helou K, Adra N, Hwalla N. Trends in nutritional intakes and nutrition-related cardiovascular disease risk factors in Lebanon: the need for immediate action. *J Med Liban* 2014; **62**: 83-91 [PMID: 25011369 DOI: 10.12816/0004102]

65 **Nasreddine L**, Tamim H, Itani L, Nasrallah MP, Isma'eel H, Nakhoul NF, Abou-Rizk J, Naja F. A minimally processed dietary pattern is associated with lower odds of metabolic syndrome among Lebanese adults. *Public Health Nutr* 2018; **21**: 160-171 [PMID: 28965534 DOI: 10.1017/S1368980017002130]

66 **World Health Organization**. Global Recommendations on Physical Activity for Health. [cited 25 Jan 2021]. In: World Health Organization [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/44399/9789241599979_eng.pdf

67 **Sherif S**, Sumpio BE. Economic development and diabetes prevalence in MENA countries: Egypt

and Saudi Arabia comparison. *World J Diabetes* 2015; **6**: 304-311 [PMID: 25789111 DOI: 10.4239/wjd.v6.i2.304]

68 **Al-Nozha MM**, Al-Hazzaa HM, Arafah MR, Al-Khadra A, Al-Mazrou YY, Al-Maatouq MA, Khan NB, Al-Marzouki K, Al-Harthi SS, Abdullah M, Al-Shahid MS. Prevalence of physical activity and inactivity among Saudis aged 30-70 years. A population-based cross-sectional study. *Saudi Med J* 2007; **28**: 559-568 [PMID: 17457478]

69 **Chan JM**, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994; **17**: 961-969 [PMID: 7988316 DOI: 10.2337/diacare.17.9.961]

70 **World Health Organization**. Noncommunicable Diseases. [cited 26 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/health-topics/noncommunicable-diseases

71 **World Health Organization**. The top 10 causes of death. [cited 26 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

72 **Yusuf S**, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; **364**: 937-952 [PMID: 15364185 DOI: 10.1016/S0140-6736(04)17018-9]

73 **Selvin E**, Juraschek SP. Diabetes Epidemiology in the COVID-19 Pandemic. *Diabetes Care* 2020; **43**: 1690-1694 [PMID: 32540920 DOI: 10.2337/dc20-1295]

74 **World Health Organization**. Global Report on Diabetes. [cited 25 Jan 2021]. In: World Health Organization [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf

75 **World Health Organization**. Noncommunicable diseases: Mortality. [cited 25 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/ncd-mortality

76 **Anjana RM**, Mohan V, Rangarajan S, Gerstein HC, Venkatesan U, Sheridan P, Dagenais GR, Lear SA, Teo K, Karsidag K, Alhabib KF, Yusoff K, Ismail N, Mony PK, Lopez-Jaramillo P, Chifamba J, Palileo-Villanueva LM, Iqbal R, Yusufali A, Kruger IM, Rosengren A, Bahonar A, Zatonska K, Yeates K, Gupta R, Li W, Hu L, Rahman MO, Lakshmi PVM, Iype T, Avezum A, Diaz R, Lanas F, Yusuf S. Contrasting Associations Between Diabetes and Cardiovascular Mortality Rates in Low-, Middle-, and High-Income Countries: Cohort Study Data From 143,567 Individuals in 21 Countries in the PURE Study. *Diabetes Care* 2020; **43**: 3094-3101 [PMID: 33060076 DOI: 10.2337/dc20-0886]

77 **GBD 2015 Eastern Mediterranean Region Diabetes and Chronic Kidney Disease Collaborators**. Diabetes mellitus and chronic kidney disease in the Eastern Mediterranean Region: findings from the Global Burden of Disease 2015 study. *Int J Public Health* 2018; 177-186 [PMID: 28776240 DOI: 10.1007/s00038-017-1014-1]

78 **Emerging Risk Factors Collaboration**, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK, Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; **375**: 2215-2222 [PMID: 20609967 DOI: 10.1016/S0140-6736(10)60484-9]

79 **Robert AA**, Al Dawish MA. Cardiovascular Disease among Patients with Diabetes: The Current Scenario in Saudi Arabia. *Curr Diabetes Rev* 2021; **17**: 180-185 [PMID: 32459609 DOI: 10.2174/1573399816666200527135512]

80 **Afsharian S**, Akbarpour S, Abdi H, Sheikholeslami F, Moeini AS, Khalili D, Momenan AA, Azizi F, Hadaegh F. Risk factors for cardiovascular disease and mortality events in adults with type 2 diabetes - a 10-year follow-up: Tehran Lipid and Glucose Study. *Diabetes Metab Res Rev* 2016; **32**: 596-606 [PMID: 26787367 DOI: 10.1002/dmrr.2776]

81 **Abu Al-Halaweh A**, Davidovitch N, Almdal TP, Cowan A, Khatib S, Nasser-Eddin L, Baradia Z. Prevalence of type 2 diabetes mellitus complications among palestinians with T2DM. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S783-S787 [PMID: 28599963 DOI: 10.1016/j.dsx.2017.05.017]

82 **Maziak W**, Nakkash R, Bahelah R, Husseini A, Fanous N, Eissenberg T. Tobacco in the Arab world: old and new epidemics amidst policy paralysis. *Health Policy Plan* 2014; **29**: 784-794 [PMID: 23958628 DOI: 10.1093/heapol/czt055]

83 **Jelwan YA**, Asbeutah AAA, Welty FK. Comprehensive Review of Cardiovascular Diseases, Diabetes, and Hypercholesterolemia in Lebanon. *Cardiol Rev* 2020; **28**: 73-83 [PMID: 31985525 DOI: 10.1097/CRD.0000000000000290]

84 **Al Rasadi K**, Almahmeed W, AlHabib KF, Abifadel M, Farhan HA, AlSifri S, Jambart S, Zubaid M, Awan Z, Al-Waili K, Barter P. Dyslipidaemia in the Middle East: Current status and a call for action. *Atherosclerosis* 2016; **252**: 182-187 [PMID: 27522462 DOI: 10.1016/j.atherosclerosis.2016.07.925]

85 **Alrawahi AH**, Lee P. Validation of the cardiovascular risk model developed for Omanis with type 2 diabetes. *Diabetes Metab Syndr* 2018; **12**: 387-391 [PMID: 29397365 DOI: 10.1016/j.dsx.2018.01.004]

86 **Boulton AJ**, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot

disease. *Lancet* 2005; **366**: 1719-1724 [PMID: 16291066 DOI: 10.1016/S0140-6736(05)67698-2]
87 **Mairghani M**, Elmusharaf K, Patton D, Burns J, Eltahir O, Jassim G, Moore Z. The prevalence and incidence of diabetic foot ulcers among five countries in the Arab world: a systematic review. *J Wound Care* 2017; **26**: S27-S34 [PMID: 28880754 DOI: 10.12968/jowc.2017.26.Sup9.S27]
88 **Assaad-Khalil SH**, Zaki A, Abdel Rehim A, Megallaa MH, Gaber N, Gamal H, Rohoma KH. Prevalence of diabetic foot disorders and related risk factors among Egyptian subjects with diabetes. *Prim Care Diabetes* 2015; **9**: 297-303 [PMID: 25543864 DOI: 10.1016/j.pcd.2014.10.010]
89 **Al-Rubeaan K**, Al Derwish M, Ouizi S, Youssef AM, Subhani SN, Ibrahim HM, Alamri BN. Diabetic foot complications and their risk factors from a large retrospective cohort study. *PLoS One* 2015; **10**: e0124446 [PMID: 25946144 DOI: 10.1371/journal.pone.0124446]
90 **Akram J**, Aamir AU, Basit A, Qureshi MS, Mehmood T, Shahid SK, Khoso IA, Ebrahim MA, Omair A. Prevalence of peripheral arterial disease in type 2 diabetics in Pakistan. *J Pak Med Assoc* 2011; **61**: 644-648 [PMID: 22204237]
91 **Ababneh A**, Bakri FG, Khader Y, Lazzarini P, Ajlouni K. Prevalence and Associates of Foot Deformities among Patients with Diabetes in Jordan. *Curr Diabetes Rev* 2020; **16**: 471-482 [PMID: 31573891 DOI: 10.2174/1573399815666191001101910]
92 **Yazdanpanah L**, Shahbazian H, Nazari I, Arti HR, Ahmadi F, Mohammadianinejad SE, Cheraghian B, Latifi SM. Prevalence and related risk factors of diabetic foot ulcer in Ahvaz, south west of Iran. *Diabetes Metab Syndr* 2018; **12**: 519-524 [PMID: 29602761 DOI: 10.1016/j.dsx.2018.03.018]
93 **AlAyed MY**, Younes N, Al-Smady M, Khader YS, Robert AA, Ajlouni K. Prevalence of Foot Ulcers, Foot at Risk and Associated Risk Factors Among Jordanian Diabetics. *Curr Diabetes Rev* 2017; **13**: 182-191 [PMID: 26652612 DOI: 10.2174/1573399812666151210143140]
94 **Al-Rubeaan K**, Almashouq MK, Youssef AM, Al-Qumaidi H, Al Derwish M, Ouizi S, Al-Shehri K, Masoodi SN. All-cause mortality among diabetic foot patients and related risk factors in Saudi Arabia. *PLoS One* 2017; **12**: e0188097 [PMID: 29176889 DOI: 10.1371/journal.pone.0188097]
95 **Yau JW**, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; **35**: 556-564 [PMID: 22301125 DOI: 10.2337/dc11-1909]
96 **Arej N**, Antoun J, Waked R, Saab C, Saleh M, Waked N. [Screening for diabetic retinopathy by non-mydriatic fundus photography: First national campaign in Lebanon]. *J Fr Ophtalmol* 2019; **42**: 288-294 [PMID: 30857804 DOI: 10.1016/j.jfo.2018.12.005]
97 **Elshafei M**, Gamra H, Khandekar R, Al Hashimi M, Pai A, Ahmed MF. Prevalence and determinants of diabetic retinopathy among persons ≥ 40 years of age with diabetes in Qatar: a community-based survey. *Eur J Ophthalmol* 2011; **21**: 39-47 [PMID: 20602322 DOI: 10.5301/ejo.2010.2699]
98 **Heydari B**, Yaghoubi G, Yaghoubi MA, Miri MR. Prevalence and risk factors for diabetic retinopathy: an Iranian eye study. *Eur J Ophthalmol* 2012; **22**: 393-397 [PMID: 21928255 DOI: 10.5301/ejo.5000044]
99 **Uddin F**, Ali B, Junaid N. Prevalence Of Diabetic Complications In Newly Diagnosed Type 2 Diabetes Patients In Pakistan: Findings From National Registry. *J Ayub Med Coll Abbottabad* 2018; **30(Suppl** 1): S652-S658 [PMID: 30838825]
100 **Al-Rubeaan K**, Abu El-Asrar AM, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Alguwaihes A, Alotaibi MS, Al-Ghamdi A, Ibrahim HM. Diabetic retinopathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi National Diabetes Registry-based study. *Acta Ophthalmol* 2015; **93**: e140-e147 [PMID: 25270515 DOI: 10.1111/aos.12532]
101 **Macky TA**, Khater N, Al-Zamil MA, El Fishawy H, Soliman MM. Epidemiology of diabetic retinopathy in Egypt: a hospital-based study. *Ophthalmic Res* 2011; **45**: 73-78 [PMID: 20714195 DOI: 10.1159/000314876]
102 **Kahloun R**, Jelliti B, Zaouali S, Attia S, Ben Yahia S, Resnikoff S, Khairallah M. Prevalence and causes of visual impairment in diabetic patients in Tunisia, North Africa. *Eye (Lond)* 2014; **28**: 986-991 [PMID: 24924439 DOI: 10.1038/eye.2014.131]
103 **Mohammadi M**, Raiegani AAV, Jalali R, Ghobadi A, Salari N. The prevalence of retinopathy among type 2 diabetic patients in Iran: A systematic review and meta-analysis. *Rev Endocr Metab Disord* 2019; **20**: 79-88 [PMID: 30887405 DOI: 10.1007/s11154-019-09490-3]
104 **Jammal H**, Khader Y, Alkhatib S, Abujbara M, Alomari M, Ajlouni K. Diabetic retinopathy in patients with newly diagnosed type 2 diabetes mellitus in Jordan: prevalence and associated factors. *J Diabetes* 2013; **5**: 172-179 [PMID: 23163974 DOI: 10.1111/1753-0407.12015]
105 **Harb W**, Harb G, Chamoun N, Kanbar A, Harb M, Chanbour W. Severity of diabetic retinopathy at the first ophthalmological examination in the Lebanese population. *Ther Adv Ophthalmol* 2018; **10**: 2515841418791950 [PMID: 30140789 DOI: 10.1177/2515841418791950]
106 **Alashek WA**, McIntyre CW, Taal MW. Epidemiology and aetiology of dialysis-treated end-stage kidney disease in Libya. *BMC Nephrol* 2012; **13**: 33 [PMID: 22682181 DOI: 10.1186/1471-2369-13-33]
107 **Al-Rubeaan K**, Siddiqui K, Alghonaim M, Youssef AM, AlNaqeb D. The Saudi Diabetic Kidney Disease study (Saudi-DKD): clinical characteristics and biochemical parameters. *Ann Saudi Med*

2018; **38**: 46-56 [PMID: 29295969 DOI: 10.5144/0256-4947.2018.03.01.1010]
108 **Shahwan MJ**, Gacem SA, Zaidi SK. Prevalence of Diabetic Nephropathy and associated risk factors among type 2 diabetes mellitus patients in Ramallah, Palestine. *Diabetes Metab Syndr* 2019; **13**: 1491-1496 [PMID: 31336511 DOI: 10.1016/j.dsx.2019.02.017]
109 **Ali AA**, Al Lami FH. Prevalence and determinants of microalbuminurea among type 2 diabetes mellitus patients, Baghdad, Iraq, 2013. *Saudi J Kidney Dis Transpl* 2016; **27**: 348-355 [PMID: 26997390 DOI: 10.4103/1319-2442.178561]
110 **Zakkerkish M**, Shahbazian HB, Shahbazian H, Latifi SM, Moravej Aleali A. Albuminuria and its correlates in type 2 diabetic patients. *Iran J Kidney Dis* 2013; **7**: 268-276 [PMID: 23880803]
111 neuropathy. *Nat Rev Dis Primers* 2019; **5**: 42 [PMID: 31197183 DOI: 10.1038/s41572-019-0097-9]
112 **Garoushi S**, Johnson MI, Tashani OA. A cross-sectional study to estimate the point prevalence of painful diabetic neuropathy in Eastern Libya. *BMC Public Health* 2019; **19**: 78 [PMID: 30654784 DOI: 10.1186/s12889-018-6374-9]
113 **Kiani J**, Moghimbeigi A, Azizkhani H, Kosarifard S. The prevalence and associated risk factors of peripheral diabetic neuropathy in Hamedan, Iran. *Arch Iran Med* 2013; **16**: 17-19 [PMID: 23273229]
114 **Ghandour R**, Mikki N, Abu Rmeileh NME, Jerdén L, Norberg M, Eriksson JW, Husseini A. Complications of type 2 diabetes mellitus in Ramallah and al-Bireh: The Palestinian Diabetes Complications and Control Study (PDCCS). *Prim Care Diabetes* 2018; **12**: 547-557 [PMID: 30072279 DOI: 10.1016/j.pcd.2018.07.002]
115 **Chahbi Z**, Lahmar B, Hadri SE, Abainou L, Kaddouri S, Qacif H, Baizri H, Zyani M. The prevalence of painful diabetic neuropathy in 300 Moroccan diabetics. *Pan Afr Med J* 2018; **31**: 158 [PMID: 31086614 DOI: 10.11604/pamj.2018.31.158.14687]
116 **Al-Kandari H**, Al-Abdulrazzaq D, Davidsson L, Sharma P, Al-Tararwa A, Mandani F, Al-Shawaf F, Al-Hussaini F, Qabazard M, Haddad D, Al-Mahdi M, Al-Jasser F, Al-Anzi A, Al-Sanea H, Al-Basari I, Al-Adsani A, Shaltout A, AbdulRasoul M. Incidence of Type 2 Diabetes in Kuwaiti Children and Adolescents: Results From the Childhood-Onset Diabetes Electronic Registry (CODeR). *Front Endocrinol (Lausanne)* 2019; **10**: 836 [PMID: 31866943 DOI: 10.3389/fendo.2019.00836]
117 **Alyafei F**, Soliman A, Alkhalaf F, Sabt A, De Sanctis V, Waseef R, Elsayed N. Incidence of type 1 and type 2 diabetes, between 2012-2016, among children and adolescents in Qatar. *Acta Biomed* 2018; **89**: 7-10 [PMID: 30049926 DOI: 10.23750/abm.v89iS4.7360]
118 **Al-Rubeaan K**. National surveillance for type 1, type 2 diabetes and prediabetes among children and adolescents: a population-based study (SAUDI-DM). *J Epidemiol Community Health* 2015; **69**: 1045-1051 [PMID: 26085648 DOI: 10.1136/jech-2015-205710]
119 **Mirbolouk M**, Derakhshan A, Charkhchi P, Guity K, Azizi F, Hadaegh F. Incidence and predictors of early adulthood pre-diabetes/type 2 diabetes, among Iranian adolescents: the Tehran Lipid and Glucose Study. *Pediatr Diabetes* 2016; **17**: 608-616 [PMID: 26764014 DOI: 10.1111/pedi.12343]
120 **Salameh P**, Barbour B. Pattern of obesity and associated diabetes in Lebanese adolescents: a pilot study. *East Mediterr Health J* 2011; **17**: 226-230 [PMID: 21735963]
121 **Al Amiri E**, Abdullatif M, Abdulle A, Al Bitar N, Afandi EZ, Parish M, Darwiche G. The prevalence, risk factors, and screening measure for prediabetes and diabetes among Emirati overweight/obese children and adolescents. *BMC Public Health* 2015; **15**: 1298 [PMID: 26704130 DOI: 10.1186/s12889-015-2649-6]
122 **Farrag NS**, Cheskin LJ, Farag MK. A systematic review of childhood obesity in the Middle East and North Africa (MENA) region: Health impact and management. *Adv Pediatr Res* 2017; **4** [PMID: 29104941 DOI: 10.12715/apr.2017.4.6]
123 **World Health Organization**. Prevalence of obesity among children and adolescents, BMI>+2 standard deviation above the median, crude Estimates by country, among children aged 5-19 years. [cited 27 Jan 2021]. In: World Health Organization [Internet]. Available from: https://apps.who.int/gho/data/view.main.BMIPLUS2C05-19v
124 **Nasreddine L**, Naja F, Chamieh MC, Adra N, Sibai AM, Hwalla N. Trends in overweight and obesity in Lebanon: evidence from two national cross-sectional surveys (1997 and 2009). *BMC Public Health* 2012; **12**: 798 [PMID: 22984791 DOI: 10.1186/1471-2458-12-798]
125 **Afzal W**. Type 2 diabetes and weight dynamics in young: current view. *J Pak Med Assoc* 2019; **69**: 1234-1236 [PMID: 31511704]
126 **Sharara E**, Akik C, Ghattas H, Makhlouf Obermeyer C. Physical inactivity, gender and culture in Arab countries: a systematic assessment of the literature. *BMC Public Health* 2018; **18**: 639 [PMID: 29776343 DOI: 10.1186/s12889-018-5472-z]
127 **Temneanu OR**, Trandafir LM, Purcarea MR. Type 2 diabetes mellitus in children and adolescents: a relatively new clinical problem within pediatric practice. *J Med Life* 2016; **9**: 235-239 [PMID: 27974926]
128 **American Diabetes Association**. 13. Children and Adolescents: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S163-S182 [PMID: 31862756 DOI: 10.2337/dc20-S013]
129 **United Nations High Commissioner for Refugees**. UNHCR Global appeal 2021 update. [cited 26 Jan 2021]. In: United Nations High Commissioner for Refugees [Internet]. Available from: https://reporting.unhcr.org/sites/default/files/ga2021/pdf/Chapter_MENA.pdf
130 **The World Bank**. MENA: Global Action is Urgently Needed to Reverse Damaging Jumps in Extreme Poverty. [cited 26 Jan 2021]. In: The World Bank [Internet]. Available from:

https://www.worldbank.org/en/news/press-release/2020/10/09/mena-global-action-is-urgently-needed-to-reverse-damaging-jumps-in-extreme-poverty

131 **Global Peace Index**. Global Peace Index 2018. [cited 26 Jan 2021]. In: Wikipedia [Internet]. Available from: https://en.wikipedia.org/wiki/Global_Peace_Index#/media/File:Global_Peace_Index.svg

132 **Wagner EH**, Austin BT, Davis C, Hindmarsh M, Schaefer J, Bonomi A. Improving chronic illness care: translating evidence into action. *Health Aff (Millwood)* 2001; **20**: 64-78 [PMID: 11816692 DOI: 10.1377/hlthaff.20.6.64]

133 **World Health Organization**. WHO stepwise approach for non-communicable diseases risk factor surveillance: Lebanon, 2016-2017. [cited 26 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/ncds/surveillance/steps/Lebanon_STEPS_report_2016-2017.pdf

134 **Zhu L**, She ZG, Cheng X, Qin JJ, Zhang XJ, Cai J, Lei F, Wang H, Xie J, Wang W, Li H, Zhang P, Song X, Chen X, Xiang M, Zhang C, Bai L, Xiang D, Chen MM, Liu Y, Yan Y, Liu M, Mao W, Zou J, Liu L, Chen G, Luo P, Xiao B, Zhang Z, Lu Z, Wang J, Lu H, Xia X, Wang D, Liao X, Peng G, Ye P, Yang J, Yuan Y, Huang X, Guo J, Zhang BH. Association of Blood Glucose Control and Outcomes in Patients with COVID-19 and Pre-existing Type 2 Diabetes. *Cell Metab* 2020; **31**: 1068-1077.e3 [PMID: 32369736 DOI: 10.1016/j.cmet.2020.04.021]

135 **Soliman AT**, Prabhakaran Nair A, Al Masalamani MS, De Sanctis V, Abu Khattab MA, Alsaud AE, Sasi S, Ali EA, Ola A H, Iqbal FM, Nashwan AJ, Fahad J, El Madhoun I, Yassin MA. Prevalence, clinical manifestations, and biochemical data of type 2 diabetes mellitus *vs* nondiabetic symptomatic patients with COVID-19: A comparative study. *Acta Biomed* 2020; **91**: e2020010

136 **Alshareef R**, Al Zahrani A, Alzahrani A, Ghandoura L. Impact of the COVID-19 Lockdown on diabetes patients in Jeddah, Saudi Arabia. *Diabetes Metab Syndr* 2020; **14**: 1583-1587 [PMID: 32947759 DOI: 10.1016/j.dsx.2020.07.051]

137 **Rawshani A**, Rawshani A, Franzén S, Sattar N, Eliasson B, Svensson AM, Zethelius B, Miftaraj M, McGuire DK, Rosengren A, Gudbjörnsdottir S. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2018; **379**: 633-644 [PMID: 30110583 DOI: 10.1056/NEJMoa1800256]

138 **The Lancet**. Turning evidence into action on diabetes. *Lancet* 2020; **396**: 1535 [PMID: 33189162 DOI: 10.1016/S0140-6736(20)32412-0]

139 **Al-Rifai RH**, Majeed M, Qambar MA, Ibrahim A, AlYammahi KM, Aziz F. Type 2 diabetes and pre-diabetes mellitus: a systematic review and meta-analysis of prevalence studies in women of childbearing age in the Middle East and North Africa, 2000-2018. *Syst Rev* 2019; **8**: 268 [PMID: 31703716 DOI: 10.1186/s13643-019-1187-1]

140 **Baker DW**, Parker RM, Williams MV, Clark WS, Nurss J. The relationship of patient reading ability to self-reported health and use of health services. *Am J Public Health* 1997; **87**: 1027-1030 [PMID: 9224190 DOI: 10.2105/ajph.87.6.1027]

141 **Al Alawi E**, Ahmed AA. Screening for diabetic retinopathy: the first telemedicine approach in a primary care setting in Bahrain. *Middle East Afr J Ophthalmol* 2012; **19**: 295-298 [PMID: 22837622 DOI: 10.4103/0974-9233.97928]

142 **Shivashankar R**, Kirk K, Kim WC, Rouse C, Tandon N, Narayan KM, Ali MK. Quality of diabetes care in low- and middle-income Asian and Middle Eastern countries (1993-2012): 20-year systematic review. *Diabetes Res Clin Pract* 2015; **107**: 203-223 [PMID: 25529849 DOI: 10.1016/j.diabres.2014.11.004]

143 **World Health Organization**. WHO announces the Global Diabetes Compact. [cited 26 Jan 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news/item/17-11-2020-who-announces-the-global-diabetes-compact

144 **NCD Risk Factor Collaboration (NCD-RisC)**. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**: 1513-1530 [PMID: 27061677 DOI: 10.1016/S0140-6736(16)00618-8]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1426-1441

DOI: 10.4239/wjd.v12.i9.1426

ISSN 1948-9358 (online)

REVIEW

Dipeptidyl peptidase-4 inhibitor-induced autoimmune diseases: Current evidence

Ayan Roy, Jayaprakash Sahoo, Niya Narayanan, Chandhana Merugu, Sadishkumar Kamalanathan, Dukhabandhu Naik

ORCID number: Ayan Roy 0000-0003-4419-9376; Jayaprakash Sahoo 0000-0002-8805-143X; Niya Narayanan 0000-0001-8403-7756; Chandhana Merugu 0000-0003-1911-0026; Sadishkumar Kamalanathan 0000-0002-2371-0625; Dukhabandhu Naik 0000-0003-4568-877X.

Author contributions: Sahoo J and Kamalanathan S conceptualized the work; Roy A wrote the first draft and provided critical inputs; Narayanan N and Merugu C prepared the first draft of the arthritis and the inflammatory bowel disease sections, respectively; Sahoo J, Naik D, and Kamalanathan S supervised the writing and critically revised the manuscript; Roy A, Sahoo J, and Kamalanathan S edited the final draft; all authors have read and approved the final version of the manuscript.

Conflict-of-interest statement: Dr. Sahoo has nothing to disclose.

Ayan Roy, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur 342005, India

Ayan Roy, Jayaprakash Sahoo, Niya Narayanan, Chandhana Merugu, Sadishkumar Kamalanathan, Dukhabandhu Naik, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

Corresponding author: Jayaprakash Sahoo, MD, DM, Additional Professor, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Room No. 5444, 4th Floor, Super Specialty Block, Puducherry 605006, India. jppgi@yahoo.com

Abstract

Dipeptidyl peptidase-4 inhibitors (DPP-4i) have an important place in the management of type 2 diabetes. The DPP-4 enzyme is ubiquitously distributed throughout the human body and has multiple substrates through which it regulates several important physiological functions. DPP-4 regulates several immune functions, including T-cell activation, macrophage function, and secretion of cytokines. Studies have reported an increase in autoimmune diseases like bullous pemphigoid, inflammatory bowel disease, and arthritis with DPP-4i use. The relationship of DPP-4i and autoimmune diseases is a complex one and warrants further research into the effect of DPP-4 inhibition on the immune system to understand the pathogenesis more clearly. Whether a particular cluster of autoimmune diseases is associated with DPP-4i use remains an important contentious issue. Nevertheless, a heightened awareness from the clinicians is required to identify and treat any such diseases. Through this review, we explore the clinical and pathophysiological characteristics of this association in light of recent evidence.

Key Words: Autoimmune disease; Bullous pemphigoid; Diabetes; Dipeptidyl peptidase-4 inhibitors; Gliptins; Inflammation

Core Tip: Dipeptidyl peptidase-4 (DPP-4) has an important role in the function of the

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: India

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 15, 2021
Peer-review started: February 15, 2021
First decision: March 30, 2021
Revised: April 12, 2021
Accepted: July 19, 2021
Article in press: July 19, 2021
Published online: September 15, 2021

P-Reviewer: Yen FS
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Wang LYT

immune system. DPP-4 inhibitors are an important drug class for the management of type 2 diabetes mellitus. This group of drugs can have a diverse effect on immune modulation. Recently, certain autoimmune diseases are described with the use of DPP-4 inhibitors, particularly bullous pemphigoid. Clinicians should be aware of this association and take appropriate action if such an adverse event takes place.

Citation: Roy A, Sahoo J, Narayanan N, Merugu C, Kamalanathan S, Naik D. Dipeptidyl peptidase-4 inhibitor-induced autoimmune diseases: Current evidence. *World J Diabetes* 2021; 12(9): 1426-1441
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1426.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1426

INTRODUCTION

Dipeptidyl peptidase-4 inhibitors (DPP-4i), also known as gliptins, are being increasingly used as a second-line add-on therapy in diabetes mellitus[1]. They have certain advantages as an oral hypoglycaemic agent like weight neutrality, lesser risk of hypoglycaemia, and insulin independent mechanism of action compared to other medications like sulfonylureas[2]. Sitagliptin, saxagliptin, alogliptin, and linagliptin are Food and Drug Administration (FDA) approved DPP-4is[3]. However, other DPP-4is like teneligliptin, anagliptin, and vildagliptin are also in use in different countries across the globe.

DPP-4, also known as cluster of differentiation 26 (CD26) molecule, is expressed in many tissues and known for its role in diverse physiological functions of the human body. The interaction of the DPP-4 molecule and the immune system is complex. This includes regulation of a various subset of the immune cells including T cells and antigen presenting cells. Therefore, DPP-4i has the potential to modulate various immunological functions. Indeed, the therapeutic role of DPP-4i has been studied in autoimmune diseases (AD) like type 1 diabetes mellitus[4], latent autoimmune diabetes of the adult[5], acute graft *vs* host disease[6], autoimmune encephalomyelitis [7], and multiple sclerosis[8]. However, in recent times several studies have shown an increased risk of certain AD like bullous pemphigoid (BP) among DPP-4i users. Thus, through this review, we summarise the currently available literature in the field of DPP-4i induced AD.

SEARCH STRATEGY

The keywords and combination of keywords for literature search are summarised in the Table 1. The initial literature search was carried out by three authors (AR, NN, and CM) independently in PubMed. The search was performed from the date of inception until January 15, 2021 to find relevant articles. The studies available in the English language were selected for this review. Relevant references in the individual articles were also scrutinised for their suitability and included in this review if found to be appropriate. The studies that evaluated the development of AD in patients treated with DPP-4i were selected by the authors (JS, SK, and DN) and were included in this review. We have given preferences to the most recent studies published in the last 5 years.

THE INTERFACE BETWEEN IMMUNE SYSTEM AND DPP-4 ENZYME

DPP-4 is an enzyme that has a ubiquitous presence throughout the human body. The most important metabolic function is to cleave various gut peptides known as 'incretin hormones' like glucagon-like peptide-1, glucose-dependent insulinotropic peptide, and neuropeptide Y[9]. Incretins have several metabolic benefits like enhanced insulin secretion from the pancreatic beta cells and thus help in controlling blood glucose in subjects with diabetes[10]. DPP-4i prolongs the half-life of different incretins by inhibiting intestinal DPP-4 enzyme activity.

Table 1 List of the keywords used for literature search

No.	
1	Dipeptidyl peptidase 4 inhibitor
2	DPP-4 inhibitor
3	Gliptins
4	'Autoimmune disease'
5	[1] and [4]
6	[2] and [4]
7	[3] and [4]
8	[2] and [3] and [4]
9	Inflammatory bowel disease
10	[1] and [9]
11	Arthritis
12	Arthralgia
13	'Rheumatoid arthritis'
14	[1] and [11]
15	[1] and [12]
16	[1] and [13]

DPP-4: Dipeptidyl peptidase-4.

The details of the role of DPP-4/CD26 in immune system is beyond the scope of this review and many elegant reviews are already there in this area[11,12]. The CD26 molecule, also known as the 'moonlight protein' is a cell surface protein having significant DPP-4 activity. DPP-4 is expressed in several cell lines involved in the pathway of immune regulation. These include T helper cells type 17 (Th17), natural killer cells, activated B cells, macrophages, and myeloid cells[12]. DPP-4 is a transmembrane protein having three parts: a small intracellular part, a transmembrane part carrying the DPP-4 activity, and a large extracellular part[12]. There is also a soluble form of DPP-4 (sDPP-4) that carries a significant amount of enzyme catalytic activity[9]. Recent evidence suggests that circulating lymphocytes are an important source of the sDPP-4[13]. sDPP-4 is used as a biomarker of several diseases and a reduced serum level has been described in rheumatoid arthritis (RA), systemic lupus erythematosus, and psoriasis[12,14]. An elevated sDPP-4 has been shown in type 1 diabetes suggesting its role in the pathogenesis[15].

DPP-4 promotes activation and proliferation of both T cells (Th1, Th17, and regulatory T cells)[16,17], and macrophages[18]. It also has a role in the immunoglobulin synthesis regulation like isotype switching of B cells[19]. Moreover, evidence also suggests that DPP-4 significantly modulates secretion of different cytokines and chemokines, thus regulating tissue response to injury[20,21]. DPP-4i increases stromal derived factor-1 (or CXCL12) levels, which has several pleiotropic effects[22], *e.g.*, beneficial effects in ischemic myocardium[23], diabetic nephropathy[24] and stroke [25]. Interestingly, a recent study demonstrated a decrease in certain chemokines (CCL11/Eotaxin, CCL22/MDC, and CXCL10/IP-10) following a mixed meal test after 6 mo of teneligliptin treatment in diabetes patients[26]. Nevertheless, the exact role of different cytokines/chemokines cleaved by the DPP-4 enzyme in immune regulation remains an unexplored area[27].

Several experimental studies have shown that DPP-4i suppresses various markers of inflammation[28] and/or fibrosis[29] and thus is regarded as an attractive therapeutic option in AD. Several animal research studies have shown the beneficial effects of DPP-4i in obesity-related inflammation[30], hepatic fibrosis[31], myocarditis[32], and diabetic nephropathy[25,26,33], as well as chemotherapy induced renal injury[34] through its immune-modulatory action. Earlier studies also showed the potential beneficial role of DPP-4i in different inflammatory central nervous system disorders, including multiple sclerosis[7,35]. However, several other studies have reported an

increased risk of a few specific AD with DPP-4i use, which are described below.

DPP-4I USE AND RISK OF OVERALL AD

In recent times, several studies have evaluated the association of different DPP-4i with overall AD. However, these studies are limited by the fact that most of them are either retrospective or cross-sectional in nature, and few studies assessed the full autoimmune spectrum. Kridin *et al*[36] from Israel reported that the prevalence of 3 AD (Crohn's disease, psoriasis and Hashimoto's thyroiditis) out of nearly 15 AD was significantly higher in the DPP-4i-treated group as compared to age, gender, and ethnicity-matched diabetes control subjects. This study suggested that a cluster of AD might be associated with DPP-4i treatment. On the other hand, a Japanese study based on the analysis of the adverse drug reaction database showed an increased risk of overall AD in the older age group (> 60 years)[37]. However, a recent population-based study[38] performed in Asian people found that DPP-4i treatment was significantly associated with a reduction in the prevalence of overall AD [adjusted hazard ratio (aHR): 0.56 (95% confidence interval (CI): 0.53–0.60; $P < 0.001$)]. The AD included RA, systemic lupus erythematosus, inflammatory bowel disease (IBD), Sjogren's syndrome, psoriasis, and ankylosing spondylitis. Similar results indicating a lower risk of overall AD had been demonstrated by a few other studies[39,40]. Chen *et al*[38] also showed that the risk of AD was significantly lower in the younger population. This finding signifies the importance of age as an important determining factor of autoimmunity. Clinicians must be vigilant about the risk of different AD in DPP-4i treated patients as summarised in Table 2.

DPP-4I USE AND RISK OF BP

BP is the commonest skin AD associated with DPP-4i use, as described in the literature. However, pemphigus vulgaris has been reported rarely[41] in patients using DPP-4i. BP is a blistering skin condition that occurs commonly in the elderly population. It is an AD characterised by the presence of circulating autoantibodies directed against BP180 and BP230 autoantigens in basal keratinocytes. BP is caused by several drugs and carries a significant risk of mortality[42]. The recent addition to the list of the drugs is DPP-4i. Since 2011, many case reports[43-45] and case series[46-48] have reported the association between the use of DPP-4i and development of BP. In recent times, both observational and retrospective studies[49-54] and adverse drug event-based registries[55,56] have also shown this association (Tables 3 and 4).

Estimating the risk of BP with DPP-4i use

After the initial information obtained from the case reports and case series, adverse drug reaction-based databases have increasingly reported the association of BP with the use of DPP-4i[49,57-59]. Similarly, nation-wide population-based studies[50,60,61] also strengthened this association further as summarised in Table 3. However, it is important to note that most of the pharmacovigilance and adverse database studies have mentioned reporting odds ratio (ROR) to gather early signals of the association between DPP-4i use and BP. However, ROR neither allows to establish any association nor proves causality[62]. Moreover, a few meta-analyses[63,64] tried to sum-up the available data. DPP-4i use in diabetes is associated with both de novo development of BP as well as exacerbation of the already existing BP[65]. It is also important to note that studies have reported increasing diabetes prevalence in BP patients[66], so one should be cautious while prescribing DPP-4i in these patients.

How much is the risk? The answer is not a straightforward one. There is definite evidence that increased risk of BP is a class effect of DPP-4i use, and it varies from molecule to molecule. Studies have reported a 2-3 times risk (as reported by aHR) of developing BP in diabetes patients receiving DPP-4i (Tables 3 and 4). The meta-analysis performed by Phan *et al*[64] on five case-control studies reported overall OR of 2.13 (95%CI: 1.59-2.86) for developing BP in DPP-4i users. Furthermore, a recent meta-analysis including randomized controlled trials (RCTs) as performed by Silverii *et al*[63] reported a Mantel-Haenszel OR of 4.44 (95%CI: 1.31-15.00) for overall DPP-4i use and development of BP. However, the included number of BP cases was low (n = 17), and most of the data came from linagliptin trials, thus drawing conclusions about other DPP-4is was not possible from this study. It also underscores the importance of

Table 2 Summary of the studies that assessed risk of overall autoimmune diseases in dipeptidyl peptidase-4 inhibitor users

Ref.	Population	Study design	Composite outcome	Individual autoimmune disease outcome
Kridin *et al*[36], 2018	T2DM patients receiving DPP-4i (n = 283) *vs* matched controls (n = 5660)	Cross-sectional retrospective study using patient database	OR 1.44 (95%CI: 1.06–1.96) for any disease from the cluster of AD (Crohn's disease, psoriasis, Hashimoto's thyroiditis, MS, ulcerative colitis)	Crohn's disease OR 3.56 (95%CI: 1.04–12.21). Psoriasis OR 2.12 (95%CI: 0.99–4.66). Hashimoto's thyroiditis OR 1.38 (95%CI: 1.00–1.91). No difference in the following ADs: Addison's disease, Arthropathy, Celiac disease, Idiopathic thrombocytopenic Purpura, Myasthenia gravis, Pernicious anaemia, RA, Sarcoidosis, Scleroderma, SLE
Noguchi *et al*[37], 2019	Diabetes patients receiving DPP-4i and other antidiabetic drugs (n = 38887)	Adverse Drug Event Report database analysis	PRR 4.09 for overall autoimmune disease	Increased risk was noted in the following AD: RA, pemphigoid, autoimmune pancreatitis, and polymyalgia rheumatica
Chen *et al*[38], 2020	T2DM patients (age ≥ 20 yr) receiving DPP-4i *vs* non-DPP-4i medications (n = 387099 in each group)	Retrospective cohort study using insurance claim data	HR 0.56 (95%CI: 0.53–0.60) for overall AD like RA, SLE, IBD, Sjogren syndrome, psoriasis and ankylosing spondylitis	RA: HR 0.56 (95%CI: 0.46–0.68). Psoriasis: HR 0.56 (95%CI: 0.52–0.61). Ankylosing spondylitis: HR 0.56 (95%CI: 0.50–0.63). SLE: HR 0.55 (95%CI: 0.35–0.88). IBD: HR 0.66 (95%CI: 0.11–3.95). Sjogren syndrome: HR 0.58 (95%CI: 0.46–0.75)
Kim *et al*[39], 2015	T2DM patients (age ≥ 40 yr) started on DPP-4i as a part of combination therapy (n = 73928) *vs* non-DPP-4i combination therapy (n = 163062)	Cohort study using insurance claim data	HR 0.68 (95%CI: 0.52-0.89) for AD like RA, SLE, psoriasis, psoriatic arthritis, MS and IBD	RA: HR 0.66, (95%CI: 0.44-0.99). Other AD (excluding RA): HR 0.73 (95%CI: 0.51-1.03)
Seong *et al*[40], 2019	New T2DM patients (age ≥ 18 yr) using DPP-4i (n = 497619) or non-DPP-4i (n = 643165) oral combination therapy	Active comparator new-user cohort study	aHR 0.82 (95%CI: 0.68–0.99) for AD like RA, IBD, MS and SLE	RA: aHR 0.67 (95%CI: 0.49–0.92). IBD: aHR 0.81 (95%CI: 0.61-1.08). SLE + MS: aHR 0.67 (95%CI: 0.37-1.19)

AD: Autoimmune disease; aHR: Adjusted hazard ratio; CI: Confidence interval; DPP-4i: Dipeptidyl peptidase-4 inhibitor; HR: Hazard ratio; IBD: Inflammatory bowel disease; MS: Multiple sclerosis; OR: Odds ratio; PRR: Proportional reporting ratio; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; T2DM: Type 2 diabetes mellitus.

systematic reporting of AD including BP as an adverse event in large clinical trials involving other DPP-4is.

Individual DPP-4i and risk of BP

Almost all of the DPP-4is are associated with the development of BP. However, vildagliptin is the most commonly implicated drug in the published literature. Both pharmacovigilance[64] and observational studies[57,67,68] reported vildagliptin use as a risk factor for development of BP with a OR varying from 1.81 to 10.40. Moreover, the meta-analysis by Phan *et al*[64] concluded that vildagliptin has the highest risk (OR 5.08) followed by linagliptin (OR 2.87). But sitagliptin was not associated with BP (OR 1.29, 95%CI: 0.79-2.08). Linagliptin had a similar propensity (HR 4.90, 95%CI: 2.68–8.96) to cause BP as vildagliptin (HR 4.56, 95%CI: 1.42–14.64) in a recently published study[57]. The conclusion of a recently conducted meta-analysis[63] also revealed an increased risk of BP in linagliptin-treated patients (Mantel-Haenszel-OR 4.69, 95%CI: 1.09-20.22). Additionally, few reports have also revealed teneligliptin[58,69] and saxagliptin[46,56-58,70] induced BP. However, there is a possibility of reporting bias present since BP is not systematically reported in large clinical trials involving various DPP-4i.

Risk factors for the development of DPP-4i induced BP

Older age is one of the important risk factors, and most of the studies have reported the mean age of subjects with DPP-4i induced BP > 70-75 years (Table 5). But Lee *et al*[57] has shown that the risk was similar between patients with more than or less than 75 years of age. The second important risk factor is male gender. Kridin and Bergman[71] reported a higher risk in males compared to females (OR 4.46 *vs* 1.88). In the same way, other studies also reported a male predilection[72]. On the contrary, Varpuluoma *et al*[60] reported that females are more likely to develop DPP-4i induced BP. Phan *et al*[64] in their pooled meta-analysis found that both genders are susceptible to develop BP with higher propensity in males (OR 2.35 *vs* 1.88). The third risk factor is the DPP-4i with less selective DPP-4 enzyme inhibition like vildagliptin. The fourth is a recently discovered association of specific human leucocyte antigen (HLA) HLA-DQB1*03:01 with DPP-4i induced BP in Japanese population[73]. However, Lindgren *et al*[74] did

Table 3 Summary of the pharmacovigilance and population-based studies reporting dipeptidyl peptidase-4 inhibitor induced bullous pemphigoid

Ref.	Country	Population	Pooled odds ratio	Individual DPP-4i	Remarks
Reolid *et al*[55], 2020	Spanish Pharmacovigilance System	Overall reported adverse events	NA	ROR: linagliptin 69.42 (95%CI: 21.17–227.57), saxagliptin 46.45 (6.26-344.25), vildagliptin 123.38 (95%CI: 68.72–221.15), sitagliptin 12.42 (95%CI: 3.89–39.63)	Vildagliptin was the DPP-4i that most frequently induced BP
García *et al*[56], 2016	European pharmacovigilance database	Overall reported adverse events	NA	PRR: Vildagliptin 85.98 (95%CI: 70.98–104.15), sitagliptin 4.55 (95%CI: 3.32–6.24), saxagliptin 8.36 (95%CI: 3.14–22.28), linagliptin 24.32 (95%CI: 14.11–41.92)	Alogliptin was not associated with development of BP
Lee *et al*[57], 2019	Korea (Retrospective, nationwide, population-based, case-control study)	670 patients with diabetes with BP and 670 control patients with only diabetes	aOR, 1.58 (95%CI: 1.25-2.00)	Vildagliptin aOR 1.81 (95%CI: 1.31-2.50), sitagliptin aOR, 1.70 (95%CI: 1.19-2.43), linagliptin aOR 1.64 (95%CI: 1.15-2.33)	Male gender was associated with higher risk of development of BP
Carnovale *et al* [58], 2019	World Health Organization global Individual Case Safety Reports database	Overall reported adverse events	ROR 179.48 (95%CI: 166.41–193.58)	Teneligliptin 975.04 (95%CI: 801.70–1185.87), sitagliptin 46.52 (95%CI: 40.57–53.36), vildagliptin 399.70 (95%CI: 362.26–441.02), linagliptin 143.23 (95%CI: 122.60–167.33)	The highest ROR was found for teneligliptin
Béné *et al*[59], 2016	French Pharmacovigilance Database	Among 1297 spontaneous ADR reports, 42 were DPP-4i induced BP	ROR 67·5 (95%CI: 47.1-96.9)	Vildagliptin ROR 225·3 (95%CI: 148.9-340.9), sitagliptin ROR 17.0 (95%CI: 8.9-32.5), saxagliptin ROR 16.5 (95%CI: 2.3-119.1)	Vildagliptin had higher ROR
Varpuluoma *et al* [60], 2018	Finland (Nationwide Registry Study)	3397 BP cases and 12941 controls	aOR 2.13 (95%CI: 1.51–3.00)	aOR vildagliptin 8.66 (95%CI: 4.06-18.50), aOR sitagliptin 1.36 (95%CI: 0.93-1.99)	A significantly increased risk of BP after the use of vildagliptin
Hung *et al*[61], 2020	Taiwan (Nationwide, population-based, cohort study)	6340 patients with DM on DPP-4i and 25360 DM patients without DPP-4i	aHR 2.382 (95%CI: 1.163-4.883)	Vildagliptin aHR, 2.849 (95%CI: 1.893-4.215), saxagliptin aHR, 2.657 (95%CI: 1.770-3.934), sitagliptin aHR, 2.585 (95%CI: 1.723–3.829), linagliptin aHR, 2.360 (95%CI: 1.567–3.477), alogliptin aHR, 1.450 (95%CI: 0.965–2.152)	Vildagliptin was significantly associated with an increased risk of BP, and alogliptin was not associated with development of BP
Arai *et al*[49], 2018	Japanese Adverse Drug Event Report database	392 BP cases in DPP-4i user and 12811 without BP as control	ROR 87.56 (95%CI: 72.61–105.59)	ROR: alogliptin 8.02 (95%CI: 4.87–13.22), anagliptin 10.84 (95%CI: 3.46–33.96), sitagliptin 12.59 (95%CI: 9.86–16.06), trelagliptin 13.77 (95%CI: 3.40–55.85), saxagliptin 15.85 (95%CI: 5.87–42.79), linagliptin 28.96 (95%CI: 21.38–39.23), omarigliptin 43.79 (95%CI: 5.85–327.70), teneligliptin 58.52 (95%CI: 42.75–80.10), vildagliptin 105.33 (95%CI: 88.54–125.30)	The highest ROR was found with vildagliptin
MolinaGuarneros *et al*[70], 2020	Spain (pharmacovigilance data)	Case/non-case analysis (1998 DPP-4i induced ADR where 45 were DPP-4i induced BP)	ROR 70.0 (47.1–104.1)	Vildagliptin 113.9 (95%CI: 73.4–177), linagliptin 55.2 (95%CI: 28.2–108.0), sitagliptin 9.1 (95%CI: 3.7–22.6), saxagliptin 27.4 (95%CI: 3.7–200.1)	Highest risk of BP with vildagliptin
Douros *et al*[80], 2019	United Kingdom Clinical Practice Research Datalink	Cohort study among 168774 patients started on antidiabetic drugs	HR 2.21 (95%CI: 1.45-3.38)	Linagliptin HR 4.90 (95%CI: 2.68–8.96), vildagliptin HR 4.56 (95%CI: 1.42–14.64), saxagliptin HR 2.16 (95%CI: 0.86–5.46), sitagliptin HR 1.42 (95%CI: 0.79–2.53)	HRs for development of BP gradually increased with longer durations of DPP-4i use

ADR: Adverse drug reaction; aHR: Adjusted hazard ratio; aOR: Adjusted odds ratio; BP: Bullous pemphigoid; CI: Confidence interval; DPP-4i: Dipeptidyl peptidase-4 inhibitor; DM: Diabetes mellitus; NA: Not available; PRR: Proportional reporting ratio; ROR: Reporting odds ratio.

not find a similar association in Caucasians. The other possible associated risk factors are mentioned in Table 5[75-77].

Table 4 Clinical characteristics of case-control studies reporting dipeptidyl peptidase-4 inhibitor-induced bullous pemphigoid

Ref.	Type of the study	Population	Effect of gender	Latency period	Age	Outcome
Plaquevent *et al*[50], 2019	Multicentre case-control study	Out of 1787 patients with BP, 108 subjects were gliptin users. Comparison with a large general population data base	NA	14.8 mo (interquartile range 6.0-26.7 mo)	77.9 ± 9.3 yr	No difference in outcome between gliptin withdrawal *vs* continued groups
Schaffer *et al* [51], 2017	Retrospective case-control study	Patients with diabetes and BP (n = 23) compared with patients with only diabetes (n = 170)	NA	Range: 5-48 mo	77.6 yr	Favourable outcome after gliptin withdrawal; however topical and systemic therapy were required in most of the cases
Béné *et al*[59], 2016	Case/non case analysis from database	Patients with BP (n = 150) compared with other spontaneous adverse drug reactions	NA	10 mo (range 8 d-37 mo)	74 yr (range 45-91)	Favourable outcome in patients when DPP-4is were discontinued. Median time to improvement was 10 d (interquartile range : 5-15 d)
Benzaquen *et al*[68], 2018	Retrospective case-control study with 1:2 design	Patients with diabetes and BP (n = 61) compared with patients with only diabetes (n = 122)	Male aOR 4.36 (95%CI: 1.38-13.83), females 1.64 (95%CI: 0.53-5.11)	Median 8.2 mo (range 10 d to 3 yr)	79.1 ± 7.0 yr	Favourable outcome when DPP-4is were discontinued
Kridin and Bergman[71], 2018	Retrospective case-control study	Diabetes patients with BP (n = 82) *vs* age and gender matched control population with only diabetes (n = 328)	Male OR 4.46 (95%CI: 2.11-9.40), female OR 1.88 (95%CI: 0.92-3.86)	Median 10.4 mo (range 1.0-26.5 mo)	79.1 ± 9.1 yr	Favourable outcome in gliptin withdrawal group

aOR: Adjusted odds ratio; BP: Bullous pemphigoid; CI: Confidence interval; DPP-4is: Dipeptidyl peptidase-4 inhibitors; NA: Not available; OR: Odds ratio.

Table 5 Emerging risk factors for development of dipeptidyl peptidase-4 inhibitor-induced bullous pemphigoid

Risk factors	Possible risk/trigger factor[1]
Older age (> 70 yr of age)[57,59,68]	Longer duration of DPP-4i use[64]
Male gender[64,71]	Patients with dementia[53,54]
Specific HLA like HLA-DQB1*03:01 (In Japanese population)[73]	Concomitant use of spironolactone[53]
Certain DPP-4i[63,64] (*i.e.* vildagliptin, linagliptin)[2]	Chronic kidney disease[54,77] and haemodialysis[76]
	Thermal Burn[75]

[1]Based on small studies and case reports.
[2]High likelihood of modifications of the list as new data emerges. DPP-4i: Dipeptidyl peptidase-4 inhibitor; HLA: Human leucocyte antigen.

Clinical course of DPP-4i induced BP

Latency period: The reported range of latency period for the development of DPP-4i induced BP varies widely. It ranges from 8 d to 4 years[59,63,64,78] (Table 4). A very recent case series[79] reported a median latency period of 64 mo (range 20-128 mo); however, such an association is deemed as 'possible,' and causality is difficult to establish in such cases. Douros *et al*[80] showed that the risk of development of BP increases with longer duration of use of DPP-4i, and the peak reaches around 20 mo after exposure to DPP-4i. Molina-Guarneros *et al*[70] reported a variable latency period between different DPP-4i, where linagliptin has the shortest (3.5 mo) and sitagliptin has the longest (12 mo) latency period.

Clinical characteristics of DPP-4i induced BP: As more evidence is emerging, the clinical characteristics of DPP-4i induced BP are becoming clearer[81,82]. Moreover, studies have started differentiating this disease from the more common classical BP. The BP lesions in DPP-4i treated patients are described as a predominantly 'non-inflammatory' phenotype and often exhibit lesser erythema when compared to the classical BP lesions[83,84]. Furthermore, a study performed in an Israeli cohort reported that DPP-4i induced BP had more extensive involvement and a predominant distribution of the lesion in the cephalic and truncal region of the body when

compared with other non-DPP-4i associated variants of BP[85]. A predominant mucosal involvement in DPP-4i induced BP was also reported by Kridin and Bergman [71]. But this finding was not duplicated in other studies[64,72,73,86,87]. Another interesting feature of DPP-4i associated BP is lower peripheral eosinophil count[71] as well as less eosinophilic infiltrate in the skin lesion[83,88]. But Bellinato *et al*[89] did not find any such difference. These conflicting results warrant further research to look into this area, preferably in long-term follow-up studies.

Is DPP-4i induced BP a distinct immunological phenomenon?: BP is an immunological disease characterized by the development of autoimmunity against the BP180 and BP230 protein, both of which are hemi-desmosomal protein present in the dermo-epidermal junction[81]. BP180 is also known as collagen XVII. The principal autoantibody involved in the pathogenesis of the classic variant of BP is the one that acts against the extracellular non-collagenous part named the NC16A domain[90]. However, there is some evidence that DPP-4i induced BP has a different autoantibody profile compared to its classic counterpart. In an earlier report, Izumi *et al*[83] reported that a significant proportion of the DPP-4i induced BP patients had immunoglobulin G autoantibody against epitope other than the known NC16A region. Moreover, patients with non-NC16A antibodies had less inflammation and erythema, which is often-described in patients with DPP-4i induced BP. A lesser prevalence of anti-NC16A antibody in DPP-4i induced BP was also described by Horikawa *et al*[84]. Interestingly, they reported that the majority of the anti-NC16A antibody-negative patients had antibody against the full-length BP180 antigen. Another study from Japan also described a similar finding[52]. However, this specific antibody profile that could differentiate DPP-4i induced BP from the classical variety was not demonstrated in studies performed in the European population[87,89,91]. Further research is needed in this area to better characterize the role of certain autoantibodies in the pathogenesis and to use them as markers for DPP-4i induced BP.

Effect of DPP-4i withdrawal on the outcome of BP: It is expected that DPP-4i withdrawal will lead to an improvement in BP. Studies had reported a favourable outcome when DPP-4i was withdrawn after the diagnosis of BP[59,68,71]. Moreover, mortality remained significant in few studies if DPP-4i was not withdrawn[68,71]. The outcome had been measured in terms of achievement of complete or partial remission. However, even after DPP-4i withdrawal, some patients may require topical or systemic glucocorticoids depending upon the severity of the lesions[59,68,71]. Contrarily, one study found no difference in the outcome of BP lesions irrespective of the withdrawal status of DPP-4i[50]. In fact, the effect of DPP-4i withdrawal in the natural history of BP is often complicated by the fact that concomitant topical and/or systemic glucocorticoids are already being used as a therapy of BP. Despite withdrawal of DPP-4i, BP may not remit fully and require glucocorticoid therapy. Therefore, DPP-4i might play a role of aggravator of BP rather than independently inducing BP in some cases. The time taken for the improvement of BP lesions also varies in different studies. One study reported a median time for improvement of 10 d after drug withdrawal[59], whereas other studies reported months to improve[68]. Re-challenge or replacement with another DPP-4i carries a high risk of relapse of BP[59, 68] and thus preferably should be avoided. A very recent retrospective study reported that linagliptin induced BP might be difficult to treat, and it requires a higher dosage of systemic glucocorticoid compared to vildagliptin-induced BP[85].

DPP-4I USE AND RISK OF IBD

IBD is a chronic, relapsing, intestinal inflammatory condition in which various genetic, immunological, and environmental factors play a critical role. Earlier, the experimental studies showed a beneficial effect of DPP-4i use in animal models of colitis[92]. Thus, it was suggested that DPP-4i can be used as a potential therapeutic agent in IBD[8]. Studies have shown that DPP-4 levels in the plasma as well as in tissue are decreased in IBD patients compared to healthy controls[93], and the lower DPP-4 level correlates with higher disease activity and serum inflammatory markers like C-reactive proteins [94,95]. Thus DPP-4i use is expected to have a potential impact on the immunopathogenesis of IBD. DPP-4i can also have an indirect effect on IBD by increasing the levels of different incretin hormones like glucagon-like peptide-1, glucagon-like peptide-2, and vaso-active intestinal peptide[93], though a direct effect of the DPP-4 molecule is still a possibility[96]. The clinical data are quite contrary to this basic science research.

In a population-based cohort study by Abrahami *et al*[97] in the United Kingdom, it was shown that the use of DPP-4i was associated with an increased risk of IBD with an HR of 1.75 (95%CI: 1.22- 2.49; the estimated risk was 53.4 *vs* 34.5 *per* 100000 person years in DPP-4i users *vs* non-users). The maximum risk was seen after 3-4 years of DPP-4i use (HR 2.90, 95%CI: 1.31-6.41), and the risk declined thereafter. Another population-based study by Kridin *et al*[36] showed a three and half times increased risk of Crohn's disease in DPP-4i users (OR 3.56; 95%CI: 1.04-12.21, $P = 0.031$). Wang *et al*[98] also demonstrated the increased risk of IBD in DPP-4i users while assessing the FDA's Adverse Event Reporting System database. Radel *et al*[99] performed a meta-analysis that included 16 studies (including major cardiovascular outcome trials of DPP-4i like EXAMINE, SAVOR-TIMI, and TECOS trial; $n = 198404$) and found a significantly increased relative risk (RR) 3.01 (95%CI: 2.30-3.93) of IBD using a fixed-effects model. However, the most important limitation of the analysis was that the data was driven mainly by the study of Abrahami *et al*[97]. Moreover, a random effect analysis did not reveal any elevation in the IBD risk among DPP-4i users, and the duration of most of the trials included in the analysis were less than 4 years.

On the other hand, another meta-analysis (included 13 RCTs) performed by Li *et al*[100] did not show any increase in the IBD risk among the DPP-4i users as compared to control population (RR 1.01, 95%CI: 0.30-3.41). The reported heterogeneity of the studies was low($I^2 = 0\%$). However, the mean follow-up period was only 1.5 years. Wang *et al*[101] also evaluated this association in the real-world setting using the insurance databases and compared the risk of IBD between DPP-4i with sulfonylurea and thiazolidinedione users. During a median duration of 1.09–1.69 years, DPP-4i was not found to be associated with a risk of IBD. The population-based studies that evaluated the overall AD composite outcomes also did not find increased risk of IBD [39,40].

To summarise, the data suggest a modest association of DPP-4i use and the development of IBD in studies that specifically looked for it, whereas pooled analysis of the RCT data failed to confirm this finding. Since the duration of the studies including many of the RCTs are short, a continued and watchful observation is required, particularly during the post-marketing surveillance. Future RCTs on DPP-4i should also systematically report development of IBD as an adverse event. Importantly, pathophysiological studies should be undertaken to further elucidate the underlying mechanism behind any such association. Clinicians should be aware of this association and a cautious approach should be undertaken while prescribing DPP-4i in a predisposed individual or those who show clinical features suggestive of IBD.

DPP-4I USE AND RISK OF AUTOIMMUNE JOINT DISEASES

The relationship between use of DPP-4i and different joint disorders is a complex one. The joint involvement can be either arthritis or arthralgia, which is not attributable to a specific autoimmune pathology.

Nonspecific autoimmune arthritis/arthralgia

The FDA's Adverse Event Reporting System database found 33 cases of severe arthralgia reported with the use of DPP-4i. The reported DPP-4is were sitagliptin followed by saxagliptin, linagliptin, vildagliptin, and alogliptin suggesting a class effect of these drugs. In five cases, arthralgia was also reported even after switching to another DPP-4i. Following this data, the FDA published a safety warning declaring that DPP-4i may cause severe joint pain, with a time to event ranging from 1 d to years in August 2015[3]. Mascolo *et al*[102] summarised 22 published cases of DPP-4i induced arthralgia/arthritis. The duration of DPP-4i therapy before joint symptoms ranged from 2 wk to 31 mo. All these cases developed arthralgia following initiation of DPP-4i, and resolution of clinical features was achieved in most cases after discontinuation of the drug. Similar to the FDA review, few of these described patients experienced joint symptoms following reinstitution of the DPP-4i. The joints that were involved were small joints of the hands/feet, knee, and ankle. A study by Saito *et al*[103] identified 13 cases of multiple joint involvements in DPP-4i users and also noted improvement of symptoms within 3 mo of drug discontinuation. No patient required treatment with glucocorticoids. But 4 patients required non-steroidal anti-inflammatory drugs. A lower level of stromal derived factor-1α was noted during the active phase of joint involvement with normalisation of the values following clinical resolution. The levels of other cytokines and chemokines were not different between the groups thus warranting further research into the mechanism of DPP-4i induced

joint involvement. Moreover, in the absence of further study, clinical utility of measuring stromal derived factor-1α remains inconclusive at present.

Another study demonstrated a 3.77 times increased risk of arthralgia/arthritis among DPP-4i users, and interestingly different inflammatory markers were negative in a significant number (66%, n = 27/41) of such patients[104]. On the contrary, few studies negated the finding of an association between DPP-4i use and severe joint disease[105,106]. A meta-analysis including a total of 67 RCTs (79110 patients) showed that DPP-4is were associated with a small but statistically significant increased risk of arthralgia (RR: 1.13, 95%CI: 1.04-1.22; P = 0.003)[107]. However, the risk of development of serious arthralgia was not significant (RR: 1.44, 95%CI: 0.83–2.51; P = 0.20). Also, subgroup analyses disclosed that add-on or combination therapy and diabetes duration (> 5 years) were possible predictive factors associated with the increased risk of overall arthralgia[107]. Thus, it remains to be proven that DPP-4i induced joint involvement is truly an autoimmune phenomenon, but clinicians should be alert to this association. Importantly, thorough investigation is required to rule out specific AD when drug discontinuation does not result in relief of joint symptoms.

RA

The relationship between DPP-4i and RA is complex. In recent times, multiple population-based cohort studies evaluated the onset of RA in DPP-4i users. The United States of America health claim data from 2005 to 2012 showed that DPP-4i was associated with a 34% decreased risk of RA (HR = 0.66, 95%CI: 0.44-0.99) compared with other oral antidiabetic drugs (sulfonylureas and thiazolidinediones)[39]. This was similar to the study findings by Seong *et al*[40], who also showed a 33% decreased risk of RA (HR = 0.67; 95%CI: 0.49-0.92)[40]. In contrast, a recent large United Kingdom population-based study by Douros *et al*[108] who specifically looked for the association of DPP-4i use and the new development of RA found that DPP-4i use was not associated with a risk of incident RA compared with the use of other antidiabetic drugs (HR 1.0, 95%CI: 0.8-1.3). These findings were consistent irrespective of the duration of drug use or the types of DPP-4i[108]. Kathe *et al*[109] also reported a similar finding in their study. Indeed, a recent meta-analysis revealed a hazard ratio of 0.72 (95%CI: 0.54–0.96) for the development of RA in DPP-4i users[110]. However, this analysis had a limitation in the form of very high heterogeneity (I^2 = 75%).

On the other hand, there are few case reports of flaring of RA in remitted patients with DPP-4i use. Sasaki *et al*[111] had reported relapse of RA in a patient using sitagliptin in 2010[111]. Yokota and Igaki[112] also reported the onset of RA with sitagliptin use in an HLA predisposed (HLA-DRB1 allele) individual[112]. In a recent report, Padron *et al*[113] reported sitagliptin induced sero-negative RA in a 56-year-old patient with a long duration of diabetes. Hence, caution should be exercised while prescribing DPP-4i to a person with a history of prior RA or at risk of RA.

CONCLUSION

In summary, the relationship between DPP-4i use and the development of AD is complex and evolving. While recent studies have suggested that DPP-4i use may be associated with decreases in the incidence of composite AD, they can also result in the development of certain AD. BP is one AD that can be induced by DPP-4i, particularly in the elderly population. The increment in IBD risk is modest, but evidence is mixed and requires further studies to confirm this finding. DPP-4i can increase the risk of nonspecific arthritis and arthralgia along with flaring up of RA. However, data regarding this finding needs further validation. The association with other AD is mostly uncertain due to lack of evidence, but an astute clinician should be alert to any such events in a patient receiving DPP-4i. Future studies, particularly long-term follow-up studies, should clarify the relationship between AD and DPP-4i use. More basic research is also needed to find the exact underlying pathogenesis behind this association.

REFERENCES

1 **American Diabetes Association**. 9. Pharmacologic Approaches to Glycemic Treatment: *Standards of Medical Care in Diabetes-2021*. *Diabetes Care* 2021; **44**: S111-S124 [PMID: 33298420 DOI: 10.2337/dc21-S009]

2 **Deacon CF**, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and

sulphonylureas. *Diabetes Obes Metab* 2016; **18**: 333-347 [PMID: 26597596 DOI: 10.1111/dom.12610]
3 **Kim H**, Pfeiffer CM, Gray MP, Stottlemyer BA, Boyce RD, Kane-Gill SL. Assessing Adverse Drug Reactions Reported for New Respiratory Medications in the FDA Adverse Event Reporting System Database. *Respir Care* 2021 [PMID: 34103383 DOI: 10.4187/respcare.08809]
4 **Griffin KJ**, Thompson PA, Gottschalk M, Kyllo JH, Rabinovitch A. Combination therapy with sitagliptin and lansoprazole in patients with recent-onset type 1 diabetes (REPAIR-T1D): 12-month results of a multicentre, randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2014; **2**: 710-718 [PMID: 24997559 DOI: 10.1016/S2213-8587(14)70115-9]
5 **Zhao Y**, Yang L, Xiang Y, Liu L, Huang G, Long Z, Li X, Leslie RD, Wang X, Zhou Z. Dipeptidyl peptidase 4 inhibitor sitagliptin maintains β-cell function in patients with recent-onset latent autoimmune diabetes in adults: one year prospective study. *J Clin Endocrinol Metab* 2014; **99**: E876-E880 [PMID: 24432999 DOI: 10.1210/jc.2013-3633]
6 **Farag SS**, Abu Zaid M, Schwartz JE, Thakrar TC, Blakley AJ, Abonour R, Robertson MJ, Broxmeyer HE, Zhang S. Dipeptidyl Peptidase 4 Inhibition for Prophylaxis of Acute Graft-versus-Host Disease. *N Engl J Med* 2021; **384**: 11-19 [PMID: 33406328 DOI: 10.1056/NEJMoa2027372]
7 **Reinhold D**, Bank U, Entz D, Goihl A, Stoye D, Wrenger S, Brocke S, Thielitz A, Stefin S, Nordhoff K, Heimburg A, Täger M, Ansorge S. PETIR-001, a dual inhibitor of dipeptidyl peptidase IV (DP IV) and aminopeptidase N (APN), ameliorates experimental autoimmune encephalomyelitis in SJL/J mice. *Biol Chem* 2011; **392**: 233-237 [PMID: 21194377 DOI: 10.1515/BC.2011.024]
8 **Yazbeck R**, Howarth GS, Abbott CA. Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol Sci* 2009; **30**: 600-607 [PMID: 19837468 DOI: 10.1016/j.tips.2009.08.003]
9 **Mulvihill EE**, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev* 2014; **35**: 992-1019 [PMID: 25216328 DOI: 10.1210/er.2014-1035]
10 **Nauck MA**, Meier JJ. Incretin hormones: Their role in health and disease. *Diabetes Obes Metab* 2018; **20** Suppl 1: 5-21 [PMID: 29364588 DOI: 10.1111/dom.13129]
11 **Zhao Y**. CD26 in autoimmune diseases: The other side of "moonlight protein". *Int Immunopharmacol* 2019; **75**: 105757 [PMID: 31357088 DOI: 10.1016/j.intimp.2019.105757]
12 **Klemann C**, Wagner L, Stephan M, von Hörsten S. Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. *Clin Exp Immunol* 2016; **185**: 1-21 [PMID: 26919392 DOI: 10.1111/cei.12781]
13 **Casrouge A**, Sauer AV, Barreira da Silva R, Tejera-Alhambra M, Sánchez-Ramón S, ICAReB, Cancrini C, Ingersoll MA, Aiuti A, Albert ML. Lymphocytes are a major source of circulating soluble dipeptidyl peptidase 4. *Clin Exp Immunol* 2018; **194**: 166-179 [PMID: 30251416 DOI: 10.1111/cei.13163]
14 **Yazbeck R**, Jaenisch SE, Abbott CA. Potential disease biomarkers: dipeptidyl peptidase 4 and fibroblast activation protein. *Protoplasma* 2018; **255**: 375-386 [PMID: 28620698 DOI: 10.1007/s00709-017-1129-5]
15 **Osawa S**, Kawamori D, Katakami N, Takahara M, Sakamoto F, Katsura T, Yasuda T, Kaneto H, Matsuhisa M, Matsuoka TA, Shimomura I. Significant elevation of serum dipeptidyl peptidase-4 activity in young-adult type 1 diabetes. *Diabetes Res Clin Pract* 2016; **113**: 135-142 [PMID: 26827118 DOI: 10.1016/j.diabres.2015.12.022]
16 **Ohnuma K**, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, Kina S, Tanaka H, Lin X, Dang NH, Morimoto C. Caveolin-1 triggers T-cell activation *via* CD26 in association with CARMA1. *J Biol Chem* 2007; **282**: 10117-10131 [PMID: 17287217 DOI: 10.1074/jbc.M609157200]
17 **Reinhold D**, Hemmer B, Gran B, Steinbrecher A, Brocke S, Kähne T, Wrenger S, Born I, Faust J, Neubert K, Martin R, Ansorge S. Dipeptidyl peptidase IV (CD26): role in T cell activation and autoimmune disease. *Adv Exp Med Biol* 2000; **477**: 155-160 [PMID: 10849742 DOI: 10.1007/0-306-46826-3_17]
18 **Zhong J**, Rao X, Deiuliis J, Braunstein Z, Narula V, Hazey J, Mikami D, Needleman B, Satoskar AR, Rajagopalan S. A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation. *Diabetes* 2013; **62**: 149-157 [PMID: 22936179 DOI: 10.2337/db12-0230]
19 **Bühling F**, Junker U, Reinhold D, Neubert K, Jäger L, Ansorge S. Functional role of CD26 on human B lymphocytes. *Immunol Lett* 1995; **45**: 47-51 [PMID: 7622187 DOI: 10.1016/0165-2478(94)00230-o]
20 **Shao S**, Xu Q, Yu X, Pan R, Chen Y. Dipeptidyl peptidase 4 inhibitors and their potential immune modulatory functions. *Pharmacol Ther* 2020; **209**: 107503 [PMID: 32061923 DOI: 10.1016/j.pharmthera.2020.107503]
21 **Mortier A**, Gouwy M, Van Damme J, Proost P, Struyf S. CD26/dipeptidylpeptidase IV-chemokine interactions: double-edged regulation of inflammation and tumor biology. *J Leukoc Biol* 2016; **99**: 955-969 [PMID: 26744452 DOI: 10.1189/jlb.3MR0915-401R]
22 **Papazafiropoulou AK**, Papanas N, Trikkalinou A, Fousteris E, Melidonis A. The Oral Dipeptidyl-Peptidase-4 Inhibitor Sitagliptin Increases Circulating Levels Of Stromal-Derived Factor-1 Alpha. *Exp Clin Endocrinol Diabetes* 2018; **126**: 367-370 [PMID: 28931178 DOI: 10.1055/s-0043-118748]
23 **Anderluh M**, Kocic G, Tomovic K, Kocic R, Deljanin-Ilic M, Smelcerovic A. Cross-talk between

the dipeptidyl peptidase-4 and stromal cell-derived factor-1 in stem cell homing and myocardial repair: Potential impact of dipeptidyl peptidase-4 inhibitors. *Pharmacol Ther* 2016; **167**: 100-107 [PMID: 27484974 DOI: 10.1016/j.pharmthera.2016.07.009]

24 **Takashima S**, Fujita H, Fujishima H, Shimizu T, Sato T, Morii T, Tsukiyama K, Narita T, Takahashi T, Drucker DJ, Seino Y, Yamada Y. Stromal cell-derived factor-1 is upregulated by dipeptidyl peptidase-4 inhibition and has protective roles in progressive diabetic nephropathy. *Kidney Int* 2016; **90**: 783-796 [PMID: 27475229 DOI: 10.1016/j.kint.2016.06.012]

25 **Chiazza F**, Tammen H, Pintana H, Lietzau G, Collino M, Nyström T, Klein T, Darsalia V, Patrone C. The effect of DPP-4 inhibition to improve functional outcome after stroke is mediated by the SDF-1α/CXCR4 pathway. *Cardiovasc Diabetol* 2018; **17**: 60 [PMID: 29776406 DOI: 10.1186/s12933-018-0702-3]

26 **Aso Y**, Kase M, Sagara M, Sakurai S, Iijima T, Tomaru T, Jojima T, Usui I. Teneligliptin, a DPP-4 Inhibitor, Decreases Plasma Levels of Inflammatory Chemokines During a Standard Meal Test in Patients With Type 2 Diabetes. *Am J Med Sci* 2020; **360**: 261-267 [PMID: 32540146 DOI: 10.1016/j.amjms.2020.05.005]

27 **Elmansi AM**, Awad ME, Eisa NH, Kondrikov D, Hussein KA, Aguilar-Pérez A, Herberg S, Periyasamy-Thandavan S, Fulzele S, Hamrick MW, McGee-Lawrence ME, Isales CM, Volkman BF, Hill WD. What doesn't kill you makes you stranger: Dipeptidyl peptidase-4 (CD26) proteolysis differentially modulates the activity of many peptide hormones and cytokines generating novel cryptic bioactive ligands. *Pharmacol Ther* 2019; **198**: 90-108 [PMID: 30759373 DOI: 10.1016/j.pharmthera.2019.02.005]

28 **Reinhold D**, Biton A, Goihl A, Pieper S, Lendeckel U, Faust J, Neubert K, Bank U, Täger M, Ansorge S, Brocke S. Dual inhibition of dipeptidyl peptidase IV and aminopeptidase N suppresses inflammatory immune responses. *Ann N Y Acad Sci* 2007; **1110**: 402-409 [PMID: 17911455 DOI: 10.1196/annals.1423.042]

29 **Pinheiro MM**, Stoppa CL, Valduga CJ, Okuyama CE, Gorjão R, Pereira RM, Diniz SN. Sitagliptin inhibit human lymphocytes proliferation and Th1/Th17 differentiation in vitro. *Eur J Pharm Sci* 2017; **100**: 17-24 [PMID: 28065853 DOI: 10.1016/j.ejps.2016.12.040]

30 **Zhuge F**, Ni Y, Nagashimada M, Nagata N, Xu L, Mukaida N, Kaneko S, Ota T. DPP-4 Inhibition by Linagliptin Attenuates Obesity-Related Inflammation and Insulin Resistance by Regulating M1/M2 Macrophage Polarization. *Diabetes* 2016; **65**: 2966-2979 [PMID: 27445264 DOI: 10.2337/db16-0317]

31 **Zheng W**, Zhou J, Song S, Kong W, Xia W, Chen L, Zeng T. Dipeptidyl-Peptidase 4 Inhibitor Sitagliptin Ameliorates Hepatic Insulin Resistance by Modulating Inflammation and Autophagy in ob/ob Mice. *Int J Endocrinol* 2018; **2018**: 8309723 [PMID: 30123267 DOI: 10.1155/2018/8309723]

32 **Aroor AR**, Habibi J, Kandikattu HK, Garro-Kacher M, Barron B, Chen D, Hayden MR, Whaley-Connell A, Bender SB, Klein T, Padilla J, Sowers JR, Chandrasekar B, DeMarco VG. Dipeptidyl peptidase-4 (DPP-4) inhibition with linagliptin reduces western diet-induced myocardial TRAF3IP2 expression, inflammation and fibrosis in female mice. *Cardiovasc Diabetol* 2017; **16**: 61 [PMID: 28476142 DOI: 10.1186/s12933-017-0544-4]

33 **Kodera R**, Shikata K, Takatsuka T, Oda K, Miyamoto S, Kajitani N, Hirota D, Ono T, Usui HK, Makino H. Dipeptidyl peptidase-4 inhibitor ameliorates early renal injury through its anti-inflammatory action in a rat model of type 1 diabetes. *Biochem Biophys Res Commun* 2014; **443**: 828-833 [PMID: 24342619 DOI: 10.1016/j.bbrc.2013.12.049]

34 **Iwakura T**, Zhao Z, Marschner JA, Devarapu SK, Yasuda H, Anders HJ. Dipeptidyl peptidase-4 inhibitor teneligliptin accelerates recovery from cisplatin-induced acute kidney injury by attenuating inflammation and promoting tubular regeneration. *Nephrol Dial Transplant* 2019; **34**: 1669-1680 [PMID: 30624740 DOI: 10.1093/ndt/gfy397]

35 **Reinhold D**, Bank U, Täger M, Ansorge S, Wrenger S, Thielitz A, Lendeckel U, Faust J, Neubert K, Brocke S. DP IV/CD26, APN/CD13 and related enzymes as regulators of T cell immunity: implications for experimental encephalomyelitis and multiple sclerosis. *Front Biosci* 2008; **13**: 2356-2363 [PMID: 17981717 DOI: 10.2741/2849]

36 **Kridin K**, Amber K, Khamaisi M, Comaneshter D, Batat E, Cohen AD. Is there an association between dipeptidyl peptidase-4 inhibitors and autoimmune disease? *Immunol Res* 2018; **66**: 425-430 [PMID: 29855994 DOI: 10.1007/s12026-018-9005-8]

37 **Noguchi Y**, Toda Y, Esaki H, Matsuyama T, Tachi T, Tsuchiya T, Teramachi H. Association between dipeptidyl peptidase-4 inhibitors and autoimmune disorders: Data mining of the spontaneous reporting system in Japan. *Pharmazie* 2019; **74**: 305-309 [PMID: 31109402 DOI: 10.1691/ph.2019.8972]

38 **Chen YC**, Chen TH, Sun CC, Chen JY, Chang SS, Yeung L, Tsai YW. Dipeptidyl peptidase-4 inhibitors and the risks of autoimmune diseases in type 2 diabetes mellitus patients in Taiwan: a nationwide population-based cohort study. *Acta Diabetol* 2020; **57**: 1181-1192 [PMID: 32318876 DOI: 10.1007/s00592-020-01533-5]

39 **Kim SC**, Schneeweiss S, Glynn RJ, Doherty M, Goldfine AB, Solomon DH. Dipeptidyl peptidase-4 inhibitors in type 2 diabetes may reduce the risk of autoimmune diseases: a population-based cohort study. *Ann Rheum Dis* 2015; **74**: 1968-1975 [PMID: 24919467 DOI: 10.1136/annrheumdis-2014-205216]

40 **Seong JM**, Yee J, Gwak HS. Dipeptidyl peptidase-4 inhibitors lower the risk of autoimmune disease in patients with type 2 diabetes mellitus: A nationwide population-based cohort study. *Br J Clin*

Pharmacol 2019; **85**: 1719-1727 [PMID: 30964554 DOI: 10.1111/bcp.13955]
41 **Okauchi Y**, Tomoda Y, Takata M, Deguchi A, Takenoshita Y, Yokomi A, Mineo I. Pemphigus vulgaris developing after 6-month treatment with a dipeptidyl peptidase-4 inhibitor: A case report. *J Dermatol* 2018; **45**: e39-e40 [PMID: 28971524 DOI: 10.1111/1346-8138.14076]
42 **Langan SM**, Smeeth L, Hubbard R, Fleming KM, Smith CJ, West J. Bullous pemphigoid and pemphigus vulgaris--incidence and mortality in the UK: population based cohort study. *BMJ* 2008; **337**: a180 [PMID: 18614511 DOI: 10.1136/bmj.a180]
43 **Pasmatzi E**, Monastirli A, Habeos J, Georgiou S, Tsambaos D. Dipeptidyl peptidase-4 inhibitors cause bullous pemphigoid in diabetic patients: report of two cases. *Diabetes Care* 2011; **34**: e133 [PMID: 21788636 DOI: 10.2337/dc11-0804]
44 **Skandalis K**, Spirova M, Gaitanis G, Tsartsarakis A, Bassukas ID. Drug-induced bullous pemphigoid in diabetes mellitus patients receiving dipeptidyl peptidase-IV inhibitors plus metformin. *J Eur Acad Dermatol Venereol* 2012; **26**: 249-253 [PMID: 21466592 DOI: 10.1111/j.1468-3083.2011.04062.x]
45 **Aouidad I**, Fite C, Marinho E, Deschamps L, Crickx B, Descamps V. A case report of bullous pemphigoid induced by dipeptidyl peptidase-4 inhibitors. *JAMA Dermatol* 2013; **149**: 243-245 [PMID: 23426497 DOI: 10.1001/jamadermatol.2013.1073]
46 **Yoshiji S**, Murakami T, Harashima SI, Ko R, Kashima R, Yabe D, Ogura M, Doi K, Inagaki N. Bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors: A report of five cases. *J Diabetes Investig* 2018; **9**: 445-447 [PMID: 28520234 DOI: 10.1111/jdi.12695]
47 **Béné J**, Jacobsoone A, Coupe P, Auffret M, Babai S, Hillaire-Buys D, Jean-Pastor MJ, Vonarx M, Vermersch A, Tronquoy AF, Gautier S. Bullous pemphigoid induced by vildagliptin: a report of three cases. *Fundam Clin Pharmacol* 2015; **29**: 112-114 [PMID: 24861252 DOI: 10.1111/fcp.12083]
48 **García-Díez I**, Ivars-Lleó M, López-Aventín D, Ishii N, Hashimoto T, Iranzo P, Pujol RM, España A, Herrero-Gonzalez JE. Bullous pemphigoid induced by dipeptidyl peptidase-4 inhibitors. Eight cases with clinical and immunological characterization. *Int J Dermatol* 2018; **57**: 810-816 [PMID: 29682739 DOI: 10.1111/ijd.14005]
49 **Arai M**, Shirakawa J, Konishi H, Sagawa N, Terauchi Y. Bullous Pemphigoid and Dipeptidyl Peptidase 4 Inhibitors: A Disproportionality Analysis Based on the Japanese Adverse Drug Event Report Database. *Diabetes Care* 2018; **41**: e130-e132 [PMID: 30002201 DOI: 10.2337/dc18-0210]
50 **Plaquevent M**, Tétart F, Fardet L, Ingen-Housz-Oro S, Valeyrie-Allanore L, Bernard P, Hebert V, Roussel A, Avenel-Audran M, Chaby G, D'Incan M, Ferrier-Le-Bouedec MC, Duvert-Lehembre S, Picard-Dahan C, Jeudy G, Collet E, Labeille B, Morice C, Richard MA, Bourgault-Villada I, Litrowski N, Bara C, Mahe E, Prost-Squarcioni C, Alexandre M, Quereux G, Bernier C, Soria A, Thomas-Beaulieu D, Pauwels C, Dereure O, Benichou J, Joly P; French Investigators for Skin Adverse Reaction to Drugs; French Study Group on Autoimmune Bullous Skin Diseases. Higher Frequency of Dipeptidyl Peptidase-4 Inhibitor Intake in Bullous Pemphigoid Patients than in the French General Population. *J Invest Dermatol* 2019; **139**: 835-841 [PMID: 30543900 DOI: 10.1016/j.jid.2018.10.045]
51 **Schaffer C**, Buclin T, Jornayvaz FR, Cazzaniga S, Borradori L, Gilliet M, Feldmeyer L. Use of Dipeptidyl-Peptidase IV Inhibitors and Bullous Pemphigoid. *Dermatology* 2017; **233**: 401-403 [PMID: 29040970 DOI: 10.1159/000480498]
52 **Kawaguchi Y**, Shimauchi R, Nishibori N, Kawashima K, Oshitani S, Fujiya A, Shibata T, Ohashi N, Izumi K, Nishie W, Shimizu H, Arima H, Sobajima H. Dipeptidyl peptidase-4 inhibitors-associated bullous pemphigoid: A retrospective study of 168 pemphigoid and 9,304 diabetes mellitus patients. *J Diabetes Investig* 2019; **10**: 392-398 [PMID: 29920976 DOI: 10.1111/jdi.12877]
53 **Guo JY**, Chen HH, Yang YC, Wu PY, Chang MP, Chen CC. The association of dipeptidyl peptidase IV inhibitors and other risk factors with bullous pemphigoid in patients with type 2 diabetes mellitus: A retrospective cohort study. *J Diabetes Complications* 2020; **34**: 107515 [PMID: 31932172 DOI: 10.1016/j.jdiacomp.2019.107515]
54 **Bukvić Mokos Z**, Petković M, Balić A, Marinović B. The association between clinical and laboratory findings of bullous pemphigoid and dipeptidyl peptidase-4 inhibitors in the elderly: a retrospective study. *Croat Med J* 2020; **61**: 93-99 [PMID: 32378375]
55 **Reolid A**, Muñoz-Aceituno E, Rodríguez-Jiménez P, González-Rojano E, Llamas-Velasco M, Fraga J, Daudén E. Bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors. A case series and analysis of cases reported in the Spanish pharmacovigilance database. *Int J Dermatol* 2020; **59**: 197-206 [PMID: 31605541 DOI: 10.1111/ijd.14658]
56 **García M**, Aranburu MA, Palacios-Zabalza I, Lertxundi U, Aguirre C. Dipeptidyl peptidase-IV inhibitors induced bullous pemphigoid: a case report and analysis of cases reported in the European pharmacovigilance database. *J Clin Pharm Ther* 2016; **41**: 368-370 [PMID: 27191539 DOI: 10.1111/jcpt.12397]
57 **Lee SG**, Lee HJ, Yoon MS, Kim DH. Association of Dipeptidyl Peptidase 4 Inhibitor Use With Risk of Bullous Pemphigoid in Patients With Diabetes. *JAMA Dermatol* 2019; **155**: 172-177 [PMID: 30624566 DOI: 10.1001/jamadermatol.2018.4556]
58 **Carnovale C**, Mazhar F, Arzenton E, Moretti U, Pozzi M, Mosini G, Leoni O, Scatigna M, Clementi E, Radice S. Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: a pharmacovigilance-pharmacodynamic/pharmacokinetic assessment through an analysis of the vigibase®. *Expert Opin Drug Saf* 2019; **18**: 1099-1108 [PMID: 31519110 DOI:

10.1080/14740338.2019.1668373]
59 **Béné J**, Moulis G, Bennani I, Auffret M, Coupe P, Babai S, Hillaire-Buys D, Micallef J, Gautier S; French Association of Regional PharmacoVigilance Centres. Bullous pemphigoid and dipeptidyl peptidase IV inhibitors: a case-noncase study in the French Pharmacovigilance Database. *Br J Dermatol* 2016; **175**: 296-301 [PMID: 27031194 DOI: 10.1111/bjd.14601]
60 **Varpuluoma O**, Försti AK, Jokelainen J, Turpeinen M, Timonen M, Huilaja L, Tasanen K. Vildagliptin Significantly Increases the Risk of Bullous Pemphigoid: A Finnish Nationwide Registry Study. *J Invest Dermatol* 2018; **138**: 1659-1661 [PMID: 29427585 DOI: 10.1016/j.jid.2018.01.027]
61 **Hung CT**, Liu JS, Cheng CY, Chung CH, Chiang CP, Chien WC, Wang WM. Increased risk of bullous pemphigoid in dipeptidyl peptidase 4 inhibitors: A nationwide, population-based, cohort study in Taiwan. *J Dermatol* 2020; **47**: 245-250 [PMID: 31885117 DOI: 10.1111/1346-8138.15195]
62 **Montastruc JL**, Sommet A, Bagheri H, Lapeyre-Mestre M. Benefits and strengths of the disproportionality analysis for identification of adverse drug reactions in a pharmacovigilance database. *Br J Clin Pharmacol* 2011; **72**: 905-908 [PMID: 21658092 DOI: 10.1111/j.1365-2125.2011.04037.x]
63 **Silverii GA**, Dicembrini I, Nreu B, Montereggi C, Mannucci E, Monami M. Bullous pemphigoid and dipeptidyl peptidase-4 inhibitors: a meta-analysis of randomized controlled trials. *Endocrine* 2020; **69**: 504-507 [PMID: 32236820 DOI: 10.1007/s12020-020-02272-x]
64 **Phan K**, Charlton O, Smith SD. Dipeptidyl peptidase-4 inhibitors and bullous pemphigoid: A systematic review and adjusted meta-analysis. *Australas J Dermatol* 2020; **61**: e15-e21 [PMID: 31215644 DOI: 10.1111/ajd.13100]
65 **Nishiyama C**, Tateishi C, Hashimoto T, Nishida M, Imanishi A, Shiratori T, Maekawa N, Tsuruta D, Fukai K. Exacerbation of well-controlled bullous pemphigoid by the administration of a dipeptidyl peptidase-4 inhibitor. *Clin Exp Dermatol* 2019; **44**: 830-832 [PMID: 30859617 DOI: 10.1111/ced.13962]
66 **Fania L**, Di Zenzo G, Didona B, Pilla MA, Sobrino L, Panebianco A, Mazzanti C, Abeni D. Increased prevalence of diabetes mellitus in bullous pemphigoid patients during the last decade. *J Eur Acad Dermatol Venereol* 2018; **32**: e153-e154 [PMID: 29055144 DOI: 10.1111/jdv.14649]
67 **Stoian AP**, Sachinidis A, Stoica RA, Nikolic D, Patti AM, Rizvi AA. The efficacy and safety of dipeptidyl peptidase-4 inhibitors compared to other oral glucose-lowering medications in the treatment of type 2 diabetes. *Metabolism* 2020; **109**: 154295 [PMID: 32553739 DOI: 10.1016/j.metabol.2020.154295]
68 **Benzaquen M**, Borradori L, Berbis P, Cazzaniga S, Valero R, Richard MA, Feldmeyer L. Dipeptidyl peptidase IV inhibitors, a risk factor for bullous pemphigoid: Retrospective multicenter case-control study from France and Switzerland. *J Am Acad Dermatol* 2018; **78**: 1090-1096 [PMID: 29274348 DOI: 10.1016/j.jaad.2017.12.038]
69 **Carnovale C**, Tombetti E, Battini V, Mazhar F, Radice S, Nivuori M, Negro E, Tamanini S, Brucato A. Inflammasome Targeted Therapy in Pregnancy: New Insights From an Analysis of Real-World Data From the FAERS Database and a Systematic Review. *Front Pharmacol* 2020; **11**: 612259 [PMID: 33551814 DOI: 10.3389/fphar.2020.612259]
70 **Molina-Guarneros JA**, Sainz-Gil M, Sanz-Fadrique R, García P, Rodríguez-Jiménez P, Navarro-García E, Martin LH. Bullous pemphigoid associated with the use of dipeptidil peptidase-4 inhibitors: analysis from studies based on pharmacovigilance databases. *Int J Clin Pharm* 2020; **42**: 713-720 [PMID: 32140915 DOI: 10.1007/s11096-020-01003-6]
71 **Kridin K**, Bergman R. Association of Bullous Pemphigoid With Dipeptidyl-Peptidase 4 Inhibitors in Patients With Diabetes: Estimating the Risk of the New Agents and Characterizing the Patients. *JAMA Dermatol* 2018; **154**: 1152-1158 [PMID: 30090931 DOI: 10.1001/jamadermatol.2018.2352]
72 **Murakami T**, Yabe D, Inagaki N. Bullous pemphigoid with dipeptidyl peptidase-4 inhibitors: Clinical features and pathophysiology. *J Diabetes Investig* 2019; **10**: 1168-1170 [PMID: 30989811 DOI: 10.1111/jdi.13060]
73 **Ujiie H**, Muramatsu K, Mushiroda T, Ozeki T, Miyoshi H, Iwata H, Nakamura A, Nomoto H, Cho KY, Sato N, Nishimura M, Ito T, Izumi K, Nishie W, Shimizu H. HLA-DQB1*03:01 as a Biomarker for Genetic Susceptibility to Bullous Pemphigoid Induced by DPP-4 Inhibitors. *J Invest Dermatol* 2018; **138**: 1201-1204 [PMID: 29203362 DOI: 10.1016/j.jid.2017.11.023]
74 **Lindgren O**, Varpuluoma O, Tuusa J, Ilonen J, Huilaja L, Kokkonen N, Tasanen K. Gliptin-associated Bullous Pemphigoid and the Expression of Dipeptidyl Peptidase-4/CD26 in Bullous Pemphigoid. *Acta Derm Venereol* 2019; **99**: 602-609 [PMID: 30848289 DOI: 10.2340/00015555-3166]
75 **Mai Y**, Nishie W, Sato K, Hotta M, Izumi K, Ito K, Hosokawa K, Shimizu H. Bullous Pemphigoid Triggered by Thermal Burn Under Medication With a Dipeptidyl Peptidase-IV Inhibitor: A Case Report and Review of the Literature. *Front Immunol* 2018; **9**: 542 [PMID: 29706950 DOI: 10.3389/fimmu.2018.00542]
76 **Usami J**, Takezawa Y. DPP-4 Inhibitor-associated Bullous Pemphigoid in a Hemodialysis Patient. *Intern Med* 2020; **59**: 593 [PMID: 31611529 DOI: 10.2169/internalmedicine.3461-19]
77 **Sánchez López-Muelas B**, Muray Cases S, Illán Gómez F, García Guzmán G, Arjonilla Sampedro ME. Bullous pemphigoid associated with linagliptin treatment in diabetic patients with chronic kidney disease. *Endocrinol Diabetes Nutr (Engl Ed)* 2019; **66**: 338-339 [PMID: 30630688 DOI: 10.1016/j.endinu.2018.11.003]
78 **Mai Y**, Nishie W, Izumi K, Shimizu H. Preferential Reactivity of Dipeptidyl Peptidase-IV Inhibitor-

Associated Bullous Pemphigoid Autoantibodies to the Processed Extracellular Domains of BP180. *Front Immunol* 2019; **10**: 1224 [PMID: 31191560 DOI: 10.3389/fimmu.2019.01224]

79 **Thewjitcharoen Y**, Wanothayaroj E, Thammawiwat C, Porramatikul S, Vorayingyong C, Nakasatien S, Krittiyawong S, Chanprapaph K, Himathongkam T. Clinical Features and Outcomes of Dipeptidyl Peptidase-4 Inhibitor-Associated Bullous Pemphigoid (DPP4i-Associated BP) in Thai Patients. *Case Rep Endocrinol* 2020; **2020**: 8832643 [PMID: 33101737 DOI: 10.1155/2020/8832643]

80 **Douros A**, Rouette J, Yin H, Yu OHY, Filion KB, Azoulay L. Dipeptidyl Peptidase 4 Inhibitors and the Risk of Bullous Pemphigoid Among Patients With Type 2 Diabetes. *Diabetes Care* 2019; **42**: 1496-1503 [PMID: 31182489 DOI: 10.2337/dc19-0409]

81 **Tasanen K**, Varpuluoma O, Nishie W. Dipeptidyl Peptidase-4 Inhibitor-Associated Bullous Pemphigoid. *Front Immunol* 2019; **10**: 1238 [PMID: 31275298 DOI: 10.3389/fimmu.2019.01238]

82 **Nishie W**. Dipeptidyl peptidase IV inhibitor-associated bullous pemphigoid: a recently recognized autoimmune blistering disease with unique clinical, immunological and genetic characteristics. *Immunol Med* 2019; **42**: 22-28 [PMID: 31169082 DOI: 10.1080/25785826.2019.1619233]

83 **Izumi K**, Nishie W, Mai Y, Wada M, Natsuga K, Ujiie H, Iwata H, Yamagami J, Shimizu H. Autoantibody Profile Differentiates between Inflammatory and Noninflammatory Bullous Pemphigoid. *J Invest Dermatol* 2016; **136**: 2201-2210 [PMID: 27424319 DOI: 10.1016/j.jid.2016.06.622]

84 **Horikawa H**, Kurihara Y, Funakoshi T, Umegaki-Arao N, Takahashi H, Kubo A, Tanikawa A, Kodani N, Minami Y, Meguro S, Itoh H, Izumi K, Nishie W, Shimizu H, Amagai M, Yamagami J. Unique clinical and serological features of bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors. *Br J Dermatol* 2018; **178**: 1462-1463 [PMID: 29478242 DOI: 10.1111/bjd.16479]

85 **Kridin K**. Dipeptidyl-peptidase IV inhibitors (DPP4i)-associated bullous pemphigoid: Estimating the clinical profile and exploring intraclass differences. *Dermatol Ther* 2020; **33**: e13790 [PMID: 32506731 DOI: 10.1111/dth.13790]

86 **Gaudin O**, Seta V, Alexandre M, Bohelay G, Aucouturier F, Mignot-Grootenboer S, Ingen-Housz-Oro S, Bernardeschi C, Schneider P, Mellottee B, Caux F, Prost-Squarcioni C. Gliptin Accountability in Mucous Membrane Pemphigoid Induction in 24 Out of 313 Patients. *Front Immunol* 2018; **9**: 1030 [PMID: 29881377 DOI: 10.3389/fimmu.2018.01030]

87 **Patsatsi A**, Kyriakou A, Meltzanidou P, Trigoni A, Lamprou F, Kokolios M, Giannakou A. Bullous pemphigoid in patients with DPP-4 inhibitors at the onset of disease: does this differ from common bullous pemphigoid? *Eur J Dermatol* 2018; **28**: 711-713 [PMID: 30325322 DOI: 10.1684/ejd.2018.3371]

88 **Chijiwa C**, Takeoka S, Kamata M, Tateishi M, Fukaya S, Hayashi K, Fukuyasu A, Tanaka T, Ishikawa T, Ohnishi T, Watanabe S, Tada Y. Decrease in eosinophils infiltrating into the skin of patients with dipeptidyl peptidase-4 inhibitor-related bullous pemphigoid. *J Dermatol* 2018; **45**: 596-599 [PMID: 29411416 DOI: 10.1111/1346-8138.14245]

89 **Bellinato F**, Maurelli M, Schena D, Gisondi P, Girolomoni G. Clinical and immunological profile of patients with dipeptidyl peptidase-4 inhibitor-associated bullous pemphigoid. *G Ital Dermatol Venereol* 2020 [PMID: 32545941 DOI: 10.23736/S0392-0488.20.06562-1]

90 **Schmidt E**, Zillikens D. Pemphigoid diseases. *Lancet* 2013; **381**: 320-332 [PMID: 23237497 DOI: 10.1016/S0140-6736(12)61140-4]

91 **Fania L**, Salemme A, Provini A, Pagnanelli G, Collina MC, Abeni D, Didona B, Di Zenzo G, Mazzanti C. Detection and characterization of IgG, IgE, and IgA autoantibodies in patients with bullous pemphigoid associated with dipeptidyl peptidase-4 inhibitors. *J Am Acad Dermatol* 2018; **78**: 592-595 [PMID: 28987492 DOI: 10.1016/j.jaad.2017.09.051]

92 **Bank U**, Heimburg A, Helmuth M, Stefin S, Lendeckel U, Reinhold D, Faust J, Fuchs P, Sens B, Neubert K, Täger M, Ansorge S. Triggering endogenous immunosuppressive mechanisms by combined targeting of Dipeptidyl peptidase IV (DPIV/CD26) and Aminopeptidase N (APN/ CD13)--a novel approach for the treatment of inflammatory bowel disease. *Int Immunopharmacol* 2006; **6**: 1925-1934 [PMID: 17161345 DOI: 10.1016/j.intimp.2006.09.014]

93 **Melo FJ**, Pinto-Lopes P, Estevinho MM, Magro F. The Role of Dipeptidyl Peptidase 4 as a Therapeutic Target and Serum Biomarker in Inflammatory Bowel Disease: A Systematic Review. *Inflamm Bowel Dis* 2021; **27**: 1153-1165 [PMID: 33295607 DOI: 10.1093/ibd/izaa324]

94 **Moran GW**, O'Neill C, Padfield P, McLaughlin JT. Dipeptidyl peptidase-4 expression is reduced in Crohn's disease. *Regul Pept* 2012; **177**: 40-45 [PMID: 22561447 DOI: 10.1016/j.regpep.2012.04.006]

95 **Pinto-Lopes P**, Afonso J, Pinto-Lopes R, Rocha C, Lago P, Gonçalves R, Tavares De Sousa H, Macedo G, Camila Dias C, Magro F. Serum Dipeptidyl Peptidase 4: A Predictor of Disease Activity and Prognosis in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2020; **26**: 1707-1719 [PMID: 31912883 DOI: 10.1093/ibd/izz319]

96 **Zatorski H**, Sałaga M, Fichna J. Role of glucagon-like peptides in inflammatory bowel diseases-current knowledge and future perspectives. *Naunyn Schmiedebergs Arch Pharmacol* 2019; **392**: 1321-1330 [PMID: 31359088 DOI: 10.1007/s00210-019-01698-z]

97 **Abrahami D**, Douros A, Yin H, Yu OHY, Renoux C, Bitton A, Azoulay L. Dipeptidyl peptidase-4 inhibitors and incidence of inflammatory bowel disease among patients with type 2 diabetes: population based cohort study. *BMJ* 2018; **360**: k872 [PMID: 29563098 DOI: 10.1136/bmj.k872]

98 **Wang T**, Lu W, Li D, Tang H, Yang JY, Buse JB, Stürmer T. Assessing the Association Between

Dipeptidyl Peptidase 4 Inhibitor Use and Inflammatory Bowel Disease Through Drug Adverse Event Reporting. *Diabetes Care* 2019; **42**: e89-e91 [PMID: 31110120 DOI: 10.2337/dc18-1609]

99 **Radel JA**, Pender DN, Shah SA. Dipeptidyl Peptidase-4 Inhibitors and Inflammatory Bowel Disease Risk: A Meta-analysis. *Ann Pharmacother* 2019; **53**: 697-704 [PMID: 30700100 DOI: 10.1177/1060028019827852]

100 **Li G**, Crowley MJ, Tang H, Yang JY, Sandler RS, Wang T. Dipeptidyl Peptidase 4 Inhibitors and Risk of Inflammatory Bowel Disease Among Patients With Type 2 Diabetes: A Meta-analysis of Randomized Controlled Trials. *Diabetes Care* 2019; **42**: e119-e121 [PMID: 31221707 DOI: 10.2337/dc18-1578]

101 **Wang T**, Yang JY, Buse JB, Pate V, Tang H, Barnes EL, Sandler RS, Stürmer T. Dipeptidyl Peptidase 4 Inhibitors and Risk of Inflammatory Bowel Disease: Real-world Evidence in U.S. Adults. *Diabetes Care* 2019; **42**: 2065-2074 [PMID: 31471377 DOI: 10.2337/dc19-0162]

102 **Mascolo A**, Rafaniello C, Sportiello L, Sessa M, Cimmaruta D, Rossi F, Capuano A. Dipeptidyl Peptidase (DPP)-4 Inhibitor-Induced Arthritis/Arthralgia: A Review of Clinical Cases. *Drug Saf* 2016; **39**: 401-407 [PMID: 26873369 DOI: 10.1007/s40264-016-0399-8]

103 **Saito T**, Ohnuma K, Suzuki H, Dang NH, Hatano R, Ninomiya H, Morimoto C. Polyarthropathy in type 2 diabetes patients treated with DPP4 inhibitors. *Diabetes Res Clin Pract* 2013; **102**: e8-e12 [PMID: 23937822 DOI: 10.1016/j.diabres.2013.07.010]

104 **Sayiner ZA**, Okyar B, Kısacık B, Akarsu E, Özkaya M, Araz M. DPP-4 INHIBITORS INCREASE THE INCIDENCE OF ARTHRITIS/ARTHRALGIA BUT DO NOT AFFECT AUTOIMMUNITY. *Acta Endocrinol (Buchar)* 2018; **14**: 473-476 [PMID: 31149299 DOI: 10.4183/aeb.2018.473]

105 **Rai P**, Zhao X, Sambamoorthi U. The Association of Joint Pain and Dipeptidyl Peptidase-4 Inhibitor Use Among U.S. Adults With Type-2 Diabetes Mellitus. *J Pain Palliat Care Pharmacother* 2018; **32**: 90-97 [PMID: 30676844 DOI: 10.1080/15360288.2018.1546789]

106 **Hou WH**, Chang KC, Li CY, Ou HT. Dipeptidyl peptidase-4 inhibitor use is associated with decreased risk of fracture in patients with type 2 diabetes: a population-based cohort study. *Br J Clin Pharmacol* 2018; **84**: 2029-2039 [PMID: 29766544 DOI: 10.1111/bcp.13636]

107 **Men P**, He N, Song C, Zhai S. Dipeptidyl peptidase-4 inhibitors and risk of arthralgia: A systematic review and meta-analysis. *Diabetes Metab* 2017; **43**: 493-500 [PMID: 28778563 DOI: 10.1016/j.diabet.2017.05.013]

108 **Douros A**, Abrahami D, Yin H, Yu OHY, Renoux C, Hudson M, Azoulay L. Use of Dipeptidyl Peptidase-4 Inhibitors and New-onset Rheumatoid Arthritis in Patients with Type 2 Diabetes. *Epidemiology* 2018; **29**: 904-912 [PMID: 30028343 DOI: 10.1097/EDE.0000000000000891]

109 **Kathe N**, Shah A, Said Q, Painter JT. DPP-4 Inhibitor-Induced Rheumatoid Arthritis Among Diabetics: A Nested Case-Control Study. *Diabetes Ther* 2018; **9**: 141-151 [PMID: 29236221 DOI: 10.1007/s13300-017-0353-5]

110 **Charoenngam N**, Rittiphairoj T, Ponvilawan B, Ungprasert P. Use of dipeptidyl peptidase-4 inhibitors is associated with a lower risk of rheumatoid arthritis in patients with type 2 diabetes mellitus: A systematic review and meta-analysis of cohort studies. *Diabetes Metab Syndr* 2021; **15**: 249-255 [PMID: 33465685 DOI: 10.1016/j.dsx.2020.12.042]

111 **Sasaki T**, Hiki Y, Nagumo S, Ikeda R, Kimura H, Yamashiro K, Gojo A, Saito T, Tomita Y, Utsunomiya K. Acute onset of rheumatoid arthritis associated with administration of a dipeptidyl peptidase-4 (DPP-4) inhibitor to patients with diabetes mellitus. *Diabetol Int* 2010; 90-92 [DOI: 10.1007/s13340-010-0010-y]

112 **Yokota K**, Igaki N. Sitagliptin (DPP-4 inhibitor)-induced rheumatoid arthritis in type 2 diabetes mellitus: a case report. *Intern Med* 2012; **51**: 2041-2044 [PMID: 22864134 DOI: 10.2169/internalmedicine.51.7592]

113 **Padron S**, Rogers E, Demory Beckler M, Kesselman M. DPP-4 inhibitor (sitagliptin)-induced seronegative rheumatoid arthritis. *BMJ Case Rep* 2019; **12** [PMID: 31444259 DOI: 10.1136/bcr-2018-228981]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1442-1462

DOI: 10.4239/wjd.v12.i9.1442

ISSN 1948-9358 (online)

REVIEW

Cellular targets in diabetic retinopathy therapy

María Lucía Rodríguez, Iván Millán, Ángel Luis Ortega

ORCID number: María Lucía Rodríguez 0000-0003-1809-4693; Iván Millán 0000-0003-2049-5875; Ángel Luis Ortega 0000-0002-9901-3383.

Author contributions: Ortega AL was responsible for the conceptualization and edition of the manuscript; Rodríguez ML, Millán I and Ortega AL contributed to the literature review, writing and revision of the manuscript; All authors approved the final version.

Conflict-of-interest statement: The authors declare that they have no conflicting interests.

Manuscript source: Invited manuscript

Specialty type: Ophthalmology

Country/Territory of origin: Spain

María Lucía Rodríguez, Ángel Luis Ortega, Department of Physiology, Faculty of Pharmacy, University of Valencia, Burjassot 46100, Valencia, Spain

Iván Millán, Neonatal Research Group, Health Research Institute La Fe, Valencia 46026, Valencia, Spain

Corresponding author: Ángel Luis Ortega, PhD, Associate Professor, Department of Physiology, Faculty of Pharmacy, University of Valencia, Av. Vicente Andrés Estellés, s/n, Burjassot 46100, Valencia, Spain. angel.ortega@uv.es

Abstract

Despite the existence of treatment for diabetes, inadequate metabolic control triggers the appearance of chronic complications such as diabetic retinopathy. Diabetic retinopathy is considered a multifactorial disease of complex etiology in which oxidative stress and low chronic inflammation play essential roles. Chronic exposure to hyperglycemia triggers a loss of redox balance that is critical for the appearance of neuronal and vascular damage during the development and progression of the disease. Current therapies for the treatment of diabetic retinopathy are used in advanced stages of the disease and are unable to reverse the retinal damage induced by hyperglycemia. The lack of effective therapies without side effects means there is an urgent need to identify an early action capable of preventing the development of the disease and its pathophysiological consequences in order to avoid loss of vision associated with diabetic retinopathy. Therefore, in this review we propose different therapeutic targets related to the modulation of the redox and inflammatory status that, potentially, can prevent the development and progression of the disease.

Key Words: Diabetic retinopathy; Oxidative stress; Inflammation; Cellular target; Diabetic macular edema

Core Tip: The identification of potential therapeutic targets related to oxidative stress and low chronic inflammation induced in diabetic retinopathy (DR) may be crucial in developing therapeutic approaches for preventing the development of DR. Hence, we focus on the antioxidant role of nuclear factor erythroid 2-related factor 2, low and chronic inflammatory conditions developed in DR, modulation of lipid peroxidation,

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 26, 2021
Peer-review started: February 26, 2021
First decision: April 20, 2021
Revised: May 8, 2021
Accepted: August 3, 2021
Article in press: August 3, 2021
Published online: September 15, 2021

P-Reviewer: Duerfeldt AS
S-Editor: Gong ZM
L-Editor: Filipodia
P-Editor: Xing YX

activation of glucagon-like peptide-1 receptor, the classical biochemical pathways altered under hyperglycemia, and epigenetic alterations.

Citation: Rodríguez ML, Millán I, Ortega ÁL. Cellular targets in diabetic retinopathy therapy. *World J Diabetes* 2021; 12(9): 1442-1462
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1442.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1442

INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with hyperglycemia. The global prevalence of diabetes in adults 20-79 years of age, including both type 1 and type 2 diabetes, diagnosed, and undiagnosed, was estimated at 463 million in 2019. Based on the estimation, by 2045 a projected 700 million adults will have diabetes[1]. Although diabetes is a pathology with multiple systemic consequences, the loss of metabolic control in particular is not effectively controlled in many patients and that triggers the development of long-term damage of various organs, including the retina. In fact, diabetic retinopathy (DR) is the greatest cause of preventable blindness in the working age population and the most frequent ocular pathology caused by diabetes[2]. Its prevalence increases as the number of diabetic patients increases, depends on the duration of the disease, and on inadequate glycemic control. It has also been associated with the presence of hypertension, and was estimated to affect 2.6 million people in 2015 and projected to affect 3.2 million adults by 2020[2,3].

Cellular aerobic metabolism induces the physiological production of reactive oxygen species (ROS), which are molecular actors in the regulation of normal cell signaling. The production of ROS is countered by antioxidant enzymatic and nonenzymatic machinery enabling a homeostatic redox balance. However, the balance may be easily altered by a pathological condition. Glucose metabolism linked to reduction in antioxidant defenses triggers an oxidant environment in body tissues exposed to chronic hyperglycemia[4]. Although the blood-retinal barrier (BRB) makes the tissue a privileged place, as the retina is protected from the escape of circulating toxins, its cellular components are extremely sensitive to alterations in oxygen level[5]. In fact, the imbalance in redox homeostasis induced by diabetes triggers neuronal retinal cell death and pericyte cell death followed by an increase in the vascular permeability, and cumulative molecular damage leading to development and progression of DR to advanced stages[2,6-8]. Because of this, oxidative stress is considered a major cause of DR development.

The complex and extensive harmful effects of ROS contribute to the neurovascular complications observed in the retina. In this review, we focus on the main cellular targets affected by oxidative stress. The affects lead to cellular dysfunction and are potential therapeutic targets to avoid the development and progression of DR. Among hyperglycemia abnormalities closely associated with oxidative stress we highlight the key role of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and its importance in the modulation of oxidative stress, the increased accumulation of advanced glycation end products (AGEs), polyol and hexosamine pathways and protein kinase C activation, lipid peroxidation, activation of glucagon-like peptide-1 receptor (GLP1R), and alteration of the epigenetic status[2,9].

THE IMPORTANCE OF LOOKING FOR NEW THERAPEUTIC TARGETS IN DIABETIC RETINOPATHY: ACTUAL THERAPIES

As DR is most often asymptomatic, the pathology can be significantly advanced when the patients suffer a loss of vision. Therefore, an early diagnosis is necessary to detect the first signs before the disease progresses to more serious stages[10]. In the early stages of DR, with the objective being to prevent its development or stop its progression, the only therapeutic strategy is a strict control of risk factors, mainly blood glucose and blood pressure[11]. Overall, treatment is applicable in very advanced stages of the pathology and when DR affects the macula, triggering diabetic

macular edema (DME), which is the most common cause of blindness induced by chronic hyperglycemia. The main interventions for DR and DME include ocular and systemic pharmacotherapy, with conventional laser therapy as the secondary treatment option, although it remains the first-line option when the cost and burden of drug treatment are considered, and vitreoretinal surgery[12,13]. The decision to use one or other of the treatments depends on the specific clinical situation of the patient.

Pharmacotherapy

The evidence that inflammation plays a critical role when DR affects the macula, triggering DME, has opened new avenues and targets for developing new treatments. There are many anti-inflammatory therapies, such as intravitreal glucocorticoids, topical nonsteroidal anti-inflammatory drugs (NSAIDs), inflammatory molecule inhibitors, renin-angiotensin system blockers, and natural anti-inflammatory therapies that can reduce the use of anti-neovascularizing agents in the treatment of DR, but more studies are needed[6]. Despite these therapies, the most important class of drugs are those that decrease the effects of vascular endothelial growth factor (VEGF), and corticosteroids[14].

Anti-VEGF treatment

Intravitreal injections of anti-VEGF drugs are the treatment par excellence for DR and its angiogenic complications. The monoclonal antibody ranibizumab (Lucentis®), the long-acting antibody bevacizumab (Avastin®), the aptamer pegaptanib (Macugen®), and the recombinant fusion protein aflibercept (Eylea®) are the anti-VEGF agents most frequently used to treat DME. The drugs, do not affect the pathogenesis of DR and must be administered for years as frequent intravitreal injections, estimated to be around 12-15 injections in the first 3 years of treatment[15-17]. They are also associated with adverse effects such as susceptibility to the development of endophthalmitis, vitreous floaters, and transient increase in intraocular pressure[18].

Administration of corticosteroids

Acknowledging the role of inflammatory processes in the pathogenesis of DR, anti-inflammatory drugs are an attractive option for the treatment of the disease[19]. Hence, the anti-inflammatory and anti-angiogenic effects associated with corticosteroids have led to their inclusion in the treatment of DR and DME. Several mediators of inflammation are upregulated in DR. The mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and VEGF have a key role in pathogenesis and can be modulated by corticosteroids[20]. The effects of corticosteroids include the reduction of vascular permeability and the breakdown of the BRB, prevention of leukocyte adhesion to vascular walls, suppression of VEGF gene transcription and translation, and the rapid decrease of DME[21].

The main mode of administration is intravitreal injection, which avoids the limitations of BRB. However, treatment-associated adverse effects of steroids include cataracts, high intraocular pressure, and glaucoma. Less frequent side effects, such as vitreous hemorrhage, retinal detachment, and endophthalmitis are related to the injection[22,23]. Moreover, short-term effects and transient efficacy are limiting factors in the application of this treatment, and new injections it is often required at various time intervals based on the steroid half-life. Currently, DME is treated with several different steroids, including fluocinolone, triamcinolone, and dexamethasone[24]. Side effects associated with chronic use and the need for repeat injections have brought about the development of new methods of intraocular administration, such as sustained release from an intravitreal implant. Slow-release formulations are used to avoid reinjection, which allows the use small quantities of corticosteroids, which results in fewer side effects[25]. Both nonbiodegradable and biodegradable devices are available. In biodegradable devices, the polymers degrade slowly over time, thus avoiding the need for surgery to remove the implant, in contrast to the nonbiodegradable ones[26].

Laser therapy

Over the past 30 years, the most successful means of delaying the progression of DR has been focal, grid, or panretinal photocoagulation (PRP) laser treatment[27]. In the treatment of proliferative DR, the use of PRP reduces oxygen requirements and decreases retinal neovascularization. PRP eliminates the hypoxic retina and/or increases the diffusion of O_2 found in the choroid to supplement the affected retinal circulation. Furthermore, laser therapy decreases the formation of vasoproliferative agents and inhibits neovascularization. The procedure uses scattered laser spots of

200-500 μm in the peripheral retina, avoiding the central macula. In the case of DME, the laser spots are applied in the regions of the macular area with microaneurysms in order to decrease exudation[28].

The use of laser therapy plays an important role in controlling diabetes mellitus-related retinal disease and is generally used in situations in which the use of pharmacotherapy is contraindicated, there is poor monitoring of patient visits, if the response to anti-VEGF treatment is ineffective, or if the patient is pregnant[13]. Although PRP treatment can effectively control neovascularization and prevent blindness, it is unable to restore vision and has its own damaging effects on vision[29]. The destructive capacity of laser therapy permanently damages the cells, thus producing side effects that affect the deterioration of vision, such as loss of contrast sensitivity, decreased night vision, color vision, visual field, and the appearance of DME[30]. In certain situations, the prior use of laser photocoagulation and intravitreal anti-VEGF agents induce fibrotic changes in preexisting retinal neovascularization, causing tractional retinal detachment with the need for early surgery to avoid permanent blindness[31].

Surgical intervention

Surgical intervention is used in cases that show no response to pharmacological treatment, laser, or combined therapy, as well as in the most severe cases of DME. Therefore, vitrectomy is indicated in situations such as vitreous hemorrhages that do not disappear, tractional detachment of the retina in proliferative DR, and anomalies in the vitreoretinal interface that prevent the resolution of DME[32]. To facilitate the intervention, an intravitreal injection of an anti-VEGF agent like bevacizumab, ranibizumab, or aflibercept, is included as a preoperative complement in patients with no contraindications, as they cause a rapid involution of active neovascularization[33].

Surgical vitrectomy entailing the removal of most of the vitreous body and hyaloid membrane has shown a series of benefits, such as decreased growth of fibrovascular membranes caused by the absence of proliferation in scaffolds, increased intraocular cytokine turnover, and removal of mechanical barriers that hinder the exit of metabolites and fluids and obstruct intravitreal drug delivery through intraretinal penetration[34]. However, because of individual variability in the surgical anatomy that each case presents, diabetic vitrectomy continues to be one of the most difficult conditions to treat. In addition, it has postoperative consequences such as rhegmatogenous retinal detachment, development of cataracts, proliferation of diabetic fibrovascular membranes, vitreous hemorrhage, appearance of epiretinal membranes, elevated intraocular pressure, and neurovascular glaucoma[35-37].

All these treatments are expensive, uncomfortable for the patient, have limited effectiveness because of the administration protocols, and are associated with a significant number of side effects[38]. Despite benefits in slowing the progression of DR and improving vision, damage to the retinal blood vessels the function of neuronal cells is irreversible[2]. Even after the advances made in the treatment of retinopathy, many patients still progress to advanced stages of disease. It is necessary, therefore, to investigate new therapeutic approaches capable of both delaying and preventing the appearance of the first stages of DR.

RETINOCELLULAR ALTERATIONS IN DIABETIC RETINOPATHY DEVELOPMENT

Oxidative stress has been defined as an imbalance between the production and the removal of free radicals, which leads to their accumulation. The most common free radicals are ROS, such as the superoxide anion (O_2•−), hydrogen peroxide (H_2O_2), the peroxyl radical (ROO•), and the hydroxyl radical (•OH). These oxygen-derived molecules are very reactive and generally toxic to cells[39,40]. Under physiological conditions, free radicals are normally and continuously produced. Low to moderate levels of free radicals support normal cellular metabolism, proliferation, differentiation, immune system regulation, and vascular remodeling[2,41]. Intracellular ROS levels are controlled by enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and nonenzymatic species like glutathione (GSH), thioredoxin, NADPH, α-tocopherol, ascorbic acid and β-carotene, which constitute an antioxidant defenses system. Oxidative stress leads to the accumulation of ROS because of excessive production or inefficient removal. ROS can modify the structure of proteins, lipids, carbohydrates, and nucleic acids, thus affecting their function[2].

Oxidative stress plays a critical role in the pathogenesis of DR. The retina has high metabolic activity, high oxygen partial pressure from the blood in the choroid, and it is highly exposed to bright light. All these factors, together with the oxidative environment induced by hyperglycemia in diabetes, cause an increased level of ROS in the retina[42-44]. ROS overproduction in the retina triggers cell death, retinal ischemia, retinal neovascularization, and DME[45]. Furthermore, various mutations of detoxifying enzymes that have a significant role in DR development, such as CAT or SOD, have been reported[46]. This suggest that hyperglycemia-induced oxidative stress is one of the main causes of DR[45,47,48]. Therefore, some treatments of DR are based in the inhibition of ROS generation, neutralization of free radicals, or the reinforcement of the antioxidant defense system[39].

Oxidative stress and Nrf2

Nrf2 is a transcription factor that activates the expression of various detoxifying and antioxidant defense genes in response to oxidative stress[49,50]. The functional activity of Nrf2 depends on whether it is located in the nucleus or in the cytoplasm. Under physiological conditions and in the absence of oxidative stress, Kelch-like enoyl-CoA hydratase-associated protein 1 (Keap1) sequesters Nrf2 in the cytoplasm and mediates its rapid ubiquitination and degradation, suppressing its transcriptional activity[51, 52]. When there is an accumulation of ROS, Keap1 changes its conformational structure and releases Nrf2, which then translocates from the cytoplasm to the nucleus. Once there, Nrf2 binds to the antioxidant response element of a promoter region to initiate transcription of several genes encoding heme oxygenase 1 (HO-1), NAD(P)H dehydrogenase (quinone) 1, thioredoxin reductase, peroxiredoxins, SOD, CAT, GPx, GSH reductase (GR), GSH S-transferase (GST), and glutamate-cysteine ligase (GCL). These enzymes contribute to elimination of ROS and play a critical defensive role in cell homeostasis[2,50,53]. Nrf2 is an important cellular pathway that protects against oxidative stress in the retina[54,55]. In diabetes, Nrf2 increases in the retina but so does keap-1, which prevents Nrf2 from reaching the nucleus. Thus, Nrf2 nuclear level is decreased and the antioxidant defense system is compromised[55-57]. As a result, the activity of Nrf2-associated antioxidant enzymes like SOD, GR, GPx, and CAT in diabetes patients or glutamate-cysteine ligase in rat diabetes models[55,58,59]. Thus, the increased risk of developing DR in diabetes patients results from reduced antioxidant capability and the oxidative environment generated by hyperglycemia[2]. These studies also suggest that Keap1 knockdown would release Nrf2, which would move to the nucleus and activate the antioxidant defense system[54,55]. In addition to the regulation of the antioxidant response, Nrf2 regulates the inflammatory response in diabetes[60]. The response is mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclooxygenase-2 (COX-2). When Nrf2 activity is reduced, there is an increase of proinflammatory cytokines because of the induction of NF-κB, which is associated with capillary cell apoptosis in diabetes *via* the overexpression of proapoptotic Bax or TNF-α[61-63]. In an experimental model of streptozotocin-induced diabetes, rutin, a flavonoid derivative of quercetin, protected against neuron damage in diabetes *via* the Nrf2/HO-1 and NF-κB signaling pathway, together with its anti-inflammatory action *via* COX-2 inhibition[2,64]. The data suggest that Nrf2 activation could be an important protective mechanism for diabetic complications, making it an especially attractive pharmacological target in the progression of DR[54]. Many studies suggest that natural compounds, including polyphenols, can reduce oxidative stress and inflammation through activating Nrf2 and the consequent antioxidant response[57].

Several publications have described the therapeutic potential of various polyphenols in diabetes, including those in green tea,, resveratrol, curcumin, quercetin, and tannins[65-73]. Pterostilbene (Pter), is a phenol that been shown to prevent early DR alterations *via* Nrf2 activation in an experimental rabbit model[47]. In addition to natural antioxidants, other molecules have been shown to activate Nrf2 in DR. One example is RS9, a derivative of the triterpenoid bardoxolone methyl, which was found to delay retinal degeneration by inhibiting inflammatory responses and increasing intrinsic antioxidant enzymes *via* activation of Nrf2[74]. Another triterpenoid derivative, dihydro-CDDO-trifluoroethyl amide (dh404) has been shown to protect the retina against diabetes-induced damage through the activation of Nrf2[75].

Another therapeutic approach in the treatment of DR is, as suggested above, is the inactivation of Keap1. Triterpenoids, salvianolic acids, and sulforaphane[76-79] have been shown to inactivate Keap1 by covalently modifying its reactive cysteine residues. As a consequence, Nrf2 is activated by its translocation into the nucleus and its downstream target genes are then activated, which prevents or reverts ROS-mediated toxicity[50].

Inflammatory response

Inflammation is a defensive process mediated by the host immune system in response to injury or stress. In DR, acute inflammation normally produces beneficial effects like tissue defense and repair. Chronic inflammation produces structural and molecular alterations in the retina that usually cause tissue damage and cell death[2]. The inflammatory response in the retina is caused by various factors like hyperglycemia, growth factors, AGEs, high levels of circulating or vitreous cytokines and chemokines, and ROS[80]. These factors induce intracellular signaling pathways, including the transcription factor NF-κB, which translocates into the nucleus to initiate the transcription of proinflammatory cytokines *i.e.* TNF-α, IL-1β, and IL-6; proinflammatory proteins such as COX-2 or the inducible isoform of nitric oxide synthetase (iNOS), and chemokines such as monocyte chemoattractant protein-1. The proinflammatory molecules play an important role in the recruitment and activation of monocytes and leukocytes[81,82]. Adhesion of leukocytes to the capillaries of the retina (leukostasis), together with the release of ROS and proinflammatory cytokines, leads to vascular permeability, BRB breakdown, and capillary pericyte loss. Thus, it is clear that chronic inflammation is critical for the development of DR, principally in the early stages[2,27,81,83].

Several studies have shown that there is an increase of proinflammatory molecules in the retina or vitreous humor of diabetic animals and patients. Those reported are VEGF, TNF-α, iNOS, COX-2, prostacyclin, insulin-like growth factor 1, NF-κB, placental growth factor, intercellular adhesion molecule-1, IL-1β, IL-2, IL-6 and IL-8[81,84-86]. The findings highlight the key role of inflammation in the development of DR. The detailed mechanisms involved in the inflammatory response in DR are not clear, but inhibition of some of the inflammatory mediators mentioned in the previous paragraphs has been shown to block DR development in animal models of diabetes[82,87-92]. NSAIDs, anti-VEGF, and anti-TNF-α agents diminish the progression of DR in humans because of their anti-inflammatory properties[93]. Systemic administration of specific COX-2 inhibitors could be a possible therapy, although COX-2 inhibitors increase the incidence of heart attack and stroke[94]. Nevertheless, in preclinical studies, topical administration was shown to reduce the signs of DR[95-97]. More studies on the beneficial effects of these molecules are needed.

Tetracyclines, such as minocycline and doxycycline, have immunomodulatory properties that include inhibiting the production of NO, COX, prostaglandins, IL-1β, TNF-α, and caspases[98-100]. In a single-center phase I/II clinical trial in five patients with DME, treatment with minocycline resulted in improved visual function, reduced central DME, and vascular leakage[101]. In another clinical trial, patients with severe nonproliferative or non-high-risk proliferative DR were treated with doxycycline, which resulted in an improvement of perimetric parameters compared with patients who received a placebo[102]. IL-6 is one of the most important proinflammatory cytokines present in the vitreous of DR patients. Various clinical studies have investigated the effect on DR of two IL-6 inhibitors, an antibody against IL-6 (EBI-031, clinicaltrials.gov ID: NCT02842541) and an antibody against the IL-6 receptor (tocilizumab, clinicaltrials.gov ID: NCT02511067) in patients with DME. Although they have not yet concluded, the studies have shown that IL-6 inhibitors can be effective in the management of non-infectious uveitis. Therefore, the roles of IL-6 inhibition could be more widely investigated in the management of retinal vascular diseases and non-uveitic DME[103]. The effect of anti-TNF-α therapy has also been studied in a few clinical cases but there are no conclusive data about the effects of these inhibitors in DR or DME[104]. The same is true of canakinumab, a selective IL-1β antibody[105].

Alteration of biochemical pathways

It has long been accepted that hyperglycemia induces the alteration of the biochemical pathways, such as an increased flux of advanced glycation end products/receptors (AGE/RAGE), the polyol pathway, protein kinase C (PKC) activation, the hexosamine pathway, and unbalancing redox status. The induction of ROS stimulates a low chronic inflammatory state that contributes to the development and progression of neurovascular dysfunction in DR[2]. The regulation of these molecular pathways therefore offers potential targets against DR.

Glucose and products generated by carbohydrate metabolism are able to transform proteins, lipids, or nucleic acids by glycation, triggering the formation of AGEs, a synthesis that is accelerated in the presence of ROS and redox-active transition metals [106,107]. In addition, the production of AGEs stimulates increased formation of oxidative species, resulting in positive feedback that contributes to the progression of the complications of diabetes[108]. AGEs have severe effects on retinal tissue, such as

aberrant extracellular crosslinking of extracellular matrix proteins and increased vascular stiffness, which disturbs normal vascular function. AGEs also bind to various receptors in the plasma membrane (RAGE) and activate intracellular signaling cascades that trigger the release of proinflammatory cytokines and proangiogenic factors, with evident damage of neurovascular retinal structures[109]. As AGEs formation is closely related to oxidative stress, modulation of the antioxidant machinery is an attractive approach for preventing the development and progression of DR. The administration of curcumin to diabetic rats was shown to improve redox imbalance in the retina[110] and protect against effects of glycation[111]. Epigallocatechin 3-gallate, quercetin, kaempferol, and resveratrol are other examples of natural antioxidants able to diminish the production of AGEs[112-115]. In addition, drugs such as aminoguanidine have been shown to be effective inhibitors of AGE formation and to inhibit the development of DR[116,117]. However, adverse side- effects preclude their use in humans[108]. Aragonès *et al*[108] in their latest excellent paper, review the benefits of enhancing the detoxifying activity of the glyoxalase system, a main mechanism for detoxifying the intermediates and precursors of AGEs formation, to avoid glycation-derived damage in DR.

Under normoglycemic conditions, glucose is metabolized by the glycolytic pathway. However, in chronic hyperglycemia, excess glucose is reduced to sorbitol by the enzymatic action of aldose reductase. Sorbitol is then converted to fructose by sorbitol dehydrogenase. The two enzymes constitute an alternative route of glucose metabolism known as the polyol pathway, which is an important source of oxidative stress and AGE production[2]. In addition, sorbitol increases cellular osmolarity, triggering osmotic damage and cell death in retinal capillaries[118,119]. Although clinical trials have been inconclusive in the use of polyol pathway inhibitors to treat DR, its use as a potential therapeutic target in DR should not be ruled out[120,121]. In fact, the benefits of polyphenols for DR treatment is extended to inhibition of the polyol pathway. For example, Pter, a natural stilbene analog of resveratrol, in addition to promoting antioxidant defenses *via* Nrf2, inhibited aldose reductase and AGEs formation in a galactosemic rat model[47,122]. Another alternative route to glycolysis in hyperglycemia is the hexosamine pathway. Glutamine fructose-6-phosphate amidotransferase (GFAT) converts fructose-6-phosphate to N-acetylglucosamine-6-phosphate, which is a substrate of O-N-Acetyl-GluN transferase (OGT) and converted to uridine-5-diphosphate-N-acetylglucosamine (UDP-GlucNAc), a precursor of glycoproteins, glycolipids, proteoglycans, and glycosaminoglycans[123]. High levels of glucose and N-acetylglucosamine-6-phosphate activity inhibit glucose-6-phosphate dehydrogenase and low NADPH-dependent GSH production, triggering an increase in the level of H_2O_2[124]. Glucosamine administration or overexpression of GFAT also leads to H_2O_2 accumulation, highlighting the role of the hexosamine pathway in oxidative stress[125]. Moreover, OGT activity has been associated with altered *TGFβ* gene expression, which induces NADPH oxidase (NOX) activation, suppression of the antioxidant system, and mitochondrial ROS production[126-128]. In fact, antioxidant treatment has shown beneficial effects against some adverse consequences of the hexosamine pathway[125]. Various inhibitors of the hexosamine pathway, such as the antineoplastic azaserine, the anthraquinone rhein, and the lipid-soluble thiamine derivative benfotiamine, have been evaluated in experimental animal models. In addition to the hexosamine pathway, those agents inhibit AGE formation and the PKC pathway[129-131]. However, the effectiveness of this therapeutic approach in DR has not been shown in clinical trials.

Inhibition of the PKC pathway is of interest. PKCs comprise a family of cAMP-dependent protein kinases with multiple isoforms involved in the regulation of other proteins[2]. PKCs are activated when the second messenger is bound to its regulatory domain. Phosphatidylserine, calcium, and diacylglycerol (DAG) or phorbol esters are activators of PKC-α, β1, β2, and γ. Phosphatidylserine, DAG or phorbol 12-myristate 13-acetate (PMA) activate PKC-δ, ε, θ, and η, while PKC-ζ and -ι/λ are not activated by calcium, DAG or PMA[132]. Cysteine residues are abundant in the PKC structure which makes the regulatory domain susceptible to redox modulation[2]. In fact, hyperglycemia can activate some PKC isoforms directly through DAG, or indirectly by the oxidative stress generated through AGE production and the polyol pathway[133, 134]. PKC contributes to redox injury of retinas exposed to chronic hyperglycemia at different levels, triggering the signs of DR. For example, PKC-β is an activator of NOX, and the overproduction of $O_2^{\bullet-}$ increases the formation of peroxynitrite to induce endothelial changes[135-137]. PKC-δ is involved in the death of capillary cells and perycites, with subsequent formation of microaneurysms[138,139]. PKC-β and PKC-ζ are involved in VEGF-dependent changes of the retinal barrier[140]. Moreover, PKC induces the overexpression of plasminogen activator-1 and the activation of NF- κB in

vascular smooth muscle and endothelial cells, pericytes, and mesangial cells[134]. Inhibition of PKC has been considered as an effective approach to treat DR. The highly selective PKC-β inhibitor, ruboxistaurin mesylate, is one of the most studied. Initial clinical studies showed its potential in the prevention of vision loss induced by DR [141]. However, in 2007 the European Medicines Agency declared a minimum benefit in the treatment of moderately severe to severe non-proliferative DR[142]. In any case, knowledge of the role of the various isoforms of PKC is incomplete and offers another therapeutic target to be considered.

Lipid alterations

Lipids play a crucial role in the maintenance and development of retinal functions. The plasma membranes of the outer segments of retina photoreceptors contain high levels of polyunsaturated fatty acids (PUFAs). The most abundant PUFAs in the retina are ω3-docohexaenoic (DHA), ω3-eicosapentaenoic (EPA), and ω6-arachidonic (AA), with DHA being predominant[143-146]. The functions of PUFAs in the retina have been demonstrated in numerous studies. PUFA supplementation has protective and therapeutic effects against proliferative and degenerative retinal diseases, possibly resulting from their antioxidant and anti-inflammatory properties[147-150]. In addition, DHA deficiency has been associated with structural and functional abnormalities in the visual system[149]. ROS formed during oxidative stress can oxidize PUFAs because of the presence of susceptible carbon double bonds in the molecular structure[44,150]. The free radical chain reaction results in lipid peroxidation and acts to amplify the generation of lipid radical species, causing PUFA degradation into a variety of potentially harmful oxidation products[42,146]. The increase of ROS in DR, together with the high PUFA content in the membranes of the photoreceptors, triggers an increase of lipid peroxidation[42,44,151]. In fact, patients with DR have higher lipid peroxidation than those without retinal disease[151-153]. Moreover, a number of published papers indicate that lipid peroxidation has serious pathophysiological effects that contribute to the development of DR[149,154-158], and there is increasing evidence of the importance of products of lipid peroxidation as mediators in the development of neovascularization in DR[149,159,160].

The role of lipid peroxidation in DR has been extensively studied, the determination of lipid peroxidation products, including aldehydes such as 4-hydroxynonenal (4-HNE) or malondialdehyde (MDA), and F_2-isoprostanes (F_2-IsoP) such as 8-iso-$PGF_{2\alpha}$ in plasma, urine, or the retina[161]. 4-HNE, an end product of nonenzymatic lipid peroxidation of ω6 PUFAs like linoleic acid and amino acids, has been shown to be extremely reactive with DNA, RNA, and proteins in the retina[39,162-165]. Zhou *et al* [166] reported that 4-HNE activates the canonical WNT pathway through oxidative stress in a rat model, playing a pathogenic role in the development of DR. Previous studies by that group have shown that blockade of WNT signaling attenuated retinal inflammation and neovascularization in DR in humans and animal models[167]. In fact, inhibition of the WNT pathway by peroxisome proliferator-activated receptor alpha (PPARα) overexpression induced anti-inflammatory and antifibrosis effects [168]. The retinal protective role of PPARα has been demonstrated both *in vitro* and *in vivo*. Chronic hyperglycemia in experimental animal models of diabetes or treatment of retinal cell lines with high glucose concentrations reduces PPARα mRNA and protein expression levels. The use of PPARα agonists, such as fenofibrate, have been discussed as a treatment of DR by preventing microvascular damage[169,170]. Overexpression of PPARα was found to reduce ROS production, apoptosis induced by oxidative stress, and downregulation of NOX4 expression[171]. It also inhibited cell proliferation, migration, and had anti-angiogenic effects[172]. The data suggest that the WNT pathway and PPARα represent a new target for therapeutical intervention of DR[167].

Other studies suggest that 4-HNE retinal damage in DR could result from the induction of p53-mediated apoptosis in retinal pigment epithelial cells[173]. It has also been shown that 4-HNE attenuated β_2-adrenoceptor-mediated vasodilation of rat retinal arterioles, which would contribute to the retinal vascular dysfunction observed in patients with diabetes mellitus[174].

Several studies of possible new treatments of DR have focusing on protecting effects damage associated with 4-HNE. Chiang *et al*[175] reported that fucoxanthin, a marine carotenoid extracted from seaweed, effectively protected against the effects of 4-HNE- and high glucose-induced DR in ARPE-19 human retinal epithelial cells through the antioxidant ability of this compound. Pter was also shown to reduce 4HNE levels in the retina of a rabbit model of type 1 diabetes mellitus, preventing early DR alterations [47]. MDA is a product of the peroxidative decomposition of PUFAs. It is a highly reactive molecule that forms covalent bonds with the amino acids of endogenous

proteins[42,48]. MDA possesses cytotoxic, hepatotoxic, mutagenic, and genotoxic properties, and can alter proteins, DNA, RNA, and many other biomolecules[176,177]. MDA concentration as a final product of lipid oxidation is routinely determined by thiobarbituric acid assay or chromatography-mass spectrometry[176-178]. There are no studies of its mechanism of action in DR. It has only been used as a biomarker of lipid peroxidation in biological samples.

Since its discovery, F_2-IsoP has become one of the most reliable biomarkers of lipid peroxidation and oxidative stress in in vitro studies and in animal models[179-181]. F_2-IsoP comprises a family of prostaglandin-like compounds produced by nonenzymatic peroxidation of amino acids in membrane phospholipids[181]. One of the most studied F_2-IsoP is 8-iso-$PGF_{2\alpha}$ (also known as 8-epi-$PGF_{2\alpha}$ or 15-F_{2t}-isoprostane), which has been shown to be involved in inflammation and immunity in various diseases[48,181]. In DR, 8-iso-$PGF_{2\alpha}$ is produced by COX activity and enzymatic oxidation of $PGF_{2\alpha}$[182]. It has been shown to be a potent vasoconstrictor in the retina by increasing thromboxane A_2 formation through the activation of Ca^{2+} influx[182-184].

Further research is needed to clarify the pathophysiological activity of PUFA derivatives in DR. Nevertheless, it seems that inhibition of the formation of these highly cytotoxic molecules could be a possible therapeutic strategy for the management of DR. In fact, Pter has been recently reported to be able to restore the control levels of a large group of specific neuronal and retinal lipid peroxidation markers in diabetic rabbits[185]. This suggests that this polyphenol could protect the retina, preventing early lipid peroxidation damage in DR development.

GLP1R

In recent years, new pharmacological therapies have been developed as effective treatments for type 2 diabetes. Glucagon-like peptide 1 receptor agonists (GLP1RAs) have emerged as a safe treatment, and some agonists have been incorporated into the clinical guidelines of the American Diabetes Association and the European Association for the Study of Diabetes. Furthermore, preclinical studies have shown the benefits of GLP1R activation on diabetic vascular complications such as DR[186]. Actually, the benefits are broad. GLP1R activation, independent of homeostatic glycemic control, can reduce the harmful consequences of diabetes on the retina, such as oxidative stress, neurodegeneration, inflammation, BRB breakdown, or angiogenesis[187-190].

The AKT pathway is a target of GLP1R activation and is essential for retinal neuroprotection in early DR development[188]. AKT phosphorylates a number of heterogeneous substrates including E2 ubiquitin ligases, transcription factors, protein and lipid kinases, metabolic enzymes, *etc.*, showing that AKT not only regulates a physiological process, but also controls multiple cellular functions. The first AKT substrate reported was GSK3[191]. Inactivation of GSK3 by AKT-phosphorylation has been shown to regulate transcription factors such as Nrf2, which is needed for DR development[192]. Moreover, in vitro and in vivo studies have demonstrated the ability of GLP1 to protect neurons from aggregation by β-amyloid peptide and against AGEs, as well as being able to reduce hyperphosphorylation of the *tau* protein by regulating GSK3β. It is believed that the mechanism of action of GLP1 is the activation of the PI3K/AKT signaling pathway, which is capable of phosphorylating and inactivating GSK3β[193]. Although further studies are needed to understand the importance and possible modulation of PI3K/AKT/GSK3β/Nrf2 pathway by GLP1R, these observations allow us to develop hypotheses of the key effects that modulation of Nrf2 by GLP1R agonists have on DR development.

Epigenetic modifications

Although glycemic control may be achieved, chronic hyperglycemia during the first few months may be enough exposure to develop stable and heritable epigenetic modifications capable of altering gene expression and becoming a potential major factor of DR development[194]. The alterations occur on chromosomes without changes in the DNA sequence and are the basis of the known "metabolic memory". The identified molecular mechanisms underlying these long-term effects act at different levels that include DNA methylation, post translational modifications of histones or regulation by noncoding (nc)RNAs[195]. For example, the low retinal histone acetylation of H3 induced by hyperglycemia for 6 mo did not recover after 6 mo of good glycemic control[196]. Likewise, euglycemia was unable to recover the DNA hypomethylation and unusual gene expression induced by hyperglycemia[197].

DNA methylation status is controlled by the activity of DNA methyltransferase (DNMT) enzymes that catalyze the transfer of a methyl group from S-adenosyl-L-methinione, and DNA demethylases. Imbalanced activity in diabetes, induces alterations in specific genes that triggers aberrant expression related to DR. For

example, chronic hyperglycemia in the retina stimulates the binding of DNMT1 and the DNA demethylase ten-eleven-translocation (TET) 2 to the promoter of Ras-related C3 botulinum toxin substrate (Rac1)[198]. Methylation induced by DNMT1 is rapidly reversed by TET2, triggering hypomethylation of the promoter and allowing Rac1 transcription, which induces NOX, and relevant effectors in DR development[199]. In fact, the mitochondrial damage initiated by NOX-2 activation has been associated with early DR development while its inhibition protects endothelial retinal cells from diabetes-induced apoptosis[200].

Although diabetes induces a global state of DNA hypomethylation, different states of methylation for specific CpG islands are closely related to DR development. An increase in the expression and activity of DNMTs has been observed in DR[201-203]. Based on that, inhibition of DNMTs can be a possible protective therapy against the development of DR. For example, 5-aza-2'-deoxycytidine, a nonselective inhibitor of DNMTs, re-establishes the expression of genes hypermethylated by hyperglycemia and related to DR development, such as SOD2 and glutathione S-transferase theta 1 (GSTT1), which protects against oxidative stress[203].

Changes in the pattern of acetylation and methylation are the most studied post translational modifications of histones. Overall, the acetylation of histones H3 and H4 and di or tri-methylation of H3K4 are related to euchromatin status. Low acetylation and high methylation levels are associated with silent heterochromatin. Experimental models of DR have provided contradictory results for histone acetylation. For example, Zhong and Kowluru[196] revealed reduced global acetylation, but Kadiyala *et al*[204] observed augmented histone acetylation in diabetic retinas. So far, in vivo experimental results for histone acetylation in DR remain contradictory[194,205].

Histone methylation is associated with transcriptional activation or repression depending on the type of residue and the number of methyl groups. Hence, the methylation of H3K4, H3K48, and H3K79 have been considered activation marks, while that of H3K9 and H3K27 are associated with transcriptional repression[206]. For example, decreased levels of H3K4me1 and H3K4me3 at the GCL promoter in diabetic rats compromised Nrf2 binding, triggering low transcription of the enzyme and reduced levels of GSH in the retina[207]. Moreover, the overexpression of matrix metalloproteinase-9, a proapoptotic enzyme in the development of DR, is caused by a decrease in H3K9me2 and an increase in acetyl H3K9, which facilitates the binding of NF-κB p65[208].

Thus, hyperglycemia-induced differential histone methylation or acetylation appears to regulate expression of several genes in cellular pathways that contribute to the development of diabetic retinopathy. In fact, the polyisoprenylated benzophenone derivative garcinol, prevents histone acetylation involved in the metabolic memory in DR[209]. In that sense, histone deacetylase inhibitors like resveratrol, curcumin, and genistein are also being considered as targets for treatment of DR[210].

A low percentage of cellular transcribed RNA is ncRNA, RNA sequences with different but important cell functions. Long ncRNA and small ncRNA, such as circular RNA, or miRNA, are essential in the pathological processes of diabetic complications, including atherosclerosis, microvascular dysfunction, and DR[211]. The most well-studied are miRNAs[212], sequences of approximately 18-25 nucleotides partially complementary to mRNAs able to block their translation and activate their degradation in collaboration with the ribonucleoprotein complex RNA-induced silencing complex[213]. There are numerous examples of the importance of their role in DR. Experimental models of DR have shown that downregulation of miR126, miR-146a, and miR200b is associated with retinal neovascularization through increased VEGF production[214]. The expression of miR-20b-5p, a modulator of cell proliferation, apoptosis, differentiation, and angiogenesis, is upregulated in the retinal endothelial cells of diabetic rats and patients with DR, inducing a decrease in tight junction proteins that increases BRB permeability and the microvascular leakage observed in DR[215]. Although the expression and physiological function of circular RNA is not yet fully elucidated, the molecules serve as miRNA or RNA-binding protein sponges to modulate expression or translation of regulatory proteins[216]. Circular DNMT3B, a reducer of the expression of miR-20b-5p, is downregulated in diabetes and its overexpression improves the vascular dysfunction induced in diabetic retinas, an interesting potential strategy for treatment of DR[215]. The possibility of using siRNAs to target some miRNAs mentioned above has also been considered. However, no methods are currently available for in vivo treatments[209]. In addition, double-stranded miRNA mimics and anti-mRNA antisense oligodeoxyribonucleotide are being used to target specific miRNA in other diseases, and therefore can also be studied for the treatment of DR[210].

Table 1 Summary of alterations, targets, and novel therapies

Contributors in DR development	Retinal alterations	Targets	Possible novel therapies
ROS accumulation	Low nuclear levels of Nrf2, antioxidant enzymes activities, and GLP1R expression. Retinal cell death, retinal ischemia, retinal neovascularization, DME	Nrf2 activation, Keap1 knockdown, inhibition and/or neutralization of ROS generation, GLP1R activation, reinforcement of the antioxidant defense system	Green tea polyphenols, resveratrol, curcumin, quercetin, tannins, pterostilbene, GLP1R agonist, RS9, dh404, triterpenoids, salvianolic acids, sulforaphane
Synthesis of proinflammatory molecules	Vascular permeability, BRB breakdown, capillary pericyte loss, neovascularization	Inhibition of inflammatory pathways	COX-2 inhibitors, tetracyclines (minocycline and doxycycline), IL-6 inhibitors (EBI-031 and tocilizumab), anti-TNF-α therapy, canakinumab (selective IL-1β antibody), fenofibrate (PPARα agonist)
Increased production of AGE/RAGE	Aberrant extracellular crosslinking of extracellular matrix proteins, increased vascular stiffness, release of proinflammatory cytokines and proangiogenic factors	Low the production of AGEs	Curcumin, epigallocatechin 3-gallate, quercetin, kaempferol and resveratrol
Activation of the polyol pathway	Retinal capillary osmotic damage and cell death	Inhibition of the polyol pathway	Pterostilbene
Increased flux through the hexosamine pathway	Neuro-vascular dysfunctions	Inhibition of the hexosamine pathway	Azaserine (antineoplastic), rhein (anthraquinone), benfotiamine (lipid-soluble thiamine derivative)
Activation of the PKC pathway	Endothelial alterations, cell demise of capillary cells and pericytes, formation of microaneurysms, VEGF-dependent retinal barrier alterations	Inhibition of PKC pathway	Ruboxistaurin mesylate (PKC-β inhibitor)
Lipid peroxidation	Generation of lipid radical species, apoptosis in retinal pigment epithelial cells, retinal vascular dysfunction, development of neovascularization	Inhibition of the formation of lipid peroxides in the retina	Fucoxanthin, pterostilbene
DNA methylation	Increased expression and activity of DNMTs	Inhibition of DNMTs	5-aza-2'-deoxycytidine
Histone methylation and acetylation	Decreased levels of H3K4me1 and H3K4me3 at glutamate-cysteine ligase promoter or decreased levels of H3K9me2 and increased levels in acetyl H3K9	Regulation of histone methylation/acetylation	Garcinol, resveratrol, curcumin, genistein
Regulation by ncRNA (miRNA and circular RNA)	Downregulation of miR126, miR-146a, and miR200b; retinal upregulation of miR-20b-5p, neovascularization and microvascular leakage	Modulation of miRNAs expression, overexpression of circular DNMT3B	siRNAs,double-stranded miRNA mimics and anti-mRNA antisense oligodeoxyribonucleotide

AGE/RAGE: Advanced glycation end products/receptors; BRB: Blood-retinal barrier; COX-2: Cyclooxygenase-2; dh404: Dihydro-CDDO-trifluoroethyl amide; DME: Diabetic macular edema; DNMT: DNA methyltransferases; GLP1R: Glucagon-like peptide-1 receptor; IL: Interleukin; Keap1: Kelch-like enoyl-CoA hydratase associated protein 1; miRNA: microRNA; ncRNA: noncoding RNAs; Nrf2: Nuclear factor erythroid 2-related factor 2; PKC: Protein kinase C; PPARα: Peroxisome proliferator-activated receptor α; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor α; VEGF: Vascular endothelial growth factor.

With the increase in evidence on the importance of epigenetic modifications in DR, a better understanding of their effects has great potential for establishing new targets against this pathology. Fortunately, advances are being made in the use of mimics and inhibitors in different chronic diseases and cancer that will undoubtedly contribute to a better understanding of the role of epigenetic changes in DR.

CONCLUSION

With the global increase in the prevalence of diabetes, an increase in associated complications such as DR is expected. Although in recent decades considerable advances have been made in the treatment of the disease, current therapeutic approaches focus on advanced stages in which the retina can present irreparable

damage at the neuronal and vascular level. Furthermore, the recommended treatments for DR have serious limitations such us long-term side effects, the high cost involved, or patient discomfort. Hence the need for the development of new therapeutic approaches (Table 1). Considering the current state of knowledge, treatments for diabetic retinopathy should go beyond acting on a single etiological cause such as neovascularization. New treatments should present a set of advantages that facilitate their administration without the need for special facilities. Ideal treatments would be noninvasive, effective, affordable, and accessible to the global population. Recognizing the importance of redox imbalance in the development and progression of DR offers a new direction for tackling the condition. One such option that should be explored is action directed at cellular targets that participate in modulating or altering the pathology, so that the progression of the disease can be delayed or even prevented.

REFERENCES

1 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

2 **Rodríguez ML**, Pérez S, Mena-Mollá S, Desco MC, Ortega ÁL. Oxidative Stress and Microvascular Alterations in Diabetic Retinopathy: Future Therapies. *Oxid Med Cell Longev* 2019; **2019**: 4940825 [PMID: 31814880 DOI: 10.1155/2019/4940825]

3 **Flaxman SR**, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR; Vision Loss Expert Group of the Global Burden of Disease Study. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health* 2017; **5**: e1221-e1234 [PMID: 29032195 DOI: 10.1016/S2214-109X(17)30393-5]

4 **Asmat U**, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. *Saudi Pharm J* 2016; **24**: 547-553 [PMID: 27752226 DOI: 10.1016/j.jsps.2015.03.013]

5 **Wangsa-Wirawan ND**, Linsenmeier RA. Retinal oxygen: fundamental and clinical aspects. *Arch Ophthalmol* 2003; **121**: 547-557 [PMID: 12695252 DOI: 10.1001/archopht.121.4.547]

6 **Semeraro F**, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C. Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. *Diabetes Metab* 2019; **45**: 517-527 [PMID: 31005756 DOI: 10.1016/j.diabet.2019.04.002]

7 **Rangasamy S**, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. *Middle East Afr J Ophthalmol* 2012; **19**: 52-59 [PMID: 22346115 DOI: 10.4103/0974-9233.92116]

8 **Ishida S**, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP. VEGF164 is proinflammatory in the diabetic retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 2155-2162 [PMID: 12714656 DOI: 10.1167/iovs.02-0807]

9 **Sinclair SH**, Schwartz SS. Diabetic Retinopathy-An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes. *Front Endocrinol (Lausanne)* 2019; **10**: 843 [PMID: 31920963 DOI: 10.3389/fendo.2019.00843]

10 **Stitt AW**, Lois N, Medina RJ, Adamson P, Curtis TM. Advances in our understanding of diabetic retinopathy. *Clin Sci (Lond)* 2013; **125**: 1-17 [PMID: 23485060 DOI: 10.1042/CS20120588]

11 **Hernández C**, Simó-Servat A, Bogdanov P, Simó R. Diabetic retinopathy: new therapeutic perspectives based on pathogenic mechanisms. *J Endocrinol Invest* 2017; **40**: 925-935 [PMID: 28357783 DOI: 10.1007/s40618-017-0648-4]

12 **Calderon GD**, Juarez OH, Hernandez GE, Punzo SM, De la Cruz ZD. Oxidative stress and diabetic retinopathy: development and treatment. *Eye (Lond)* 2017; **31**: 1122-1130 [PMID: 28452994 DOI: 10.1038/eye.2017.64]

13 **Mansour SE**, Browning DJ, Wong K, Flynn HW Jr, Bhavsar AR. The Evolving Treatment of Diabetic Retinopathy. *Clin Ophthalmol* 2020; **14**: 653-678 [PMID: 32184554 DOI: 10.2147/OPTH.S236637]

14 **Bandello F**, Toni D, Porta M, Varano M. Diabetic retinopathy, diabetic macular edema, and cardiovascular risk: the importance of a long-term perspective and a multidisciplinary approach to optimal intravitreal therapy. *Acta Diabetol* 2020; **57**: 513-526 [PMID: 31749046 DOI: 10.1007/s00592-019-01453-z]

15 **Fogli S**, Del Re M, Rofi E, Posarelli C, Figus M, Danesi R. Clinical pharmacology of intravitreal anti-VEGF drugs. *Eye (Lond)* 2018; **32**: 1010-1020 [PMID: 29398697 DOI: 10.1038/s41433-018-0021-7]

16 **Sultan MB**, Zhou D, Loftus J, Dombi T, Ice KS; Macugen 1013 Study Group. A phase 2/3, multicenter, randomized, double-masked, 2-year trial of pegaptanib sodium for the treatment of diabetic macular edema. *Ophthalmology* 2011; **118**: 1107-1118 [PMID: 21529957 DOI: 10.1016/j.ophtha.2011.02.045]

17 **Diabetic Retinopathy Clinical Research Network**, Elman MJ, Qin H, Aiello LP, Beck RW, Bressler NM, Ferris FL 3rd, Glassman AR, Maturi RK, Melia M. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. *Ophthalmology* 2012; **119**: 2312-2318 [PMID: 22999634 DOI: 10.1016/j.ophtha.2012.08.022]

18 **Fallico M**, Maugeri A, Lotery A, Longo A, Bonfiglio V, Russo A, Avitabile T, Pulvirenti A, Furino C, Cennamo G, Barchitta M, Agodi A, Reibaldi M. Intravitreal anti-vascular endothelial growth factors, panretinal photocoagulation and combined treatment for proliferative diabetic retinopathy: a systematic review and network meta-analysis. *Acta Ophthalmol* 2020 [PMID: 33326183 DOI: 10.1111/aos.14681]

19 **Gologorsky D**, Thanos A, Vavvas D. Therapeutic interventions against inflammatory and angiogenic mediators in proliferative diabetic retinopathy. *Mediators Inflamm* 2012; **2012**: 629452 [PMID: 23028203 DOI: 10.1155/2012/629452]

20 **Kumar B**, Gupta SK, Saxena R, Srivastava S. Current trends in the pharmacotherapy of diabetic retinopathy. *J Postgrad Med* 2012; **58**: 132-139 [PMID: 22718058 DOI: 10.4103/0022-3859.97176]

21 **Kaštelan S**, Tomić M, Gverović Antunica A, Salopek Rabatić J, Ljubić S. Inflammation and pharmacological treatment in diabetic retinopathy. *Mediators Inflamm* 2013; **2013**: 213130 [PMID: 24288441 DOI: 10.1155/2013/213130]

22 **Quiram PA**, Gonzales CR, Schwartz SD. Severe steroid-induced glaucoma following intravitreal injection of triamcinolone acetonide. *Am J Ophthalmol* 2006; **141**: 580-582 [PMID: 16490518 DOI: 10.1016/j.ajo.2005.10.004]

23 **Pai A**, El Shafei MM, Mohammed OA, Al Hashimi M. Current concepts in intravitreal drug therapy for diabetic retinopathy. *Saudi J Ophthalmol* 2010; **24**: 143-149 [PMID: 23960892 DOI: 10.1016/j.sjopt.2010.06.003]

24 **Stewart MW**. Corticosteroid use for diabetic macular edema: old fad or new trend? *Curr Diab Rep* 2012; **12**: 364-375 [PMID: 22581206 DOI: 10.1007/s11892-012-0281-8]

25 **Kompella UB**, Kadam RS, Lee VH. Recent advances in ophthalmic drug delivery. *Ther Deliv* 2010; **1**: 435-456 [PMID: 21399724 DOI: 10.4155/TDE.10.40]

26 **Kuno N**, Fujii S. Biodegradable intraocular therapies for retinal disorders: progress to date. *Drugs Aging* 2010; **27**: 117-134 [PMID: 20104938 DOI: 10.2165/11530970-000000000-00000]

27 **Wang W**, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. *Int J Mol Sci* 2018; **19** [PMID: 29925789 DOI: 10.3390/ijms19061816]

28 **Ellis MP**, Lent-Schochet D, Lo T, Yiu G. Emerging Concepts in the Treatment of Diabetic Retinopathy. *Curr Diab Rep* 2019; **19**: 137 [PMID: 31748965 DOI: 10.1007/s11892-019-1276-5]

29 **Lee R**, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis (Lond)* 2015; **2**: 17 [PMID: 26605370 DOI: 10.1186/s40662-015-0026-2]

30 **Fong DS**, Girach A, Boney A. Visual side effects of successful scatter laser photocoagulation surgery for proliferative diabetic retinopathy: a literature review. *Retina* 2007; **27**: 816-824 [PMID: 17891003 DOI: 10.1097/IAE.0b013e318042d32c]

31 **Helbig H**. Surgery for diabetic retinopathy. *Ophthalmologica* 2007; **221**: 103-111 [PMID: 17380064 DOI: 10.1159/000098255]

32 **Sharma T**, Fong A, Lai TY, Lee V, Das S, Lam D. Surgical treatment for diabetic vitreoretinal diseases: a review. *Clin Exp Ophthalmol* 2016; **44**: 340-354 [PMID: 27027299 DOI: 10.1111/ceo.12752]

33 **Zhao LQ**, Zhu H, Zhao PQ, Hu YQ. A systematic review and meta-analysis of clinical outcomes of vitrectomy with or without intravitreal bevacizumab pretreatment for severe diabetic retinopathy. *Br J Ophthalmol* 2011; **95**: 1216-1222 [PMID: 21278146 DOI: 10.1136/bjo.2010.189514]

34 **Nakajima T**, Roggia MF, Noda Y, Ueta T. EFFECT OF INTERNAL LIMITING MEMBRANE PEELING DURING VITRECTOMY FOR DIABETIC MACULAR EDEMA: Systematic Review and Meta-analysis. *Retina* 2015; **35**: 1719-1725 [PMID: 26079478 DOI: 10.1097/IAE.0000000000000622]

35 **Belin PJ**, Parke DW 3rd. Complications of vitreoretinal surgery. *Curr Opin Ophthalmol* 2020; **31**: 167-173 [PMID: 32175941 DOI: 10.1097/ICU.0000000000000652]

36 **Lahey JM**, Francis RR, Kearney JJ. Combining phacoemulsification with pars plana vitrectomy in patients with proliferative diabetic retinopathy: a series of 223 cases. *Ophthalmology* 2003; **110**: 1335-1339 [PMID: 12867387 DOI: 10.1016/S0161-6420(03)00454-8]

37 **Khuthaila MK**, Hsu J, Chiang A, DeCroos FC, Milder EA, Setlur V, Garg SJ, Spirn MJ. Postoperative vitreous hemorrhage after diabetic 23-gauge pars plana vitrectomy. *Am J Ophthalmol* 2013; **155**: 757-763, 763.e1 [PMID: 23317651 DOI: 10.1016/j.ajo.2012.11.004]

38 **Stitt AW**, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 2016; **51**: 156-186 [PMID: 26297071 DOI: 10.1016/j.preteyeres.2015.08.001]

39 **Kang Q**, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biol* 2020; **37**: 101799 [PMID: 33248932 DOI: 10.1016/j.redox.2020.101799]

40 **Slater TF**. Free-radical mechanisms in tissue injury. *Biochem J* 1984; **222**: 1-15 [PMID: 6383353 DOI: 10.1042/bj2220001]

41 **Sies H**, Berndt C, Jones DP. Oxidative Stress. *Annu Rev Biochem* 2017; **86**: 715-748 [PMID:

28441057 DOI: 10.1146/annurev-biochem-061516-045037]
42 **Kersten E**, Paun CC, Schellevis RL, Hoyng CB, Delcourt C, Lengyel I, Peto T, Ueffing M, Klaver CCW, Dammeier S, den Hollander AI, de Jong EK. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol* 2018; **63**: 9-39 [PMID: 28522341 DOI: 10.1016/j.survophthal.2017.05.003]
43 **Tokarz P**, Kaarniranta K, Blasiak J. Role of antioxidant enzymes and small molecular weight antioxidants in the pathogenesis of age-related macular degeneration (AMD). *Biogerontology* 2013; **14**: 461-482 [PMID: 24057278 DOI: 10.1007/s10522-013-9463-2]
44 **Cai J**, Nelson KC, Wu M, Sternberg P Jr, Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 2000; **19**: 205-221 [PMID: 10674708 DOI: 10.1016/s1350-9462(99)00009-9]
45 **Wu Y**, Tang L, Chen B. Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives. *Oxid Med Cell Longev* 2014; **2014**: 752387 [PMID: 25180070 DOI: 10.1155/2014/752387]
46 **Prištáková P**, Minárik G, Repiská V. Candidate gene studies of diabetic retinopathy in human. *Mol Biol Rep* 2016; **43**: 1327-1345 [PMID: 27730450 DOI: 10.1007/s11033-016-4075-y]
47 **Millán I**, Desco MDC, Torres-Cuevas I, Pérez S, Pulido I, Mena-Mollá S, Mataix J, Asensi M, Ortega ÁL. Pterostilbene Prevents Early Diabetic Retinopathy Alterations in a Rabbit Experimental Model. *Nutrients* 2019; **12** [PMID: 31892189 DOI: 10.3390/nu12010082]
48 **Al-Shabrawey M**, Smith S. Prediction of diabetic retinopathy: role of oxidative stress and relevance of apoptotic biomarkers. *EPMA J* 2010; **1**: 56-72 [PMID: 23199041 DOI: 10.1007/s13167-010-0002-9]
49 **Kensler TW**, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses *via* the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007; **47**: 89-116 [PMID: 16968214 DOI: 10.1146/annurev.pharmtox.46.120604.141046]
50 **Batliwala S**, Xavier C, Liu Y, Wu H, Pang IH. Involvement of Nrf2 in Ocular Diseases. *Oxid Med Cell Longev* 2017; **2017**: 1703810 [PMID: 28473877 DOI: 10.1155/2017/1703810]
51 **Canning P**, Sorrell FJ, Bullock AN. Structural basis of Keap1 interactions with Nrf2. *Free Radic Biol Med* 2015; **88**: 101-107 [PMID: 26057936 DOI: 10.1016/j.freeradbiomed.2015.05.034]
52 **McMahon M**, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* 2003; **278**: 21592-21600 [PMID: 12682069 DOI: 10.1074/jbc.M300931200]
53 **Itoh K**, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997; **236**: 313-322 [PMID: 9240432 DOI: 10.1006/bbrc.1997.6943]
54 **Xu Z**, Wei Y, Gong J, Cho H, Park JK, Sung ER, Huang H, Wu L, Eberhart C, Handa JT, Du Y, Kern TS, Thimmulappa R, Barber AJ, Biswal S, Duh EJ. NRF2 plays a protective role in diabetic retinopathy in mice. *Diabetologia* 2014; **57**: 204-213 [PMID: 24186494 DOI: 10.1007/s00125-013-3093-8]
55 **Zhong Q**, Mishra M, Kowluru RA. Transcription factor Nrf2-mediated antioxidant defense system in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2013; **54**: 3941-3948 [PMID: 23633659 DOI: 10.1167/iovs.13-11598]
56 **Mishra M**, Zhong Q, Kowluru RA. Epigenetic modifications of Keap1 regulate its interaction with the protective factor Nrf2 in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2014; **55**: 7256-7265 [PMID: 25301875 DOI: 10.1167/iovs.14-15193]
57 **Nabavi SF**, Barber AJ, Spagnuolo C, Russo GL, Daglia M, Nabavi SM, Sobarzo-Sánchez E. Nrf2 as molecular target for polyphenols: A novel therapeutic strategy in diabetic retinopathy. *Crit Rev Clin Lab Sci* 2016; **53**: 293-312 [PMID: 26926494 DOI: 10.3109/10408363.2015.1129530]
58 **Kowluru RA**, Kennedy A. Therapeutic potential of anti-oxidants and diabetic retinopathy. *Expert Opin Investig Drugs* 2001; **10**: 1665-1676 [PMID: 11772276 DOI: 10.1517/13543784.10.9.1665]
59 **Haskins K**, Bradley B, Powers K, Fadok V, Flores S, Ling X, Pugazhenthi S, Reusch J, Kench J. Oxidative stress in type 1 diabetes. *Ann N Y Acad Sci* 2003; **1005**: 43-54 [PMID: 14679039 DOI: 10.1196/annals.1288.006]
60 **Negi G**, Kumar A, Joshi RP, Sharma SS. Oxidative stress and Nrf2 in the pathophysiology of diabetic neuropathy: old perspective with a new angle. *Biochem Biophys Res Commun* 2011; **408**: 1-5 [PMID: 21439933 DOI: 10.1016/j.bbrc.2011.03.087]
61 **Li W**, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. *Biochem Pharmacol* 2008; **76**: 1485-1489 [PMID: 18694732 DOI: 10.1016/j.bcp.2008.07.017]
62 **Kowluru RA**, Koppolu P, Chakrabarti S, Chen S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free Radic Res* 2003; **37**: 1169-1180 [PMID: 14703729 DOI: 10.1080/10715760310001604189]
63 **Romeo G**, Liu WH, Asnaghi V, Kern TS, Lorenzi M. Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 2002; **51**: 2241-2248 [PMID: 12086956 DOI: 10.2337/diabetes.51.7.2241]
64 **Mittal R**, Kumar A, Singh DP, Bishnoi M, Nag TC. Ameliorative potential of rutin in combination with nimesulide in STZ model of diabetic neuropathy: targeting Nrf2/HO-1/NF-kB and COX signalling pathway. *Inflammopharmacology* 2018; **26**: 755-768 [PMID: 29094308 DOI:

10.1007/s10787-017-0413-5]
65 **Sun W**, Liu X, Zhang H, Song Y, Li T, Liu Y, Guo L, Wang F, Yang T, Guo W, Wu J, Jin H, Wu H. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy *via* disabling KEAP1. *Free Radic Biol Med* 2017; **108**: 840-857 [PMID: 28457936 DOI: 10.1016/j.freeradbiomed.2017.04.365]
66 **Cheng AS**, Cheng YH, Chiou CH, Chang TL. Resveratrol upregulates Nrf2 expression to attenuate methylglyoxal-induced insulin resistance in Hep G2 cells. *J Agric Food Chem* 2012; **60**: 9180-9187 [PMID: 22917016 DOI: 10.1021/jf302831d]
67 **Palsamy P**, Subramanian S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines *via* Nrf2-Keap1 signaling. *Biochim Biophys Acta* 2011; **1812**: 719-731 [PMID: 21439372 DOI: 10.1016/j.bbadis.2011.03.008]
68 **Bucolo C**, Drago F, Maisto R, Romano GL, D'Agata V, Maugeri G, Giunta S. Curcumin prevents high glucose damage in retinal pigment epithelial cells through ERK1/2-mediated activation of the Nrf2/HO-1 pathway. *J Cell Physiol* 2019; **234**: 17295-17304 [PMID: 30770549 DOI: 10.1002/jcp.28347]
69 **Pugazhenthi S**, Akhov L, Selvaraj G, Wang M, Alam J. Regulation of heme oxygenase-1 expression by demethoxy curcuminoids through Nrf2 by a PI3-kinase/Akt-mediated pathway in mouse beta-cells. *Am J Physiol Endocrinol Metab* 2007; **293**: E645-E655 [PMID: 17535857 DOI: 10.1152/ajpendo.00111.2007]
70 **Zhang X**, Liang D, Guo L, Liang W, Jiang Y, Li H, Zhao Y, Lu S, Chi ZH. Curcumin protects renal tubular epithelial cells from high glucose-induced epithelial-to-mesenchymal transition through Nrf2-mediated upregulation of heme oxygenase-1. *Mol Med Rep* 2015; **12**: 1347-1355 [PMID: 25823828 DOI: 10.3892/mmr.2015.3556]
71 **Park JY**, Han X, Piao MJ, Oh MC, Fernando PM, Kang KA, Ryu YS, Jung U, Kim IG, Hyun JW. Hyperoside Induces Endogenous Antioxidant System to Alleviate Oxidative Stress. *J Cancer Prev* 2016; **21**: 41-47 [PMID: 27051648 DOI: 10.15430/JCP.2016.21.1.41]
72 **Liu XF**, Hao JL, Xie T, Malik TH, Lu CB, Liu C, Shu C, Lu CW, Zhou DD. Nrf2 as a target for prevention of age-related and diabetic cataracts by against oxidative stress. *Aging Cell* 2017; **16**: 934-942 [PMID: 28722304 DOI: 10.1111/acel.12645]
73 **Laddha AP**, Kulkarni YA. Tannins and vascular complications of Diabetes: An update. *Phytomedicine* 2019; **56**: 229-245 [PMID: 30668344 DOI: 10.1016/j.phymed.2018.10.026]
74 **Nakagami Y**, Hatano E, Inoue T, Yoshida K, Kondo M, Terasaki H. Cytoprotective Effects of a Novel Nrf2 Activator, RS9, in Rhodopsin Pro347Leu Rabbits. *Curr Eye Res* 2016; **41**: 1123-1126 [PMID: 26430824 DOI: 10.3109/02713683.2015.1078362]
75 **Deliyanti D**, Alrashdi SF, Tan SM, Meyer C, Ward KW, de Haan JB, Wilkinson-Berka JL. Nrf2 Activation Is a Potential Therapeutic Approach to Attenuate Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2018; **59**: 815-825 [PMID: 29411009 DOI: 10.1167/iovs.17-22920]
76 **Liu X**, Ward K, Xavier C, Jann J, Clark AF, Pang IH, Wu H. The novel triterpenoid RTA 408 protects human retinal pigment epithelial cells against H_2O_2-induced cell injury *via* NF-E2-related factor 2 (Nrf2) activation. *Redox Biol* 2016; **8**: 98-109 [PMID: 26773873 DOI: 10.1016/j.redox.2015.12.005]
77 **Zhang H**, Liu YY, Jiang Q, Li KR, Zhao YX, Cao C, Yao J. Salvianolic acid A protects RPE cells against oxidative stress through activation of Nrf2/HO-1 signaling. *Free Radic Biol Med* 2014; **69**: 219-228 [PMID: 24486344 DOI: 10.1016/j.freeradbiomed.2014.01.025]
78 **Guerrero-Beltrán CE**, Calderón-Oliver M, Pedraza-Chaverri J, Chirino YI. Protective effect of sulforaphane against oxidative stress: recent advances. *Exp Toxicol Pathol* 2012; **64**: 503-508 [PMID: 21129940 DOI: 10.1016/j.etp.2010.11.005]
79 **Li S**, Yang H, Chen X. Protective effects of sulforaphane on diabetic retinopathy: activation of the Nrf2 pathway and inhibition of NLRP3 inflammasome formation. *Exp Anim* 2019; **68**: 221-231 [PMID: 30606939 DOI: 10.1538/expanim.18-0146]
80 **Al-Kharashi AS**. Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy. *Saudi J Ophthalmol* 2018; **32**: 318-323 [PMID: 30581303 DOI: 10.1016/j.sjopt.2018.05.002]
81 **Rübsam A**, Parikh S, Fort PE. Role of Inflammation in Diabetic Retinopathy. *Int J Mol Sci* 2018; **19** [PMID: 29565290 DOI: 10.3390/ijms19040942]
82 **Tang J**, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011; **30**: 343-358 [PMID: 21635964 DOI: 10.1016/j.preteyeres.2011.05.002]
83 **Kern TS**. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res* 2007; **2007**: 95103 [PMID: 18274606 DOI: 10.1155/2007/95103]
84 **ValdezGuerrero AS**, Quintana-Pérez JC, Arellano-Mendoza MG, Castañeda-Ibarra FJ, Tamay-Cach F, Alemán-González-Duhart D. Diabetic Retinopathy: Important Biochemical Alterations and the Main Treatment Strategies. *Can J Diabetes* 2020 [PMID: 33341391 DOI: 10.1016/j.jcjd.2020.10.009]
85 **Rangasamy S**, McGuire PG, Franco Nitta C, Monickaraj F, Oruganti SR, Das A. Chemokine mediated monocyte trafficking into the retina: role of inflammation in alteration of the blood-retinal barrier in diabetic retinopathy. *PLoS One* 2014; **9**: e108508 [PMID: 25329075 DOI: 10.1371/journal.pone.0108508]
86 **Simó-Servat O**, Hernández C, Simó R. Usefulness of the vitreous fluid analysis in the translational research of diabetic retinopathy. *Mediators Inflamm* 2012; **2012**: 872978 [PMID: 23028204 DOI:

10.1155/2012/872978]
87 **Li Q**, Verma A, Han PY, Nakagawa T, Johnson RJ, Grant MB, Campbell-Thompson M, Jarajapu YP, Lei B, Hauswirth WW. Diabetic eNOS-knockout mice develop accelerated retinopathy. *Invest Ophthalmol Vis Sci* 2010; **51**: 5240-5246 [PMID: 20435587 DOI: 10.1167/iovs.09-5147]
88 **Vallejo S**, Palacios E, Romacho T, Villalobos L, Peiró C, Sánchez-Ferrer CF. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. *Cardiovasc Diabetol* 2014; **13**: 158 [PMID: 25518980 DOI: 10.1186/s12933-014-0158-z]
89 **Zheng L**, Du Y, Miller C, Gubitosi-Klug RA, Kern TS, Ball S, Berkowitz BA. Critical role of inducible nitric oxide synthase in degeneration of retinal capillaries in mice with streptozotocin-induced diabetes. *Diabetologia* 2007; **50**: 1987-1996 [PMID: 17583794 DOI: 10.1007/s00125-007-0734-9]
90 **Joussen AM**, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Döhmen S, Adamis AP. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy *via* TNF-alpha suppression. *FASEB J* 2002; **16**: 438-440 [PMID: 11821258 DOI: 10.1096/fj.01-0707fje]
91 **Zheng L**, Howell SJ, Hatala DA, Huang K, Kern TS. Salicylate-based anti-inflammatory drugs inhibit the early lesion of diabetic retinopathy. *Diabetes* 2007; **56**: 337-345 [PMID: 17259377 DOI: 10.2337/db06-0789]
92 **Vincent JA**, Mohr S. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. *Diabetes* 2007; **56**: 224-230 [PMID: 17192486 DOI: 10.2337/db06-0427]
93 **Rosberger DF**. Diabetic retinopathy: current concepts and emerging therapy. *Endocrinol Metab Clin North Am* 2013; **42**: 721-745 [PMID: 24286948 DOI: 10.1016/j.ecl.2013.08.001]
94 **Kim SJ**, Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. *Surv Ophthalmol* 2010; **55**: 108-133 [PMID: 20159228 DOI: 10.1016/j.survophthal.2009.07.005]
95 **Kern TS**, Miller CM, Du Y, Zheng L, Mohr S, Ball SL, Kim M, Jamison JA, Bingaman DP. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. *Diabetes* 2007; **56**: 373-379 [PMID: 17259381 DOI: 10.2337/db05-1621]
96 **Amrite AC**, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci* 2006; **47**: 1149-1160 [PMID: 16505053 DOI: 10.1167/iovs.05-0531]
97 **Yüksel B**, Karti Ö, Kusbeci T. Topical nepafenac for prevention of post-cataract surgery macular edema in diabetic patients: patient selection and perspectives. *Clin Ophthalmol* 2017; **11**: 2183-2190 [PMID: 29269999 DOI: 10.2147/OPTH.S132810]
98 **Garrido-Mesa N**, Zarzuelo A, Gálvez J. Minocycline: far beyond an antibiotic. *Br J Pharmacol* 2013; **169**: 337-352 [PMID: 23441623 DOI: 10.1111/bph.12139]
99 **Krady JK**, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, Levison SW. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes* 2005; **54**: 1559-1565 [PMID: 15855346 DOI: 10.2337/diabetes.54.5.1559]
100 **Bernardino AL**, Kaushal D, Philipp MT. The antibiotics doxycycline and minocycline inhibit the inflammatory responses to the Lyme disease spirochete Borrelia burgdorferi. *J Infect Dis* 2009; **199**: 1379-1388 [PMID: 19301981 DOI: 10.1086/597807]
101 **Cukras CA**, Petrou P, Chew EY, Meyerle CB, Wong WT. Oral minocycline for the treatment of diabetic macular edema (DME): results of a phase I/II clinical study. *Invest Ophthalmol Vis Sci* 2012; **53**: 3865-3874 [PMID: 22589436 DOI: 10.1167/iovs.11-9413]
102 **Scott IU**, Jackson GR, Quillen DA, Larsen M, Klein R, Liao J, Holfort S, Munch IC, Gardner TW. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in patients with severe nonproliferative or non-high-risk proliferative diabetic retinopathy: a randomized clinical trial. *JAMA Ophthalmol* 2014; **132**: 535-543 [PMID: 24604308 DOI: 10.1001/jamaophthalmol.2014.93]
103 **Karkhur S**, Hasanreisoglu M, Vigil E, Halim MS, Hassan M, Plaza C, Nguyen NV, Afridi R, Tran AT, Do DV, Sepah YJ, Nguyen QD. Interleukin-6 inhibition in the management of non-infectious uveitis and beyond. *J Ophthalmic Inflamm Infect* 2019; **9**: 17 [PMID: 31523783 DOI: 10.1186/s12348-019-0182-y]
104 **Tsilimbaris MK**, Panagiotoglou TD, Charisis SK, Anastasakis A, Krikonis TS, Christodoulakis E. The use of intravitreal etanercept in diabetic macular oedema. *Semin Ophthalmol* 2007; **22**: 75-79 [PMID: 17564925 DOI: 10.1080/08820530701418243]
105 **Stahel M**, Becker M, Graf N, Michels S. SYSTEMIC INTERLEUKIN 1β INHIBITION IN PROLIFERATIVE DIABETIC RETINOPATHY: A Prospective Open-Label Study Using Canakinumab. *Retina* 2016; **36**: 385-391 [PMID: 26218500 DOI: 10.1097/IAE.0000000000000701]
106 **Ahmed N**. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract* 2005; **67**: 3-21 [PMID: 15620429 DOI: 10.1016/j.diabres.2004.09.004]
107 **Gkogkolou P**, Böhm M. Advanced glycation end products: Key players in skin aging? *Dermatoendocrinol* 2012; **4**: 259-270 [PMID: 23467327 DOI: 10.4161/derm.22028]
108 **Aragonès G**, Rowan S, G Francisco S, Yang W, Weinberg J, Taylor A, Bejarano E. Glyoxalase System as a Therapeutic Target against Diabetic Retinopathy. *Antioxidants (Basel)* 2020; **9** [PMID: 33143048 DOI: 10.3390/antiox9111062]

109 **Xu J**, Chen LJ, Yu J, Wang HJ, Zhang F, Liu Q, Wu J. Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy. *Cell Physiol Biochem* 2018; **48**: 705-717 [PMID: 30025404 DOI: 10.1159/000491897]

110 **Kowluru RA**, Kanwar M. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr Metab (Lond)* 2007; **4**: 8 [PMID: 17437639 DOI: 10.1186/1743-7075-4-8]

111 **Sajithlal GB**, Chithra P, Chandrakasan G. Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. *Biochem Pharmacol* 1998; **56**: 1607-1614 [PMID: 9973181 DOI: 10.1016/s0006-2952(98)00237-8]

112 **Sampath C**, Rashid MR, Sang S, Ahmedna M. Green tea epigallocatechin 3-gallate alleviates hyperglycemia and reduces advanced glycation end products *via* nrf2 pathway in mice with high fat diet-induced obesity. *Biomed Pharmacother* 2017; **87**: 73-81 [PMID: 28040599 DOI: 10.1016/j.biopha.2016.12.082]

113 **Bhuiyan MN**, Mitsuhashi S, Sigetomi K, Ubukata M. Quercetin inhibits advanced glycation end product formation *via* chelating metal ions, trapping methylglyoxal, and trapping reactive oxygen species. *Biosci Biotechnol Biochem* 2017; **81**: 882-890 [PMID: 28388357 DOI: 10.1080/09168451.2017.1282805]

114 **Kishore L**, Kaur N, Singh R. Effect of Kaempferol isolated from seeds of Eruca sativa on changes of pain sensitivity in Streptozotocin-induced diabetic neuropathy. *Inflammopharmacology* 2018; **26**: 993-1003 [PMID: 29159712 DOI: 10.1007/s10787-017-0416-2]

115 **Hajizadeh-Sharafabad F**, Sahebkar A, Zabetian-Targhi F, Maleki V. The impact of resveratrol on toxicity and related complications of advanced glycation end products: A systematic review. *Biofactors* 2019; **45**: 651-665 [PMID: 31185146 DOI: 10.1002/biof.1531]

116 **Bolton WK**, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP; ACTION I Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004; **24**: 32-40 [PMID: 14685005 DOI: 10.1159/000075627]

117 **Hammes HP**, Brownlee M, Edelstein D, Saleck M, Martin S, Federlin K. Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. *Diabetologia* 1994; **37**: 32-35 [PMID: 8150227 DOI: 10.1007/BF00428774]

118 **Gabbay KH**. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Annu Rev Med* 1975; **26**: 521-536 [PMID: 238458 DOI: 10.1146/annurev.me.26.020175.002513]

119 **Gabbay KH**. The sorbitol pathway and the complications of diabetes. *N Engl J Med* 1973; **288**: 831-836 [PMID: 4266466 DOI: 10.1056/NEJM197304192881609]

120 **Hotta N**, Kawamori R, Fukuda M, Shigeta Y; Aldose Reductase Inhibitor-Diabetes Complications Trial Study Group. Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on progression of diabetic neuropathy and other microvascular complications: multivariate epidemiological analysis based on patient background factors and severity of diabetic neuropathy. *Diabet Med* 2012; **29**: 1529-1533 [PMID: 22507139 DOI: 10.1111/j.1464-5491.2012.03684.x]

121 A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. Sorbinil Retinopathy Trial Research Group. *Arch Ophthalmol* 1990; **108**: 1234-1244 [PMID: 2119168 DOI: 10.1001/archopht.1990.01070110050024]

122 **Dodda D**, Rama Rao A, Veeresham C. In vitro and in vivo evaluation of pterostilbene for the management of diabetic complications. *J Ayurveda Integr Med* 2020; **11**: 369-375 [PMID: 30459079 DOI: 10.1016/j.jaim.2018.01.003]

123 **Yang Y**, Hayden MR, Sowers S, Bagree SV, Sowers JR. Retinal redox stress and remodeling in cardiometabolic syndrome and diabetes. *Oxid Med Cell Longev* 2010; **3**: 392-403 [PMID: 21307645 DOI: 10.4161/oxim.3.6.14786]

124 **Horal M**, Zhang Z, Stanton R, Virkamäki A, Loeken MR. Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: involvement in diabetic teratogenesis. *Birth Defects Res A Clin Mol Teratol* 2004; **70**: 519-527 [PMID: 15329829 DOI: 10.1002/bdra.20056]

125 **Kaneto H**, Xu G, Song KH, Suzuma K, Bonner-Weir S, Sharma A, Weir GC. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J Biol Chem* 2001; **276**: 31099-31104 [PMID: 11390407 DOI: 10.1074/jbc.M104115200]

126 **Goldberg H**, Whiteside C, Fantus IG. O-linked β-N-acetylglucosamine supports p38 MAPK activation by high glucose in glomerular mesangial cells. *Am J Physiol Endocrinol Metab* 2011; **301**: E713-E726 [PMID: 21712532 DOI: 10.1152/ajpendo.00108.2011]

127 **Ighodaro OM**. Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed Pharmacother* 2018; **108**: 656-662 [PMID: 30245465 DOI: 10.1016/j.biopha.2018.09.058]

128 **Liu RM**, Desai LP. Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biol* 2015; **6**: 565-577 [PMID: 26496488 DOI: 10.1016/j.redox.2015.09.009]

129 **Rajapakse AG**, Ming XF, Carvas JM, Yang Z. The hexosamine biosynthesis inhibitor azaserine prevents endothelial inflammation and dysfunction under hyperglycemic condition through antioxidant effects. *Am J Physiol Heart Circ Physiol* 2009; **296**: H815-H822 [PMID: 19136606 DOI: 10.1152/ajpheart.00756.2008]

130 **Zheng JM**, Zhu JM, Li LS, Liu ZH. Rhein reverses the diabetic phenotype of mesangial cells over-expressing the glucose transporter (GLUT1) by inhibiting the hexosamine pathway. *Br J Pharmacol* 2008; **153**: 1456-1464 [PMID: 18264122 DOI: 10.1038/bjp.2008.26]

131 **Hammes HP**, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 2003; **9**: 294-299 [PMID: 12592403 DOI: 10.1038/nm834]

132 **Geraldes P**, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 2010; **106**: 1319-1331 [PMID: 20431074 DOI: 10.1161/CIRCRESAHA.110.217117]

133 **Zong H**, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep* 2011; **11**: 244-252 [PMID: 21590515 DOI: 10.1007/s11892-011-0198-7]

134 **Koya D**, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998; **47**: 859-866 [PMID: 9604860 DOI: 10.2337/diabetes.47.6.859]

135 **Lei S**, Su W, Liu H, Xu J, Xia ZY, Yang QJ, Qiao X, Du Y, Zhang L, Xia Z. Nitroglycerine-induced nitrate tolerance compromises propofol protection of the endothelial cells against TNF-α: the role of PKC-β2 and NADPH oxidase. *Oxid Med Cell Longev* 2013; **2013**: 678484 [PMID: 24396568 DOI: 10.1155/2013/678484]

136 **Chalupsky K**, Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 2005; **102**: 9056-9061 [PMID: 15941833 DOI: 10.1073/pnas.0409594102]

137 **Kowluru RA**, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 5327-5334 [PMID: 14638734 DOI: 10.1167/iovs.03-0353]

138 **Geraldes P**, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, Aiello LP, Kern TS, King GL. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nat Med* 2009; **15**: 1298-1306 [PMID: 19881493 DOI: 10.1038/nm.2052]

139 **Enge M**, Bjarnegård M, Gerhardt H, Gustafsson E, Kalén M, Asker N, Hammes HP, Shani M, Fässler R, Betsholtz C. Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J* 2002; **21**: 4307-4316 [PMID: 12169633 DOI: 10.1093/emboj/cdf418]

140 **Jiang Y**, Zhang Q, Steinle JJ. Beta-adrenergic receptor agonist decreases VEGF levels through altered eNOS and PKC signaling in diabetic retina. *Growth Factors* 2015; **33**: 192-199 [PMID: 26115368 DOI: 10.3109/08977194.2015.1054990]

141 **Gálvez MI**. Rubosixtaurin and other PKC inhibitors in diabetic retinopathy and macular edema. Review. *Curr Diabetes Rev* 2009; **5**: 14-17 [PMID: 19199893 DOI: 10.2174/157339909787314167]

142 **Deissler HL**, Lang GE. The Protein Kinase C Inhibitor: Ruboxistaurin. *Dev Ophthalmol* 2016; **55**: 295-301 [PMID: 26501476 DOI: 10.1159/000431204]

143 **Eynard AR**, Repossi G. Role of ω3 polyunsaturated fatty acids in diabetic retinopathy: a morphological and metabolically cross talk among blood retina barriers damage, autoimmunity and chronic inflammation. *Lipids Health Dis* 2019; **18**: 114 [PMID: 31092270 DOI: 10.1186/s12944-019-1049-9]

144 **Liu A**, Chang J, Lin Y, Shen Z, Bernstein PS. Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. *J Lipid Res* 2010; **51**: 3217-3229 [PMID: 20688753 DOI: 10.1194/jlr.M007518]

145 **Skowronska-Krawczyk D**, Chao DL. Long-Chain Polyunsaturated Fatty Acids and Age-Related Macular Degeneration. *Adv Exp Med Biol* 2019; **1185**: 39-43 [PMID: 31884586 DOI: 10.1007/978-3-030-27378-1_7]

146 **Tanito M**, Anderson RE. Dual roles of polyunsaturated fatty acids in retinal physiology and pathophysiology associated with retinal degeneration. *Clin Lipidol* 2009; **4**: 821-827 [DOI: 10.2217/clp.09.65]

147 **Suzumura A**, Terao R, Kaneko H. Protective Effects and Molecular Signaling of n-3 Fatty Acids on Oxidative Stress and Inflammation in Retinal Diseases. *Antioxidants* **9**: 920 [PMID: 32993153 DOI: 10.3390/antiox9100920]

148 **Behl T**, Kotwani A. Omega-3 fatty acids in prevention of diabetic retinopathy. *J Pharm Pharmacol* 2017; **69**: 946-954 [PMID: 28481011 DOI: 10.1111/jphp.12744]

149 **SanGiovanni JP**, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 2005; **24**: 87-138 [PMID: 15555528 DOI: 10.1016/j.preteyeres.2004.06.002]

150 **Nowak JZ**. Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacol Rep* 2013; **65**: 288-304 [PMID: 23744414 DOI: 10.1016/s1734-1140(13)71005-3]

151 **Polak M**, Zagórski Z. Lipid peroxidation in diabetic retinopathy. *Ann Univ Mariae Curie Sklodowska Med* 2004; **59**: 434-437 [PMID: 16146026]

152 **Mondal LK**, Bhaduri G, Bhattacharya B. Biochemical scenario behind initiation of diabetic retinopathy in type 2 diabetes mellitus. *Indian J Ophthalmol* 2018; **66**: 535-540 [PMID: 29582815 DOI: 10.4103/ijo.IJO_1121_17]

153 **Pan HZ**, Zhang H, Chang D, Li H, Sui H. The change of oxidative stress products in diabetes mellitus and diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 548-551 [PMID: 18369071 DOI: 10.1136/bjo.2007.130542]

154 **Al-Shabrawey M**, Bartoli M, El-Remessy AB, Ma G, Matragoon S, Lemtalsi T, Caldwell RW, Caldwell RB. Role of NADPH oxidase and Stat3 in statin-mediated protection against diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2008; **49**: 3231-3238 [PMID: 18378570 DOI:

10.1167/iovs.08-1754]
155 **Al-Shabrawey M**, Bartoli M, El-Remessy AB, Platt DH, Matragoon S, Behzadian MA, Caldwell RW, Caldwell RB. Inhibition of NAD(P)H oxidase activity blocks vascular endothelial growth factor overexpression and neovascularization during ischemic retinopathy. *Am J Pathol* 2005; **167**: 599-607 [PMID: 16049343 DOI: 10.1016/S0002-9440(10)63001-5]
156 **Armstrong D**, al-Awadi F. Lipid peroxidation and retinopathy in streptozotocin-induced diabetes. *Free Radic Biol Med* 1991; **11**: 433-436 [PMID: 1797628 DOI: 10.1016/0891-5849(91)90161-u]
157 **Davì G**, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 1999; **99**: 224-229 [PMID: 9892587 DOI: 10.1161/01.cir.99.2.224]
158 **Augustin AJ**, Spitznas M, Koch F, Grus F, Böker T. Indicators of oxidative tissue damage and inflammatory activity in epiretinal membranes of proliferative diabetic retinopathy, proliferative vitreoretinopathy and macular pucker. *Ger J Ophthalmol* 1995; **4**: 47-51 [PMID: 7728110]
159 **Ayalasomayajula SP**, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *Eur J Pharmacol* 2003; **458**: 283-289 [PMID: 12504784 DOI: 10.1016/s0014-2999(02)02793-0]
160 **Li T**, Hu J, Du S, Chen Y, Wang S, Wu Q. ERK1/2/COX-2/PGE2 signaling pathway mediates GPR91-dependent VEGF release in streptozotocin-induced diabetes. *Mol Vis* 2014; **20**: 1109-1121 [PMID: 25324681]
161 **Njie-Mbye YF**, Kulkarni-Chitnis M, Opere CA, Barrett A, Ohia SE. Lipid peroxidation: pathophysiological and pharmacological implications in the eye. *Front Physiol* 2013; **4**: 366 [PMID: 24379787 DOI: 10.3389/fphys.2013.00366]
162 **Esterbauer H**, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; **11**: 81-128 [PMID: 1937131 DOI: 10.1016/0891-5849(91)90192-6]
163 **Van Kuijk FJ**, Holte LL, Dratz EA. 4-Hydroxyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. *Biochim Biophys Acta* 1990; **1043**: 116-118 [PMID: 2138035 DOI: 10.1016/0005-2760(90)90118-h]
164 **Esterbauer H**. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am J Clin Nutr* 1993; **57**: 779S-785S; discussion 785S [PMID: 8475896 DOI: 10.1093/ajcn/57.5.779S]
165 **Csala M**, Kardon T, Legeza B, Lizák B, Mandl J, Margittai É, Puskás F, Száraz P, Szelényi P, Bánhegyi G. On the role of 4-hydroxynonenal in health and disease. *Biochim Biophys Acta* 2015; **1852**: 826-838 [PMID: 25643868 DOI: 10.1016/j.bbadis.2015.01.015]
166 **Zhou T**, Zhou KK, Lee K, Gao G, Lyons TJ, Kowluru R, Ma JX. The role of lipid peroxidation products and oxidative stress in activation of the canonical wingless-type MMTV integration site (WNT) pathway in a rat model of diabetic retinopathy. *Diabetologia* 2011; **54**: 459-468 [PMID: 20978740 DOI: 10.1007/s00125-010-1943-1]
167 **Chen Y**, Hu Y, Zhou T, Zhou KK, Mott R, Wu M, Boulton M, Lyons TJ, Gao G, Ma JX. Activation of the Wnt pathway plays a pathogenic role in diabetic retinopathy in humans and animal models. *Am J Pathol* 2009; **175**: 2676-2685 [PMID: 19893025 DOI: 10.2353/ajpath.2009.080945]
168 **Cheng R**, Ding L, He X, Takahashi Y, Ma JX. Interaction of PPARα With the Canonic Wnt Pathway in the Regulation of Renal Fibrosis. *Diabetes* 2016; **65**: 3730-3743 [PMID: 27543085 DOI: 10.2337/db16-0426]
169 **Yao PL**, Peavey J, Malek G. Leveraging Nuclear Receptors as Targets for Pathological Ocular Vascular Diseases. *Int J Mol Sci* 2020; **21** [PMID: 32326149 DOI: 10.3390/ijms21082889]
170 **ACCORD Study Group**. ACCORD Eye Study Group, Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC Jr, Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ. Effects of medical therapies on retinopathy progression in type 2 diabetes. *N Engl J Med* 2010; **363**: 233-244 [PMID: 20587587 DOI: 10.1056/NEJMoa1001288]
171 **Ding L**, Cheng R, Hu Y, Takahashi Y, Jenkins AJ, Keech AC, Humphries KM, Gu X, Elliott MH, Xia X, Ma JX. Peroxisome proliferator-activated receptor α protects capillary pericytes in the retina. *Am J Pathol* 2014; **184**: 2709-2720 [PMID: 25108226 DOI: 10.1016/j.ajpath.2014.06.021]
172 **Hu Y**, Chen Y, Ding L, He X, Takahashi Y, Gao Y, Shen W, Cheng R, Chen Q, Qi X, Boulton ME, Ma JX. Pathogenic role of diabetes-induced PPAR-α down-regulation in microvascular dysfunction. *Proc Natl Acad Sci USA* 2013; **110**: 15401-15406 [PMID: 24003152 DOI: 10.1073/pnas.1307211110]
173 **Sharma A**, Sharma R, Chaudhary P, Vatsyayan R, Pearce V, Jeyabal PV, Zimniak P, Awasthi S, Awasthi YC. 4-Hydroxynonenal induces p53-mediated apoptosis in retinal pigment epithelial cells. *Arch Biochem Biophys* 2008; **480**: 85-94 [PMID: 18930016 DOI: 10.1016/j.abb.2008.09.016]
174 **Mori A**, Takei T, Sakamoto K, Nakahara T, Ishii K. 4-Hydroxy-2-nonenal attenuates β2-adrenoceptor-mediated vasodilation of rat retinal arterioles. *Naunyn Schmiedebergs Arch Pharmacol* 2015; **388**: 575-582 [PMID: 25693977 DOI: 10.1007/s00210-015-1099-0]
175 **Chiang YF**, Chen HY, Chang YJ, Shih YH, Shieh TM, Wang KL, Hsia SM. Protective Effects of Fucoxanthin on High Glucose- and 4-Hydroxynonenal (4-HNE)-Induced Injury in Human Retinal Pigment Epithelial Cells. *Antioxidants (Basel)* 2020; **9** [PMID: 33255669 DOI: 10.3390/antiox9121176]

176 **Esterbauer H**, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol* 1990; **186**: 407-421 [PMID: 2233308 DOI: 10.1016/0076-6879(90)86134-h]

177 **Ayala A**, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; **2014**: 360438 [PMID: 24999379 DOI: 10.1155/2014/360438]

178 **Cheeseman KH**, Beavis A, Esterbauer H. Hydroxyl-radical-induced iron-catalysed degradation of 2-deoxyribose. Quantitative determination of malondialdehyde. *Biochem J* 1988; **252**: 649-653 [PMID: 3421915 DOI: 10.1042/bj2520649]

179 **Morrow JD**, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci USA* 1990; **87**: 9383-9387 [PMID: 2123555 DOI: 10.1073/pnas.87.23.9383]

180 **Gopaul NK**, Anggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett* 1995; **368**: 225-229 [PMID: 7628610 DOI: 10.1016/0014-5793(95)00649-t]

181 **Galano JM**, Lee YY, Oger C, Vigor C, Vercauteren J, Durand T, Giera M, Lee JC. Isoprostanes, neuroprostanes and phytoprostanes: An overview of 25years of research in chemistry and biology. *Prog Lipid Res* 2017; **68**: 83-108 [PMID: 28923590 DOI: 10.1016/j.plipres.2017.09.004]

182 **Van't Erve TJ**, Lih FB, Jelsema C, Deterding LJ, Eling TE, Mason RP, Kadiiska MB. Reinterpreting the best biomarker of oxidative stress: The 8-iso-prostaglandin F2α/prostaglandin F2α ratio shows complex origins of lipid peroxidation biomarkers in animal models. *Free Radic Biol Med* 2016; **95**: 65-73 [PMID: 26964509 DOI: 10.1016/j.freeradbiomed.2016.03.001]

183 **Lahaie I**, Hardy P, Hou X, Hasséssian H, Asselin P, Lachapelle P, Almazan G, Varma DR, Morrow JD, Roberts LJ 2nd, Chemtob S. A novel mechanism for vasoconstrictor action of 8-isoprostaglandin F2 alpha on retinal vessels. *Am J Physiol* 1998; **274**: R1406-R1416 [PMID: 9612409 DOI: 10.1152/ajpregu.1998.274.5.R1406]

184 **Hou X**, Roberts LJ 2nd, Gobeil F Jr, Taber D, Kanai K, Abran D, Brault S, Checchin D, Sennlaub F, Lachapelle P, Varma D, Chemtob S. Isomer-specific contractile effects of a series of synthetic f2-isoprostanes on retinal and cerebral microvasculature. *Free Radic Biol Med* 2004; **36**: 163-172 [PMID: 14744628 DOI: 10.1016/j.freeradbiomed.2003.10.024]

185 **Torres-Cuevas I**, Millán I, Asensi M, Vento M, Oger C, Galano JM, Durand T, Ortega ÁL. Analysis of Lipid Peroxidation by UPLC-MS/MS and Retinoprotective Effects of the Natural Polyphenol Pterostilbene. *Antioxidants (Basel)* 2021; **10** [PMID: 33498744 DOI: 10.3390/antiox10020168]

186 **Simó R**, Hernández C. GLP-1R as a Target for the Treatment of Diabetic Retinopathy: Friend or Foe? *Diabetes* 2017; **66**: 1453-1460 [PMID: 28533296 DOI: 10.2337/db16-1364]

187 **Hölscher C**. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. *CNS Drugs* 2012; **26**: 871-882 [PMID: 22938097 DOI: 10.2165/11635890-000000000-00000]

188 **Hernández C**, Bogdanov P, Corraliza L, García-Ramírez M, Solà-Adell C, Arranz JA, Arroba AI, Valverde AM, Simó R. Topical Administration of GLP-1 Receptor Agonists Prevents Retinal Neurodegeneration in Experimental Diabetes. *Diabetes* 2016; **65**: 172-187 [PMID: 26384381 DOI: 10.2337/db15-0443]

189 **Zeng Y**, Yang K, Wang F, Zhou L, Hu Y, Tang M, Zhang S, Jin S, Zhang J, Wang J, Li W, Lu L, Xu GT. The glucagon like peptide 1 analogue, exendin-4, attenuates oxidative stress-induced retinal cell death in early diabetic rats through promoting Sirt1 and Sirt3 expression. *Exp Eye Res* 2016; **151**: 203-211 [PMID: 27212443 DOI: 10.1016/j.exer.2016.05.002]

190 **Fan Y**, Liu K, Wang Q, Ruan Y, Ye W, Zhang Y. Exendin-4 alleviates retinal vascular leakage by protecting the blood-retinal barrier and reducing retinal vascular permeability in diabetic Goto-Kakizaki rats. *Exp Eye Res* 2014; **127**: 104-116 [PMID: 24910901 DOI: 10.1016/j.exer.2014.05.004]

191 **Cross DA**, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995; **378**: 785-789 [PMID: 8524413 DOI: 10.1038/378785a0]

192 **Manning BD**, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell* 2017; **169**: 381-405 [PMID: 28431241 DOI: 10.1016/j.cell.2017.04.001]

193 **Rowlands J**, Heng J, Newsholme P, Carlessi R. Pleiotropic Effects of GLP-1 and Analogs on Cell Signaling, Metabolism, and Function. *Front Endocrinol (Lausanne)* 2018; **9**: 672 [PMID: 30532733 DOI: 10.3389/fendo.2018.00672]

194 **Kowluru RA**. Diabetic retinopathy, metabolic memory and epigenetic modifications. *Vision Res* 2017; **139**: 30-38 [PMID: 28700951 DOI: 10.1016/j.visres.2017.02.011]

195 **García-Giménez JL**, Mena-Mollá S, Beltrán-García J, Sanchis-Gomar F. Challenges in the analysis of epigenetic biomarkers in clinical samples. *Clin Chem Lab Med* 2017; **55**: 1474-1477 [PMID: 28301317 DOI: 10.1515/cclm-2016-1162]

196 **Zhong Q**, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. *J Cell Biochem* 2010; **110**: 1306-1313 [PMID: 20564224 DOI: 10.1002/jcb.22644]

197 **Olsen AS**, Sarras MP Jr, Leontovich A, Intine RV. Heritable transmission of diabetic metabolic memory in zebrafish correlates with DNA hypomethylation and aberrant gene expression. *Diabetes* 2012; **61**: 485-491 [PMID: 22228713 DOI: 10.2337/db11-0588]

198 **Duraisamy AJ**, Mishra M, Kowluru A, Kowluru RA. Epigenetics and Regulation of Oxidative Stress in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2018; **59**: 4831-4840 [PMID: 30347077 DOI: 10.1167/iovs.18-24548]

199 **Sahajpal N**, Kowluru A, Kowluru RA. The Regulatory Role of Rac1, a Small Molecular Weight GTPase, in the Development of Diabetic Retinopathy. *J Clin Med* 2019; **8** [PMID: 31277234 DOI: 10.3390/jcm8070965]

200 **Kowluru RA**, Kowluru A, Veluthakal R, Mohammad G, Syed I, Santos JM, Mishra M. TIAM1-RAC1 signalling axis-mediated activation of NADPH oxidase-2 initiates mitochondrial damage in the development of diabetic retinopathy. *Diabetologia* 2014; **57**: 1047-1056 [PMID: 24554007 DOI: 10.1007/s00125-014-3194-z]

201 **Kreuz S**, Fischle W. Oxidative stress signaling to chromatin in health and disease. *Epigenomics* 2016; **8**: 843-862 [PMID: 27319358 DOI: 10.2217/epi-2016-0002]

202 **Mishra M**, Kowluru RA. The Role of DNA Methylation in the Metabolic Memory Phenomenon Associated With the Continued Progression of Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2016; **57**: 5748-5757 [PMID: 27787562 DOI: 10.1167/iovs.16-19759]

203 **Xie MY**, Yang Y, Liu P, Luo Y, Tang SB. 5-aza-2'-deoxycytidine in the regulation of antioxidant enzymes in retinal endothelial cells and rat diabetic retina. *Int J Ophthalmol* 2019; **12**: 1-7 [PMID: 30662833 DOI: 10.18240/ijo.2019.01.01]

204 **Kadiyala CS**, Zheng L, Du Y, Yohannes E, Kao HY, Miyagi M, Kern TS. Acetylation of retinal histones in diabetes increases inflammatory proteins: effects of minocycline and manipulation of histone acetyltransferase (HAT) and histone deacetylase (HDAC). *J Biol Chem* 2012; **287**: 25869-25880 [PMID: 22648458 DOI: 10.1074/jbc.M112.375204]

205 **Corso-Díaz X**, Jaeger C, Chaitankar V, Swaroop A. Epigenetic control of gene regulation during development and disease: A view from the retina. *Prog Retin Eye Res* 2018; **65**: 1-27 [PMID: 29544768 DOI: 10.1016/j.preteyeres.2018.03.002]

206 **Gensous N**, Franceschi C, Santoro A, Milazzo M, Garagnani P, Bacalini MG. The Impact of Caloric Restriction on the Epigenetic Signatures of Aging. *Int J Mol Sci* 2019; **20** [PMID: 31022953 DOI: 10.3390/ijms20082022]

207 **Mishra M**, Zhong Q, Kowluru RA. Epigenetic modifications of Nrf2-mediated glutamate-cysteine ligase: implications for the development of diabetic retinopathy and the metabolic memory phenomenon associated with its continued progression. *Free Radic Biol Med* 2014; **75**: 129-139 [PMID: 25016074 DOI: 10.1016/j.freeradbiomed.2014.07.001]

208 **Kowluru RA**, Mishra M. Contribution of epigenetics in diabetic retinopathy. *Sci China Life Sci* 2015; **58**: 556-563 [PMID: 26025281 DOI: 10.1007/s11427-015-4853-0]

209 **Zhang X**, Zhao L, Hambly B, Bao S, Wang K. Diabetic retinopathy: reversibility of epigenetic modifications and new therapeutic targets. *Cell Biosci* 2017; **7**: 42 [PMID: 28815013 DOI: 10.1186/s13578-017-0167-1]

210 **Kowluru RA**, Kowluru A, Mishra M, Kumar B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. *Prog Retin Eye Res* 2015; **48**: 40-61 [PMID: 25975734 DOI: 10.1016/j.preteyeres.2015.05.001]

211 **Jin J**, Wang X, Zhi X, Meng D. Epigenetic regulation in diabetic vascular complications. *J Mol Endocrinol* 2019; **63**: R103-R115 [PMID: 31600719 DOI: 10.1530/JME-19-0170]

212 **Lambert M**, Benmoussa A, Provost P. Small Noncoding RNAs Derived From Eukaryotic Ribosomal RNA. *Noncoding RNA* 2019; **5** [PMID: 30720712 DOI: 10.3390/ncrna5010016]

213 **Creugny A**, Fender A, Pfeffer S. Regulation of primary microRNA processing. *FEBS Lett* 2018; **592**: 1980-1996 [PMID: 29683487 DOI: 10.1002/1873-3468.13067]

214 **McArthur K**, Feng B, Wu Y, Chen S, Chakrabarti S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* 2011; **60**: 1314-1323 [PMID: 21357793 DOI: 10.2337/db10-1557]

215 **Zhu K**, Hu X, Chen H, Li F, Yin N, Liu AL, Shan K, Qin YW, Huang X, Chang Q, Xu GZ, Wang Z. Downregulation of circRNA DMNT3B contributes to diabetic retinal vascular dysfunction through targeting miR-20b-5p and BAMBI. *EBioMedicine* 2019; **49**: 341-353 [PMID: 31636010 DOI: 10.1016/j.ebiom.2019.10.004]

216 **Yu CY**, Kuo HC. The emerging roles and functions of circular RNAs and their generation. *J Biomed Sci* 2019; **26**: 29 [PMID: 31027496 DOI: 10.1186/s12929-019-0523-z]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1463-1478

DOI: 10.4239/wjd.v12.i9.1463

ISSN 1948-9358 (online)

REVIEW

Holistic perspective of the role of gut microbes in diabetes mellitus and its management

Kannayiram Alagiakrishnan, Tyler Halverson

ORCID number: Kannayiram Alagiakrishnan 0000-0003-1444-2451; Tyler Halverson 0000-0001-5841-7659.

Author contributions: Alagiakrishnan K and Halverson T contributed equally by reviewing the literature and drafting the manuscript; both the authors have read and approved the final version.

Conflict-of-interest statement: The authors declare no potential conflict of interest.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Kannayiram Alagiakrishnan, Tyler Halverson, Department of Medicine, University of Alberta, Edmonton T6G 2G3, Alberta, Canada

Corresponding author: Kannayiram Alagiakrishnan, FRCPC, MD, Full Professor, Medicine, University of Alberta, B139-152 University Hospital Clinical Sciences Building, Edmonton T6G 2G3, Alberta, Canada. kalagiakri@aol.com

Abstract

The gut microbiota (GM) plays a role in the development and progression of type 1 and type 2 diabetes mellitus (DM) and its complications. Gut dysbiosis contributes to the pathogenesis of DM. The GM has been shown to influence the efficacy of different antidiabetic medications. Intake of gut biotics, like prebiotics, probiotics and synbiotics, can improve the glucose control as well as the metabolic profile associated with DM. There is some preliminary evidence that it might even help with the cardiovascular, ophthalmic, nervous, and renal complications of DM and even contribute to the prevention of DM. More large-scale research studies are needed before wide spread use of gut biotics in clinical practice as an adjuvant therapy to the current management of DM.

Key Words: Probiotics; Prebiotics; Synbiotics; Diabetes mellitus; Microbial dysbiosis; Antidiabetic drugs

Core Tip: The emerging role of the gut microbiome on diabetes development, progression as well as prevention has been discussed in this manuscript. The significance of gut dysbiosis in the aetiopathogenesis of diabetes mellitus and its complications has been reviewed. A bidirectional relationship exists between the antidiabetic drugs and the gut microbiome. Faecal transplantation, and bariatric surgery, typically used to treat morbid obesity, have also been shown to improve commensal gut microbiota changes. Diabetic outcomes and management can improve with better understanding of the drug-gut microbiome interactions. There is emerging evidence pointing out that gut biotics can be an add-on therapy with the antidiabetic management. To our knowledge, there is no evidence about the role of gut microbes of diabetic patients who had pancreatic cell transplantation, as well as the role of gut

Country/Territory of origin: Canada

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 26, 2021
Peer-review started: February 26, 2021
First decision: April 20, 2021
Revised: April 24, 2021
Accepted: August 13, 2021
Article in press: August 13, 2021
Published online: September 15, 2021

P-Reviewer: Park SC
S-Editor: Wang JL
L-Editor: A
P-Editor: Xing YX

biotics influencing the management in this group.

Citation: Alagiakrishnan K, Halverson T. Holistic perspective of the role of gut microbes in diabetes mellitus and its management. *World J Diabetes* 2021; 12(9): 1463-1478
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1463.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1463

INTRODUCTION

Globally diabetes mellitus (DM) is a common medical disorder and is seen in pandemic proportions[1] with the global prevalence in adult subjects is roughly 10%[2]. The International Diabetes Federation projected by 2035, there will be 592 million cases of diabetes in the world[3]. DM type 1 is secondary to auto-immune- mediated loss of beta-cell function and is seen in 5% of the diabetic population. DM type 2 is mainly due to insulin resistance and is seen in 95% of diabetic subjects[4]. The 2016 US National Health Interview Survey data showed roughly 8.58% of the population had type 2 DM and 0.55% had type 1 DM[5].

Various research has been done in the last decade since the study of the human microbiome in 2012[6,7]. Microbes contribute to 2% of human body weight and the bacterial genomes exceeds human genes by a factor of 150[8,9]. Gut microbiota (GM) varies with age, diet, geographical location, life style, and the use of xenobiotics[10-12]. In the recent years there have been more focus on the GM in the development, progression, and distant organ complications due to DM[13]. Many studies have shown the role of the gut microbiome in DM[14-17].

The gut microbiome starts to develop with the mode of birth and it is influenced by environmental factors, diet, as well as certain medications, including antibiotics[18]. There are differences between the gut microbes seen between non-diabetic and diabetic subjects[20] (Table 1). Gut dysbiosis plays a role in numerous diseases including DM. Both altered GM and endocrine disrupters can influence the development of DM[21]. In this literature review, we analyzed the evidence for the role of GM in the development, pathogenesis, complications, management, and prevention of DM.

LITERATURE SEARCH

A literature search was performed using the electronic databases MEDLINE (1966–February 2021), EMBASE and SCOPUS (1965–February 2021), and DARE (1966–February 2021). The main search items were gut bacteria, GM, intestinal flora, gut dysbiosis, type 1 DM, type 2 DM, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, probiotics, prebiotics, synbiotics, bariatric surgery, and faecal transplantation. Non-English articles were excluded.

GM IN TYPE 1 DM

Studies have shown that Firmicutes/Bacteroides ratio is altered in type 1 DM[22]. In the study by Huang *et al*[23] (2018) negative association was seen with gut microbe *Faecalibacterium* and *Ruminococcacea* and hemoglobin A1c (HbA1c), whereas in the study by Fassatoui *et al*[24]. (2019) a negative association was seen between HbA1c and *Akkermansia muciciniphia*. A systematic review of studies done in Hispanic populations showed that patients with newly diagnosed type 1 DM have high levels of *Bacteroides* with a reduced proportion of *Prevotella*, *Megamonas*, and *Acidaminococcus*. With the initiation of insulin treatment these subjects showed an increase of *Prevotella* levels. Prior to the development of type 1 DM, inverse relationship of Firmicutes/Bacteroidetes ratio has been reported[25].

Table 1 Changes in the microbiome in type 1 and type 2 diabetes mellitus

Location	Change in microbiome	Ref.
Type 1 diabetes		
Gastrointestinal tract	(1) Decreased: *Prevotella*; *Megamona*; *Acidaminococcus*; and (2) Increased: *Bacteriodes*	Elena *et al*[25], 2019
Gastrointestinal tract	(1) Decreased: *Bifidobacterium adolescentis*; *Bifidobacteria*; and (2) Increased: *Clostridium perfingens*; *Bacteroides*	De Goffau *et al*[122], 2013
Gastrointestinal tract	Increased: *Leptotrichia goodfellowii*; *Bacillus cerus*; *Enterobacter mori* LMG 25706	Tai *et al*[123], 2016
Gastrointestinal tract	Increased: *Bacteroidetes/Firmicutes*	Giongo *et al*[124], 2011
Gastrointestinal tract	(1) Decreased: *Faecalibacterium prausnitzii*; and (2) Increased: *Bacteroides dorei*; *Bacteroides vulgatus*	De Goffa *et al*[125], 2014
Gastrointestinal tract	(1) Decreased: *Prevotella*; *Akkermansia*; *Bifidobacterium adolescentis*; *Roseburia faecis*; *Faecalibacterium prausnitzii*; and (2) Increased: *Dialister invisus*; *Gemella sanguinis*; *Difidobacterium longum*	Brown *et al*[126], 2011
Type 2 diabetes		
Gastrointestinal tract	(1) Decreased: *Clostridium coccoides*; *Clostridium leptum*; and (2) Increased: *Lactobacillus*	Chen *et al*[28], 2019
Gastrointestinal tract	(1) Decreased: *Bifidobacterium*; *Bacteroides*; *Faecalibacterium*; *Akkermansia*; *Roseburia*; and (2) Increased: *Ruminococcus*; *Fusobacterium*; *Blautia*	Gurung *et al*[30], 2020
Gastrointestinal tract	(1) Decreased: *Bifidobacterium*; *Akkermansia*; and (2) Increased: *Dorea*	Li *et al*[127], 2020
Gastrointestinal tract	(1) Decreased: *Bifdobacterium*; and (2) Increased: *Lactobacillus*	Sedighi *et al*[31], 2017
Blood	(1) Decreased: *Aquabacterium*; *Xanthomonas*; *Pseudonocardia*; and (2) Increased: *Actinotalea*; *Alishewanella*; *Seiminibacterium*; *Pseudoclavibacter*	Qiu *et al*[38], 2019

GM IN TYPE 2 DM

The type of gut microbes and the changes seen with them influence the development of DM. The prominent GM seen in the intestine are the gram-positive Firmicutes and gram-negative Bacteroidetes and it is influenced by dietary changes[26]. A change in the ratio of Bacteroidetes to Firmicutes is associated with DM[27]. A case-control study done by Chen *et al*[28] (2019) in newly diagnosed type 2 DM subjects, *Lactobacillus* faecal count was significantly higher whereas *Clostridium coccoides* and *Clostridium leptum* was lower, and these changes in the microbes was positively correlated with glycated hemoglobin with higher *Lactobacillus* count subjects, and negatively correlated with decreased *Clostridium* count subjects when compared with healthy controls. Another study found that patients with DM showed an affiliation with the following phyla of bacteria: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria[29]. Alterations in the gut microbe population may be related to DM, and gut microbes *Ruminococcus* and *Fusobacterium* has been shown with the development of type 2 DM, when compared to healthy adults[30]. A study by Sedighi *et al*[31] (2017) found that patients with type 2 DM has increased levels of *Lactobacillus,* while healthy controls showed increased *Bifidobacterium*. With respect to the *Lactobacillus* genus, there are various mixed results suggesting its association with type 2 DM. Certain strain such as *L. acidophilus, L. gasseri, and L. salivarius* have been increased where as *L. amyloyorus* has been decreased[30]. However, many species from this genus, such as *L. plantarum, L. casei,* and *L. rhamnosus* are often involved in probiotic preparation and have shown to be beneficial in diabetic mice models[30]. Overall, it looks that there may be a strain-specific association with DM.

Further changes in the microbiome in patients with DM are listed in Table 1. Nutrient imbalance by affecting the GM can influence the development of type 2 DM. With newly diagnosed type 2 DM different measurement parameters like age, blood lipids, body-mass index, blood pressure, and dietary nutrient intake was related to the gut microbiome composition[32].

RELATIONSHIP BETWEEN GUT AND BLOOD MICROBIOME AND ITS ASSOCIATION WITH TYPE 2 DM

Cani *et al*[33] (2008) in their animal study showed lipopolysaccharide produced by gram negative intestinal bacteria can translocate into systemic circulation through a leaky gut and can result in endotoxemia causing metabolic dysfunction and obesity. Recent evidence points out in addition to gut microbiome, the blood microbiome plays a role in DM. Blood is usually considered to be sterile, but the research suggests the presence of a microbe or microbial component in healthy humans is known as a blood microbiome. The evidence for blood human microbiome is slowly growing[34-36].

In a study by Sato *et al*[37] (2014) with Japanese type 2 DM subjects, blood microbiome translocation from gut microbiome was detected at a higher rate (28%) in type 2 diabetic subjects when compared with healthy controls (4%) ($P < 0.01$). A recent nested case control study by Qiu *et al*[38] (2019) showed the blood microbe *Sediminibacterium* is associated with increased risk of type 2 DM [Odd ratio (OR) = 14.098, 95%CI: 1.358-146.330] where as the microbe *Bacteroides* in blood have a reduced risk for type 2 DM (OR = 0.367, 95%CI: 0.151- 0.894).

GM AS A COMPLEX ENDOCRINE ORGAN

The regulation of the GI system is done by short-chain fatty acids (SCFA) derived from the metabolism of carbohydrates, and GM plays a role in this function. In addition, the gut microbes produce hormone like chemicals that can act at distant targets. Neuroactive compounds like tryptophan and neurotransmitters like serotonin, dopamine, noradrenaline, GABA, and hormones like leptin, ghrelin and glucagon-like peptide 1 (GLP-1) are indirectly regulated by SCFAs *via* enteroendocrine cells. Overall, the gut microbes produce several substances of a hormonal nature into the circulation which act as distant sites. Because of the GM's ability to influence distant organs and systems as mentioned above it is considered as an endocrine organ. Overall GM functions as an autonomous endocrine organ and plays a role in bodily endocrine actions including neuroendocrine and immunoendocrine regulations[39-42].

DM AND GUT DYSBIOSIS

Gut dysbiosis, is a state of increased or altered prevalence of gut bacteria which might in turn result in many disorders such as gastrointestinal, obesity, DM, immunological, and neurobehavioral diseases[43]. Shifts in the GM's composition with more pathogenic species and phyla can contribute to the above-mentioned diseases. Hyperglycemia was associated with changes of microbiota composition, preferring the non-commensal ones, on the detriment of beneficial phyla such as Bacteroidetes, Proteobacteria, and Actinobacteria. The ratio of Firmicutes/Bacteroidetes has been found to be correlated with plasma glucose concentration. Microbiota are capable to ferment undigested carbohydrates, fiber, and other dietary and xenobiotic compounds to produce SCFAs, which through their ubiquitous receptor play an important role in host glucose metabolism[37,44,45]. The Human Microbiome plays a role in gut permeability, modification of bile acids, glucose breakdown and in the absorption of nutrients[46,47].

Normal commensal bacteria are helpful in maintaining the gut wall integrity, innate immunity, insulin sensitivity, metabolism, and in communication with the brain functions, as well as help to prevent the penetration of harmful microorganisms in the bowel. Bidirectional relationship exists between the GM and the brain. This chain of communication depends on the interaction of gut microbe through immune and neuroendocrine system with the central nervous system. Short-chain fatty acids, such as butyrates, acetates and propionates, produced by the GM are beneficial to different metabolic processes. The imbalance between the microbiome and host organism lead to dysbiosis. Gut microbiome dysbiosis through inflammation and metabolic dysregulation increases insulin resistance and influence the development of type 2 DM[48] (Figure 1).

Microbial dysbiosis can also be the result of nutritional imbalance which can lead to a low-grade inflammatory state, obesity, and other metabolic disorders[49]. Gut microbes affect gut permeability, glucose and lipid metabolism, energy homeostasis, and insulin sensitivity. Like any other medical conditions, gut microbes play a role in

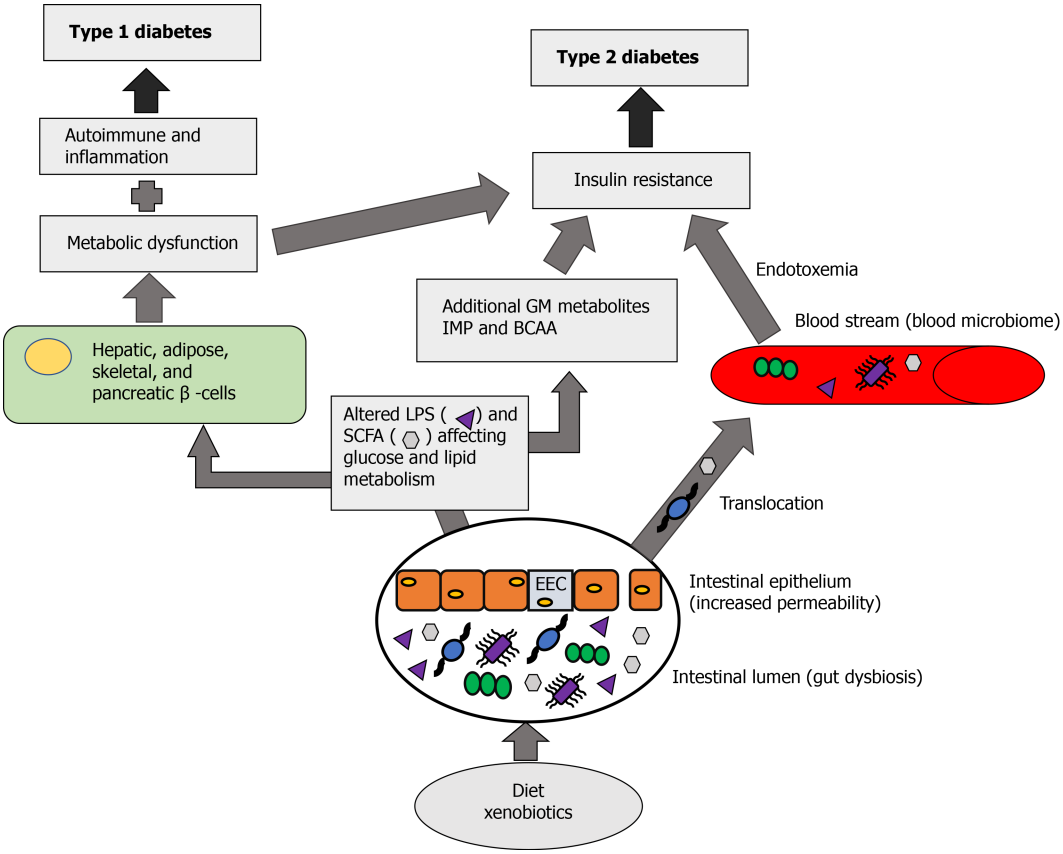

Figure 1 The role of gut dysbiosis in diabetes mellitus. The ingestion of a diet rich in carbohydrates and fats along with certain xenobiotics can lead to a disruption of the gut microbiome (dysbiosis). Under normal conditions, the gut bacteria produce metabolic products such as short chain fatty acids (SCFA) (Hexagons) that act locally and have a positive benefit on metabolism. Under conditions of dysbiosis there can be a disruption to the enteroendocrine cells and lead to gut permeability. This can lead to an increase in these metabolic products as well as bacterial translocation to the bloodstream, leading to endotoxemia resulting in metabolic dysfunction and insulin resistance contributing to type 2 diabetes. Gut dysbiosis also results in altered production of SCFA and release of lipopolysaccharides (LPS) (Triangles) and an increase production of other metabolites such as imidazole propionate and bacteria derived amino acids. These metabolites can act directly to affect insulin resistance. Excess SCFA and LPS can act on hepatic, skeletal, adipose, and pancreatic cells leading to metabolic dysfunction, altered inflammation and immune response which can influence insulin resistance. These factors can contribute to the development of type 1 and type 2 diabetes. SCFA: Short chain fatty acids; EEC: Enteroendocrine cells; LPS: Lipopolysaccharides; BCAA: Bacteria derived amino acids; IMP: Imidazole propionate; GM: Gut microbiota.

inflammation and immunity[50]. A diet rich in fat and sugar may lead to an abundance of lipopolysaccharide (LPS) release from GM and this LPS, by entering into systemic circulation, can affect β-cells, leading to decreased insulin release, and thereby altering systemic insulin sensitivity, resulting in insulin resistance, and potentially leading to DM[51].

Diets rich in carbohydrates and fat as well as xenobiotics (medications affecting the gut microbes) can cause gut dysbiosis. Normally GM produces metabolic products like SCFA, acetate, butyrate and propionate which acts locally leading to beneficial effects on different metabolic process. When there is gut dysbiosis, it can affect the enteroendocrine L-type cells in the intestinal epithelium and increase the gut permeability (leaky gut) causing these metabolic products to enter into the systemic circulation, as well as translocation of the gut microbiome into the circulation leading to the formation of the blood microbiome. This blood microbiome can cause endotoxemia and affect both metabolic dysfunction and insulin resistance. Gut dysbiosis results in excessive production of SCFA and LPS, as well as additional GM metabolites like imidazole propionate (IMP), derived from histidine, and bacteria derived amino acids. Excessive SCFA and LPS by acting on hepatic, adipose, skeletal and pancreatic cells causes metabolic dysfunction, inflammation and altered immune response. When there is a metabolic dysfunction due to gut dysbiosis combined with inflammatory and altered immune response it can cause type 1 DM, and when combined with insulin resistance due to gut dysbiosis as well as the effect of blood microbiome it can lead to the development of type 2 DM (Figure 1).

GUT MICROBES AND METABOLIC NETWORKS

The human gut contains a wide variety of microbial communities that carry out a wide range of biochemical functions that can influence the human body through metabolite production, physiological regulation, and interacting with the host's cellular response and immunity[52]. It has also been found that the host's own genetics can influence the composition of their gut microbiome, making each host a unique ecosystem[53]. Dynamic changes in the gut microbiome have been seen within individuals often in various disease states, such as obesity, and DM[19,54-56]. The GM has been found to cause enhanced transcriptional changes in the intestinal cells and protein biosynthesis in the crypts within the intestine[57].

SCFAs produced by GM can serve as signaling molecules that can influence the host's lipid and glucose levels, liver, skeletal muscle, and even immunity[52]. When there is a disruption of the gut microbiome, the altered mixture of SCFA may influence obesity, insulin sensitivity, weight gain and other comorbidities[58,59]. Obese individuals with type 2 DM have shown changes in the GM that are distinct, from non-diabetic subjects. It was found that individuals with type 2 DM showed an increase level of *Proteobacteria* and *Bacteroides* with a decreased level of *Firmicutes*[19].

The GM has been found to influence the host's metabolism and show great adaptability to the changing environment within the intestines based on diet, genetics, and various physiological cues from the host[52]. The human gut microbiome can modulate absorption as well as nutrient availability within the host. This can be achieved through gene expression changes, alteration of hormones and immunity[52].

ASSOCIATION BETWEEN MICROBIOME, OBESITY AND DM

Microbial diversity and the production of SCFA as well as products such as butyrate, propionate, and acetate have been found to have a protective role against obesity and insulin resistance[60,61]. SCFAs are able to act as signaling molecule that can activate a variety of pathways that are involved in cholesterol, lipid, and glucose metabolism [58]. Modifications of the microbiome can influence metabolic parameters, in particular when there is a higher abundance of Firmicutes leading to a higher Firmicutes/Bacteroidetes ratio that may be linked to obesity[62]. This may in part be due to the fact that Firmicutes are more efficient at promoting the nutrient absorption leading to subsequent weight gain compared to Bacteroidetes[63].

A study showed the GM composition is different in obese subjects with and without type 2 DM[20]. A recent study also showed for the first time in subjects with type 2 DM the relationship between body composition and GM[64]. Faecal microbiota of obese subjects without DM had increased numbers of SCFA producing microbes, whereas obese subjects with type 2 DM had less beneficial SCFA butyrate producing microbes[65].

ROLE OF GUT MICROBES IN THE PROGRESSION OF DM

The progression of DM is seen as macrovascular[66] and microvascular complications like retinopathy, nephropathy, and neuropathy[67]. Gut microbes seem to play a role in the progression of DM and also shown to play a role in these complications. Diet induced diabetic animal models helps to study these complications[68]. Studies have shown that subjects with DM and eye complications have higher bacterial conjunctival flora when compared to subjects without DM[69-72]. Beli *et al*[73] (2018) in their animal study showed the association between the GM and diabetic retinopathy (Table 2). More research is needed to understand the mechanism how GM causes diabetic retinopathy[74].

Diabetic neuropathy is seen as autonomic neuropathy as well as distal sensory and motor neuropathy and correlate with diabetic control, and GM also seems to play a role[75]. In a human study with early diabetic nephropathy, Barrios *et al*[76] (2015) showed an increase in colonic GM, whereas with end-stage renal disease patients microbes producing urease, uricase, p-cresol and indole-forming enzymes were seen [77]. The proposed mechanisms for progression of kidney disease could be due to GM imbalance, metabolic shifts, immunosuppression, inflammation, as well as accumulation of uremic toxins[78].

Table 2 Selected animal studies showing the effect of various interventions on the gut microbiome and the role of gut microbiota in diabetes mellitus management

Intervention	Organism	Health benefit	Change in microbiome	Ref.
Intermittent fasting	Mice	Protection from diabetic retinopathy by increasing Tauroursodeoxycholate (a neuroprotective bile acid) producing microbes	Increased Firmicutes and decreased Bacteroidetes and Verrucomicrobia in diabetic mice undergoing intermittent fasting	Beli *et al* [73], 2018
Antibiotic treatment (ampicillin, metronidazole, neomycin, vancomycin, or their cocktail)	Mice	Reduction in fasting glucose. Change in glucose tolerance (seen with ampicillin, vancomycin, or cocktail)	Alterations in the α- and β- diversity. An association with *Akkermansia muciniphila* with decrease fasting glucose. The effect is mediated through systemic changes in glucose metabolism	Rodrigues *et al*[94], 2017
Prebiotic: Acorn and sago	Mice	Mice fed acorn and sago derived prebiotics had an amelioration of the glucose intolerance and insulin resistance induced by a high-fat diet feeding. Intake of both novel prebiotics as well as inulin increases SCFAs levels in the mouse gut		Ahmadi *et al*[103], 2019
Combination of a functional fibre [PolyGlycopleX (PGX) with metformin (MET) or sitagliptin and metformin (S/MET)]	Mice	PGX + MET and PGX + S/MET showed reduced glycemia compared to controls and single treatment (P = 0.001). HbA1c was lower in PGX + S/MET compared to all other treatments (P = 0.001)		Reimer *et al* [93], 2014
Artificial sweetener (Neotame)	Mice	Decreased butyrate synthetic genes in Neotame group. Higher concentrations of cholesterol (P < 0.05) and fatty acids (P < 0.05) in Neotame treated mice feces	Reduction in α-diversity and altered β-diversity. Reduced Firmicutes (P < 0.01) and increased Bacteroides (P < 0.01)	Chi *et al* [85], 2018
Combination of metformin and a prebiotic [konjac mannan-oligosaccharides (MOS)]	Mice	Combination of metformin and MOS help ameliorate insulin resistance and improved glycemic control (P < 0.05) and repair islet and hepatic histology	Metformin and MOS change the microbiome (P < 0.0001) with: Decreased: Rikenellaceae and Clostridiales; Increased: *Akkermansia muciniphila* and *Bifidobacterium pseudolongum*	Zheng *et al* [96], 2018

MANAGEMENT

In DM, normal GM can be restored using diet, gut biotics, faecal transplantation, and bariatric surgery, which may help with the proper management of DM.

Faecal transplant, bariatric surgery

There is some evidence from human studies, that both faecal transplant and bariatric surgery improved the glucose and metabolic parameters by altering the GM[48]. A meta-analysis done by Magouliotis *et al*[79] (2017) showed some discrepancy between the human studies and the benefits witnessed from bariatric surgery. Another study looking at obese insulin resistant subjects who received allogenic faecal transplants from a lean insulin sensitive donor show improved insulin sensitivity for a short period of 6 weeks, however the benefit was not seen past 12 weeks[80] (Table 3).

Nutritional therapy

Diet can modulate the GM and play a role in the management of DM by preventing gut dysbiosis[81] (Table 2). Fruits and vegetables contain polyphenols which can increase beneficial GM like *A. muciniphila*, *Lactobacilli* and *Bifidobacteria*[82]. Unbalanced dietary intake can affect the structure and abundance of GM which can play a role in the development of DM[83].

Artificial sweeteners

Artificial sweeteners are no-calorie sugar substitutes, may induce glucose intolerance by affecting the gut microbes. In an animal study with saccharin-fed mice showed an increase in Bacteroides and a reduction in *Lactobacillus reuteri* leading to GM dysbiosis and glucose intolerance[84]. Similar effects were seen in another study by Chi *et al*[85] (2018) using the artificial sweetener, Neotame. In a cross-sectional human study by Frankenfeld *et al*[86] (2015), showed sweeteners like aspartame or acesulfame-K found no effect on gut bacterial abundance. A recent randomized-blinded crossover study in healthy participants did not demonstrate measurable changes in the GM or in SCFAs after 14 d daily intake of aspartame and sucralose[87]. These preliminary observations

Table 3 Selected human studies showing the effect of diet, gut biotics, faecal transplantation and bariatric surgery on gut microbiome and the role of gut microbiota in diabetes mellitus management

Intervention	Organism	Health benefit	Change in microbiome	Ref.
Probiotics	Human	Decreased fasting blood glucose and HbA1c levels. Increased HDL levels, however no significant effect on BMI and LDL levels were found		Kocsis *et al*[112], 2020
Artificial sweeteners (aspartame and acesulfame-K)	Human		Compared to controls, aspartame and acesulfame-K had different bacterial diversity ($P < 0.01$, $P = 0.03$ respectively), compared to controls	Frankenfeld *et al* [86], 2015
Probiotics, Prebiotics, or synbiotics	Human (meta-analysis)	The use of probiotics, prebiotics, or synbiotics showed a decrease in FBG ($P < 0.01$), total cholesterol ($P = 0.02$), triacylglycerols ($P = 0.01$) and insulinaemia ($P < 0.01$), as well as increased HDL-cholesterol levels ($P < 0.01$. Even though HbA1c reduction is seen it is not statistically significant. No effect on LDL-cholesterol was seen		Bock *et al*[115], 2020
Laparoscopic sleeve gastrectomy	Human	Decreased weight and BMI. Restored insulin tolerance and type 2 DM remission	Increased: *Bacteroidetes/Firmicutes* ratio at 1- and 3-months post surgery. *Lactobacillales*	Kikuchi *et al* [128], 2018;Li *et al*[129], 2013
Roux-en-Y gastric bypass	Human	Type 2 DM remission and improved BMI and weight loss. Improved gastric emptying and bile acid metabolism	Decreased: *Bacteroidetes/Firmicutes* ratio. Improved probiotic supplementation effects due to lowered pH environment	Selber-Hnatiw *et al*[52], 2020; Li *et al*[129], 2013

BMI: Body mass index; HbA1c: Hemoglobin A1c; FBG: Fasting blood glucose; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

needed to be established in future human research studies.

ALTERATION OF GM BY ANTIDIABETIC DRUGS AND ITS ROLE IN DM MANAGEMENT

Antidiabetic drugs can influence the gut microbiome by affecting the drug microbiome interface, whereas the gut microbiome also influences the metabolism and play a role in the efficacy of antidiabetic drugs. The interactions of antidiabetic drugs and microbiota is getting more attention as it may play a role in the management of DM [88]. Antidiabetic agents cause alteration of the specific gut microbes. Metformin increases the population of *Akkermansia muciniphila* by 18-fold, enhancing the digestion of mucin and increasing SCFA[89]. Metformin, in addition to *Akkermasia*, causes increase in *Lactobacillus* and *Bifidobacterium*, whereas insulin increase *Fusobacterium* [90]. This first line antidiabetic agent in type 2 DM modifies the GM, alter the bile acid circulation and thereby a possibility that primary site of action may be gut and the GM [91].

Understanding the pharmacokinetics, pharmacodynamics and pharmacomicrobiomics of antidiabetic medications and gut microbes can help to understand drug-gut microbiome and its potential benefit with antidiabetic drugs. Overall, it may help to better manage the DM management[92].

Antidiabetic drugs have been shown to affect the different gut microbes and their metabolic effects through the medication-microbiome-metabolism axis. GM can influence the pharmacokinetics of various antidiabetic drugs such as drug absorption, drug metabolism which can affect the potency of these medications. Overall, there is a bidirectional relationship exist between antidiabetic drugs and gut microbes[88].

Different combinations of antidiabetic drugs are used to better control DM. The commonly used combination is metformin with sulphonylureas, thiazolidinediones, DPP-4 inhibitors and insulin. One animal study showed some delay in the progression of DM when sitagliptin/metformin combination given with a prebiotic fibre[93]. Currently, there is a need for more research of different combination therapies on GM.

GUT BIOTICS AND DM

Animal studies

Several animal studies have showed that gut biotics, like prebiotics and probiotics, can improve the efficacy of antidiabetic drugs. Treatment with individual or a cocktail of antibiotics reduced dysbiosis and decrease fasting glucose but did not affect body weight, as well as antibiotic treatment also changed gene expression in the ileum and liver, and shifted the alpha (α) and beta (β) diversities of GM[94]. In an animal study with mice, combining probiotics and/or prebiotics with antidiabetic medications showed an improvement in glycemic control and insulin sensitivity[95]. A study by Reimer *et al*[93] (2014) found that using a combination of sitagliptin and metformin with a functional fiber can delay DM progression. In an animal study usage of mannan-oligosaccharides by altering the GM increased the hypoglycemic effects of metformin[96]. Yang *et al*[97] (2020) found that Genistein found in soybeans and soy derived foods (prebiotic) helped to improve glucose and lipid metabolism by altering GM composition[97]. In another animal study, certain GM like *Bacteroides fragilis, A. muciniphila, L. plantarum, L. casei* can induce interleukin 10 (IL-10), which has been shown to improve both insulin resistance and glucose metabolism[98] (Table 2).

Human studies

Many gut microbes have been shown to have antidiabetic effect in humans by different mechanisms including effect on insulin sensitivity[99]. *Roseburia intestinalis* can improve insulin sensitivity by increasing IL-22 production[100]. Some strains of *Lactobacilli* act like acarbose and have been shown to inhibit alpha glucosidase[101]. Prebiotics can feed the gut microbiome and increase the population of L-cells in the intestine and thereby increase the amount of GLP-1[102] and prevent high fat diet induced insulin resistance[103]. In the recent PREMOTE randomized control trial (RTC) study, probiotics showed antidiabetic effect by altering metabolic homeostasis [104]. Thus, GM may be useful in the management of DM[105]. Jafarnejad *et al*[106] (2015) and Asemi *et al*[107] (2014), in their two studies showed multi-probiotic supplement as well as synbiotic *(L. sporogenes* plus inulin) product helps to reduce glucose and other metabolic parameters. Tonucci *et al*[108] (2015) in their double-blind RCT study comparing fermented milk containing *L. acidophilus (LA-5)* plus *B. animalis (Lactis BB-12)* with plain fermented milk in 45 type 2 DM subjects showed decreased in HbA1c as well as low-density lipoproteins cholesterol and inflammatory cytokines. Multiple RTCs and the meta-analysis of these RCT's with different gut microbes demonstrated antidiabetic effect as well as effect on different metabolic parameters [109-111] (Table 3).

A recent meta-analysis of 14 RCTs showed significant decrease in HbA1c in the probiotic group compared to placebo controls, weighted mean difference (WMD) is -0.33%, 95%CI -0.53 to –0.13, P = 0.001. In this meta-analysis, probiotics significantly reduced fasting blood glucose, insulin, lipid profile and inflammatory marker in addition to blood pressure levels[112]. Another meta-analysis showed similar result with reduction in HbA1c% (WMD = - 0.24, 95%CI: - 0.44 to - 0.04, P = 0.02), fasting blood glucose (WMD = - 0.44 mmol/L, 95%CI: - 0.74 to - 0.15, P = 0.003)[113,114]. A meta-analysis study done in 2021 with probiotics, prebiotics or synbiotics on type 2 DM also showed significant improvement in glucose and other metabolic parameters [115]. Prebiotic inulin improves glycemic control in young adults with type 1 DM [116]. Certain specific species of probiotic microbes as well as certain prebiotics by altering the GM was shown to improve the auto-immune condition, which plays a major role in the pathogenesis of type 1 DM[117].

A study by Didari *et al*[118] (2014) looked at the safety of probiotics and synbiotics and found that certain populations, such as patients who are immunocompromised, with cardiac valvular disease, having a central venous catheter, or those with short-bowel syndrome may have an increased risk for systemic infections. Thus, caution may be warranted when using these products in diabetic patients and a risk-benefit analysis should be considered.

GUT MICROBES AND THE PREVENTION OF DM

Some preliminary evidence in animal studies indicates altering GM may help to prevent DM[119,120]. A recent study by Gurung *et al*[30] (2020) showed with certain gut microbes like *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Akkermansia* and *Roseburia* have a negative association with DM and appears to be protective. In 42

healthy adults, GM *Lactobacillus johnsonii* seems to reduce the risk of type 1 DM[121].

CONCLUSION

Gut dysbiosis plays a role in the development and progression of DM. The current evidence also points out that the GM can play a role in DM related complications. Modulation of the gut bacteria or dysbiosis can be corrected by fibre, diet, antidiabetic medications, and by using gut biotics like prebiotics, probiotics, and synbiotics as well as by bariatric surgery and faecal transplantation. The interaction between gut microbes and antidiabetic agents is a promising field that may change the landscape of DM management in the future. There is some preliminary evidence to show that GM may play a role in the prevention of DM. More research is needed on a large scale to confirm these findings.

REFERENCES

1 **Toniolo A**, Cassani G, Puggioni A, Rossi A, Colombo A, Onodera T, Ferrannini E. The diabetes pandemic and associated infections: suggestions for clinical microbiology. *Rev Med Microbiol* 2019; **30**: 1-17 [PMID: 30662163 DOI: 10.1097/MRM.0000000000000155]

2 **NCD Risk Factor Collaboration (NCD-RisC)**. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**: 1513-1530 [PMID: 27061677 DOI: 10.1016/S0140-6736(16)00618-8]

3 **Ayadurai S**, Hattingh HL, Tee LB, Md Said SN. A Narrative Review of Diabetes Intervention Studies to Explore Diabetes Care Opportunities for Pharmacists. *J Diabetes Res* 2016; **2016**: 5897452 [PMID: 27247949 DOI: 10.1155/2016/5897452]

4 **American Diabetes Association**. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018; **41**: S13-S27 [PMID: 29222373 DOI: 10.2337/dc18-S002]

5 **Bullard KM**, Cowie CC, Lessem SE, Saydah SH, Menke A, Geiss LS, Orchard TJ, Rolka DB, Imperatore G. Prevalence of Diagnosed Diabetes in Adults by Diabetes Type - United States, 2016. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 359-361 [PMID: 29596402 DOI: 10.15585/mmwr.mm6712a2]

6 **Human Microbiome Project Consortium**. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]

7 **Chattopadhyay A**, Mythili S. The journey of gut microbiome – an introduction and its influence on metabolic disorders. *Front Biol* 2018; **5**: 327-341 [DOI: 10.1007/s11515-018-1490-6]

8 **Molina DK**, DiMaio VJ. Normal organ weights in men: part I-the heart. *Am J Forensic Med Pathol* 2012; **33**: 362-367 [PMID: 22182983 DOI: 10.1097/PAF.0b013e31823d298b]

9 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Jian M, Zhou Y, Li Y, Zhang X, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

10 **Conlon MA**, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014; **7**: 17-44 [PMID: 25545101 DOI: 10.3390/nu7010017]

11 **He Y**, Wu W, Zheng HM, Li P, McDonald D, Sheng HF, Chen MX, Chen ZH, Ji GY, Zheng ZD, Mujagond P, Chen XJ, Rong ZH, Chen P, Lyu LY, Wang X, Wu CB, Yu N, Xu YJ, Yin J, Raes J, Knight R, Ma WJ, Zhou HW. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med* 2018; **24**: 1532-1535 [PMID: 30150716 DOI: 10.1038/s41591-018-0164-x]

12 **Maier L**, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P, Typas A. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018; **555**: 623-628 [PMID: 29555994 DOI: 10.1038/nature25979]

13 **Schroeder BO**, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med* 2016; **22**: 1079-1089 [PMID: 27711063 DOI: 10.1038/nm.4185]

14 **Barlow GM**, Yu A, Mathur R. Role of the Gut Microbiome in Obesity and Diabetes Mellitus. *Nutr Clin Pract* 2015; **30**: 787-797 [PMID: 26452391 DOI: 10.1177/0884533615609896]

15 **Bouter KE**, van Raalte DH, Groen AK, Nieuwdorp M. Role of the Gut Microbiome in the Pathogenesis of Obesity and Obesity-Related Metabolic Dysfunction. *Gastroenterology* 2017; **152**: 1671-1678 [PMID: 28192102 DOI: 10.1053/j.gastro.2016.12.048]

16 **Chen X**, Devaraj S. Gut Microbiome in Obesity, Metabolic Syndrome, and Diabetes. *Curr Diab Rep* 2018; **18**: 129 [PMID: 30338410 DOI: 10.1007/s11892-018-1104-3]

17 **Vallianou NG**, Stratigou T, Tsagarakis S. Microbiome and diabetes: Where are we now? *Diabetes Res Clin Pract* 2018; **146**: 111-118 [PMID: 30342053 DOI: 10.1016/j.diabres.2018.10.008]

18 **Purkait D**, Hameed S, Fatima Z. Chapter 15 - gut microbiome: Current development, challenges, and perspectives. In: Rastegari AA, Yadav AN, Yadav N. New and future developments in microbial biotechnology and bioengineering: Elsevier, 2020: 227-241

19 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]

20 **Thingholm LB**, Rühlemann MC, Koch M, Fuqua B, Laucke G, Boehm R, Bang C, Franzosa EA, Hübenthal M, Rahnavard A, Frost F, Lloyd-Price J, Schirmer M, Lusis AJ, Vulpe CD, Lerch MM, Homuth G, Kacprowski T, Schmidt CO, Nöthlings U, Karlsen TH, Lieb W, Laudes M, Franke A, Huttenhower C. Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition. *Cell Host Microbe* 2019; **26**: 252-264.e10 [PMID: 31399369 DOI: 10.1016/j.chom.2019.07.004]

21 **Nowak K**, Jabłońska E, Ratajczak-Wrona W. Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. *Environ Int* 2019; **125**: 350-364 [PMID: 30743143 DOI: 10.1016/j.envint.2019.01.078]

22 **Mrozinska S**, Kapusta P, Gosiewski T, Sroka-Oleksiak A, Ludwig-Słomczyńska AH, Matejko B, Kiec-Wilk B, Bulanda M, Malecki MT, Wolkow PP, Klupa T. The Gut Microbiota Profile According to Glycemic Control in Type 1 Diabetes Patients Treated with Personal Insulin Pumps. *Microorganisms* 2021; **9** [PMID: 33445500 DOI: 10.3390/microorganisms9010155]

23 **Huang Y**, Li SC, Hu J, Ruan HB, Guo HM, Zhang HH, Wang X, Pei YF, Pan Y, Fang C. Gut microbiota profiling in Han Chinese with type 1 diabetes. *Diabetes Res Clin Pract* 2018; **141**: 256-263 [PMID: 29733871 DOI: 10.1016/j.diabres.2018.04.032]

24 **Fassatoui M**, Lopez-Siles M, Díaz-Rizzolo DA, Jmel H, Naouali C, Abdessalem G, Chikhaoui A, Nadal B, Jamoussi H, Abid A, Gomis R, Abdelhak S, Martinez-Medina M, Kefi R. Gut microbiota imbalances in Tunisian participants with type 1 and type 2 diabetes mellitus. *Biosci Rep* 2019; **39** [PMID: 31147456 DOI: 10.1042/BSR20182348]

25 **Elena RM**, Gabriela GD, Arnulfo GC, Enrique CA. Studying the Gut Microbiome of Latin America and Hispanic/Latino Populations. Insight into Obesity and Diabetes: Systematic Review. *Curr Diabetes Rev* 2019; **15**: 294-301 [PMID: 30058496 DOI: 10.2174/1573399814666180730124817]

26 **Walker AW**, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A, Louis P, McIntosh F, Johnstone AM, Lobley GE, Parkhill J, Flint HJ. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011; **5**: 220-230 [PMID: 20686513 DOI: 10.1038/ismej.2010.118]

27 **Blandino G**, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. *Diabetes Metab* 2016; **42**: 303-315 [PMID: 27179626 DOI: 10.1016/j.diabet.2016.04.004]

28 **Chen PC**, Chien YW, Yang SC. The alteration of gut microbiota in newly diagnosed type 2 diabetic patients. *Nutrition* 2019; **63-64**: 51-56 [PMID: 30933725 DOI: 10.1016/j.nut.2018.11.019]

29 **Das T**, Jayasudha R, Chakravarthy S, Prashanthi GS, Bhargava A, Tyagi M, Rani PK, Pappuru RR, Sharma S, Shivaji S. Alterations in the gut bacterial microbiome in people with type 2 diabetes mellitus and diabetic retinopathy. *Sci Rep* 2021; **11**: 2738 [PMID: 33531650 DOI: 10.1038/s41598-021-82538-0]

30 **Gurung M**, Li Z, You H, Rodrigues R, Jump DB, Morgun A, Shulzhenko N. Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* 2020; **51**: 102590 [PMID: 31901868 DOI: 10.1016/j.ebiom.2019.11.051]

31 **Sedighi M**, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, Amirmozafari N. Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. *Microb Pathog* 2017; **111**: 362-369 [PMID: 28912092 DOI: 10.1016/j.micpath.2017.08.038]

32 **Nuli R**, Cai J, Kadeer A, Zhang Y, Mohemaiti P. Integrative Analysis Toward Different Glucose Tolerance-Related Gut Microbiota and Diet. *Front Endocrinol (Lausanne)* 2019; **10**: 295 [PMID: 31191448 DOI: 10.3389/fendo.2019.00295]

33 **Cani PD**, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; **57**: 1470-1481 [PMID: 18305141 DOI: 10.2337/db07-1403]

34 **Païssé S**, Valle C, Servant F, Courtney M, Burcelin R, Amar J, Lelouvier B. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. *Transfusion* 2016; **56**: 1138-1147 [PMID: 26865079 DOI: 10.1111/trf.13477]

35 **Olde Loohuis LM**, Mangul S, Ori APS, Jospin G, Koslicki D, Yang HT, Wu T, Boks MP, Lomen-Hoerth C, Wiedau-Pazos M, Cantor RM, de Vos WM, Kahn RS, Eskin E, Ophoff RA. Transcriptome analysis in whole blood reveals increased microbial diversity in schizophrenia. *Transl Psychiatry* 2018; **8**: 96 [PMID: 29743478 DOI: 10.1038/s41398-018-0107-9]

36 **Whittle E**, Leonard MO, Harrison R, Gant TW, Tonge DP. Multi-Method Characterization of the Human Circulating Microbiome. *Front Microbiol* 2018; **9**: 3266 [PMID: 30705670 DOI: 10.3389/fmicb.2018.03266]

37 **Sato J**, Kanazawa A, Ikeda F, Yoshihara T, Goto H, Abe H, Komiya K, Kawaguchi M, Shimizu T, Ogihara T, Tamura Y, Sakurai Y, Yamamoto R, Mita T, Fujitani Y, Fukuda H, Nomoto K,

Takahashi T, Asahara T, Hirose T, Nagata S, Yamashiro Y, Watada H. Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. *Diabetes Care* 2014; **37**: 2343-2350 [PMID: 24824547 DOI: 10.2337/dc13-2817]

38 **Qiu J**, Zhou H, Jing Y, Dong C. Association between blood microbiome and type 2 diabetes mellitus: A nested case-control study. *J Clin Lab Anal* 2019; **33**: e22842 [PMID: 30714640 DOI: 10.1002/jcla.22842]

39 **Evans JM**, Morris LS, Marchesi JR. The gut microbiome: the role of a virtual organ in the endocrinology of the host. *J Endocrinol* 2013; **218**: R37-R47 [PMID: 23833275 DOI: 10.1530/JOE-13-0131]

40 **Lyte M**. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *Bioessays* 2011; **33**: 574-581 [PMID: 21732396 DOI: 10.1002/bies.201100024]

41 **Hampl R**, Stárka L. Endocrine disruptors and gut microbiome interactions. *Physiol Res* 2020; **69**: S211-S223 [PMID: 33094620 DOI: 10.33549/physiolres.934513]

42 **Clarke G**, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut microbiota: the neglected endocrine organ. *Mol Endocrinol* 2014; **28**: 1221-1238 [PMID: 24892638 DOI: 10.1210/me.2014-1108]

43 **Rosenfeld CS**. Gut Dysbiosis in Animals Due to Environmental Chemical Exposures. *Front Cell Infect Microbiol* 2017; **7**: 396 [PMID: 28936425 DOI: 10.3389/fcimb.2017.00396]

44 **Velmurugan G**, Ramprasath T, Gilles M, Swaminathan K, Ramasamy S. Gut Microbiota, Endocrine-Disrupting Chemicals, and the Diabetes Epidemic. *Trends Endocrinol Metab* 2017; **28**: 612-625 [PMID: 28571659 DOI: 10.1016/j.tem.2017.05.001]

45 **Needell JC**, Zipris D. The Role of the Intestinal Microbiome in Type 1 Diabetes Pathogenesis. *Curr Diab Rep* 2016; **16**: 89 [PMID: 27523648 DOI: 10.1007/s11892-016-0781-z]

46 **Singer-Englar T**, Barlow G, Mathur R. Obesity, diabetes, and the gut microbiome: an updated review. *Expert Rev Gastroenterol Hepatol* 2019; **13**: 3-15 [PMID: 30791839 DOI: 10.1080/17474124.2019.1543023]

47 **Yu F**, Han W, Zhan G, Li S, Jiang X, Wang L, Xiang S, Zhu B, Yang L, Luo A, Hua F, Yang C. Abnormal gut microbiota composition contributes to the development of type 2 diabetes mellitus in db/db mice. *Aging (Albany NY)* 2019; **11**: 10454-10467 [PMID: 31760385 DOI: 10.18632/aging.102469]

48 **Halmos T**, Suba I. [Physiological patterns of intestinal microbiota. The role of dysbacteriosis in obesity, insulin resistance, diabetes and metabolic syndrome]. *Orv Hetil* 2016; **157**: 13-22 [PMID: 26708682 DOI: 10.1556/650.2015.30296]

49 **Fallucca F**, Porrata C, Fallucca S, Pianesi M. Influence of diet on gut microbiota, inflammation and type 2 diabetes mellitus. First experience with macrobiotic Ma-Pi 2 diet. *Diabetes Metab Res Rev* 2014; **30** Suppl 1: 48-54 [PMID: 24532292 DOI: 10.1002/dmrr.2518]

50 **Aw W**, Fukuda S. Understanding the role of the gut ecosystem in diabetes mellitus. *J Diabetes Investig* 2018; **9**: 5-12 [PMID: 28390093 DOI: 10.1111/jdi.12673]

51 **Acosta-Montaño P**, Rodríguez-Velázquez E, Ibarra-López E, Frayde-Gómez H, Mas-Oliva J, Delgado-Coello B, Rivero IA, Alatorre-Meda M, Aguilera J, Guevara-Olaya L, García-González V. Fatty Acid and Lipopolysaccharide Effect on Beta Cells Proteostasis and its Impact on Insulin Secretion. *Cells* 2019; **8** [PMID: 31412623 DOI: 10.3390/cells8080884]

52 **Selber-Hnatiw S**, Sultana T, Tse W, Abdollahi N, Abdullah S, Al Rahbani J, Alazar D, Alrumhein NJ, Aprikian S, Arshad R, Azuelos JD, Bernadotte D, Beswick N, Chazbey H, Church K, Ciubotaru E, D'Amato L, Del Corpo T, Deng J, Di Giulio BL, Diveeva D, Elahie E, Frank JGM, Furze E, Garner R, Gibbs V, Goldberg-Hall R, Goldman CJ, Goltsios FF, Gorjipour K, Grant T, Greco B, Guliyev N, Habrich A, Hyland H, Ibrahim N, Iozzo T, Jawaheer-Fenaoui A, Jaworski JJ, Jhajj MK, Jones J, Joyette R, Kaudeer S, Kelley S, Kiani S, Koayes M, Kpata AJAL, Maingot S, Martin S, Mathers K, McCullogh S, McNamara K, Mendonca J, Mohammad K, Momtaz SA, Navaratnarajah T, Nguyen-Duong K, Omran M, Ortiz A, Patel A, Paul-Cole K, Plaisir PA, Porras Marroquin JA, Prevost A, Quach A, Rafal AJ, Ramsarun R, Rhnima S, Rili L, Safir N, Samson E, Sandiford RR, Secondi S, Shahid S, Shahroozi M, Sidibé F, Smith M, Sreng Flores AM, Suarez Ybarra A, Sénéchal R, Taifour T, Tang L, Trapid A, Tremblay Potvin M, Wainberg J, Wang DN, Weissenberg M, White A, Wilkinson G, Williams B, Wilson JR, Zoppi J, Zouboulakis K, Gamberi C. Metabolic networks of the human gut microbiota. *Microbiology (Reading)* 2020; **166**: 96-119 [PMID: 31799915 DOI: 10.1099/mic.0.000853]

53 **Gordon JI**, Hooper LV, McNevin MS, Wong M, Bry L. Epithelial cell growth and differentiation. III. Promoting diversity in the intestine: conversations between the microflora, epithelium, and diffuse GALT. *Am J Physiol* 1997; **273**: G565-G570 [PMID: 9316460 DOI: 10.1152/ajpgi.1997.273.3.G565]

54 **Karlsson F**, Tremaroli V, Nielsen J, Bäckhed F. Assessing the human gut microbiota in metabolic diseases. *Diabetes* 2013; **62**: 3341-3349 [PMID: 24065795 DOI: 10.2337/db13-0844]

55 **Tremaroli V**, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**: 242-249 [PMID: 22972297 DOI: 10.1038/nature11552]

56 **Ussar S**, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, Deng L, Bry L, Gordon JI, Kahn CR. Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. *Cell Metab* 2015; **22**: 516-530 [PMID: 26299453 DOI: 10.1016/j.cmet.2015.07.007]

57 **El Aidy S**, Hooiveld G, Tremaroli V, Bäckhed F, Kleerebezem M. The gut microbiota and mucosal homeostasis: colonized at birth or at adulthood, does it matter? *Gut Microbes* 2013; **4**: 118-124 [PMID: 23333858 DOI: 10.4161/gmic.23362]

58 **den Besten G**, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**: 2325-2340 [PMID: 23821742 DOI: 10.1194/jlr.R036012]

59 **Canfora EE**, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015; **11**: 577-591 [PMID: 26260141 DOI: 10.1038/nrendo.2015.128]

60 **Lin HV**, Frassetto A, Kowalik EJ Jr, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, Marsh DJ. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones *via* free fatty acid receptor 3-independent mechanisms. *PLoS One* 2012; **7**: e35240 [PMID: 22506074 DOI: 10.1371/journal.pone.0035240]

61 **Gao Z**, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; **58**: 1509-1517 [PMID: 19366864 DOI: 10.2337/db08-1637]

62 **Rácz B**, Dušková M, Stárka L, Hainer V, Kunešová M. Links between the circadian rhythm, obesity and the microbiome. *Physiol Res* 2018; **67**: S409-S420 [PMID: 30484668 DOI: 10.33549/physiolres.934020]

63 **Koliada A**, Syzenko G, Moseiko V, Budovska L, Puchkov K, Perederiy V, Gavalko Y, Dorofeyev A, Romanenko M, Tkach S, Sineok L, Lushchak O, Vaiserman A. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol* 2017; **17**: 120 [PMID: 28532414 DOI: 10.1186/s12866-017-1027-1]

64 **Hung WC**, Hung WW, Tsai HJ, Chang CC, Chiu YW, Hwang SJ, Kuo MC, Chen SC, Dai CY, Tsai YC. The Association of Targeted Gut Microbiota with Body Composition in Type 2 Diabetes Mellitus. *Int J Med Sci* 2021; **18**: 511-519 [PMID: 33390820 DOI: 10.7150/ijms.51164]

65 **Meijnikman AS**, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating Causality of Gut Microbiota in Obesity and Diabetes in Humans. *Endocr Rev* 2018; **39**: 133-153 [PMID: 29309555 DOI: 10.1210/er.2017-00192]

66 **Leustean AM**, Ciocoiu M, Sava A, Costea CF, Floria M, Tarniceriu CC, Tanase DM. Implications of the Intestinal Microbiota in Diagnosing the Progression of Diabetes and the Presence of Cardiovascular Complications. *J Diabetes Res* 2018; **2018**: 5205126 [PMID: 30539026 DOI: 10.1155/2018/5205126]

67 **Zhao S**, Jang S, Lee YK, Kim DG, Jin Z, Koh HJ. Genetic Basis of Tiller Dynamics of Rice Revealed by Genome-Wide Association Studies. *Plants (Basel)* 2020; **9** [PMID: 33276582 DOI: 10.3390/nu12123719]

68 **Preguiça I**, Alves A, Nunes S, Gomes P, Fernandes R, Viana SD, Reis F. Diet-Induced Rodent Models of Diabetic Peripheral Neuropathy, Retinopathy and Nephropathy. *Nutrients* 2020; **12** [PMID: 31963709 DOI: 10.3390/nu12010250]

69 **Ham B**, Hwang HB, Jung SH, Chang S, Kang KD, Kwon MJ. Distribution and Diversity of Ocular Microbial Communities in Diabetic Patients Compared with Healthy Subjects. *Curr Eye Res* 2018; **43**: 314-324 [PMID: 29172724 DOI: 10.1080/02713683.2017.1406528]

70 **Rowan S**, Taylor A. The Role of Microbiota in Retinal Disease. *Adv Exp Med Biol* 2018; **1074**: 429-435 [PMID: 29721973 DOI: 10.1007/978-3-319-75402-4_53]

71 **Baim AD**, Movahedan A, Farooq AV, Skondra D. The microbiome and ophthalmic disease. *Exp Biol Med (Maywood)* 2019; **244**: 419-429 [PMID: 30463439 DOI: 10.1177/1535370218813616]

72 **Floyd JL**, Grant MB. The Gut-Eye Axis: Lessons Learned from Murine Models. *Ophthalmol Ther* 2020; **9**: 499-513 [PMID: 32617914 DOI: 10.1007/s40123-020-00278-2]

73 **Beli E**, Yan Y, Moldovan L, Vieira CP, Gao R, Duan Y, Prasad R, Bhatwadekar A, White FA, Townsend SD, Chan L, Ryan CN, Morton D, Moldovan EG, Chu FI, Oudit GY, Derendorf H, Adorini L, Wang XX, Evans-Molina C, Mirmira RG, Boulton ME, Yoder MC, Li Q, Levi M, Busik JV, Grant MB. Restructuring of the Gut Microbiome by Intermittent Fasting Prevents Retinopathy and Prolongs Survival in *db/db* Mice. *Diabetes* 2018; **67**: 1867-1879 [PMID: 29712667 DOI: 10.2337/db18-0158]

74 **St Leger AJ**, Caspi RR. Visions of Eye Commensals: The Known and the Unknown About How the Microbiome Affects Eye Disease. *Bioessays* 2018; **40**: e1800046 [PMID: 30289987 DOI: 10.1002/bies.201800046]

75 **Grasset E**, Burcelin R. The gut microbiota to the brain axis in the metabolic control. *Rev Endocr Metab Disord* 2019; **20**: 427-438 [PMID: 31656993 DOI: 10.1007/s11154-019-09511-1]

76 **Barrios C**, Beaumont M, Pallister T, Villar J, Goodrich JK, Clark A, Pascual J, Ley RE, Spector TD, Bell JT, Menni C. Gut-Microbiota-Metabolite Axis in Early Renal Function Decline. *PLoS One* 2015; **10**: e0134311 [PMID: 26241311 DOI: 10.1371/journal.pone.0134311]

77 **Wong J**, Piceno YM, DeSantis TZ, Pahl M, Andersen GL, Vaziri ND. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am J Nephrol* 2014; **39**: 230-237 [PMID: 24643131 DOI: 10.1159/000360010]

78 **Stavropoulou E**, Kantartzi K, Tsigalou C, Konstantinidis T, Romanidou G, Voidarou C, Bezirtzoglou E. Focus on the Gut-Kidney Axis in Health and Disease. *Front Med (Lausanne)* 2020; **7**: 620102 [PMID: 33553216 DOI: 10.3389/fmed.2020.620102]

79 **Magouliotis DE**, Tasiopoulou VS, Sioka E, Chatedaki C, Zacharoulis D. Impact of Bariatric Surgery on Metabolic and Gut Microbiota Profile: a Systematic Review and Meta-analysis. *Obes Surg* 2017; **27**: 1345-1357 [PMID: 28265960 DOI: 10.1007/s11695-017-2595-8]

80 **Vrieze A**, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; **143**: 913-6.e7 [PMID: 22728514 DOI: 10.1053/j.gastro.2012.06.031]

81 **Candela M**, Biagi E, Soverini M, Consolandi C, Quercia S, Severgnini M, Peano C, Turroni S, Rampelli S, Pozzilli P, Pianesi M, Fallucca F, Brigidi P. Modulation of gut microbiota dysbioses in type 2 diabetic patients by macrobiotic Ma-Pi 2 diet. *Br J Nutr* 2016; **116**: 80-93 [PMID: 27151248 DOI: 10.1017/S0007114516001045]

82 **Quddus MR**, Lawrence WD. A Seminal Observation! *Int J Surg Pathol* 2016; **24**: 47 [PMID: 26338717 DOI: 10.1177/1066896915604025]

83 **Krezalek MA**, Yeh A, Alverdy JC, Morowitz M. Influence of nutrition therapy on the intestinal microbiome. *Curr Opin Clin Nutr Metab Care* 2017; **20**: 131-137 [PMID: 27997410 DOI: 10.1097/MCO.0000000000000348]

84 **Suez J**, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, Kuperman Y, Harmelin A, Kolodkin-Gal I, Shapiro H, Halpern Z, Segal E, Elinav E. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 2014; **514**: 181-186 [PMID: 25231862 DOI: 10.1038/nature13793]

85 **Chi L**, Bian X, Gao B, Tu P, Lai Y, Ru H, Lu K. Effects of the Artificial Sweetener Neotame on the Gut Microbiome and Fecal Metabolites in Mice. *Molecules* 2018; **23** [PMID: 29425148 DOI: 10.3390/molecules23020367]

86 **Frankenfeld CL**, Sikaroodi M, Lamb E, Shoemaker S, Gillevet PM. High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. *Ann Epidemiol* 2015; **25**: 736-42.e4 [PMID: 26272781 DOI: 10.1016/j.annepidem.2015.06.083]

87 **Ahmad SY**, Friel J, Mackay D. The Effects of Non-Nutritive Artificial Sweeteners, Aspartame and Sucralose, on the Gut Microbiome in Healthy Adults: Secondary Outcomes of a Randomized Double-Blinded Crossover Clinical Trial. *Nutrients* 2020; **12** [PMID: 33171964 DOI: 10.3390/nu12113408]

88 **Whang A**, Nagpal R, Yadav H. Bi-directional drug-microbiome interactions of anti-diabetics. *EBioMedicine* 2019; **39**: 591-602 [PMID: 30553752 DOI: 10.1016/j.ebiom.2018.11.046]

89 **Wu H**, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Fèlix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernàndez-Real JM, Bäckhed F. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017; **23**: 850-858 [PMID: 28530702 DOI: 10.1038/nm.4345]

90 **Zhang F**, Wang M, Yang J, Xu Q, Liang C, Chen B, Zhang J, Yang Y, Wang H, Shang Y, Wang Y, Mu X, Zhu D, Zhang C, Yao M, Zhang L. Response of gut microbiota in type 2 diabetes to hypoglycemic agents. *Endocrine* 2019; **66**: 485-493 [PMID: 31410749 DOI: 10.1007/s12020-019-02041-5]

91 **Buse JB**, DeFronzo RA, Rosenstock J, Kim T, Burns C, Skare S, Baron A, Fineman M. The Primary Glucose-Lowering Effect of Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic and 12-Week Dose-Ranging Studies. *Diabetes Care* 2016; **39**: 198-205 [PMID: 26285584 DOI: 10.2337/dc15-0488]

92 **Rizkallah MR**, Saad R, Aziz RK. The human microbiome project, personalized medicine and the birth of pharmacomicrobiomics. *Curr Pharmacogenomics Person Med* 2010; **8**: 182-193 [DOI: 10.2174/187569210792246326]

93 **Reimer RA**, Grover GJ, Koetzner L, Gahler RJ, Lyon MR, Wood S. Combining sitagliptin/metformin with a functional fiber delays diabetes progression in Zucker rats. *J Endocrinol* 2014; **220**: 361-373 [PMID: 24389593 DOI: 10.1530/JOE-13-0484]

94 **Rodrigues RR**, Greer RL, Dong X, DSouza KN, Gurung M, Wu JY, Morgun A, Shulzhenko N. Antibiotic-Induced Alterations in Gut Microbiota Are Associated with Changes in Glucose Metabolism in Healthy Mice. *Front Microbiol* 2017; **8**: 2306 [PMID: 29213261 DOI: 10.3389/fmicb.2017.02306]

95 **Stenman LK**, Waget A, Garret C, Briand F, Burcelin R, Sulpice T, Lahtinen S. Probiotic B420 and prebiotic polydextrose improve efficacy of antidiabetic drugs in mice. *Diabetol Metab Syndr* 2015; **7**: 75 [PMID: 26366205 DOI: 10.1186/s13098-015-0075-7]

96 **Zheng J**, Li H, Zhang X, Jiang M, Luo C, Lu Z, Xu Z, Shi J. Prebiotic Mannan-Oligosaccharides Augment the Hypoglycemic Effects of Metformin in Correlation with Modulating Gut Microbiota. *J Agric Food Chem* 2018; **66**: 5821-5831 [PMID: 29701959 DOI: 10.1021/acs.jafc.8b00829]

97 **Yang R**, Jia Q, Mehmood S, Ma S, Liu X. Genistein ameliorates inflammation and insulin resistance through mediation of gut microbiota composition in type 2 diabetic mice. *Eur J Nutr* 2021; **60**: 2155-2168 [PMID: 33068158 DOI: 10.1007/s00394-020-02403-0]

98 **Dagdeviren S**, Jung DY, Friedline RH, Noh HL, Kim JH, Patel PR, Tsitsilianos N, Inashima K, Tran DA, Hu X, Loubato MM, Craige SM, Kwon JY, Lee KW, Kim JK. IL-10 prevents aging-

associated inflammation and insulin resistance in skeletal muscle. *FASEB J* 2017; **31**: 701-710 [PMID: 27811060 DOI: 10.1096/fj.201600832R]

99 **Andreasen AS**, Larsen N, Pedersen-Skovsgaard T, Berg RM, Møller K, Svendsen KD, Jakobsen M, Pedersen BK. Effects of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. *Br J Nutr* 2010; **104**: 1831-1838 [PMID: 20815975 DOI: 10.1017/S0007114510002874]

100 **Wang X**, Ota N, Manzanillo P, Kates L, Zavala-Solorio J, Eidenschenk C, Zhang J, Lesch J, Lee WP, Ross J, Diehl L, van Bruggen N, Kolumam G, Ouyang W. Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. *Nature* 2014; **514**: 237-241 [PMID: 25119041 DOI: 10.1038/nature13564]

101 **Panwar H**, Calderwood D, Grant IR, Grover S, Green BD. Lactobacillus strains isolated from infant faeces possess potent inhibitory activity against intestinal alpha- and beta-glucosidases suggesting anti-diabetic potential. *Eur J Nutr* 2014; **53**: 1465-1474 [PMID: 24414142 DOI: 10.1007/s00394-013-0649-9]

102 **Napolitano A**, Miller S, Nicholls AW, Baker D, Van Horn S, Thomas E, Rajpal D, Spivak A, Brown JR, Nunez DJ. Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus. *PLoS One* 2014; **9**: e100778 [PMID: 24988476 DOI: 10.1371/journal.pone.0100778]

103 **Ahmadi S**, Nagpal R, Wang S, Gagliano J, Kitzman DW, Soleimanian-Zad S, Sheikh-Zeinoddin M, Read R, Yadav H. Prebiotics from acorn and sago prevent high-fat-diet-induced insulin resistance *via* microbiome-gut-brain axis modulation. *J Nutr Biochem* 2019; **67**: 1-13 [PMID: 30831458 DOI: 10.1016/j.jnutbio.2019.01.011]

104 **Zhang Y**, Gu Y, Ren H, Wang S, Zhong H, Zhao X, Ma J, Gu X, Xue Y, Huang S, Yang J, Chen L, Chen G, Qu S, Liang J, Qin L, Huang Q, Peng Y, Li Q, Wang X, Kong P, Hou G, Gao M, Shi Z, Li X, Qiu Y, Zou Y, Yang H, Wang J, Xu G, Lai S, Li J, Ning G, Wang W. Gut microbiome-related effects of berberine and probiotics on type 2 diabetes (the PREMOTE study). *Nat Commun* 2020; **11**: 5015 [PMID: 33024120 DOI: 10.1038/s41467-020-18414-8]

105 **Adeshirlarijaney A**, Gewirtz AT. Considering gut microbiota in treatment of type 2 diabetes mellitus. *Gut Microbes* 2020; **11**: 253-264 [PMID: 32005089 DOI: 10.1080/19490976.2020.1717719]

106 **Jafarnejad S**, Saremi S, Jafarnejad F, Arab A. Effects of a Multispecies Probiotic Mixture on Glycemic Control and Inflammatory Status in Women with Gestational Diabetes: A Randomized Controlled Clinical Trial. *J Nutr Metab* 2016; **2016**: 5190846 [PMID: 27429803 DOI: 10.1155/2016/5190846]

107 **Asemi Z**, Khorrami-Rad A, Alizadeh SA, Shakeri H, Esmaillzadeh A. Effects of synbiotic food consumption on metabolic status of diabetic patients: a double-blind randomized cross-over controlled clinical trial. *Clin Nutr* 2014; **33**: 198-203 [PMID: 23786900 DOI: 10.1016/j.clnu.2013.05.015]

108 **Tonucci LB**, Olbrich Dos Santos KM, Licursi de Oliveira L, Rocha Ribeiro SM, Duarte Martino HS. Clinical application of probiotics in type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled study. *Clin Nutr* 2017; **36**: 85-92 [PMID: 26732026 DOI: 10.1016/j.clnu.2015.11.011]

109 **Zhang Q**, Wu Y, Fei X. Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. *Medicina (Kaunas)* 2016; **52**: 28-34 [PMID: 26987497 DOI: 10.1016/j.medici.2015.11.008]

110 **Yao K**, Zeng L, He Q, Wang W, Lei J, Zou X. Effect of Probiotics on Glucose and Lipid Metabolism in Type 2 Diabetes Mellitus: A Meta-Analysis of 12 Randomized Controlled Trials. *Med Sci Monit* 2017; **23**: 3044-3053 [PMID: 28638006 DOI: 10.12659/msm.902600]

111 **Kasińska MA**, Drzewoski J. Effectiveness of probiotics in type 2 diabetes: a meta-analysis. *Pol Arch Med Wewn* 2015; **125**: 803-813 [PMID: 26431318 DOI: 10.20452/pamw.3156]

112 **Kocsis T**, Molnár B, Németh D, Hegyi P, Szakács Z, Bálint A, Garami A, Soós A, Márta K, Solymár M. Probiotics have beneficial metabolic effects in patients with type 2 diabetes mellitus: a meta-analysis of randomized clinical trials. *Sci Rep* 2020; **10**: 11787 [PMID: 32678128 DOI: 10.1038/s41598-020-68440-1]

113 **Tao YW**, Gu YL, Mao XQ, Zhang L, Pei YF. Correction to: Effects of probiotics on type II diabetes mellitus: a meta-analysis. *J Transl Med* 2020; **18**: 105 [PMID: 32111256 DOI: 10.1186/s12967-020-02274-3]

114 **Tao YW**, Gu YL, Mao XQ, Zhang L, Pei YF. Effects of probiotics on type II diabetes mellitus: a meta-analysis. *J Transl Med* 2020; **18**: 30 [PMID: 31952517 DOI: 10.1186/s12967-020-02213-2]

115 **Bock PM**, Telo GH, Ramalho R, Sbaraini M, Leivas G, Martins AF, Schaan BD. The effect of probiotics, prebiotics or synbiotics on metabolic outcomes in individuals with diabetes: a systematic review and meta-analysis. *Diabetologia* 2021; **64**: 26-41 [PMID: 33047170 DOI: 10.1007/s00125-020-05295-1]

116 **Ho J**, Reimer RA, Doulla M, Huang C. Effect of prebiotic intake on gut microbiota, intestinal permeability and glycemic control in children with type 1 diabetes: study protocol for a randomized controlled trial. *Trials* 2016; **17**: 347 [PMID: 27456494 DOI: 10.1186/s13063-016-1486-y]

117 **Mishra SP**, Wang S, Nagpal R, Miller B, Singh R, Taraphder S, Yadav H. Probiotics and Prebiotics for the Amelioration of Type 1 Diabetes: Present and Future Perspectives. *Microorganisms* 2019; **7** [PMID: 30832381 DOI: 10.3390/microorganisms7030067]

118 **Didari T**, Solki S, Mozaffari S, Nikfar S, Abdollahi M. A systematic review of the safety of

probiotics. *Expert Opin Drug Saf* 2014; **13**: 227-239 [PMID: 24405164 DOI: 10.1517/14740338.2014.872627]

119 **Matsuzaki T**, Nagata Y, Kado S, Uchida K, Kato I, Hashimoto S, Yokokura T. Prevention of onset in an insulin-dependent diabetes mellitus model, NOD mice, by oral feeding of Lactobacillus casei. *APMIS* 1997; **105**: 643-649 [PMID: 9298104 DOI: 10.1111/j.1699-0463.1997.tb05066.x]

120 **Xie Y**, Xiao M, Ni Y, Jiang S, Feng G, Sang S, Du G. *Alpinia oxyphylla* Miq. Extract Prevents Diabetes in Mice by Modulating Gut Microbiota. *J Diabetes Res* 2018; **2018**: 4230590 [PMID: 29967794 DOI: 10.1155/2018/4230590]

121 **Marcial GE**, Ford AL, Haller MJ, Gezan SA, Harrison NA, Cai D, Meyer JL, Perry DJ, Atkinson MA, Wasserfall CH, Garrett T, Gonzalez CF, Brusko TM, Dahl WJ, Lorca GL. *Lactobacillus johnsonii* N6.2 Modulates the Host Immune Responses: A Double-Blind, Randomized Trial in Healthy Adults. *Front Immunol* 2017; **8**: 655 [PMID: 28659913 DOI: 10.3389/fimmu.2017.00655]

122 **de Goffau MC**, Luopajärvi K, Knip M, Ilonen J, Ruohtula T, Härkönen T, Orivuori L, Hakala S, Welling GW, Harmsen HJ, Vaarala O. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. *Diabetes* 2013; **62**: 1238-1244 [PMID: 23274889 DOI: 10.2337/db12-0526]

123 **Tai N**, Peng J, Liu F, Gulden E, Hu Y, Zhang X, Chen L, Wong FS, Wen L. Microbial antigen mimics activate diabetogenic CD8 T cells in NOD mice. *J Exp Med* 2016; **213**: 2129-2146 [PMID: 27621416 DOI: 10.1084/jem.20160526]

124 **Giongo A**, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; **5**: 82-91 [PMID: 20613793 DOI: 10.1038/ismej.2010.92]

125 **de Goffau MC**, Fuentes S, van den Bogert B, Honkanen H, de Vos WM, Welling GW, Hyöty H, Harmsen HJ. Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia* 2014; **57**: 1569-1577 [PMID: 24930037 DOI: 10.1007/s00125-014-3274-0]

126 **Brown CT**, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 2011; **6**: e25792 [PMID: 22043294 DOI: 10.1371/journal.pone.0025792]

127 **Li Q**, Chang Y, Zhang K, Chen H, Tao S, Zhang Z. Implication of the gut microbiome composition of type 2 diabetic patients from northern China. *Sci Rep* 2020; **10**: 5450 [PMID: 32214153 DOI: 10.1038/s41598-020-62224-3]

128 **Kikuchi R**, Irie J, Yamada-Goto N, Kikkawa E, Seki Y, Kasama K, Itoh H. The Impact of Laparoscopic Sleeve Gastrectomy with Duodenojejunal Bypass on Intestinal Microbiota Differs from that of Laparoscopic Sleeve Gastrectomy in Japanese Patients with Obesity. *Clin Drug Investig* 2018; **38**: 545-552 [PMID: 29508311 DOI: 10.1007/s40261-018-0638-0]

129 **Li JF**, Lai DD, Ni B, Sun KX. Comparison of laparoscopic Roux-en-Y gastric bypass with laparoscopic sleeve gastrectomy for morbid obesity or type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. *Can J Surg* 2013; **56**: E158-E164 [PMID: 24284156 DOI: 10.1503/cjs.026912]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1479-1493

DOI: 10.4239/wjd.v12.i9.1479

ISSN 1948-9358 (online)

REVIEW

Non-alcoholic fatty liver disease in diabetes: When to refer to the hepatologist?

Reshu Khandelwal, Anuradha S Dassanayake, Hari S Conjeevaram, Shivaram P Singh

ORCID number: Reshu Khandelwal 0000-0003-3541-8119; Anuradha S Dassanayake 0000-0001-5567-3542; Hari S Conjeevaram 0000-0003-1311-9863; Shivaram P Singh 0000-0002-8197-2674.

Author contributions: Khandelwal R performed the majority of writing and prepared the table and figures; Singh SP provided input and designed the outline of this paper; Dassanayake AS and Conjeevaram HS were responsible for the revision of the manuscript and editing of the language; All authors approved the submission of the final version.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Reshu Khandelwal, Shivaram P Singh, Department of Gastroenterology, Srirama Chandra Bhanja (SCB) Medical College and Hospital, Cuttack 753007, Odisha, India

Anuradha S Dassanayake, Department of Medicine, Colombo North Centre for Liver Disease, University of Kelaniya, Kelaniya 11600, Sri Lanka

Hari S Conjeevaram, Division of Gastroenterology and Hepatology, University of Michigan Medical School, Ann Arbor, MI 48109, United States

Corresponding author: Shivaram P Singh, FACG, FRCP, MBBS, MD, Professor, Department of Gastroenterology, Srirama Chandra Bhanja (SCB) Medical College and Hospital, 3rd Floor, Cuttack 753007, Odisha, India. scb_gastro_dept@hotmail.com

Abstract

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases worldwide. A strong relationship exists between NAFLD and diabetes mellitus. There is growing evidence of a mechanistically complex and strong association between the two diseases. Current data also shows that one disease actually leads to worsening of the other and *vice versa*. Understanding of the various pathophysiological mechanisms involved, natural history and spectrum of these two diseases is essential not only for early diagnosis and management but also for prevention of severe disease forms. Despite the tremendous progress made in recent times in acquiring knowledge about these highly prevalent diseases, the guidelines and recommendations for screening and management of diabetics with NAFLD remain ambiguous. An interdisciplinary approach is required to not only raise awareness of the prevalence of NAFLD in diabetics but also for better patient management. This can help attenuate the development of significant complications, such as cirrhosis, decompensation and hepatocellular carcinoma in these patients, thereby halting NAFLD in its tracks. This review focuses on the pivotal role of primary care physicians and endocrinologists in identification of NAFLD in diabetics in early stages and the role of proactive screening for prompt referral to hepatologist.

Key Words: Fibrosis; Diabetes; Insulin resistance; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Steatosis

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: India

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

Received: May 17, 2021
Peer-review started: May 17, 2021
First decision: June 5, 2021
Revised: June 18, 2021
Accepted: August 3, 2021
Article in press: August 3, 2021
Published online: September 15, 2021

P-Reviewer: Ji G, Wang CR
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Li X

Core Tip: With prevalence of non-alcoholic fatty liver disease (NAFLD) in diabetics being substantial, there is a need for its increased awareness and knowledge in the primary care physicians and endocrinologists. It is important to understand that these patients have the propensity to develop more severe forms of liver diseases, and their early identification and management can help in providing a stitch in time. We have reviewed in detail, the currently available societal guidance on screening of NAFLD in diabetics, especially with regards to high-risk patients that require hepatologist's referral. We have even proposed a screening protocol for these patients based on available literature. This will not only help the treating physicians in identifying the disease in its incipient stages but also help in patient's timely referral.

Citation: Khandelwal R, Dassanayake AS, Conjeevaram HS, Singh SP. Non-alcoholic fatty liver disease in diabetes: When to refer to the hepatologist? *World J Diabetes* 2021; 12(9): 1479-1493
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1479.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1479

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has assumed the status of major global health concern in recent times. It has become the most common chronic liver disease (CLD) worldwide, with prevalence among the general population being 25%-35%[1]. NAFLD is increasingly being recognized not only in adults but also in children and adolescents, adding further to the disease burden[2]. An individual is said to have NAFLD if the liver biopsy or imaging shows evidence of hepatic steatosis (≥ 5% liver fat) with background history of little or no alcohol consumption and in the absence of other liver diseases/conditions leading to hepatic steatosis[3]. The disease spectrum varies from simple steatosis (simple fatty liver) to the more severe and progressive non-alcoholic steatohepatitis (NASH) and cirrhosis[4,5]. Cirrhosis due to NASH is currently the second leading etiology for liver transplantation in the United States as well as in Europe and is projected to become the leading indication in the next decade [6,7]. The risk factors associated with NAFLD are also linked with other manifestations of metabolic syndrome viz diabetes, dyslipidemia, obesity and hypertension suggesting a relationship between these metabolic traits and the likelihood of developing NAFLD and also advanced fibrosis[8-10]. Studies have shown that 70%-80% of diabetics have NAFLD[11,12]. The presence of diabetes and obesity in patients with NAFLD has consistently been shown to be a key predictor of inflammatory disease progression leading to NASH and advanced fibrosis[13-15]. It is noteworthy that NAFLD is also frequent in subjects at increased risk for developing diabetes, including patients with the metabolic syndrome[13,16] and women with gestational diabetes or polycystic ovary syndrome[17], further underscoring the relationship between the two diseases. The strong association, as highlighted by various studies, is mechanistically complex, and NAFLD may precede or succeed diabetes onset[18,19]. Despite this, ambiguity remains in the guidelines and recommendations for screening of diabetic patients for NAFLD and *vice versa*, and their management. As most of the diabetic patients and patients with metabolic syndrome are under long-term treatment from an endocrinologist or a general physician, they are unlikely to visit a hepatologist for liver assessment and risk stratification, before they develop significant liver-related morbidity. This article represents an effort to understand and examine not only the link between the two diseases but to also review the available guidelines and screening strategies, and suggest future directives for these patients. Timely referral to a specialist can surely help nip 'the epidemic of this liver disease' in the bud.

ASSOCIATION BETWEEN NAFLD AND DIABETES - ARE THEY CO-PREVALENT OR CORRELATED?

NAFLD has often been referred to as the "hepatic manifestation" of metabolic syndrome[20]. It is known that lipid accumulation is the hallmark of NAFLD. In order

to understand if NAFLD is the cause or consequence of diabetes or are they both just co-passengers, we need to delve deeper into the pathophysiology of the two entities.

Cross-talk between adipose tissue, insulin and liver in normal individuals

Adipose tissue present in the body not only acts as a storage depot but also prevents ectopic lipid deposition in muscle, liver, heart, and other tissues. Besides, it also acts as an endocrine gland, secreting many hormones, cytokines, and vasoactive substances [21]. It has been found that upon eating a meal, there is an increase in the rate of insulin secretion, that facilitates the entry of glucose into the adipocytes[22] enabling the generation of L-α-glycerophosphate needed for triglyceride formation[23]. Insulin also increases the lipoprotein lipase activity in adipose tissue, thus promoting the generation of free fatty acids (FFAs) from chylomicron-triglyceride which leads to increase in the rate of entry of FFAs into adipocytes[24]. Besides, insulin is known to inhibit the action of hormone-sensitive lipase, the enzyme that causes hydrolysis of the triglycerides already stored in the adipocytes, further reducing the levels of circulating FFAs and glycerol[25]. The various adipokines released from adipose tissue, including adiponectin and cytokines, regulate liver energy metabolism[26]. In addition, adiponectin also stimulates β oxidation in the liver and improves liver insulin sensitivity[27].

When carbohydrates are abundant, the liver not only utilizes glucose as the main metabolic fuel but also converts glucose into fatty acids[28]. Hepatocytes derive FFAs either from diet or from adipose tissue *via* lipolysis and/or from hepatic *de novo* lipogenesis (DNL)[28]. Once inside the hepatocytes, the FFAs are acted on by acyl-CoA synthases to form fatty acyl-CoAs, which may enter either esterification and/or β-oxidation pathways[29]. Studies have shown that 59% triacylglycerols (TAGs) that tend to accumulate in liver, come from circulating FFAs; DNL, which is the process in which carbohydrates are converted to lipids, contributes to 26% and the rest 14% is from the diet[30]. The TAGs and cholesterol esters are either stored in lipid droplets within hepatocytes or secreted into the circulation as very-low density lipoprotein (VLDL) particles. Insulin has an important action in liver as it potently suppresses gluconeogenesis in liver and stimulates lipogenesis[28]. TAG accumulation is not hepatotoxic *per se* and could represent a defensive mechanism to balance FFA excess, as demonstrated in mouse models[31]. Studies have shown that increased TAG concentration is an epiphenomenon which happens simultaneously with toxic metabolite generation, lipotoxicity and liver injury[32].

Normally, in the liver, there is a fine balance between lipid uptake (in the form of FFAs/DNL, and esterification) and lipid disposal (in the form of metabolism/β-oxidation and elimination as VLDLs)[33]. In patients with NAFLD, it has been demonstrated that VLDL removal, at times, is unable to keep pace with the increased rate of TAG uptake and intrahepatic production[33], leading to metabolic disturbances.

Insulin resistance: The key player in NAFLD and diabetes

The pathogenesis of NAFLD was earlier explained by the 'two-hits hypothesis', according to which the 'first hit' was hepatic accumulation of lipids, occurring secondary to sedentary lifestyle, high fat diet, obesity and insulin resistance (IR). This sensitized the liver to further insults, which acted as 'second hit', thereby leading to activation of inflammatory cascades and fibrogenesis[34]. The dictum that steatosis always precedes inflammation has now largely been discarded, as it was discovered that NASH can also present in liver '*de novo*'. Indeed, the timing and combination of genetic, external and intracellular events, rather than the simple sum of hepatic insults, result in different pathways leading to steatosis or NASH[35]. In order to overcome the shortcomings, the 'multiple hit' theory for pathogenesis of NAFLD has been proposed. Such hits include IR, hormones from the adipose tissue, nutritional factors, gut microbiota, and genetic and epigenetic factors[36].

IR has been highlighted as one of the key events occurring in NAFLD and diabetes, but difficulty lies in establishing if it is the cause or the consequence[37]. The link between diabetes and NAFLD can be described by a spectrum of metabolic changes represented by IR, defective hepatic lipid profile and TAG metabolism causing fat accumulation, immune responses, and/or subsequent hyperinsulinemia as determined by the β-cell dysfunction in diabetes[37].

Presence of IR in NAFLD has also been substantiated in one study which showed that lean non-diabetic men with increased liver fat (as quantified by MRS) had both hepatic and adipose tissue IR along with impaired insulin suppression of glucose production and serum FFAs, when compared with subjects matched for both body mass index (BMI) and intra-abdominal fat but having low levels of hepatic fat[38].

IR and the liver

IR causes decrease in the rate of glycogen synthesis, along with increased rate of gluconeogenesis in liver[30]. The increase in intrahepatic glucose and resultant glycolysis provide substrates for DNL. There is increased production of acetylCoA which gets converted to malonylCoA that gets sequestered towards DNL as a substrate, thereby leading to hepatic steatosis[30]. Transcriptional regulation of DNL is primarily orchestrated by the sterol regulating element binding protein 1c (SREBP1c)[39]. Glucose and insulin promote lipogenesis through activation of the carbohydrate response element binding protein (ChREBP) and SREBP1c[39]. In states of IR, SREBP-1c is over-expressed and DNL is up-regulated[40]. SREBP1c can enhance the generation of harmful lipid molecules, such as diacylglycerol and ceramides, which further enhance IR. This results in a positive feedback loop in which hepatic DNL helps IR and IR stimulates hepatic DNL[41]. Also, β-oxidation of FFAs is inhibited in IR states, further promoting accumulation of hepatic lipids[42]. Excess of stored fat leads to abnormal lipid peroxidation and release of pro-inflammatory cytokines, high reactive oxygen species, and reactive nitrogen species causing liver disease[37]. The role of abnormal adipocyte and liver macrophage activity has also been highlighted in research models[37].

FFAs in the hepatocytes can induce defects in insulin signaling pathways through serine-kinase activation, thereby contributing to the IR[43]. Further, the increased FFAs released, due to excessive intra-hepatic TAGs, cause hepatic IR and inflammation[44], and localized intrahepatic inflammation can contribute to peripheral IR[45]. In addition to this, an increase in circulating FFAs impairs the ability of insulin to suppress endogenous glucose production and may directly enhance hepatic glucose production[25]. This explains why hepatic steatosis resolution can prevent diabetes onset[46].

IR and adipose tissue

IR by its action on adipose tissue, causes lipolysis, increasing the flux of FFAs to the liver. The adipocyte tissue becomes inflamed and dysfunctional and releases adipokines and inflammatory cytokines, such asinterleukin-6 and tumor necrosis factor (TNF)α-1, and there is decreased release of anti-inflammatory adiponectin[47]. The imbalance between pro-insulin (adiponectin, leptin) and anti-insulin (TNFα) cytokines further helps in the development of IR[48]. A novel adipokine, Gremlin 1, that antagonizes insulin signaling is positively correlated with the percentage of body fat and IR in diabetes and NAFLD/NASH subjects, and may become a potential biomarker or therapeutic target in the future[49].

Other mechanisms behind association of NAFLD and diabetes

Some researchers have proposed that chronic hyperglycemia ("glucose toxicity" or glucotoxicity), especially in patients with diabetes, may play an important role in development of NASH, further underscoring the interplay between NAFLD and diabetes[50]. Proposed mechanisms that need to be validated include hepatic inflammation and oxidative stress due to hyperglycemia, accelerated production of advanced glycosylation end-products and development of inflammation in Kupffer and hepatic stellate cells, alteration of the hepatocyte microenvironment by glucotoxicity, up-regulation by hyperglycemia of genes involved in key lipogenic and glycolytic pathways (such as the transcription factor ChREBP, stimulation of liver-pyruvate kinase, and many others), activation by high-fructose diets of DNL, along with up-regulation of inflammatory pathways[50]. Some theories also suggest that gut microbiome alteration and dietary habits are other mechanisms that induce and maintain diabetes and/or NAFLD[51].

As research to unravel the pathophysiological correlation between NAFLD and diabetes continues, the role of a new 'liver-pancreas' axis, existing between the liver and pancreatic α-cells, has emerged[52]. Pancreatic α-cells are known to secrete glucagon[53]. A study has shown that normal glucose-tolerant obese patients have fasting hyperglucagonemia, which is related to liver steatosis[53]. It has also been shown that besides hepatic IR, glucagon resistance can also putatively contribute to diabetes development in NAFLD patients[54].

Figure 1 schematically illustrates the pathophysiologic association between NAFLD and diabetes.

Many studies in NAFLD patients have also highlighted that both genetic and environmental factors interfere with the insulin signaling cascade and become cardinal in maintaining and worsening of IR[36]. Various molecular mechanisms that may be involved include serine phosphorylation of 'insulin receptor substrate' by inflam-

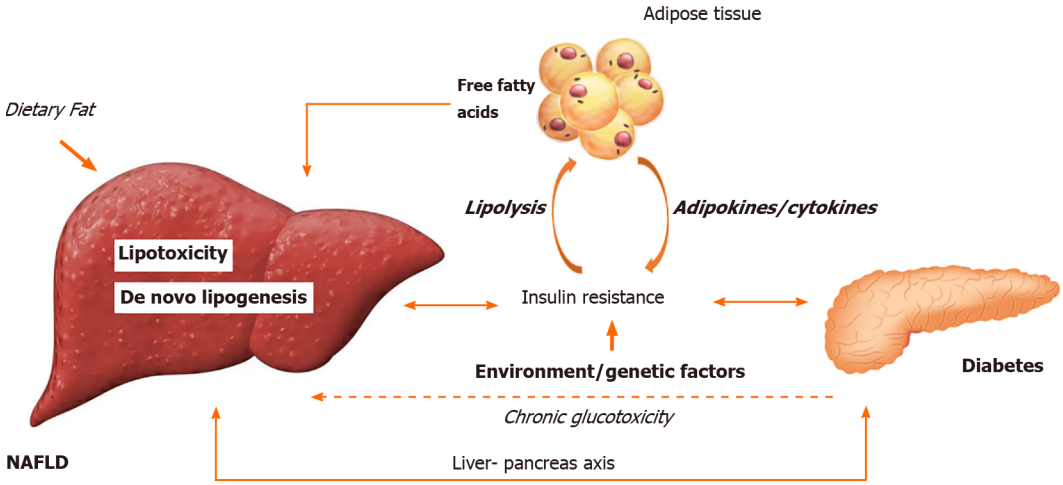

Figure 1 Pathophysiologic association between diabetes and non-alcoholic fatty liver disease. NAFLD: Non-alcoholic fatty liver disease.

matory signal transducers, such as c-Jun N-terminal protein kinase 1 or inhibitor of nuclear factor-jB kinase-b, activation of nuclear factor kappa B and suppressors of cytokine signaling[36].

It is interesting to note that not all studies show the positive relationship of diabetes and NAFLD. Recently, few genotype/phenotype-related studies have shown a disproportional development of diabetes in patients of NAFLD having specific gene variants, such as the patatin-like phospholipase domain-containing 3 (*PNPLA3 rs738409 GG*) and transmembrane 6 superfamily member 2 protein (*TM6SF2 rs58542926 C>T gene*) [55]. Other NAFLD-related gene variants, such as *LYPLAL1* and *MBOAT7*, have also shown no increase in the risk of diabetes in these patients[55].

NATURAL HISTORY AND DISEASE SPECTRUM OF NAFLD AND DIABETES

While the pathophysiologic association of diabetes and NAFLD is partly because of the "common soil", the clinical course of the two entities appears to be like an inextricably intertwined vine. NAFLD, as a disease, has been studied for many years and is broadly divided into two pathologically distinct conditions with different prognoses, namely: non-alcoholic fatty liver (NAFL) (*i.e.* pure steatosis or steatosis with mild lobular inflammation) and NASH[15]. The latter encompasses a wide spectrum of disease, including fibrosis and cirrhosis, and may be associated with hepatocellular carcinoma (HCC). NASH is further sub-classified as early NASH with no or mild (F0-F1) fibrosis, fibrotic NASH with significant (≥ F2) or advanced (≥ F3, bridging) fibrosis and NASH-cirrhosis with F4 fibrosis[15]. It is often difficult to differentiate NAFL from the progressive NASH, as patients are usually asymptomatic with normal liver enzyme levels, and imaging tests may at times fail to identify the steatosis and fibrosis[56,57]. Various studies have highlighted that about one-third patients with NAFL and NASH have progressive fibrosis and 20% may have some regression over an average follow-up between 2.2 and 13.8 years[58]. The rate of fibrosis progression has been found to be characteristically slow, with an average progression of one stage taking 7.7 years[58]. While the rate of progression in NASH subjects may be twice as high, there exists a sub-group of both NASH and NAFL patients who may progress rapidly from no fibrosis to advanced fibrosis over an average period of 6 years[58].

Diabetes in patients with NAFLD

It has been observed that individuals with diagnosed NAFLD have a 2-fold increased risk of developing diabetes[59]. The prevalence of diabetes among diagnosed NAFLD and NASH patients is estimated to be 22.51% and 43.63%, respectively, which is much higher as compared to the prevalence of diabetes in the general population (8.5%)[60]. A study from India, conducted on 515 NAFLD patients, showed that the prevalence of diabetes and prediabetes in the cohort was about 24% and 23% respectively[61]. Diabetes seems to accelerate the course of NAFLD, and has also been found to be one

of the strongest clinical predictors of progression of NAFLD to NASH and cirrhosis [62]. Furthermore, a strong pathophysiological link also exists between diabetes and HCC. The increased levels of inflammatory biomarkers and hyperinsulinemia, that are found in diabetics, may be responsible for the increased risk of HCC[63].

NAFLD in patients with diabetes

Increasing epidemiological evidence suggests that there is a bidirectional relationship between NAFLD and diabetes and that NAFLD may precede and/or promote the development of diabetes[64]. On evaluating and analyzing patients with diabetes, several studies have reported that the prevalence of NAFLD in these patients ranges broadly, between 34%-94%[65]. The prevalence of NAFLD is higher not just in diabetics but also in those at risk of developing diabetes[66]. These patients can be identified as having glycosylated hemoglobin A1c (HbA1c) values of 5.7%–6.4% (38.8–46.4 mmoL/moL), impaired fasting glucose (fasting glucose: 100–125 mg/dL [5.55–6.94 mmoL/L]) and/or impaired glucose tolerance (glucose: 140–199 mg/dL [7.77–11.04 mmoL/L]) at 2 h of the standardized 75 g oral glucose tolerance test (OGTT)[66]. Interestingly, insulin treatment, that increases body fat, does not appear to promote or worsen NAFLD in diabetics[67]. A study has shown the estimated prevalence of NASH and advanced fibrosis in individuals with coexisting NAFLD and diabetes to be 37.3% (95% confidence interval [CI]: 24.7–50.0) and 17.0% (95%CI: 7.2–34.8), respectively[68]. Further, the overall mortality ratio in 5–10 years was found to be of 585 per 100000, which was greater than mortality from other CLDs[68].

In patients with diabetes, NAFLD is believed to increase the risk of cardiovascular events by 1.87-fold after adjusting for confounders[69]. Co-existent NAFLD may also increase the risk of microvascular complications of diabetes, including chronic kidney disease and retinopathy[70]. Growing evidence shows that besides its effect on the liver, NAFLD in diabetics may also lead to development of sensory-motor and autonomic neuropathy[71,72].

Thus, a careful consideration and evaluation of diabetes in patients with NAFLD (NAFL/NASH) and *vice versa* not only helps in prognostication and therapy but also has a potential to prevent associated complications.

EVALUATION OF DIABETICS WITH NAFLD

Before discussing the moot point of when to refer a diabetic with NAFLD to a hepatologist, it is essential to understand that NAFLD and diabetes are like a "two-way road"; detection of one entity in patients of the other can immensely help in reducing the disease burden of both. Patients with NAFLD have a significantly higher prevalence of abnormal glucose tolerance (prediabetes or diabetes) than those without NAFLD (20.6% *vs* 11%)[73]. It is therefore possible to decrease incident diabetes with improvement of NAFLD[46]. In view of this, European associations (EASL-EASD-EASO) in their guidelines have recommended mandatory screening for diabetes in all persons with NAFLD, by fasting or random blood glucose or HbA1c and if available, by the standardized 75-g OGTT in high-risk groups[15].

It was observed that diabetics with other components of metabolic syndrome were at a higher risk of having advanced CLD, thereby requiring further liver assessment [74]. It was found that development of NAFLD and, in particular, NASH-related fibrosis can have profound effects on morbidity and mortality in diabetics[60,75]. Studies have shown that steatosis in diabetics is associated with increased prevalence of altered albumin excretion rate, thus playing an important role in the development of diabetic nephropathy[76]. The earlier NICE NAFLD Guidelines of 2016 did not give any recommendations for screening of patients with diabetes for liver fibrosis, but with mounting evidence, this saw a gradual change.

Various non-invasive indicators were approved by the EASD for NAFLD diagnosis. These include NAFLD liver fat score, the fatty liver index (FLI)[77], fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS)[78]. Besides, SteatoTest, NashTest, ActiTest and FibroTest too are handy tools in quantifying liver steatosis and fibrosis[79]. FibroScan®, a frequently used non-invasive test for liver stiffness measurement (LSM), can not only help to detect and stage fibrosis in NAFLD/NASH but can also predict macrovascular and microvascular complications of diabetes[80,81]. Experts have also proposed the use of intrahepatic TAG measurement with magnetic resonance imaging-derived proton density fat fraction as a standard test for detecting and grading hepatic steatosis[82,83]. Separation of diabetes patients with the relatively benign form of the disease (NAFL), from those who have NASH (with or without

moderate-to severe fibrosis [F2]) requiring early intervention, has always been seen as a big challenge[84].

The gold standard for NAFLD diagnosis is liver biopsy. But this procedure, which requires specialist referral, being invasive and having several drawbacks, such as sampling error, high cost, inter- and intra- observer variability and risk of complications[85], cannot be applied to all the patients. In order to not miss at-risk patients while focusing on suitable and sustainable hepatologist referral, in 2016, the EASL-EASD-EASO guidelines were published[15]. It was recommended that in case of presence of obesity/diabetes or the incidental finding of raised liver enzymes with metabolic risk factors, patients should promptly be evaluated with non-invasive screening tests to identify steatosis, NASH, and fibrosis[15]. They suggested ultrasound evaluation along with application of steatosis biomarkers like FLI, SteatoTest, NAFLD Fat score to identify steatosis. Surrogate markers of fibrosis (NFS, FIB-4, ELF or FibroTest) are then to be calculated, in order to rule out significant fibrosis (≥ F2), which, if found, would mandate specialist referral for evaluation with or without liver biopsy[15].

In 2020, the American Diabetes Association also recommended checking for NASH and fibrosis in patients with elevated liver enzymes[86]. It was proposed that patients with type 2 diabetes or prediabetes and elevated liver enzymes (alanine aminotransferase [ALT]) or fatty liver on ultrasound should be evaluated for presence of NASH and liver fibrosis[86].

One study evaluated the performance of international (EASL-EASD-EASO) and national (DGVS) guidelines for NAFLD risk stratification in diabetic patients and compared it to their simplified referral strategy using Fibroscan-AST (FAST) score[87]. LSM values by FibroScan® in diabetics were defined as low (< 7.9/7.2kPa M/XL probe), intermediate (7.9–9.6/7.2–9.3kPaM/XL-probe) and high (> 9.6/9.3kPaM/XL probe)[87]. EASL-EASD-EASO recommended specialist referral for 60%–77% of the subjects depending on the fibrosis score, whereas the DGVS algorithm required LSM for 76%; 25% were referred for specialized care. The sensitivities of the diagnostic pathways were 47%–96%. The FAST score, when compared to these, revealed a sensitivity/specificity of 46V/88% for fibrosis risk and a specialist referral rate of 35% [87].

Thus, despite the societal guidance, debate continues as there is still no clarity as to which test is to be applied first and the suitable cut-offs that can be used so that the screening is simple, inexpensive with reasonable sensitivity and specificity.

With regards to the cut-off values used for plasma ALT concentration, studies have shown that although a threshold of 40 IU/L is frequently used in clinical practice and trials, lower cut-off points for normal (*i.e.* 30 IU/L for males and 19 IU/L for females) can improve the sensitivity of diagnosing prevalence of NAFLD[88]. Also, despite studies showing association of increased ALT values with increased risk of NASH, it has been found that, in some patients, especially those with diabetes and IR, NASH with/without advanced fibrosis may occur even when the ALT values are normal[89]. Thus, we cannot consider liver function test as a surrogate for disease severity in diabetics and relying solely on it may lead to missing of advanced liver disease cases.

As for abdominal ultrasonography, it is operator-dependent and is insensitive to mild steatosis[90]. When compared to this, Controlled Attenuation Parameter measurement by FibroScan can detect liver fat involving as little as 10% of the hepatocytes[91-93], thereby avoiding underreporting or missing cases. Various indices, such as FLI and hepatic steatosis index (HSI), have also been found useful in predicting hepatic steatosis. It has been shown that FLI < 30 can rule out hepatic steatosis with a sensitivity of 87% and a value of FLI ≥ 60 can help rule in hepatic steatosis with a specificity of 86%[77]. HSI, on the other hand, at values of < 30 or > 36.0, can rule out NAFLD with a sensitivity of 93.1% or detect NAFLD with a specificity of 92.4%, respectively[94].

Studies have also evaluated performance of other non-invasive scores such as aspartate aminotransferase (AST) to platelet ratio, FIB-4 and NFS that are used for evaluation of fibrosis while considering referral to hepatologist. Table 1 depicts a few of these non-invasive models that have been used for the evaluation of steatosis and fibrosis in patients of diabetes with NAFLD. In one study, FIB-4 and NFS models had a high negative predictive value (NPV) of 93.48% and 93.61%, respectively in patients with severe liver fibrosis (stages 3 and 4), thereby indicating that these models should actually only be used for excluding severe liver disease[95]. These scores also show lower specificity among older adults and lower accuracy in young adults[96]. It was found that the use of age-adjusted FIB-4 cut-offs can lead to appropriate referrals[97]. It was proposed that for those over the age of 65 years, a FIB-4 score of > 2.0 should be used as the cut-off for referral[96,98].

Table 1 Few available non-invasive models for evaluating steatosis and fibrosis in patients of diabetes with non-alcoholic fatty liver disease

Index	Components	Cut-offs	Sensitivity, %	Specificity, %
Steatosis				
FLI[77]	WC, BMI, TG and GGT	< 30	87.0	64.0
		≥ 60	61.0	86.0
HSI[94]	AST, ALT, BMI, diabetes, female sex	< 30	93.1	39.6
		> 36	45.1	92.4
Fibrosis (stage 2, 3 or 4)				
APRI[95]	AST and platelet count	0.518	50.00	89.19
FIB-4[95]	Age, AST, ALT and platelet count	1.743	63.33	94.59
NFS[95]	Age, BMI, IFG and diabetes, AST-to-ALT ratio, platelet count and albumin	-0.054	50.00	86.21

Formulae: FLI = ($e^{0.953*\log e (TG) + 0.139*BMI + 0.718*\log e (GGT) + 0.053*WC - 15.745}$)/(1 + $e^{0.953*\log e (TG) + 0.139*BMI + 0.718*\log e (GGT) + 0.053*WC - 15.745}$)*100[77]; HSI = 8 × (ALT/AST ratio) + BMI (+ 2, if female; + 2, if diabetes mellitus)[94]; APRI = {[AST(U/L)/upper limit of normal AST (U/L)] × 100/platelet count (10^9/L)}[95]; FIB-4 = Age (years) × AST (U/L)/[platelet count (10^9/L) × $ALT^{1/2}$ (U/L)][95]; NFS = − 1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio– 0.013 × platelet count (10^9/L) – 0.66 × albumin (g/dL)[95]. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio; BMI: Body mass index; FIB-4: Fibrosis-4 score; FLI: Fatty liver index; GGT: Gamma-glutamyltransferase; HG: Haptoglobins; HSI: Hepatic steatosis index; IFG: Impaired fasting glucose; NFS: Non-alcoholic fatty liver disease fibrosis score; TG: Triglyceride; WC: Waist circumference.

The NASH Council, in their study, proposed that a patient who has a FIB-4 score of > 1.3 be referred to a specialist and for those that have a score ≤ 1.3 undergo lifestyle intervention with their primary provider[97,99]. Indian researchers have found that among patients of NAFLD, a FIB-4 cut-off of 1.0 instead of 1.3, showed 100% sensitivity and 94.3% specificity to rule out any fibrosis (F0 *vs* F1-F4) validated *vs* MRE in a cohort of 239 NAFLD patients, thus leading to inclusion of patients with F2 fibrosis in the primary care referral pathway[100]. However, validation of this is needed in diabetics and a larger patient cohort is needed to be further evaluated[100].

Another study that derived data from NASH Clinical Research Network studies and included patients with biopsy-proven NAFLD with diabetes, proposed a different model for NASH evaluation[101]. The parameters included were White race, BMI, waist circumference, ALT, AST, albumin, HbA1c, HOMA-IR and ferritin[101]. The specificity, sensitivity, NPV and pars plana vitrectomy (PPV) were 90.0%, 56.8%, 47.7%, and 93.2%, respectively, and the model correctly classified 67% of patients as having NASH[101]. The researchers also proposed a model for predicting advanced fibrosis using the parameters- age, Hispanic ethnicity, BMI, waist-to-hip ratio, hypertension, ALT/AST ratio, alkaline phosphatase, isolated abnormal alkaline phosphatase, bilirubin (total and direct), globulin, albumin, serum insulin, hematocrit, international normalized ratio, and platelet count. The specificity, sensitivity, NPV, and PPV were 90.0%, 57%, 75.1%, and 80.2%, respectively, and the model correctly classified 76.6% of patients as having advanced fibrosis[101]. It was concluded that proposed model performed better than the NAFLD fibrosis score in detecting advanced fibrosis[101].

Based on the extensive review of the literature and pertaining to this ongoing debate of how best to manage diabetic patients with NAFLD, we propose a screening protocol that takes into account not only the current societal guidance but also the results of ongoing research. We believe that instead of only those diabetics who are at intermediate or high risk of having NAFLD, all diabetics must be evaluated using a baseline ultrasound abdomen and liver function test along with non-invasive markers as per the algorithm shown in Figure 2. However, more studies with larger patient cohorts are needed to further explore this simplified algorithm and for further re-strategizing the screening protocols.

Surveys in the Netherlands[102] and an urban western United States population [103] have shown that 84% of general practitioners and 83% of largely primary care providers respectively have endorsed the need for increased awareness and knowledge on NAFLD.

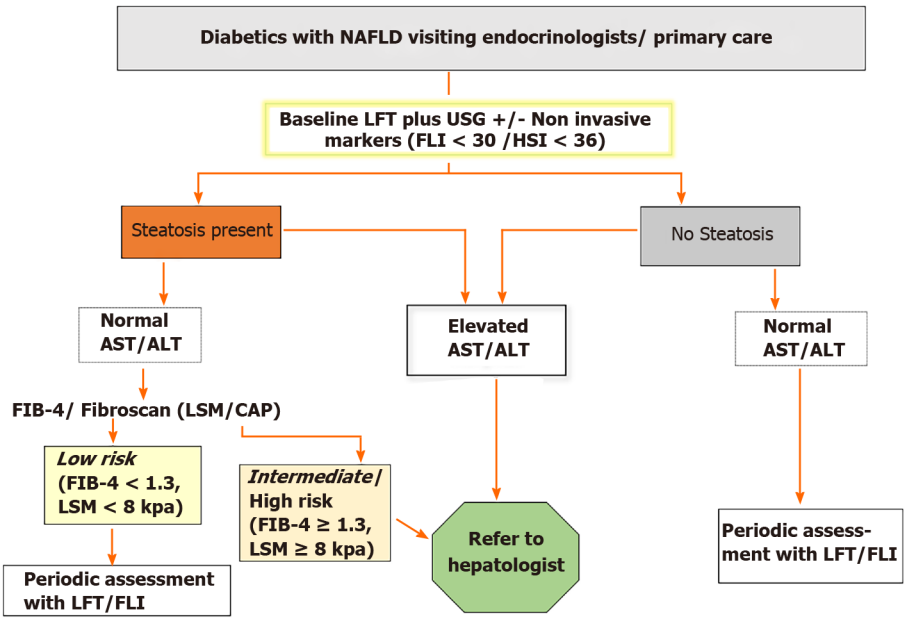

Figure 2 Algorithm for referral of diabetics with non-alcoholic fatty liver disease to hepatologist. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CAP: Controlled attenuation parameter; FIB-4: Fibrosis-4 score; FLI: Fatty liver index; HSI: Hepatic steatosis index; LFT: Liver function test; LSM: Liver stiffness measurement; NAFLD: Non-alcoholic fatty liver disease; USG: Ultrasonography.

In diabetics with NAFLD, lifestyle interventions and weight loss have been found to be the most beneficial therapeutic strategies[104]. In a few randomized controlled trials, drugs such as pioglitazone, a potent and selective agonist for peroxisome proliferator-activated receptor-gamma, have also been consistently found to induce resolution of NASH and have shown modest effects on liver fibrosis[50,105]. Also, contrary to the popular belief that statins cannot be used in diabetics with NASH with elevated liver enzymes due to potential risk of hepatotoxicity, it has been shown that statin therapy is safe in these patients[106]. Trials are underway for evaluating efficacy of other treatment options.

CONCLUSION

As the prevalence of NAFLD in diabetics is substantial, and due to the influence of each disease on the other with regards to disease progression, the role of primary care physicians and diabetologists becomes pivotal. A knowledge of preventive measures and available treatment is needed in order to manage the milder disease forms at primary care level only. Diabetics must be monitored for NAFLD/NASH with a vigilant eye, similar to the way the other complications of diabetes, like retinopathy and nephropathy, are screened. This proactive approach of screening will surely help in not only prevention but also in early detection of the more sinister and progressive disease form, despite its benign phenotype. Where indicated, a prompt referral to hepatologists can make the patient turn the corner and can thereby attenuate the development of more severe forms of NASH, including cirrhosis and even HCC.

ACKNOWLEDGEMENTS

We sincerely thank Dr. Ankush R Pawar (Liverpool University Hospitals NHS Foundation Trust) for his helpful suggestions.

REFERENCES

1 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

2 **Wiegand S**, Keller KM, Röbl M, L'Allemand D, Reinehr T, Widhalm K, Holl RW; APV-Study Group and the German Competence Network Adipositas. Obese boys at increased risk for nonalcoholic liver disease: evaluation of 16,390 overweight or obese children and adolescents. *Int J Obes (Lond)* 2010; **34**: 1468-1474 [PMID: 20531349 DOI: 10.1038/ijo.2010.106]

3 **Puri P**, Sanyal AJ. Nonalcoholic fatty liver disease: Definitions, risk factors, and workup. *Clin Liver Dis (Hoboken)* 2012; **1**: 99-103 [PMID: 31186860 DOI: 10.1002/cld.81]

4 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]

5 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1053/gast.2002.34168]

6 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]

7 **Adam R**, Karam V, Cailliez V, O Grady JG, Mirza D, Cherqui D, Klempnauer J, Salizzoni M, Pratschke J, Jamieson N, Hidalgo E, Paul A, Andujar RL, Lerut J, Fisher L, Boudjema K, Fondevila C, Soubrane O, Bachellier P, Pinna AD, Berlakovich G, Bennet W, Pinzani M, Schemmer P, Zieniewicz K, Romero CJ, De Simone P, Ericzon BG, Schneeberger S, Wigmore SJ, Prous JF, Colledan M, Porte RJ, Yilmaz S, Azoulay D, Pirenne J, Line PD, Trunecka P, Navarro F, Lopez AV, De Carlis L, Pena SR, Kochs E, Duvoux C; all the other 126 contributing centers (www. eltr.org) and the European Liver and Intestine Transplant Association (ELITA). 2018 Annual Report of the European Liver Transplant Registry (ELTR) - 50-year evolution of liver transplantation. *Transpl Int* 2018; **31**: 1293-1317 [PMID: 30259574 DOI: 10.1111/tri.13358]

8 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511 DOI: 10.1002/hep.510300604]

9 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129 [PMID: 19827166 DOI: 10.1002/hep.23276]

10 **Neuschwander-Tetri BA**, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV; NASH Clinical Research Network. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 913-924 [PMID: 20648476 DOI: 10.1002/hep.23784]

11 **Targher G**, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212-1218 [PMID: 17277038 DOI: 10.2337/dc06-2247]

12 **Williamson RM**, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, Frier BM, Van Look LA, Johnston GI, Reynolds RM, Strachan MW; Edinburgh Type 2 Diabetes Study Investigators. Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2011; **34**: 1139-1144 [PMID: 21478462 DOI: 10.2337/dc10-2229]

13 **Hamaguchi M**, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, Omatsu T, Nakajima T, Sarui H, Shimazaki M, Kato T, Okuda J, Ida K. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* 2005; **143**: 722-728 [PMID: 16287793 DOI: 10.7326/0003-4819-143-10-200511150-00009]

14 **Ong JP**, Elariny H, Collantes R, Younoszai A, Chandhoke V, Reines HD, Goodman Z, Younossi ZM. Predictors of nonalcoholic steatohepatitis and advanced fibrosis in morbidly obese patients. *Obes Surg* 2005; **15**: 310-315 [PMID: 15826462 DOI: 10.1381/0960892053576820]

15 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* 2016; **59**: 1121-1140 [PMID: 27053230 DOI: 10.1007/s00125-016-3902-y]

16 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]

17 **Cerda C**, Pérez-Ayuso RM, Riquelme A, Soza A, Villaseca P, Sir-Petermann T, Espinoza M, Pizarro M, Solis N, Miquel JF, Arrese M. Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *J Hepatol* 2007; **47**: 412-417 [PMID: 17560682 DOI: 10.1016/j.jhep.2007.04.012]

18 **Tilg H**, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 32-42 [PMID: 27729660 DOI: 10.1038/nrgastro.2016.147]

19 **Stefan N**, Häring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol* 2019; **7**: 313-324 [PMID: 30174213 DOI: 10.1016/S2213-8587(18)30154-2]

20 **Anstee QM**, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? *BMJ* 2011; **343**: d3897 [PMID: 21768191 DOI: 10.1136/bmj.d3897]

21 **Kahn BB**. Adipose Tissue, Inter-Organ Communication, and the Path to Type 2 Diabetes: The 2016

Banting Medal for Scientific Achievement Lecture. *Diabetes* 2019; **68**: 3-14 [PMID: 30573674 DOI: 10.2337/dbi18-0035]

22 **Unger RH**, Eisentraut AM. Entero-insular axis. *Arch Intern Med* 1969; **123**: 261-266 [PMID: 4885674]

23 **Renold AE**. A brief and fragmentary introduction to some aspects of adipose tissue metabolism, with emphasis on glucose uptake. *Ann N Y Acad Sci* 1965; **131**: 7-12 [PMID: 5217003 DOI: 10.1111/j.1749-6632.1965.tb34774.x]

24 **Garfinkel AG**, Nilsson-ehle P, Schotz MC. Regulation of lipoprotein lipase.Induction by insulin. *Biochim Biophys Acta* 1976; **424**: 264-273 [PMID: 1252491 DOI: 10.1016/0005-2760(76)90194-6]

25 **Roden M**, Stingl H, Chandramouli V, Schumann WC, Hofer A, Landau BR, Nowotny P, Waldhäusl W, Shulman GI. Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. *Diabetes* 2000; **49**: 701-707 [PMID: 10905476 DOI: 10.2337/diabetes.49.5.701]

26 **Rajala MW**, Scherer PE. Minireview: The adipocyte--at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* 2003; **144**: 3765-3773 [PMID: 12933646 DOI: 10.1210/en.2003-0580]

27 **Yamauchi T**, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288-1295 [PMID: 12368907 DOI: 10.1038/nm788]

28 **Rui L**. Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]

29 **Ferramosca A**, Zara V. Modulation of hepatic steatosis by dietary fatty acids. *World J Gastroenterol* 2014; **20**: 1746-1755 [PMID: 24587652 DOI: 10.3748/wjg.v20.i7.1746]

30 **du Plessis J**, van Pelt J, Korf H, Mathieu C, van der Schueren B, Lannoo M, Oyen T, Topal B, Fetter G, Nayler S, van der Merwe T, Windmolders P, Van Gaal L, Verrijken A, Hubens G, Gericke M, Cassiman D, Francque S, Nevens F, van der Merwe S. Association of Adipose Tissue Inflammation With Histologic Severity of Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 635-48.e14 [PMID: 26028579 DOI: 10.1053/j.gastro.2015.05.044]

31 **Koliwad SK**, Streeper RS, Monetti M, Cornelissen I, Chan L, Terayama K, Naylor S, Rao M, Hubbard B, Farese RV Jr. DGAT1-dependent triacylglycerol storage by macrophages protects mice from diet-induced insulin resistance and inflammation. *J Clin Invest* 2010; **120**: 756-767 [PMID: 20124729 DOI: 10.1172/JCI36066]

32 **Neuschwander-Tetri BA**. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology* 2010; **52**: 774-788 [PMID: 20683968 DOI: 10.1002/hep.23719]

33 **Mendez-Sanchez N**, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP, Barranco-Fragoso B, Cordova-Gallardo J. New Aspects of Lipotoxicity in Nonalcoholic Steatohepatitis. *Int J Mol Sci* 2018; **19** [PMID: 30011790 DOI: 10.3390/ijms19072034]

34 **Peverill W**, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci* 2014; **15**: 8591-8638 [PMID: 24830559 DOI: 10.3390/ijms15058591]

35 **Yilmaz Y**. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? *Aliment Pharmacol Ther* 2012; **36**: 815-823 [PMID: 22966992 DOI: 10.1111/apt.12046]

36 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: 26823198 DOI: 10.1016/j.metabol.2015.12.012]

37 **Tanase DM**, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, Ouatu A, Floria M. The Intricate Relationship between Type 2 Diabetes Mellitus (T2DM), Insulin Resistance (IR), and Nonalcoholic Fatty Liver Disease (NAFLD). *J Diabetes Res* 2020; **2020**: 3920196 [PMID: 32832560 DOI: 10.1155/2020/3920196]

38 **Seppälä-Lindroos A**, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijärvi A, Halavaara J, Yki-Järvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002; **87**: 3023-3028 [PMID: 12107194 DOI: 10.1210/jcem.87.7.8638]

39 **Liu W**, Baker RD, Bhatia T, Zhu L, Baker SS. Pathogenesis of nonalcoholic steatohepatitis. *Cell Mol Life Sci* 2016; **73**: 1969-1987 [PMID: 26894897 DOI: 10.1007/s00018-016-2161-x]

40 **Stefan N**, Kantartzis K, Häring HU. Causes and metabolic consequences of Fatty liver. *Endocr Rev* 2008; **29**: 939-960 [PMID: 18723451 DOI: 10.1210/er.2008-0009]

41 **Xia JY**, Holland WL, Kusminski CM, Sun K, Sharma AX, Pearson MJ, Sifuentes AJ, McDonald JG, Gordillo R, Scherer PE. Targeted Induction of Ceramide Degradation Leads to Improved Systemic Metabolism and Reduced Hepatic Steatosis. *Cell Metab* 2015; **22**: 266-278 [PMID: 26190650 DOI: 10.1016/j.cmet.2015.06.007]

42 **Postic C**, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008; **118**: 829-838 [PMID: 18317565 DOI: 10.1172/JCI34275]

43 **Hotamisligil GS**. Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes* 2005; **54** Suppl 2: S73-S78 [PMID:

16306344 DOI: 10.2337/diabetes.54.suppl_2.s73]
44 **Savage DB**, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev* 2007; **87**: 507-520 [PMID: 17429039 DOI: 10.1152/physrev.00024.2006]
45 **Cai D**, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 2005; **11**: 183-190 [PMID: 15685173 DOI: 10.1038/nm1166]
46 **Sung KC**, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. *J Clin Endocrinol Metab* 2013; **98**: 3637-3643 [PMID: 23873989 DOI: 10.1210/jc.2013-1519]
47 **Godoy-Matos AF**, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr* 2020; **12**: 60 [PMID: 32684985 DOI: 10.1186/s13098-020-00570-y]
48 **Gastaldelli A**, Gaggini M, DeFronzo RA. Role of Adipose Tissue Insulin Resistance in the Natural History of Type 2 Diabetes: Results From the San Antonio Metabolism Study. *Diabetes* 2017; **66**: 815-822 [PMID: 28052966 DOI: 10.2337/db16-1167]
49 **Hedjazifar S**, Khatib Shahidi R, Hammarstedt A, Bonnet L, Church C, Boucher J, Blüher M, Smith U. The Novel Adipokine Gremlin 1 Antagonizes Insulin Action and Is Increased in Type 2 Diabetes and NAFLD/NASH. *Diabetes* 2020; **69**: 331-341 [PMID: 31882566 DOI: 10.2337/db19-0701]
50 **Cusi K**. A diabetologist's perspective of non-alcoholic steatohepatitis (NASH): Knowledge gaps and future directions. *Liver Int* 2020; **40** Suppl 1: 82-88 [PMID: 32077613 DOI: 10.1111/liv.14350]
51 **Campo L**, Eiseler S, Apfel T, Pyrsopoulos N. Fatty Liver Disease and Gut Microbiota: A Comprehensive Update. *J Clin Transl Hepatol* 2019; **7**: 56-60 [PMID: 30944821 DOI: 10.14218/JCTH.2018.00008]
52 **Knop FK**. EJE PRIZE 2018: A gut feeling about glucagon. *Eur J Endocrinol* 2018; **178**: R267-R280 [PMID: 29678923 DOI: 10.1530/EJE-18-0197]
53 **Suppli MP**, Bagger JI, Lund A, Demant M, van Hall G, Strandberg C, Kønig MJ, Rigbolt K, Langhoff JL, Wewer Albrechtsen NJ, Holst JJ, Vilsbøll T, Knop FK. Glucagon Resistance at the Level of Amino Acid Turnover in Obese Subjects With Hepatic Steatosis. *Diabetes* 2020; **69**: 1090-1099 [PMID: 31974144 DOI: 10.2337/db19-0715]
54 **Wewer Albrechtsen NJ**, Pedersen J, Galsgaard KD, Winther-Sørensen M, Suppli MP, Janah L, Gromada J, Vilstrup H, Knop FK, Holst JJ. The Liver-α-Cell Axis and Type 2 Diabetes. *Endocr Rev* 2019; **40**: 1353-1366 [PMID: 30920583 DOI: 10.1210/er.2018-00251]
55 **Xia MF**, Bian H, Gao X. NAFLD and Diabetes: Two Sides of the Same Coin? *Front Pharmacol* 2019; **10**: 877 [PMID: 31447675 DOI: 10.3389/fphar.2019.00877]
56 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]
57 **Vuppalanchi R**, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. *Hepatology* 2009; **49**: 306-317 [PMID: 19065650 DOI: 10.1002/hep.22603]
58 **Calzadilla Bertot L**, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17** [PMID: 27213358 DOI: 10.3390/ijms17050774]
59 **Ballestri S**, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, Roverato A, Guaraldi G, Lonardo A. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 936-944 [PMID: 26667191 DOI: 10.1111/jgh.13264]
60 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
61 **Singh SP**, Singh A, Pati GK, Misra B, Misra D, Kar SK, Panigrahi MK. A Study of Prevalence of Diabetes and Prediabetes in Patients of Non-Alcoholic Fatty Liver Disease and the Impact of Diabetes on Liver Histology in Coastal Eastern India. *J Diab Mel* 2014; **4**: 290-296 [DOI: 10.4236/jdm.2014.44040]
62 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]
63 **Aleksandrova K**, Boeing H, Nöthlings U, Jenab M, Fedirko V, Kaaks R, Lukanova A, Trichopoulou A, Trichopoulos D, Boffetta P, Trepo E, Westhpal S, Duarte-Salles T, Stepien M, Overvad K, Tjønneland A, Halkjaer J, Boutron-Ruault MC, Dossus L, Racine A, Lagiou P, Bamia C, Benetou V, Agnoli C, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita B, Peeters PH, Gram IT, Lund E, Weiderpass E, Quirós JR, Agudo A, Sánchez MJ, Gavrila D, Barricarte A, Dorronsoro M, Ohlsson B, Lindkvist B, Johansson A, Sund M, Khaw KT, Wareham N, Travis RC, Riboli E, Pischon T. Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. *Hepatology* 2014; **60**: 858-871 [PMID: 24443059 DOI: 10.1002/hep.27016]
64 **Targher G**, Marchesini G, Byrne CD. Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: Causal association or epiphenomenon? *Diabetes Metab* 2016; **42**: 142-156 [PMID: 27142870 DOI: 10.1016/j.diabet.2016.04.002]
65 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]
66 **Loomba R**, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, Bass NM; Nonalcoholic Steatohepatitis Clinical Research Network. Association between diabetes, family history of diabetes,

and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012; **56**: 943-951 [PMID: 22505194 DOI: 10.1002/hep.25772]

67 **Juurinen L**, Tiikkainen M, Häkkinen AM, Hakkarainen A, Yki-Järvinen H. Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes. *Am J Physiol Endocrinol Metab* 2007; **292**: E829-E835 [PMID: 17090752 DOI: 10.1152/ajpendo.00133.2006]

68 **Younossi ZM**, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A, Nader F. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* 2019; **71**: 793-801 [PMID: 31279902 DOI: 10.1016/j.jhep.2019.06.021]

69 **Targher G**, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, Arcaro G. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. *Diabetes Care* 2007; **30**: 2119-2121 [PMID: 17519430 DOI: 10.2337/dc07-0349]

70 **Targher G**, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, Muggeo M. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. *Diabetologia* 2008; **51**: 444-450 [PMID: 18058083 DOI: 10.1007/s00125-007-0897-4]

71 **Williams KH**, Burns K, Constantino M, Shackel NA, Prakoso E, Wong J, Wu T, George J, McCaughan GW, Twigg SM. An association of large-fibre peripheral nerve dysfunction with non-invasive measures of liver fibrosis secondary to non-alcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2015; **29**: 1240-1247 [PMID: 26297218 DOI: 10.1016/j.jdiacomp.2015.06.015]

72 **Ziegler D**, Strom A, Kupriyanova Y, Bierwagen A, Bönhof GJ, Bódis K, Müssig K, Szendroedi J, Bobrov P, Markgraf DF, Hwang JH, Roden M; GDS Group. Association of Lower Cardiovagal Tone and Baroreflex Sensitivity With Higher Liver Fat Content Early in Type 2 Diabetes. *J Clin Endocrinol Metab* 2018; **103**: 1130-1138 [PMID: 29267946 DOI: 10.1210/jc.2017-02294]

73 **Nobili V**, Mantovani A, Cianfarani S, Alisi A, Mosca A, Sartorelli MR, Maffeis C, Loomba R, Byrne CD, Targher G. Prevalence of prediabetes and diabetes in children and adolescents with biopsy-proven non-alcoholic fatty liver disease. *J Hepatol* 2019; **71**: 802-810 [PMID: 31279904 DOI: 10.1016/j.jhep.2019.06.023]

74 **Kwok R**, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut* 2016; **65**: 1359-1368 [PMID: 25873639 DOI: 10.1136/gutjnl-2015-309265]

75 **Non-alcoholic Fatty Liver Disease Study Group**, Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, Caldwell SH, Cortez-Pinto H, Grieco A, Machado MV, Miele L, Targher G. Epidemiological modifiers of non-alcoholic fatty liver disease: Focus on high-risk groups. *Dig Liver Dis* 2015; **47**: 997-1006 [PMID: 26454786 DOI: 10.1016/j.dld.2015.08.004]

76 **Ciardullo S**, Muraca E, Perra S, Bianconi E, Zerbini F, Oltolini A, Cannistraci R, Parmeggiani P, Manzoni G, Gastaldelli A, Lattuada G, Perseghin G. Screening for non-alcoholic fatty liver disease in type 2 diabetes using non-invasive scores and association with diabetic complications. *BMJ Open Diabetes Res Care* 2020; **8** [PMID: 32049637 DOI: 10.1136/bmjdrc-2019-000904]

77 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]

78 **Patel PJ**, Cheng JC, Banh X, Gracen L, Radford-Smith D, Hossain F, Horsfall LU, Hayward KL, Williams S, Johnson T, Brown NN, Saad N, Stuart KA, Russell AW, Valery PC, Clouston AD, Irvine KM, Bernard A, Powell EE. Clinically Significant Fibrosis Is Associated With Longitudinal Increases in Fibrosis-4 and Nonalcoholic Fatty Liver Disease Fibrosis Scores. *Clin Gastroenterol Hepatol* 2020; **18**: 710-718.e4 [PMID: 31352092 DOI: 10.1016/j.cgh.2019.07.036]

79 **Poynard T**, Lassailly G, Diaz E, Clement K, Caïazzo R, Tordjman J, Munteanu M, Perazzo H, Demol B, Callafe R, Pattou F, Charlotte F, Bedossa P, Mathurin P, Ratziu V; FLIP consortium. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. *PLoS One* 2012; **7**: e30325 [PMID: 22431959 DOI: 10.1371/journal.pone.0030325]

80 **Eddowes PJ**, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, Bedossa P, Newsome PN. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **156**: 1717-1730 [PMID: 30689971 DOI: 10.1053/j.gastro.2019.01.042]

81 **Lombardi R**, Airaghi L, Targher G, Serviddio G, Maffi G, Mantovani A, Maffeis C, Colecchia A, Villani R, Rinaldi L, Orsi E, Pisano G, Adinolfi LE, Fargion S, Fracanzani AL. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. *Liver Int* 2020; **40**: 347-354 [PMID: 31612634 DOI: 10.1111/liv.14274]

82 **Caussy C**, Alquiraish MH, Nguyen P, Hernandez C, Cepin S, Fortney LE, Ajmera V, Bettencourt R, Collier S, Hooker J, Sy E, Rizo E, Richards L, Sirlin CB, Loomba R. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. *Hepatology* 2018; **67**: 1348-1359 [PMID: 29108123 DOI: 10.1002/hep.29639]

83 **Runge JH**, Smits LP, Verheij J, Depla A, Kuiken SD, Baak BC, Nederveen AJ, Beuers U, Stoker J. MR Spectroscopy-derived Proton Density Fat Fraction Is Superior to Controlled Attenuation Parameter for Detecting and Grading Hepatic Steatosis. *Radiology* 2018; **286**: 547-556 [PMID: 28915103 DOI: 10.1148/radiol.2017162931]

84 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

85 **Sumida Y**, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**: 475-485 [PMID: 24574716 DOI: 10.3748/wjg.v20.i2.475]

86 **American Diabetes Association**. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020; **43**: S37-S47 [PMID: 31862747 DOI: 10.2337/dc20-S004]

87 **Blank V**, Petroff D, Beer S, Böhlig A, Heni M, Berg T, Bausback Y, Dietrich A, Tönjes A, Hollenbach M, Blüher M, Keim V, Wiegand J, Karlas T. Current NAFLD guidelines for risk stratification in diabetic patients have poor diagnostic discrimination. *Sci Rep* 2020; **10**: 18345 [PMID: 33110165 DOI: 10.1038/s41598-020-75227-x]

88 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10 [PMID: 12093239 DOI: 10.7326/0003-4819-137-1-200207020-00006]

89 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]

90 **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750 [PMID: 12198701 DOI: 10.1053/gast.2002.35354]

91 **Sasso M**, Beaugrand M, de Ledinghen V, Douvin C, Marcellin P, Poupon R, Sandrin L, Miette V. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound Med Biol* 2010; **36**: 1825-1835 [PMID: 20870345 DOI: 10.1016/j.ultrasmedbio.2010.07.005]

92 **Sasso M**, Tengher-Barna I, Ziol M, Miette V, Fournier C, Sandrin L, Poupon R, Cardoso AC, Marcellin P, Douvin C, de Ledinghen V, Trinchet JC, Beaugrand M. Novel controlled attenuation parameter for noninvasive assessment of steatosis using Fibroscan(®): validation in chronic hepatitis C. *J Viral Hepat* 2012; **19**: 244-253 [PMID: 22404722 DOI: 10.1111/j.1365-2893.2011.01534.x]

93 **de Lédinghen V**, Vergniol J, Capdepont M, Chermak F, Hiriart JB, Cassinotto C, Merrouche W, Foucher J, Brigitte le B. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. *J Hepatol* 2014; **60**: 1026-1031 [PMID: 24378529 DOI: 10.1016/j.jhep.2013.12.018]

94 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]

95 **Nones RB**, Ivantes CP, Pedroso MLA. Can FIB4 and NAFLD fibrosis scores help endocrinologists refer patients with non-alcoholic fat liver disease to a hepatologist? *Arch Endocrinol Metab* 2017; **61**: 276-281 [PMID: 28225987 DOI: 10.1590/2359-3997000000233]

96 **McPherson S**, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, Oliveira CP, Francque S, Van Gaal L, Schattenberg JM, Tiniakos D, Burt A, Bugianesi E, Ratziu V, Day CP, Anstee QM. Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. *Am J Gastroenterol* 2017; **112**: 740-751 [PMID: 27725647 DOI: 10.1038/ajg.2016.453]

97 **Younossi ZM**, Corey KE, Alkhouri N, Noureddin M, Jacobson I, Lam B, Clement S, Basu R, Gordon SC, Ravendhra N, Puri P, Rinella M, Scudera P, Singal AK, Henry L; US Members of the Global Nash Council. Clinical assessment for high-risk patients with non-alcoholic fatty liver disease in primary care and diabetology practices. *Aliment Pharmacol Ther* 2020; **52**: 513-526 [PMID: 32598051 DOI: 10.1111/apt.15830]

98 **Tapper EB**, Krajewski K, Lai M, Challies T, Kane R, Afdhal N, Lau D. Simple non-invasive biomarkers of advanced fibrosis in the evaluation of non-alcoholic fatty liver disease. *Gastroenterol Rep (Oxf)* 2014; **2**: 276-280 [PMID: 25002154 DOI: 10.1093/gastro/gou034]

99 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

100 **Shah S**, Dhami-Shah H, Kamble S, Shukla A. FIB-4 cut-off of 1.3 may be inappropriate in a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol* 2020; **73**: 216-217 [PMID: 32276730 DOI: 10.1016/j.jhep.2019.12.025]

101 **Bazick J**, Donithan M, Neuschwander-Tetri BA, Kleiner D, Brunt EM, Wilson L, Doo E, Lavine J, Tonascia J, Loomba R. Clinical Model for NASH and Advanced Fibrosis in Adult Patients With

Diabetes and NAFLD: Guidelines for Referral in NAFLD. *Diabetes Care* 2015; **38**: 1347-1355 [PMID: 25887357 DOI: 10.2337/dc14-1239]

102 **van Asten M**, Verhaegh P, Koek G, Verbeek J. The increasing burden of NAFLD fibrosis in the general population: Time to bridge the gap between hepatologists and primary care. *Hepatology* 2017; **65**: 1078 [PMID: 27862126 DOI: 10.1002/hep.28940]

103 **Wieland AC**, Quallick M, Truesdale A, Mettler P, Bambha KM. Identifying practice gaps to optimize medical care for patients with nonalcoholic fatty liver disease. *Dig Dis Sci* 2013; **58**: 2809-2816 [PMID: 23843097 DOI: 10.1007/s10620-013-2740-8]

104 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

105 **Cusi K**, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med* 2016; **165**: 305-315 [PMID: 27322798 DOI: 10.7326/M15-1774]

106 **Bril F**, Portillo Sanchez P, Lomonaco R, Orsak B, Hecht J, Tio F, Cusi K. Liver Safety of Statins in Prediabetes or T2DM and Nonalcoholic Steatohepatitis: Post Hoc Analysis of a Randomized Trial. *J Clin Endocrinol Metab* 2017; **102**: 2950-2961 [PMID: 28575232 DOI: 10.1210/jc.2017-00867]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1494-1506

DOI: 10.4239/wjd.v12.i9.1494

ISSN 1948-9358 (online)

MINIREVIEWS

Improving nutrition for the prevention of gestational diabetes: Current status and perspectives

Polina V Popova, Evgenii A Pustozerov, Aleksandra S Tkachuk, Elena N Grineva

ORCID number: Polina V Popova 0000-0002-3697-7791; Evgenii A Pustozerov 0000-0001-8109-1319; Aleksandra S Tkachuk 0000-0002-1384-8743; Elena N Grineva 0000-0003-0042-7680.

Author contributions: Popova PV and Grineva EN contributed conceptualization; Popova PV and Pustozerov EA contributed methodology; Popova PV and Tkachuk AS wrote original draft preparation; Popova PV, Pustozerov EA and Grineva EN reviewed and edited the manuscript; all authors have read and agreed to the published version of the manuscript.

Supported by Governmental Research Topic from the Ministry of Health Care of Russian Federation, No. 121031100288-5.

Conflict-of-interest statement: The authors declare no conflict of interest.

Polina V Popova, Evgenii A Pustozerov, Aleksandra S Tkachuk, Elena N Grineva, Institute of Endocrinology, Almazov National Medical Research Centre, Saint Petersburg 194156, Russia

Polina V Popova, Department of Faculty Therapy, Saint Petersburg Pavlov State Medical University, Saint Petersburg 197022, Russia

Evgenii A Pustozerov, Department of Biomedical Engineering, Saint Petersburg State Electrotechnical University, Saint Petersburg 197341, Russia

Corresponding author: Polina V Popova, MD, PhD, Associate Professor, Institute of Endocrinology, Almazov National Medical Research Centre, 2, Akkuratova street, Saint Petersburg 194156, Russia. pvpopova@yandex.ru

Abstract

Gestational diabetes mellitus (GDM) is a common complication of pregnancy and a serious public health problem. It carries significant risks of short-term and long-term adverse health effects for both mothers and their children. Risk factors, especially modifiable risk factors, must be considered to prevent GDM and its consequences. Observational studies have identified several nutritional and lifestyle factors associated with the risk of GDM. The results of intervention studies examining the effects of diet and lifestyle on the prevention of GDM are contradictory. Differences in the study populations, types and intensity of intervention, time frame of the intervention, and diagnostic criteria for GDM may explain the heterogeneity in the results of intervention studies. This review provides an overview of new diets and other factors that may help prevent GDM. The main results of epidemiological studies assessing the risk factors for GDM, as well as the results and methodological problems of intervention studies on the prevention of GDM and their meta-analyses, are discussed. In addition, the evidence that gene and lifestyle interactions influence the development of GDM, as well as prospects for increasing the effectiveness of interventions designed to prevent GDM, including new data on the possible uses of personalized diet therapy, are highlighted.

Key Words: Gestational diabetes mellitus; Risk factors; Nutrition; Prevention; Personalized medicine; Postprandial glycemic response

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Russia

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 28, 2021
Peer-review started: February 28, 2021
First decision: March 30, 2021
Revised: April 20, 2021
Accepted: August 16, 2021
Article in press: August 16, 2021
Published online: September 15, 2021

P-Reviewer: Alkhayyat M, Nassar G, Zhang W
S-Editor: Gao CC
L-Editor: A
P-Editor: Xing YX

Core Tip: Gestational diabetes mellitus (GDM) is a common complication of pregnancy and a serious public health problem. This review provides an overview of new diets and other factors that may help prevent GDM. The main results of epidemiological studies assessing the risk factors for GDM, as well as the results and methodological problems of intervention studies on the prevention of GDM and their meta-analyses, are discussed. In addition, prospects for increasing the effectiveness of interventions designed to prevent GDM, including new data on the possible use of personalized diet therapy, are highlighted.

Citation: Popova PV, Pustozerov EA, Tkachuk AS, Grineva EN. Improving nutrition for the prevention of gestational diabetes: Current status and perspectives. *World J Diabetes* 2021; 12(9): 1494-1506
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1494.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1494

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common complication of pregnancy affecting approximately one in five pregnant women, according to the criteria of the International Association of the Diabetes and Pregnancy Study Groups (IADPSG)[1].

The problem of GDM prevention has attracted increasing attention from researchers in recent years, given the numerous short-term and long-term adverse effects associated with GDM on both mothers and their offspring. For women, GDM is associated with an increased risk of preeclampsia during pregnancy[2] and a significantly increased risk of type 2 DM (T2D) and comorbidities such as cardiovascular disease after pregnancy[3]. Intrauterine hyperglycemia in pregnancy potentially affects many aspects of offspring health throughout their lives. For example, babies born to mothers with GDM are more likely to be large for gestational age and thus are more likely to suffer from birth trauma[2]. Intrauterine hyperglycemia in mothers with GDM is an important factor in programming the predisposition to obesity, DM and cardiovascular disease in offspring[4-6]. The maintenance of a normal glycemic level in pregnancy is necessary to prevent adverse pregnancy outcomes and to interrupt the vicious cycle of the transmission of a predisposition to metabolic diseases in subsequent generations[7].

RISK FACTORS FOR GDM

Changes in hormones and glucose metabolism associated with the development of GDM during pregnancy must be understood to prevent adverse outcomes such as T2D [8]. GDM occurs when insulin receptors are unable to respond adequately to changes in blood glucose levels due to the influence of hormones produced by the placenta during pregnancy, such as human placental lactogen. This insufficient response, in turn, causes an increase in blood glucose levels. Because of the similarities in the underlying pathophysiological and risk factors for GDM and T2D, factors that are effective in preventing T2D may also be successful in preventing GDM.

Some risk factors for GDM, such as advanced maternal age[9], a family history of T2D[10], polycystic ovarian syndrome[11], hypothyroidism[12], previous diagnosis of GDM, history of fetal macrosomia, overweight and obesity, are well known[13].

Fasting glycemia in the first trimester of 5.1 mmol/L or higher is a diagnostic criterion for GDM, according to the IADPSG recommendations[14], which have subsequently been adopted by most international organizations, although they are not recognized by influential medical organizations in some countries[15]. In a prospective observational study of pregnant women, we found that in 33% of women presenting first trimester fasting glycemia in the range of 5.1 mmol/L to 5.6 mmol/L, the diagnosis of GDM was confirmed by a subsequent oral glucose tolerance test (OGTT) at 24-32 wk of pregnancy[16].

Genetic factors also contribute to the etiology of GDM. Several genes have been identified as associated with the development of GDM, including polymorphic

variants of the melatonin receptor 1B (*MTNR1B*) gene, glucokinase (*GCK*), transcription factor 7 (*TCF7L2*), potassium internal rectifying channel (*KCNJ11*), regulatory subunit 1 related protein (CDKAL1), insulin-like growth factor 2 binding protein 2 (*IGF2BP2*), fat mass and obesity-associated protein (*FTO*), and insulin receptor substrate 1 (*IRS1*)[17-21]. However, different genes may play a predominant role in the pathogenesis of GDM in different populations. Our previous study has confirmed the association of the rs10830963 variant in the *MTNR1B* gene and rs1799884 variant in the *GCK* gene with GDM in Russian women[22].

New data also suggest a possible contribution of environmental factors to the etiology of GDM. For example, exposure to perfluorooctanoic acid, an endocrine-disrupting substance often present in some carpet-cleaning fluids, microwave corn packets, and some culinary products, has been shown to be positively associated with the risk of GDM[23].

In addition to these risk factors, data from numerous epidemiological studies indicate that dietary and lifestyle factors, both before and during pregnancy, are associated with the risk of developing GDM and play a key role in the treatment of GDM. This review will discuss the results of prospective cohort studies and randomized clinical trials (RCTs) on the effectiveness of dietary modifications in preventing GDM.

EVIDENCE FROM OBSERVATIONAL STUDIES ON THE ASSOCIATION OF PRENATAL NUTRITION WITH THE RISK OF GDM

A major contribution to the accumulation of data on the association of preconceptional nutrition with the risk of GDM was the Nurses' Health Study II, which included 14437 nurses who have been followed in the United States since 1989 and became pregnant during the follow-up period[24-32]. A number of prepregnancy nutritional parameters were significantly associated with the risk of developing GDM: Sugary drinks[24], heme iron intake[25], fried foods[26], animal fat[27], animal protein[28], a diet low in carbohydrates but high in animal fat and protein[29], and a general Western diet high in red meat and processed meat, refined grain products, sweets, fries, and pizza[30]. For example, the risk of developing GDM increased 1.6-fold [relative risk (RR) 1.61; 95% confidence interval (CI): 1.25-2.07] with the consumption of one serving of red meat per day. Potential factors for reducing the risk of GDM included a “prudent diet” characterized by a high intake of fruit, green leafy vegetables, poultry, and fish[30], a Mediterranean diet[31], nut consumption[28] and fiber intake[32].

Similar data were obtained in the Australian population, where the "meat, snacks and sweets" type of diet was associated with an increased risk of developing GDM, and the Mediterranean type of diet was associated with a decreased risk of developing GDM[33].

Our data obtained in a survey of Russian women are consistent with the results of the aforementioned studies: high consumption of processed meat in the form of sausages was associated with an increased risk of GDM, and higher consumption of legumes and fruit was associated with a decreased risk of GDM[22].

Women who develop GDM have impaired β-cell function and insulin resistance, which limits their ability to cope with the metabolic problems of pregnancy[34]. Additionally, iron is an active transition metal and a strong pro-oxidant that promotes the formation of hydroxyl radicals, increasing oxidative stress. Pancreatic b-cells are particularly sensitive to oxidative stress because of their weak antioxidant defenses [35]. Nevertheless, following a healthy diet, such as a Mediterranean diet, may reduce the risk of GDM. Common components of healthy dietary options include fruits and vegetables, relatively small amounts of red and processed meats, and high-quality, slow-absorbing carbohydrates. Fruits and vegetables, in particular, have many antioxidant properties, in addition to providing fiber and micronutrients such as magnesium and vitamin C. The combination of all these factors has been shown to protect against metabolic disorders by counteracting free radicals and reducing systemic oxidative stress[36]. The main results of the reviewed observational studies on the association between prenatal nutrition and the risk of GDM are summarized in Table 1.

Table 1 Summary of observational studies on the association of prenatal nutrition with the risk of gestational diabetes mellitus

Ref.	Population, sample size	Nutritional factors/diet pattern	Comparison	RR/OR of GDM (95%CI)
Zhang *et al*[32], 2006	13110 United States women	Fiber intake	Highest *vs* lowest quintile	RR 0.67 (0.51-0.90)
Zhang *et al*[30], 2006	13110 United States women	Western diet high in red meat and processed meat, refined grain products, sweets, fries, and pizza	Highest *vs* lowest quintile	RR 1.63 (1.20-2.21)
		"Prudent diet" characterized by high intake of fruit, green leafy vegetables, poultry, and fish	Lowest *vs* highest quintile	RR 1.39 (1.08-1.80)
Chen *et al*[24], 2009	13475 United States women	Sugar-sweetened cola	5 servings per week *vs* < 1 serving per month	RR 1.22 (1.01-1.47)
Bowers *et al*[25], 2011	13475 United States women	Heme iron intake	Highest *vs* lowest quintile	RR 1.58 (1.21-2.08)
Bowers *et al*[27], 2012	13475 United States women	Animal fat	Highest *vs* lowest quintile	RR 1.88 (1.36-2.60)
Tobias *et al*[31], 2012	15254 United States women	Mediterranean diet	Highest *vs* lowest quartile	RR 0.76 (0.60-0.95)
Bao *et al*[28], 2013	15294 United States women	Animal protein	Highest *vs* lowest quintile	RR 1.49 (1.03-2.17)
Bao *et al*[26], 2014	15027 United States women	Fried foods	> 7 times per week *vs* < 1 time per week	RR 1.88 (1.34-2.64)
Bao *et al*[29], 2014	15265 United States women	Diet low in carbohydrates but high in animal fat and protein	Highest *vs* lowest quartile	RR 1.36 (1.13-1.64)
Schoenaker *et al*[33], 2015	3853 Australian women	'Meats, snacks and sweets' pattern	Bottom and top tertiles of dietary pattern scores	RR 1.35 (0.98-1.81).
		'Mediterranean-style' pattern	Bottom and top tertiles of dietary pattern scores	RR 0.85 (0.76-0.98)
Popova *et al*[22], 2017	457 Russian women	Sausage	> 3 times per week *vs* less than once per week	OR 2.2 (1.2-4.1)
		Legumes	1-2 times per week *vs* less frequent consumption	OR 0.58 (0.36-0.94)

RR: Relative risk; OR: Odds ratio; CI: Confidence interval; GDM: Gestational diabetes mellitus.

EVIDENCE FROM OBSERVATIONAL STUDIES ON THE RELATIONSHIP BETWEEN NUTRITION DURING PREGNANCY AND THE RISK OF GDM

The evidence from observational studies on the relationship between diet during pregnancy and the risk of GDM is mixed, and a wide variety of methods for assessing dietary habits have been developed (isolating certain types of diet and consumption of specific nutrients or foods). However, several studies suggest that adherence to a Mediterranean diet during pregnancy may reduce the risk of developing GDM by 15-38%. In a multicenter study of 10 Mediterranean countries, the incidence of GDM was lower in women with better adherence to the Mediterranean diet during pregnancy (with a higher Mediterranean diet index score) by approximately 35%-38%: 8.0% *vs* 12.3%, odds ratio (OR) = 0.618, P = 0.030 when using the American Diabetes Association (ADA 2010) criteria and 24.3% *vs* 32.8%, OR = 0.655, P = 0.004 using the IADPSG 2012 criteria[37].

The St. Carlos GDM Prevention Study[38] also showed that among 874 Spanish women, high adherence to the Mediterranean diet was associated with a reduced risk of GDM (OR 0.35; 95%CI: 0.18-0.67) compared with women with low adherence.

However, differences in the definition of what exactly constitutes a "traditional" Mediterranean diet exist because of the differences between different Mediterranean regions. Typically, a "traditional" Mediterranean diet is characterized by large amounts of fruits, vegetables, legumes, nuts, unprocessed grains and cereals, extra virgin olive oil, moderate amounts of fish and wine, and small amounts of meat with few foods containing "empty calories"[39]. Higher consumption of red or processed meat prior to pregnancy is associated with an increased risk of developing GDM. Two meta-analyses in a healthy adult population showed that the consumption of processed

meat was associated with a higher risk of coronary heart disease (42%) and T2D (19%-32%)[40,41]. The proposed mechanism of coronary heart disease and T2D includes excess sodium and oxidative stress due to high levels of iron and glycation end products[41], but it requires further study. Because the traditional Mediterranean diet is characterized by low meat intake, the diet has been shown to be beneficial for preventing GDM.

A large cohort study from China including 3,063 pregnant women analyzed the association of four diets (vegetable, protein-rich, "prudent", and "sweets and seafood") with the risk of GDM. The vegetable type of diet was associated with a decreased risk of GDM, whereas the "sweets and seafood" type of diet was associated with an increased risk of GDM[42].

Similarly, in another Chinese prospective cohort study of 1014 women [mean prepregnancy body mass index (BMI) < 23 kg/m^2], a "traditional dietary pattern" (high consumption of vegetables, fruits, and rice) was associated with a lower risk of GDM [0.40 (95%CI: 0.23-0.70)][41]. Meanwhile, a diet rich in whole-grain foods and seafood was associated with an increased risk of developing GDM [OR 1.73 (95%CI: 1.10-274)][43]. This finding contradicts the results of previous studies[44] and may be due to the older age of the women who followed this type of diet, which is generally considered healthier.

Another study from China including 6,299 pregnant women showed that higher intake of total protein and animal protein in mid-pregnancy was associated with an increased risk of GDM (RR 1.92, 95%CI: 1.10-3.14, $P = 0.04$)[45].

In our study, which included 266 women with GDM and 414 pregnant women without GDM, only higher fruit consumption (more than 12 servings per week) was associated with a reduced risk of GDM among the nutritional factors analyzed during pregnancy[22]. The association between high fruit consumption and a lower risk of developing GDM may be explained by several potential mechanisms. First, fruit is rich in fiber, which may reduce obesity and improve insulin sensitivity[46]. In addition, fiber consumption may delay gastric emptying and delay digestion and assimilation, resulting in lower plasma glucose levels after a meal[46,47]. In addition, fruits are rich in polyphenols and other antioxidant components, such as vitamin C, vitamin E and carotenoids[48,49]. These compounds may reduce the risk of GDM by reducing oxidative stress, which interferes with glucose uptake by cells.

Meanwhile, a Norwegian prospective study did not observe differences in diet among 702 women (of whom 40 had GDM) who developed GDM and those who did not[50]. Similarly, Looman *et al*[51] found no consistent correlation between diet quality and glycemia (as assessed using the 2015 Dutch Healthy Diet Index). Only a weak correlation was observed between fasting glucose levels, diet quality, and total iron intake (both P values < 0.05).

The Project Viva study in the United States, which included 1733 pregnant women who were evaluated for the association of diet type and frequency of red and processed meat consumption in the first trimester with the risk of developing GDM, obtained similar results[52]. Nutritional habits and the consumption of red and processed meat during pregnancy were not predictors of GDM diagnosis. The authors concluded that prepregnancy dietary patterns, as reflected by BMI before pregnancy, were probably more important contributors to the development of GDM than the diet during pregnancy.

The summary of observational studies on the relationship between nutrition during pregnancy and the risk of GDM is depicted in Table 2.

An important limitation of all observational studies remains the use of food frequency questionnaires to assess food intake. Nevertheless, collectively, the results of most observational studies indicate the important role of lifestyle factors during pregnancy in the development of GDM.

What is the next step in this area of research? The obvious next step appears to be a shift from the results of large observational studies to effective interventions designed to prevent GDM. Interventional studies on GDM prevention have emerged in the last 10 years.

RANDOMIZED TRIALS OF THE EFFECT OF DIET DURING PREGNANCY ON THE RISK OF GDM

A large number of RCTs have evaluated different lifestyle interventions during pregnancy to prevent GDM. Individual studies have limited power and ability to prove the effect of diet on the risk of developing GDM. Therefore, this review will

Table 2 Summary of observational studies on the relationship between nutrition during pregnancy and the risk of gestational diabetes mellitus

Ref.	Population, sample size	Nutritional factors/diet pattern	Comparison	RR/OR of GDM (95%CI)
Radesky *et al* [52], 2008	1733 pregnant United States women	Diet type and frequency of red and processed meat consumption	Macronutrient energy partition and nutrient density substitution models	No association
Karamanos *et al*[37], 2014	Multicenter study of 10 Mediterranean countries, 1076 pregnant women	Mediterranean diet index (MDI), reflecting the degree of adherence to the MedDiet pattern of eating	Lower tertile of MDI (poor adherence) *vs* the upper tertile (good adherence)	OR 0.655(0.495-0.867)
He *et al*[42], 2015	3063 pregnant Chinese women	Vegetable pattern	Highest tertile *vs* lowest tertile	RR 0.79 (0.64-0.97)
		Protein-rich pattern		No association
		"Prudent" pattern		No association
		Sweets and seafood pattern		RR 1.23 (1.02-1.49)
Popova *et al* [22], 2017	680 pregnant Russian women	Fruit consumption	> 12 servings per week *vs* less consumption	OR 0.5 (0.3-0.8)
Elvebakk *et al* [50], 2018	702 pregnant Norwegian women	Intake of food groups	Women who developed GDM and women who did not develop GDM	No association
Liang *et al*[45], 2018	6299 Chinese pregnant women	Total protein	Highest tertile *vs* lowest tertile	RR 1.92 (1.10-3.14)
		Animal protein		RR 1.67 (1.19-2.93)
		Vegetable protein intake		No association
Assaf-Balut *et al*[38], 2018	874 Spanish women	Degree of adherence to a MedDiet pattern based on six food targets	High adherence (complying with 5-6 targets); moderate adherence (2-4 targets); low adherence (0-1 targets)	OR 0.35 (0.18-0.67)
Hu *et al*[43], 2019	1014 pregnant Chinese women	"Traditional pattern" (high vegetable, fruit, and rice intake)	Quartile 4 versus quartile 1	OR 0.44 (0.27-0.70)
		Whole grain-seafood pattern		OR 1.73, (1.10-2.74)

RR: Relative risk; OR: Odds ratio; CI: Confidence interval; GDM: Gestational diabetes mellitus.

mainly discuss the results of meta-analyses, which combine the results of individual studies and clarify the presence of an effect with greater certainty.

A meta-analysis by Tieu *et al*[53] that included 11 RCTs (2786 women) evaluated the effectiveness of dietary recommendations for the prevention of GDM. Five of the included studies compared dietary recommendations with standard treatment, four studies compared a low-glycemic index (GI) diet with medium or high-GI dietary recommendations, and one study compared a high-fiber diet with standard dietary recommendations. A trend toward a reduced risk of GDM (RR 0.60, 95%CI: 0.35-1.04; $P = 0.07$) was observed in women who received dietary recommendations compared with the standard treatment. A subgroup analysis showed a more significant effect of dietary recommendations on reducing the risk of GDM in overweight and obese women (RR 0.39, 95%CI: 0.19-0.79)[53].

Song *et al*[54] included studies of the effects of diet, physical activity (PA) or a combination of the two during pregnancy on the risk of GDM (a total of 27 RCTs and 11487 women) in a systematic review and meta-analysis. In the pooled analysis, diet or PA resulted in an 18% (95%CI: 5-30, $P = 0.009$) reduction in the risk of GDM. However, in separate analyses, the effect of PA combined with diet, diet alone, or PA alone on the risk of GDM did not reach statistical significance. In subgroup analyses, the intervention was only effective if initiated before 15 wk of gestation (RR 0.8, 95%CI: 0.66-0.97)[54].

In a systematic review involving 23 RCTs and approximately 9000 women from the Cochrane Database, Shepherd *et al*[55] compared the effect of a combination of diet and exercise with no intervention (standard management) on preventing GDM in pregnant women. This analysis showed a possible reduction in the risk of GDM (RR 0.85, 95%CI: 0.71-1.01, $P = 0.07$) in the intervention group with a moderate level of

evidence.

In a recently published meta-analysis, Guo *et al*[56] examined the effectiveness of lifestyle interventions, including diet, exercise, or a combination of the two, in preventing GDM and were able to include 47 RCTs (15745 participants). As a result, the authors were able to show a significant reduction in the risk of GDM in the lifestyle intervention groups (RR 0.77, 95%CI: 0.69-0.87) and separately in studies of the effect of diet alone on GDM risk (n = 11, RR 0.75, 95%CI: 0.59-0.95). In addition, the authors were able to assess the contributions of different factors to the effectiveness of preventive interventions and identified four key aspects: high-risk intervention, early intervention, appropriate intensity and frequency of exercise, and control of weight gain during pregnancy. Interestingly, in overweight or obese women, BMI was not a predictor of intervention effectiveness. However, interventions were most effective in populations with a high prevalence of GDM rather than only overweight or obese women[56].

The summary of randomized trials on the effect of diet during pregnancy on the risk of GDM is depicted in Table 3.

RANDOMIZED TRIALS OF THE EFFECT OF DIET BEFORE PREGNANCY ON THE RISK OF GDM

Given the data from meta-analyses concerning the benefits of early intervention, a logical assumption is that the optimal approach is to start the intervention before pregnancy. To date, few studies have been published on the effectiveness of diet and/or lifestyle changes before pregnancy on the risk of GDM. To the best of our knowledge, only two such studies have been published[57,58].

In the study by Mutsaerts *et al*[57] evaluating the effect of a 6-month lifestyle change before pregnancy on the rate of live birth in obese and infertile women, greater weight loss was achieved in the intervention group. However, no difference was observed in the incidence of GDM between groups.

In a study from Finland, high-risk women (n = 228) planning a pregnancy were randomized into 2 groups: Lifestyle intervention or standard management[58]. The prepregnancy lifestyle intervention did not reduce the incidence of GDM. However, the lifestyle intervention was very mild. It included individual lifestyle counseling only once every 3 mo and only one group session with a nutritionist. No prepregnancy weight change was indicated, and pregnancy weight gain did not differ between the intervention group and the control group[58]. The intensity of the intervention did not appear sufficient to cause prepregnancy weight loss, and a real change in lifestyle may not have occurred. Consequently, the lack of a reduction in the risk of GDM was not surprising.

Additional studies evaluating preconception lifestyle interventions are needed. Longer, more intensive, and more frequent preconception lifestyle interventions in larger study groups might affect the incidence of GDM and perinatal and neonatal outcomes.

PROSPECTS FOR IMPROVING THE EFFECTIVENESS OF GDM PREVENTION INTERVENTIONS

Dietary interventions aimed at reducing the risk of GDM in most studies follow a "one size fits all" approach, which provides uniform dietary recommendations for all participants in the same group. However, data on the effectiveness of these interventions for GDM prevention are inconsistent, as described above.

Among factors that potentially improve the effectiveness of GDM prevention interventions, approaches that personalize dietary recommendations are promising.

A reduction in the consumption of foods with a high GI and high glycemic load (GL) has consistently been shown to increase weight loss by reducing the postprandial glycemic response (PPGR) and insulin secretion[59-62]. In addition, minimizing the PPGR attenuates the decrease in resting energy expenditure associated with weight loss[63].

Standard dietary interventions based on GI/GL may not be sufficiently effective for weight loss and GDM prevention because people vary in their glycemic response to the same food[64], and, as shown in our studies, the addition of GI and GL to models predicting PPGR in pregnant women only marginally improved the prediction

Table 3 Summary of randomized trials on the effect of diet during pregnancy on the risk of gestational diabetes mellitus

Ref.	Design	Comparison	No. of participants (studies)	RR of GDM (95%CI)
Song *et al*[54], 2016	Meta-analysis, 27 RCTs (11487 women)	Lifestyle intervention of diet, PA or both *vs* standard management	11487 (27)	0.82 (0.70-0.95)
		PA plus diet *vs* standard management	6047 (14)	0.85 (0.70-1.03)
		Diet only *vs* standard management	1279 (5)	0.80 (0.58-1.10)
Tieu *et al*[53], 2017	Meta-analysis, 11 RCTs (2786 women)	Dietary recommendations *vs* standard treatment	1279 (5 RCTs)	0.60 (0.35-1.04); in overweight and obese women RR 0.39 (0.19-0.79)
		Low-glycemic index (GI) diet *vs* medium- or high-GI dietary recommendations	912 (4 RCTs)	0.91 (0.63-1.31)
		High-fiber diet *vs* standard dietary recommendations	25 (1)	No association
Shepherd *et al* [55], 2017	Meta-analysis, 23 RCTs (8918 women)	Combination of diet and exercise *vs* standard management	6633 (19)	0.85 (0.71-1.01)
Guo *et al*[56], 2019	Meta-analysis, 47 RCTs (15745 women)	Lifestyle intervention (diet, exercise, and mixed interventions) *vs* standard management	15745 (47)	0.77 (0.69-0.87)
		Diet alone *vs* standard management	2838 (11)	0.75 (0.60-0.95),

RR: Relative risk; PA: Physical activity; RCTs: Randomized clinical trials; CI: Confidence interval; GDM: Gestational diabetes mellitus.

accuracy[65]. Consequently, a proportion of individuals may experience postprandial hyperglycemia despite eating low GI/GL foods. The mismatch between lifestyle change efforts (*e.g.*, low GI/GL diet) and outcome (*e.g.*, weight loss or blood glucose control) may reduce motivation and adherence to the diet.

The reasons for differences in metabolic response are complex and widely studied. Genetic parameters and the microbiome may play a role and have great potential to explain at least part of the individual metabolic differences in food intake.

T2D is known to develop through an interaction between genetic predisposition and lifestyle, as has been confirmed in several studies [66,67]. The pathogenesis of GDM and T2D shares many factors. Therefore, researchers have assumed that GDM results from a combination of genetic risk factors and an unfavorable lifestyle. A number of new studies support the hypothesis that gene and lifestyle interactions influence the development of GDM[68]. Our study found that the association of sausage consumption with the risk of developing GDM is determined by the number of risk alleles for rs10830963 in the *MTNR1B* gene and rs1799884 in the *GCK* gene. Both genes are involved in the regulation of pancreatic islet beta-cell function and glucose homeostasis. Restriction of fatty food consumption (including sausage and sausage products) is one of the components of lifestyle changes in GDM prevention programs. Our results confirm the data reported by Grotenfelt *et al*[68] on the interaction between the rs10830963 allele and lifestyle interventions in modifying the risk of GDM. According to their study, the relative risk of GDM among women homozygous for the rs10830963 C allele was significantly lower in the intervention group than in the control group (OR = 0.16, 95%CI: 0.03-0.85, P = 0.014). This difference was not observed in women with the G risk allele. Further studies are needed to clarify the effects of genetic factors on the effectiveness of lifestyle changes designed to prevent GDM.

Researchers are also very interested in the microbiome as a determinant of individual metabolic differences in food intake.

In 2015, Zeevi *et al*[69] described a new machine learning algorithm that predicts individual PPGR per meal in healthy volunteers based on food intake composition, history, anthropometry, and a gut microbiome analysis. The authors showed high variability in the PPGR for the same foods among participants and suggested that universal dietary recommendations have limited utility for postprandial glucose control. Zeevi *et al*[69] also reported that individually tailored dietary recommendations based on the predicted response significantly improved the PPGR. We used a similar approach to develop models for predicting PPGR in pregnant women and obtained comparable results for accuracy, although we have not yet included microbiome data as input parameters in the models[65,70].

A promising direction to improve dietary effects is the use of mobile technology, which allows remote consultation and self-monitoring in a manner that is convenient for patients and has great potential for dissemination. We have developed a system for the remote monitoring of patients with GDM, which is a combination of software that includes a mobile app for the patient and programs to perform calculations and data analysis for the physician[71]. This system can also be used for GDM prevention programs, especially in high-risk groups.

The combination of mobile technology and providing participants with specific food recommendations tailored to their unique physiological response to food intake may increase their adherence to lifestyle changes and improve the success of weight loss and GDM prevention.

CONCLUSION

Most observational studies have shown an association between dietary patterns before and during pregnancy and the risk of developing GDM. However, the results of randomized trials of the effect of dietary and/or lifestyle interventions on pregnant women and women planning pregnancy have been inconsistent.

The lack of effect of dietary recommendations before and during pregnancy on the risk of GDM in a number of studies can be explained by reasons such as insufficient intensity of the intervention, changes during pregnancy that prevent adherence to the recommendations (nausea, change in taste, and fatigue), and a late start and short time for lifestyle changes. In addition, individual studies have insufficient sample sizes and statistical power. Perhaps the problem of small sample size also explained the lack of reliable associations in meta-analyses published before 2019. Only the recently published and largest meta-analysis by Guo *et al*[56] showed a significant reduction in the risk of GDM in the lifestyle intervention groups and separately in studies of the effect of diet alone on GDM risk.

Most RCTs that included dietary recommendations for the prevention of GDM compared the effectiveness of the studied diet with standard dietary recommendations. Therefore, data comparing the effectiveness of different dietary options for preventing GDM are currently unavailable. Based on the data from observational studies, the benefits of the Mediterranean diet have been confirmed. Further studies are needed to clarify the optimal variant of the diet or a personalized approach to the formation of dietary recommendations to prevent the development of GDM.

REFERENCES

1 **Sacks DA**, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, Lowe LP, Coustan DR, Hod M, Oats JJ, Persson B, Trimble ER; HAPO Study Cooperative Research Group. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. *Diabetes Care* 2012; **35**: 526-528 [PMID: 22355019 DOI: 10.2337/dc11-1641]

2 **HAPO Study Cooperative Research Group**, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; **358**: 1991-2002 [PMID: 18463375 DOI: 10.1056/NEJMoa0707943]

3 **Ben-Haroush A**, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabet Med* 2004; **21**: 103-113 [PMID: 14984444 DOI: 10.1046/j.1464-5491.2003.00985.x]

4 **Silverman BL**, Rizzo TA, Cho NH, Metzger BE. Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes Care* 1998; **21** Suppl 2: B142-B149 [PMID: 9704242]

5 **Popova P**, Vasilyeva L, Tkachuck A, Puzanov M, Golovkin A, Bolotko Y, Pustozerov E, Vasilyeva E, Li O, Zazerskaya I, Dmitrieva R, Kostareva A, Grineva E. A Randomised, Controlled Study of Different Glycaemic Targets during Gestational Diabetes Treatment: Effect on the Level of Adipokines in Cord Blood and ANGPTL4 Expression in Human Umbilical Vein Endothelial Cells. *Int J Endocrinol* 2018; **2018**: 6481658 [PMID: 29861725 DOI: 10.1155/2018/6481658]

6 **El Hajj N**, Pliushch G, Schneider E, Dittrich M, Müller T, Korenkov M, Aretz M, Zechner U, Lehnen H, Haaf T. Metabolic programming of MEST DNA methylation by intrauterine exposure to gestational diabetes mellitus. *Diabetes* 2013; **62**: 1320-1328 [PMID: 23209187 DOI: 10.2337/db12-0289]

7 **El Hajj N**, Schneider E, Lehnen H, Haaf T. Epigenetics and life-long consequences of an adverse nutritional and diabetic intrauterine environment. *Reproduction* 2014; **148**: R111-R120 [PMID:

25187623 DOI: 10.1530/REP-14-0334]
8 **Ramírez-Torres MA**. The importance of gestational diabetes beyond pregnancy. *Nutr Rev* 2013; **71** Suppl 1: S37-S41 [PMID: 24147923 DOI: 10.1111/nure.12070]
9 **Li G**, Wei T, Ni W, Zhang A, Zhang J, Xing Y, Xing Q. Incidence and Risk Factors of Gestational Diabetes Mellitus: A Prospective Cohort Study in Qingdao, China. *Front Endocrinol (Lausanne)* 2020; **11**: 636 [PMID: 33042010 DOI: 10.3389/fendo.2020.00636]
10 **Moosazadeh M**, Asemi Z, Lankarani KB, Tabrizi R, Maharlouei N, Naghibzadeh-Tahami A, Yousefzadeh G, Sadeghi R, Khatibi SR, Afshari M, Khodadost M, Akbari M. Family history of diabetes and the risk of gestational diabetes mellitus in Iran: A systematic review and meta-analysis. *Diabetes Metab Syndr* 2017; **11** Suppl 1: S99-S104 [PMID: 28017634 DOI: 10.1016/j.dsx.2016.12.016]
11 **Kjerulff LE**, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. *Am J Obstet Gynecol* 2011; **204**: 558.e1-558.e6 [PMID: 21752757 DOI: 10.1016/j.ajog.2011.03.021]
12 **Giannakou K**, Evangelou E, Yiallouros P, Christophi CA, Middleton N, Papatheodorou E, Papatheodorou SI. Risk factors for gestational diabetes: An umbrella review of meta-analyses of observational studies. *PLoS One* 2019; **14**: e0215372 [PMID: 31002708 DOI: 10.1371/journal.pone.0215372]
13 **Zhang C**, Ning Y. Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence. *Am J Clin Nutr* 2011; **94**: 1975S-1979S [PMID: 21613563 DOI: 10.3945/ajcn.110.001032]
14 **International Association of Diabetes and Pregnancy Study Groups Consensus Panel**, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva Ad, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010; **33**: 676-682 [PMID: 20190296 DOI: 10.2337/dc09-1848]
15 **Popova P**, Castorino K, Grineva EN, Kerr D. Gestational diabetes mellitus diagnosis and treatment goals: measurement and measures. *Minerva Endocrinol* 2016 [PMID: 26824326]
16 **Popova P**, Tkachuk A, Dronova A, Gerasimov A, Kravchuk E, Bolshakova M, Rozdestvenskaya O, Demidova K, Nikolaeva A, Grineva E. Fasting glycemia at the first prenatal visit and pregnancy outcomes in Russian women. *Minerva Endocrinol* 2016; **41**: 477-485 [PMID: 27600641]
17 **Mao H**, Li Q, Gao S. Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. *PLoS One* 2012; **7**: e45882 [PMID: 23029294 DOI: 10.1371/journal.pone.0045882]
18 **Zhang C**, Bao W, Rong Y, Yang H, Bowers K, Yeung E, Kiely M. Genetic variants and the risk of gestational diabetes mellitus: a systematic review. *Hum Reprod Update* 2013; **19**: 376-390 [PMID: 23690305 DOI: 10.1093/humupd/dmt013]
19 **Wu L**, Cui L, Tam WH, Ma RC, Wang CC. Genetic variants associated with gestational diabetes mellitus: a meta-analysis and subgroup analysis. *Sci Rep* 2016; **6**: 30539 [PMID: 27468700 DOI: 10.1038/srep30539]
20 **Ding M**, Chavarro J, Olsen S, Lin Y, Ley SH, Bao W, Rawal S, Grunnet LG, Thuesen ACB, Mills JL, Yeung E, Hinkle SN, Zhang W, Vaag A, Liu A, Hu FB, Zhang C. Genetic variants of gestational diabetes mellitus: a study of 112 SNPs among 8722 women in two independent populations. *Diabetologia* 2018; **61**: 1758-1768 [PMID: 29947923 DOI: 10.1007/s00125-018-4637-8]
21 **Cao M**, Zhang L, Chen T, Shi A, Xie K, Li Z, Xu J, Chen Z, Ji C, Wen J. Genetic Susceptibility to Gestational Diabetes Mellitus in a Chinese Population. *Front Endocrinol (Lausanne)* 2020; **11**: 247 [PMID: 32390949 DOI: 10.3389/fendo.2020.00247]
22 **Popova PV**, Klyushina AA, Vasilyeva LB, Tkachuk AS, Bolotko YA, Gerasimov AS, Pustozerov EA, Kravchuk EN, Predeus A, Kostareva AA, Grineva EN. Effect of gene-lifestyle interaction on gestational diabetes risk. *Oncotarget* 2017; **8**: 112024-112035 [PMID: 29340108 DOI: 10.18632/oncotarget.22999]
23 **Zhang C**, Sundaram R, Maisog J, Calafat AM, Barr DB, Buck Louis GM. A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. *Fertil Steril* 2015; **103**: 184-189 [PMID: 25450302 DOI: 10.1016/j.fertnstert.2014.10.001]
24 **Chen L**, Hu FB, Yeung E, Willett W, Zhang C. Prospective study of pre-gravid sugar-sweetened beverage consumption and the risk of gestational diabetes mellitus. *Diabetes Care* 2009; **32**: 2236-2241 [PMID: 19940226 DOI: 10.2337/dc09-0866]
25 **Bowers K**, Yeung E, Williams MA, Qi L, Tobias DK, Hu FB, Zhang C. A prospective study of prepregnancy dietary iron intake and risk for gestational diabetes mellitus. *Diabetes Care* 2011; **34**: 1557-1563 [PMID: 21709294 DOI: 10.2337/dc11-0134]
26 **Bao W**, Tobias DK, Olsen SF, Zhang C. Pre-pregnancy fried food consumption and the risk of gestational diabetes mellitus: a prospective cohort study. *Diabetologia* 2014; **57**: 2485-2491 [PMID: 25303998 DOI: 10.1007/s00125-014-3382-x]
27 **Bowers K**, Tobias DK, Yeung E, Hu FB, Zhang C. A prospective study of prepregnancy dietary fat intake and risk of gestational diabetes. *Am J Clin Nutr* 2012; **95**: 446-453 [PMID: 22218158 DOI: 10.3945/ajcn.111.026294]
28 **Bao W**, Bowers K, Tobias DK, Hu FB, Zhang C. Prepregnancy dietary protein intake, major dietary protein sources, and the risk of gestational diabetes mellitus: a prospective cohort study. *Diabetes*

Care 2013; **36**: 2001-2008 [PMID: 23378620 DOI: 10.2337/dc12-2018]
29 **Bao W**, Bowers K, Tobias DK, Olsen SF, Chavarro J, Vaag A, Kiely M, Zhang C. Prepregnancy low-carbohydrate dietary pattern and risk of gestational diabetes mellitus: a prospective cohort study. *Am J Clin Nutr* 2014; **99**: 1378-1384 [PMID: 24717341 DOI: 10.3945/ajcn.113.082966]
30 **Zhang C**, Schulze MB, Solomon CG, Hu FB. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. *Diabetologia* 2006; **49**: 2604-2613 [PMID: 16957814 DOI: 10.1007/s00125-006-0422-1]
31 **Tobias DK**, Zhang C, Chavarro J, Bowers K, Rich-Edwards J, Rosner B, Mozaffarian D, Hu FB. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. *Am J Clin Nutr* 2012; **96**: 289-295 [PMID: 22760563 DOI: 10.3945/ajcn.111.028266]
32 **Zhang C**, Liu S, Solomon CG, Hu FB. Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus. *Diabetes Care* 2006; **29**: 2223-2230 [PMID: 17003297 DOI: 10.2337/dc06-0266]
33 **Schoenaker DA**, Soedamah-Muthu SS, Callaway LK, Mishra GD. Pre-pregnancy dietary patterns and risk of gestational diabetes mellitus: results from an Australian population-based prospective cohort study. *Diabetologia* 2015; **58**: 2726-2735 [PMID: 26358582 DOI: 10.1007/s00125-015-3742-1]
34 **Buchanan TA**. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. *J Clin Endocrinol Metab* 2001; **86**: 989-993 [PMID: 11238474 DOI: 10.1210/jcem.86.3.7339]
35 **Buchanan TA**, Xiang A, Kjos SL, Watanabe R. What is gestational diabetes? *Diabetes Care* 2007; **30** Suppl 2: S105-S111 [PMID: 17596457 DOI: 10.2337/dc07-s201]
36 **Hamer M**, Chida Y. Intake of fruit, vegetables, and antioxidants and risk of type 2 diabetes: systematic review and meta-analysis. *J Hypertens* 2007; **25**: 2361-2369 [PMID: 17984654 DOI: 10.1097/HJH.0b013e3282efc214]
37 **Karamanos B**, Thanopoulou A, Anastasiou E, Assaad-Khalil S, Albache N, Bachaoui M, Slama CB, El Ghomari H, Jotic A, Lalic N, Lapolla A, Saab C, Marre M, Vassallo J, Savona-Ventura C; MGSD-GDM Study Group. Relation of the Mediterranean diet with the incidence of gestational diabetes. *Eur J Clin Nutr* 2014; **68**: 8-13 [PMID: 24084515 DOI: 10.1038/ejcn.2013.177]
38 **Assaf-Balut C**, García de la Torre N, Fuentes M, Durán A, Bordiú E, Del Valle L, Valerio J, Jiménez I, Herraiz MA, Izquierdo N, Torrejón MJ, de Miguel MP, Barabash A, Cuesta M, Rubio MA, Calle-Pascual AL. A High Adherence to Six Food Targets of the Mediterranean Diet in the Late First Trimester is Associated with a Reduction in the Risk of Materno-Foetal Outcomes: The St. Carlos Gestational Diabetes Mellitus Prevention Study. *Nutrients* 2018; **11** [PMID: 30602688 DOI: 10.3390/nu11010066]
39 **Radd-Vagenas S**, Kouris-Blazos A, Singh MF, Flood VM. Evolution of Mediterranean diets and cuisine: concepts and definitions. *Asia Pac J Clin Nutr* 2017; **26**: 749-763 [PMID: 28802282 DOI: 10.6133/apjcn.082016.06]
40 **Micha R**, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* 2010; **121**: 2271-2283 [PMID: 20479151 DOI: 10.1161/CIRCULATIONAHA.109.924977]
41 **Feskens EJ**, Sluik D, van Woudenbergh GJ. Meat consumption, diabetes, and its complications. *Curr Diab Rep* 2013; **13**: 298-306 [PMID: 23354681 DOI: 10.1007/s11892-013-0365-0]
42 **He JR**, Yuan MY, Chen NN, Lu JH, Hu CY, Mai WB, Zhang RF, Pan YH, Qiu L, Wu YF, Xiao WQ, Liu Y, Xia HM, Qiu X. Maternal dietary patterns and gestational diabetes mellitus: a large prospective cohort study in China. *Br J Nutr* 2015; **113**: 1292-1300 [PMID: 25821944 DOI: 10.1017/S0007114515000707]
43 **Hu J**, Oken E, Aris IM, Lin PD, Ma Y, Ding N, Gao M, Wei X, Wen D. Dietary Patterns during Pregnancy Are Associated with the Risk of Gestational Diabetes Mellitus: Evidence from a Chinese Prospective Birth Cohort Study. *Nutrients* 2019; **11** [PMID: 30769927 DOI: 10.3390/nu11020405]
44 **Schoenaker DA**, Mishra GD, Callaway LK, Soedamah-Muthu SS. The Role of Energy, Nutrients, Foods, and Dietary Patterns in the Development of Gestational Diabetes Mellitus: A Systematic Review of Observational Studies. *Diabetes Care* 2016; **39**: 16-23 [PMID: 26696657 DOI: 10.2337/dc15-0540]
45 **Liang Y**, Gong Y, Zhang X, Yang D, Zhao D, Quan L, Zhou R, Bao W, Cheng G. Dietary Protein Intake, Meat Consumption, and Dairy Consumption in the Year Preceding Pregnancy and During Pregnancy and Their Associations With the Risk of Gestational Diabetes Mellitus: A Prospective Cohort Study in Southwest China. *Front Endocrinol (Lausanne)* 2018; **9**: 596 [PMID: 30364240 DOI: 10.3389/fendo.2018.00596]
46 **Wang T**, Heianza Y, Sun D, Zheng Y, Huang T, Ma W, Rimm EB, Manson JE, Hu FB, Willett WC, Qi L. Improving fruit and vegetable intake attenuates the genetic association with long-term weight gain. *Am J Clin Nutr* 2019; **110**: 759-768 [PMID: 31301130 DOI: 10.1093/ajcn/nqz136]
47 **McIntosh M**, Miller C. A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. *Nutr Rev* 2001; **59**: 52-55 [PMID: 11310776 DOI: 10.1111/j.1753-4887.2001.tb06976.x]
48 **Du H**, Li L, Bennett D, Guo Y, Turnbull I, Yang L, Bragg F, Bian Z, Chen Y, Chen J, Millwood IY, Sansome S, Ma L, Huang Y, Zhang N, Zheng X, Sun Q, Key TJ, Collins R, Peto R, Chen Z; China Kadoorie Biobank study. Fresh fruit consumption in relation to incident diabetes and diabetic vascular

complications: A 7-y prospective study of 0.5 million Chinese adults. *PLoS Med* 2017; **14**: e1002279 [PMID: 28399126 DOI: 10.1371/journal.pmed.1002279]

49 **Bahadoran Z**, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J Diabetes Metab Disord* 2013; **12**: 43 [PMID: 23938049 DOI: 10.1186/2251-6581-12-43]

50 **Elvebakk T**, Mostad IL, Mørkved S, Salvesen KÅ, Stafne SN. Dietary Intakes and Dietary Quality during Pregnancy in Women with and without Gestational Diabetes Mellitus-A Norwegian Longitudinal Study. *Nutrients* 2018; **10** [PMID: 30463394 DOI: 10.3390/nu10111811]

51 **Looman M**, Geelen A, Samlal RAK, Heijligenberg R, Klein Gunnewiek JMT, Balvers MGJ, Wijnberger LDE, Brouwer-Brolsma EM, Feskens EJM. Changes in Micronutrient Intake and Status, Diet Quality and Glucose Tolerance from Preconception to the Second Trimester of Pregnancy. *Nutrients* 2019; **11** [PMID: 30813281 DOI: 10.3390/nu11020460]

52 **Radesky JS**, Oken E, Rifas-Shiman SL, Kleinman KP, Rich-Edwards JW, Gillman MW. Diet during early pregnancy and development of gestational diabetes. *Paediatr Perinat Epidemiol* 2008; **22**: 47-59 [PMID: 18173784 DOI: 10.1111/j.1365-3016.2007.00899.x]

53 **Tieu J**, Shepherd E, Middleton P, Crowther CA. Dietary advice interventions in pregnancy for preventing gestational diabetes mellitus. *Cochrane Database Syst Rev* 2017; **1**: CD006674 [PMID: 28046205 DOI: 10.1002/14651858.CD006674.pub3]

54 **Song C**, Li J, Leng J, Ma RC, Yang X. Lifestyle intervention can reduce the risk of gestational diabetes: a meta-analysis of randomized controlled trials. *Obes Rev* 2016; **17**: 960-969 [PMID: 27417680 DOI: 10.1111/obr.12442]

55 **Shepherd E**, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. *Cochrane Database Syst Rev* 2017; **11**: CD010443 [PMID: 29129039 DOI: 10.1002/14651858.CD010443.pub3]

56 **Guo XY**, Shu J, Fu XH, Chen XP, Zhang L, Ji MX, Liu XM, Yu TT, Sheng JZ, Huang HF. Improving the effectiveness of lifestyle interventions for gestational diabetes prevention: a meta-analysis and meta-regression. *BJOG* 2019; **126**: 311-320 [PMID: 30216635 DOI: 10.1111/1471-0528.15467]

57 **Mutsaerts MA**, van Oers AM, Groen H, Burggraaff JM, Kuchenbecker WK, Perquin DA, Koks CA, van Golde R, Kaaijk EM, Schierbeek JM, Oosterhuis GJ, Broekmans FJ, Bemelmans WJ, Lambalk CB, Verberg MF, van der Veen F, Klijn NF, Mercelina PE, van Kasteren YM, Nap AW, Brinkhuis EA, Vogel NE, Mulder RJ, Gondrie ET, de Bruin JP, Sikkema JM, de Greef MH, ter Bogt NC, Land JA, Mol BW, Hoek A. Randomized Trial of a Lifestyle Program in Obese Infertile Women. *N Engl J Med* 2016; **374**: 1942-1953 [PMID: 27192672 DOI: 10.1056/NEJMoa1505297]

58 **Rönö K**, Stach-Lempinen B, Eriksson JG, Pöyhönen-Alho M, Klemetti MM, Roine RP, Huvinen E, Andersson S, Laivuori H, Valkama A, Meinilä J, Kautiainen H, Tiitinen A, Koivusalo SB. Prevention of gestational diabetes with a prepregnancy lifestyle intervention - findings from a randomized controlled trial. *Int J Womens Health* 2018; **10**: 493-501 [PMID: 30214318 DOI: 10.2147/IJWH.S162061]

59 **Radulian G**, Rusu E, Dragomir A, Posea M. Metabolic effects of low glycaemic index diets. *Nutr J* 2009; **8**: 5 [PMID: 19178721 DOI: 10.1186/1475-2891-8-5]

60 **Chaput JP**, Tremblay A, Rimm EB, Bouchard C, Ludwig DS. A novel interaction between dietary composition and insulin secretion: effects on weight gain in the Quebec Family Study. *Am J Clin Nutr* 2008; **87**: 303-309 [PMID: 18258618 DOI: 10.1093/ajcn/87.2.303]

61 **Ebbeling CB**, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load *vs* low-fat diet in obese young adults: a randomized trial. *JAMA* 2007; **297**: 2092-2102 [PMID: 17507345 DOI: 10.1001/jama.297.19.2092]

62 **Pawlak DB**, Kushner JA, Ludwig DS. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *Lancet* 2004; **364**: 778-785 [PMID: 15337404 DOI: 10.1016/S0140-6736(04)16937-7]

63 **Ebbeling CB**, Swain JF, Feldman HA, Wong WW, Hachey DL, Garcia-Lago E, Ludwig DS. Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA* 2012; **307**: 2627-2634 [PMID: 22735432 DOI: 10.1001/jama.2012.6607]

64 **Matthan NR**, Ausman LM, Meng H, Tighiouart H, Lichtenstein AH. Estimating the reliability of glycemic index values and potential sources of methodological and biological variability. *Am J Clin Nutr* 2016; **104**: 1004-1013 [PMID: 27604773 DOI: 10.3945/ajcn.116.137208]

65 **Pustozerov E**, Tkachuk A, Vasukova E, Dronova A, Shilova E, Anopova A, Piven F, Pervunina T, Vasilieva E, Grineva E, Popova P. The Role of Glycemic Index and Glycemic Load in the Development of Real-Time Postprandial Glycemic Response Prediction Models for Patients With Gestational Diabetes. *Nutrients* 2020; **12** [PMID: 31979294 DOI: 10.3390/nu12020302]

66 **Neel JV**. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet* 1962; **14**: 353-362 [PMID: 13937884]

67 **Sonestedt E**, Lyssenko V, Ericson U, Gullberg B, Wirfält E, Groop L, Orho-Melander M. Genetic variation in the glucose-dependent insulinotropic polypeptide receptor modifies the association between carbohydrate and fat intake and risk of type 2 diabetes in the Malmo Diet and Cancer cohort. *J Clin Endocrinol Metab* 2012; **97**: E810-E818 [PMID: 22399504 DOI: 10.1210/jc.2011-2444]

68 **Grotenfelt NE**, Wasenius NS, Rönö K, Laivuori H, Stach-Lempinen B, Orho-Melander M, Schulz CA, Kautiainen H, Koivusalo SB, Eriksson JG. Interaction between rs10830963 polymorphism in MTNR1B and lifestyle intervention on occurrence of gestational diabetes. *Diabetologia* 2016; **59**:

1655-1658 [PMID: 27209463 DOI: 10.1007/s00125-016-3989-1]

69 **Zeevi D**, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M, Suez J, Mahdi JA, Matot E, Malka G, Kosower N, Rein M, Zilberman-Schapira G, Dohnalová L, Pevsner-Fischer M, Bikovsky R, Halpern Z, Elinav E, Segal E. Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 2015; **163**: 1079-1094 [PMID: 26590418 DOI: 10.1016/j.cell.2015.11.001]

70 **Pustozerov EA**, Tkachuk AS, Vasukova EA, Anopova AD, Kokina MA, Gorelova IV, Pervunina TM, Grineva EN, Popova PV. Machine Learning Approach for Postprandial Blood Glucose Prediction in Gestational Diabetes Mellitus. *IEEE Access* 2020; **8**: 219308-219321 [DOI: 10.1109/access.2020.3042483]

71 **Pustozerov EA**, Yuldashev ZM, Popova PV, Bolotko YA, Tkachuk AS. Information Support System for Patients with Gestational Diabetes Mellitus. *Biomed Eng* 2018; **51**: 407-410 [DOI: 10.1007/s10527-018-9759-2]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1507-1517

DOI: 10.4239/wjd.v12.i9.1507

ISSN 1948-9358 (online)

MINIREVIEWS

Salivary resistin level and its association with insulin resistance in obese individuals

Mona Mohamed Ibrahim Abdalla

ORCID number: Mona Mohamed Ibrahim Abdalla 00000-0002-4987-9517.

Author contributions: Abdalla MMI collected the data and wrote the paper.

Conflict-of-interest statement: The author declares no conflict of interest for this article.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Malaysia

Peer-review report's scientific quality classification

Mona Mohamed Ibrahim Abdalla, Human Biology Department, School of Medicine, International Medical University, Kuala Lumpur 57000, Malaysia

Corresponding author: Mona Mohamed Ibrahim Abdalla, MBBS, MSc, MD, PhD, Senior Lecturer, Human Biology Department, School of Medicine, International Medical University, 126, Jln Jalil Perkasa 19, Bukit Jalil, Kuala Lumpur 57000, Malaysia.
monamohamed@imu.edu.my

Abstract

The escalating global burden of type 2 diabetes mellitus necessitates the implementation of strategies that are both more reliable and faster in order to improve the early identification of insulin resistance (IR) in high-risk groups, including overweight and obese individuals. The use of salivary biomarkers offers a promising alternative to serum collection because it is safer, more comfortable, and less painful to obtain saliva samples. As obesity is the foremost contributory factor in IR development, the adipocytokines such as leptin, adiponectin, resistin, and visfatin secreted from the adipose tissue have been studied as potential reliable biomarkers for IR. Measurement of salivary adipokines as predictors for IR has attracted widespread attention because of the strong correlation between their blood and salivary concentrations. One of the adipokines that is closely related to IR is resistin. However, there are conflicting findings on resistin's potential role as an etiological link between obesity and IR and the reliability of measuring salivary resistin as a biomarker for IR. Hence this study reviewed the available evidence on the potential use of salivary resistin as a biomarker for IR in order to attempt to gain a better understanding of the role of resistin in the development of IR in obese individuals.

Key Words: Homeostatic model assessment of insulin resistance; Insulin resistance; Obesity; Salivary resistin; Diabetes; Adipocytokines

Core Tip: The worldwide increased prevalence of obesity-induced insulin resistance (IR) highlights the limitations of the long-term, invasive methods currently being used in detecting and monitoring IR. Measurement of salivary concentrations of adipokines

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 22, 2021
Peer-review started: January 22, 2021
First decision: May 3, 2021
Revised: May 11, 2021
Accepted: July 15, 2021
Article in press: July 15, 2021
Published online: September 15, 2021

P-Reviewer: Geng TY
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Liu JH

such as resistin offers a good alternative to serum collection for early detection and monitoring of glycaemic control among obese individuals. However, there are conflicting findings on the association between resistin and IR. Hence this review of the available evidence aims to provide a better understanding of the role of resistin in the development of IR and the potential use of salivary resistin as a biomarker for IR.

Citation: Abdalla MMI. Salivary resistin level and its association with insulin resistance in obese individuals. *World J Diabetes* 2021; 12(9): 1507-1517
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1507.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1507

INTRODUCTION

The World Health Organization reported a marked increase in the number of diabetic patients from 108 million in 1980 to 422 million in 2014[1]. There was also a 5% increase in premature mortality from diabetes between 2000 and 2016, and the World Health Organization estimated that diabetes was the seventh leading cause of death in 2016[1]. More recently, in 2020, an epidemiological study estimated that 462 million individuals or 6.28% of the global population are affected by type 2 diabetes mellitus (T2DM), a condition that seems to be more prevalent in developed countries despite the promotion and implementation of a range of public health measures[2].

T2DM is a non-insulin-dependent type of diabetes that was previously largely considered to be a disease of middle and old age. However, during recent decades, there has been a global rise in the prevalence of T2DM among children and young adults[3,4]. This rise has coincided with an increased prevalence of obesity, the foremost contributory factor to insulin resistance (IR) and T2DM[5,6]. The earlier the onset of the disease, the longer its duration and the higher the incidence of complications, which subsequently leads to higher mortality among the younger generation [7].

T2DM is characterized by increased insulin secretion, IR, and impaired glucose tolerance[8]. Early detection of impaired glycaemic control among prediabetics as well as maintenance of good control of the blood glucose level is, therefore, crucial in reducing mortality and delaying the onset of complications[9]. The glucose tolerance test and measurement of glycated haemoglobin are the methods most commonly used for early detection of IR in high-risk individuals[10]. However, the global burdens of T2DM and obesity highlight the limitations of the long-term, invasive screening methods currently employed in the early identification of individuals at high-risk of these two conditions.

PATHOGENESIS OF IR IN OBESITY

The chronic inflammatory state in obesity is known to be a significant pathogenic mechanism for obesity-associated complications[11]. Several polypeptides known as adipokines or pro-inflammatory cytokines that are secreted from adipocytes and adipose tissue macrophages have been found to play a role in inflammatory response as well as in the regulation of energy balance, food intake, and insulin sensitization [12]. Among the known adipokines, adiponectin, leptin, resistin, interleukin-6, tumour necrosis factor-alpha, plasminogen activator inhibitor-1, and monocyte chemo attractant protein-1 have been found to be directly related to the pathogenesis of IR in obesity[13].

Moreover, the chronic inflammatory state in obesity is found to induce a state of oxidative stress that is caused by enhanced production of reactive oxygen species, which is induced by pro-inflammatory cytokines such as tumour necrosis factor-alpha and resistin. It has therefore been suggested that a combination of inflammation and oxidative stress is involved in the process of the pathogenesis of IR[14], as illustrated in Figure 1.

The chronic inflammation, macrophage infiltration, increased leptin level, decreased adiponectin, mitochondrial dysfunction, endoplasmic reticulum stress, and adipocyte apoptosis could be attributed to the adipose tissue hypoxia response and adipocyte

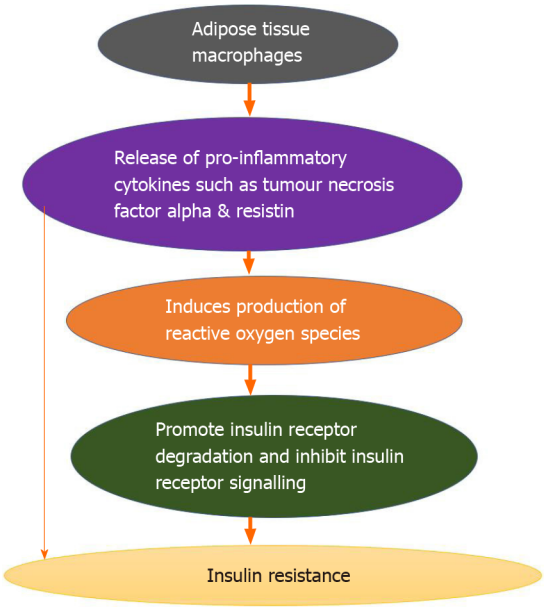

Figure 1 Simplified mechanism of obesity-induced insulin resistance.

dysfunction[15], which are found to be associated with the downregulation of insulin receptors resulting in systemic IR[16]. In addition, the inflammation and oxidative stress associated with obesity may be attributed to the aging of the adipose tissue. This process includes molecular changes in the cells, such as deactivation of P53 tumour suppressor and inflammation[17,18]. Oxidative stress is also associated with endoplasmic reticulum stress, which occurs due to excess nutrient intake in obesity [19].

Even with little macrophage infiltrates, lipotoxicity caused due to excess and ectopic fat accumulation in adipocytes, liver, and muscle is found to damage the pancreatic beta cells, leading to T2DM[20]. In addition, lipid overload leads to cellular dysfunction, endoplasmic reticulum stress, activation of pro-inflammatory stress pathways, and occurrence of IR, which may be attributed to the increased production of biologically active lipid intermediates such as ceramides and diacylglycerol[21-23].

IR

Insulin, a pleiotropic peptide secreted by beta cells in the pancreatic islets, regulates the blood glucose level by increasing glucose uptake and utilization in muscles and adipose tissues through stimulating the translocation of glucose transporter 4 to the plasma membrane, inhibiting glucose production in the liver through inhibiting the expression of key gluconeogenic enzymes and promoting lipolysis[24]. The effects of insulin are mediated by the binding of insulin-to-insulin receptors and insulin-like growth factor-1 receptors, which results in phosphorylation of the receptor substrate, followed by activation of the intracellular signalling pathways phosphoinositide 3-kinase/protein kinase B pathway and the mitogen-activated protein kinase pathways [25].

IR is a disease condition in which insulin-dependent cells, such as those found in skeletal muscle, the liver, and adipocytes, are unable to respond properly to the normal circulatory levels of insulin[14]. This inability to respond results in hyperglycaemia, which is caused by decreased removal of glucose from the blood and by increased production of glucose in the liver, the latter of which is associated with decreased fatty acid release from adipose tissues[26].

Studies have proved the usefulness of assessing the serum levels of many of the adipokines, including resistin and adiponectin, as biomarkers for IR[9,27]. The positive correlation between serum and salivary proteome levels[28,29] has attracted attention because of the implication that salivary biomarkers could be used in preference to serum due to the potential benefits of the former in reducing the suffering, pain, and stress associated with serum sampling[30]. The use of salivary biomarkers in diabetes is further supported by the fact that the increased permeability of the basement membrane in diabetes is associated with increased leakage of proteins from serum into

saliva[30,31].

RESISTIN STRUCTURE AND DISTRIBUTION

Resistin is a cysteine-rich polypeptide that was discovered by Steppan *et al*[32]. It has been proposed that resistin is a potential link between obesity and T2DM because it is upregulated in rodent models of obesity and IR and downregulated by insulin sensitizers. Resistin is also known as an adipose-tissue-specific secretory factor, which in humans is encoded by the *RETN* gene located on chromosome 19[33].

The normal serum level of human resistin ranges from 7 to 22 ng/mL[34]. There are two circulating forms of resistin: high molecular weight resistin, which is the predominant form, and low molecular weight resistin, which is the bioactive form in which bioactivity is initiated by disulphide cleavage in its hexametric structure[35].

Human resistin is quite different from rodent resistin in terms of both its structure and distribution. Murine resistin is a 114 amino acid polypeptide that is produced primarily in white adipose tissue[36], whereas human resistin is a 108 amino acid polypeptide expressed by adipose tissue, particularly visceral fat, pre-adipocytes, adipocytes[37,38], peripheral blood mononuclear cells[39], skeletal muscle[40], the pancreas[41], hypothalamus, adrenal gland, spleen, bone marrow, gastrointestinal tract, lungs[42], pituitary gland[43], and placenta[44]. Here it is worth mentioning that several studies have proved the role of peripheral blood mononuclear cells – the primary producers of human resistin[39,45] – in the inflammatory process involved in the pathogenesis of obesity-induced IR[46,47]. The dissimilar genetic organization of murine and human resistin[48] may be the reason for the conflicting findings on the potential role of resistin as an aetiological link between obesity and diabetes reported in studies on murine *vs* human resistin.

RESISTIN AS A LINK BETWEEN OBESITY AND IR

Since the discovery of resistin two decades ago, many research studies have been conducted on humans and rodents in order to investigate its potential role as a link between obesity and IR. Higher circulating levels of resistin have been reported in murine and rodent models of obesity compared to lean[32,49], and higher circulating levels of resistin have also been reported in obese individuals compared with lean[50,51] and positively correlated with body mass index (BMI) and visceral fat[51,52]. The increase in resistin in obese rodents may represent a negative feedback mechanism that acts to control adipocyte differentiation[49]. It has been suggested that an increase in the accumulation of adipose tissue, which reflects an increase in adipocyte differentiation and the pool of adipocytes, results in an increase in the secretion of resistin from the adipocytes, where the secreted resistin acts as a paracrine polypeptide that autoregulates its secretion by inhibiting adipocyte differentiation[53]. It has also been reported that resistin increases in parallel with increases in insulin and glucose and decreases in parallel with their decrease, hence the serum level of resistin seems to be regulated by the levels of insulin and glucose[53]. Other regulators of resistin include age, gender, thyroid hormones, and gonadal hormones[54].

Human studies have revealed contradictory findings on the correlation between circulating resistin and IR in T2DM and obesity. Some studies have reported a positive correlation[55-60], whereas others have found a negative[61] or lack of correlation[62,63].

A recent systematic review and meta-analysis conducted in 2019 on the correlation between serum resistin and IR in T2DM and obesity concluded that, overall, the results were in favour of there being a positive correlation between circulating resistin and IR in T2DM and obese individuals with hyperresistinaemia but not in those with normal circulating levels of resistin[64], which implies that resistin needs to reach a certain critical level to cause IR[60]. The meta-analysis was performed on 15 studies that were undertaken during the period 2005–2017 and involved a total of 1227 patients of diverse age, gender, and ethnicity. In the meta-analysis, these patients were classified into 20 clinical groups: 10 with simple T2DM, 7 with simple obesity, 2 with T2DM and obesity, and 1 group of T2DM patients with or without obesity[64]. The difference in resistin concentration among these different groups, which led to conflicting results on the association between resistin and IR, may be explained by the several single nucleotide polymorphisms of the resistin *RETN* gene in the different ethnic groups studied[65], differences in the levels of insulin and leptin, which have

been found to stimulate resistin expression[66], and differences in the methods used to assess resistin levels, specifically, commercially available enzyme-linked immunosorbent assays have the potential to cross-react with circulating resistin-like molecules, and not all studies assessed for this cross-reactivity before measuring the resistin levels [67,68].

EFFECTS OF INCREASED RESISTIN ON GLUCOSE HOMEOSTASIS

Overexpression of resistin in transgenic mice has been found to result in the impairment of insulin-dependent glucose transport and uptake by muscles and adipose tissue, which seems to be caused by a reduction in the intrinsic activity of cell-membrane glucose transporters that does not affect insulin receptor signalling[69]. The insulin-independent effect of resistin on glucose homeostasis is supported by a previous study that has shown that resistin induces the expression of a suppressor of cytokine-signalling-3, which functions to inhibit insulin signalling[70]. Resistin stimulates gluconeogenesis in the liver, an action that is evidenced by low hepatic glucose production in resistin gene knockout mice, an effect that is reversed by resistin infusion that causes an increase in the glucose level of approximately 25%[71]. In addition, hyperresistinaemia increased level of fasting glucose, hepatic glucose production, and induced activity of gluconeogenic enzymes[72].

High levels of resistin stimulate the expression of tumour necrosis factor-alpha and interleukin-6 in both human and murine macrophages *via* the NF-kB-dependent pathway, which results in IR[73]. In addition, an increased resistin level leads to leptin resistance[74], which contributes to the development of IR[75], as illustrated in Figure 2.

SALIVARY RESISTIN AND IR IN OBESE INDIVIDUALS

Due to the increased global and economic burden of T2DM, IR, and obesity across age groups, including children and young adults, the need for effective, easy, and non-invasive methods for early detection and monitoring of IR has increased. The use of saliva as a diagnostic tool is evolving not only due to the rapid advances that are being made in the fields of nanotechnology and molecular diagnostics, but also because saliva contains biomarkers that are ideal for early detection and monitoring of oral as well as systemic diseases[30,76].

To review the available evidence on the association between salivary resistin and IR in obese individuals, the scientific literature published up to 31 December 2020 was searched in the following databases: PubMed, ProQuest, Scopus, Ovid, Science Direct, Springer Link, and Trip. The search was limited to papers published in the English language. A search of the references in the identified papers was also done to identify any additional relevant papers. At the end of the search process, a total of six papers were identified for review. These papers, which were published between 2011 and 2020, are discussed in ascending chronological order.

The first selected study, conducted by Mamali *et al*[77] in Patras, Greece and published in 2011, involved the measurement of resistin in saliva and an assessment of the association between its salivary and serum levels. Salivary and serum resistin was measured using a commercial enzyme immunoassay method. Samples were measured in duplicate. Serum samples were diluted five-fold while saliva samples were diluted three-fold. The study reported a strong positive correlation between the serum and salivary levels of resistin (r = 0.441, P = 0.003) with no significant correlation between their levels with age, body fat percentage, or BMI. The ratio of the serum level of resistin to its salivary level was 0.2[77]. The positive correlation between the salivary and serum levels of resistin that was reported indicated that resistin was transported from the blood to saliva, which supports the potential use of the salivary levels of resistin rather than its serum levels for early detection of IR[78]. The absence of a correlation between the salivary as well as the serum levels of resistin with age, BMI, and body fat percentage could be attributed to the characteristics of the participants, who were healthy with almost normal BMI and body fat. It could also be attributed to the measurement method that was used.

A positive correlation between saliva and serum levels of resistin was further evidenced in a 2012 study conducted in China by Yin *et al*[29] who investigated for the first time the differences in the serum and salivary levels of resistin in a sample of 38 patients who were newly diagnosed with T2DM (18 males/20 females) compared with

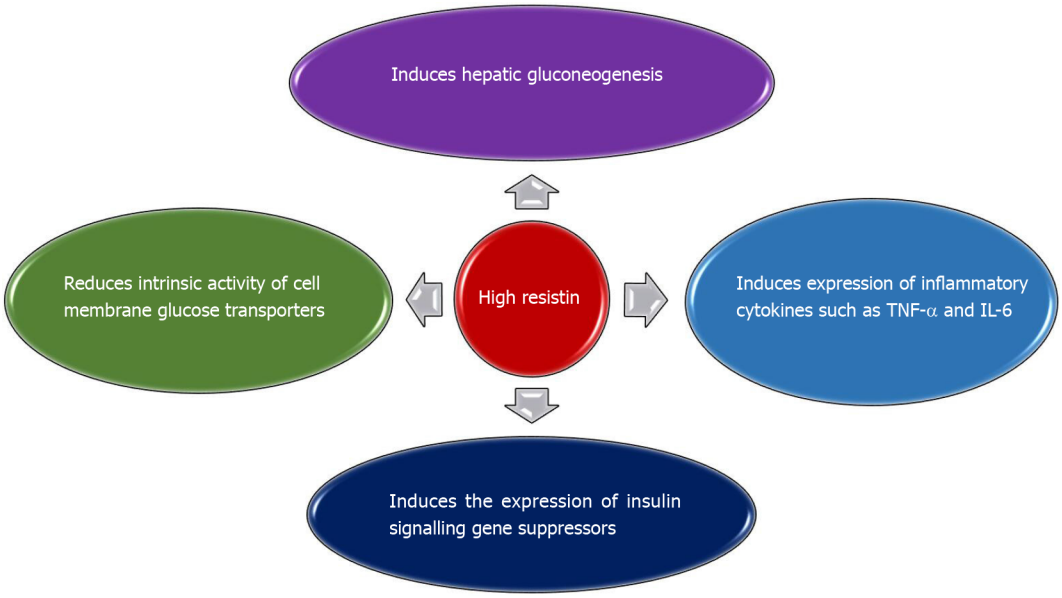

Figure 2 Mechanisms of resistin-induced insulin resistance and glucose intolerance. TNF-α: Tumour necrosis factor-alpha; IL-6: Interleukin-6

a control group of 35 non-diabetic individuals (18 males/17 females). The study revealed a significantly higher level of serum resistin as compared with salivary resistin in both diabetic and control groups. It also found significantly higher levels of both serum and salivary resistin in T2DM patients as compared with the control group. Furthermore, the study revealed significant correlations between salivary resistin and BMI (r = 0.39), glycated haemoglobin (r = 0.31) and the homeostatic model assessment of IR (r = 0.20)[29]. Collectively, these findings along with the presence of a consistent fluctuating trend of salivary and serum resistin levels during the oral glucose tolerance test provide evidence that indicates that the source of resistin in the saliva of newly diagnosed T2DM is mainly derived from the blood[29] rather than from local production by the salivary glands[79].

The above findings are further supported by a study conducted by Sarhat *et al*[80] in Iraq in which a significantly higher concentration of resistin was found in the saliva of patients with T2DM as compared with a healthy control group.

In 2017, Al-Rawi and Al-Marzooq[81] undertook a study in the United Arab Emirates to assess concentrations of resistin in the saliva of 26 obese diabetics, 26 obese non-diabetics, and 26 non-obese non-diabetics. The study found no difference in resistin concentration between obese diabetics (14.7 ± 2.8 ng/mL) and obese non-diabetics (14.4 ± 3.6 ng/mL), but the concentration level in these two groups was significantly higher than in non-obese non-diabetics (10.8 ± 6.1 ng/mL, P = 0.01). The study also reported a significant correlation between salivary resistin and BMI. However, there was no correlation between salivary resistin and glucose level[81].

A study by Srinivasan *et al*[82] in 2018 in the United States assessed not only the level of resistin, but also the levels of adiponectin, visfatin, and ghrelin in unstimulated whole saliva as biomarkers for T2DM. The study involved two groups: 20 periodontally healthy patients with self-reported T2DM and a control group of 20 individuals with no known oral or systemic diseases. Salivary resistin was measured using an enzyme-linked immunosorbent assay kit. The study found that the glycated haemoglobin values of the diabetic group were consistent with the diagnosis of T2DM. It also revealed a significantly higher level of salivary resistin in the T2DM group (9.2 ± 2.3 ng/mL) as compared with the control group (5.7 ± 1.3 ng/mL). However, the study did not assess the correlation between salivary resistin and IR. Based on their findings, Srinivasan *et al*[82] supported the potential use of salivary resistin as a biomarker for T2DM. However, they emphasized that some caution needs to be exercised during the collection of saliva for this purpose because certain factors may affect the interpretation of the results. Specifically, they noted that it was important to consider whether the saliva is stimulated or unstimulated and to account for the existence of circadian variations. They also recommended that pre-processing of saliva should be performed in order to reduce the possibility of the presence of confounding factors such as oral or systemic diseases[82].

Table 1 Salivary resistin concentration in association with body mass index and the homeostatic model assessment of insulin resistance

Study group	BMI (kg/m^2)	Salivary resistin (ng/mL)	Correlation with BMI	Correlation with blood glucose	Correlation with HOMA-IR	Correlation test used	Ref.
Healthy	22.39 ± 3.65	1.69	NS	-	-	Partial correlation	Mamali *et al*[77]
T2DM	25.5 ± 4.9	3.4± 0.4[1]	-0.391	-0.14	-0.201	Pearson's test	Yin *et al*[29]
Control	23.9 ± 3.3	1.5 ± 0.3	-0.17	-0.281	-0.19		
T2DM	-	4 ± 0.45[1]	-	-	-	-	Sarhat *et al*[80]
Control	-	1.73 ± 0.34	-	-	-	-	
Obese diabetic	34.3 ± 3.9	14.7 ± 2.8	Significant	NS			Al-Rawi and Al-Marzooq[81]
Obese non-diabetic	34.2 ± 2.9	14.4 ± 3.6	Significant				
Control "Non-obese-non - diabetic	7.1 ± 2.1	10.8 ± 6.1	Significant		-		
T2DM	-	9.2 ± 2.3[1]	-	-	-		Srinivasan *et al* [82]
Control	-	5.7 ± 1.3	-	-	-		
GDM	32 (20.4–43.7)	13.11	-	-	-		Gürlek and Çolak [83]
Control	27.2 (19.4–42)	5.9	-	-	-		

[1]Significant difference between the study groups, significant correlation. BMI: Body mass index; HOMA-IR: Homeostatic model assessment of insulin resistance; NS: Not significant; T2DM: Type 2 diabetes mellitus; GDM: Gestational diabetes mellitus.

More recently, in 2020, Gürlek and Çolak[83] investigated the effectiveness of evaluating salivary resistin concentrations as a screening marker for gestational diabetes mellitus (GDM). Gestational diabetes mellitus is a type of diabetes that occurs during pregnancy with an incidence that varies from 1% to 25%[84,85]. Studies have revealed that GDM is associated with decreased insulin sensitivity and release of pro-inflammatory cytokines such as resistin[86]. Also, a recent meta-analysis supported the use of serum resistin concentrations to screen for GDM[87]. Studies have also shown that the risk of GDM is far higher among overweight and obese pregnant women, especially those with central obesity[88-90]. Gürlek and Çolak[83] included 81 pregnant women in their study: 41 with newly diagnosed GDM and 40 with normal pregnancy without GDM. Fasting blood and unstimulated saliva samples were collected, and resistin was estimated using an enzyme-linked immunosorbent assay. The study revealed that pregnant women with GDM had significantly higher pre-gestational BMI, BMI at the time of sampling, and salivary resistin concentrations as compared with the pregnant women without GDM. Hence their study provided evidence for the first time that resistin concentrations in saliva might be a useful screening marker for GDM[83].

A summary of the findings related to the concentration of resistin in saliva and its correlation with BMI, homeostatic model assessment of IR, and blood glucose level are presented in Table 1.

CONCLUSION

The available evidence indicates that resistin plays a role in the development of IR. Prior studies also support the use of serum resistin as a reliable marker for IR. The evidence also supports the potential use of salivary resistin as a reliable biomarker for IR. However, the number of studies that assessed the correlation of salivary resistin with IR among obese, newly diagnosed T2DM patients is still limited.

REFERENCES

1 **World Health Organization**. Global report on diabetes. 2016. Available from: https://apps.who.int/iris/handle/10665/204871
2 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]
3 **Zheng Y**, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* 2018; **14**: 88-98 [PMID: 29219149 DOI: 10.1038/nrendo.2017.151]
4 **Molnár D**. The prevalence of the metabolic syndrome and type 2 diabetes mellitus in children and adolescents. *Int J Obes Relat Metab Disord* 2004; **28** Suppl 3: S70-S74 [PMID: 15543223 DOI: 10.1038/sj.ijo.0802811]
5 **Caceres M**, Teran CG, Rodriguez S, Medina M. Prevalence of insulin resistance and its association with metabolic syndrome criteria among Bolivian children and adolescents with obesity. *BMC Pediatr* 2008; **8**: 31 [PMID: 18700035 DOI: 10.1186/1471-2431-8-31]
6 **Pulungan AB**, Puspitadewi A, Sekartini R. Prevalence of insulin resistance in obese adolescents. *Paediatr Indones* 2013; **53**: 167-172
7 **Huo L**, Magliano DJ, Rancière F, Harding JL, Nanayakkara N, Shaw JE, Carstensen B. Impact of age at diagnosis and duration of type 2 diabetes on mortality in Australia 1997-2011. *Diabetologia* 2018; **61**: 1055-1063 [PMID: 29473119 DOI: 10.1007/s00125-018-4544-z]
8 **Rodger W**. Non-insulin-dependent (type II) diabetes mellitus. *CMAJ* 1991; **145**: 1571-1581 [PMID: 1742694]
9 **Meigs JB**. Multiple biomarker prediction of type 2 diabetes. *Diabetes Care* 2009; **32**: 1346-1348 [PMID: 19564478 DOI: 10.2337/dc09-0754]
10 **Faerch K**, Borch-Johnsen K, Holst JJ, Vaag A. Pathophysiology and aetiology of impaired fasting glycaemia and impaired glucose tolerance: does it matter for prevention and treatment of type 2 diabetes? *Diabetologia* 2009; **52**: 1714-1723 [PMID: 19590846 DOI: 10.1007/s00125-009-1443-3]
11 **Trayhurn P**, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* 2004; **92**: 347-355 [PMID: 15469638 DOI: 10.1079/bjn20041213]
12 **Rabe K**, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med* 2008; **14**: 741-751 [PMID: 19009016 DOI: 10.2119/2008-00058.Rabe]
13 **Fain JN**. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. *Mediators Inflamm* 2010; **2010**: 513948 [PMID: 20508843 DOI: 10.1155/2010/513948]
14 **Yaribeygi H**, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: Review of the underlying molecular mechanisms. *J Cell Physiol* 2019; **234**: 8152-8161 [PMID: 30317615 DOI: 10.1002/jcp.27603]
15 **Wondmkun YT**. Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabetes Metab Syndr Obes* 2020; **13**: 3611-3616 [PMID: 33116712 DOI: 10.2147/DMSO.S275898]
16 **Arcidiacono B**, Chiefari E, Foryst-Ludwig A, Currò G, Navarra G, Brunetti FS, Mirabelli M, Corigliano DM, Kintscher U, Britti D, Mollace V, Foti DP, Goldfine ID, Brunetti A. Obesity-related hypoxia *via* miR-128 decreases insulin-receptor expression in human and mouse adipose tissue promoting systemic insulin resistance. *EBioMedicine* 2020; **59**: 102912 [PMID: 32739259 DOI: 10.1016/j.ebiom.2020.102912]
17 **Ahima RS**. Connecting obesity, aging and diabetes. *Nat Med* 2009; **15**: 996-997 [PMID: 19734871 DOI: 10.1038/nm0909-996]
18 **Liu Z**, Wu KKL, Jiang X, Xu A, Cheng KKY. The role of adipose tissue senescence in obesity- and ageing-related metabolic disorders. *Clin Sci (Lond)* 2020; **134**: 315-330 [PMID: 31998947 DOI: 10.1042/CS20190966]
19 **Burgos-Morón E**, Abad-Jiménez Z, Marañón AM, Iannantuoni F, Escribano-López I, López-Domènech S, Salom C, Jover A, Mora V, Roldan I, Solá E, Rocha M, Víctor VM. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *J Clin Med* 2019; **8** [PMID: 31487953 DOI: 10.3390/jcm8091385]
20 **Lee Y**, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci USA* 1994; **91**: 10878-10882 [PMID: 7971976 DOI: 10.1073/pnas.91.23.10878]
21 **Chaurasia B**, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol Metab* 2015; **26**: 538-550 [PMID: 26412155 DOI: 10.1016/j.tem.2015.07.006]
22 **Han J**, Kaufman RJ. The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* 2016; **57**: 1329-1338 [PMID: 27146479 DOI: 10.1194/jlr.R067595]
23 **Hauck AK**, Bernlohr DA. Oxidative stress and lipotoxicity. *J Lipid Res* 2016; **57**: 1976-1986 [PMID: 27009116 DOI: 10.1194/jlr.R066597]
24 **Dimitriadis G**, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S52-S59 [PMID: 21864752 DOI: 10.1016/S0168-8227(11)70014-6]
25 **Ieronymaki E**, Daskalaki MG, Lyroni K, Tsatsanis C. Insulin Signaling and Insulin Resistance

Facilitate Trained Immunity in Macrophages Through Metabolic and Epigenetic Changes. *Front Immunol* 2019; **10**: 1330 [PMID: 31244863 DOI: 10.3389/fimmu.2019.01330]

26 **Smith U**, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. *J Intern Med* 2016; **280**: 465-475 [PMID: 27699898 DOI: 10.1111/joim.12540]

27 **Lau CH**, Muniandy S. Novel adiponectin-resistin (AR) and insulin resistance (IRAR) indexes are useful integrated diagnostic biomarkers for insulin resistance, type 2 diabetes and metabolic syndrome: a case control study. *Cardiovasc Diabetol* 2011; **10**: 8 [PMID: 21251282 DOI: 10.1186/1475-2840-10-8]

28 **Loo JA**, Yan W, Ramachandran P, Wong DT. Comparative human salivary and plasma proteomes. *J Dent Res* 2010; **89**: 1016-1023 [PMID: 20739693 DOI: 10.1177/0022034510380414]

29 **Yin J**, Gao H, Yang J, Xu L, Li M. Measurement of salivary resistin level in patients with type 2 diabetes. *Int J Endocrinol* 2012; **2012**: 359724 [PMID: 22969799 DOI: 10.1155/2012/359724]

30 **Desai GS**, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. *World J Diabetes* 2014; **5**: 730-738 [PMID: 25512775 DOI: 10.4239/wjd.v5.i6.730]

31 **Williamson S**, Munro C, Pickler R, Grap MJ, Elswick RK Jr. Comparison of biomarkers in blood and saliva in healthy adults. *Nurs Res Pract* 2012; **2012**: 246178 [PMID: 22619709 DOI: 10.1155/2012/246178]

32 **Steppan CM**, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307-312 [PMID: 11201732 DOI: 10.1038/35053000]

33 **Steppan CM**, Lazar MA. The current biology of resistin. *J Intern Med* 2004; **255**: 439-447 [PMID: 15049878 DOI: 10.1111/j.1365-2796.2004.01306.x]

34 **Jamaluddin MS**, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. *Br J Pharmacol* 2012; **165**: 622-632 [PMID: 21545576 DOI: 10.1111/j.1476-5381.2011]

35 **Patel SD**, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family hormones. *Science* 2004; **304**: 1154-1158 [PMID: 15155948 DOI: 10.1126/science.1093466]

36 **Muse ED**, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, Scherer PE, Rossetti L. Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 2004; **114**: 232-239 [PMID: 15254590 DOI: 10.1172/JCI21270]

37 **Fain JN**, Cheema PS, Bahouth SW, Lloyd Hiler M. Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* 2003; **300**: 674-678 [PMID: 12507502 DOI: 10.1016/s0006-291x(02)02864-4]

38 **McTernan PG**, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker J, Barnett AH, Kumar S. Increased resistin gene and protein expression in human abdominal adipose tissue. *J Clin Endocrinol Metab* 2002; **87**: 2407 [PMID: 11994397 DOI: 10.1210/jcem.87.5.8627]

39 **Patel L**, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003; **300**: 472-476 [PMID: 12504108 DOI: 10.1016/s0006-291x(02)02841-3]

40 **Dietze D**, Koenen M, Röhrig K, Horikoshi H, Hauner H, Eckel J. Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes. *Diabetes* 2002; **51**: 2369-2376 [PMID: 12145147 DOI: 10.2337/diabetes.51.8.2369]

41 **Minn AH**, Patterson NB, Pack S, Hoffmann SC, Gavrilova O, Vinson C, Harlan DM, Shalev A. Resistin is expressed in pancreatic islets. *Biochem Biophys Res Commun* 2003; **310**: 641-645 [PMID: 14521959 DOI: 10.1016/j.bbrc.2003.09.061]

42 **Nohira T**, Nagao K, Kameyama K, Nakai H, Fukumine N, Okabe K, Kitano S, Hisatomi H. Identification of an alternative splicing transcript for the resistin gene and distribution of its mRNA in human tissue. *Eur J Endocrinol* 2004; **151**: 151-154 [PMID: 15248836 DOI: 10.1530/eje.0.1510151]

43 **Morash BA**, Ur E, Wiesner G, Roy J, Wilkinson M. Pituitary resistin gene expression: effects of age, gender and obesity. *Neuroendocrinology* 2004; **79**: 149-156 [PMID: 15103228 DOI: 10.1159/000077273]

44 **Yura S**, Sagawa N, Itoh H, Kakui K, Nuamah MA, Korita D, Takemura M, Fujii S. Resistin is expressed in the human placenta. *J Clin Endocrinol Metab* 2003; **88**: 1394-1397 [PMID: 12629135 DOI: 10.1210/jc.2002-011926]

45 **Kaser S**, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. *Biochem Biophys Res Commun* 2003; **309**: 286-290 [PMID: 12951047 DOI: 10.1016/j.bbrc.2003.07.003]

46 **Bokarewa M**, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005; **174**: 5789-5795 [PMID: 15843582 DOI: 10.4049/jimmunol.174.9.5789]

47 **Kapłon-Cieślicka A**, Postuła M, Rosiak M, Peller M, Kondracka A, Serafin A, Trzepla E, Opolski G, Filipiak KJ. Association of adipokines and inflammatory markers with lipid control in type 2 diabetes. *Pol Arch Med Wewn* 2015; **125**: 414-423 [PMID: 25978118 DOI: 10.20452/pamw.2880]

48 **Ghosh S**, Singh AK, Aruna B, Mukhopadhyay S, Ehtesham NZ. The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. *Gene* 2003; **305**: 27-34 [PMID: 12594039 DOI: 10.1016/s0378-1119(02)01213-1]

49 **Asensio C**, Cettour-Rose P, Theander-Carrillo C, Rohner-Jeanrenaud F, Muzzin P. Changes in glycemia by leptin administration or high- fat feeding in rodent models of obesity/type 2 diabetes suggest a link between resistin expression and control of glucose homeostasis. *Endocrinology* 2004; **145**: 2206-2213 [PMID: 14962997 DOI: 10.1210/en.2003-1679]

50 **Vendrell J**, Broch M, Vilarrasa N, Molina A, Gómez JM, Gutiérrez C, Simón I, Soler J, Richart C. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obes Res* 2004; **12**: 962-971 [PMID: 15229336 DOI: 10.1038/oby.2004.118]

51 **Degawa-Yamauchi M**, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 2003; **88**: 5452-5455 [PMID: 14602788 DOI: 10.1210/jc.2002-021808]

52 **Yannakoulia M**, Yiannakouris N, Blüher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003; **88**: 1730-1736 [PMID: 12679465 DOI: 10.1210/jc.2002-021604]

53 **Rajala MW**, Qi Y, Patel HR, Takahashi N, Banerjee R, Pajvani UB, Sinha MK, Gingerich RL, Scherer PE, Ahima RS. Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. *Diabetes* 2004; **53**: 1671-1679 [PMID: 15220189 DOI: 10.2337/diabetes.53.7.1671]

54 **Nogueiras R**, Gualillo O, Caminos JE, Casanueva FF, Diéguez C. Regulation of resistin by gonadal, thyroid hormone, and nutritional status. *Obes Res* 2003; **11**: 408-414 [PMID: 12634438 DOI: 10.1038/oby.2003.55]

55 **Al-Harithy RN**, Al-Ghamdi S. Serum resistin, adiposity and insulin resistance in Saudi women with type 2 diabetes mellitus. *Ann Saudi Med* 2005; **25**: 283-287 [PMID: 16212119 DOI: 10.5144/0256-4947.2005.283]

56 **Jung HS**, Youn BS, Cho YM, Yu KY, Park HJ, Shin CS, Kim SY, Lee HK, Park KS. The effects of rosiglitazone and metformin on the plasma concentrations of resistin in patients with type 2 diabetes mellitus. *Metabolism* 2005; **54**: 314-320 [PMID: 15736108 DOI: 10.1016/j.metabol.2004.05.019]

57 **Mojiminiyi OA**, Abdella NA. Associations of resistin with inflammation and insulin resistance in patients with type 2 diabetes mellitus. *Scand J Clin Lab Invest* 2007; **67**: 215-225 [PMID: 17366001 DOI: 10.1080/00365510601032532]

58 **Tokuyama Y**, Osawa H, Ishizuka T, Onuma H, Matsui K, Egashira T, Makino H, Kanatsuka A. Serum resistin level is associated with insulin sensitivity in Japanese patients with type 2 diabetes mellitus. *Metabolism* 2007; **56**: 693-698 [PMID: 17445546 DOI: 10.1016/j.metabol.2006.12.019]

59 **Mabrouk R**, Ghareeb H, Shehab A, Omar K, El-Kabarity RH, Soliman DA, Mohamed NA. Serum visfatin, resistin and IL-18 in A group of Egyptian obese diabetic and non diabetic individuals. *Egypt J Immunol* 2013; **20**: 1-11 [PMID: 23888552]

60 **Zaidi SI**, Shirwany TA. Relationship of serum resistin with insulin resistance and obesity. *J Ayub Med Coll Abbottabad* 2015; **27**: 552-555 [PMID: 26721005]

61 **Bu J**, Feng Q, Ran J, Li Q, Mei G, Zhang Y. Visceral fat mass is always, but adipokines (adiponectin and resistin) are diversely associated with insulin resistance in Chinese type 2 diabetic and normoglycemic subjects. *Diabetes Res Clin Pract* 2012; **96**: 163-169 [PMID: 22244787 DOI: 10.1016/j.diabres.2011.12.014]

62 **Owecki M**, Miczke A, Nikisch E, Pupek-Musialik D, Sowiński J. Serum resistin concentrations are higher in human obesity but independent from insulin resistance. *Exp Clin Endocrinol Diabetes* 2011; **119**: 117-121 [PMID: 20827661 DOI: 10.1055/s-0030-1263111]

63 **Park H**, Hasegawa G, Obayashi H, Fujinami A, Ohta M, Hara H, Adachi T, Tamaki S, Nakajima Y, Kimura F, Ogata M, Fukui M, Yoshikawa T, Nakamura N. Relationship between insulin resistance and inflammatory markers and anti-inflammatory effect of losartan in patients with type 2 diabetes and hypertension. *Clin Chim Acta* 2006; **374**: 129-134 [PMID: 16857181 DOI: 10.1016/j.cca.2006.06.004]

64 **Su KZ**, Li YR, Zhang D, Yuan JH, Zhang CS, Liu Y, Song LM, Lin Q, Li MW, Dong J. Relation of Circulating Resistin to Insulin Resistance in Type 2 Diabetes and Obesity: A Systematic Review and Meta-Analysis. *Front Physiol* 2019; **10**: 1399 [PMID: 31803062 DOI: 10.3389/fphys.2019.01399]

65 **Hivert MF**, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples LA, Meigs JB, Florez JC. Association of variants in RETN with plasma resistin levels and diabetes-related traits in the Framingham Offspring Study. *Diabetes* 2009; **58**: 750-756 [PMID: 19074981 DOI: 10.2337/db08-1339]

66 **Tsiotra PC**, Boutati E, Dimitriadis G, Raptis SA. High insulin and leptin increase resistin and inflammatory cytokine production from human mononuclear cells. *Biomed Res Int* 2013; **2013**: 487081 [PMID: 23484124 DOI: 10.1155/2013/487081]

67 **Youn BS**, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, Cho YM, Park YJ, Kim SY, Lee HK, Park KS. Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2004; **89**: 150-156 [PMID: 14715842 DOI: 10.1210/jc.2003-031121]

68 **Fujinami A**, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H, Kawahara Y, Yamazaki M, Ogata M, Hasegawa G, Nakamura N, Yoshikawa T, Nakano K, Ohta M. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. *Clin Chim Acta* 2004; **339**: 57-63 [PMID: 14687894 DOI: 10.1016/j.cccn.2003.09.009]

69 **Moon B**, Kwan JJ, Duddy N, Sweeney G, Begum N. Resistin inhibits glucose uptake in L6 cells

independently of changes in insulin signaling and GLUT4 translocation. *Am J Physiol Endocrinol Metab* 2003; **285**: E106-E115 [PMID: 12618360 DOI: 10.1152/ajpendo.00457.2002]

70 **Steppan CM**, Wang J, Whiteman EL, Birnbaum MJ, Lazar MA. Activation of SOCS-3 by resistin. *Mol Cell Biol* 2005; **25**: 1569-1575 [PMID: 15684405 DOI: 10.1128/MCB.25.4.1569-1575.2005]

71 **Banerjee RR**, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, Wang J, Rajala MW, Pocai A, Scherer PE, Steppan CM, Ahima RS, Obici S, Rossetti L, Lazar MA. Regulation of fasted blood glucose by resistin. *Science* 2004; **303**: 1195-1198 [PMID: 14976316 DOI: 10.1126/science.1092341]

72 **Rangwala SM**, Rich AS, Rhoades B, Shapiro JS, Obici S, Rossetti L, Lazar MA. Abnormal glucose homeostasis due to chronic hyperresistinemia. *Diabetes* 2004; **53**: 1937-1941 [PMID: 15189975 DOI: 10.2337/diabetes.53.8.1937]

73 **Silswal N**, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. *Biochem Biophys Res Commun* 2005; **334**: 1092-1101 [PMID: 16039994 DOI: 10.1016/j.bbrc.2005.06.202]

74 **Asterholm IW**, Rutkowski JM, Fujikawa T, Cho YR, Fukuda M, Tao C, Wang ZV, Gupta RK, Elmquist JK, Scherer PE. Elevated resistin levels induce central leptin resistance and increased atherosclerotic progression in mice. *Diabetologia* 2014; **57**: 1209-1218 [PMID: 24623101 DOI: 10.1007/s00125-014-3210-3]

75 **Zhang J**, Pronyuk K, Kuliesh O, Chenghe S. Adiponectin, resistin and leptin: Possible markers of metabolic syndrome. *Endocrinol Metab Syndr* 2015; **4**: 2161-1017.1000212 [DOI: 10.4172/2161-1017.1000212]

76 **Streckfus CF**, Bigler LR. Saliva as a diagnostic fluid. *Oral Dis* 2002; **8**: 69-76 [PMID: 11991307 DOI: 10.1034/j.1601-0825.2002.1o834.x]

77 **Mamali I**, Roupas ND, Armeni AK, Theodoropoulou A, Markou KB, Georgopoulos NA. Measurement of salivary resistin, visfatin and adiponectin levels. *Peptides* 2012; **33**: 120-124 [PMID: 22108712 DOI: 10.1016/j.peptides.2011.11.007]

78 **Gröschl M**. [Current status of salivary hormone analysis]. *Ann Biol Clin (Paris)* 2009; **67**: 493-504 [PMID: 19789121 DOI: 10.1684/abc.2009.0357]

79 **Boström EA**, d'Elia HF, Dahlgren U, Simark-Mattsson C, Hasséus B, Carlsten H, Tarkowski A, Bokarewa M. Salivary resistin reflects local inflammation in Sjögren's syndrome. *J Rheumatol* 2008; **35**: 2005-2011 [PMID: 18709689]

80 **Sarhat ER**, Najim RS, Abdulla EH. Estimation of salivary resistin, malondialdehyde and lipid profile levels in patients with diabetes mellitus. *Tikrit J Pure Sci* 2018; **20**: 167-170

81 **Al-Rawi N**, Al-Marzooq F. The Relation between Periodontopathogenic Bacterial Levels and Resistin in the Saliva of Obese Type 2 Diabetic Patients. *J Diabetes Res* 2017; **2017**: 2643079 [PMID: 29138754 DOI: 10.1155/2017/2643079]

82 **Srinivasan M**, Meadows ML, Maxwell L. Assessment of Salivary Adipokines Resistin, Visfatin, and Ghrelin as Type 2 Diabetes Mellitus Biomarkers. *Biochem Res Int* 2018; **2018**: 7463796 [PMID: 29487749 DOI: 10.1155/2018/7463796]

83 **Gürlek B**, Çolak S. Saliva resistin as a screening marker of gestational diabetes mellitus. *Gynecol Endocrinol* 2021; **37**: 324-327 [PMID: 32804001 DOI: 10.1080/09513590.2020.1807504]

84 **Barbour LA**. New concepts in insulin resistance of pregnancy and gestational diabetes: long-term implications for mother and offspring. *J Obstet Gynaecol* 2003; **23**: 545-549 [PMID: 12963518 DOI: 10.1080/0144361031000156500]

85 **Moyer VA**; U. S. Preventive Services Task Force. Screening for gestational diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2014; **160**: 414-420 [PMID: 24424622 DOI: 10.7326/M13-2905]

86 **Lappas M**, Yee K, Permezel M, Rice GE. Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. *J Endocrinol* 2005; **186**: 457-465 [PMID: 16135665 DOI: 10.1677/joe.1.06227]

87 **Hu SM**, Chen MS, Tan HZ. Maternal serum level of resistin is associated with risk for gestational diabetes mellitus: A meta-analysis. *World J Clin Cases* 2019; **7**: 585-599 [PMID: 30863758 DOI: 10.12998/wjcc.v7.i5.585]

88 **Chu SY**, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, Dietz PM. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care* 2007; **30**: 2070-2076 [PMID: 17416786 DOI: 10.2337/dc06-2559a]

89 **Sathyapalan T**, Mellor D, Atkin SL. Obesity and gestational diabetes. *Semin Fetal Neonatal Med* 2010; **15**: 89-93 [PMID: 19875346 DOI: 10.1016/j.siny.2009.09.002]

90 **Yao D**, Chang Q, Wu QJ, Gao SY, Zhao H, Liu YS, Jiang YT, Zhao YH. Relationship between Maternal Central Obesity and the Risk of Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis of Cohort Studies. *J Diabetes Res* 2020; **2020**: 6303820 [PMID: 32337296 DOI: 10.1155/2020/6303820]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1518-1529

DOI: 10.4239/wjd.v12.i9.1518

ISSN 1948-9358 (online)

MINIREVIEWS

Psychosocial factors affecting the etiology and management of type 1 diabetes mellitus: A narrative review

Anja Turin, Maja Drobnič Radobuljac

ORCID number: Anja Turin 0000-0001-9877-0158; Maja Drobnič Radobuljac 0000-0002-8632-9725.

Author contributions: Turin A and Radobuljac MD both made substantial contributions to conception and design of the article; Turin A drafted the article; Radobuljac MD made critical revisions related to important intellectual content of the manuscript; Turin A and Radobuljac MD approved the final version of the article to be published.

Supported by Slovenian Research Agency Grant, No. P3-0343.

Conflict-of-interest statement: The authors declare that the manuscript was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Anja Turin, Department for Child Psychiatry, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana 1000, Slovenia

Anja Turin, Maja Drobnič Radobuljac, Department of Psychiatry, Faculty of Medicine, University of Ljubljana, Ljubljana 1000, Slovenia

Maja Drobnič Radobuljac, Unit for Intensive Child and Adolescent Psychiatry, University Psychiatric Clinic Ljubljana, Ljubljana 1000, Slovenia

Corresponding author: Maja Drobnič Radobuljac, MD, PhD, Associate Professor, Chief Physician, Unit for Intensive Child and Adolescent Psychiatry, University Psychiatric Clinic Ljubljana, Grabloviceva 44a, Ljubljana 1000, Slovenia. maja.radobuljac@psih-klinika.si

Abstract

Type 1 diabetes (T1D) is one of the most common chronic diseases in children and adolescents worldwide. Its etiopathogenesis results from the interplay of genetic and environmental variables. Among the latter, psychological stress has been implicated in disease onset as well as disease management. Various studies, including large population-based studies, have highlighted the role of stressful life events in the etiopathogenesis of T1D. In this article, we also emphasize the importance of attachment in the early child-caregiver relationship, which can be seen as a measure of the quality of the relationship and is crucial for stress and emotional regulation. It serves as a model for all subsequent relationships in one's life. We summarize some of the few studies performed in the field of attachment and T1D etiopathogenesis or management. T1D management demands a lifelong therapeutic regimen to prevent acute and chronic complications. In addition to psychological stress, psychological factors such as family functioning, developmental adjustment, autonomy, mental health problems and other factors have been found to relate to metabolic control. Psychological factors need to be understood not as a single directional causality-based principle but as a dynamic bi- or multidirectional system that is affected by the normal developmental transitions of childhood and adolescence.

Key Words: Type 1 diabetes; Psychosocial factors; Stressful life events; Etiology; Disease management; Attachment

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Slovenia

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: May 3, 2021
Revised: May 26, 2021
Accepted: July 26, 2021
Article in press: July 26, 2021
Published online: September 15, 2021

P-Reviewer: He X, Klobucar Majanovic S
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Liu JH

Core Tip: The incidence of type 1 diabetes is increasing worldwide. Its diagnosis and management present a major burden for the child as well as the family. Different psychological factors affecting the development and course of type 1 diabetes need to be understood not as a single directional causality-based principle but as a dynamic bi- or multidirectional system that is affected by the normal developmental transitions of childhood and adolescence. The current article summarizes some of these factors, especially those related to stress and its regulation, both in an attachment context and in relation to family dynamics and psychopathology.

Citation: Turin A, Drobnič Radobuljac M. Psychosocial factors affecting the etiology and management of type 1 diabetes mellitus: A narrative review. *World J Diabetes* 2021; 12(9): 1518-1529
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1518.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1518

INTRODUCTION

Type 1 diabetes (T1D) is the result of autoimmune-mediated destruction of insulin producing beta cells and is considered one of the most common pediatric illnesses with an increasing incidence of 2%-5% annually. It peaks in presentation between the ages of five to seven and puberty[1,2]. Its management demands a lifelong therapeutic regimen to prevent acute and chronic complications[3]. Psychosocial factors have been shown to play a role in both its etiopathogenesis and in disease management. However, recent studies have highlighted the importance of bidirectionality in the relationship between T1D and psychosocial well-being[4]. Stressful life events have been shown to be related to the onset and the course of T1D in various studies[5-9]. It is important to understand the effect of stress not only as an external factor influencing an individual, but to consider its effect in the light of a person's distinct and unique way of stress regulation[10]. Younger children depend highly on their parents for disease management and stress regulation[11,12]. On the other hand, older children and adolescents transition from solely depending on their parents to achieving more independence and autonomy in stress regulation and disease management[13].

We conducted an electronic literature search through PubMed. The search was performed in four sessions using the keywords: "type 1 diabetes" and "attachment" (first session), "type 1 diabetes" and "glycemic control" or "management" and "stress" or "serious life events" and "children" or "adolescent" and "mother" or "parent" (second session), "type 1 diabetes" and "family functioning" (third session) and "type 1 diabetes" and "mental health" or "psychiatric" or "psychopathology" (fourth session). From the first session only 7 articles from 251 were relevant for the researched topic, in the second session from 175 articles, 14 were relevant, in third session from 4771, 24 were relevant and in the fourth session 25 were relevant from 1909 articles. Only articles related to child or adolescent T1D etiopathogenesis or management were included. Those that did not contain an abstract or were not in English were excluded. Subsequently, articles from the authors' personal archives considered important were added.

INFLUENCES ON THE ETIOLOGY

Stressful life events

Several studies, including large population-based prospective studies, have highlighted the role of stressful life events in the manifestation of T1D. The All Babies in Southeast Sweden study included 10495 non-diabetic children, and data was collected at the ages of 2–3, 5–6, 8 and 10–13 years. Fifty-eight children were subsequently diagnosed with T1D. Family psychological stress was measured *via* questionnaires given to the parents assessing serious life events, parenting stress, parental worries and the parental social support. The results showed that serious life events experienced in childhood, such as death or illness in the family, family conflicts, violence and unemployment were associated with a higher risk of future diagnosis of

T1D independent of heredity[5]. In another study, based on the same registry, 4400 consecutive 1-year-old children were included. Parents completed questionnaires at birth and at 1 year, including various measures of psychosocial stress (*e.g.*, parenting stress) and sociodemographic background. Blood samples drawn from the children at 1 year were analyzed for T1D-associated autoantibodies toward tyrosine phosphatase and glutamic acid decarboxylase. The results showed that psychological stress, measured as psychosocial strain in the family, was involved in the induction or progression of diabetes-related autoimmunity[6]. Lundgren *et al*[14] reported a significantly increased risk of T1D in children with stress and severe life events (such as severe disease in the family, death of a close relative, serious accident, violence, divorce, unemployment, *etc.*) occurring during the child's first 2 years of life as reported by the parents (n = 3784).

Virk *et al*[15] conducted a population-based follow-up study on subjects from Danish national registers. They categorized 1740245 children as exposed to bereavement if they lost a mother, a father or a sibling from the age of 5 years onwards. The children were then followed until a first diagnosis of diabetes, death or emigration. According to their results, bereavement was associated with an increased rate of T1D when the exposure began after the age of 11 years. However, a Swedish nationwide study did not support the hypothesis that psychosocial stressful life events were involved in the development of autoimmune T1D in young adults. The study included 349 newly diagnosed patients aged 15-34 years and 979 control subjects. They used questionnaires asking about diabetes heredity, social environment, educational level and life events experienced during the 12 mo before diagnosis. No major stress factors were detected in the patients with T1D. Nevertheless, in contrast with the control subjects, the T1D group had experienced fewer conflicts with their parents and had less often broken contacts with friends[16].

Stress regulation and the role of attachment

To understand the role of stress we must consider how it is regulated and managed inside the family dynamics. Individual stressful life events and the effect of these events on an individual in terms of stress regulation and resilience are important. The impact of stressful life events on the individual is influenced by genetic vulnerability, coping mechanisms, personality type and social support[10]. A dysregulated individual response to psychological stress was proposed as one of the factors contributing in a complex way to increased insulin demands and pancreatic beta-cell overload[17-21], which in turn is believed to mediate their destruction and consequently the emergence of T1D[22].

A rather interesting approach to understanding the role of stress is *via* the theory of attachment, an early theory of psychological development, which has received a lot of attention in recent years. Attachment is described as a behavioral and physiological system, which enables an individual's dynamic adaptation to his or her environment. It develops in close interaction between an infant and his/her caretaker and influences the quality of interpersonal relationships throughout life. It is inextricably linked to the way one regulates stress, at the beginning with the help of the primary caretaker and later by oneself in accordance with the internal attachment representations. These are categorized as secure or insecure, the latter being related to a more intensive stress response compared to the former[23]. Few studies have been carried out to determine the role of attachment in the risk of T1D. Sepa *et al*[22] explored the connection between the mothers' attachment insecurity and diabetes-related autoimmunity in early childhood using Adult Attachment Interviews with a group of mothers with antibody-positive infants and a group of mothers with antibody-negative infants. Their results showed a larger proportion of insecure mothers in the antibody-positive group, although the association was not statistically significant. They concluded that if an association between mothers' attachment and diabetes-related autoimmunity in children exists, it is not very strong, acknowledging their small sample size as well as a generally imperfect correlation between mother and child attachment. Based on their findings, our group conducted a study that included 101 dyads of children with T1D and their caretakers and 106 healthy control pairs. Attachment between the children and their parents was evaluated *via* a questionnaire (the parents) and the Child Attachment Interview (the children). The results showed no correlation between the attachment of the children to their parents and T1D. However, a correlation between higher caretaker attachment anxiety and child's T1D diagnosis was revealed[24].

INFLUENCES ON THE DISEASE MANAGEMENT

Stressful life events

Various studies have highlighted the role of stress in T1D management and thus metabolic control. In a cross-sectional study, Commissariat *et al*[7] studied the association between stressful life events, T1D management and psychological measures in adolescents with T1D. One hundred and seventy-eight teens and their parents were included in the research. Results showed that teens with more stressful life events such as the hospitalization of a family member, getting a bad report card, witnessing serious arguments between parents or a serious illness or injury of a family member, reported lower self-efficacy, poorer adherence, poorer quality of life and higher glycated hemoglobin (HbA1c).

In a prospective study of 128 families, Stanek *et al*[8] investigated the occurrence of stressful life events within the first year of T1D diagnosis in children, assessing correlations with family functioning and parental psychosocial measures and diabetes management. More than half of the families reported one or more stressful life events, such as the child attending a different school, caregiver job change, a change in household income, a change in caregiver marital status and/or a significant change in the health of a family member. Baseline active avoidance coping, parental depression and diabetes-related family conflict correlated with a higher number of stressful life events. There were also cross-sectional associations between HbA1c, a decrease in household income, a school change and/or a job change at various time points in the study. Rechenberg *et al*[9] included 320 adolescents in their cross-sectional research examining the associations between general stress (the degree to which an individual considers his or her life to be stressful over the previous month), diabetes-specific stress (such as telling others about the diabetes diagnosis or others noticing the insulin pump, stress about "bad numbers", stress about parental involvement in diabetes care and stress about interference of diabetes in daily activities), glycemic control (HbA1c), self-management and diabetes-specific quality of life. Higher general and diabetes-specific stress were significantly associated with higher HbA1c, poorer self-management activities and lower diabetes-specific quality of life. Diabetes-specific stress accounted for a significant proportion of the variance in HbA1c, while general stress did not. On the other hand, in a study with 132 children with T1D, Helgeson *et al* [25] found that only general parental stress was associated with poorer child outcomes, whereas diabetes-specific parental stress was associated with better child outcomes. Both types of stress, however, were associated with poorer parental mental health.

Attachment and metabolic control

Rosenberg and Shields[26] conducted a pilot study on 31 families that explored the associations between parent and adolescent reports of adolescent attachment and glycemic control in adolescents with T1D. Adolescents and parents reported on their perceptions of the adolescents' attachment to their mothers and fathers. The mothers' perceptions of adolescents' attachment were significantly correlated with adolescents' HbA1c, indicating that maternal perceptions of more secure attachment were associated with better glycemic control. Neither fathers' perceptions nor adolescents' reports of attachment significantly correlated with glycemic control.

Ciechanowski *et al*[27] conducted their attachment-based research on an adult sample and presented evidence that dismissing attachment (a type of insecure attachment) was related to poor glycemic control. Costa-Cordella *et al*[28] assessed the role of attachment in T1D management in children. The results on 77 mother-child dyads showed a negative correlation of secure child attachment and HbA1c, which was seen only in boys but not in girls. The same author conducted a study in which mentalization (assessed by the Reflective Functioning Scale) was determined in two groups of mother-son dyads (with good *vs* poor diabetes control). Reflective functioning refers to an essential human capacity to understand other's behavior considering their underlying mental states and intentions. Better reflective functioning correlates with more secure attachment. Their results showed that both maternal and child reflective functioning were higher in the good diabetes control group compared to the poor diabetes control group and were negatively correlated with HbA1c in the total sample. Meaning the higher the reflective functioning, the lower the HbA1c[4].

Family functioning

The diagnosis of T1D is a source of stress for the family and can have a negative impact on family functioning[29-31]. Additionally, numerous studies have linked

various aspects of family functioning to metabolic control[32-42]. Factors such as positive parental emotional support, family communication and sufficient parental guidance with diabetes-related care have been linked to improved metabolic control, while a high level of family conflict as well as negative and unsupportive parental behavior are linked to poorer metabolic control and adherence[32,34,43-47].

In a study by Lewin *et al*[32] of 109 children, two dimensions of family functioning, warm/caring and guidance/control, were assessed. The results demonstrated that family functioning and adherence were strongly associated with metabolic control and accounted for as much as 34% of its variance. The research highlighted the role of adherence as a mediating factor between the family functioning and metabolic control.

A Danish population-based study included all families with a child diagnosed with T1D. They assessed seven dimensions of family functioning and their relationship to metabolic control: problem solving; communication; roles; affective responsiveness; affective involvement; behavior control; and overall family functioning. Their results showed that discrepancies in family functioning were associated with higher HbA1c levels[34].

However, other studies have found no association between aspects of family life and metabolic control[48,49]. For example, Kovacs *et al*[48] conducted a study of 85 children with T1D and assessed the relationship between two aspects of family functioning: parental perception of overall quality of family life and quality of the parent's marriage. Their results showed no relationship between family functioning and the child's glycemic control. Similarly, Gowers *et al*[49] found no association between various aspects of family functioning and metabolic control, among a sample of 60 children and adolescents with T1D. They found, however, a significant positive association of the parental involvement in administering injections.

Role of development

Patients with T1D need the support of their family and the diabetes team to keep up with their diabetes management. The roles of the young patients and their families change throughout the developmental span from childhood to adolescence. Over time, the responsibility for diabetes management transitions from the parents (caregivers) to a shared responsibility between youth and caregivers, with older teens ultimately taking on the majority of self-care responsibilities[13].

Berg *et al*[12] suggested a developmental model for understanding the interplay of the child's self-regulation skills, the parents' involvement and diabetes management as a dynamic bidirectional system changing throughout development. Each developmental period has its unique developmental challenges that affect diabetes management. Very young children, for example, are completely dependent on parental caretaking. Their capacity to self-regulate behavior, emotions, sleeping, eating and physical activity is labile, which may complicate TD1 care[11,12]. Parental characteristics, such as marital status, socioeconomic status and coping style also influence diabetes management, as does the individual child's character, such as temperament. For example, higher activity levels and shorter attention span were associated with poor child cooperation with daily T1D care in a study performed on 34 children with T1D[11,50].

Many studies have shown deterioration of metabolic control when children transition into adolescence, with a subsequent improvement during adulthood[25,51-54]. Therefore, adolescence itself is a stage that poses a specific risk to the management of diabetes. It is a period of complex physiological and psychological changes that can all influence diabetes management. Teens move from complete dependence on their caregivers to a more independent lifestyle. This occurs against a background of major changes in the hormonal mediators of puberty, the latter representing a biological factor contributing to deteriorated metabolic control due to insulin resistance[25]. As such, adolescents not only have to master the skills of diabetes self-management but also negotiate a new balance between autonomy and connectedness with their caregivers[12]. However, family support in terms of the caregiver's involvement in diabetes management continues to be important[55]. Different studies have shown that supportive, cohesive families with low levels of conflict were more likely to have adolescents with strong adherence and good metabolic control than families without such cohesion[36]. Despite being less involved in the management of T1D in adolescence, greater parental monitoring was related to better adherence and lower levels of HbA1c[43,56,57].

Mental health problems

A higher incidence of mental health problems such as depression, anxiety and eating disorders were reported in children or adolescents with T1D compared to their healthy

peers[58-65].

Butwicka *et al*[59] conducted a population-based case-cohort study on individuals born in Sweden between 1973 and 2009. Children with T1D (n = 17122) and their healthy siblings (n = 18847) were identified and followed until their 18th birthday. Their results showed that the risk of psychiatric morbidity in children with T1D compared with the general population was tripled within 6 mo of diabetes onset and doubled within the total observation period. They also reported an increased risk of attempted suicide[59].

Another population-based national register study, conducted on 5084 Danish children and adolescents with T1D and 35588 healthy controls, reported an increased risk of eating disorders, anxiety and mood disorders, substance misuse and personality disorders in the years following T1D onset. The highest risk was seen in subjects with diabetes onset between the ages of 10 and 14 years. The risk increased with the duration of T1D, with the highest risk occurring 5 or more years after the diagnosis[60].

Contrary to the research mentioned above, there are also studies that have shown a lower or equal prevalence of mental health problems in youth with T1D compared to healthy populations[66-69].

Silverstein *et al*[67] found no evidence of increased psychopathology across a wide range of mental health measures in youth with T1D in a large Norwegian population study that included 9883 adolescents aged 16-19 years, 40 of whom were diagnosed with T1D.

Another large population-based study on Brazilian adolescents aged 12-17 years compared 116 youth with T1D and 73508 healthy youth. The results showed that mental health symptoms but not disorders were more common in youth with T1D[68].

A case-control study on a Slovene cohort of 126 adolescents with T1D and 499 healthy controls reported a lower prevalence of suicidal thoughts, suicide attempts and self-injurious and other risk-taking behaviors in adolescents with T1D (especially males) as compared to the general population of adolescents and a higher prevalence of disturbed eating behavior in females with T1D[70,71]. Suicidal behavior in adolescents with T1D, however, was related to poorer metabolic control in the same cohort[71].

According to the research, mental health problems are related to poor glycemic control[61,64,71-75]. However, the results of studies in this area are mixed, as some studies have found only a weak association, or no association at all, between mental health problems and glycemic control[76-79].

In a Danish population-based study of 4725 children and adolescents with T1D, 1035 had at least one psychiatric disorder. A high average HbA1c level during the first 2 years predicted a higher risk of psychiatric diagnosis. Patients with psychiatric comorbidities had higher HbA1c levels and an increased risk of hospitalization with diabetic ketoacidosis[72].

On the other hand, T1D is also related to an increased risk of neurodevelopmental disorders such as attention-deficit/hyperactivity disorder and autism spectrum disorders. The risk, according to a recent Swedish population-based cohort study, increases with HbA1c levels[80]. Previous studies have shown a relationship between attention-deficit/hyperactivity disorder and poor metabolic control suggesting that patients with T1D should be assessed for attention-deficit/hyperactivity disorder symptoms[81,82].

Having T1D necessarily means having to conform to a strict behavioral and dietary regime, which is a source of stress for the children and their parents, especially the mothers, who are usually responsible for most of the diabetes management[83]. Post-traumatic stress symptoms have been commonly described in children and mothers as well as clinically significant levels of symptoms of anxiety and depression[30,84-86]. While post-traumatic stress symptoms were most severe at disease onset, they often persisted until 1 to 5 years after the diagnosis of T1D[83,84]. Together with diabetes-specific stress, the post-traumatic stress symptoms following the diagnosis of T1D adversely affect children's health[83]. Landolt *et al*[87] evaluated the rates of post-traumatic stress disorder and symptoms in mothers and fathers of children with newly diagnosed T1D. Parents of 38 children with newly diagnosed T1D were included in the assessment. Twenty-four percent of the mothers and 22% of the fathers met full diagnostic criteria for current post-traumatic stress disorder. In addition, 51% of the mothers and 41% of the fathers met criteria for partial or subclinical post-traumatic stress disorder.

A study by Rumburg *et al*[86] included 81 mothers of youth aged 10-16 with T1D duration of at least 1 year. They measured diabetes distress and maternal depressive symptoms. The results showed that mothers' overall diabetes distress was strongly

related to maternal depressive symptoms, and relationship-related diabetes distress was significantly associated with adolescents' HbA1c. Fear of hypoglycemia, one of the most disturbing acute complications, is an important source of parental stress, which is in turn related to worse metabolic control. Nevertheless, some studies propose that the fear of hypoglycemia is to a certain extent adaptive because it leads to more frequent diabetes monitoring[88,89].

DYNAMIC BIDIRECTIONAL SYSTEM BETWEEN T1D AND PSYCHOSOCIAL VARIABLES

So far, we have presented the role of psychosocial factors such as stress, security of attachment to parents, family dynamics and mental disorder in the etiology of T1D and metabolic control. The recent literature, however, emphasizes the bidirectionality between psychosocial variables and T1D: T1D affects psychosocial functioning in a negative way, which in turn negatively impacts the course of T1D[4]. An attempt of a diagrammatic presentation of these influences is shown in Figure 1.

According to the transactional stress and coping model, a chronic childhood illness is seen as a stressor to which children and families attempt to adapt. The ways in which mothers cope with the stress has an important impact on both maternal and adolescent adjustment to the disease[90]. Many studies have shown that T1D poses diabetes-specific risk to the mental health of mothers who usually bear higher responsibility for their child's diabetes care. The stress often results in clinical symptoms of depression and anxiety, which are related to poor glycemic control of the child's T1D [86,91].

Jaser *et al*[90] measured diabetes-related stress and coping in 118 mothers of adolescents aged 10-16 years with T1D. The mothers were asked how often they experienced diabetes-related stress (*e.g.*, taking care of diabetes, frequently reminding an adolescent to take care of him/herself), and they then completed a questionnaire asking how they responded to these stressors. Three ways of coping were subsequently identified: Primary control engagement coping (problem solving, emotional modulation, emotional expression); secondary control engagement coping (positive thinking, cognitive restructuring, acceptance, distraction); and disengagement coping (avoidance, denial, wishful thinking). Further, they evaluated depressive and anxiety symptoms in the mothers, diabetes-related family conflict, depressive symptoms and the quality of life of the adolescents. Their results showed that diabetes-related stress in the mothers was significantly associated with their symptoms of depression, anxiety, family conflict and secondary control coping. Furthermore, the mothers' use of primary or secondary control coping strategies was related to fewer symptoms of depression, anxiety and less family conflict. On the other hand, disengagement coping was related to higher symptoms of depression and anxiety. Maternal coping was not related to any adolescent outcomes. However, maternal depressive symptoms were associated with poorer adolescent quality of life. In addition, family conflict was related to higher depressive symptoms in adolescents and, notably, to their glycemic control. Their results support the idea that ways of coping or attempts to adapt to the stressor may mediate the relationship between diabetes-related stress and maternal symptoms of depression and anxiety, which in turn may influence the family functioning, adolescent quality of life and finally the T1D outcomes.

This bidirectional system must also be understood as a dynamic system that moves through different developmental stages. Young children depend mostly on their parents in diabetes care[11,12], while adolescents gradually take over the management control[13]. According to this, diabetes-specific family stress was shown to be negatively associated to the age of the affected children[92].

CONCLUSION

The present narrative review summarizes some of the psychosocial factors that influence the etiology and management of T1D in children and adolescents, with an emphasis on stressful life events and stress regulation inside the family dynamic and highlighting the role of the attachment. Stressful life events have been associated with T1D etiology and T1D management in many studies, as have family dynamics and the child and parent psychiatric morbidity. Many clinical guidelines thus emphasize the importance of family support as well as performing an assessment of psychosocial

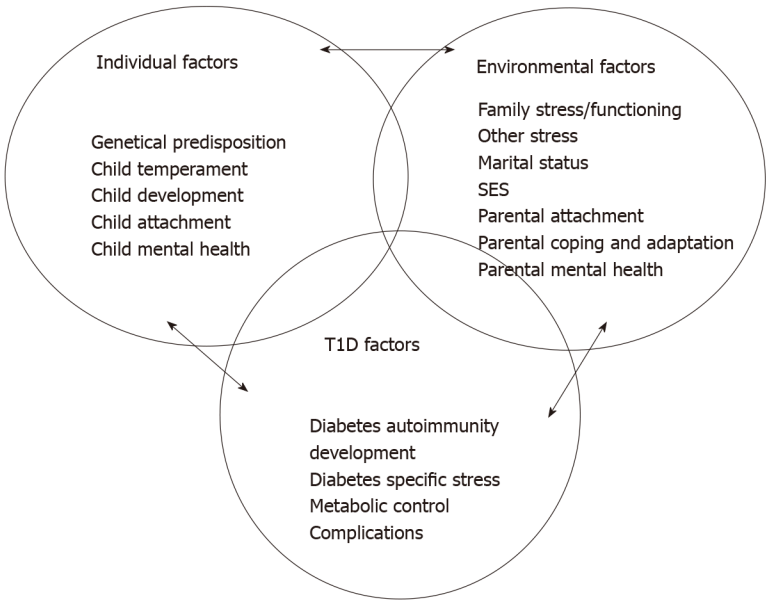

Figure 1 Dynamic multidirectional system of psychosocial factors in type 1 diabetes. SES: Socioeconomic status; T1D: Type 1 diabetes.

functioning and screening for psychiatric disorders. There is also a vast literature considering the impact of development and normal transitions throughout a child's life on the management of T1D. Less research has been performed in the field of attachment and understanding the significance of the primary relationships between the children and their caregivers for the regulation of stress and successful management of T1D. To better understand the complex multidirectional interplay of psychosocial factors between the individual and the family in the induction, progression and management of T1D, more longitudinal and interdisciplinary studies combining the fields of (neuro)endocrinology, stress regulation and developmental psychology are needed.

ACKNOWLEDGEMENTS

The authors wish to thank all the children, adolescents and their parents as well as the staff from the Department of Endocrinology, Diabetes and Metabolism, University Medical Centre Ljubljana for their kind cooperation and dedicated work on the diabetes research.

REFERENCES

1 **Atkinson MA**, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014; **383**: 69-82 [PMID: 23890997 DOI: 10.1016/S0140-6736(13)60591-7]

2 **Patterson CC**, Harjutsalo V, Rosenbauer J, Neu A, Cinek O, Skrivarhaug T, Rami-Merhar B, Soltesz G, Svensson J, Parslow RC, Castell C, Schoenle EJ, Bingley PJ, Dahlquist G, Jarosz-Chobot PK, Marčiulionytė D, Roche EF, Rothe U, Bratina N, Ionescu-Tirgoviste C, Weets I, Kocova M, Cherubini V, Rojnic Putarek N, deBeaufort CE, Samardzic M, Green A. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989-2013: a multicentre prospective registration study. *Diabetologia* 2019; **62**: 408-417 [PMID: 30483858 DOI: 10.1007/s00125-018-4763-3]

3 **American Diabetes Association**. 13. Children and Adolescents: *Standards of Medical Care in Diabetes-2021*. *Diabetes Care* 2021; **44**: S180-S199 [PMID: 33298424 DOI: 10.2337/dc21-S013]

4 **Costa-Cordella S**, Luyten P, Cohen D, Mena F, Fonagy P. Mentalizing in mothers and children with type 1 diabetes. *Dev Psychopathol* 2021; **33**: 216-225 [PMID: 31931891 DOI: 10.1017/S0954579419001706]

5 **Nygren M**, Carstensen J, Koch F, Ludvigsson J, Frostell A. Experience of a serious life event increases the risk for childhood type 1 diabetes: the ABIS population-based prospective cohort study. *Diabetologia* 2015; **58**: 1188-1197 [PMID: 25870022 DOI: 10.1007/s00125-015-3555-2]

6 **Sepa A**, Wahlberg J, Vaarala O, Frodi A, Ludvigsson J. Psychological stress may induce diabetes-related autoimmunity in infancy. *Diabetes Care* 2005; **28**: 290-295 [PMID: 15677781 DOI:

10.2337/diacare.28.2.290]
7 **Commissariat PV**, Volkening LK, Guo Z, ElBach JL, Butler DA, Laffel LM. Associations between major life events and adherence, glycemic control, and psychosocial characteristics in teens with type 1 diabetes. *Pediatr Diabetes* 2018; **19**: 85-91 [PMID: 28425183 DOI: 10.1111/pedi.12523]
8 **Stanek KR**, Noser AE, Patton SR, Clements MA, Youngkin EM, Majidi S. Stressful life events, parental psychosocial factors, and glycemic management in school-aged children during the 1 year follow-up of new-onset type 1 diabetes. *Pediatr Diabetes* 2020; **21**: 673-680 [PMID: 32227565 DOI: 10.1111/pedi.13012]
9 **Rechenberg K**, Whittemore R, Holland M, Grey M. General and diabetes-specific stress in adolescents with type 1 diabetes. *Diabetes Res Clin Pract* 2017; **130**: 1-8 [PMID: 28551480 DOI: 10.1016/j.diabres.2017.05.003]
10 **Sharif K**, Watad A, Coplan L, Amital H, Shoenfeld Y, Afek A. Psychological stress and type 1 diabetes mellitus: what is the link? *Expert Rev Clin Immunol* 2018; **14**: 1081-1088 [PMID: 30336709 DOI: 10.1080/1744666X.2018.1538787]
11 **Pierce JS**, Kozikowski C, Lee JM, Wysocki T. Type 1 diabetes in very young children: a model of parent and child influences on management and outcomes. *Pediatr Diabetes* 2017; **18**: 17-25 [PMID: 26712357 DOI: 10.1111/pedi.12351]
12 **Berg CA**, Butner J, Wiebe DJ, Hughes Lansing A, Osborn P, King PS, Palmer DL, Butler JM. Developmental model of parent-child coordination for self-regulation across childhood and into emerging adulthood: Type 1 diabetes management as an example. *Dev Rev* 2017; **46**: 1-26 [DOI: 10.1016/j.dr.2017.09.001]
13 **Markowitz JT**, Garvey KC, Laffel LM. Developmental changes in the roles of patients and families in type 1 diabetes management. *Curr Diabetes Rev* 2015; **11**: 231-238 [PMID: 25901503 DOI: 10.2174/1573399811666150421114146]
14 **Lundgren M**, Ellström K, Elding Larsson H; DiPiS study group. Influence of early-life parental severe life events on the risk of type 1 diabetes in children: the DiPiS study. *Acta Diabetol* 2018; **55**: 797-804 [PMID: 29752553 DOI: 10.1007/s00592-018-1150-y]
15 **Virk J**, Ritz B, Li J, Obel C, Olsen J. Childhood Bereavement and Type 1 Diabetes: a Danish National Register Study. *Paediatr Perinat Epidemiol* 2016; **30**: 86-92 [PMID: 26444317 DOI: 10.1111/ppe.12247]
16 **Littorin B**, Sundkvist G, Nyström L, Carlson A, Landin-Olsson M, Ostman J, Arnqvist HJ, Björk E, Blohmé G, Bolinder J, Eriksson JW, Scherstén B, Wibell L; Diabetes Incidence Study in Sweden (DISS). Family characteristics and life events before the onset of autoimmune type 1 diabetes in young adults: a nationwide study. *Diabetes Care* 2001; **24**: 1033-1037 [PMID: 11375366 DOI: 10.2337/diacare.24.6.1033]
17 **de Kloet ER**, de Kloet SF, de Kloet CS, de Kloet AD. Top-down and bottom-up control of stress-coping. *J Neuroendocrinol* 2019; **31**: e12675 [PMID: 30578574 DOI: 10.1111/jne.12675]
18 **Godoy LD**, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, de Lima Umeoka EH. A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. *Front Behav Neurosci* 2018; **12**: 127 [PMID: 30034327 DOI: 10.3389/fnbeh.2018.00127]
19 **Dahlquist G**. Can we slow the rising incidence of childhood-onset autoimmune diabetes? *Diabetologia* 2006; **49**: 20-24 [PMID: 16362279 DOI: 10.1007/s00125-005-0076-4]
20 **Ludvigsson J**. Why diabetes incidence increases--a unifying theory. *Ann N Y Acad Sci* 2006; **1079**: 374-382 [PMID: 17130582 DOI: 10.1196/annals.1375.058]
21 **Drobnič Radobuljac M**, Shmueli-Goetz Y. Attachment to Caregivers and Type 1 Diabetes in Children. *Zdr Varst* 2015; **54**: 126-130 [PMID: 27646919 DOI: 10.1515/sjph-2015-0019]
22 **Sepa A**, Frodi A, Ludvigsson J. Mothers' attachment insecurity and diabetes-related autoantibodies in their infants. *Ann N Y Acad Sci* 2004; **1037**: 110-113 [PMID: 15699501 DOI: 10.1196/annals.1337.016]
23 **Schore AN**. Attachment and the regulation of the right brain. *Attach Hum Dev* 2000; **2**: 23-47 [PMID: 11707891 DOI: 10.1080/146167300361309]
24 **Turin A**, Dovč K, Klemenčič S, Bratina N, Battelino T, Lipovšek JK, Uršič K, Shmueli-Goetz Y, Drobnič-Radobuljac M. Carer's Attachment Anxiety, Stressful Life-Events and the Risk of Childhood-Onset Type 1 Diabetes. *Front Psychiatry* 2021; **12**: 657982 [PMID: 34140900 DOI: 10.3389/fpsyt.2021.657982]
25 **Helgeson VS**, Siminerio L, Escobar O, Becker D. Predictors of metabolic control among adolescents with diabetes: a 4-year longitudinal study. *J Pediatr Psychol* 2009; **34**: 254-270 [PMID: 18667479 DOI: 10.1093/jpepsy/jsn079]
26 **Rosenberg T**, Shields CG. The role of parent-adolescent attachment in the glycemic control of adolescents with Type 1 diabetes: a pilot study. *Fam Syst Health* 2009; **27**: 237-248 [PMID: 19803618 DOI: 10.1037/a0017117]
27 **Ciechanowski PS**, Hirsch IB, Katon WJ. Interpersonal predictors of HbA(1c) in patients with type 1 diabetes. *Diabetes Care* 2002; **25**: 731-736 [PMID: 11919133 DOI: 10.2337/diacare.25.4.731]
28 **Costa-Cordella S**, Luyten P, Giraudo F, Mena F, Shmueli-Goetz Y, Fonagy P. Attachment and stress in children with type 1 Diabetes and their mothers. *Rev Chil Pediatr* 2020; **91**: 68-75 [PMID: 32730415 DOI: 10.32641/rchped.v91i1.1197]
29 **Grey M**. Coping and Psychosocial Adjustment in Mothers of Young Children with Type 1 Diabetes. *Child Health Care* 2009; **38**: 91-106 [PMID: 19412355 DOI: 10.1080/02739610902813229]
30 **Şişmanlar ŞG**, Demirbaş-Çakir E, Karakaya I, Çizmecioğlu F, Yavuz CI, Hatun Ş, Ağaoğlu B.

Posttraumatic stress symptoms in children diagnosed with type 1 diabetes. *Ital J Pediatr* 2012; **38**: 13 [PMID: 22537813 DOI: 10.1186/1824-7288-38-13]

31 **Piazza-Waggoner C**, Modi AC, Powers SW, Williams LB, Dolan LM, Patton SR. Observational assessment of family functioning in families with children who have type 1 diabetes mellitus. *J Dev Behav Pediatr* 2008; **29**: 101-105 [PMID: 18285720 DOI: 10.1097/DBP.0b013e31815f24ce]

32 **Lewin AB**, Heidgerken AD, Geffken GR, Williams LB, Storch EA, Gelfand KM, Silverstein JH. The relation between family factors and metabolic control: the role of diabetes adherence. *J Pediatr Psychol* 2006; **31**: 174-183 [PMID: 16467317 DOI: 10.1093/jpepsy/jsj004]

33 **Leonard BJ**, Jang YP, Savik K, Plumbo MA. Adolescents with type 1 diabetes: family functioning and metabolic control. *J Fam Nurs* 2005; **11**: 102-121 [PMID: 16287821 DOI: 10.1177/1074840705275152]

34 **Pedersen MAM**, Kristensen LJ, Sildorf SM, Kreiner S, Svensson J, Mose AH, Thastum M, Birkebaek N. Assessment of family functioning in families with a child diagnosed with type 1 diabetes: Validation and clinical relevance of the general functioning subscale of the McMaster family assessment device. *Pediatr Diabetes* 2019; **20**: 785-793 [PMID: 31099112 DOI: 10.1111/pedi.12866]

35 **Tsiouli E**, Alexopoulos EC, Stefanaki C, Darviri C, Chrousos GP. Effects of diabetes-related family stress on glycemic control in young patients with type 1 diabetes: Systematic review. *Can Fam Physician* 2013; **59**: 143-149 [PMID: 23418238]

36 **Luo D**, Xu JJ, Cai X, Zhu M, Wang H, Yan D, Li MZ. The effects of family functioning and resilience on self-management and glycaemic control among youth with type 1 diabetes. *J Clin Nurs* 2019; **28**: 4478-4487 [PMID: 31410916 DOI: 10.1111/jocn.15033]

37 **Cameron FJ**, Skinner TC, de Beaufort CE, Hoey H, Swift PG, Aanstoot H, Aman J, Martul P, Chiarelli F, Daneman D, Danne T, Dorchy H, Kaprio EA, Kaufman F, Kocova M, Mortensen HB, Njølstad PR, Phillip M, Robertson KJ, Schoenle EJ, Urakami T, Vanelli M, Ackermann RW, Skovlund SE; Hvidoere Study Group on Childhood Diabetes. Are family factors universally related to metabolic outcomes in adolescents with Type 1 diabetes? *Diabet Med* 2008; **25**: 463-468 [PMID: 18294223 DOI: 10.1111/j.1464-5491.2008.02399.x]

38 **Burroughs TE**, Harris MA, Pontious SL, Santiago JV. Research on social support in adolescents with IDDM: a critical review. *Diabetes Educ* 1997; **23**: 438-448 [PMID: 9305010 DOI: 10.1177/014572179702300409]

39 **Jacobson AM**, Hauser ST, Lavori P, Willett JB, Cole CF, Wolfsdorf JI, Dumont RH, Wertlieb D. Family environment and glycemic control: a four-year prospective study of children and adolescents with insulin-dependent diabetes mellitus. *Psychosom Med* 1994; **56**: 401-409 [PMID: 7809339 DOI: 10.1097/00006842-199409000-00004]

40 **Marteau TM**, Bloch S, Baum JD. Family life and diabetic control. *J Child Psychol Psychiatry* 1987; **28**: 823-833 [PMID: 3436991 DOI: 10.1111/j.1469-7610.1987.tb00671.x]

41 **Anderson BJ**, Miller JP, Auslander WF, Santiago JV. Family characteristics of diabetic adolescents: relationship to metabolic control. *Diabetes Care* 1981; **4**: 586-594 [PMID: 7347668 DOI: 10.2337/diacare.4.6.586]

42 **Lawler MK**, Volk R, Viviani N, Mengel MB. Individual and family factors impacting diabetic control in the adolescent: a preliminary study. *Matern Child Nurs J* 1990; **19**: 331-345 [PMID: 2136583]

43 **Hilliard ME**, Wu YP, Rausch J, Dolan LM, Hood KK. Predictors of deteriorations in diabetes management and control in adolescents with type 1 diabetes. *J Adolesc Health* 2013; **52**: 28-34 [PMID: 23260831 DOI: 10.1016/j.jadohealth.2012.05.009]

44 **Vaid E**, Lansing AH, Stanger C. Problems With Self-Regulation, Family Conflict, and Glycemic Control in Adolescents Experiencing Challenges With Managing Type 1 Diabetes. *J Pediatr Psychol* 2018; **43**: 525-533 [PMID: 29077875 DOI: 10.1093/jpepsy/jsx134]

45 **Rybak TM**, Ali JS, Berlin KS, Klages KL, Banks GG, Kamody RC, Ferry RJ, Alemzadeh R, Diaz-Thomas AM; Guest Editors: Cynthia A. Gerhardt, Cynthia A. Berg, Deborah J. Wiebe and Grayson N. Holmbeck. Patterns of Family Functioning and Diabetes-Specific Conflict in Relation to Glycemic Control and Health-Related Quality of Life Among Youth With Type 1 Diabetes. *J Pediatr Psychol* 2017; **42**: 40-51 [PMID: 28173113 DOI: 10.1093/jpepsy/jsw071]

46 **Anderson BJ**, Vangsness L, Connell A, Butler D, Goebel-Fabbri A, Laffel LM. Family conflict, adherence, and glycaemic control in youth with short duration Type 1 diabetes. *Diabet Med* 2002; **19**: 635-642 [PMID: 12147143 DOI: 10.1046/j.1464-5491.2002.00752.x]

47 **Drotar D**, Ittenbach R, Rohan JM, Gupta R, Pendley JS, Delamater A. Diabetes management and glycemic control in youth with type 1 diabetes: test of a predictive model. *J Behav Med* 2013; **36**: 234-245 [PMID: 22569775 DOI: 10.1007/s10865-012-9426-0]

48 **Kovacs M**, Kass RE, Schnell TM, Goldston D, Marsh J. Family functioning and metabolic control of school-aged children with IDDM. *Diabetes Care* 1989; **12**: 409-414 [PMID: 2731460 DOI: 10.2337/diacare.12.6.409]

49 **Gowers SG**, Jones JC, Kiana S, North CD, Price DA. Family functioning: a correlate of diabetic control? *J Child Psychol Psychiatry* 1995; **36**: 993-1001 [PMID: 7593406 DOI: 10.1111/j.1469-7610.1995.tb01345.x]

50 **Garrison WT**, Biggs D, Williams K. Temperament characteristics and clinical outcomes in young children with diabetes mellitus. *J Child Psychol Psychiatry* 1990; **31**: 1079-1088 [PMID: 2289945 DOI: 10.1111/j.1469-7610.1990.tb00847.x]

51 **Borus JS**, Laffel L. Adherence challenges in the management of type 1 diabetes in adolescents:

prevention and intervention. *Curr Opin Pediatr* 2010; **22**: 405-411 [PMID: 20489639 DOI: 10.1097/MOP.0b013e32833a46a7]

52 **Daneman D**, Hamilton J. Is poor metabolic control inevitable in adolescents with type 1 diabetes? *An Pediatr* 2001; **54**: 40-44

53 **Demirel F**, Tepe D, Esen I, Buber N, Boztepe H. Individual and familial factors associated with metabolic control in children with type 1 diabetes. *Pediatr Int* 2013; **55**: 710-713 [PMID: 23919500 DOI: 10.1111/ped.12183]

54 **Urbach SL**, LaFranchi S, Lambert L, Lapidus JA, Daneman D, Becker TM. Predictors of glucose control in children and adolescents with type 1 diabetes mellitus. *Pediatr Diabetes* 2005; **6**: 69-74 [PMID: 15963032 DOI: 10.1111/j.1399-543X.2005.00104.x]

55 **La Greca AM**. It's "all in the family": responsibility for diabetes care. *J Pediatr Endocrinol Metab* 1998; **11** Suppl 2: 379-385 [PMID: 9642668]

56 **Berg CA**, King PS, Butler JM, Pham P, Palmer D, Wiebe DJ. Parental involvement and adolescents' diabetes management: the mediating role of self-efficacy and externalizing and internalizing behaviors. *J Pediatr Psychol* 2011; **36**: 329-339 [PMID: 20926405 DOI: 10.1093/jpepsy/jsq088]

57 **Horton D**, Berg CA, Butner J, Wiebe DJ. The role of parental monitoring in metabolic control: effect on adherence and externalizing behaviors during adolescence. *J Pediatr Psychol* 2009; **34**: 1008-1018 [PMID: 19420225 DOI: 10.1093/jpepsy/jsp022]

58 **Buchberger B**, Huppertz H, Krabbe L, Lux B, Mattivi JT, Siafarikas A. Symptoms of depression and anxiety in youth with type 1 diabetes: A systematic review and meta-analysis. *Psychoneuroendocrinology* 2016; **70**: 70-84 [PMID: 27179232 DOI: 10.1016/j.psyneuen.2016.04.019]

59 **Butwicka A**, Frisén L, Almqvist C, Zethelius B, Lichtenstein P. Risks of psychiatric disorders and suicide attempts in children and adolescents with type 1 diabetes: a population-based cohort study. *Diabetes Care* 2015; **38**: 453-459 [PMID: 25650362 DOI: 10.2337/dc14-0262]

60 **Dybdal D**, Tolstrup JS, Sildorf SM, Boisen KA, Svensson J, Skovgaard AM, Teilmann GK. Increasing risk of psychiatric morbidity after childhood onset type 1 diabetes: a population-based cohort study. *Diabetologia* 2018; **61**: 831-838 [PMID: 29242985 DOI: 10.1007/s00125-017-4517-7]

61 **Jones JM**, Lawson ML, Daneman D, Olmsted MP, Rodin G. Eating disorders in adolescent females with and without type 1 diabetes: cross sectional study. *BMJ* 2000; **320**: 1563-1566 [PMID: 10845962 DOI: 10.1136/bmj.320.7249.1563]

62 **Blanz BJ**, Rensch-Riemann BS, Fritz-Sigmund DI, Schmidt MH. IDDM is a risk factor for adolescent psychiatric disorders. *Diabetes Care* 1993; **16**: 1579-1587 [PMID: 8299453 DOI: 10.2337/diacare.16.12.1579]

63 **Kovacs M**, Goldston D, Obrosky DS, Bonar LK. Psychiatric disorders in youths with IDDM: rates and risk factors. *Diabetes Care* 1997; **20**: 36-44 [PMID: 9028691 DOI: 10.2337/diacare.20.1.36]

64 **Hannonen R**, Eklund K, Tolvanen A, Komulainen J, Riikonen R, Delamater AM, Ahonen T. Psychological distress of children with early-onset type 1 diabetes and their mothers' well-being. *Acta Paediatr* 2015; **104**: 1144-1149 [PMID: 26234344 DOI: 10.1111/apa.13144]

65 **Sendela J**, Zduńczyk B, Trippenbach-Dulska H, Szypowska A. Prevalence of depressive symptoms in school aged children with type 1 diabetes - a questionnaire study. *Psychiatr Pol* 2015; **49**: 1005-1016 [PMID: 26688850 DOI: 10.12740/PP/35649]

66 **Peveler RC**, Fairburn CG, Boller I, Dunger D. Eating disorders in adolescents with IDDM. A controlled study. *Diabetes Care* 1992; **15**: 1356-1360 [PMID: 1425101 DOI: 10.2337/diacare.15.10.1356]

67 **Sivertsen B**, Petrie KJ, Wilhelmsen-Langeland A, Hysing M. Mental health in adolescents with Type 1 diabetes: results from a large population-based study. *BMC Endocr Disord* 2014; **14**: 83 [PMID: 25303963 DOI: 10.1186/1472-6823-14-83]

68 **Telo GH**, Cureau FV, Lopes CS, Schaan BD. Common mental disorders in adolescents with and without type 1 diabetes: Reported occurrence from a countrywide survey. *Diabetes Res Clin Pract* 2018; **135**: 192-198 [PMID: 29155124 DOI: 10.1016/j.diabres.2017.10.027]

69 **Striegel-Moore RH**, Nicholson TJ, Tamborlane WV. Prevalence of eating disorder symptoms in preadolescent and adolescent girls with IDDM. *Diabetes Care* 1992; **15**: 1361-1368 [PMID: 1425102 DOI: 10.2337/diacare.15.10.1361]

70 **Radobuljac MD**, Bratina NU, Battelino T, Tomori M. Lifetime prevalence of suicidal and self-injurious behaviors in a representative cohort of Slovenian adolescents with type 1 diabetes. *Pediatr Diabetes* 2009; **10**: 424-431 [PMID: 19490494 DOI: 10.1111/j.1399-5448.2009.00501.x]

71 **Radobuljac MD**, Tomori M, Battelino T, Bratina N. Adolescent Risk Behavior is Less Frequent in Patients with Type 1 Diabetes. *J Diabetes Metab* 2013; **S12**: 007 [DOI: 10.4172/2155-6156.S12-007]

72 **Sildorf SM**, Breinegaard N, Lindkvist EB, Tolstrup JS, Boisen KA, Teilmann GK, Skovgaard AM, Svensson J. Poor Metabolic Control in Children and Adolescents With Type 1 Diabetes and Psychiatric Comorbidity. *Diabetes Care* 2018; **41**: 2289-2296 [PMID: 30270201 DOI: 10.2337/dc18-0609]

73 **Young V**, Eiser C, Johnson B, Brierley S, Epton T, Elliott J, Heller S. Eating problems in adolescents with Type 1 diabetes: a systematic review with meta-analysis. *Diabet Med* 2013; **30**: 189-198 [PMID: 22913589 DOI: 10.1111/j.1464-5491.2012.03771.x]

74 **Northam EA**, Matthews LK, Anderson PJ, Cameron FJ, Werther GA. Psychiatric morbidity and health outcome in Type 1 diabetes--perspectives from a prospective longitudinal study. *Diabet Med* 2005; **22**: 152-157 [PMID: 15660731 DOI: 10.1111/j.1464-5491.2004.01370.x]

75 **Bernstein CM**, Stockwell MS, Gallagher MP, Rosenthal SL, Soren K. Mental health issues in adolescents and young adults with type 1 diabetes: prevalence and impact on glycemic control. *Clin Pediatr (Phila)* 2013; **52**: 10-15 [PMID: 22988007 DOI: 10.1177/0009922812459950]

76 **Kovacs M**, Mukerji P, Iyengar S, Drash A. Psychiatric disorder and metabolic control among youths with IDDM. A longitudinal study. *Diabetes Care* 1996; **19**: 318-323 [PMID: 8729153 DOI: 10.2337/diacare.19.4.318]

77 **Eaton WW**, Mengel M, Mengel L, Larson D, Campbell R, Montague RB. Psychosocial and psychopathologic influences on management and control of insulin-dependent diabetes. *Int J Psychiatry Med* 1992; **22**: 105-117 [PMID: 1517018 DOI: 10.2190/DF4H-9HQW-NJEC-Q54E]

78 **Colton PA**, Olmsted MP, Daneman D, Rodin GM. Depression, disturbed eating behavior, and metabolic control in teenage girls with type 1 diabetes. *Pediatr Diabetes* 2013; **14**: 372-376 [PMID: 23418901 DOI: 10.1111/pedi.12016]

79 **Engström I**, Kroon M, Arvidsson CG, Segnestam K, Snellman K, Aman J. Eating disorders in adolescent girls with insulin-dependent diabetes mellitus: a population-based case-control study. *Acta Paediatr* 1999; **88**: 175-180 [PMID: 10102151 DOI: 10.1080/08035259950170358]

80 **Liu S**, Kuja-Halkola R, Larsson H, Lichtenstein P, Ludvigsson JF, Svensson AM, Gudbjörnsdottir S, Tideman M, Serlachius E, Butwicka A. Poor glycaemic control is associated with increased risk of neurodevelopmental disorders in childhood-onset type 1 diabetes: a population-based cohort study. *Diabetologia* 2021; **64**: 767-777 [PMID: 33454829 DOI: 10.1007/s00125-020-05372-5]

81 **Nylander C**, Lindström K, Khalifa N, Fernell E. Previously undiagnosed attention-deficit/hyperactivity disorder associated with poor metabolic control in adolescents with type 1 diabetes. *Pediatr Diabetes* 2018; **19**: 816-822 [PMID: 29575401 DOI: 10.1111/pedi.12651]

82 **Macek J**, Battelino T, Bizjak M, Zupanc C, Bograf AK, Vesnic S, Klemencic S, Volk E, Bratina N. Impact of attention deficit hyperactivity disorder on metabolic control in adolescents with type1 diabetes. *J Psychosom Res* 2019; **126**: 109816 [PMID: 31493719 DOI: 10.1016/j.jpsychores.2019.109816]

83 **Rechenberg K**, Grey M, Sadler L. Stress and Posttraumatic Stress in Mothers of Children With Type 1 Diabetes. *J Fam Nurs* 2017; **23**: 201-225 [PMID: 28795899 DOI: 10.1177/1074840716687543]

84 **Schiaffini R**, Carducci C, Cianfarani S, Mauti M, Nicolais G. Post-traumatic Stress Disorder in Children Affected by Type 1 Diabetes and Their Parents. *SN Compr Clin Med* 2019; **1**: 349-353 [DOI: 10.1007/s42399-019-0049-4]

85 **Bassi G**, Mancinelli E, Di Riso D, Salcuni S. Parental Stress, Anxiety and Depression Symptoms Associated with Self-Efficacy in Paediatric Type 1 Diabetes: A Literature Review. *Int J Environ Res Public Health* 2020; **18** [PMID: 33379307 DOI: 10.3390/ijerph18010152]

86 **Rumburg TM**, Lord JH, Savin KL, Jaser SS. Maternal diabetes distress is linked to maternal depressive symptoms and adolescents' glycemic control. *Pediatr Diabetes* 2017; **18**: 67-70 [PMID: 26712240 DOI: 10.1111/pedi.12350]

87 **Landolt MA**, Ribi K, Laimbacher J, Vollrath M, Gnehm HE, Sennhauser FH. Posttraumatic stress disorder in parents of children with newly diagnosed type 1 diabetes. *J Pediatr Psychol* 2002; **27**: 647-652 [PMID: 12228336 DOI: 10.1093/jpepsy/27.7.647]

88 **Viaene AS**, Van Daele T, Bleys D, Faust K, Massa GG. Fear of Hypoglycemia, Parenting Stress, and Metabolic Control for Children with Type 1 Diabetes and Their Parents. *J Clin Psychol Med Settings* 2017; **24**: 74-81 [PMID: 28280962 DOI: 10.1007/s10880-017-9489-8]

89 **Driscoll KA**, Raymond J, Naranjo D, Patton SR. Fear of Hypoglycemia in Children and Adolescents and Their Parents with Type 1 Diabetes. *Curr Diab Rep* 2016; **16**: 77 [PMID: 27370530 DOI: 10.1007/s11892-016-0762-2]

90 **Jaser SS**, Linsky R, Grey M. Coping and psychological distress in mothers of adolescents with type 1 diabetes. *Matern Child Health J* 2014; **18**: 101-108 [PMID: 23420308 DOI: 10.1007/s10995-013-1239-4]

91 **Whittemore R**, Jaser S, Chao A, Jang M, Grey M. Psychological experience of parents of children with type 1 diabetes: a systematic mixed-studies review. *Diabetes Educ* 2012; **38**: 562-579 [PMID: 22581804 DOI: 10.1177/0145721712445216]

92 **Stallwood L**. Influence of caregiver stress and coping on glycemic control of young children with diabetes. *J Pediatr Health Care* 2005; **19**: 293-300 [PMID: 16202837 DOI: 10.1016/j.pedhc.2005.04.003]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1530-1538

DOI: 10.4239/wjd.v12.i9.1530

ISSN 1948-9358 (online)

MINIREVIEWS

Sugar intake from sweetened beverages and diabetes: A narrative review

Tung-Sung Tseng, Wei-Ting Lin, Gabrielle V Gonzalez, Yu-Hsiang Kao, Lei-Shih Chen, Hui-Yi Lin

ORCID number: Tung-Sung Tseng 0000-0001-6266-9891; Wei-Ting Lin 0000-0001-5556-2754; Gabrielle V Gonzalez 0000-0002-8553-4591; Yu-Hsiang Kao 0000-0003-3560-5889; Lei-Shih Chen 0000-0001-5320-6350; Hui-Yi Lin 0000-0002-3307-5549.

Author contributions: Tseng TS reviewed and drafted the first manuscript; Lin WT, and Gonzalez GV contributed to the study conception and literature review; Kao YH checked the review quality and results; Tseng TS, Lin HY and Chen LS contributed to the content analysis and the interpretation of the study; all authors contributed to edit and revise the manuscript critically and approve the final version of the article to be published.

Conflict-of-interest statement: Authors have nothing to disclose.

Tung-Sung Tseng, Gabrielle V Gonzalez, Yu-Hsiang Kao, Behavioral and Community Health Sciences, School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA 70112, United States

Wei-Ting Lin, Department of Global Community Health and Behavioral Sciences, School of Public Health & Tropical Medicine, Tulane University, New Orleans, LA 70112, United States

Lei-Shih Chen, Department of Health and Kinesiology, Texas A&M University, College Station, TX 77843, United States

Hui-Yi Lin, Biostatistics Program, School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA 70112, United States

Corresponding author: Tung-Sung Tseng, MSc, PhD, Associate Professor, Behavioral and Community Health Sciences, School of Public Health, Louisiana State University Health Sciences Center, 2020 Gravier Street, Room 213, New Orleans, LA 70112, United States. ttseng@lsuhsc.edu

Abstract

Type 2 diabetes mellitus (T2DM) is one of the fastest growing public health concerns around the world. Sugar-sweetened beverage (SSB) consumption has been proven to be associated with adverse health consequences in the diabetic population. Reducing SSB consumption, body weight control, healthy diets, and increased physical activity have been suggested as strategies to improve diabetes prevention and management. This literature review provides an overview of: (1) The association between SSB consumption and the risk of T2DM; (2) Types of SSB consumption and T2DM; (3) The effect of obesity and inflammation on the association between SSB consum-ption and risk of T2DM; and (4) SSB consumption in T2DM patients. There is still work to be done to determine how SSB consumption is related to T2DM, but the current research on identifying the association between SSB consumption and T2DM is promising, with the most promising studies confirming the connection between SSBs, T2DM risk, and diabetes management. Future studies should explore more effective SSB related diabetes prevention and management interventions.

Key Words: Sugar-sweetened beverages; Type 2 diabetes mellitus; Inflammation; Obesity; Diabetes management

Manuscript source: Invited manuscript

Specialty type: Behavioral sciences

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 27, 2021
Peer-review started: January 27, 2021
First decision: April 20, 2021
Revised: May 5, 2021
Accepted: August 3, 2021
Article in press: August 3, 2021
Published online: September 15, 2021

P-Reviewer: Mao RF
S-Editor: Liu M
L-Editor: A
P-Editor: Guo X

Core Tip: Sugar-sweetened beverage (SSB) consumption has been proven to be associated with adverse health consequences in the diabetic population. This literature review provides an overview of: (1) The association between SSB consumption and the risk of type 2 diabetes mellitus (T2DM); (2) Types of SSB consumption and T2DM; (3) The effect of obesity and inflammation on the association between SSB consumption and risk of T2DM; and (4) SSB consumption in T2DM patients. The current research on identifying the association between SSB consumption and T2DM is promising, with the most promising studies confirming the connection between SSBs, T2DM risk, and diabetes management.

Citation: Tseng TS, Lin WT, Gonzalez GV, Kao YH, Chen LS, Lin HY. Sugar intake from sweetened beverages and diabetes: A narrative review. *World J Diabetes* 2021; 12(9): 1530-1538
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1530.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1530

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the fastest growing public health concerns around the world, and has a high prevalence in the United States as one in every ten Americans has diabetes[1]. Many diabetes-related research studies, including those in the basic, clinical, translational, and public health sciences have identified the mechanisms of the disease, its risk factors, and intervention strategies to prevent or manage T2DM. Weight or diet management, physical activity, glycated hemoglobin level control, and diabetic retinopathy screening are useful approaches for the prevention and management of diabetes. Among these strategies, reducing sugar-sweetened beverages (SSB; beverages with high sugar content) consumption has been identified as one of the most cost-effective diabetes prevention and management approaches in the last decade[2]. Because SSB consumption plays an important role in both diabetes prevention and control, researchers are urging the public to avoid consuming large amounts of SSB's.

Although SSB consumption can lead to diabetes, there is limited information on both how SSB consumption is associated with diabetes, and on how the underlying mechanisms of obesity and inflammation affect the association between SSB consumption and T2DM. This manuscript reviews recent studies investigating the mechanisms and impact of SSB consumption on T2DM. In addition, the impact of SSB consumption on diabetic patients is a critical factor in successfully managing this chronic disease. Many studies have focused on SSB consumption and the risk of T2DM. However, the effect of SSB consumption upon adults with diabetes or prediabetes is unclear. The objective of this literature review is to provide an update on: (1) The association between SSB consumption and T2DM risk; (2) The effect of different types of SSB consumption and T2DM; (3) The effect of obesity and inflammation on the association between SSB consumption and risk of T2DM; and (4) SSB consumption in T2DM patients.

SSB AND RISK OF T2DM

Previous longitudinal and large cohort studies found a strong association between SSB consumption and subsequent T2DM risk. Previous large cohorts that were monitored for long periods (from 4-20 years) found that SSB consumption increased the risk of developing T2DM[3]. It is known that SSB's are the main source of added sugars in American diets. Previous literature and epidemiological evidence supports the fact that SSBs contribute to T2DM[4]. This study combined three cohorts of United States women and men and suggested that increased consumption of sugary beverages and artificially sweetened SSBs was associated with a higher risk of diabetes[4]. Similarly, the other three cohort studies consistently demonstrated that both SSB, and artificially

sweetened SSB consumption were observed to contribute to an increased risk of diabetes development[5-7].

The association of SSB and T2DM can also be observed in SSB related policy. Increased taxation of SSBs was recently implemented to evaluate associated changes in SSB consumption[8-12]. Some studies have suggested that increased taxation of SSBs is likely to reduce SSB consumption, lower the incidence of diabetes, and decrease diabetes related morbidity and mortality. Findings from a South African nationwide survey found an estimated decrease of approximately 108000 cumulative TD2M cases in South African adults after a 20% SSB tax increase was imposed for 20 years. Over the same period, the increased tax on SSB could also have been responsible for an 860 million dollars reduction in T2DM-related healthcare costs in South Africa[11]. A 1.6% and a 0.37% lowering of the incidence rate of diabetes in the populations of India and Ireland respectively was predicted after the imposition of a 20% SSB tax increase for ten years[9]. Although support of taxation on SSBs varies significantly across populations with differing demographic characteristics and health conditions, an SSB tax may have the potential to reduce both obesity-related disease incidence and health care costs.

TYPES OF SSB AND T2DM

SSBs include soda, energy drinks, sweetened tea, sweetened juices, and vitamin water drinks[13]. Although studies have indicated that SSBs are associated with diabetes, it is important to understand the associations that different types of SSBs have upon T2DM. Different types of sweetened beverages may be associated with different risks of diabetes or diabetic-related markers, such as beta-cell function and insulin sensitivity[14]. However, controversial findings were proposed from cohort results including one 14 year longitudinal cohort targeting women that showed both SSB consumption, and non-soda artificially sweetened drink consumption were associated with a higher risk of diabetes, with the exception of 100% fruit juice consumption[5].

The effect of low-calorie-sweetened beverages (LCSB) consumption has been studied to assess its efficacy in assisting in weight control. The American Diabetes Association, the American Heart Association, and the Academy of Nutrition and Dietetics have released statements that LCSBs can be used in a structured diet to replace SSBs and reduce energy intake[15]. Another large cohort study of men collected beverage intake information every 4 years over 20 years of follow-up using food frequency questionnaires. An increased risk of diabetes was found in men who consumed either carbonated or noncarbonated SSB, but not in those who consumed low-calorie sweetened beverages. Artificial sweeteners such as in diet soda, are an alternative to SSBs but they have no nutritional value and their health consequences are still being evaluated. Particularly, consumption of SSBs or artificially sweetened SSBs was associated with a higher risk of diabetes not only in middle-aged or older adults but also in young adults. One multi-city study observed that the long-term consumption of soda or fruit drinks sweetened with non-caloric sweeteners was associated with diabetes development. After adjusting for body mass index (BMI), these two associations were attenuated. However, total SSB (sugar-sweetened soft drinks and fruit drinks) consumption was still significantly associated with a higher risk of diabetes development in United States young adults[16].

Some studies focused on exploring whether the association between diabetes and regular and diet soda consumption was due to the presence of high fructose levels in these beverages. The association between consumption of regular soda and diabetes development, and decreased beta-cell function has been reported[14,17,18]. However, it is still unclear whether the consumption of regular and diet sodas is associated with different diabetes related outcomes. One study conducted by Gardener *et al*[19] showed that the substitution of diet soda or artificially sweetened diet beverages in place of regular soda was not associated with a reduced incidence of diabetes. Consistent results were reported from studies that compared regular and diet sodas. Consumption of SSB or diet sodas were both observed to result in an increased risk of diabetes in middle-aged Japanese men[6].

Reducing SSB consumption to lower diabetes incidence is suggested in the findings of all of the above-mentioned studies. Of these, findings from one cohort study conducted using 3.99 million person-years of data collected in multiple European countries (*e.g.* France, Italy, Spain, the United Kingdom, the Netherlands, Germany, Sweden, and Denmark) suggested that replacing SSBs with coffee or tea may result in a decrease of approximately 20% in diabetes development. This effect was not

observed by substituting SSB or diet soda consumption with fruit juice or milk. However, the consumption of sweetened or unsweetened coffee and tea were not distinguished in this study[20]. Another 10-year follow-up study reported that the replacement of all types of SSB and sweetened milk consumption with unsweetened tea, coffee, or water had a positive effect on diabetes prevention[21].

SSB, OBESITY, INFLAMMATION, AND T2DM

SSBs are a well known contributor to an unhealthy diet that can contribute to obesity due to high sugar consumption and low satiety. Added sugar intake in a liquid form was also found to be associated with higher levels of inflammatory markers[22,23]. In addition to adiposity, inflammation is another factor associated with diabetes. Accumulation of adiposity is one of the risk factors linked with higher inflammation levels. Controlling body weight, maintaining a healthy diet, reducing SSB consumption, and increased physical activity have been reported to be have a positive effect on diabetes prevention. In the past decade, some review studies have reported that SSB consumption is likely to contribute to the accumulation of adiposity and a higher risk of future diabetes[3,24,25]. Most of these studies considered adiposity as a confounder to evaluate the association between SSB consumption and diabetes. However, how adiposity influences the association between SSB consumption and diabetes is unclear. It has been hypothesized that a high intake level of added sugars and SSBs can increase chronic inflammation[23]. SSBs have been found to be a contributor to high dietary glycemic load (GL) that can lead to inflammation independent of obesity[3]. The consumption of large amounts of SSBs can increase blood glucose and insulin concentrations, while leading to a high GL. Diets with a high GL can stimulate hunger and lead to weight gain as well as induce glucose intolerance and insulin resistance. High GL can increase inflammatory biomarkers like the C-reactive protein (CRP), which is associated with T2DM.

Aeberli *et al*[26] reported how low to moderate consumption of fructose, glucose, and sucrose-containing beverages can cause an inflammatory response in young men. Fructose, which can trigger inflammation, is a major component of SSBs and has been observed to activate inflammatory pathways. This evidence supports the hypothesis of a positive association between high SSB consumption and inflammation[26,27]. A short 3-wk period of SSB consumption can cause changes in glucose metabolism that can lead to long-term insulin resistance. This study supports other research that found that consumption of SSBs had an adverse effect on lipid and glucose metabolism and raised inflammation levels[26]. Other studies reported fructose as the main component in chronic inflammation, and that there were no observable differences in the triggering of low-grade chronic inflammation due to either short-term or long-term consumption of SSBs[27]. Some review studies have proposed that higher levels of inflammatory markers are associated with an increased risk of diabetes[28,29]. One recent study identified the role of inflammatory markers in the association between SSB consumption and diabetes. Findings showed no significant linear association between SSB and T2DM risk, or CRP levels because of the U-shaped association between SSB consumption or added sugar intake, and diabetes risk and CRP level. However, six plasma proteins, including HGF, tPA, CHI3L1, IL1ra, PRSS8 and FUR, were identified as being associated with SSB consumption[23]. CRP are most commonly studied in relation to added sugars and SSBs, but there is currently a dearth of research on other plasma proteins[23]. SSB's with the familiar caramel coloring found in cola soft drinks are known to have high levels of advanced glycation end products, which can also increase inflammation[3].

Obesity plays an important role in diabetes prevention and management. One survey study reported that the association between SSB consumption and diabetes was marginally significant after adjusting for adiposity indexes, such as body fatness, BMI, and waist circumference[30]. Another cross-sectional study that analyzed data from 75 countries found that sweetened soft drink consumption was related to diabetes prevalence. Findings from one nationwide eight-year cohort study found that BMI mediated the relationship between SSB consumption and diabetes incidence[31], while one case-control study demonstrated that excess weight might partially mediate the association between SSB intake and diabetes development[32].

SSB CONSUMPTION IN T2DM PATIENTS

Identifying which variables are associated with SSB intake among people with T2DM may provide vital evidence for the effective management of diabetes. Two studies assessed large United States nationwide survey datasets (*i.e.*, Behavioral Risk Factor Surveillance System and National Health and Nutrition Examination Survey) to explore associations between sociodemographic and behavioral characteristics and SSB consumption among T2DM patients. Around 16% of adults with diabetes, and about 30% of adults without diabetes reported consuming SSBs at least once per day. Diabetic adults who were younger, male, non–Hispanic Black, lower education, lower income, not married, and current smokers were significantly more likely to consume more SSBs[2,33]. In addition, diabetes management behaviors were also observed to have a significant association with SSB intake. Adults with T2DM who had a shorter duration of diabetes, checked their blood sugar less frequently, and did not attend a diabetes self-management course reported higher SSB consumption[2].

SSB consumption has been proven to be associated with adverse health consequences in both the overall and the diabetic populations. Previous literature has reported that adults with T2DM who consumed more SSBs had an elevated risk of developing numerous adverse health outcomes such as abdominal obesity[34], cardiovascular diseases[35], gout[36], poor cognitive function[37], and tooth loss[34-38]. Although these studies applied a cross-sectional design using a survey questionnaire, a positive association between SSB intake and risk of adverse health outcomes was identified, which should motivate T2DM patients to reduce SSB intake or stop consuming SSBs altogether to decrease the risk of adverse outcomes.

IMPACT OF SSB CONSUMPTION ON T2DM

Our review of the literature found trends in research results connecting SSB consumption to the risk of developing T2DM. Findings from a computer simulation study demonstrated that a 10% reduction in SSB consumption could decrease diabetes incidence over a 10-year period in adults aged 35 and older[39]. The majority of beverages containing high levels of sugar included soft drinks, juices, energy drinks, and vitamin water drinks. Studies showed that current SSB consumption levels resulted in possible increased incidence of T2DM. The added sugar content in these beverages is a major factor contributing to T2DM related adverse health outcomes, though fruit juices can also contribute to increased T2DM risk due to high levels of added sugar in these beverages that were once thought to be healthier alternatives.

Different types of sweetened beverages may be associated with different risks of diabetes or the appearance of diabetic-related markers; however, inconsistent findings were reported. Some studies showed that fruit juice and low-calorie sweetened beverage consumption were not associated with a higher risk of diabetes. The effect of the replacement of SSB's with diet or no-caloric sweetened beverages on diabetes incidence is still uncertain. None of the studies we reviewed suggested diet or zero calorie sweetened beverage consumption as an strategy to prevent diabetes development. The more effective strategy to lower diabetes risk is to substitute water, unsweetened tea, or coffee for SSBs. These are simple and low-cost options to improve glycemic and weight control.

In concluding this review, most studies found that SSB consumption increased the risk of developing T2DM (Figure 1). SSB consumption is thought to add to the risk of individuals developing T2DM as it can lead to obesity. SSBs are likely to contribute to the accumulation of adiposity and a higher risk of developing diabetes in the future. There was a consensus that the obesity rate in the United States could be partly due to the number of individuals consuming more than the recommended daily intake of sugar. Excess adiposity in the body is known to promote inflammation flare-ups, but SSB's can increase the effects of inflammation due to the higher levels of added sugars being absorbed in the body. There was a call for more research into exactly what types of inflammation related proteins are increasing due to excess sugar consumption. CRP are the main area of study related to how inflammation is caused in the body, but broader ranging research has the potential to contribute insight into all sources of inflammation, and how inflammation can be combated it in the future. Scientists agree that high levels of sugar consumed on a daily basis increases inflammation, so the reduction of added sugar in the diet has to potential to significantly improve the health outcomes of individuals living with T2DM and chronic inflammation.

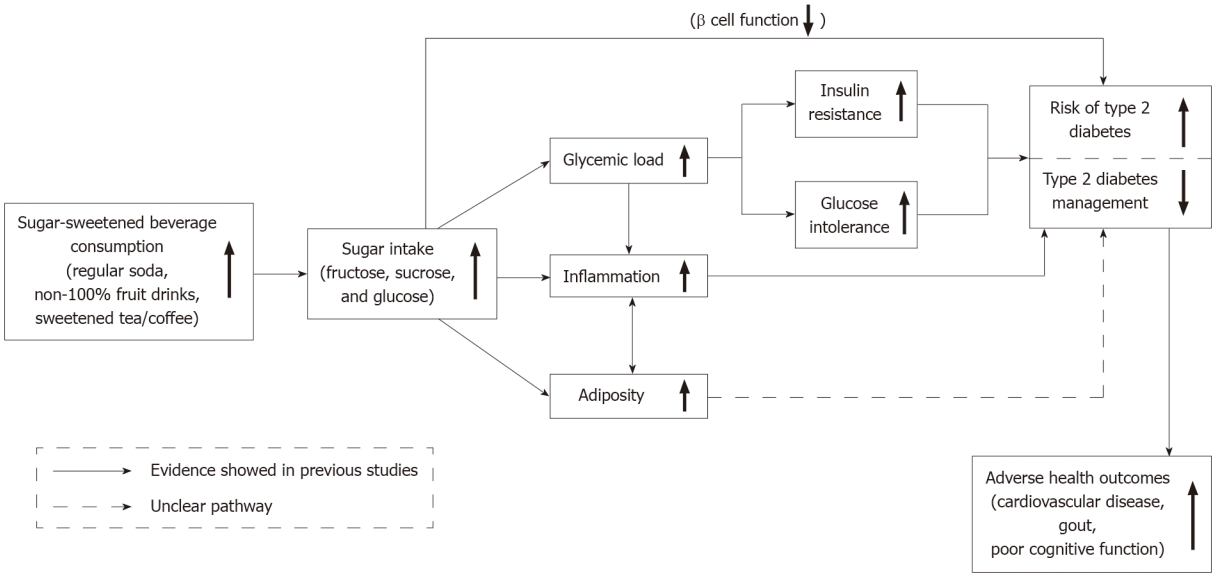

Figure 1 The association between sugar-sweetened beverages and diabetes.

SSBs have been found to be a contributor to high dietary GL that can lead to inflammation independent of obesity. Fructose, which is a major component of SSBs and can trigger inflammation, was observed to activate inflammatory pathways, evidence that supports the hypothesis of a positive association between high SSB consumption and inflammation. SSBs can also cause changes in glucose metabolism that can have the potential to lead to long-term insulin resistance. Large amounts of rapidly absorbable carbohydrates that are found in sugars, a significant component of SSBs, can lead to diabetes independent of obesity. SSB consumption has been found to be an factor in the incidence of T2DM, meaning that sugar content in the diet is more detrimental to health than was previously understood. Different sugars had different effects, but all had an adverse effect on inflammation levels. Thus, the consumption of SSBs has been associated with an increased risk of chronic disease, which may be mediated by low-grade chronic inflammation[27]. SSB consumption can lead to T2DM, weight gain, increased inflammation, impaired beta-cell function, and insulin sensitivity[36]. When SSBs are consumed in large amounts, the possibility of developing glucose intolerance increases along with inflammation levels[40]. This means that SSBs can lead to diabetes independently of obesity.

The literature agreed that persons living with diabetes need to take action to reduce their intake of SSBs. A consensus was seen that a reduction in SSB consumption would improve overall health, including reduction of body fat, inflammation, and insulin sensitivity. Alternatives to SSBs are an option for those living with T2DM, but their lack of nutritional benefits is leading researchers to instead support the consumption of water, tea, and coffee rather than low-calorie or zero-calorie beverages. Patients were found to understand the health benefits of drinking affordable and no-sugar-added beverages, but there is still a large portion of the population that continues to drink SSBs. Education is needed to reduce the consumption of SSBs and reduce the risk of developing T2DM. It is recommended that T2DM patients limit SSB consumption, and increase exercise levels to become physically fit and lose weight [36]. T2DM patients should avoid SSBs due to the adverse health outcomes associated with their consumption, and the effects that dietary sugar consumption has on obesity, glycemic control, and inflammation. Reduced SSB consumption in the daily diet can benefit individuals with T2DM by improving lipid profiles and insulin sensitivity, and by reducing blood pressure, inflammation, and excess visceral adiposity[3]. Recent studies have reported that replacing SSBs with artificially sweetened beverages (ASBs) can positively affect body weight and possibly reduce the risk of diabetes long-term [4]. However, researchers suggest caution in replacing SSBs with ASBs due to findings of weight gain, insulin resistance, and appetite stimulation. In terms of diabetes diagnosis, approximately 7.3 million Americans are undiagnosed[41], which means those adults had a fasting plasma glucose level of ≥ 126 mg/dL but did not be told by the physician. A One study revealed that undiagnosed adults were significantly more likely to consume more SSBs than those who were diagnosed with T2DM[33]. Therefore, undiagnosed adults should be targeted and diagnosed as early as possible,

which may improve their ability to manage their diabetes successfully.

LIMITATIONS

Several limitations of this literature review should be noted. Although this review employed bibliographic search strategies to minimize bias, not all relevant published papers have been included. Second, cross-sectional studies cannot provide evidence of causal linkage. Future studies are needed to clarify the causal relationship between SSB consumption, adiposity, and diabetes. However, the scope of this review provided an update on what is currently know regarding the association between SSBs and T2DM. The literature surveyed mostly relied on large cohorts to generate valid data, the analysis of which resulted in the conclusion that those who consume large amounts of SSB's have a higher chance of developing T2DM, but more research needs to be done to see if that association is a significant factor in disease incidence. An individual's lifestyle and eating habits may also contribute to a T2DM diagnosis just as much or more than consuming SSBs.

CONCLUSION

Overall, consumption of beverages with added sweeteners, including soda, sweetened tea, coffee, juices, and milk is related to a higher risk of diabetes development. However, the interactions between the incidence of diabetes, the type of SSBs consumed, and adiposity is, as yet, unclear. SSB consumption can lead to weight gain, inflammation, and T2DM. The consumption of SSBs was associated with a higher risk of adverse health outcomes in adults with T2DM.

Future perspectives

It is important to continue to educate diabetic patients and those at risk of developing diabetes that high levels of SSB consumption has the potential to lead to T2DM and obesity. There is still more work to be done to definitively determine the effect of SSB consumption on the health of those who have T2DM, and those who are at risk for T2DM. The majority of the studies we reviewed confirmed that large-scale cohorts with long study periods show the most promise in demonstrating the connection between SSB consumption and T2DM. Future work is needed to conduct innovative and effective SSB-related behavioral and community-based research focused on discovering data-driven intervention strategies aimed at both reducing the risk of contracting diabetes, and the successful management of diabetes in those who have been diagnosed.

REFERENCES

1 **Centers for Disease Control and Prevention**, National Diabetes Statistics Report, 2020. [cited 20 December 2020]. Available from: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf

2 **Xu F**, Park S, Siegel KR. Factors Associated With Frequency of Sugar-Sweetened Beverage Consumption Among US Adults With Diabetes or Prediabetes. *Am J Health Promot* 2018; **32**: 1489-1497 [PMID: 29254359 DOI: 10.1177/0890117117746187]

3 **Malik VS**, Popkin BM, Bray GA, Després JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* 2010; **33**: 2477-2483 [PMID: 20693348 DOI: 10.2337/dc10-1079]

4 **Drouin-Chartier JP**, Zheng Y, Li Y, Malik V, Pan A, Bhupathiraju SN, Tobias DK, Manson JE, Willett WC, Hu FB. Changes in Consumption of Sugary Beverages and Artificially Sweetened Beverages and Subsequent Risk of Type 2 Diabetes: Results From Three Large Prospective U.S. Cohorts of Women and Men. *Diabetes Care* 2019; **42**: 2181-2189 [PMID: 31582428 DOI: 10.2337/dc19-0734]

5 **Fagherazzi G**, Vilier A, Saes Sartorelli D, Lajous M, Balkau B, Clavel-Chapelon F. Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Epidemiologique aupres des femmes de la Mutuelle Generale de l'Education Nationale-European Prospective Investigation into Cancer and Nutrition cohort. *Am J Clin Nutr* 2013; **97**: 517-523 [PMID: 23364017 DOI: 10.3945/ajcn.112.050997]

6 **Sakurai M**, Nakamura K, Miura K, Takamura T, Yoshita K, Nagasawa SY, Morikawa Y, Ishizaki M, Kido T, Naruse Y, Suwazono Y, Sasaki S, Nakagawa H. Sugar-sweetened beverage and diet soda

consumption and the 7-year risk for type 2 diabetes mellitus in middle-aged Japanese men. *Eur J Nutr* 2014; **53**: 251-258 [PMID: 23575771 DOI: 10.1007/s00394-013-0523-9]

7 **Huang M**, Quddus A, Stinson L, Shikany JM, Howard BV, Kutob RM, Lu B, Manson JE, Eaton CB. Artificially sweetened beverages, sugar-sweetened beverages, plain water, and incident diabetes mellitus in postmenopausal women: the prospective Women's Health Initiative observational study. *Am J Clin Nutr* 2017; **106**: 614-622 [PMID: 28659294 DOI: 10.3945/ajcn.116.145391]

8 **Mangera KAS**, Adams OP. Knowledge, attitudes and practices with regard to sugar sweetened beverages and taxation among people with type 2 diabetes mellitus in the Caribbean island of Barbados - A cross sectional survey in primary care. *Prim Care Diabetes* 2021; **15**: 69-73 [PMID: 32471769 DOI: 10.1016/j.pcd.2020.04.002]

9 **O'Neill KN**, Fitzgerald AP, Kearney PM. Impact of population distribution shifts in sugar-sweetened beverage consumption on type II diabetes incidence in Ireland. *Ann Epidemiol* 2020; **41**: 1-6 [PMID: 31928896 DOI: 10.1016/j.annepidem.2019.12.007]

10 **Sánchez-Romero LM**, Penko J, Coxson PG, Fernández A, Mason A, Moran AE, Ávila-Burgos L, Odden M, Barquera S, Bibbins-Domingo K. Projected Impact of Mexico's Sugar-Sweetened Beverage Tax Policy on Diabetes and Cardiovascular Disease: A Modeling Study. *PLoS Med* 2016; **13**: e1002158 [PMID: 27802278 DOI: 10.1371/journal.pmed.1002158]

11 **Manyema M**, Veerman JL, Chola L, Tugendhaft A, Labadarios D, Hofman K. Decreasing the Burden of Type 2 Diabetes in South Africa: The Impact of Taxing Sugar-Sweetened Beverages. *PLoS One* 2015; **10**: e0143050 [PMID: 26575644 DOI: 10.1371/journal.pone.0143050]

12 **Basu S**, Vellakkal S, Agrawal S, Stuckler D, Popkin B, Ebrahim S. Averting obesity and type 2 diabetes in India through sugar-sweetened beverage taxation: an economic-epidemiologic modeling study. *PLoS Med* 2014; **11**: e1001582 [PMID: 24409102 DOI: 10.1371/journal.pmed.1001582]

13 **U.S. Department of Health and Human Services and U.S. Department of Agriculture**. 2015–2020 Dietary Guidelines for Americans. 8th Edition. [cited 20 December 2020]. Available from: https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015

14 **den Biggelaar LJCJ**, Sep SJS, Mari A, Ferrannini E, van Dongen MCJM, Wijckmans NEG, Schram MT, van der Kallen CJ, Schaper N, Henry RMA, van Greevenbroek MM, Stehouwer CDA, Eussen SJPM. Association of artificially sweetened and sugar-sweetened soft drinks with β-cell function, insulin sensitivity, and type 2 diabetes: the Maastricht Study. *Eur J Nutr* 2020; **59**: 1717-1727 [PMID: 31486878 DOI: 10.1007/s00394-019-02026-0]

15 **Leahy M**, Ratliff JC, Riedt CS, Fulgoni VL. Consumption of Low-Calorie Sweetened Beverages Compared to Water Is Associated with Reduced Intake of Carbohydrates and Sugar, with No Adverse Relationships to Glycemic Responses: Results from the 2001-2012 National Health and Nutrition Examination Surveys. *Nutrients* 2017; **9**: 928 [PMID: 28837084 DOI: 10.3390/nu9090928]

16 **Hirahatake KM**, Jacobs DR, Shikany JM, Jiang L, Wong ND, Steffen LM, Odegaard AO. Cumulative intake of artificially sweetened and sugar-sweetened beverages and risk of incident type 2 diabetes in young adults: the Coronary Artery Risk Development In Young Adults (CARDIA) Study. *Am J Clin Nutr* 2019; **110**: 733-741 [PMID: 31374564 DOI: 10.1093/ajcn/nqz154]

17 **Stern D**, Mazariegos M, Ortiz-Panozo E, Campos H, Malik VS, Lajous M, López-Ridaura R. Sugar-Sweetened Soda Consumption Increases Diabetes Risk Among Mexican Women. *J Nutr* 2019; **149**: 795-803 [PMID: 31050751 DOI: 10.1093/jn/nxy298]

18 **Eshak ES**, Iso H, Mizoue T, Inoue M, Noda M, Tsugane S. Soft drink, 100% fruit juice, and vegetable juice intakes and risk of diabetes mellitus. *Clin Nutr* 2013; **32**: 300-308 [PMID: 22917499 DOI: 10.1016/j.clnu.2012.08.003]

19 **Gardener H**, Moon YP, Rundek T, Elkind MSV, Sacco RL. Diet Soda and Sugar-Sweetened Soda Consumption in Relation to Incident Diabetes in the Northern Manhattan Study. *Curr Dev Nutr* 2018; **2**: nzy008 [PMID: 29955723 DOI: 10.1093/cdn/nzy008]

20 **Imamura F**, Schulze MB, Sharp SJ, Guevara M, Romaguera D, Bendinelli B, Salamanca-Fernández E, Ardanaz E, Arriola L, Aune D, Boeing H, Dow C, Fagherazzi G, Franks PW, Freisling H, Jakszyn P, Kaaks R, Khaw KT, Kühn T, Mancini FR, Masala G, Chirlaque MD, Nilsson PM, Overvad K, Pala VM, Panico S, Perez-Cornago A, Quirós JR, Ricceri F, Rodríguez-Barranco M, Rolandsson O, Sluijs I, Stepien M, Spijkerman AMW, Tjønneland A, Tong TYN, Tumino R, Vissers LET, Ward HA, Langenberg C, Riboli E, Forouhi NG, Wareham NJ. Estimated Substitution of Tea or Coffee for Sugar-Sweetened Beverages Was Associated with Lower Type 2 Diabetes Incidence in Case-Cohort Analysis across 8 European Countries in the EPIC-InterAct Study. *J Nutr* 2019; **149**: 1985-1993 [PMID: 31396627 DOI: 10.1093/jn/nxz156]

21 **O'Connor L**, Imamura F, Lentjes MA, Khaw KT, Wareham NJ, Forouhi NG. Prospective associations and population impact of sweet beverage intake and type 2 diabetes, and effects of substitutions with alternative beverages. *Diabetologia* 2015; **58**: 1474-1483 [PMID: 25944371 DOI: 10.1007/s00125-015-3572-1]

22 **O'Connor L**, Imamura F, Brage S, Griffin SJ, Wareham NJ, Forouhi NG. Intakes and sources of dietary sugars and their association with metabolic and inflammatory markers. *Clin Nutr* 2018; **37**: 1313-1322 [PMID: 28711418 DOI: 10.1016/j.clnu.2017.05.030]

23 **Ramne S**, Drake I, Ericson U, Nilsson J, Orho-Melander M, Engström G, Sonestedt E. Identification of Inflammatory and Disease-Associated Plasma Proteins that Associate with Intake of Added Sugar and Sugar-Sweetened Beverages and Their Role in Type 2 Diabetes Risk. *Nutrients* 2020; **12**: 3129 [PMID: 33066363 DOI: 10.3390/nu12103129]

24 **Basu S**, McKee M, Galea G, Stuckler D. Relationship of soft drink consumption to global

overweight, obesity, and diabetes: a cross-national analysis of 75 countries. *Am J Public Health* 2013; **103**: 2071-2077 [PMID: 23488503 DOI: 10.2105/AJPH.2012.300974]

25 **Greenwood DC**, Threapleton DE, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, Burley VJ. Association between sugar-sweetened and artificially sweetened soft drinks and type 2 diabetes: systematic review and dose-response meta-analysis of prospective studies. *Br J Nutr* 2014; **112**: 725-734 [PMID: 24932880 DOI: 10.1017/S0007114514001329]

26 **Aeberli I**, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, Berthold HK, Spinas GA, Berneis K. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. *Am J Clin Nutr* 2011; **94**: 479-485 [PMID: 21677052 DOI: 10.3945/ajcn.111.013540]

27 **Kuzma JN**, Cromer G, Hagman DK, Breymeyer KL, Roth CL, Foster-Schubert KE, Holte SE, Weigle DS, Kratz M. No differential effect of beverages sweetened with fructose, high-fructose corn syrup, or glucose on systemic or adipose tissue inflammation in normal-weight to obese adults: a randomized controlled trial. *Am J Clin Nutr* 2016; **104**: 306-314 [PMID: 27357093 DOI: 10.3945/ajcn.115.129650]

28 **Donath MY**, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011; **11**: 98-107 [PMID: 21233852 DOI: 10.1038/nri2925]

29 **Wang X**, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 2013; **36**: 166-175 [PMID: 23264288 DOI: 10.2337/dc12-0702]

30 **Jing Y**, Han TS, Alkhalaf MM, Lean MEJ. Attenuation of the association between sugar-sweetened beverages and diabetes risk by adiposity adjustment: a secondary analysis of national health survey data. *Eur J Nutr* 2019; **58**: 1703-1710 [PMID: 29766286 DOI: 10.1007/s00394-018-1716-z]

31 **Papier K**, D'Este C, Bain C, Banwell C, Seubsman S, Sleigh A, Jordan S. Consumption of sugar-sweetened beverages and type 2 diabetes incidence in Thai adults: results from an 8-year prospective study. *Nutr Diabetes* 2017; **7**: e283 [PMID: 28628126 DOI: 10.1038/nutd.2017.27]

32 **Löfvenborg JE**, Ahlqvist E, Alfredsson L, Andersson T, Dorkhan M, Groop L, Tuomi T, Wolk A, Carlsson S. Genotypes of HLA, TCF7L2, and FTO as potential modifiers of the association between sweetened beverage consumption and risk of LADA and type 2 diabetes. *Eur J Nutr* 2020; **59**: 127-135 [PMID: 30656477 DOI: 10.1007/s00394-019-01893-x]

33 **Bleich SN**, Wang YC. Consumption of sugar-sweetened beverages among adults with type 2 diabetes. *Diabetes Care* 2011; **34**: 551-555 [PMID: 21273500 DOI: 10.2337/dc10-1687]

34 **Anari R**, Amani R, Veissi M. Sugar-sweetened beverages consumption is associated with abdominal obesity risk in diabetic patients. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S675-S678 [PMID: 28487104 DOI: 10.1016/j.dsx.2017.04.024]

35 **Anari R**, Amani R, Veissi M. Sugary beverages are associated with cardiovascular risk factors in diabetic patients. *J Diabetes Metab Disord* 2019; **18**: 7-13 [PMID: 31275869 DOI: 10.1007/s40200-019-00383-5]

36 **Murphy R**, Thornley S, de Zoysa J, Stamp LK, Dalbeth N, Merriman TR. Sugar Sweetened Beverage Consumption among Adults with Gout or Type 2 Diabetes. *PLoS One* 2015; **10**: e0125543 [PMID: 25978428 DOI: 10.1371/journal.pone.0125543]

37 **Crichton GE**, Elias MF, Torres RV. Sugar-sweetened soft drinks are associated with poorer cognitive function in individuals with type 2 diabetes: the Maine-Syracuse Longitudinal Study. *Br J Nutr* 2016; **115**: 1397-1405 [PMID: 26940176 DOI: 10.1017/S0007114516000325]

38 **Wiener RC**, Shen C, Findley PA, Sambamoorthi U, Tan X. The association between diabetes mellitus, sugar-sweetened beverages, and tooth loss in adults: Evidence from 18 states. *J Am Dent Assoc* 2017; **148**: 500-509.e4 [PMID: 28483048 DOI: 10.1016/j.adaj.2017.03.012]

39 **Salgado MV**, Penko J, Fernandez A, Konfino J, Coxson PG, Bibbins-Domingo K, Mejia R. Projected impact of a reduction in sugar-sweetened beverage consumption on diabetes and cardiovascular disease in Argentina: A modeling study. *PLoS Med* 2020; **17**: e1003224 [PMID: 32722677 DOI: 10.1371/journal.pmed.1003224]

40 **Audain K**, Levy L, Ellahi B. Sugar-sweetened beverage consumption in the early years and implications for type-2 diabetes: a sub-Saharan Africa context. *Proc Nutr Soc* 2019; **78**: 547-553 [PMID: 30816084 DOI: 10.1017/S0029665118002860]

41 **American Diabetes Association**. Statistics About Diabetes. 2018 [cited 20 December 2020]. Available from: https://www.diabetes.org/resources/statistics/statistics-about-diabetes

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1539-1549

DOI: 10.4239/wjd.v12.i9.1539

ISSN 1948-9358 (online)

MINIREVIEWS

Role of an acidic environment in the treatment of diabetic foot infections: A review

Basavraj Nagoba, Ajay Gavkare, Abhijit Rayate, Sachin Mumbre, Arunkumar Rao, Basavraj Warad, Neeta Nanaware, Nawab Jamadar

ORCID number: Basavraj Nagoba 0000-0001-5625-3777; Ajay Gavkare 0000-0003-4711-5596; Abhijit Rayate 0000-0002-6183-7029; Sachin Mumbre 0000-0002-9169-6001; Arunkumar Rao 0000-0003-2315-4478; Basavraj Warad 0000-0002-4450-6025; Neeta Nanaware 0000-0002-3176-4930; Nawab Jamadar 0000-0002-3483-4949.

Author contributions: Nagoba B contributed to the idea behind the manuscript, writing the paper, modification of content and final approval of the draft; Gavkare A, Rayate A and Nanaware N contributed to the literature search, collection of the data and writing the paper; Mumbre S, Rao A, Warad B and Jamadar N contributed to the writing the paper, modification of content and final approval of the draft.

Conflict-of-interest statement: Authors declare that they have no conflicting interests.

Basavraj Nagoba, Department of Microbiology, MIMSR Medical College, Latur 413512, Maharashtra, India

Ajay Gavkare, Department of Physiology, MIMSR Medical College, Latur 413512, Maharashtra, India

Abhijit Rayate, Basavraj Warad, Department of Surgery, MIMSR Medical College, Latur 413512, Maharashtra, India

Sachin Mumbre, Department of Community Medicine, Ashwini Rural Medical College, Solapur 413001, Maharashtra, India

Arunkumar Rao, Department of Orthopedics, MIMSR Medical College, Latur 413512, India

Neeta Nanaware, Department of Physiology, Government Medical College, Latur 413512, Maharashtra, India

Nawab Jamadar, Department of Anesthesiology, MIMSR Medical College, Latur 413512, Maharashtra, India

Corresponding author: Basavraj Nagoba, PhD, Assistant Dean, Research, Professor, Department of Microbiology, MIMSR Medical College, Vishwanathpuram, Ambejogai Road, Latur 413512, Maharashtra, India. dr_bsnagoba@yahoo.com

Abstract

Management of diabetic foot ulcers is the biggest challenge to the clinician, as conventional antibiotic therapies and local wound care have their own limitations. They are not effective for control of infections and promotion of healing because of cytotoxic effects. In view of cytotoxicity of routinely used topical antiseptic agents, this article focuses on the search of an ideal topical antiseptic agent that is safe and effective in controlling infectious agents and also in promoting the healing process. This review focuses on the use of various acids such as citric, acetic, hyaluronic, and hypochlorous acids as topical agents in diabetic foot infections. This article also focuses on the different roles of acids in the treatment of diabetic foot infections.

Key Words: Diabetic foot ulcer; Infection; Management; Topical agents; Acids; Role of acids

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: India

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 28, 2021
Peer-review started: January 29, 2021
First decision: June 16, 2021
Revised: June 21, 2021
Accepted: August 5, 2021
Article in press: August 5, 2021
Published online: September 15, 2021

P-Reviewer: He Z, Korkmaz P
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Ma YJ

Core Tip: Diabetic foot ulcer is the most serious complication of diabetes mellitus. The biggest challenge is to find an ideal topical antiseptic agent that is safe and effective in controlling infectious agents and promoting the healing process. This article focuses on the use of acids as topical agents to control diabetic foot infections, with special emphasis on the different roles of citric, acetic, hyaluronic, and hypochlorous acids in the effective management of diabetic foot ulcers.

Citation: Nagoba B, Gavkare A, Rayate A, Mumbre S, Rao A, Warad B, Nanaware N, Jamadar N. Role of an acidic environment in the treatment of diabetic foot infections: A review. *World J Diabetes* 2021; 12(9): 1539-1549
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1539.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1539

INTRODUCTION

Diabetes mellitus is a global public health problem. The global diabetes prevalence in 2019 was estimated to be 9.3%and is expected to rise to 10.2% by 2030 and 10.9% by 2045[1].Development of foot ulcers is one of the most serious complications of diabetes mellitus. The annual risk of foot ulceration in diabetic patients is 2% whereas the lifetime risk is 12%-25%, which increases further in the presence of peripheral neuropathy[2-6]. Intrinsic factors such as loss of sensation because of peripheral neuropathy, vascular insufficiency because of microvascular disease, and impaired immune response along with mechanical factors such as increased plantar pressure associated with foot deformity and calluses, local trauma, and infection are the important risk factors[3,7,8].

Infection is the most common sequela of diabetic foot ulceration, and once established, it becomes progressively severe and more difficult to treat. An infected foot ulcer is the most common cause of diabetes-related hospital admission, and if not treated well in time, it is the most common cause leading to lower extremity amputation[8]. It has been reported that nearly 28% of patients with diabetes require lower limb amputations and majority of amputations (50%) are needed because of uncontrolled infections. Thus, diabetic foot infection is the leading cause of nontraumatic lower extremity amputation[10,11]. Eradication of the infectious agent to control infection and sepsis, especially in a chronic diabetic foot ulcer, is paramount to the success of healing. Hence, finding a safe and effective antiseptic agent to control/eradicate infection as well as hasten the healing process should be the prime objective in the management of diabetic foot infections.

MICROBIOLOGY OF DIABETIC FOOT INFECTIONS

Foot infections in diabetics are most commonly caused by bacteria. Both aerobes and anaerobes have been shown to cause infection[12-17]. Fungi are also known to be associated with foot infections in diabetics[18,19]. Polymicrobial etiology has been reported to be more common than monomicrobial infection. Bacteriological analysis shows a predominance of both Gram-positive and Gram-negative bacteria. Among the Gram-positive aerobic bacteria, *Staphylococcus aureus* (*S. aureus*) is the most common bacterium. Coagulase-negative Staphylococci and Streptococci are the other bacterial pathogens isolated from foot infections. Among the Gram-negative aerobes, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., *etc.* are common isolates. Among anaerobic bacteria, Peptostreptococci, Clostridia and Peptococci are the common Gram-positive isolates and *Bacteroides* spp., *Prevotella* spp., *Fusobacterium* spp., *etc.* are common Gram-negative anaerobic isolates. Among fungi, *Candida* spp., *C. albicans* in particular, has been reported to be the most common[12-19].

MANAGEMENT OF DIABETIC FOOT INFECTIONS

A critical part of the management of diabetic foot ulcers is to treat the infection to reduce microbial load quantitatively to a level that can be resolved by the host immune system. As most of the etiological agents associated with diabetic foot infections are known to form biofilms and are resistant to multiple antimicrobial agents, they are difficult to eliminate from the infection site. It has been observed that biofilm formation is associated with increased virulence and delayed wound healing [20]. Biofilms release a variety of toxic components during the taxis of neutrophils and discourage the process of phagocytosis. In chronic wounds, formation of biofilms discourages wound healing by increasing the inflammatory response[21].

The ideal management of diabetic foot infections should positively and potentially reduce the incidence of infection-related morbidities, duration of hospital stay, the cost of treatment, and most importantly, reduce limb amputations[22]. Compared with other wounds, diabetic foot ulcers are more prone to infection, and infection is one of the most important factors that delay wound healing. Hence, good wound care for control of infection is critical for successful wound healing[23], but successful treatment of diabetic foot infections is the biggest challenge for the following reasons: (1) Parenterally or orally administered antimicrobial agents have been shown not to reach adequate levels in chronic granulation tissue and have no effect on growing bacterial populations in granulating wounds[24]. Biofilm formation by infecting agents in diabetic foot ulcers makes wound healing and infections difficult to resolve by hampering local access of antimicrobial agents and because diabetes hampers the immune system[25]. Biofilm formation not only helps to prevent phagocytosis but also helps to increase the resistance of infecting agents to antimicrobial agents[26,27]. In a previous study, we found that in spite of in vitro susceptibility of infecting agent isolated from patients to antimicrobial agents, administration of the antimicrobial agent to patients did not result in successful outcomes. The result indicates that systemic antimicrobial therapy may not have practical and potential value in such cases, making local wound care the backbone of treatment[28]. And (2) Many topical antiseptic agents are used for wound care in diabetics. Some are good in controlling infections but their cytotoxic effect on the cells involved in the wound healing process and other cells like dermal and epidermal cells limit their use. Available experimental data show that majority of the agents retard healing by interfering with the normal process and can be harmful rather than useful. Studies show that these agents should be avoided, especially in the treatment of diabetic foot ulcers[29-32].

Hydrogen peroxide is the most commonly used antiseptic for washing diabetic foot ulcers, but is toxic to newly formed epithelium[33], because it kills fibroblasts, which have an important role in healing and epithelialization. In addition, it may also destroy normal cells surrounding the wound[34]. Povidone-iodine (betadine) is another commonly used antiseptic agent, but because it is also cytotoxic to fibroblasts and other cells involved in wound healing, it fails to promote good wound healing. Most studies show that it impairs wound healing and reduces wound strength[30,35-37]. Sodium hypochlorite (Dakin's solution) has also been reported to be toxic to fibroblasts and keratinocytes and has been found to delay the process of epithelialization and neovascularization. It has also been reported to retard collagen synthesis and inhibit migration of neutrophils in a wound bed[38-42]. Silver nitrate has also been reported to slow down the process of epithelialization and may delay wound healing[30,38]. Many other antiseptic agents such as iodine, alcohol, chlorhexidine, mafenide acetate, silver compounds, and benzalkonium chloride, *etc.* have been reported to retard wound healing[31,38,43-45]. In view of these observations, the treatment of diabetic foot ulcers has always been a big challenge to the clinician as conventional therapies (antibiotic therapy and local wound care) have limitations. Infection is the most common and most important reason for nonhealing/poor healing of diabetic foot ulcers. Infecting organisms are most difficult to eliminate from the infection site. Infections of diabetic foot ulcers need special attention, and if not controlled well in time, may become limb threatening and sometimes life threatening by progressing to osteomyelitis or gangrene, which can lead to septicemia, amputation and death. The biggest challenge is the search of an ideal topical antiseptic agent that is safe and effective in controlling/eradicating infectious agents from the infection site and as well as promoting/hastening the healing process.

Use of acids as topical agents in diabetic foot infections

Various acids, such as citric, acetic, hyaluronic, and hypochlorous (HOCl) acids are topical agents used in the treatment of diabetic foot infections. Citric acid (2%-3%) has been used to treat a variety of infected wounds and ulcers such as necrotizing fasciitis,

lepromatous ulcers, burns infections, surgical site infections, post-operative wounds in HIV/AIDS patients, traumatic wounds, diabetic foot ulcers, and many others[46-55]. In our initial study, citric acid was successfully used to treat diabetic foot ulcers infected with multiple antibiotic-resistant strains of *P. aeruginosa*[52]. Later on, citric acid was found to be effective against *S. aureus* also. Considering its activity against *S. aureus,* a case report published in 2000 described the successful treatment of a diabetic foot infected with multiple antibiotic-resistant *P. aeruginosa* and *S. aureus* and not responding to conventional antibiotic therapy and local wound care by application of a 2% citric acid solution[53]. A subsequent study reported the activity of citric acid against multiple antibiotic-resistant *E. coli* (MAREC). The in vitro sensitivity of *E. coli* to citric acid was reported in 2008, with successful use of 3% citric acid gel to treat diabetic foot ulcers infected with MAREC, with complete elimination of MAREC from infected sites and successful healing following 29-42 applications of citric acid[54]. A study published in 2010, found that citric acid was effective against almost all aerobic bacterial pathogens commonly associated with diabetic foot ulcers, *i.e. S. aureus*, *P. aeruginosa, E. coli, Klebsiella* spp., *S. albus, Citrobacter* spp., Streptococci and *Proteus vulgaris*. That study reported that citric acid was found effective in control of diabetic foot infections and successful management of Wagner grades I and II ulcers, and even Wagner grade III ulcers without deep osteomyelitis. The success rate was more than 94% in Wagner grade I and II ulcers, and 86.21% in Wagner grade III ulcers[13]. A recent case study described the treatment of a 70-year-old man with a diabetic leg ulcer that developed at the operative site 2 years after coronary artery bypass graft surgery. The ulcer was infected with methicillin-resistant *S. aureus* (MRSA) and had not responded to conventional treatment for months. It was successfully treated by application of 3% citric acid once daily for 30 d[55]. These studies of infected diabetic foot ulcers did not report any adverse effects, which shows that citric acid was found to be a safe and most effective topical antimicrobial agent for the treatment of diabetic foot ulcers. Citric acid has been found effective in chronic plantar ulcers in diabetic individuals with uncontrolled blood sugar levels and infected with multiple antibiotic-resistant bacteria not responding to conventional therapies for months (See Figure 1).

Acetic acid has been used for the treatment of skin and soft tissue infections and burn wound infections caused by *P. aeruginosa*[56-58]. It is rarely used in the treatment of diabetic foot infections and infections caused by other microbial agents. Agrawal *et al*[59] reported that acetic acid controlled the overgrowth of many common isolates in addition to *P. aeruginosa*, including *Streptococcus*, *S. aureus, Proteus mirabilis, Citrobacter* spp., *C. albicans, Aspergillus niger, A. fumigatus* and *Cryptococcus neoformans*. In a previous study, 52-year-old man with diabetic foot ulcer infected with *P. aeruginosa* was successfully treated with 3% acetic acid. Application of acetic acid once daily for 12 d successfully eliminated *P. aeruginosa* and resulted in successful healing of the ulcer[60]. Agrawal *et al*[59] reported remarkable improvement in raw areas in 7-14 d. They noted similar results even in cases with exposed tendons and crush injuries in diabetic patients, and even in infections caused by antibiotic-resistant strains. Fejfarová *et al*[61]also reported favorable outcomes of reduced ulcer dimensions using 1% acetic acid in diabetic foot ulcers, but the difference was not statistically significant.

Hyaluronic acid has been used in the management of diabetic foot ulcers in previous studies[62-65]. Lee *et al*[62] reported higher mean percentages of wound area reduction, wound depth reduction, and increase of healthy granulation tissue in the experimental group than in the control group indicating the potential of hyaluronic acid dressings to accelerate diabetic wound healing. A meta-analysis by Chen *et al*[63] found that hyaluronic acid was beneficial in treating diabetic foot ulcers by increasing the rate of wound healing, evidence that further supports the use of hyaluronic acid in the treatment of diabetic foot ulcers. In a study conducted by Lee *et al*[64], hyaluronic acid treatment achieved a significantly higher complete healing rate(84.6%) than was observed in the control group(41.6%). Healing was faster in the hyaluronic acid group and had a shorter mean duration of achieving a 50% ulcer size reduction without any adverse events, indicating that hyaluronic acid was safe and effective in treating diabetic foot ulcers. A study by Hwang *et al*[65] also concluded that hyaluronic acid dressing without additional substances was a safe and effective treatment for diabetic foot ulcers.

HOCl is another option for acid treatment of infected diabetic foot ulcers. HOCl has been reported to be effective against *Candida* spp., *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and MRSA. It was found comparatively more effective than hydrogen peroxide and povidone-iodine as a potent antimicrobial agent as well as being a better wound healing agent in diabetic foot ulcers, as evidenced by the formation of healthy granulation tissue and a significant reduction in number of organisms on quantitative culture. HOCl has also been reported to soften the wound

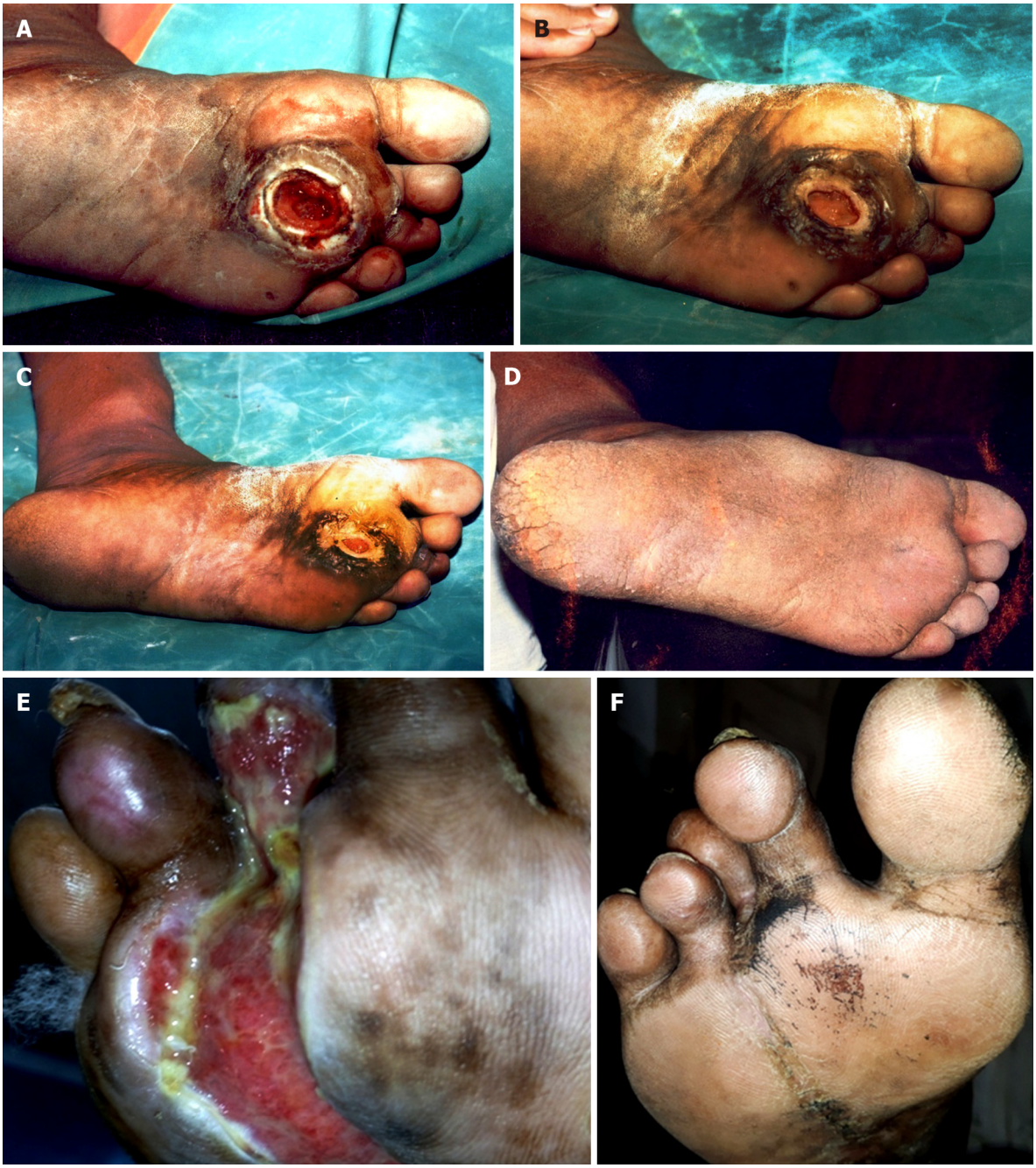

Figure 1 Diabetic foot ulcer. A: Before application of citric acid ointment; B: After 6 applications of citric acid ointment; C: After 16 applications of citric acid ointment; D: After 43 applications of citric acid ointment; E: Before application of citric acid ointment; F: After 25 applications of citric acid ointment.

surface eschar, clean and remove necrotic tissue and biofilms from diabetic foot infections. When compared with hydrogen peroxide and povidone-iodine, HOCl was found to cause a significant reduction in the quantity of exudate, had broad spectrum antimicrobial activity against a variety of microbes, and caused significant reductions in bacterial count and bacterial burden. HOCl appears to be a potent topical antiseptic to treat diabetic foot ulcers. It effectively controls the bioburden without impeding the process of wound healing[66-70].

THE ROLE OF ACIDS IN THE TREATMENT OF DIABETIC FOOT INFECTIONS

The various acids used to treat diabetic foot infections are known to have different roles in controlling infections caused by a variety of microbes and in promoting healing by participating in different stages in the healing of diabetic foot ulcers. Apart

from the specific roles of different acids, the acidic environment created by all acids helps in the following ways. (1) Antimicrobial property: Application of acids to the wound surface creates an acidic environment. A pH of < 6.0 at the wound surface makes it an environment unsuitable for the growth and multiplication of most pathogenic bacteria, which require an optimum pH of 7. Acids thus have antimicrobial property that helps in rapid cleaning of infected surfaces[71]. (2) Inhibition of enzyme activity: The acidic environment inhibits the activity of proteolytic enzymes such as elastase and plasmin produced by various bacteria and by wound itself. The proteases are highly active in alkaline conditions. The acidic environment slows/inhibits their activity and formation of their end products, which are toxic to the healing process[71,72]. (3) Increase in oxygenation: The acidic environ-ment improves tissue oxygenation, which increases resistance to infection, promotes wound healing, and boosts the immune response as well. Improvement of oxygenation increases the production of oxygen radicals that kill bacteria[71,73,74]. (4) Decrease in the toxicity of bacterial end products: The acidic environment helps to reduce the toxicity of bacterial end products, *e.g.*, ammonia, which are toxic to the process of wound healing[71,75]. And (5) Promotion of angiogenesis: The acidic environ-ment promotes angiogenesis, which increases the microcirculation of nutrients and oxygen and boosts fibroblast growth, thereby enhancing epithelialization that leads to faster wound healing[71,76].

SPECIFIC ROLES OF ACIDS

Citric acid

Citric acid is known to have key roles in the process of wound healing. As shown in Table 1, it helps in the management of a variety of infected wounds, including diabetic foot ulcers, in a number of ways.

Antibacterial activity: Citric acid is inhibitory to all bacterial pathogens commonly associated with diabetic foot ulcers. However, it has not been found effective against fungal pathogens. MICs (minimum inhibitory concentrations) in the range of 500-2500 μg/mL against different bacterial isolates from diabetic foot ulcers and nondiabetic traumatic wounds have been reported. *P. aeruginosa* (MIC 500-1000 μg/mL) has been found to be the most susceptible and *Klebsiella* spp. (MIC 2000-2500 μg/mL) least susceptible to citric acid[13,51].

Decrease in the wound surface pH: Decrease in pH has an important role in wound healing. It is a biochemical indicator of wound healing processes and can be used to monitor the progression of wound healing. Wounds treated with citric acid show a pH ranging from 4 to 6, which is not suitable for the growth and multiplication of most bacterial pathogens that cause infection, and thus helps in effective elimination of bacteria from the infection site, leading to rapid cleaning up of infected surfaces. In addition, lowering the pH of wound surfaces also helps to reduce bacterial toxicity (*e.g.*, endotoxins and metalloproteinases), altering protease activity, *etc.* Microbiological evaluation after application of citric acid shows significant reductions in bacterial counts, or no growth, suggesting that citric acid effectively controls the infection[13,76-79].

Fibroblastic growth, neovascularization, and epithelialization: Histopathological studies show that application of citric acid boosts fibroblastic growth and promotes neovascularization, which increases the microcirculation of nutrients and improves oxygenation. This enhances epithelialization and increases the migration of epithelial cells from the surrounding skin. Epithelialization in turn acts as stimulus for the deposition of ground substance and formation of healthy granulation tissue, thereby leading to faster wound healing[13,51,76]. A significant increase in granulation tissue compared with control treatment has been reported after application of citric acid[77].

Notable clinical changes: Application of citric acid results in significant reduction in wound size, early reduction in the amount of discharge and sloughing, and reduces hospital stay[76,77,79]. Significant reductions in common signs of inflammation such as edema, wound discharge, and erythema were noted in a study by Tandon *et al*[77].

Role of acetic acid

In most of the studies, acetic acid has been reported to be inhibitory to *P. aeruginosa* only[56-58]. However, Agrawal *et al*[59] found that it has antibacterial activity against bacterial pathogens commonly associated with wound infections, and antifungal

Table 1 Important roles of various acids in treating diabetic foot ulcers

Order of efficacy	Name of acid	Roles
1	Citric acid	Antibacterial activity[13,51]; Decrease in pH-preventing growth and multiplication[13,76,79]; Fibroblastic growth, neovascularization and epithelialization[13,51,76,77]; Notable clinical changes[76,77,79]
2	Acetic acid	Mainly antipseudomonal activity[55-57]; Anti biofilm activity[81-83]
3	Hyaluronic acid	Reduces inflammatory response[22,84,85]; Increases angiogenesis and promotes granulation[22,84,85]; Proliferation of keratin cells[22,84,85]; Contributes to scarring[22,84,85]; Scavenger of free radicals and tissue degrading enzymes[85,86]; Controls tissue hydration[87]
4	Hypochlorous acid	Antimicrobial activity[70]; Wound debridement[70]; Anti-biofilm activity[70]; Promotes granulation[66]

activity as well. An in vitro study by Lineaweaver *et al*[80] showed that 0.25% acetic acid was toxic to fibroblasts, slowed wound epithelialization, and limited neutrophil function. It is well tolerated in vivo and gives superior results in the treatment of wounds infected with *P. aeruginosa*[57]. Bjarnsholt *et al*[81] reported that acetic acid lowered the pH and was effective in removing biofilms. It kills planktonic bacteria as well as helps eradicate bacteria growing in biofilms. Halstead *et al*[82] found that acetic acid was active against drug-resistant and biofilm-producing bacteria. In a study by Bjarnsholt *et al*[81], acetic acid was found effective against planktonic cells as well as biofilms of *P. aeruginosa* and *S. aureus*, and was found to have potential clinical use as a topical agent to eradicate biofilms in chronic infections caused by *P. aeruginosa.*

Role of hyaluronic acid

Hyaluronic acid is known to have a key role in every phase of wound healing. During the inflammatory phase, it binds to fibrinogen to initiate the clotting pathway, allows inflammatory cell migration, creates edema to allow cell infiltration and inhibits migration of neutrophils to reduce inflammatory response. During the granulation phase, it promotes cell mitosis and increases cell migration and angiogenesis. During re-epithelialization, it is associated with the proliferation of keratin cells and facilitates their migration. During the remodeling phase, it contributes to normal and pathological scarring[22,83,84]. Hyaluronic acid also serves as a scavenger of free radicals and tissue degrading enzymes that cause prolonged inflammation in chronic wounds[84,85]. It has been also reported to have important role in controlling tissue hydration[86].

Role of HOCl

HOCl has antibacterial as well as antifungal activity. It kills pathogens without causing cytotoxicity to keratinocytes or fibroblasts. The killing of pathogens promotes the natural healing process. It has been reported to be an effective wound cleaning and debriding agent by softening the wound surface eschar and removing necrotic tissue and biofilms from infected diabetic foot ulcers[70]. It significantly reduces the number of microbes in wounds and promotes rapid formation of healthy granulation tissue [66].

CONCLUSION

The results of various studies show that conventional antibiotic treatment and local wound care with routinely used topical antiseptic agents have limitations. In view of cytotoxic effects on the cells involved in the process of wound healing, various acids are better options to treat diabetic foot infections. Citric acid, hyaluronic acid, and HOCl in a decreasing order of efficacy, and to a lesser extent acetic acid can be used as better alternatives to control infection and promote the healing of diabetic foot ulcers.

ACKNOWLEDGEMENTS

Authors wish to thank Mr. Jogdand V and Mr. Badne D from Department of Medical Education for their assistance in preparation of manuscript. Thanks are also due to Mr. Ghante D, Mr. Borgaonkar P and Mr. Kothiwale N from Department of Art and

Photography, MIMSR Medical College, Latur, India for photographs of clinically treated diabetic foot ulcers.

REFERENCES

1 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

2 **World Health Organization**. Global report on diabetes. France: 2016. [cited 20 January 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/publications/i/item/9789241565257

3 **Kavitha KV**, Tiwari S, Purandare VB, Khedkar S, Bhosale SS, Unnikrishnan AG. Choice of wound care in diabetic foot ulcer: A practical approach. *World J Diabetes* 2014; **5**: 546-556 [PMID: 25126400 DOI: 10.4239/wjd.v5.i4.546]

4 **Singh N**, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005; **293**: 217-228 [PMID: 15644549 DOI: 10.1001/jama.293.2.217]

5 **International Diabetes Federation**. IDF Diabetes Atlas.9th ed. 2019. [cited 2 January 2021]. In: International Diabetes Federation [Internet].Available from: https://www.diabetesatlas.org

6 **International Diabetes Federation**. Clinical Practice Recommendation on the Diabetic Foot: A guide for health care professionals. 2017:1-70 [cited 18 January 2021]. In: International Diabetes Federation [Internet]. Available from: https://www.idf.org/component/attachments/?task=download&id=1152

7 **Bakker K**, Apelqvist J, Schaper NC; International Working Group on Diabetic Foot Editorial Board. Practical guidelines on the management and prevention of the diabetic foot 2011. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 225-231 [PMID: 22271742 DOI: 10.1002/dmrr.2253]

8 **Farahani RM**, Kloth LC. The hypothesis of 'biophysical matrix contraction': wound contraction revisited. *Int Wound J* 2008; **5**: 477-482 [PMID: 18593398 DOI: 10.1111/j.1742-481X.2007.00402.x]

9 **Boulton AJ**, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005; **366**: 1719-1724 [PMID: 16291066 DOI: 10.1016/S0140-6736(05)67698-2]

10 **Richard JL**, Sotto A, Lavigne JP. New insights in diabetic foot infection. *World J Diabetes* 2011; **2**: 24-32 [PMID: 21537457 DOI: 10.4239/wjd.v2.i2.24]

11 **Viswanathan V**, Rao VN. Managing diabetic foot infection in India. *Int J Low Extrem Wounds* 2013; **12**: 158-166 [PMID: 23667101 DOI: 10.1177/1534734613486153]

12 **Jaju K**, Pichare A, Davane M, Nagoba B. Profile and Antibiotic Susceptibility of Bacterial Pathogens Associated With Diabetic Foot Ulcers From a Rural Area. *Wounds* 2019; **31**: 158-162 [PMID: 31184594]

13 **Nagoba BS**, Gandhi RC, Wadher BJ, Rao A, Hartalkar AR, Selkar SP. A simple and effective approach for the treatment of diabetic foot ulcers with different Wagner grades. *Int Wound J* 2010; **7**: 153-158 [PMID: 20455958 DOI: 10.1111/j.1742-481X.2010.00666.x]

14 **Gadepalli R**, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care* 2006; **29**: 1727-1732 [PMID: 16873771 DOI: 10.2337/dc06-0116]

15 **Viswanathan V**, Jasmine JJ, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. *J Assoc Physicians India* 2002; **50**: 1013-1016 [PMID: 12421021]

16 **Gerding DN**. Foot infections in diabetic patients: the role of anaerobes. *Clin Infect Dis* 1995; **20** Suppl 2: S283-S288 [PMID: 7548576 DOI: 10.1093/clinids/20.Supplement_2.S283]

17 **Johnson S**, Lebahn F, Peterson LR, Gerding DN. Use of an anaerobic collection and transport swab device to recover anaerobic bacteria from infected foot ulcers in diabetics. *Clin Infect Dis* 1995; **20** Suppl 2: S289-S290 [PMID: 7548577 DOI: 10.1093/clinids/20.supplement_2.s289]

18 **Raja NS**. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. *J Microbiol Immunol Infect* 2007; **40**: 39-44 [PMID: 17332905]

19 **Bansal E**, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol* 2008; **51**: 204-208 [PMID: 18603682 DOI: 10.4103/0377-4929.41685]

20 **Banu A**, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. *Australas Med J* 2015; **8**: 280-285 [PMID: 26464584 DOI: 10.4066/AMJ.2015.2422]

21 **Damir A**. Why Diabetic foot ulcers do not heal. *J Int Med Sci Acad* 2011; **24**: 205

22 **Lipsky BA**, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS; Infectious Diseases Society of America. Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2004; **39**: 885-910 [PMID: 15472838 DOI: 10.1086/424846]

23 **Niezgoda JA**, Sordi PJ, Hermans MH. Evaluation of Vashe Wound Therapy in the clinical management of patients with chronic wounds. *Adv Skin Wound Care* 2010; **23**: 352-357 [PMID: 20664326 DOI: 10.1097/01.ASW.0000383198.35815.a2]

24 **Knighton DR**, Halliday B, Hunt TK. Oxygen as an antibiotic. A comparison of the effects of inspired oxygen concentration and antibiotic administration on *in vivo* bacterial clearance. *Arch Surg* 1986; **121**: 191-195 [PMID: 3511888 DOI: 10.1001/archsurg.1986.01400020077009]

25 **Mah TF**, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001; **9**: 34-39 [PMID: 11166241 DOI: 10.1016/s0966-842x(00)01913-2]

26 **Najafi RB**, Samani SM, Pishva N, Moheimani F. Formulation and Clinical Evaluation of Povidone-Iodine Ophthalmic Drop. *Iran J Pharm Res* 2003; **2**: 157-160 [DOI: 10.22037/ijpr.2010.44]

27 **Simmons TJ**, Hashim D, Vajtai R, Ajayan PM. Large area-aligned arrays from direct deposition of single-wall carbon nanotube inks. *J Am Chem Soc* 2007; **129**: 10088-10089 [PMID: 17663555 DOI: 10.1021/ja073745e]

28 **Nagoba B**, Kolhe S, Wadher B. Antibiotic Treatment Failure in Chronic Nosocomial Wound Infections. *Eur J Gen Med* 2009; **6**: 60 [DOI: 10.29333/ejgm/82641]

29 **Brånemark PI**, Albrektsson B, Lindström J, Lundborg G. Local tissue effects of wound disinfectants. *Acta ChirScand Suppl* 1966; **357**: 166-176 [PMID: 5335618]

30 **Kramer SA**. Effect of povidone-iodine on wound healing: a review. *J VascNurs* 1999; **17**: 17-23 [PMID: 10362983 DOI: 10.1016/s1062-0303(99)90004-3]

31 **Pathare NA**. Infections and diabetes mellitus. *J Diabet Asso Ind* 1998; **38**: 4-6

32 **Robson MC**, Edstrom LE, Krizek TJ, Groskin MG. The efficacy of systemic antibiotics in the treatment of granulating wounds. *J Surg Res* 1974; **16**: 299-306 [PMID: 4208080 DOI: 10.1016/0022-4804(74)90046-8]

33 **Wilson JR**, Mills JG, Prather ID, Dimitrijevich SD. A toxicity index of skin and wound cleansers used on *in vitro* fibroblasts and keratinocytes. *Adv Skin Wound Care* 2005; **18**: 373-378 [PMID: 16160464 DOI: 10.1097/00129334-200509000-00011]

34 **Wang L**, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, Hwong W, Barati E, Belisle B, Celeri C, Robson MC. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. *J Burns Wounds* 2007; **6**: e5 [PMID: 17492050]

35 **Agarwal R**, Khan A, Aggarwal AN, Gupta D. Efficacy & safety of iodopovidone pleurodesis: a systematic review & meta-analysis. *Indian J Med Res* 2012; **135**: 297-304 [PMID: 22561614]

36 **Dumville JC**, McFarlane E, Edwards P, Lipp A, Holmes A, Liu Z. Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. *Cochrane Database Syst Rev* 2015; CD003949 [PMID: 25897764 DOI: 10.1002/14651858.CD003949.pub4]

37 **Cooper ML**, Laxer JA, Hansbrough JF. The cytotoxic effects of commonly used topical antimicrobial agents on human fibroblasts and keratinocytes. *J Trauma* 1991; **31**: 775-82; discussion 782 [PMID: 2056541 DOI: 10.1097/00005373-199106000-00007]

38 **Dumville JC**, Lipsky BA, Hoey C, Cruciani M, Fiscon M, Xia J. Topical antimicrobial agents for treating foot ulcers in people with diabetes. *Cochrane Database Syst Rev* 2017; **6**: CD011038 [PMID: 28613416 DOI: 10.1002/14651858.CD011038.pub2]

39 **Miller JM**, Creazzo J, Witt C. Wound healing: an introductory clinical approach. *Contemp Pediatr Physic* 1992; **1**: 38-42

40 **Dakin HD**. THE ANTISEPTIC ACTION OF HYPOCHLORITES: The Ancient History of the "New Antiseptic.". *Br Med J* 1915; **2**: 809-810 [PMID: 20767916 DOI: 10.1136/bmj.2.2866.809]

41 **Kozol RA**, Gillies C, Elgebaly SA. Effects of sodium hypochlorite (Dakin's solution) on cells of the wound module. *Arch Surg* 1988; **123**: 420-423 [PMID: 3348732 DOI: 10.1001/archsurg.1988.01400280026004]

42 **Kjolseth D**, Frank JM, Barker JH, Anderson GL, Rosenthal AI, Acland RD, Schuschke D, Campbell FR, Tobin GR, Weiner LJ. Comparison of the effects of commonly used wound agents on epithelialization and neovascularization. *J Am Coll Surg* 1994; **179**: 305-312 [PMID: 7520807]

43 **Bellinger CG**, Conway H. Effects of silver nitrate and sulfamylon on epithelial regeneration. *PlastReconstr Surg* 1970; **45**: 582-585 [PMID: 4910557 DOI: 10.1097/00006534-197006000-00009]

44 **Lineaweaver W**, McMorris S, Soucy D, Howard R. Cellular and bacterial toxicities of topical antimicrobials. *PlastReconstr Surg* 1985; **75**: 394-396 [PMID: 3975287 DOI: 10.1097/00006534-198503000-00016]

45 **Ramirez-Acuña JM**, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, Flores-Morales V, Martinez-Fierro ML. Diabetic Foot Ulcers: Current Advances in Antimicrobial Therapies and Emerging Treatments. *Antibiotics (Basel)* 2019; **8** [PMID: 31652990 DOI: 10.3390/antibiotics8040193]

46 **Nagoba BS**, Gandhi RC, Wadher BJ, Gandhi SP, Selkar SP. Citric acid treatment of necrotizing fasciitis: a report of two cases. *Int Wound J* 2010; **7**: 536-538 [PMID: 20666856 DOI: 10.1111/j.1742-481X.2010.00721.x]

47 **Nagoba BS**, Wadher BJ, Rao A, Selkar SP, Gandhi RC. Treatment of lepromatous ulcers using citric acid as a sole antimicrobial agent. *Int Wound J* 2012; **9**: 553-556 [PMID: 22264346 DOI: 10.1111/j.1742-481X.2011.00914.x]

48 **Nagoba BS**, Gandhi RC, Hartalkar AR, Wadher BJ, Selkar SP. Simple, effective and affordable approach for the treatment of burns infections. *Burns* 2010; **36**: 1242-1247 [PMID: 20554394 DOI: 10.1016/j.burns.2010.05.011]

49 **Nagoba B**, Raju R, Wadher B, Gandhi R, Rao AK, Selkar S, Hartalkar A. Citric acid treatment of surgical site infections: a prospective open study. *Wound Pract Res* 2011; **19**: 82-87

50 **Nagoba B**, Patil Dawale C, Raju R, Wadher B, Chidrawar S, Selkar S, Suryawanshi N. Citric acid

treatment of post operative wound infections in HIV/AIDS patients. *J Tissue Viability* 2014; **23**: 24-28 [PMID: 24411100 DOI: 10.1016/j.jtv.2013.12.004]

51 **Nagoba B**, Gandhi R, Wadher B, Rao A, Selkar S. Simple and effective approach for the treatment of traumatic wounds in non-diabetic patients: a prospective open study. *Int Wound J* 2013; **10**: 585-589 [PMID: 22781002 DOI: 10.1111/j.1742-481X.2012.01026.x]

52 **Nagoba BS**, Deshmukh SR, Wadher BJ, Mahabaleshwar L, Gandhi RC, Kulkarni PB, Mane VA, Deshmukh JS. Treatment of superficial pseudomonal infections with citric acid: an effective and economical approach. *J Hosp Infect* 1998; **40**: 155-157 [PMID: 9819695 DOI: 10.1016/s0195-6701(98)90095-0]

53 **Nagoba BS**, Kulkarni PB, Wadher BJ, Kulkarni UP, Mahabaleshwar L. Citric acid treatment of diabetic foot: a simple and effective approach. *J Assoc Physicians India* 2000; **48**: 739-741 [PMID: 11273514]

54 **Nagoba BS**, Wadher BJ, Rao AK, Kore GD, Gomashe AV, Ingle AB. A simple and effective approach for the treatment of chronic wound infections caused by multiple antibiotic resistant Escherichia coli. *J Hosp Infect* 2008; **69**: 177-180 [PMID: 18485531 DOI: 10.1016/j.jhin.2008.03.014]

55 **Nagoba BS**, Rawal C, Davane M. Citric acid treatment of diabetic leg ulcer infected with methicillin resistant Staphylococcus aureus. *J Wound Care* 2021; In press

56 **Sloss JM**, Cumberland N, Milner SM. Acetic acid used for the elimination of Pseudomonas aeruginosa from burn and soft tissue wounds. *J R Army Med Corps* 1993; **139**: 49-51 [PMID: 8355236 DOI: 10.1136/jramc-139-02-04]

57 **Nagoba BS**, Deshmukh SR, Wadher BJ, Patil SB. Acetic acid treatment of pseudomonal postoperative wound infection. *J Hosp Infect* 1997; **36**: 243-244 [PMID: 9253707 DOI: 10.1016/s0195-6701(97)90201-2]

58 **Nagoba BS**, Selkar SP, Wadher BJ, Gandhi RC. Acetic acid treatment of pseudomonal wound infections--a review. *J Infect Public Health* 2013; **6**: 410-415 [PMID: 23999348 DOI: 10.1016/j.jiph.2013.05.005]

59 **Agrawal KS**, Sarda AV, Shrotriya R, Bachhav M, Puri V, Nataraj G. Acetic acid dressings: Finding the Holy Grail for infected wound management. *Indian J Plast Surg* 2017; **50**: 273-280 [PMID: 29618862 DOI: 10.4103/ijps.IJPS_245_16]

60 **Nagoba B**, Wadher B, Kulkarni P, Kolhe S. Acetic acid treatment of pseudomonal wound infections. *Eur J Gen Med* 2008; **5**: 104-106 [DOI: 10.29333/ejgm/82586]

61 **Fejfarová V**, Tibenská H, Niklová J, Bém R, Dubský M, Wosková V, Němcová A, Jirkovská A, Jude E, Lánská V. Benefits of Acidifying Agents in Local Therapy of Diabetic Foot Ulcers Infected by *Pseudomonas*sp: A Pilot Study. *Int J Low Extrem Wounds* 2019; **18**: 262-268 [PMID: 31155991 DOI: 10.1177/1534734619848573]

62 **Lee Y**, Yun TK, Han S. Effect of Hyaluronic Acid Dressing on Diabetic Ulcer Healing -A Pilot Study Effect of Hyaluronic Acid Dressing on Diabetic Ulcer Healing - A Pilot Study. *J Korean Wound Manag Soc* 2014; **10**: 67-74

63 **Chen CP**, Hung W, Lin SH. Effectiveness of hyaluronic acid for treating diabetic foot: a systematic review and meta-analysis. *Dermatol Ther* 2014; **27**: 331-336 [PMID: 25039587 DOI: 10.1111/dth.12153]

64 **Lee M**, Han SH, Choi WJ, Chung KH, Lee JW. Hyaluronic acid dressing (Healoderm) in the treatment of diabetic foot ulcer: A prospective, randomized, placebo-controlled, single-center study. *Wound Repair Regen* 2016; **24**: 581-588 [PMID: 26972358 DOI: 10.1111/wrr.12428]

65 **Hwang Y**, Park YJ, Shim D-W, Lee M, Park KH, Suh JW, Han SH, Choi WJ, Lee JW. Hyaluronic Acid Dressing in the Treatment of Diabetic Foot Ulcer: A Prospective, Randomized, Placebo-Controlled, Single-Center Study. *Foot Ankle Orthop* 2016; **1**: 2473011416S0007 [DOI: 10.1177/2473011416S00078]

66 **Ragab II**, Kamal A. The Effectiveness of Hypochlorous Acid Solution on Healing of Infected Diabetic Foot Ulcers. *J Educ Pract* 2017; **8**: 58-71

67 **Payne WG**, Wright TE, Ko F, Wheeler C, Wang X, Robson MC. Bacterial Degradation of Growth Factors. *J Appl Res* 2003; **3**: 35-40

68 **Chopra I**. Efflux-based antibiotic resistance mechanisms: the evidence for increasing prevalence. *J Antimicrob Chemother* 1992; **30**: 737-739 [PMID: 1289349 DOI: 10.1093/jac/30.6.737]

69 **Chopra I**, Johnson SC, Bennett PM. Inhibition of Providencia stuartii cell envelope enzymes by chlorhexidine. *J Antimicrob Chemother* 1987; **19**: 743-751 [PMID: 2956230 DOI: 10.1093/jac/19.6.743]

70 **Mekkawy MM**, Kamal A. A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound. *J Educ Pract* 2014; **5**: 89-101

71 **Nagoba B**, Suryawanshi N, Wadher B, Selkar S. Acidic Environment and Wound Healing: A Review. *Wounds* 2015; **27**: 5-11

72 **Greener B**, Hughes AA, Bannister NP, Douglass J. Proteases and pH in chronic wounds. *J Wound Care* 2005; **14**: 59-61 [PMID: 15739652 DOI: 10.12968/jowc.2005.14.2.26739]

73 **Hunt TK**, Hopf HW. Wound healing and wound infection. What surgeons and anesthesiologists can do. *Surg Clin North Am* 1997; **77**: 587-606 [PMID: 9194882 DOI: 10.1016/s0039-6109(05)70570-3]

74 **Hunt TK**, Beckert S. Therapeutical and practical aspects of oxygen in wound healing. In: Lee BY. The Wound Management Manual. New York: McGraw-Hill Professional, 2004: 44-54

75 **Gethin G**. The significance of surface pH in chronic wounds. *Wounds UK* 2007; **3**: 52-56

76 **Nagoba BS**, Gandhi RC, Wadher BJ, Potekar RM, Kolhe SM. Microbiological, histopathological and clinical changes in chronic infected wounds after citric acid treatment. *J Med Microbiol* 2008; **57**: 681-682 [PMID: 18436609 DOI: 10.1099/jmm.0.47647-0]

77 **Tandon S**, Singh B, Kapoor S, Mangal S. Comparison of Effect of pH Modulation on Wound Healing with Topical Application of Citric Acid Versus Superoxide Ions. *Niger J Surg* 2020; **26**: 122-126 [PMID: 33223809]

78 **Bennison LR**, Miller CN, Summers RJ, Minnis AMB, Sussman G, Mcguiness W, Bennison LR. The pH of wounds during healing and infection: a descriptive literature review. *Wound Pract Res* 2017; **25**: 63-69

79 **Prabhu V**, Prasadi S, Shivani A, Gore A, Pawar V. Does wound pH modulation with 3% citric acid solution dressing help in wound healing: A pilot study. *Saudi Surg J* 2014; **2**: 38-46 [DOI: 10.4103/2320-3846.140690]

80 **Lineaweaver W**, Howard R, Soucy D, McMorris S, Freeman J, Crain C, Robertson J, Rumley T. Topical antimicrobial toxicity. *Arch Surg* 1985; **120**: 267-270 [PMID: 3970664 DOI: 10.1001/archsurg.1985.01390270007001]

81 **Bjarnsholt T**, Alhede M, Jensen PØ, Nielsen AK, Johansen HK, Homøe P, Høiby N, Givskov M, Kirketerp-Møller K. Antibiofilm Properties of Acetic Acid. *Adv Wound Care (New Rochelle)* 2015; **4**: 363-372 [PMID: 26155378 DOI: 10.1089/wound.2014.0554]

82 **Halstead FD**, Rauf M, Moiemen NS, Bamford A, Wearn CM, Fraise AP, Lund PA, Oppenheim BA, Webber MA. The Antibacterial Activity of Acetic Acid against Biofilm-Producing Pathogens of Relevance to Burns Patients. *PLoS One* 2015; **10**: e0136190 [PMID: 26352256 DOI: 10.1371/journal.pone.0136190]

83 **Frenkel JS**. The role of hyaluronan in wound healing. *Int Wound J* 2014; **11**: 159-163 [PMID: 22891615 DOI: 10.1111/j.1742-481X.2012.01057.x]

84 **Chen WY**, Abatangelo G. Functions of hyaluronan in wound repair. *Wound Repair Regen* 1999; **7**: 79-89 [PMID: 10231509 DOI: 10.1046/j.1524-475x.1999.00079.x]

85 **Kvam BJ**, Fragonas E, Degrassi A, Kvam C, Matulova M, Pollesello P, Zanetti F, Vittur F. Oxygen-derived free radical (ODFR) action on hyaluronan (HA), on two HA ester derivatives, and on the metabolism of articular chondrocytes. *Exp Cell Res* 1995; **218**: 79-86 [PMID: 7737382 DOI: 10.1006/excr.1995.1133]

86 **Laurent TC**, Laurent UB, Fraser JR. The structure and function of hyaluronan: An overview. *Immunol Cell Biol* 1996; **74**: A1-A7 [PMID: 8724014 DOI: 10.1038/icb.1996.32]

87 **Nelson CM**, Bissell MJ. Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. *Annu Rev Cell Dev Biol* 2006; **22**: 287-309 [PMID: 16824016 DOI: 10.1146/annurev.cellbio.22.010305.104315]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1550-1562

DOI: 10.4239/wjd.v12.i9.1550

ISSN 1948-9358 (online)

MINIREVIEWS

Diabetes and COVID-19: Role of insulin resistance as a risk factor for COVID-19 severity

Charitha Gangadharan, Rupa Ahluwalia, Alben Sigamani

ORCID number: Charitha Gangadharan 0000-0002-4623-1694; Rupa Ahluwalia 0000-0002-6885-0875; Alben Sigamani 0000-0002-6927-1947.

Author contributions: Sigamani A conceived the idea; Gangadharan C wrote the first draft; Ahluwalia R contributed to the review, restructuring and modifications of the final draft; All the authors contributed for structuring and improvement of the final draft, and all approved the final draft.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Manuscript source: Invited manuscript

Charitha Gangadharan, Department of Clinical Research, Narayana Hrudayalaya Limited, Bangalore 560099, Karnataka, India

Rupa Ahluwalia, Consultant in Diabetes and Endocrinology, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich NR4 7UY, United Kingdom

Alben Sigamani, Chief Scientific Officer, Numen Health, Bangalore 560095, Karnataka, India

Corresponding author: Alben Sigamani, MBBS, MD, MSc, Professor, Chief Scientific Officer, Numen Health, No. 19, 4th C Cross Koramangala Industrial, 5th Block, Area, Bengaluru 560095, Karnataka, India. dralbens@numenhealth.com

Abstract

Patients with diabetes are more susceptible to coronavirus disease 2019 (COVID-19), and as a consequence, develop more severe form of disease. This is partly due to a systemic inflammatory state and pro thrombotic milieu seen in metabolic syndrome. In this review, we attempt to explore the pathogenetic links between insulin resistance and COVID-19 disease severity. Insulin resistance is an underlying condition for metabolic syndromes, including type 2 diabetes, which impairs insulin signaling pathways affecting metabolic and cardiovascular homeostasis. A high concentration of circulating insulin shifts the balance to mitogen activated protein kinase (MAPK)-dependent signaling and causes endothelial cell damage. The phosphatidylinositol 3 kinase and MAPK dependent signaling pathways maintain a balance between nitric oxide-dependent vasodilator and endothelin-1 dependent vasoconstriction actions of insulin. Vascular smooth muscle cell dysfunction is responsible for inflammation and blood coagulation leading to microvascular and macrovascular complications in diabetes. Hyperactivity in renin-angiotensin system is implicated in development of islet oxidative stress and subsequent β-cell dysfunction, as it alters the islet blood flow. These deleterious effects of insulin resistance involving altered blood pressure, vascular dysfunction, and inflammation could be associated with increased severity in COVID-19 patients. We conclude that clinical and/or biochemical markers of insulin resistance should be included as prognostic markers in assessment of acute COVID-19 disease.

Key Words: Insulin resistance; Renin–angiotensin system; Blood flow measurements; Inflammation; Thrombosis; Severity of COVID-19

Specialty type: Endocrinology and metabolism

Country/Territory of origin: India

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

Received: February 19, 2021
Peer-review started: February 19, 2021
First decision: May 12, 2021
Revised: June 11, 2021
Accepted: July 30, 2021
Article in press: July 30, 2021
Published online: September 15, 2021

P-Reviewer: Balbaa ME, Lee KS, Ro S, Wu QN
S-Editor: Fan JR
L-Editor: A Filipodia
P-Editor: Wang LYT

Core Tip: Diabetes has been associated with an increased risk of developing coronavirus disease 2019 (COVID-19) as well as more severe outcomes as a consequence. The pathogenetic link between insulin resistance and COIVD-19 disease severity is not fully understood. Establishing an association between insulin resistance and COVID-19 severity can help to develop targeted therapeutic interventions and potentially improve outcomes amongst the at-risk group.

Citation: Gangadharan C, Ahluwalia R, Sigamani A. Diabetes and COVID-19: Role of insulin resistance as a risk factor for COVID-19 severity. *World J Diabetes* 2021; 12(9): 1550-1562
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1550.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1550

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral infection caused by a single stranded RNA virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and first identified in Wuhan, China. World Health Organization declared COVID-19 a global pandemic on March 11, 2020, with confirmed positive cases reported from more than 200 countries[1]. The most common signs and symptoms of SARS-CoV-2 infections range from asymptomatic to mild (20%-80% restricted to upper respiratory tract), while some may rapidly develop acute respiratory distress syndrome (ARDS), regional inflammation leading to pneumonia, respiratory failure, arrhythmias, acute cardiac injury, shock, multiple organ failure, and death. The severity of infection is manifested particularly in patients with concomitant conditions, including diabetes, cardiovascular disease, hypertension, obesity, and chronic obstructive pulmonary disease[2]. Epidemiological data has revealed that the mortality rate is higher amongst the older population with pre-existing comorbidities, including diabetes[3]. The clinical features of hospitalized COVID-19 patients reported by Liu *et al*[3] showed that elderly patients had underlying comorbidities including hypertension (27.78%), diabetes (16.67%), coronary heart disease (11.11%), and liver disease (5.56%). Elderly patients (median age 68) have had more complications following hospital admission, including ARDS (22.22%) and need for invasive ventilator support (22.22%)[3].

COVID-19 AND DIABETES MELLITUS

During the first wave, compared to Asia, a greater percentage of people in Europe had worse outcomes from COVID-19[4]. An analysis on comorbidities amongst Italian subjects infected with COVID-19 revealed diabetes mellitus as the highest-ranking condition, followed by systemic hypertension and ischemic heart disease[1]. A study from Wuhan, China on the characteristics of COVID-19 patients showed that those with pre-existing diabetes (prevalence of 9.7%)[5] were more likely to require admission to an intensive care unit (20%) or to die as a result of severe COVID-19[6]. A study on glycemic characteristics and clinical outcomes of COVID-19 inpatients in United States showed that poor glycemic control correlated with longer stays in the hospital and a higher mortality rate (28.8% *vs* 6.2%; $P < 0.001$) in the diabetic group compared to nondiabetic group[7]. It is now well recognized that advanced age, presence of diabetes mellitus, hypertension, and severe obesity (body mass index > 40 kg/m^2) are associated with increased hospital admissions, morbidity, and mortality in patients with COIVD-19[8].

POTENTIAL MECHANISMS

Individuals with type 2 diabetes as part of a metabolic syndrome are characterized by increased activation of the renin angiotensin system, resulting in the development of diabetic complications, including micro and macro vascular diseases[9]. Renin

angiotensin system activation also triggers pro-inflammatory and procoagulant pathways, which further contribute to endothelial dysfunction and impaired vascular tone. Circulating levels of renin-angiotensin system (RAS) components, especially angiotensin II (ATII), have a potential role in endothelial cell dysfunction, insulin resistance, inflammation, and proliferation. Evidence has shown that insulin resistance is also positively associated with high prevalence of subclinical coronary artery disease and an altered adaptive immune response[10]. In addition, immune dysregulation and hyper inflammatory response induced by SARS-CoV-2 causes a delayed and impaired interferon response, lymphocyte exhaustion and cytokine storm in patients with diabetes and underlying insulin resistance[11,12]. However, the extent to which insulin resistance contributes to COVID-19 disease severity, along the underlying mechanisms involved, remain largely unexplored. In this review we explore the potential mechanisms linking insulin resistance and disease severity of COVID-19.

COVID-19–INFLAMMATORY PATHWAYS

SARS-CoV-2 is transmitted primarily *via* respiratory droplets, direct and indirect contact, with possible, but unproven, fecal-oral route. Following infection of SARS-CoV-2 in patients, the onset of symptoms occurs within 4 to 5 d, and 97.5% of symptomatic patients develop the disease within 2 wk. A common feature in a subgroup of severely ill patients admitted to hospital exhibit severe respiratory failure with dyspnea and bilateral lung infiltration as observed on chest computerized tomography scans, lymphopenia, diarrhea, and hemoptysis[12]. Thus, the spectrum of disease is broad, including a few reported cases from China involving neurological symptoms, including strokes[13]. It is believed that the disease severity and mortality could be due to the cytokine storm or an imbalance in the function of angiotensin converting enzyme 2 (ACE2) caused by the virus, which disrupts the RAS[14].

The entry of SARS-CoV-2 into the human body is facilitated by the RAS and it's regulator ACE2[15]. The RAS and ACE2 play a key role in maintaining physiological functions of kidneys, heart, and lungs. The kidneys, lungs, heart, and endothelium abundantly express ACE2 protein. Further, ACE2 regulates and maintains homeostasis of local concentration of ATII, a potent vasoconstrictor and pro-inflammatory agent, which enhances fibrosis and is a vital component of the RAS system in maintaining cardiovascular functions[16]. Two independent research groups proved that the viral spike (S) protein binds to ACE2 protein on tissue cells leading to direct membrane fusion between the host cell and the virus, facilitating the viral entry and release of viral RNA genome into the host cell[15,17]. Binding of SARS-CoV-2 to ACE2 protein results in the loss of ACE2 expression due to internalization and shedding with decreased degradation of ATII, which leads to several pro-inflammatory and pro-fibrotic actions with lung injury[18]. The viral entry is also dependent on the expression of transmembrane serine protease 2 (TMPRSS2), and the endosomal cysteine protease cathepsin B and L (CatB/L). In vitro studies have shown that the TMPRSS2 inhibitor camostat mesylate, along with the CatB/L inhibitor E-64d, inhibited viral entry into Caco-2 cells[19]. During the late phase of infection, SARS-CoV-2 can infect cells with Fc receptors which are involved in antibody mediated internalization in macrophages ,monocytes, or B cells, even without ACE2 protein and TMPRSS2 expression[20], contributing further to a dampened immune response.

During an acute infection, an effective immune response is elicited for optimal pathogen clearance. In most individuals with SARS-CoV-2 infection, both innate and adaptive immune responses are activated leading to successful recovery. However, in severe cases of COVID-19, an excessive inflammatory innate response and deregulated adaptive host immune defense may cause tissue damage at both the viral entry site and systemic level. Autopsy findings in COVID-19 related deaths shows interstitial mononuclear inflammatory infiltrates lymphocytes in the lung and severe lymphopenia, with hyper activated T cells in the peripheral blood[21]. In addition, a decreased level of regulatory T cells was observed in severe COVID-19 cases. In severe cases of COVID-19 requiring intensive care in hospitals showed higher levels of inflammatory cytokines, including interleukin (IL)-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), tumour necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) in serum, suggest a dysfunctional immune response triggering a cytokine storm that mediates wide-spread lung inflammation[22]. The release of free radicals along with cytokine storm causes damage to the host with multiple organ failure and severe ARDs. The circulating levels of G-CSF, interferon gamma-induced protein 10, MCP-1, macrophage inflammatory protein1α, and TNF-α

are elevated in intensive care unit patients compared to those not requiring intensive care, further confirming the relation between cytokine storm, severity of COVID-19, mortality, and multiple organ failure[23].

Thromboembolism is another complication and cause of death in COVID-19. The main function of thrombin is to promote clot formation by activating platelets and converting fibrinogen to fibrin. Thrombin can augment inflammation *via* proteinase activated receptor-1, and its production is tightly controlled by the level of physiological anticoagulants like anti thrombin III, tissue factor pathway inhibitor and protein C system. During hyper inflammation, an imbalance in pro-coagulant-anticoagulant systems, which predisposes the patient to the development of microthrombosis, disseminated intravascular coagulation, and multi organ failure, were reported in severe COIVD-19 pneumonia[24]. Disseminated intravascular coagulation, lower platelet count, and an increased d-Dimer evidenced in non survivors of COVID-19 is associated with poor prognosis[24]. Notably, endothelial cell death caused by COVID-19 leads to vascular leakage and induces cytopathic effect on airway epithelial cells[25]. Thus, the inflammatory mediators lead to vascular hyper permeability and stimulate endothelial cells that express ACE2 protein on blood vessels, which together with viral particles cause systemic inflammation, higher concentration of ATII, and induces tissue factor and plasminogen activator inhibitor 1 expression by endothelial cells *via* ATI receptors, leading to a hypercoagulable state. Thus, it is likely that dysregulation in RAS pathways contribute to cross talk between inflammation and thrombosis contributing to increased mortality. Patients with diabetes are now known to be at higher risk of severe clinical outcomes of COVID-19. Furthermore, insulin therapy itself has proven fatal in patients with COVID-19 and concurrent diabetes[26]. It is becoming increasingly apparent that an impaired adaptive immune response, characterized by chronic inflammation as seen amongst type 2 diabetes as well as obesity, can lead to abrupt systemic metabolic alterations contributing to increased production of inflammatory cytokines, fueling a cytokine storm resulting in poor outcomes[27]. Recent evidence also suggests that COVID-19 infection could precipitate acute metabolic complications of diabetes such as diabetic ketoacidosis and hyperglycemia[28]. The proposed mechanism is likely to involve ACE2 protein, which is expressed in pancreas including β-cells, and serve as a binding site or receptor for the SARS-CoV-2[29]. It is assumed that elevated circulating insulin levels with insulin resistance in type 2 diabetes underpins increased ACE2 expression in lung epithelial cells, and hence, contributes to severe disease associated with COVID-19 infection. Insulin resistance, a hallmark feature of type 2 diabetes, is known to elevate inflammatory cytokines[30], endothelial dysfunction[31] and procoagulant state[30] in this high-risk subgroup even before SARS-CoV-2 infection. Hence, as a result, insulin resistance potentially contributes to severity of COVID-19 associated with poorer outcomes amongst patients with pre-existing diabetes.

INSULIN AND VASCULAR INFLAMMATION

Insulin is a potent anabolic hormone, involved in stimulating glucose uptake in skeletal muscles and adipocytes, promoting glycogen synthesis in skeletal muscles, suppressing hepatic glucose production, and inhibiting lipolysis in adipocytes. The biological actions of insulin are initiated by binding to its insulin receptor, a heterotetrameric tyrosine receptor kinase which phosphorylates intracellular substrates like insulin receptor substrate (IRS-1). Interaction of tyrosine phosphorylated IRS-1 creates Src Homology, recruit's phosphatidylinositol 3 kinase (PI3K) and growth factor receptor-bound protein 2 (Grb-2). Upon phosphorylation and activation of PI3K, it subsequently phosphorylates and activates other downstream serine/threonine kinase, including Akt and atypical protein kinase C, culminating in many metabolic actions of insulin including glucose uptake through insulin responsive glucose transporter (GLUT) 4[32]. In addition to PI3K dependent insulin signaling, activation of Src homology Grb-2 results in the activation of GTP binding protein Ras, Raf, mitogen activated protein kinase (MAPK). MAPK dependent insulin signaling regulates the biological actions related to growth, mitogenesis, and differentiation. Thus, there are two major insulin signaling pathways: PI3K dependent signaling mediates metabolic actions and MAPK dependent signaling regulates non-metabolic mitogenic cardiovascular physiology. In the presence of insulin resistance and hyperglycaemia, shared insulin signaling pathways are impaired in metabolic and cardiovascular tissues, contributing to reciprocal relationship between insulin resistance and endothelial dysfunction[33].

The vascular functions of insulin are complex, with either protective or deleterious effects on vasculature[34]. A normal endothelial cell function is important in maintaining vascular tone and homeostasis by regulating vasodilation and constriction, thrombosis and fibrinolysis, platelet activation and leukocyte recruitment, and smooth muscle function. In addition to metabolic actions of insulin, PI3K signaling regulates the production of vasodilator nitric oxide (NO)[35]. Vasodilation increases blood flow and augments metabolic actions of insulin in skeletal muscle, regulates sodium homeostasis by enhancing sodium reabsorption in kidneys and thereby regulating blood pressure. A counterbalance is established by MAPK signaling in the endothelium, secreting vasoconstrictor endothelin-1, vascular smooth muscle cell (VSMC) proliferation, and pro-inflammatory activity. Expression of cellular adhesion molecules, including intercellular adhesion molecule-1 vascular cell adhesion molecule, are regulated by MAPK for modulating cell-cell interactions between vascular endothelium and circulatory inflammatory cells[36].

Under physiological conditions, insulin maintains a quiescent phenotype in VSMC [37]. VSMCs express both insulin receptor and insulin like growth factor receptor-1 (IGF-IR). At physiological concentrations of insulin stimulate translocation of insulin receptor and IGF-IR on VSMCs and increases cyclic guanosine monophosphate levels by releasing endothelial NO synthase 3 that evoke vasorelaxation. Insulin further attenuates VSMC contractility by regulating Rho induced increases in cytosolic calcium through calcium channels and inactivates myosin light chain phosphatase through PI3K/Akt insulin signaling. Insulin maintains a dedifferentiating state on VSMC through PI3K pathway and mediates VSMC migration through MAPK dependent pathway. In insulin resistant states, PI3K/Akt pathway is impaired and MAPK/RAS/Raf is increased, which preferentially signals to the mitogenic pathway as demonstrated in endothelial VSMCs[31]. A study in murine models has shown that both hyperglycemia and insulin resistance downregulate IRS-1 in VSMC and blood vessels with enhanced VSMC migration and proliferation in diabetic mice aorta promoting the formation of atherosclerotic lesions[38]. Hyperinsulinemia, in insulin resistant conditions can activate inflammatory pathways through enhanced advanced glycated end products (AGE) formation, reactive oxygen species (ROS) production, and elevated levels of circulating free fatty acids. Increased ROS and free fatty acids activate nuclear factor Kappa B (NF-kB) signaling pathway, which stimulates the production of pro-inflammatory cytokines, including TNF-α and IL-6. TNF-α through activation of Jun N terminal kinase pathway reduces insulin stimulated activation of PI3K/Akt/NO in endothelial cells and promotes atherosclerotic lesions. Furthermore, TNF-α stimulates expression of inflammatory proteins like C-reactive protein (CRP), an important marker of vascular inflammation and IL-6[38]. Thus, glucotoxicity and lipotoxicity associated with insulin resistance induce a pro-inflammatory milieu impairing vascular and endothelial function, promoting coronary heart diseases and atherosclerosis.

Insulin resistant states with impaired sensitivity to insulin mediated glucose disposal display impaired insulin mediated vasodilation, as well as endothelial dysfunction. In type 2 diabetes mellitus with underlying insulin resistance, insulin stimulated PI3K/NO pathway is selectively impaired and the compensatory hyperinsulinemia activate MAPK pathway, leading to enhanced vasoconstriction, pro-inflammation, increased sodium and water retention, and elevated blood pressure. Insulin resistance is also characterized by the presence of free fatty acids and AGE products, both are pro-inflammatory in nature. Hyperglycemia and insulin resistance supports viral proliferation in human monocytes, involves glycolysis with subsequent ROS production, and cytokine release including IL-1β[38]. No studies have evaluated surrogate markers of insulin resistance for prognostic scoring in COVID-19 patients or the underlying mechanisms that contribute to disease severity in this group.

PORTAL CIRCULATION, RAS SYSTEM AND INFLAMMATION

The pancreas is a highly vascularized salivary gland which produces an array of digestive enzymes (acinar or exocrine cells), such as amylase, pancreatic lipase and trypsinogen, (released into duodenum) and pancreatic hormones (released into blood stream *via* splenic artery). The endocrine cells are distributed in cellular aggregates forming the islet of Langerhans, which are small, island-like structures within exocrine pancreatic tissue. The exocrine compartment constitutes 98% to 99% of the gland and the remaining 1% to 2% constitute endocrine cells with five different cell types, α-cells producing glucagon (15%-20%), β-cells producing amylin-, C-peptide and insulin-

(65%-80%), γ-cells producing pancreatic polypeptide (3%-5%), δ-cells producing somatostatin (3%-10%), and ε-cells producing ghrelin (< 1%)[39]. The dissimilar functions between the two compartments manifests different vascular organization, including vascular morphology and blood flow. The pancreatic islet blood flow (IBF), both basal and stimulated, is 5-10 times higher than that of exocrine pancreas, which is regulated autonomously from one another. The blood flow through islet capillaries significantly impacts nutrient sensing, paracrine communication, and final hormonal output, and hence any alterations in blood perfusion, either induced physiologically (*e.g.*, nervous input) or because of pathological changes (*e.g.*, fibrosis), could affect islets function[40].

The pancreas receives 1% of the cardiac output supplied by celiac artery (70%) and the superior mesenteric artery (30%). The venous pancreatic blood is drained into the portal vein. The islets are supplied with arterioles, branching into fenestrated capillaries constituting 7% to 8% of islet volume. The acini are drained through venules into intra lobular veins and islet into ductal venous system. A part of the venous blood enters the insula-acinar portal system where venules bridge islet capillaries to acinar capillaries to provide an interface[41]. There is compelling evidence that metabolic regulation of islet blood perfusion is done by metabolites like adenosine and adenosine triphosphate/adenosine diphosphate[42]. The perfusion of blood in islets and exocrine compartments are modified by local endothelial mediators, the nervous system (the parasympathetic and sympathetic nerves), and gastrointestinal hormones. The islets are more sensitive to endothelial mediators especially NO and the incretin hormones, and adipokines preferentially act on islet vasculature. The evidence from literature so far suggests that insulin present in high local concentrations stimulates IBF and is independently regulated from the whole pancreatic blood flow (PBF)[42].

The changes in the PBF are clinically relevant as changes in the blood perfusion may affect the pathogenesis of diabetes mellitus and other pancreatic diseases. The effects of other hormone system, like the RAS, in the pathogenesis of glucose intolerance, insulin resistance, and hypertension, are well reported. The presence of local RAS is reported in many tissues including heart, vasculature, brain, retina, liver, and pancreas [9]. Evidence from human and animal studies suggests that the local hyperactive RAS/ATII signaling pathways contribute to pathogenesis of diabetes and related complications. The relative risk of developing diabetes mellitus is reduced 25% by inhibiting RAS[43]. RAS components are found in acini, ducts, islets, endothelial cells, and its expression is modulated in different conditions, including hypoxia, pancreatitis, hyperglycemia, and diabetes mellitus. The conversion of angiotensinogen to vasoactive peptide angiotensin I (ATI) by renin, and further to ATII by ACE, occurs in most vascular systems in the body. The physiological activity of ATII is mediated by ATI and ATII receptors. The ATI receptors cause sympathetic activation facilitating norepinephrine release, vasoconstriction, sodium water retention, oxidative stress, and cell growth stimulation, while the ATII receptors cause anti-proliferative effects through kinins and vasodilation[9].

Under physiological concentrations, ATII constricts both exocrine and endocrine blood vessels and regulate the release of enzymes and hormones. Elevated levels of ATII in islet micro vessels, decreases IBF in healthy nondiabetic Sprague Dawley rats, and RAS blockade by ACE/ATI inhibitors increases blood flow to islet micro vessels, suggesting a role played by pancreatic RAS in maintaining islet perfusion and regulation of glucose stimulated insulin secretion[41]. Infusion of ATII in *in vivo* models, such as rats, caused vasoconstriction and delayed the first phase of insulin release after stimulus, the earliest detectable defect (insulin resistance) in development of diabetes mellitus. ATII has shown to induce vasoconstriction in a dose-dependent manner in islet arterioles and islet blood vessels regulating IBF. Furthermore, ATII stimulates the release of pancreatic juice *via* cholinergic afferent pathway thus regulating perfusion in exocrine pancreas as well[44].

Chronic hyper-glycemia or insulin resistance is characterized by vascular dysfunction especially with regard to the endothelium. The decreased bioavailability of NO diminishes endothelium dependent vasodilation, further favoring vasoconstriction in vascular beds. The mechanisms that diminish NO production are partially regulated by increasing the NO synthesis inhibitor, and increased formation of ROS leads to degradation of NO and dysfunction of VSMC, which lead to macro and micro vascular complications of diabetes[31]. In addition, chronic hyperglycemia causes endothelial cell death by affecting the serine threonine kinase Akt pathway. Diabetes mellitus is often associated with islet inflammation, and reduction in β-cell mass/cell number and cytoarchitecture, which of course may affect IBF. Studies on islet vasculature in insulin resistance mice models (ob/ob positive mice and GLUT4

negative mice) revealed a vascular plasticity with increased islet vessel area and decreased intra islet vessel density affecting β-cells and increased IBF is proposed to contribute to this [45]. Experimental studies in mice models have found that initial stages of disturbed metabolism increase IBF, as disease progresses β-cell mass decreases followed by decreased islet volume and IBF. Insulin resistance would stimulate an increase in IBF and increase delivery of insulin to the systemic circulation and insulin biosynthesis, a compensatory mechanism adopted by β-cells to restore euglycemic state seen as a prodrome to overt diabetes mellitus[45]. The secondary changes in the pancreatic and IBF associated with glucose intolerance could modulate the pathophysiology of diabetes mellitus.

Blood flow measurements have shown that the local inflammation mediated by the release of adipokines, and macrophage derive cytokines may augment IBF. EvidenceIfrom vagotomised rats after portal vein infusion of Intralipid® increases PBF 2-3-fold and IBF > 10-fold[46]. Administration of inhibitors of inflammation like palmitate decreases both PBF and IBF. Thus, pathological activation of innate immune system and inflammation may alter IBF as a secondary phenomenon[46]. Thus, therapies aiming to decrease augmented islet blood perfusion would improve clinical outcomes in these patients.

INSULIN RESISTANCE INCREASES THE SEVERITY OF COIVD-19 IN DIABETES PATIENTS

Hyperinsulinemia in patients with insulin resistance and diabetes can lead to increased SARS-CoV-2 viral load, as insulin increases membrane expression of ACE2, which functions as a viral dock for entry into cells[47]. ACE2 is upregulated in initial stages of diabetes as an adaptive mechanism to counter ACE over activity. ACE2 controls blood pressure in a stable microenvironment by transforming ATII to AT1-7, lowering insulin resistance, oxidative stress, and increasing GLUT4 activity. In the later phases of diabetes ACE2 is downregulated due to glycosylation. However it is markedly increased in patients with diabetes and hypertension being treated with ACE inhibitors. Thus, ACE2 expression is increased in insulin resistant states, including type 2 diabetes. This in turn facilitates SARS-CoV-2 viral entry, which contributes to increased propensity to infection[18]. However, the SARS-CoV-2 infection decreases ACE2 expression as it is internalized along with virus, further leading to exaggerated ATII. The SARS-CoV-2 viral infection enhances ACE2 deficiency, dysregulation between the 'adverse' *ACE→ATII→ATI* axis, and the "protective" *ACE2→AT1-7→MAS1* receptor axis would contribute to strengthening the progression of inflammatory and thrombotic processes. The underlying insulin resistance in patients with type 2 diabetes predispose them to COVID-19 by creating more affinity to spike proteins and an increased inflammatory response, leading to more severe forms of infection with increased mortality[47].

Insulin resistance by itself can cause inflammation. Several COVID-19 studies show a strong association between the severity of the disease and the degree of dysregulated systemic inflammation biomarkers. A total of 56 individuals were included in an observational study and 18 (32.14%) of them were elderly patients, median age ≥ 68 years. The CRP level was considerably greater in the older group ($P < 0.001$) than in the younger and middle-aged groups. This study suggests that severe COVID-19 was observed amongst older patients with diabetes where interrelation between aging and inflammation is well established[3]. IL-6 a pro-inflammatory cytokine circulation elevation of 2 to 3-fold is observed in conditions of insulin resistance[31]. In comparison to non-diabetic and diabetic patients without COVID-19 infection, patients with COVID-19 infection with diabetes mellitus have higher IL-6 levels[48]. The accumulation of activated immune cells in metabolic tissues and inflammatory mediators, in particular IL-1β and TNF-α, contribute to the onset and progression of insulin resistance. Hyperglycemia produces ROS *via* advanced glycation end products and activates immune response in diabetes patients. CRP, an acute-phase reactant usually associated with serious infection and inflammation is strongly correlated in insulin resistance and a powerful predictor of future cardiovascular event. Patients with COVID-19 and coexistent diabetes characterized by low grade chronic inflammation are more likely to develop a cytokine storm, leading to high risk of vascular hyper permeability state, multi-organ failure, and death[49]. Whether COVID-19 accelerates the existing metabolic perturbations associated with diabetes and insulin resistance or virally induced inflammation leads to insulin resistance resulting in poor prognosis needs to be explored further.

The findings of rapidly worsening glycemic control in patients with diabetes and COVID-19, requiring high doses of insulin to control elevated blood glucose, increased ketosis in older individuals indicate the possibility of pancreatic invasion by the SARS-CoV-2[50]. Investigation of ACE2 expression confirmed a higher expression in pancreas than lungs, and single cell RNA sequencing confirmed the expression in both exocrine and endocrine glands of pancreas[50]. Thus, SARS-CoV-2 may cause direct damage to insulin secreting pancreatic beta cells, and this may partly explain the worsening of glucose control in people with diabetes who do have some functional β-cells in reserve. According to Wang *et al*[51], 9 (17%) of 52 admitted COVID-19 pneumonia patients in Wuhan experienced pancreatic injury as evidenced by irregular serum amylase or lipase levels. Six of the 9 pancreatic injury patients had developed glucose intolerance[51]. Following viral entry into beta cells, ACE2 is downregulated, resulting in an increase in angiotensin levels and impaired insulin secretion. The direct cytopathic effect of SARS-CoV-2 replication, the systemic reaction to respiratory failure, and the harmful immune response caused by SARS-CoV-2 infection are all possible mechanisms of pancreatic injury[51].

Diabetes is linked with coagulopathy and thrombosis contributing to long term micro and macro vascular pathological complications. Higher d-Dimer levels are found in COVID-19 patients with diabetes[23]. Additionally, individuals with both obesity and diabetes are reported to have worse prognosis associated with COVID-19 infection, including potential thrombotic events such as stroke[49]. Considering 80% of patients with diabetes have coexistent hypertension, infection by SARS-CoV-2 may lead to deregulated blood pressure, with further increased risk of cardiovascular complications. Several publications have reported an increased risk of venous thromboembolism and pulmonary embolism amongst diabetes patients compared to the control group[52].

Patients with diabetes mellitus are more susceptible to the severe form of COVID-19, which is associated with poorer outcomes. Based on the evidence so far, we hypothesize that insulin resistance could be a key facilitator driving severe disease in COVID-19 in patients with diabetes. A better understanding of clinical and biochemical markers of insulin resistance for evaluating diabetes status in patients with COVID-19 might help in developing patient tailored treatment strategies (Figure 1). In addition real world evidence is required to test the utility of markers of insulin resistance as prognostic indicators in predicting disease severity in COVID-19.

RATIONALE AND CLINICAL IMPLICATIONS OF INSULIN RESISTANCE IN COVID-19 SEVERITY

A hypothesis that may partially explain the propensity of individuals with insulin resistance and with type 2 diabetes to develop more severe forms of COVID-19 is inflammation associated with a high concentration of insulin and activation of the RAS. The SARS-CoV-2 uses ACE2 protein for the host entry. Hyperactivity of RAS pathway due to increased ROS is implicated in the pathogenesis of diabetes. In the islets, ACE2 may modulate blood flow and morphology in order to maintain insulin secretion. A higher concentration of circulating insulin shifts the balance towards vasoconstriction, disturbing cardiovascular homeostasis and resulting in increased risk of thromboembolism and multiple organ failure as explained in Figure 2. Diabetes mellitus alters cytokine profile and aggravates a dysregulated immune response. "Cytokine storm" implicated in mortality of COVID-19 patients reflecting, at least in part, a state of insulin resistance and elevated insulin levels driving ACE2 expression, altered blood pressure, inflammation, endothelial dysfunction, and coagulation. The triglyceride glucose index (a surrogate marker for insulin resistance) was found to be associated with poor clinical outcomes in patients with COVID-19, according to a recent study[53]. Thus, COVID-19 which is otherwise a mild infection in majority of the population but much more severe and fatal in the context of people with diabetes, could be explained by above hypothesis.

Using surrogate markers of insulin resistance in clinical setting as a prognostic tool in conjunction with other disease severity markers for β-cell damage, RAS pathway, inflammation, and thrombosis would help in developing tailored management strategies for patients with COVID-19 and diabetes (Table 1). This could potentially improve outcomes in severely ill patients. The hyperinsulinemic-euglycemic clamp technique is the gold standard to measure hepatic and skeletal muscle insulin sensitivity. However, it is technically demanding, time consuming, and inappropriate to perform in acutely ill patients. Alternative methods based on fasting insulin and

Table 1 Clinical studies that reported insulin resistance markers in patients with Diabetes and coronavirus disease 2019

Ref.	Study design	Sample size	T2DM	Markers of IR	Age	Outcomes	Country
Wang *et al* [57], 2021	Retrospective	172	72	Hypertriglyceridemia (Fibrinogen, triglycerides, Serum ferritin	66 (Median)		China
Ren *et al* [53], 2020	Retrospective	151	39	Triglyceride-glucose index	59.5 (Mean)	Severity and mortality	China
Alcántara-Alonso [58], 2021	Observational cohort	43	25	Triglyceride to high density lipoprotein cholesterol	57.19 (Mean)	Incidence of acute kidney injury, requirement of invasive mechanical ventilation, vasopressor support, days of hospitalization and mortality	Mexico

IR: Insulin receptor; T2DM: Type 2 diabetes mellitus.

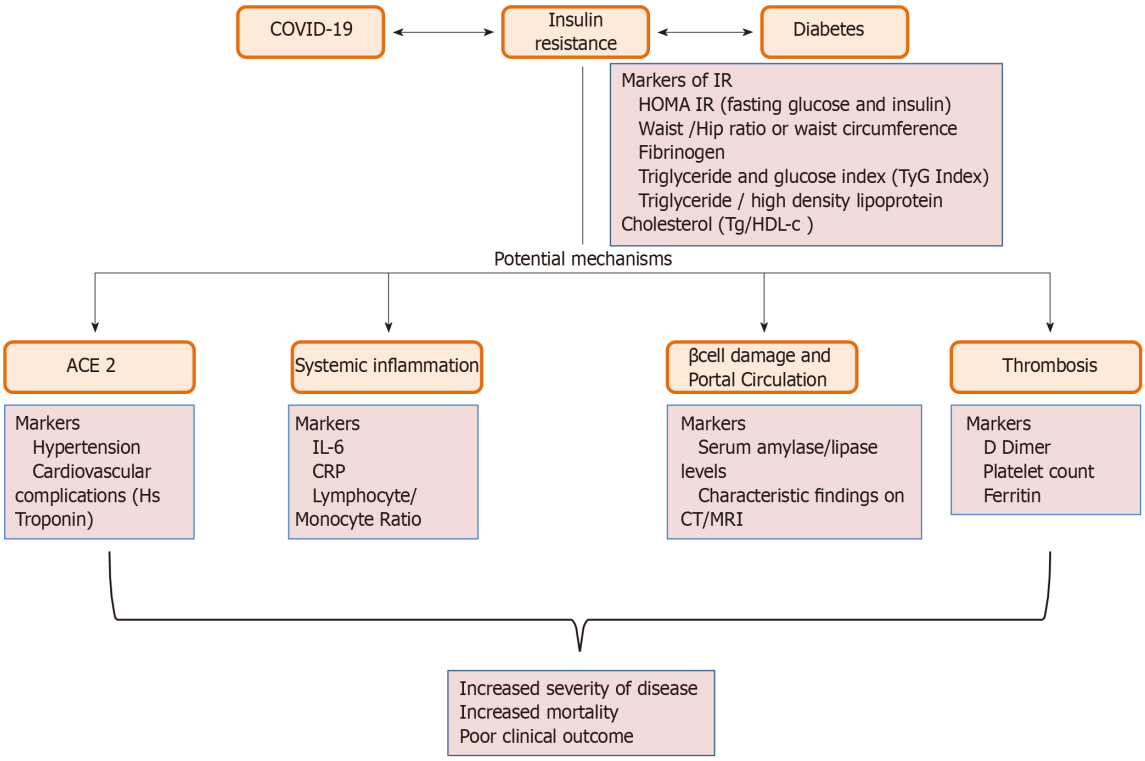

Figure 1 Insulin resistance may act as a bridging link between coronavirus disease 2019 and diabetes. The potential mechanism by which insulin resistance mediates coronavirus disease 2019 (COVID-19) severity is through angiotensin converting enzyme 2 protein, systemic inflammation, pancreatic β cell damage, and thrombosis. The potential markers of insulin resistance for evaluating diabetes status in patients with COVID-19 are also mentioned. CRP: C-reactive protein; CT: Computed tomography; IL-6: Interleukin-6; IR: Insulin receptor; MRI: Magnetic resonance imaging; T2DM: Type 2 diabetes mellitus.

glucose levels (HOMA), such as dynamic response to oral glucose loading, would also be impractical to perform in acutely unwell patients[54]. Alternatively, careful evaluation of biomarkers of inflammation (IL-6, CRP, fibrinogen, lymphocyte to monocyte ratio), thrombosis (d-Dimer, platelet number), and cardiovascular complications (Hs troponin), along with other parameters (fasting insulin and glucose) may serve as helpful prognostic tools in case of COVID-19 in the presence of diabetes. Furthermore, non-invasive strategies to assess the long-term consequences of insulin and PBF dynamics (contrast-enhanced ultrasound measurement) may also be helpful predict clinical progression[55,56].

CONCLUSION

Preliminary studies from various countries including United Kingdom and China

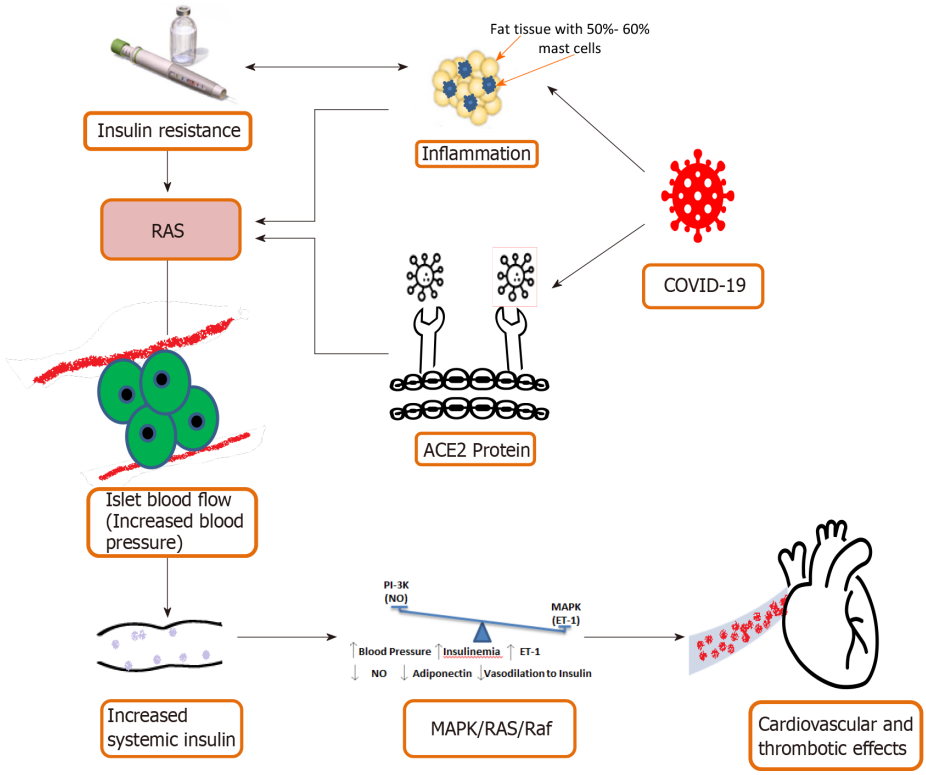

Figure 2 Inflammation linked to elevated insulin concentrations *via* portal circulation is one hypothesis that may help explain why patients with diabetes likely than others to develop extreme types of coronavirus disease 2019 diabetic patients[31,56]. The angiotensin converting enzyme (ACE) Protein is used by the severe acute respiratory syndrome coronavirus 2 to enter the host. The pathogenesis of diabetes is linked to hyperactivity of the renin-angiotensin system (RAS) pathway caused by increased reactive oxygen species (ROS). In order to maintain insulin secretion, ACE2 can modulate blood flow and morphology in the islets. A higher level of circulating insulin changes the balance from vasodilator to vasoconstrictor effects, disrupting cardiovascular homeostasis and increasing the risk of thromboembolism and multiple organ failure. COVID-19: Coronavirus disease 2019; MAPK: Mitogen activated protein kinase. Citation: Shah P, Lueschen N, Ardestani A, Oberholzer J, Olerud J, Carlsson PO, Maedler K. Angiopoetin-2 Signals Do Not Mediate the Hypervascularization of Islets in Type 2 Diabetes. PLoS One 2016; 11: e0161834. Copyright© The Authors 2016. Published by Open Access Article. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev 2007*; 28: 463-491. Copyright© The Authors 2007. Published by Oxford University Press.

suggest that COVID-19 is associated with increased severity and mortality in patients with diabetes. Insulin resistance-mediated metabolic and inflammatory processes are likely to be contributory factors. Patients with diabetes mellitus are also prone to significant dysglycaemia secondary to acute COVID-19, which in itself is detrimental and associated with poorer outcomes. Severe inflammation, either due to underlying metabolic syndromes or COVID-19 disease, can further worsen insulin resistance, which in turn would worsen dysglycaemia in diabetes mellitus, exacerbating the severity of COVID-19. We propose that surrogate markers of insulin resistance be evaluated for their prognostic value in predicting disease severity associated with COVID-19 amongst patients with diabetes. Since clinical and biochemical markers of insulin resistance are not routinely measured in COVID-19 patients, no real-world study has explicitly looked at the association between insulin resistance and disease severity. In addition, the expression of ACE2 in the pancreas is debated, and the role of ACE2 expression in the development of insulin resistance is also less explored.

The extent to which insulin resistance contributes to COVID-19 disease severity is not well known and may potentially be substantial. Population-based approaches to track individuals with insulin resistance and follow preventative treatment protocols could be a valuable method in the future to mitigate the impact of such pandemics.

REFERENCES

1 **Madabhavi I**, Sarkar M, Kadakol N. COVID-19: a review. *Monaldi Arch Chest Dis* 2020; **90** [PMID: 32498503 DOI: 10.4081/monaldi.2020.1298]

2 **Chidambaram V**, Tun NL, Haque WZ, Majella MG, Sivakumar RK, Kumar A, Hsu AT, Ishak IA, Nur AA, Ayeh SK, Salia EL, Zil-E-Ali A, Saeed MA, Sarena APB, Seth B, Ahmadzada M, Haque

EF, Neupane P, Wang KH, Pu TM, Ali SMH, Arshad MA, Wang L, Baksh S, Karakousis PC, Galiatsatos P. Factors associated with disease severity and mortality among patients with COVID-19: A systematic review and meta-analysis. *PLoS One* 2020; **15**: e0241541 [PMID: 33206661 DOI: 10.1371/journal.pone.0241541]

3 **Liu K**, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect* 2020; **80**: e14-e18 [PMID: 32171866 DOI: 10.1016/j.jinf.2020.03.005]

4 **Yamamoto N**, Bauer G. Apparent difference in fatalities between Central Europe and East Asia due to SARS-COV-2 and COVID-19: Four hypotheses for possible explanation. *Med Hypotheses* 2020; **144**: 110160 [PMID: 32795831 DOI: 10.1016/j.mehy.2020.110160]

5 **Li B**, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol* 2020; **109**: 531-538 [PMID: 32161990 DOI: 10.1007/s00392-020-01626-9]

6 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

7 **Bode B**, Garrett V, Messler J, McFarland R, Crowe J, Booth R, Klonoff DC. Glycemic Characteristics and Clinical Outcomes of COVID-19 Patients Hospitalized in the United States. *J Diabetes Sci Technol* 2020; **14**: 813-821 [PMID: 32389027 DOI: 10.1177/1932296820924469]

8 **Jain V**, Yuan JM. Predictive symptoms and comorbidities for severe COVID-19 and intensive care unit admission: a systematic review and meta-analysis. *Int J Public Health* 2020; **65**: 533-546 [PMID: 32451563 DOI: 10.1007/s00038-020-01390-7]

9 **Ramalingam L**, Menikdiwela K, LeMieux M, Dufour JM, Kaur G, Kalupahana N, Moustaid-Moussa N. The renin angiotensin system, oxidative stress and mitochondrial function in obesity and insulin resistance. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 1106-1114 [PMID: 27497523 DOI: 10.1016/j.bbadis.2016.07.019]

10 **Syed Ikmal SI**, Zaman Huri H, Vethakkan SR, Wan Ahmad WA. Potential biomarkers of insulin resistance and atherosclerosis in type 2 diabetes mellitus patients with coronary artery disease. *Int J Endocrinol* 2013; **2013**: 698567 [PMID: 24282409 DOI: 10.1155/2013/698567]

11 **Qin C**, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; **71**: 762-768 [PMID: 32161940 DOI: 10.1093/cid/ciaa248]

12 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Zhong NS. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [DOI: 10.1056/nejmoa2002032]

13 **Mao L**, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020; **77**: 683-690 [PMID: 32275288 DOI: 10.1001/jamaneurol.2020.1127]

14 **Lanza K**, Perez LG, Costa LB, Cordeiro TM, Palmeira VA, Ribeiro VT, Simões E Silva AC. Covid-19: the renin-angiotensin system imbalance hypothesis. *Clin Sci (Lond)* 2020; **134**: 1259-1264 [PMID: 32507883 DOI: 10.1042/CS20200492]

15 **Wang Q**, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, Wang Q, Zhou H, Yan J, Qi J. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* 2020; **181**: 894-904.e9 [PMID: 32275855 DOI: 10.1016/j.cell.2020.03.045]

16 **Nehme A**, Zouein FA, Zayeri ZD, Zibara K. An Update on the Tissue Renin Angiotensin System and Its Role in Physiology and Pathology. *J Cardiovasc Dev Dis* 2019; **6** [PMID: 30934934 DOI: 10.3390/jcdd6020014]

17 **Shang J**, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* 2020; **117**: 11727-11734 [PMID: 32376634 DOI: 10.1073/pnas.2003138117]

18 **Gheblawi M**, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, Raizada MK, Grant MB, Oudit GY. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Circ Res* 2020; **126**: 1456-1474 [PMID: 32264791 DOI: 10.1161/CIRCRESAHA.120.317015]

19 **Djomkam ALZ**, Olwal CO, Sala TB, Paemka L. Commentary: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Front Oncol* 2020; **10**: 1448 [PMID: 32974166 DOI: 10.3389/fonc.2020.01448]

20 **Iwasaki A**, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. *Nat Rev Immunol* 2020; **20**: 339-341 [PMID: 32317716 DOI: 10.1038/s41577-020-0321-6]

21 **Nienhold R**, Ciani Y, Koelzer VH, Tzankov A, Haslbauer JD, Menter T, Schwab N, Henkel M, Frank A, Zsikla V, Willi N, Kempf W, Hoyler T, Barbareschi M, Moch H, Tolnay M, Cathomas G, Demichelis F, Junt T, Mertz KD. Two distinct immunopathological profiles in autopsy lungs of COVID-19. *Nat Commun* 2020; **11**: 5086 [PMID: 33033248 DOI: 10.1038/s41467-020-18854-2]

22 **Chen G**, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; **130**: 2620-2629 [PMID: 32217835 DOI: 10.1172/JCI137244]

23 **Chi Y**, Ge Y, Wu B, Zhang W, Wu T, Wen T, Liu J, Guo X, Huang C, Jiao Y, Zhu F, Zhu B, Cui L. Serum Cytokine and Chemokine Profile in Relation to the Severity of Coronavirus Disease 2019 in

China. *J Infect Dis* 2020; **222**: 746-754 [PMID: 32563194 DOI: 10.1093/infdis/jiaa363]
24 **Tang N**, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020; **18**: 844-847 [PMID: 32073213 DOI: 10.1111/jth.14768]
25 **Abou-Ismail MY**, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. *Thromb Res* 2020; **194**: 101-115 [PMID: 32788101 DOI: 10.1016/j.thromres.2020.06.029]
26 **Yu B**, Li C, Sun Y, Wang DW. Insulin Treatment Is Associated with Increased Mortality in Patients with COVID-19 and Type 2 Diabetes. *Cell Metab* 2021; **33**: 65-77.e2 [PMID: 33248471 DOI: 10.1016/j.cmet.2020.11.014]
27 **Wu H**, Ballantyne CM. Metabolic Inflammation and Insulin Resistance in Obesity. *Circ Res* 2020; **126**: 1549-1564 [PMID: 32437299 DOI: 10.1161/CIRCRESAHA.119.315896]
28 **Reddy PK**, Kuchay MS, Mehta Y, Mishra SK. Diabetic ketoacidosis precipitated by COVID-19: A report of two cases and review of literature. *Diabetes Metab Syndr* 2020; **14**: 1459-1462 [PMID: 32771918 DOI: 10.1016/j.dsx.2020.07.050]
29 **The Lancet Diabetes Endocrinology**. COVID-19 and diabetes: a co-conspiracy? *Lancet Diabetes Endocrinol* 2020; **8**: 801 [PMID: 32946812 DOI: 10.1016/S2213-8587(20)30315-6]
30 **Muniyappa R**, Gubbi S. COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am J Physiol Endocrinol Metab* 2020; **318**: E736-E741 [PMID: 32228322 DOI: 10.1152/ajpendo.00124.2020]
31 **Muniyappa R**, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev* 2007; **28**: 463-491 [PMID: 17525361 DOI: 10.1210/er.2007-0006]
32 **Cohen P**. The twentieth century struggle to decipher insulin signalling. *Nat Rev Mol Cell Biol* 2006; **7**: 867-873 [PMID: 17057754 DOI: 10.1038/nrm2043]
33 **Cusi K**, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 2000; **105**: 311-320 [PMID: 10675357 DOI: 10.1172/JCI7535]
34 **Schulman IH**, Zhou MS. Vascular insulin resistance: a potential link between cardiovascular and metabolic diseases. *Curr Hypertens Rep* 2009; **11**: 48-55 [PMID: 19146801 DOI: 10.1007/s11906-009-0010-0]
35 **Hartell NA**, Archer HE, Bailey CJ. Insulin-stimulated endothelial nitric oxide release is calcium independent and mediated *via* protein kinase B. *Biochem Pharmacol* 2005; **69**: 781-790 [PMID: 15710355 DOI: 10.1016/j.bcp.2004.11.022]
36 **Formoso G**, Chen H, Kim JA, Montagnani M, Consoli A, Quon MJ. Dehydroepiandrosterone mimics acute actions of insulin to stimulate production of both nitric oxide and endothelin 1 *via* distinct phosphatidylinositol 3-kinase- and mitogen-activated protein kinase-dependent pathways in vascular endothelium. *Mol Endocrinol* 2006; **20**: 1153-1163 [PMID: 16373398 DOI: 10.1210/me.2005-0266]
37 **Frismantiene A**, Philippova M, Erne P, Resink TJ. Smooth muscle cell-driven vascular diseases and molecular mechanisms of VSMC plasticity. *Cell Signal* 2018; **52**: 48-64 [PMID: 30172025 DOI: 10.1016/j.cellsig.2018.08.019]
38 **Xi G**, Shen X, Wai C, White MF, Clemmons DR. Hyperglycemia induces vascular smooth muscle cell dedifferentiation by suppressing insulin receptor substrate-1-mediated p53/KLF4 complex stabilization. *J Biol Chem* 2019; **294**: 2407-2421 [PMID: 30578299 DOI: 10.1074/jbc.RA118.005398]
39 **Cabrera O**, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A* 2006; **103**: 2334-2339 [PMID: 16461897 DOI: 10.1073/pnas.0510790103]
40 **Almaça J**, Caicedo A. Blood Flow in the Pancreatic Islet: Not so Isolated Anymore. *Diabetes* 2020; **69**: 1336-1338 [PMID: 32561621 DOI: 10.2337/dbi20-0016]
41 **Jansson L**, Barbu A, Bodin B, Drott CJ, Espes D, Gao X, Grapensparr L, Källskog Ö, Lau J, Liljebäck H, Palm F, Quach M, Sandberg M, Strömberg V, Ullsten S, Carlsson PO. Pancreatic islet blood flow and its measurement. *Ups J Med Sci* 2016; **121**: 81-95 [PMID: 27124642 DOI: 10.3109/03009734.2016.1164769]
42 **Röder PV**, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med* 2016; **48**: e219 [PMID: 26964835 DOI: 10.1038/emm.2016.6]
43 **Andraws R**, Brown DL. Effect of inhibition of the renin-angiotensin system on development of type 2 diabetes mellitus (meta-analysis of randomized trials). *Am J Cardiol* 2007; **99**: 1006-1012 [PMID: 17398202 DOI: 10.1016/j.amjcard.2006.10.068]
44 **Jansson L**, Carlsson PO. Pancreatic Blood Flow with Special Emphasis on Blood Perfusion of the Islets of Langerhans. *Compr Physiol* 2019; **9**: 799-837 [PMID: 30892693 DOI: 10.1002/cphy.c160050]
45 **Dai C**, Brissova M, Reinert RB, Nyman L, Liu EH, Thompson C, Shostak A, Shiota M, Takahashi T, Powers AC. Pancreatic islet vasculature adapts to insulin resistance through dilation and not angiogenesis. *Diabetes* 2013; **62**: 4144-4153 [PMID: 23630302 DOI: 10.2337/db12-1657]
46 **Lai E**, Pettersson U, Verdugo AD, Carlsson PO, Bodin B, Källskog Ö, Persson AE, Sandberg M, Jansson L. Blood lipids affect rat islet blood flow regulation through β_3-adrenoceptors. *Am J Physiol Endocrinol Metab* 2014; **307**: E653-E663 [PMID: 25139049 DOI: 10.1152/ajpendo.00680.2013]
47 **Govender N**, Khaliq OP, Moodley J, Naicker T. Insulin resistance in COVID-19 and diabetes. *Prim Care Diabetes* 2021; **15**: 629-634 [PMID: 33849817 DOI: 10.1016/j.pcd.2021.04.004]

48 **Zeng Z**, Yu H, Chen H, Qi W, Chen L, Chen G, Yan W, Chen T, Ning Q, Han M, Wu D. Longitudinal changes of inflammatory parameters and their correlation with disease severity and outcomes in patients with COVID-19 from Wuhan, China. *Crit Care* 2020; **24**: 525 [PMID: 32854750 DOI: 10.1186/s13054-020-03255-0]

49 **Lim S**, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol* 2021; **17**: 11-30 [PMID: 33188364 DOI: 10.1038/s41574-020-00435-4]

50 **Bornstein SR**, Rubino F, Khunti K, Mingrone G, Hopkins D, Birkenfeld AL, Boehm B, Amiel S, Holt RI, Skyler JS, DeVries JH, Renard E, Eckel RH, Zimmet P, Alberti KG, Vidal J, Geloneze B, Chan JC, Ji L, Ludwig B. Practical recommendations for the management of diabetes in patients with COVID-19. *Lancet Diabetes Endocrinol* 2020; **8**: 546-550 [PMID: 32334646 DOI: 10.1016/S2213-8587(20)30152-2]

51 **Wang F**, Wang H, Fan J, Zhang Y, Zhao Q. Pancreatic Injury Patterns in Patients With Coronavirus Disease 19 Pneumonia. *Gastroenterology* 2020; **159**: 367-370 [PMID: 32247022 DOI: 10.1053/j.gastro.2020.03.055]

52 **Tang N**, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020; **18**: 1094-1099 [PMID: 32220112 DOI: 10.1111/jth.14817]

53 **Ren H**, Yang Y, Wang F, Yan Y, Shi X, Dong K, Yu X, Zhang S. Association of the insulin resistance marker TyG index with the severity and mortality of COVID-19. *Cardiovasc Diabetol* 2020; **19**: 58 [PMID: 32393351 DOI: 10.1186/s12933-020-01035-2]

54 **Finucane FM**, Davenport C. Coronavirus and Obesity: Could Insulin Resistance Mediate the Severity of Covid-19 Infection? *Front Public Health* 2020; **8**: 184 [PMID: 32574288 DOI: 10.3389/fpubh.2020.00184]

55 **St Clair JR**, Ramirez D, Passman S, Benninger RKP. Contrast-enhanced ultrasound measurement of pancreatic blood flow dynamics predicts type 1 diabetes progression in preclinical models. *Nat Commun* 2018; **9**: 1742 [PMID: 29717116 DOI: 10.1038/s41467-018-03953-y]

56 **Shah P**, Lueschen N, Ardestani A, Oberholzer J, Olerud J, Carlsson PO, Maedler K. Angiopoetin-2 Signals Do Not Mediate the Hypervascularization of Islets in Type 2 Diabetes. *PLoS One* 2016; **11**: e0161834 [PMID: 27617438 DOI: 10.1371/journal.pone.0161834]

57 **Wang Z**, Du Z, Zhao X, Guo F, Wang T, Zhu F. Determinants of Increased Fibrinogen in COVID-19 Patients With and Without Diabetes and Impaired Fasting Glucose. *Clin Appl Thromb Hemost* 2021; **27**: 1076029621996445 [PMID: 33760664 DOI: 10.1177/1076029621996445]

58 **Alcántara-Alonso E**. High triglyceride to HDL-cholesterol ratio as a biochemical marker of severe outcomes in COVID-19 patients. *Clin Nutr ESPEN* 2021 [DOI: 10.1016/j.clnesp.2021.04.020]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1563-1575

DOI: 10.4239/wjd.v12.i9.1563

ISSN 1948-9358 (online)

MINIREVIEWS

Obesity and bariatric surgery in kidney transplantation: A clinical review

Massimiliano Veroux, Edoardo Mattone, Matteo Cavallo, Rossella Gioco, Daniela Corona, Alessio Volpicelli, Pierfrancesco Veroux

ORCID number: Massimiliano Veroux 0000-0002-2780-6421; Edoardo Mattone 0000-0003-0248-6182; Matteo Cavallo 0000-0002-6093-6033; Rossella Gioco 0000-0003-3608-6187; Daniela Corona 0000-0002-9801-5985; Alessio Volpicelli 0000-0002-0110-6566; Pierfrancesco Veroux 0000-0003-4965-7137.

Author contributions: Veroux M and Mattone E had the idea for the review; Mattone E, Cavallo M, Gioco R, Volpicelli A and Corona D performed the literature search and data analysis; Mattone E, Cavallo M and Veroux M made the original draft preparation of the manuscript; Veroux M and Veroux P critically revised the manuscript.

Supported by FIR-14 Research Project of the University of Catania.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Massimiliano Veroux, Department of Medical and Surgical Sciences and Advanced Technologies GF Ingrassia, University of Catania, Catania 95123, Italy

Edoardo Mattone, Matteo Cavallo, Vascular Surgery and Organ Transplant Unit, University Hospital of Catania, Catania 95123, Italy

Rossella Gioco, Daniela Corona, Alessio Volpicelli, General Surgery Unit, University Hospital of Catania, Catania 95123, Italy

Pierfrancesco Veroux, Department of General Surgery and Medical Specialities, University of Catania, Catania 95123, Italy

Corresponding author: Massimiliano Veroux, MD, PhD, Associate Professor, Surgeon, Department of Medical and Surgical Sciences and Advanced Technologies GF Ingrassia, University of Catania, Via Santa Sofia 84, Catania 95123, Italy. veroux@unict.it

Abstract

Obesity is increasing worldwide, and this has major implications in the setting of kidney transplantation. Patients with obesity may have limited access to transplantation and increased posttransplant morbidity and mortality. Most transplant centers incorporate interventions aiming to target obesity in kidney transplant candidates, including dietary education and lifestyle modifications. For those failing nutritional restriction and medical therapy, the use of bariatric surgery may increase the transplant candidacy of patients with obesity and end-stage renal disease (ESRD) and may potentially improve the immediate and late outcomes. Bariatric surgery in ESRD patients is associated with weight loss ranging from 29.8% to 72.8% excess weight loss, with reported mortality and morbidity rates of 2% and 7%, respectively. The most commonly performed bariatric surgical procedures in patients with ESRD and in transplant patients are laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass. However, the correct timing of bariatric surgery and the ideal type of surgery have yet to be determined, although pretransplant LSG seems to be associated with an acceptable risk-benefit profile. We review the impact of obesity on kidney transplant candidates and recipients and in potential living kidney donors, exploring the potential impact of bariatric surgery in addressing obesity in these populations, thereby potentially improving posttransplant outcomes.

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: Italy

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: April 29, 2021
Peer-review started: April 29, 2021
First decision: June 16, 2021
Revised: June 30, 2021
Accepted: August 9, 2021
Article in press: August 9, 2021
Published online: September 15, 2021

P-Reviewer: Mavroeidis VK
S-Editor: Fan JR
L-Editor: A
P-Editor: Wang LYT

Key Words: End-stage renal disease; Living donor; Gastric bypass; Sleeve gastrectomy; Waiting list; Deceased donor

Core Tip: Many studies demonstrated that obese patients may have limited access to kidney transplantation and an increased rate of posttransplant complications. Diet and lifestyle modifications may have a limited impact in the treatment of obesity in these patients, while bariatric surgery has the potential to improve the candidacy of these patients and to improve perioperative outcomes. This review will evaluate the potential role of bariatric surgery in the setting of kidney transplantation.

Citation: Veroux M, Mattone E, Cavallo M, Gioco R, Corona D, Volpicelli A, Veroux P. Obesity and bariatric surgery in kidney transplantation: A clinical review. *World J Diabetes* 2021; 12(9): 1563-1575
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1563.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1563

INTRODUCTION

Obesity is a major public health concern, affecting more than 25% of the world population. It has been estimated that by 2030, the prevalence of the overweight population [as defined by body mass index (BMI) 25-29.9 kg/m^2] will reach 38%, while 20% will be obese (BMI > 30 kg/m^2)[1,2]. Obesity-related complications include but are not limited to cardiovascular disease, diabetes mellitus (DM) and cancer[3]. Kidney transplantation represents the best replacement therapy for patients with end-stage renal disease (ESRD), conferring a better quality of life than dialysis[4]. While the number of obese ESRD patients is increasing, obesity may limit access to kidney transplantation due to related comorbidities and posttransplant complications, including wound dehiscence, posttransplant DM and incisional hernia[4-6]. While class 1 obesity (BMI 30-34.9 kg/m^2) is not typically a contraindication, most transplant centers consider relative and absolute average BMI cutoffs of 38 and 41 kg/m^2, respectively, as contraindications to kidney transplantation[3].

Obesity may increase the risk of cardiovascular complications, DM, and metabolic syndrome, which are well-known posttransplant complications related to the chronic use of immunosuppression[4-9]. However, data on the clinical outcomes of kidney transplantation in obese patients are conflicting due to the lack of consensus on a commonly accepted definition of obesity and on a standard approach to obesity assessment[3]. Most transplant centers incorporate interventions aiming to target obesity in kidney transplant candidates, including dietary education and lifestyle modifications[3-9]. For those failing nutritional restriction and medical therapy, bariatric surgery has recently emerged as a valid therapeutic approach for improving access to kidney transplantation as well as posttransplant outcomes. However, many uncertainties remain regarding the optimal timing of bariatric surgery and the preferred surgical technique: While most transplant surgeons prefer pretransplant bariatric surgery, posttransplant surgery may theoretically reduce the impact of obesity-related complications that are magnified by immunosuppression.

In this review, we explore the impact of bariatric surgery on kidney transplant candidates and recipients and in living kidney donors, trying to address the best strategy to improve the clinical outcomes of kidney transplantation in obese individuals.

DEFINITION AND MEASUREMENT OF OBESITY

According to the 2017 Kidney Disease: Improving Global Outcomes clinical practice guidelines on the evaluation and management of candidates for kidney transplantation, obesity is usually assessed by BMI defined, according to World Health Organization, as weight in kilograms divided by height in meters squared, and by the

waist-to-hip ratio, defined as the ratio of the circumference of the waist to that of the hips[10].

Obesity is defined as a BMI ≥ 30 kg/m^2 and can be subdivided into classes I (BMI 30-34.9 kg/m^2), II (BMI 35-39.9 kg/m^2) and III (≥ 40 kg/m^2), but BMI is a surrogate measure that could have significant limitations when applied to individuals, as it does not take into account fluid status, muscle mass, body shape and weight distribution [11,12]. The waist-to-hip ratio evaluates abdominal pattern obesity, and ratios > 0.85 for women and > 0.9 for men are considered obese according to the World Health Organization[10]. Recent studies suggested that in ESRD patients on dialysis and in kidney transplant recipients, a higher BMI was associated with lower mortality after adjustment for waist circumference (WC), while a higher WC was more strongly associated with higher mortality after adjustment for BMI[13,14].

Despite these limitations, BMI is currently used for the decision-making process for kidney transplantation in obese populations.

OBESITY AND ESRD

Obesity-related comorbidities, including DM, cardiovascular disease and cancer, can all prevent access to kidney transplantation[3,10,12,15]. Some authors described an "obesity paradox" among patients with ESRD on maintenance dialysis: patients with a BMI < 20 kg/m^{2}carry the highest relative risk (RR) of mortality, while overweight patients (BMI 25-29.9 kg/m^2, RR 0.84), patients with mild obesity (BMI 30-34.9 kg/m^2; RR 0.73), and patients with moderate obesity (BMI 35-39.9 kg/m^2; RR 0.76) have significantly better outcomes[16]. A potential explanation of this effect is that patients with a higher BMI may have normal to high muscle mass or a favorable WC, and this could have a protective role[16-18]. However, this beneficial effect does not persist after transplantation, since obese transplant patients may have reduced graft and patient survival rates[6,19-23], particularly in patients older than 65 years[24]. As a consequence, obese patients may have limited access to waiting lists and transplantation: in a retrospective analysis of the United States Renal Data System (USRDS) registry including 702456 incident ESRD patients aged 18-70 years[19], women with a BMI of > 25 kg/m^2 had a lower chance of transplantation from both living and deceased donors. In contrast, in men, a BMI of 25-34.9 kg/m^2 was associated with a higher likelihood of transplantation, while a BMI > 35 kg/m^2 was associated with a lower chance of receiving a deceased donor transplantation[19]. This disparity in access to transplantation may be related to the limitations of BMI as a metric: At a given BMI, females typically have more fat than males, while males tend to have more muscle and muscle weight; in contrast, males with a higher BMI have predominantly abdominal fat, which correlates with an increased risk of wound complications.

There are several reasons for the reduced access to transplantation for obese patients: in their analysis, Segev *et al*[25] found that obese patients who were activated on the waiting list had lower access to transplantation because they were less profitable than nonobese patients, and were more frequently bypassed; moreover, United States transplant centers may be more reluctant to transplant obese patients because they may be penalized for a higher than expected rate of patient death or allograft failure in the first posttransplant year, which could occur more frequently in obese patients[25]. Obese patients on waiting lists may therefore develop a number of comorbid conditions necessitating temporary wait-list suspension[21]. On the other hand, in the United States, many insurance payers mandate a trial of medical weight management prior to approving bariatric surgery, but centers that perform bariatric surgery could be reluctant to perform bariatric surgery in these patients due to the potential high rate of complications and death[26].

Although there is no clear consensus on the highest level of BMI to be considered a contraindication to kidney transplantation, most guidelines strongly suggest that for patients with BMI > 30 kg/m^2, weight loss should be encouraged[10,25,27]. An increased risk of posttransplant death was observed in patients with a BMI of 34-36 kg/m^2[25,27], suggesting that in patients with these high levels of BMI, kidney transplantation may be associated with an unacceptably high risk, and the benefit of transplant should be balanced with the risk of remaining on dialysis[10,27,28].

However, many studies failed to demonstrate a significant survival advantage for patients who lose weight during the waiting list period[18,29,30]. In the study of Molnar *et al*[18], among 14632 waitlisted hemodialysis patients not receiving a transplant, each 1 kg/m^2 increase in BMI was associated with a death hazard ratio (HR) of 0.96. However, compared with patients with minimal weight change (± 1 kg),

patients who lost 3.0 to 4.9 kg and ≥ 5 kg had a RR of death of 1.3 and 1.51, respectively. More recently, Harhay *et al*[30] demonstrated that patients who lost ≥ 10% of their pretransplant weight had an increased risk of graft loss, mortality and longer hospitalization stay compared with those who had a < 5% weight change. Possible explanations for these adverse outcomes are the likely malnutrition status associated with weight loss and the rapid weight gain in most patients after transplantation. However, these studies did not differentiate intentional from unintentional weight loss, and only a minority of patients with higher BMI were investigated, so no conclusions about the potential benefits of intentional weight loss can be drawn.

Obese patients who lose weight may also have different access to transplantation by race and ethnicity. Ku *et al*[31] evaluated 10221 obese patients waitlisted for kidney transplantation to examine the association between weight changes and access to living or deceased donor transplantation by race/ethnicity. Death on the waiting list was more common among those who lost weight (15%) or gained weight (15%) than among those who maintained stable weight (13%). Overall, black people were more likely to lose weight and less likely to gain weight than whites. Overall, weight gain was associated with lower access to transplantation (HR 0.88) compared with maintenance of stable weight, but weight loss was not associated with better access to transplantation (HR 0.96) on the whole, although this correlation was different for recipients of living *vs* deceased donor organs. Weight loss was associated with improved access to living donor transplantation only for white recipients but not for non-Hispanic blacks or Hispanic recipients[31].

Bariatric surgery in ESRD patients

Weight loss in patients with ESRD is extremely difficult due to the restrictions of a renal diet, limited exercise tolerance due to coexisting comorbid conditions, dialysis-related fatigue, and hemodynamic instability[21]. Comprehensive weight loss programs involving regular exercise and nutrition counseling, together with pharmacotherapy, may lead to moderate weight loss among kidney transplant candidates, which could increase access to waiting lists[32]. However, medical management could have limited long-term success, while bariatric surgery could offer a reliable strategy to achieve weight loss in kidney transplant candidates[21,33], with acceptable morbidity and mortality rates[34-36]. Bariatric surgery in ESRD patients is associated with weight loss ranging from 29.8% to 72.8% excess weight loss (%EWL)[37], with reported mortality and morbidity rates of 2% and 7%, respectively[37]. Complications associated with bariatric surgery are higher in ESRD patients than in non-ESRD patients[37]: The mortality rate (2%) observed in the ESRD population is approximately 10 times higher than the mortality rate (0.18%) reported in the general population[38], while the rate of postoperative complications in ESRD patients is significantly higher than that observed in accredited hospitals for bariatric surgery (0.17%)[39]. However, many studies have consistently shown that bariatric surgery in patients with chronic kidney disease (CKD) stage 1 and 2, is associated with slower epidermal growth factor receptor (eGFR) decline and lower risk of kidney failure[40,41]. In a recent study, Kassam *et al*[40] evaluated the change in renal function in 164 patients with CKD stages 1 to 4 undergoing bariatric surgery. Metabolic surgery resulted in a significant reduction in the BMI in all patients, and 34.3% of patients with previous diabetes achieved complete remission. Kidney function, as measured by eGFR, significantly improved in patients with CKD stages 2, 3a, and 3b, while a similar result was not observed among patients with CKD stages 1 and 4[40], suggesting that the improvements in renal function are limited only to those patients with a mild reduction in kidney function.

The most commonly performed bariatric surgical procedures are laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (RYGB). The former is mainly a restrictive procedure with resection of the greater curve of the stomach, while the latter is a restrictive/malabsorptive procedure that entails creation of a gastric pouch and formation of a Roux-en-Y gastrojejunostomy[21,33]. Open surgery in ESRD patients may be associated with an increased mortality rate compared with the general population. In their registry analysis, Modanlou *et al*[34] evaluated the results of 186 ESRD patients who underwent an open bariatric surgical procedure: 72 patients underwent surgery prior to activation on the waiting list, 27 patients underwent surgery during wait-listing, and 87 patients underwent posttransplant surgery. The 30-d mortality in wait-listed and posttransplant patients was 3.5% in both groups, while the median EWL ranged between 31% and 61%[34].

Although there are few studies comparing the two surgical procedures in ESRD patients, RYGB seems to have the potential to improve access to renal transplantation and improve long-term survival compared with LSG[35]. Both LSG and RYGB achieve

significant excess body weight loss (up to 80% within 24 mo) and may increase the likelihood of being listed for kidney transplantation in up to 50.3% of patients, although a recent meta-analysis reported that only 25% of patients had access to transplant at a median follow-up of 48 mo[37]. A recent analysis of the ESRD population using a probabilistic Markov model concluded that RYGB could improve access to renal transplantation and thereby increase long-term survival[42], but it could be associated with slightly higher morbidity and mortality rates[43].

In the ESRD population, LSG could be preferable and may offer significant advantages over RYGB, including an easier and faster surgical procedure and a lower incidence of surgical complications[37,44], and may increase access to the transplant waiting list and improve posttransplant outcomes[43,44-48]. Moreover, LSG does not alter immunosuppressive pharmacokinetics, avoiding under- and overimmunosuppression[37,43,48-50]. The correct timing of bariatric surgery is still a controversial issue. Although bariatric surgery in ESRD patients could be associated with an increased rate of postsurgical complications, including an increased rate of reoperation and readmission[51], most patients could benefit from pretransplant bariatric surgery to increase access to the waiting list and to reduce obesity-related complications, including diabetes and cardiovascular disease, that could worsen after transplantation. In the largest series reported in the literature, Kassam *et al*[48] evaluated the clinical outcomes of LSG in the ESRD population and access to the transplant waiting list. LSG reduced hypertension and the need for antihypertensive medications and reduced the incidence of diabetes (59.6% *vs* 32.5%, $P < 0.01$). Sixty-three percent of patients with ESRD who achieved a BMI of ≤ 40 kg/m^2 were waitlisted and received a kidney transplant after a mean overall time from LSG to transplant of 1.9 ± 1.3 years. There was no significant difference in survival between patients who received a kidney transplant after LSG and those who remained waitlisted[48], suggesting that LSG does not increase the morbidity rate and has the potential to reduce obesity-related comorbidities, possibly improving long-term outcomes. In their retrospective study, Cohen *et al*[52] compared the outcome of pretransplant and posttransplant bariatric surgery: Compared to BMI-matched controls, pretransplant bariatric surgery was associated with a 1-year increased risk of acute rejection and a decreased risk of delayed graft function. Interestingly, there was no significant difference in BMI in the 5 years after bariatric surgery between the two groups, while both pretransplant and posttransplant bariatric surgery was associated with a decreased risk of allograft failure and mortality[52].

In summary, pretransplant bariatric surgery is safe and could increase access to transplantation for obese patients. LSG results in sustained weight loss and is associated with an improvement in obesity-related comorbidities (Figure 1). Although pretransplant bariatric surgery is associated with acceptable outcomes for patients undergoing kidney transplantation, the correct timing has yet to be determined.

OBESITY AND KIDNEY TRANSPLANTATION

Kidney transplantation offers significantly better patient survival and quality of life than remaining on dialysis for both obese and nonobese patients[20,22,53,54].

Obese patients may have an increased peritransplant risk of death, particularly for patients with a BMI > 30 kg/m^2 receiving a graft from a marginal donor, while living donor kidney transplantation seems to offer a reduced risk[20,55]. In an analysis from the USRDS including 7521 patients, Glanton *et al*[56] compared the mortality rates among transplant recipients and patients on hemodialysis with class I, II and III obesity and found that kidney transplantation from both deceased (HR 0.39, 95%CI: 0.33-0.47) and living donors (HR 0.23, 95%CI: 0.16-0.34) was associated with significant lower mortality rate of those who stayed on dialysis waiting for a kidney. Interestingly, the beneficial effect of transplantation was lost in the subgroup analysis of patients with class III obesity[57]. The beneficial effect of kidney transplantation among obese patients was recently confirmed by Gill *et al*[20], who analyzed a large cohort from the US renal registry and reported a 66% reduction in the risk of death in all BMI groups for patients receiving a living donor kidney, whereas among the deceased donor recipients, the reduction in the risk of death was 66% in patients with class I and II obesity and 48%in patients with class III obesity[20]. The reduction in the risk of death was lower for patients receiving a graft from a marginal deceased donor, while kidney transplantation did not offer a survival benefit in African Americans with class III obesity[20]. Therefore, some authors have suggested that for patients with a higher BMI, living transplantation represents the preferred choice, while kidney

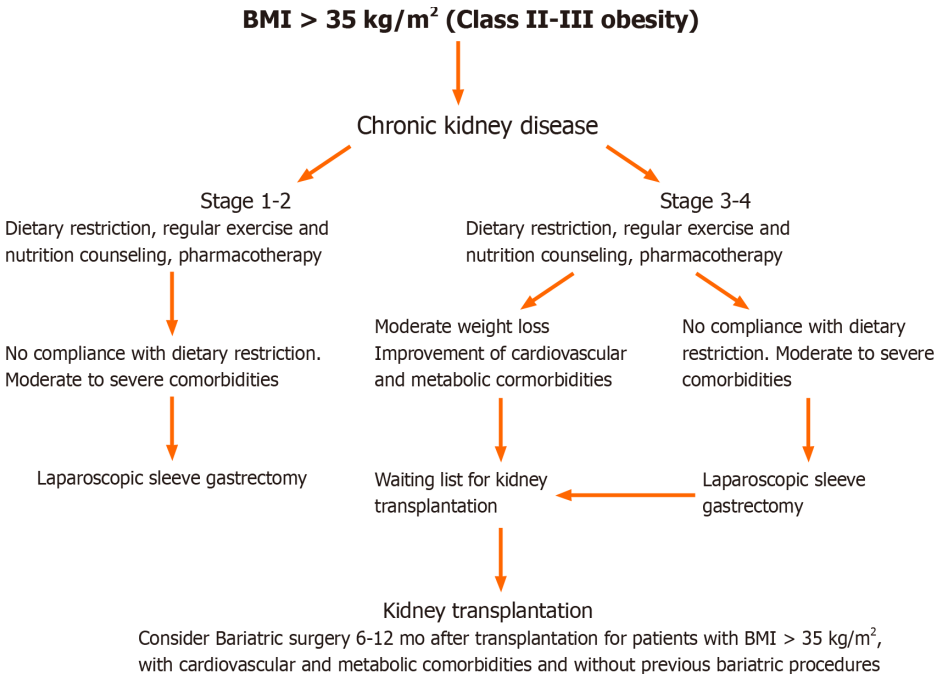

Figure 1 Proposed algorithm for the management of obesity in patients with chronic kidney disease, candidates to kidney transplantation and in kidney transplant recipients. Bariatric surgery could have a benefit in lowering the decline in renal function in patients with chronic kidney disease stages 2-3, so that it could be anticipated even in absence of significant metabolic comorbidities. In referral centers, combined laparoscopic/robotic sleeve gastrectomy with kidney transplantation could be proposed in selected patients. BMI: Body mass index.

transplantation from deceased donors could be associated with an unacceptable mortality risk[20,21].

The increased mortality risk observed in the peritransplant period in obese patients may be correlated with concomitant comorbid conditions that could worsen after transplantation or be the result of peritransplant complications.

Many studies have demonstrated that, similarly, graft survival in obese patients is inferior[20,21,55,58,59], and this pattern follows a U-shaped distribution, as patients with BMI values either lower or higher than the normal range (either ≤ 20 or ≥ 26 kg/m²) have worse posttransplant outcomes[58,60]. Moreover, significant post-transplant weight gain or weight loss (> 5%) has been associated with decreased patient survival[29,54,61].

However, the effect of BMI on posttransplant outcomes may vary by patient characteristics. In their analysis among 296807 adult kidney transplant recipients from the Scientific Registry of Transplant Recipients, Schold *et al*[60] demonstrated that BMI follows a "J-Shaped" risk profile with elevated risks for overall graft loss with low BMI and obesity. Moreover, the risk of graft loss associated with BMI is strictly dependent on the patients' characteristics: Low BMI was a relatively higher risk for older recipients (> 60 years) and males but not for younger patients, while high BMI was associated with an elevated risk for Caucasians and attenuated risk among African Americans and people with type II diabetes.

Obesity may increase the surgical complexity and is significantly associated with longer operative time and risk of wound dehiscence compared to normal-weight patients[62]. In obese patients, the risk of parietal dehiscence is significantly increased for BMI > 26 kg/m², while an increased risk of intraoperative blood loss and ureteral stenosis was observed for BMI > 32 kg/m², and the risk of abdominal wall hematoma was increased beyond a BMI of 34 kg/m²[63]. Overall, obese patients have an incidence of wound infections and incisional hernia of 4%-40% due to the longer operative time; the concomitant use of corticosteroids, sirolimus, or everolimus; and the presence of vascular disease[26,46,56]. Moreover, obesity may also increase the risk of surgical site infection (SSI), which is a well-known cause of incisional hernia[64,65]. Wound complications are significantly associated with a BMI over 30 kg/m², and in most cases, obesity is considered the most significant risk factor for the development of wound complications[65,66], although some authors did not find such an association[67].

Moreover, obese transplant recipients have an increased risk of delayed graft function, probably as a consequence of a longer operative time, a prolonged hospital stay, an increased rate of acute rejection, an increased rate of new-onset DM and hospital readmission[6,18,57,68-70].

Considering that some studies showed comparable outcomes between obese and nonobese patients in the absence of surgical complications, the adoption of a correct surgical procedure that could minimize the incidence of such complications is mandatory[33,71]. The adoption of a minimally invasive surgical approach, including robotic-assisted kidney transplantation (RAKT), has shown promising results compared to open KT and could increase access to kidney transplantation for obese patients[72,73]. Additionally, RAKT is associated with comparable patient and graft survival compared with open surgery[72], a significant reduction in SSI in obese recipients, and comparable graft and patient survival compared to the nonobese population[73,74].

Bariatric surgery after kidney transplantation

Few studies have investigated the role of bariatric surgery after kidney transplantation in morbidly obese recipients. There are many issues related to bariatric surgery after kidney transplantation: First, surgical procedures in kidney recipients may be associated with a higher risk of complications than in the general population[75-80], and second, bariatric surgery can affect immunosuppressive therapy absorption. Bariatric surgery in kidney transplant recipients may be associated with an increased operative time, length of stay, readmission, and increased SSI but not with increased mortality[75-80]. Previous diabetes and the use of corticosteroids do not increase the risk of postoperative complications after bariatric surgery in solid organ transplantation[78,80], while black race seems to be associated with an increased morbidity [79]. Bariatric surgery may be associated with an increased dose of calcineurin inhibitors needed to maintain the optimal dose, and RYGB may decrease the bioavailability of immunosuppressive drugs[37]. In their recent study, Yemini *et al*[75] analyzed the pharmacokinetic alterations in the absorption of immunosuppressive drugs in 34 kidney transplant recipients who underwent LSG or laparoscopic RYGB: Tacrolimus blood trough levels declined slightly, without significant modifications of the therapeutic range. This would reinforce the need for strict monitoring of immunosuppressive levels after bariatric surgery, particularly in the first months after surgery. The optimal timing of bariatric surgery after kidney transplantation would probably be 6-12 mo after transplantation, when immunosuppression is at its lowest level so that, as a consequence, a small variation in the trough levels would have a limited impact on graft function.

Bariatric surgery after kidney transplantation is associated with significant weight loss and a reduction in comorbidities but also with an increased risk of complications [37,52,75]. In the largest series of RYGB reported in kidney transplant recipients, Modanlou *et al*[34] reported a 3.5% mortality rate, with a median excess body weight loss of 31%-61%. Sleeve gastrectomy and RYGB have comparable outcomes with low postoperative complications[43,75-80]: A slight increase in mortality was observed in patients undergoing RYGB[43], but both LSG and RYGB were associated with improvements in comorbidities and graft function[43,75-80] and with a reduction in urinary protein excretion[76]. Cohen *et al*[52] compared the outcomes of 43 patients who underwent pretransplant bariatric surgery and 21 patients who underwent posttransplant bariatric surgery. BMI was similar between the two groups, and 5 years after bariatric surgery, there was no significant difference in BMI between the two groups (36 kg/m^2 *vs* 32 kg/m^2, P = 0.814). Compared to matched controls, post-transplant bariatric surgery was associated with a decreased risk of allograft failure and mortality[52]. In their innovative approach, Spaggiari *et al*[81] compared the safety and efficacy of combining robotic SG and RAKT (11 patients) to RAKT alone (9 patients) in candidates with class II or III obesity: At the 12-mo follow-up, there was no difference between the two groups in terms of estimated GFR, serum creatinine and graft failure rates. Patients receiving SG and RAKT had a significant reduction of BMI compared to the robotic kidney transplant group (P = 0.0041). Combined RAKT and SG was associated with a longer operative time without an increase in the incidence of surgical complications.

In summary, bariatric surgery after kidney transplantation is associated with a significant and sustained weight loss, reduction in comorbidities and improvement in graft function without significant alteration of immunosuppressive therapy absorption. The potential increase in postoperative complications and mortality warrants a careful evaluation of kidney transplant recipients scheduled for bariatric surgery.

Bariatric surgery in living kidney donors

There are approximately 2700 living-donor kidney transplants performed worldwide each year, and more than 25% of these are considered obese at the time of donation [82]. Obesity may be a relevant factor influencing clinical outcomes even in living-donor kidney transplantation and may be associated with lower preoperative kidney function and longer operative time[82]. Kinoshita *et al*[83] compared the results of living kidney transplantations from medically complex living donors, defined by the presence of older age, obesity or DM, with standard living donors; they found that kidney recipients of medically complex living donors had a higher risk of death-censored graft loss, while no significant difference in renal function in the short term was observed between standard and medically complex living donors. When compared to donors with normal BMI, kidney transplants from donors with higher BMI (> 25 kg/m^2) are associated with a higher risk of graft failure[84], and living donor obesity is associated with a 30% increased risk of long-term mortality compared with nonobese counterparts (adjusted HR: 1.32, 95%CI: 1.09-1.60, P = 0.006)[85].

Up to one-fourth of potential living kidney donors may be excluded from living donation due to obesity, which could encourage medical and surgical strategies to achieve significant weight loss. Although strongly motivated, living kidney donors are less prone to adhere to diet and lifestyle modifications for weight loss, and only 13% lose enough weight to attain a BMI < 35 kg/m^2 and then undergo donation[85]. Bariatric surgery is, therefore, a potential valid weight loss strategy for potential living donors. However, many ethical issues may arise when considering the opportunity for bariatric surgery in living kidney donors. Living kidney donors should be aware that bariatric surgery is predicated only on the potential donor's benefit and does not finalize the kidney donation or provide any future benefit to the intended recipient[86, 87], and referral to an independent bariatric surgeon to assess the potential benefits and risks of surgery is recommended. Another crucial point is the timing of living donation after bariatric surgery. Montgomery *et al*[87] suggested that kidney donation should be performed when the potential living donor meets prespecified transplant center donation eligibility requirements, such as BMI < 30 kg/m^2, and should remain stable for at least three months.

Very few studies have reported the outcomes of living kidney donation after predonation bariatric surgery. Earlier studies reported a 30%-54% decrease in BMI after bariatric surgery[88]. More recently, Nguyen *et al*[89] reported a series of 22 living kidney donors who underwent bariatric surgery 0.7-22 years before living donation. Interestingly, 18 donors would have been excluded from donation due to high BMI. All donors lost sufficient weight to subsequently become candidates for living kidney donation, and 17 donors reached a BMI < 35 kg/m^2 after bariatric surgery. No significant differences in terms of length of stay, warm ischemic time or postoperative complications were observed when compared with 37 donors with a BMI of 35–40 kg/m^2. Moreover, bariatric surgery did not significantly impact the subsequent laparoscopy for living donor nephrectomy[89]. Due to the limited cases reported in the literature, the ideal type of bariatric surgery to be performed in morbidly obese kidney donors has to be determined. RYGB has historically been the most commonly used technique since it guarantees a durable weight reduction and reversal of obesity-associated comorbidities[88,89]. However, it is associated with long-term nutritional derangements, and it has been recently supplanted by LSG as the most common bariatric surgery procedure, since it has proven comparable weight reduction with RYGB, with fewer intra- and postoperative complications, including nutritional deficiencies[88,89].

In summary, initial experience with bariatric surgery in potential living donors suggests that bariatric surgery is safe, is associated with sustained weight loss and could increase the rate of kidney donation. Sleeve gastrectomy should be preferred to RYGB due to its risk-benefit profile.

CONCLUSION

Obesity represents a major obstacle to access to kidney transplantation due to the potential increased risk of postoperative complications and mortality. However, obesity should not preclude kidney transplantation, and any efforts should be made to improve the outcomes of these patients. Bariatric surgery has been proven to be safe and helpful in reducing weight loss and obesity-related comorbidities and in increasing access to kidney transplantation. Posttransplant bariatric surgery may result in better graft survival and function but also in a high rate of postoperative complic-

ations. There is no consensus regarding the optimal timing and the ideal type of bariatric surgery, although sleeve gastrectomy seems to be associated with a reduced risk of postoperative complications. Future studies should evaluate the potential impact of bariatric surgery in the long-term reduction in cardiovascular complications and in the management of posttransplant DM in obese recipients.

REFERENCES

1 **Kelly T**, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431-1437 [PMID: 18607383 DOI: 10.1038/ijo.2008.102]

2 **Flegal KM**, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA* 2016; **315**: 2284-2291 [PMID: 27272580 DOI: 10.1001/jama.2016.6458]

3 **Diwan TS**, Lee TC, Nagai S, Benedetti E, Posselt A, Bumgardner G, Noria S, Whitson BA, Ratner L, Mason D, Friedman J, Woodside KJ, Heimbach J. Obesity, transplantation, and bariatric surgery: An evolving solution for a growing epidemic. *Am J Transplant* 2020; **20**: 2143-2155 [PMID: 31965711 DOI: 10.1111/ajt.15784]

4 **Veroux M**, Corona D, Veroux P. Kidney transplantation: future challenges. *Minerva Chir* 2009; **64**: 75-100 [PMID: 19202537]

5 **Kasiske BL**, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant* 2003; **3**: 178-185 [PMID: 12603213 DOI: 10.1034/j.1600-6143.2003.00010.x]

6 **Hill CJ**, Courtney AE, Cardwell CR, Maxwell AP, Lucarelli G, Veroux M, Furriel F, Cannon RM, Hoogeveen EK, Doshi M, McCaughan JA. Recipient obesity and outcomes after kidney transplantation: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2015; **30**: 1403-1411 [PMID: 26044837 DOI: 10.1093/ndt/gfv214]

7 **Veroux M**, Corona D, Giuffrida G, Gagliano M, Sorbello M, Virgilio C, Tallarita T, Zerbo D, Giaquinta A, Fiamingo P, Macarone M, Li Volti G, Caglia P, Veroux P. New-onset diabetes mellitus after kidney transplantation: the role of immunosuppression. *Transplant Proc* 2008; **40**: 1885-1887 [PMID: 18675079 DOI: 10.1016/j.transproceed.2008.06.005]

8 **Veroux M**, Tallarita T, Corona D, Sinagra N, Giaquinta A, Zerbo D, Guerrieri C, D'Assoro A, Cimino S, Veroux P. Conversion to sirolimus therapy in kidney transplant recipients with new onset diabetes mellitus after transplantation. *Clin Dev Immunol* 2013; **2013**: 496974 [PMID: 23762090 DOI: 10.1155/2013/496974]

9 **Xia M**, Yang H, Tong X, Xie H, Cui F, Shuang W. Risk factors for new-onset diabetes mellitus after kidney transplantation: A systematic review and meta-analysis. *J Diabetes Investig* 2021; **12**: 109-122 [PMID: 32506801 DOI: 10.1111/jdi.13317]

10 **Chadban SJ**, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, Kumar D, Oberbauer R, Pascual J, Pilmore HL, Rodrigue JR, Segev DL, Sheerin NS, Tinckam KJ, Wong G, Knoll GA. KDIGO Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. *Transplantation* 2020; **104**: S11-S103 [PMID: 32301874 DOI: 10.1097/TP.0000000000003136]

11 **Camilleri B**, Bridson JM, Sharma A, Halawa A. From chronic kidney disease to kidney transplantation: The impact of obesity and its treatment modalities. *Transplant Rev (Orlando)* 2016; **30**: 203-211 [PMID: 27534874 DOI: 10.1016/j.trre.2016.07.006]

12 **Glicklich D**, Mustafa MR. Obesity in Kidney Transplantation: Impact on Transplant Candidates, Recipients, and Donors. *Cardiol Rev* 2019; **27**: 63-72 [PMID: 29870421 DOI: 10.1097/CRD.0000000000000216]

13 **Postorino M**, Marino C, Tripepi G, Zoccali C; CREDIT (Calabria Registry of Dialysis and Transplantation) Working Group. Abdominal obesity and all-cause and cardiovascular mortality in end-stage renal disease. *J Am Coll Cardiol* 2009; **53**: 1265-1272 [PMID: 19358939 DOI: 10.1016/j.jacc.2008.12.040]

14 **Ahmadi SF**, Zahmatkesh G, Streja E, Molnar MZ, Rhee CM, Kovesdy CP, Gillen DL, Steiner S, Kalantar-Zadeh K. Body mass index and mortality in kidney transplant recipients: a systematic review and meta-analysis. *Am J Nephrol* 2014; **40**: 315-324 [PMID: 25341624 DOI: 10.1159/000367812]

15 **Dolla C**, Naso E, Mella A, Allesina A, Giraudi R, Torazza MC, Vanzino SB, Gallo E, Lavacca A, Fop F, Biancone L. Impact of type 2 diabetes mellitus on kidney transplant rates and clinical outcomes among waitlisted candidates in a single center European experience. *Sci Rep* 2020; **10**: 22000 [PMID: 33319849 DOI: 10.1038/s41598-020-78938-3]

16 **Leavey SF**, McCullough K, Hecking E, Goodkin D, Port FK, Young EW. Body mass index and mortality in 'healthier' as compared with 'sicker' haemodialysis patients: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrol Dial Transplant* 2001; **16**: 2386-2394 [PMID: 11733631 DOI: 10.1093/ndt/16.12.2386]

17 **Park J**, Ahmadi SF, Streja E, Molnar MZ, Flegal KM, Gillen D, Kovesdy CP, Kalantar-Zadeh K. Obesity paradox in end-stage kidney disease patients. *Prog Cardiovasc Dis* 2014; **56**: 415-425 [PMID: 24438733 DOI: 10.1016/j.pcad.2013.10.005]

18 **Molnar MZ**, Streja E, Kovesdy CP, Bunnapradist S, Sampaio MS, Jing J, Krishnan M, Nissenson

AR, Danovitch GM, Kalantar-Zadeh K. Associations of body mass index and weight loss with mortality in transplant-waitlisted maintenance hemodialysis patients. *Am J Transplant* 2011; **11**: 725-736 [PMID: 21446975 DOI: 10.1111/j.1600-6143.2011.03468.x]

19 **Gill JS**, Hendren E, Dong J, Johnston O, Gill J. Differential association of body mass index with access to kidney transplantation in men and women. *Clin J Am Soc Nephrol* 2014; **9**: 951-959 [PMID: 24742478 DOI: 10.2215/CJN.08310813]

20 **Gill JS**, Lan J, Dong J, Rose C, Hendren E, Johnston O, Gill J. The survival benefit of kidney transplantation in obese patients. *Am J Transplant* 2013; **13**: 2083-2090 [PMID: 23890325 DOI: 10.1111/ajt.12331]

21 **Lesage J**, Gill JS. Management of the obese kidney transplant candidate. *Transplant Rev (Orlando)* 2017; **31**: 35-41 [PMID: 28139330 DOI: 10.1016/j.trre.2016.12.002]

22 **Poggio ED**, Augustine JJ, Arrigain S, Brennan DC, Schold JD. Long-term kidney transplant graft survival-Making progress when most needed. *Am J Transplant* 2021; **21**: 2824-2832 [PMID: 33346917 DOI: 10.1111/ajt.16463]

23 **Naik AS**, Sakhuja A, Cibrik DM, Ojo AO, Samaniego-Picota MD, Lentine KL. The Impact of Obesity on Allograft Failure After Kidney Transplantation: A Competing Risks Analysis. *Transplantation* 2016; **100**: 1963-1969 [PMID: 26569067 DOI: 10.1097/TP.0000000000000983]

24 **Hatamizadeh P**, Molnar MZ, Streja E, Lertdumrongluk P, Krishnan M, Kovesdy CP, Kalantar-Zadeh K. Recipient-related predictors of kidney transplantation outcomes in the elderly. *Clin Transplant* 2013; **27**: 436-443 [PMID: 23516994 DOI: 10.1111/ctr.12106]

25 **Segev DL**, Simpkins CE, Thompson RE, Locke JE, Warren DS, Montgomery RA. Obesity impacts access to kidney transplantation. *J Am Soc Nephrol* 2008; **19**: 349-355 [PMID: 18094366 DOI: 10.1681/ASN.2007050610]

26 **Orandi BJ**, Purvis JW, Cannon RM, Smith AB, Lewis CE, Terrault NA, Locke JE. Bariatric surgery to achieve transplant in end-stage organ disease patients: A systematic review and meta-analysis. *Am J Surg* 2020; **220**: 566-579 [PMID: 32600846 DOI: 10.1016/j.amjsurg.2020.04.041]

27 **Knoll G**, Cockfield S, Blydt-Hansen T, Baran D, Kiberd B, Landsberg D, Rush D, Cole E; Kidney Transplant Working Group of the Canadian Society of Transplantation. Canadian Society of Transplantation consensus guidelines on eligibility for kidney transplantation. *CMAJ* 2005; **173**: 1181-1184 [PMID: 16275969 DOI: 10.1503/cmaj.051291]

28 **Campbell S**, Pilmore H, Gracey D, Mulley W, Russell C, McTaggart S. KHA-CARI guideline: recipient assessment for transplantation. *Nephrology (Carlton)* 2013; **18**: 455-462 [PMID: 23581832 DOI: 10.1111/nep.12068]

29 **Chang TI**, Ngo V, Streja E, Chou JA, Tortorici AR, Kim TH, Kim TW, Soohoo M, Gillen D, Rhee CM, Kovesdy CP, Kalantar-Zadeh K. Association of body weight changes with mortality in incident hemodialysis patients. *Nephrol Dial Transplant* 2017; **32**: 1549-1558 [PMID: 27789782 DOI: 10.1093/ndt/gfw373]

30 **Harhay MN**, Ranganna K, Boyle SM, Brown AM, Bajakian T, Levin Mizrahi LB, Xiao G, Guy S, Malat G, Segev DL, Reich D, McAdams-DeMarco M. Association Between Weight Loss Before Deceased Donor Kidney Transplantation and Posttransplantation Outcomes. *Am J Kidney Dis* 2019; **74**: 361-372 [PMID: 31126666 DOI: 10.1053/j.ajkd.2019.03.418]

31 **Ku E**, Whelan AM, McCulloch CE, Lee B, Niemann CU, Roll GR, Grimes BA, Johansen KL. Weighing the waitlist: Weight changes and access to kidney transplantation among obese candidates. *PLoS One* 2020; **15**: e0242784 [PMID: 33253253 DOI: 10.1371/journal.pone.0242784]

32 **MacLaughlin HL**, Cook SA, Kariyawasam D, Roseke M, van Niekerk M, Macdougall IC. Nonrandomized trial of weight loss with orlistat, nutrition education, diet, and exercise in obese patients with CKD: 2-year follow-up. *Am J Kidney Dis* 2010; **55**: 69-76 [PMID: 19926371 DOI: 10.1053/j.ajkd.2009.09.011]

33 **Di Cocco P**, Okoye O, Almario J, Benedetti E, Tzvetanov IG, Spaggiari M. Obesity in kidney transplantation. *Transpl Int* 2020; **33**: 581-589 [PMID: 31667905 DOI: 10.1111/tri.13547]

34 **Modanlou KA**, Muthyala U, Xiao H, Schnitzler MA, Salvalaggio PR, Brennan DC, Abbott KC, Graff RJ, Lentine KL. Bariatric surgery among kidney transplant candidates and recipients: analysis of the United States renal data system and literature review. *Transplantation* 2009; **87**: 1167-1173 [PMID: 19384163 DOI: 10.1097/TP.0b013e31819e3f14]

35 **Friedman AN**, Wahed AS, Wang J, Courcoulas AP, Dakin G, Hinojosa MW, Kimmel PL, Mitchell JE, Pomp A, Pories WJ, Purnell JQ, le Roux C, Spaniolas K, Steffen KJ, Thirlby R, Wolfe B. Effect of Bariatric Surgery on CKD Risk. *J Am Soc Nephrol* 2018; **29**: 1289-1300 [PMID: 29335242 DOI: 10.1681/ASN.2017060707]

36 **Sheetz KH**, Gerhardinger L, Dimick JB, Waits SA. Bariatric Surgery and Long-term Survival in Patients With Obesity and End-stage Kidney Disease. *JAMA Surg* 2020; **155**: 581-588 [PMID: 32459318 DOI: 10.1001/jamasurg.2020.0829]

37 **Guggino J**, Coumes S, Wion N, Reche F, Arvieux C, Borel AL. Effectiveness and Safety of Bariatric Surgery in Patients with End-Stage Chronic Kidney Disease or Kidney Transplant. *Obesity (Silver Spring)* 2020; **28**: 2290-2304 [PMID: 33230959 DOI: 10.1002/oby.23001]

38 **Cardoso L**, Rodrigues D, Gomes L, Carrilho F. Short- and long-term mortality after bariatric surgery: A systematic review and meta-analysis. *Diabetes Obes Metab* 2017; **19**: 1223-1232 [PMID: 28244626 DOI: 10.1111/dom.12922]

39 **De Smet J**, Van Bocxlaer J, Boussery K. The influence of bypass procedures and other anatomical changes in the gastrointestinal tract on the oral bioavailability of drugs. *J Clin Pharmacol* 2013; **53**:

361-376 [PMID: 23381905 DOI: 10.1002/jcph.65]
40 **Kassam AF**, Taylor ME, Morris MC, Watkins BM, Thompson JR, Schauer DP, Smith EP, Diwan TS. The impact of sleeve gastrectomy on renal function in patients with chronic kidney disease varies with severity of renal insufficiency. *Surg Obes Relat Dis* 2020; **16**: 607-613 [PMID: 32093996 DOI: 10.1016/j.soard.2020.01.021]
41 **Chintam K**, Chang AR. Strategies to Treat Obesity in Patients With CKD. *Am J Kidney Dis* 2021; **77**: 427-439 [PMID: 33075388 DOI: 10.1053/j.ajkd.2020.08.016]
42 **Choudhury RA**, Hoeltzel G, Prins K, Chow E, Moore HB, Lawson PJ, Yoeli D, Pratap A, Abt PL, Dumon KR, Conzen KD, Nydam TL. Sleeve Gastrectomy Compared with Gastric Bypass for Morbidly Obese Patients with End Stage Renal Disease: a Decision Analysis. *J Gastrointest Surg* 2020; **24**: 756-763 [PMID: 31044345 DOI: 10.1007/s11605-019-04225-w]
43 **Dziodzio T**, Biebl M, Öllinger R, Pratschke J, Denecke C. The Role of Bariatric Surgery in Abdominal Organ Transplantation-the Next Big Challenge? *Obes Surg* 2017; **27**: 2696-2706 [PMID: 28791580 DOI: 10.1007/s11695-017-2854-8]
44 **Freeman CM**, Woodle ES, Shi J, Alexander JW, Leggett PL, Shah SA, Paterno F, Cuffy MC, Govil A, Mogilishetty G, Alloway RR, Hanseman D, Cardi M, Diwan TS. Addressing morbid obesity as a barrier to renal transplantation with laparoscopic sleeve gastrectomy. *Am J Transplant* 2015; **15**: 1360-1368 [PMID: 25708829 DOI: 10.1111/ajt.13116]
45 **Kienzl-Wagner K**, Weissenbacher A, Gehwolf P, Wykypiel H, Öfner D, Schneeberger S. Laparoscopic sleeve gastrectomy: gateway to kidney transplantation. *Surg Obes Relat Dis* 2017; **13**: 909-915 [PMID: 28216112 DOI: 10.1016/j.soard.2017.01.005]
46 **Kim Y**, Bailey AJ, Morris MC, Kassam AF, Shah SA, Diwan TS. Kidney transplantation after sleeve gastrectomy in the morbidly obese candidate: results of a 2-year experience. *Surg Obes Relat Dis* 2020; **16**: 10-14 [PMID: 31668565 DOI: 10.1016/j.soard.2019.09.069]
47 **Kim Y**, Jung AD, Dhar VK, Tadros JS, Schauer DP, Smith EP, Hanseman DJ, Cuffy MC, Alloway RR, Shields AR, Shah SA, Woodle ES, Diwan TS. Laparoscopic sleeve gastrectomy improves renal transplant candidacy and posttransplant outcomes in morbidly obese patients. *Am J Transplant* 2018; **18**: 410-416 [PMID: 28805345 DOI: 10.1111/ajt.14463]
48 **Kassam AF**, Mirza A, Kim Y, Hanseman D, Woodle ES, Quillin RC 3rd, Johnson BL, Govil A, Cardi M, Schauer DP, Smith EP, Diwan TS. Long-term outcomes in patients with obesity and renal disease after sleeve gastrectomy. *Am J Transplant* 2020; **20**: 422-429 [PMID: 31605562 DOI: 10.1111/ajt.15650]
49 **Rogers CC**, Alloway RR, Alexander JW, Cardi M, Trofe J, Vinks AA. Pharmacokinetics of mycophenolic acid, tacrolimus and sirolimus after gastric bypass surgery in end-stage renal disease and transplant patients: a pilot study. *Clin Transplant* 2008; **22**: 281-291 [PMID: 18482049 DOI: 10.1111/j.1399-0012.2007.00783.x]
50 **Diwan TS**, Lichvar AB, Leino AD, Vinks AA, Christians U, Shields AR, Cardi MA, Fukuda T, Mizuno T, Kaiser T, Woodle ES, Alloway RR. Pharmacokinetic and pharmacogenetic analysis of immunosuppressive agents after laparoscopic sleeve gastrectomy. *Clin Transplant* 2017; **31** [PMID: 28342282 DOI: 10.1111/ctr.12975]
51 **Cohen JB**, Tewksbury CM, Torres Landa S, Williams NN, Dumon KR. National Postoperative Bariatric Surgery Outcomes in Patients with Chronic Kidney Disease and End-Stage Kidney Disease. *Obes Surg* 2019; **29**: 975-982 [PMID: 30443719 DOI: 10.1007/s11695-018-3604-2]
52 **Cohen JB**, Lim MA, Tewksbury CM, Torres-Landa S, Trofe-Clark J, Abt PL, Williams NN, Dumon KR, Goral S. Bariatric surgery before and after kidney transplantation: long-term weight loss and allograft outcomes. *Surg Obes Relat Dis* 2019; **15**: 935-941 [PMID: 31378281 DOI: 10.1016/j.soard.2019.04.002]
53 **Bennett WM**, McEvoy KM, Henell KR, Pidikiti S, Douzdjian V, Batiuk T. Kidney transplantation in the morbidly obese: complicated but still better than dialysis. *Clin Transplant* 2011; **25**: 401-405 [PMID: 20946469 DOI: 10.1111/j.1399-0012.2010.01328.x]
54 **Krishnan N**, Higgins R, Short A, Zehnder D, Pitcher D, Hudson A, Raymond NT. Kidney Transplantation Significantly Improves Patient and Graft Survival Irrespective of BMI: A Cohort Study. *Am J Transplant* 2015; **15**: 2378-2386 [PMID: 26147285 DOI: 10.1111/ajt.13363]
55 **Hoogeveen EK**, Aalten J, Rothman KJ, Roodnat JI, Mallat MJ, Borm G, Weimar W, Hoitsma AJ, de Fijter JW. Effect of obesity on the outcome of kidney transplantation: a 20-year follow-up. *Transplantation* 2011; **91**: 869-874 [PMID: 21326138 DOI: 10.1097/TP.0b013e3182100f3a]
56 **Glanton CW**, Kao TC, Cruess D, Agodoa LY, Abbott KC. Impact of renal transplantation on survival in end-stage renal disease patients with elevated body mass index. *Kidney Int* 2003; **63**: 647-653 [PMID: 12631130 DOI: 10.1046/j.1523-1755.2003.00761.x]
57 **Aziz F**, Ramadorai A, Parajuli S, Garg N, Mohamed M, Mandelbrot DA, Foley DP, Garren M, Djamali A. Obesity: An Independent Predictor of Morbidity and Graft Loss after Kidney Transplantation. *Am J Nephrol* 2020; **51**: 615-623 [PMID: 32721967 DOI: 10.1159/000509105]
58 **Meier-Kriesche HU**, Arndorfer JA, Kaplan B. The impact of body mass index on renal transplant outcomes: a significant independent risk factor for graft failure and patient death. *Transplantation* 2002; **73**: 70-74 [PMID: 11792981 DOI: 10.1097/00007890-200201150-00013]
59 **Grosso G**, Corona D, Mistretta A, Zerbo D, Sinagra N, Giaquinta A, Caglià P, Amodeo C, Leonardi A, Gula R, Veroux P, Veroux M. The role of obesity in kidney transplantation outcome. *Transplant Proc* 2012; **44**: 1864-1868 [PMID: 22974857 DOI: 10.1016/j.transproceed.2012.06.043]
60 **Schold JD**, Augustine JJ, Huml AM, Fatica R, Nurko S, Wee A, Poggio ED. Effects of body mass

index on kidney transplant outcomes are significantly modified by patient characteristics. *Am J Transplant* 2021; **21**: 751-765 [PMID: 32654372 DOI: 10.1111/ajt.16196]

61 **Chang SH**, McDonald SP. Post-kidney transplant weight change as marker of poor survival outcomes. *Transplantation* 2008; **85**: 1443-1448 [PMID: 18497685 DOI: 10.1097/TP.0b013e31816f1cd3]

62 **Chen JH**, Lee CH, Chang CM, Yin WY. Successful Management of New-Onset Diabetes Mellitus and Obesity With the Use of Laparoscopic Sleeve Gastrectomy After Kidney Transplantation-A Case Report. *Transplant Proc* 2016; **48**: 938-939 [PMID: 27234772 DOI: 10.1016/j.transproceed.2015.12.074]

63 **Gullo-Neto S**, Padoin AV, Queiroz de Carvalho JE, Wendling R, Traesel MA, Kroth L, Miranda C, Balestro AC, Siqueira R, Chao Lisot B, Lima S, Mottin CC, Saitovitch D. Metabolic surgery for the treatment of type 2 diabetes in pancreas after kidney transplant candidates. *Transplant Proc* 2014; **46**: 1741-1744 [PMID: 25131025 DOI: 10.1016/j.transproceed.2014.05.005]

64 **Valente JF**, Hricik D, Weigel K, Seaman D, Knauss T, Siegel CT, Bodziak K, Schulak JA. Comparison of sirolimus vs. mycophenolate mofetil on surgical complications and wound healing in adult kidney transplantation. *Am J Transplant* 2003; **3**: 1128-1134 [PMID: 12919093 DOI: 10.1034/j.1600-6143.2003.00185.x]

65 **Smith CT**, Katz MG, Foley D, Welch B, Leverson GE, Funk LM, Greenberg JA. Incidence and risk factors of incisional hernia formation following abdominal organ transplantation. *Surg Endosc* 2015; **29**: 398-404 [PMID: 25125093 DOI: 10.1007/s00464-014-3682-8]

66 **Shahrestani S**, Tran HM, Pleass HC, Hawthorne WJ. Optimal surgical management in kidney and pancreas transplantation to minimise wound complications: A systematic review and meta-analysis. *Ann Med Surg (Lond)* 2018; **33**: 24-31 [PMID: 30167299 DOI: 10.1016/j.amsu.2018.08.006]

67 **Howard RJ**, Thai VB, Patton PR, Hemming AW, Reed AI, Van der Werf WJ, Fujita S, Karlix JL, Scornik JC. Obesity does not portend a bad outcome for kidney transplant recipients. *Transplantation* 2002; **73**: 53-55 [PMID: 11792977 DOI: 10.1097/00007890-200201150-00009]

68 **Kwan JM**, Hajjiri Z, Metwally A, Finn PW, Perkins DL. Effect of the Obesity Epidemic on Kidney Transplantation: Obesity Is Independent of Diabetes as a Risk Factor for Adverse Renal Transplant Outcomes. *PLoS One* 2016; **11**: e0165712 [PMID: 27851743 DOI: 10.1371/journal.pone.0165712]

69 **Lentine KL**, Delos Santos R, Axelrod D, Schnitzler MA, Brennan DC, Tuttle-Newhall JE. Obesity and kidney transplant candidates: how big is too big for transplantation? *Am J Nephrol* 2012; **36**: 575-586 [PMID: 23221167 DOI: 10.1159/000345476]

70 **Erturk T**, Berber I, Cakir U. Effect of Obesity on Clinical Outcomes of Kidney Transplant Patients. *Transplant Proc* 2019; **51**: 1093-1095 [PMID: 31101178 DOI: 10.1016/j.transproceed.2019.02.012]

71 **Lynch RJ**, Ranney DN, Shijie C, Lee DS, Samala N, Englesbe MJ. Obesity, surgical site infection, and outcome following renal transplantation. *Ann Surg* 2009; **250**: 1014-1020 [PMID: 19779327 DOI: 10.1097/SLA.0b013e3181b4ee9a]

72 **Oberholzer J**, Giulianotti P, Danielson KK, Spaggiari M, Bejarano-Pineda L, Bianco F, Tzvetanov I, Ayloo S, Jeon H, Garcia-Roca R, Thielke J, Tang I, Akkina S, Becker B, Kinzer K, Patel A, Benedetti E. Minimally invasive robotic kidney transplantation for obese patients previously denied access to transplantation. *Am J Transplant* 2013; **13**: 721-728 [PMID: 23437881 DOI: 10.1111/ajt.12078]

73 **Tzvetanov IG**, Spaggiari M, Tulla KA, Di Bella C, Okoye O, Di Cocco P, Jeon H, Oberholzer J, Cristoforo Giulianotti P, Benedetti E. Robotic kidney transplantation in the obese patient: 10-year experience from a single center. *Am J Transplant* 2020; **20**: 430-440 [PMID: 31571369 DOI: 10.1111/ajt.15626]

74 **Prudhomme T**, Beauval JB, Lesourd M, Roumiguié M, Decaestecker K, Vignolini G, Campi R, Serni S, Territo A, Gausa L, Tugcu V, Sahin S, Alcaraz A, Musquera M, Stockle M, Janssen M, Fornara P, Mohammed N, Del Bello A, Kamar N, Sallusto F, Breda A, Doumerc N. Robotic-assisted kidney transplantation in obese recipients compared to non-obese recipients: the European experience. *World J Urol* 2021; **39**: 1287-1298 [PMID: 32562044 DOI: 10.1007/s00345-020-03309-6]

75 **Yemini R**, Nesher E, Winkler J, Carmeli I, Azran C, Ben David M, Mor E, Keidar A. Bariatric surgery in solid organ transplant patients: Long-term follow-up results of outcome, safety, and effect on immunosuppression. *Am J Transplant* 2018; **18**: 2772-2780 [PMID: 29569341 DOI: 10.1111/ajt.14739]

76 **Golomb I**, Winkler J, Ben-Yakov A, Benitez CC, Keidar A. Laparoscopic sleeve gastrectomy as a weight reduction strategy in obese patients after kidney transplantation. *Am J Transplant* 2014; **14**: 2384-2390 [PMID: 25139661 DOI: 10.1111/ajt.12829]

77 **Montgomery JR**, Cohen JA, Brown CS, Sheetz KH, Chao GF, Waits SA, Telem DA. Perioperative risks of bariatric surgery among patients with and without history of solid organ transplant. *Am J Transplant* 2020; **20**: 2530-2539 [PMID: 32243667 DOI: 10.1111/ajt.15883]

78 **Fagenson AM**, Mazzei MM, Zhao H, Lu X, Edwards MA. Bariatric Surgery Outcomes in Patients with Prior Solid Organ Transplantation: an MBSAQIP Analysis. *Obes Surg* 2020; **30**: 2313-2324 [PMID: 32096014 DOI: 10.1007/s11695-020-04490-8]

79 **Edwards MA**, Fagenson AM, Mazzei M, Zhao H. Bariatric Surgery in Prior Solid Organ Transplantation Patients: Is Race a Predictor of Adverse Outcomes? *Obes Surg* 2020; **30**: 4381-4390 [PMID: 32617920 DOI: 10.1007/s11695-020-04813-9]

80 **Fagenson AM**, Mazzei MM, Zhao H, Edwards MA. Bariatric surgery in posttransplantat patients: does diabetes influence outcomes? *Surg Obes Relat Dis* 2020; **16**: 1266-1274 [PMID: 32473785 DOI: 10.1016/j.soard.2020.04.015]

81 **Spaggiari M**, Di Cocco P, Tulla K, Kaylan KB, Masrur MA, Hassan C, Alvarez JA, Benedetti E, Tzvetanov I. Simultaneous robotic kidney transplantation and bariatric surgery for morbidly obese patients with end-stage renal failure. *Am J Transplant* 2021; **21**: 1525-1534 [PMID: 32976702 DOI: 10.1111/ajt.16322]

82 **Schussler L**, Khetan P, Peacock M, Dickstein E, LaPointe-Rudow D, Palese M, Arvelakis A, Herron D, Shapiro R, Florman S, Chin EH. Is obesity a contraindication for kidney donation? *Surg Endosc* 2020; **34**: 4632-4637 [PMID: 31637602 DOI: 10.1007/s00464-019-07218-7]

83 **Kinoshita Y**, Yagisawa T, Sugihara T, Hara K, Takeshima S, Kubo T, Shinzato T, Shimizu T, Suzuki M, Maeshima A, Kamei J, Fujisaki A, Ando S, Kume H, Fujimura T. Clinical outcomes in donors and recipients of kidney transplantations involving medically complex living donors - a retrospective study. *Transpl Int* 2020; **33**: 1417-1423 [PMID: 32654198 DOI: 10.1111/tri.13699]

84 **Naik AS**, Zhong Y, Parasuraman R, Doshi M, Norman S, Lu Y, Shaban E, Shahinian V, Schaubel DE. The temporal and long-term impact of donor body mass index on recipient outcomes after kidney transplantation - a retrospective study. *Transpl Int* 2020; **33**: 59-67 [PMID: 31478267 DOI: 10.1111/tri.13505]

85 **Locke JE**, Reed RD, Massie AB, MacLennan PA, Sawinski D, Kumar V, Snyder JJ, Carter AJ, Shelton BA, Mustian MN, Lewis CE, Segev DL. Obesity and long-term mortality risk among living kidney donors. *Surgery* 2019; **166**: 205-208 [PMID: 31072668 DOI: 10.1016/j.surg.2019.03.016]

86 **Sachdeva M**, Sunday S, Israel E, Varghese J, Rosen L, Bhaskaran M, Molmenti EP, Mattana J. Obesity as a barrier to living kidney donation: a center-based analysis. *Clin Transplant* 2013; **27**: 882-887 [PMID: 24102846 DOI: 10.1111/ctr.12246]

87 **Montgomery JR**, Telem DA, Waits SA. Bariatric surgery for prospective living kidney donors with obesity? *Am J Transplant* 2019; **19**: 2415-2420 [PMID: 30632698 DOI: 10.1111/ajt.15260]

88 **Branco AW**, Branco Filho AJ, Kondo W. Laparoscopic live donor nephrectomy in patients surgically treated for morbid obesity. *Int Braz J Urol* 2007; **33**: 377-9; discussion 379 [PMID: 17626654 DOI: 10.1590/s1677-55382007000300010]

89 **Nguyen MJP**, Carpenter D, Tadros J, Mathur A, Sandoval PR, Woodle ES, Diwan T, Ratner LE. Bariatric surgery prior to living donor nephrectomy: a solution to expand the living donor kidney pool - a retrospective study. *Transpl Int* 2019; **32**: 702-709 [PMID: 30721545 DOI: 10.1111/tri.13408]

World Journal of Diabetes

Submit a Manuscript: https://www.f6publishing.com

World J Diabetes 2021 September 15; 12(9): 1576-1586

DOI: 10.4239/wjd.v12.i9.1576

ISSN 1948-9358 (online)

META-ANALYSIS

Effectiveness of drug interventions in nonalcoholic fatty liver disease: A network meta-analysis

Yi-Zhou Huang, Gang-Yi Yang, Cong Wang, Xing-Yu Chen, Li-Li Zhang

ORCID number: Yi-Zhou Huang 0000-0002-7151-205X; Gang-Yi Yang 0000-0002-6458-6747; Cong Wang 0000-0002-1209-4978; Xing-Yu Chen 0000-0003-0244-3721; Li-Li Zhang 0000-0001-9007-5281.

Author contributions: Huang YZ and Zhang LL designed this study; Chen XY, Wang C and Yang GY contributed to the assessment of available studies; Huang YZ and Yang GY contributed to the writing of the manuscript and helpful discussion; Zhang LL is the person who takes full responsibility for the work as a whole, including (if applicable) access to data and the decision to submit and publish the manuscript.

Supported by National Natural Science Foundation of China, No. 81300702; and Natural Science Foundation Project of Chongqing CSTC, No. cstc2018jcyjAXO210.

Conflict-of-interest statement: All authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2020 Checklist, and the manuscript was prepared and revised according to the PRISMA 2020 Checklist.

Yi-Zhou Huang, Gang-Yi Yang, Cong Wang, Xing-Yu Chen, Li-Li Zhang, Department of Endocrinology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 404100, China

Corresponding author: Li-Li Zhang, MD, PhD, Associate Professor, Doctor, Postdoc, Research Fellow, Department of Endocrinology, The Second Affiliated Hospital, Chongqing Medical University, No. 74 Linjiang Road, Yuzhong District, Chongqing 404100, China.
zhanglili.jl@foxmail.com

Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is a major chronic liver disorder worldwide, and there is no established treatment for this disease. We conducted a network meta-analysis (NMA) to compare existing treatments, which include four classes of antidiabetic drugs, and examined the optimum treatments for NAFLD.

AIM

To compare the effectiveness of different treatments for NAFLD.

METHODS

An NMA was conducted using Stata 14.0 (Corporation LLC, College Station, United States) and R (X64 3.6.3 version) in this study. Eligible randomized controlled trials (RCTs) were searched in the PubMed, Cochrane Library, Embase, Medline and Web of Science databases from database inception to April 2021. Two researchers independently screened the available studies in strict accordance with inclusion and exclusion criteria. The Cochrane Risk of Bias tool was used to evaluate the risk of bias of the included studies. The variables with and without dimensional differences were calculated as the standardized mean difference and weighted mean difference, respectively. An inconsistency model and "node-splitting" technique were used to test for inconsistency. Funnel plots were used to evaluate publication bias.

RESULTS

Twenty-two eligible RCTs involving 1377 participants were eventually included in our analysis. Data were pooled using a random-effects model. Our NMA results revealed that glucagon-like peptide-1 receptor agonists (GLP-1RAs) were the most effective treatment, yielding improvements in hepatic fat content (HFC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum γ-glutamyl transferase (GGT) and body weight [surface under the cumulative

Manuscript source: Invited manuscript

Specialty type: Endocrinology and metabolism

Country/Territory of origin: China

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: May 6, 2021
Peer-review started: May 6, 2021
First decision: July 3, 2021
Revised: July 9, 2021
Accepted: August 12, 2021
Article in press: August 12, 2021
Published online: September 15, 2021

P-Reviewer: Abdelbasset WK, Kvit K, Seetharaman RV
S-Editor: Gao CC
L-Editor: A
P-Editor: Li X

ranking curve (SUCRA) = 99.6%, 92.6%, 82.8%, 92.3% and 99.6%, respectively], while thiazolidinediones (TZDs) were the best intervention for reducing the NAFLD activity score (NAS; SUCRA = 98.9%). In addition, moderate performance was observed for the sodium glucose cotransporter-2 inhibitors groups (SUCRA = 25.1%, 66.2%, 63.5%, 58.2% and 71.9% for HFC, ALT, AST, GGT and body weight, respectively). However, metformin performed poorly according to most indicators (SUCRA = 54.5%, 0.3%, 19.5%, 33.7%, 57.7% and 44.3% for HFC, NAS, ALT, AST, GGT and body weight, respectively).

CONCLUSION

GLP-1RAs may be the optimum choice for most patients with NAFLD. However, TZDs are considered the most effective therapies in NAFLD patients with histological disease activity.

Key Words: Antidiabetic drugs; Glucagon-like peptide-1 receptor agonists; Nonalcoholic fatty liver disease; Network meta-analysis; Thiazolidinediones

Core Tip: We performed a network meta-analysis and compared the effectiveness of different treatments for nonalcoholic fatty liver disease. In this study, glucagon-like peptide-1 receptor agonists and thiazolidinediones were revealed to be the best interventions for nonalcoholic fatty liver disease, and these findings could help clinicians make significant decisions in clinical practice. Furthermore, we address the possibility of using sodium glucose cotransporter-2 inhibitors in nonalcoholic fatty liver disease; however, trials with larger sample sizes are needed to obtain high-quality evidence.

Citation: Huang YZ, Yang GY, Wang C, Chen XY, Zhang LL. Effectiveness of drug interventions in nonalcoholic fatty liver disease: A network meta-analysis. *World J Diabetes* 2021; 12(9): 1576-1586
URL: https://www.wjgnet.com/1948-9358/full/v12/i9/1576.htm
DOI: https://dx.doi.org/10.4239/wjd.v12.i9.1576

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common forms of chronic liver diseases worldwide and encompasses a spectrum of fatty liver diseases ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis and eventually to cirrhosis and hepatocellular carcinoma[1,2]. The pathogenesis of NAFLD is not well understood; however, it has been indicated that the incidence of NAFLD often parallels the prevalence of obesity, and a large number of NAFLD patients experience metabolic disorder complications, including type 2 diabetes mellitus (T2DM), hyperlipidemia and metabolic syndrome[3,4]. These comorbidities increase the risk of adverse cardiovascular and cerebrovascular events. Therefore, it has been proposed that the term NAFLD be changed to metabolic-associated fatty liver disease for a better understanding of the disease[5]. In view of the above findings, changes to improve eating habits and lifestyle are recommended by clinicians, and this appears to be a basic strategy. To date, there have been no established pharmacotherapies for NAFLD; nonetheless, the application of antidiabetic drugs has emerged as a major therapeutic strategy.

Studies involving antidiabetic drugs in NAFLD patients have shown promising results. Thiazolidinediones (TZDs) and metformin have been confirmed to improve biochemical parameters and lipid metabolism[6,7]. Glucagon-like peptide-1 receptor agonists (GLP-1RAs), including liraglutide and exenatide, present good effects on decreasing hepatic fat content (HFC), body weight and liver enzymes. In addition, sodium glucose cotransporter-2 inhibitors (SGLT2), a new class of antidiabetic drugs, exert beneficial effects on body weight and abdominal fat area, which are accompanied by improvements in liver steatosis and fibrosis[8,9].

Although diverse interventions have been applied in an attempt to treat NAFLD, comprehensive comparisons among treatments are lacking. The aim of this network meta-analysis (NMA) research was to compare these interventions and assess drug options by analyzing the existing evidence. Based on the outcomes we defined, we identified those drugs that could improve the clinical outcomes of NAFLD. Additionally, outcomes with hierarchical ordering of interventions were determined to help clinicians make individualized treatment decisions.

MATERIALS AND METHODS

Search strategy and study selection

The protocol of this review was registered on PROSPERO (ID: CRD42021250990). The search strategy was designed and performed separately by two researchers (Huang YZ and Zhang LL). A search for all NAFLD antidiabetic drug treatment randomized controlled trials (RCTs) was conducted in the PubMed, Cochrane Library, Embase, Medline and Web of Science databases from database inception to April 2021. Without language restriction, medical subject headings combined with free terms were conducted using "nonalcoholic fatty liver disease", "nonalcoholic steatohepatitis", "glucagon-like peptide-1 receptor agonists", "metformin", "thiazolidinediones", "sodium glucose cotransporter-2 inhibitors", "randomized controlled trials" and other relevant conceptual keywords.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) Patients diagnosed with NAFLD; (2) Drug interventions including GLP-1RAs, metformin, TZDs or SGLT2; (3) Clearly reported outcome indicators; and (4) RCTs. The exclusion criteria were as follows: (1) Animal or cell models; (2) Duplicate articles; (3) Reviews, conference abstracts, retrospective studies or cross-sectional studies; and (4) Patients with fatty liver caused by alcohol or other known agents.

Data extraction and outcome indicators

Three reviewers assessed the available studies independently (Chen XY, Wang C and Yang GY). The titles and abstracts of the obtained articles were screened, and articles that did not meet the inclusion criteria were excluded. A full-text read was implemented by the reviewers if an article met the inclusion criteria. Any discrepancies between researchers were resolved by discussion or arbitrated by an experienced investigator (Zhang LL). The predefined primary outcomes included (1) HFC; (2) NAFLD activity score (NAS); (3) Alanine aminotransferase (ALT); and (4) Aspartate aminotransferase (AST). Secondary outcomes were (1) serum γ-glutamyl transferase (GGT) and (2) body weight.

Quality assessment

The Cochrane Risk of Bias tool was used to evaluate the risk of bias (ROB) of the included studies[10]. Seven domains of ROB were estimated to define the included studies as having a high, low, or unclear ROB, including "random sequence generation", "allocation concealment", "blinding of participants and personnel", "blinding of outcome assessment", "incomplete outcome data", "selective reporting", and "other bias". The judgment of ROB was carried out by two authors separately in Review Manager (Version 5.4).

Statistical analysis

First, an inconsistency model was constructed for the measurement of global inconsistency generation, which outputs a *P* value. $P < 0.05$ was considered to indicate significant inconsistency. Then, we constructed network plots of outcome indicators to exhibit all the available evidence of each treatment (Figure 1). As the indicators were continuous variables, the variables with and without dimensional differences were calculated as the standardized mean difference (SMD) and weighted mean difference (WMD), respectively. To explore whether there was a potential source of local inconsistency in our network, the "node-splitting" technique was implemented by comparing the direct evidence to the indirect evidence from the entire network (with *P* value < 0.05 indicating local inconsistency). A comparison-adjusted funnel plot was constructed to evaluate publication bias. As an estimated probability used to rank the target interventions, the surface under the cumulative ranking curve (SUCRA) was

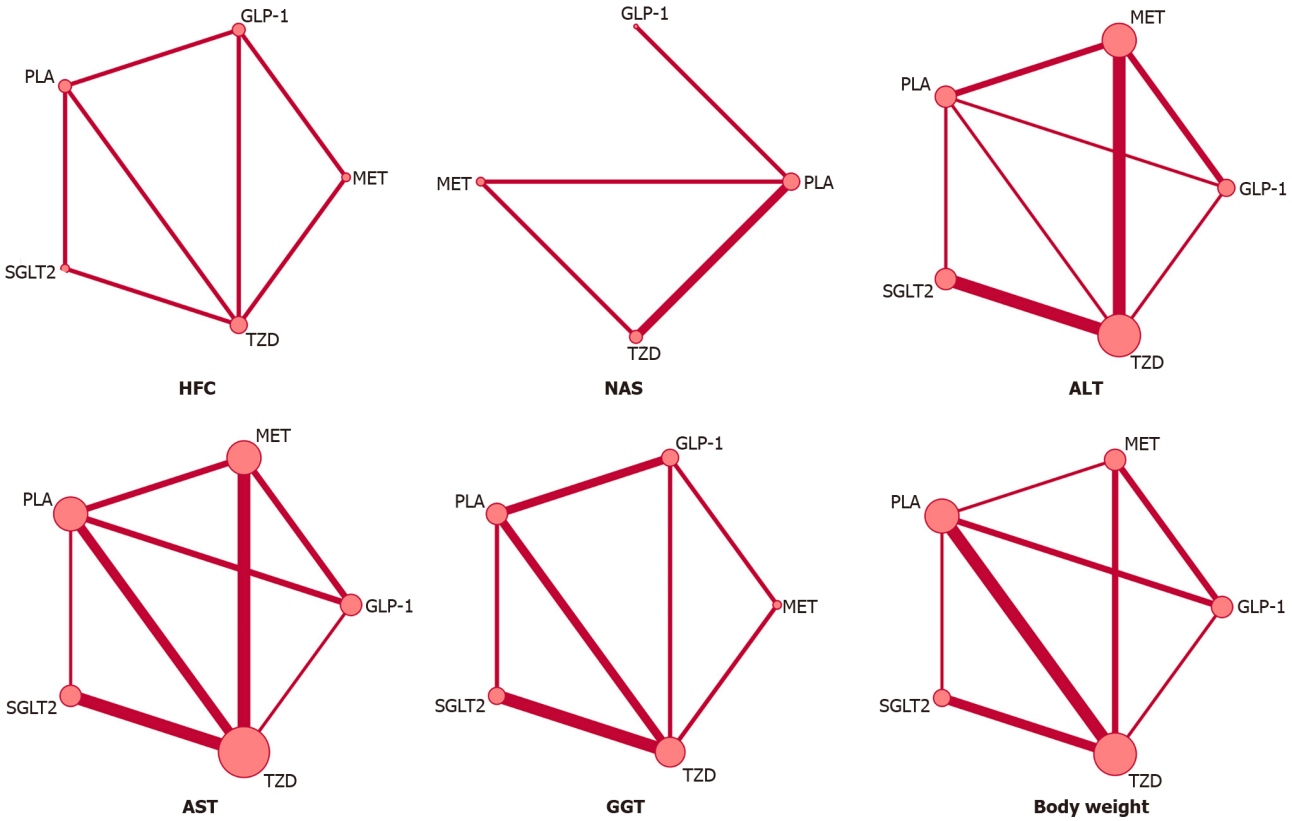

Figure 1 Network of evidence of included studies. GLP-1: Glucagon-like peptide-1 receptor agonists; TZD: Thiazolidinediones; MET: Metformin; SGLT2: Sodium glucose cotransporter-2 inhibitors; PLA: Placebo; HFC: Hepatic fat content; NAS: Nonalcoholic fatty liver disease activity score; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Serum γ-glutamyl transferase.

displayed as a simple numerical statistical cumulative ranking probability plot for various interventions. The higher the SUCRA value, the greater the possibility of a given treatment being at the highest level or highly effective; a value of zero means that the treatment is the worst. All the analyses above were performed by Stata 14.0 (Corporation LLC, College Station, United States) and R (X64 3.6.3 version). Statistical review of this study was performed by a biomedical statistician.

RESULTS

Baseline characteristics and quality assessment

A total of 1515 records were initially screened from the database, and reading the title and abstract yielded 201 articles that were initially included. Subsequently, 179 articles were eliminated based on full-text examination: 149 articles describing studies that were not RCTs, 15 articles that involved single-arm research or self-controls with different doses in the control group, 3 articles that represented duplicate research, and 12 articles that lacked outcome indicators. Finally, only 22 studies including 1377 participants were considered eligible for this NMA. The literature selection process is shown in Figure 2.

Data were retrieved from studies published from November 2006 to February 2021. All the participants in the studies were diagnosed with NAFLD, and the duration of the trials varied from 2 mo to 24 mo (Table 1). Among the 22 included trials, all trials described in detail the generation of random sequences, 16 trials described the concealment approach, and 2 trials did not describe the blinding methods related to participants, implementers, or outcome measurers. Three trials did not have complete data, and only 1 trial exhibited selective outcome reporting. The quality assessment is shown in Supplementary Figure 1.

Inconsistency and publication bias

According the inconsistency model and "node-splitting" technique, the results regarding primary and secondary outcomes presented no statistical significance,

Table 1 Characteristics of included studies

Ref.	Treatment and sample size (*n*)	Baseline age (mean ± SD, median, range)		Treatment duration (mo)	Studying area
		Intervention group	Control group		
Zhang *et al*[12], 2020	GLP-1RAs *vs* TZDs (30 *vs* 30)	50.2 ± 11.5	51.5 ± 12.1	6	China
Fan *et al*[33], 2013	GLP-1RAs *vs* MET (49 *vs* 68)	51.0 ± 10.1	54.7 ± 12.1	3	China
Feng *et al*[28], 2017	GLP-1RAs *vs* MET (29 *vs* 29)	46.8 ± 9.7	46.3 ± 12.3	6	China
Smits *et al*[34], 2016	GLP-1RAs *vs* PLA (17 *vs* 17)	60.8 ± 7.4	65.8 ± 5.8	3	Netherlands
Armstrong *et al*[15], 2016	GLP-1RAs *vs* PLA (26 *vs* 26)	50.0 ± 11.0	52.0 ± 12.0	12	United Kingdom
Hajiaghamohammadi *et al*[35], 2012	MET *vs* TZDs (22 *vs* 22)	32.6 ± 6.4	32.6 ± 6.4	2	Iran
Razavizade *et al*[31], 2013	MET *vs* TZDs (40 *vs* 40)	36.4 ± 9.0	34.2 ± 6.8	4	Iran
Shargorodsky *et al*[36], 2012	MET *vs* PLA (32 *vs* 31)	51.9 ± 10.9	55.2 ± 14.0	4	Israel
Kazemi *et al*[37], 2011	MET *vs* PLA (18 *vs* 15)	41.5 (25-58)	43.5 (26-62)	6	Iran
Haukeland *et al*[30], 2009	MET *vs* PLA (20 *vs* 24)	44.3 ± 9.0	49.9 ± 12.8	6	Norway
Omer *et al*[29], 2010	MET *vs* TZDs (22 *vs* 20)	48.0 ± 9.8	49.3 ± 6.0	12	Turkey
Anushiravani *et al*[38], 2019	MET *vs* TZDs (30 *vs* 30)	NA	NA	3	Iran
Ito *et al*[9], 2017	SGLT2 *vs* TZDs (32 *vs* 34)	57.3 ± 12.1	59.1 ± 9.8	6	Japan
Kinoshita *et al*[26], 2020	SGLT2 *vs* TZDs (32 *vs* 33)	58.7 ± 9.1	59.0 ± 10.9	7	Japan
Eriksson *et al*[39], 2018	SGLT2 *vs* PLA (21 *vs* 21)	65.0 ± 6.5	65.6 ± 6.1	3	Sweden
Chehrehgosha *et al*[8], 2021	SGLT2 *vs* TZDs (35 *vs* 34)	50.5 ± 8.4	52.5 ± 7.9	6	Iran
Yoneda *et al*[27], 2021	TZDs *vs* SGLT2 (19 *vs* 21)	58.8 ± 8.1	58.4 ± 12.2	6	Japan
Belfort *et al*[6], 2006	TZDs *vs* PLA (26 *vs* 21)	51.0 ± 7.0	51.0 ± 10.0	6	United States
Ratziu *et al*[40], 2008	TZDs *vs* PLA (32 *vs* 31)	53.1 ± 11.5	54.1 ± 10.4	12	France
Cusi *et al*[41], 2016	TZDs *vs* PLA (50 *vs* 51)	52.0 ± 10.0	49.0 ± 11.0	18	United States
Sanyal *et al*[42], 2010	TZDs *vs* PLA (80 *vs* 83)	47.0 ± 12.6	45.4 ± 11.2	24	United States
Aithal *et al*[43], 2008	TZDs *vs* PLA (37 *vs* 37)	55 (27-73)	52 (28-71)	12	United Kingdom

GLP-1RAs: Glucagon-like peptide-1 receptor agonists; TZDs: Thiazolidinediones; MET: Metformin; SGLT2: Sodium glucose co-transporter-2; PLA: Placebo.

which indicated the absence of inconsistency. Funnel plots were used to examine for publication bias, and the plots of the outcome indicators were symmetrical (Supplementary Figure 2). In addition, Begg's test for asymmetry was applied to HFC, NAS, ALT, AST, GGT and body weight and yielded *p* values of 0.548, 0.669, 0.753, 0.675, 0.902 and 0.137, respectively, which confirmed the lack of publication bias.

Primary outcomes

The league plots of primary and secondary outcomes are displayed in Figure 3. Regarding the efficacy of the interventions, all the comparisons were statistically significant in the HFC set except for one comparison [TZDs *vs* metformin, mean difference (MD) = -1.10, confidence interval (CI) (-3.56, -1.36)]. Two comparisons had no statistical significance in the NAS set [GLP-1RAs *vs* placebo, MD = -0.50, CI (-1.27, 0.27); TZDs *vs* GLP-1RAs, MD =-0.99, CI (-2.03, 0.06)]. Three comparisons were observed to be significant in the ALT set [GLP-1RAs *vs* placebo, SMD = -0.67, CI (-1.12, -0.22); TZDs *vs* placebo, SMD = -0.40, CI (-0.78, -0.03); metformin *vs* GLP-1RAs, SMD = 0.58, (CI 0.20, 0.96)], and three comparisons were found to be significant in the AST set [GLP-1RAs *vs* placebo, SMD = -0.53, CI (-0.86, -0.22); SGLT2 *vs* placebo, SMD = -0.43, CI (-0.79, -0.08); TZDs *vs* placebo, SMD = -0.45, CI (-0.70, -0.21)]. A SUCRA line was generated to rank the hierarchy of each intervention and indicated that GLP-1RAs were the most effective treatment for the outcomes (SUCRA = 99.6%, 92.6% and 82.8%

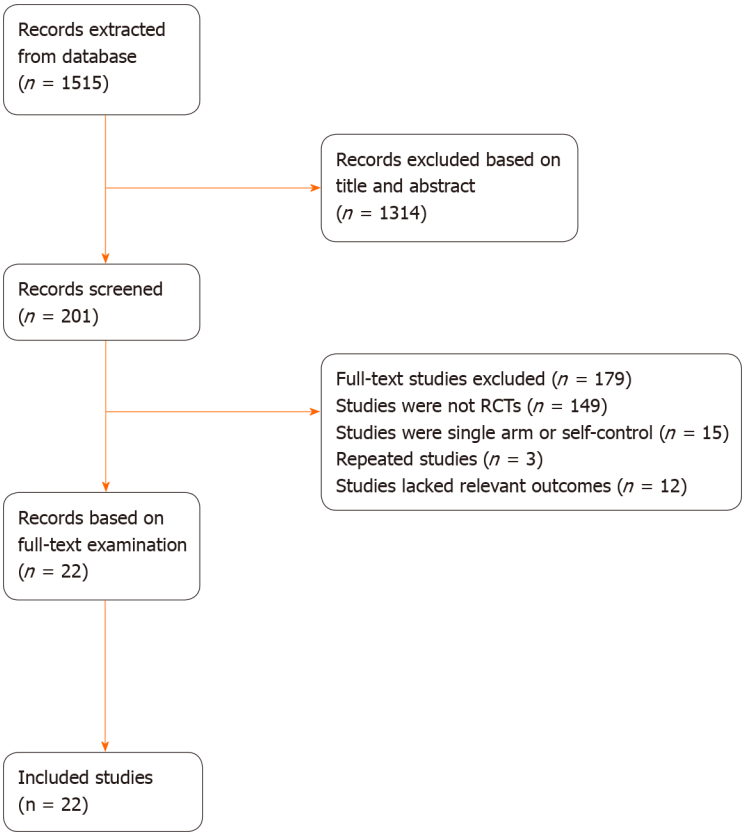

Figure 2 Literature screening flowchart. RCTs: Randomized controlled trials.

for HFC, ALT and AST, respectively). Nonetheless, TZDs were observed to satisfy rank probabilities for NAS and HFC (SUCRA = 98.9% and 70.5%, respectively) (Supplementary Figure 3).

Secondary outcomes

We performed an NMA of secondary outcomes as well. Two comparisons were observed to be significant in the GGT set [GLP-1RAs *vs* placebo, SMD = -0.89, CI (-1.57, -0.21); TZDs *vs* GLP-1RAs, SMD = 0.82, CI (0.11, 1.53)]. Two comparisons had no significance for body weight [metformin *vs* placebo, MD = -0.47, CI (-3.18, 2.24); SGLT2 *vs* metformin, MD = -1.60, CI (-4.52, 1.32)]. According to the SUCRA lines, GLP-1RAs were the most effective treatment for secondary outcomes (SUCRA = 92.3% and 99.6% for GGT and body weight, respectively).

DISCUSSION

The present NMA provides important evidence supporting the use of GLP-1RAs in treating NAFLD, with effectiveness demonstrated for both primary and secondary outcomes except NAS. The probabilities of recommendation of GLP-1RAs reached a surprisingly high priority. Moreover, promising effectiveness of TZDs with regard to the NAS set was observed. These results provide useful evidence that can help clinicians prescribe individualized drugs for patients with different stages of NAFLD.

At present, NAFLD has been considered more of a hepatic manifestation of metabolic syndrome than a class of chronic liver disease due to its association with visceral obesity and insulin resistance[11,12]. In addition, NAFLD is reported to occur in 70%-90% of patients with T2DM. Therefore, antidiabetic drugs are utilized in an attempt to improve the situation for patients with NAFLD.

GLP-1RAs are a new class of glucose-lowering drugs approved for the treatment of T2DM and obesity[13,14]. The mechanism through which GLP-1RAs improve NAFLD is not only a decrease in weight but also a promotion of the ability of hepatocytes to resolve excessive lipid status through lipid transport, beta-oxidation, and de novo lipogenesis[15]. In addition, GLP-1RAs can improve NAFLD, especially HFC, through the augmentation of adiponectin levels, and a study has demonstrated that hypoad-

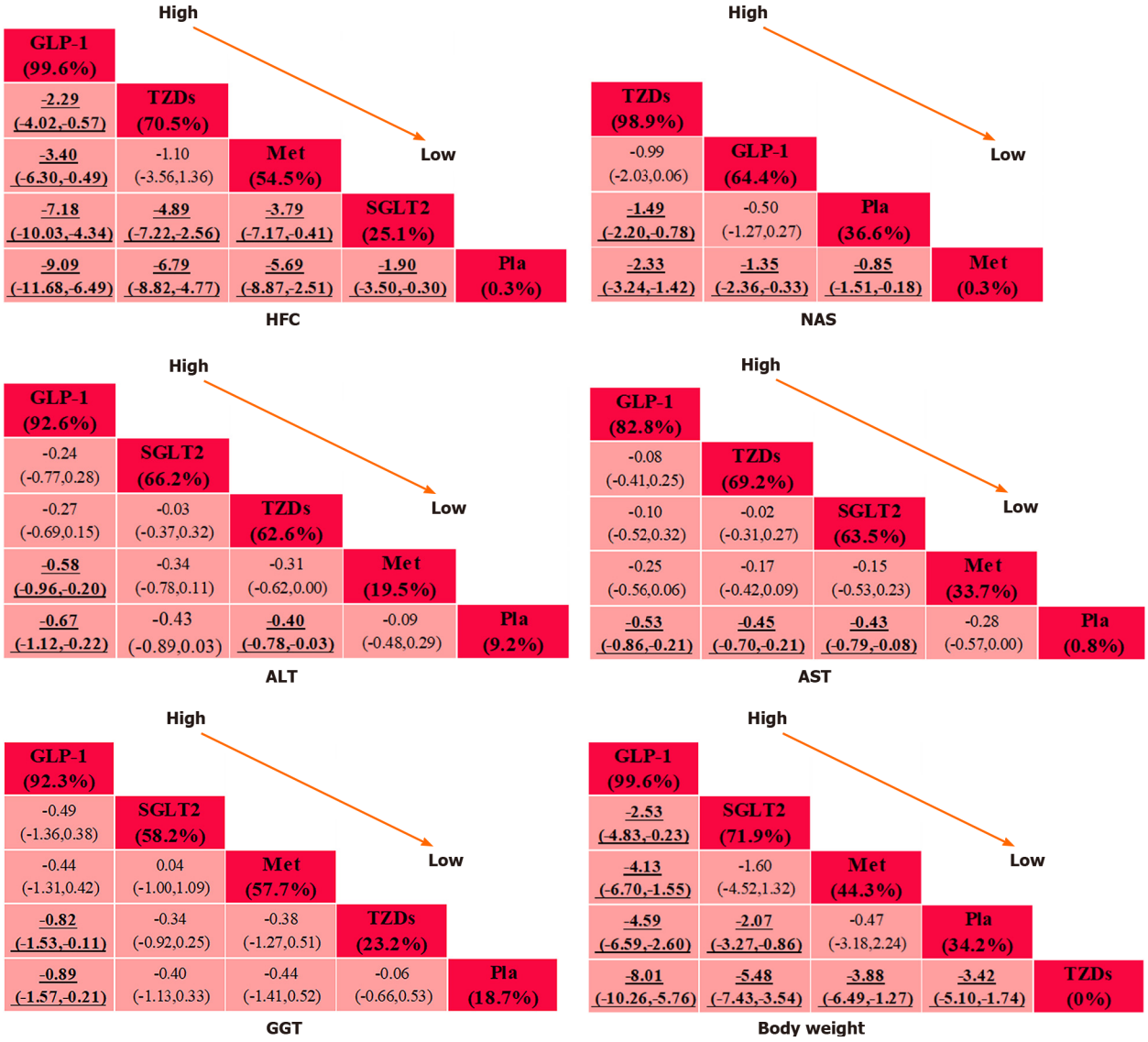

Figure 3 Relative effects of various outcomes. Treatments are ranked according to their chance of being the best treatment. "High" means the highest probability of being the best treatment, and "Low" means the lowest probability of being the best treatment. Numbers in the crimson boxes are the SUCRA (surface under the cumulative ranking curve) values, which represent the level of treatment. The higher the value, the greater the probability of being the best intervention. Significant pairwise comparisons are highlighted in TextTitle and underlined. GLP-1: Glucagon-like peptide-1 receptor agonists; TZDs: Thiazolidinediones; Met: Metformin; SGLT2: Sodium glucose cotransporter-2 inhibitors; Pla: Placebo; HFC: Hepatic fat content; NAS: Nonalcoholic fatty liver disease activity score; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Serum γ-glutamyl transferase.

iponectinemia can induce fat deposition in the liver and the progression of fatty hepatitis[16]. As an anti-inflammatory factor, adiponectin has been proven to promote fatty acid oxidation in the liver by activating AMP-activated protein kinase[17]. However, according to the current literature, weight loss is the most important factor in NAFLD improvement[18,19]. A meta-analysis showed that weight loss ≥ 5% was associated with steatosis improvements, while weight loss ≥ 7% was correlated with improved histological disease activity[20]. In our research, GLP-1RAs presented an enormous advantage in weight loss (SUCRA = 99.6%) and achieved a significant improvement compared with other interventions, which may explain their priority being highest in other sets. Furthermore, liver cells express GLP-1R, and our previous animal study showed that liraglutide could protect against inflammatory stress by inhibiting the activation of JNK, indicating that the benefit of liraglutide treatment in NAFLD is not related solely to the net effect of weight loss[21]. However, GLP-1RAs did not appear to be the best option for NAS in this study (SUCRA = 64.4%). Among the included studies involving GLP-1RA intervention, only one study performed research on NAS[15]. Although liraglutide did not demonstrate significance for NAS, a greater proportion of patients had improvements in steatosis and hepatocyte ballooning in that trial[15]. Referencing the small population in their research, Armstrong *et al*[15] speculate that a significant change in NAS could be identified in a

larger study. More studies on NAS are needed to verify the effectiveness of GLP-1RAs.

TZDs have been widely studied as a prospective treatment for NAFLD. The results of our NMA indicated that TZDs are beneficial for histological resolution (SUCRA = 98.9% and 70.5%, NAS and HFC, respectively), which is consistent with previous meta-analyses[22,23]. As insulin sensitizers, TZDs greatly reduce liver fat accumulation and inflammation by ameliorating insulin resistance[18,24]. Furthermore, the adhibition of TZDs increases serum adiponectin level and inhibits triglyceride synthesis in the liver. However, TZDs are not helpful for weight reduction. In fact, therapeutic use of TZDs has usually led to weight gain, which appears to conflict with the major goal of NAFLD treatment. The reason for this paradox may be the result of fat redistribution from visceral to subcutaneous adipose tissue[25]. In addition, research that directly compares GLP-1RAs and TZDs remains needed to assess their effectiveness regarding liver histology. Since the incidence of NAFLD in diabetic patients is high and the mechanisms of GLP-1RAs and TZDs are different, whether GLP-1RAs and TZDs in combination could have a synergistic effect on NAFLD warrants clinical study.

Moderate performance of SGLT2 regarding both primary and secondary outcomes was observed in this study. SGLT2 displayed great effects on weight loss and abdominal fat area; however, these effects were equivalent to those of TZDs in our included trials[8,9,26,27]. Moreover, there was no ranking of SGLT2 in the NAS set due to the lack of related research. Powerful evidence from high-quality, long-term and large-size studies is warranted to evaluate the effectiveness of SGLT2. Regarding metformin, it yielded poor results in our research. Although metformin has the ability to improve hepatic insulin sensitivity, it offers no advantage in improving HFC or liver histology compared with other interventions and placebo and no advantage in liver enzyme groups[28-31]. Nonetheless, new therapies, such as metformin combined with insulin, have presented promising effectiveness for HFC[32]. Further data from large multicenter RCTs are needed to assess its effectiveness.

Strengths and limitations

Our NMA combined all the eligible direct and indirect evidence to simultaneously compare interventions in patients with NAFLD, which is the greatest advantage of our study. Furthermore, our study is significant because drug interventions for NAFLD are complex and multifaceted and no established treatments for this disease exist.

The limitations of our study need to be acknowledged. First, the duration of treatment varied from 2 mo to 24 mo, which may lead to false credibility in the endpoint assessment of patients. Second, the side effects of interventions, which may influence treatment options in clinical practice, were not analyzed in this study. Finally, potential factors that could introduce bias into our results exist.

CONCLUSION

In summary, our NMA indicated that GLP-1RAs are the optimum therapeutic approach to improve HFC, abnormally elevated liver enzymes and overweight, while TZDs are the most promising intervention to ameliorate liver inflammation. The evidence from our NMA can guide the development of clinical guidelines and thus help clinicians make individualized decisions in clinical practice. Large, multicenter prospective randomized trials with liver biopsy data regarding new classes of glucose-lowering drugs are needed to confirm our results.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is becoming a major chronic liver disorder worldwide. Patients with NAFLD usually experience metabolic disorder complications, including type 2 diabetes mellitus, hyperlipidemia and metabolic syndrome. However, there are no established pharmacotherapies for NAFLD.

Research motivation

The use of antidiabetic drugs, including thiazolidinediones (TZDs), metformin, glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium glucose cotransporter-2 inhibitors (SGLT2), has emerged as a major therapeutic strategy to treat

patients with NAFLD. However, it is difficult for clinicians to decide which intervention is best for treating patients with NAFLD due to an absence of comprehensive comparisons among treatments.

Research objectives

In this study, we compared the effectiveness of different treatments for NAFLD. The results provide new evidence that can guide the development of clinical guidelines and thus help clinicians make individualized decisions in clinical practice.

Research methods

The Cochrane Risk of Bias tool was used to assess the risk of bias of the included studies. Data analysis was performed by Stata 14.0 (Corporation LLC, College Station, United States) and R (X64 3.6.3 version) and included inconsistency modeling, the "node-splitting" technique, Begg's test and the construction of plots of the surface under the cumulative ranking curve.

Research results

GLP-1RAs had a great advantage over other treatments in the improvement of liver enzymes and hepatic fat content (HFC), and promising effectiveness was observed with TZDs with regard to the NAFLD activity score (NAS) set. However, no ranking of SGLT2 was possible for the NAS set due to insufficient research. In addition, the side effects of these drugs were not analyzed in this study.

Research conclusions

GLP-1RAs are the optimum therapeutic approach to improve HFC, abnormally elevated liver enzymes and overweight, while TZDs are the most promising intervention to ameliorate liver inflammation.

Research perspectives

Large multicenter prospective randomized trials with liver biopsy data regarding new classes of glucose-lowering drugs are needed to obtain robust data and confirm our results.

REFERENCES

1 **Petroni ML**, Brodosi L, Bugianesi E, Marchesini G. Management of non-alcoholic fatty liver disease. *BMJ* 2021; **372**: m4747 [PMID: 33461969 DOI: 10.1136/bmj.m4747]

2 **Sheka AC**, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. *JAMA* 2020; **323**: 1175-1183 [PMID: 32207804 DOI: 10.1001/jama.2020.2298]

3 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

4 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

5 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

6 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

7 **Bugianesi E**, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin *vs* vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; **100**: 1082-1090 [PMID: 15842582 DOI: 10.1111/j.1572-0241.2005.41583.x]

8 **Chehrehgosha H**, Sohrabi MR, Ismail-Beigi F, Malek M, Reza Babaei M, Zamani F, Ajdarkosh H, Khoonsari M, Fallah AE, Khamseh ME. Empagliflozin Improves Liver Steatosis and Fibrosis in Patients with Non-Alcoholic Fatty Liver Disease and Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Diabetes Ther* 2021; **12**: 843-861 [PMID: 33586120 DOI: 10.1007/s13300-021-01011-3]

9 **Ito D**, Shimizu S, Inoue K, Saito D, Yanagisawa M, Inukai K, Akiyama Y, Morimoto Y, Noda M, Shimada A. Comparison of Ipragliflozin and Pioglitazone Effects on Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes: A Randomized, 24-Week, Open-Label, Active-Controlled

Trial. *Diabetes Care* 2017; **40**: 1364-1372 [PMID: 28751548 DOI: 10.2337/dc17-0518]
10 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]
11 **Luyckx FH**, Lefebvre PJ, Scheen AJ. Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. *Diabetes Metab* 2000; **26**: 98-106 [PMID: 10804323]
12 **Zhang LY**, Qu XN, Sun ZY, Zhang Y. Effect of liraglutide therapy on serum fetuin A in patients with type 2 diabetes and non-alcoholic fatty liver disease. *Clin Res Hepatol Gastroenterol* 2020; **44**: 674-680 [PMID: 32113823 DOI: 10.1016/j.clinre.2020.01.007]
13 **Vilsbøll T**, Christensen M, Junker AE, Knop FK, Gluud LL. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2012; **344**: d7771 [PMID: 22236411 DOI: 10.1136/bmj.d7771]
14 **Bethel MA**, Patel RA, Merrill P, Lokhnygina Y, Buse JB, Mentz RJ, Pagidipati NJ, Chan JC, Gustavson SM, Iqbal N, Maggioni AP, Öhman P, Poulter NR, Ramachandran A, Zinman B, Hernandez AF, Holman RR; EXSCEL Study Group. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *Lancet Diabetes Endocrinol* 2018; **6**: 105-113 [PMID: 29221659 DOI: 10.1016/S2213-8587(17)30412-6]
15 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]
16 **Arvaniti VA**, Thomopoulos KC, Tsamandas A, Makri M, Psyrogiannis A, Vafiadis G, Assimakopoulos SF, Labropoulou-Karatza C. Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. *Acta Gastroenterol Belg* 2008; **71**: 355-360 [PMID: 19317274]
17 **Yamauchi T**, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288-1295 [PMID: 12368907 DOI: 10.1038/nm788]
18 **Panunzi S**, Maltese S, Verrastro O, Labbate L, De Gaetano A, Pompili M, Capristo E, Bornstein SR, Mingrone G. Pioglitazone and bariatric surgery are the most effective treatments for non-alcoholic steatohepatitis: A hierarchical network meta-analysis. *Diabetes Obes Metab* 2021; **23**: 980-990 [PMID: 33368954 DOI: 10.1111/dom.14304]
19 **Byrne CD**, Olufadi R, Bruce KD, Cagampang FR, Ahmed MH. Metabolic disturbances in non-alcoholic fatty liver disease. *Clin Sci (Lond)* 2009; **116**: 539-564 [PMID: 19243311 DOI: 10.1042/CS20080253]
20 **Musso G**, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; **55**: 885-904 [PMID: 22278337 DOI: 10.1007/s00125-011-2446-4]
21 **Zhang L**, Yang M, Ren H, Hu H, Boden G, Li L, Yang G. GLP-1 analogue prevents NAFLD in ApoE KO mice with diet and Acrp30 knockdown by inhibiting c-JNK. *Liver Int* 2013; **33**: 794-804 [PMID: 23432843 DOI: 10.1111/liv.12120]
22 **Boettcher E**, Csako G, Pucino F, Wesley R, Loomba R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2012; **35**: 66-75 [PMID: 22050199 DOI: 10.1111/j.1365-2036.2011.04912.x]
23 **Mahady SE**, Webster AC, Walker S, Sanyal A, George J. The role of thiazolidinediones in non-alcoholic steatohepatitis - a systematic review and meta analysis. *J Hepatol* 2011; **55**: 1383-1390 [PMID: 21703200 DOI: 10.1016/j.jhep.2011.03.016]
24 **Miyazaki Y**, Matsuda M, DeFronzo RA. Dose-response effect of pioglitazone on insulin sensitivity and insulin secretion in type 2 diabetes. *Diabetes Care* 2002; **25**: 517-523 [PMID: 11874940 DOI: 10.2337/diacare.25.3.517]
25 **Shah PK**, Mudaliar S, Chang AR, Aroda V, Andre M, Burke P, Henry RR. Effects of intensive insulin therapy alone and in combination with pioglitazone on body weight, composition, distribution and liver fat content in patients with type 2 diabetes. *Diabetes Obes Metab* 2011; **13**: 505-510 [PMID: 21272186 DOI: 10.1111/j.1463-1326.2011.01370.x]
26 **Kinoshita T**, Shimoda M, Nakashima K, Fushimi Y, Hirata Y, Tanabe A, Tatsumi F, Hirukawa H, Sanada J, Kohara K, Irie S, Kimura T, Nakamura Y, Nishioka M, Obata A, Nakanishi S, Mune T, Kaku K, Kaneto H. Comparison of the effects of three kinds of glucose-lowering drugs on non-alcoholic fatty liver disease in patients with type 2 diabetes: A randomized, open-label, three-arm, active control study. *J Diabetes Investig* 2020; **11**: 1612-1622 [PMID: 32329963 DOI: 10.1111/jdi.13279]
27 **Yoneda M**, Honda Y, Ogawa Y, Kessoku T, Kobayashi T, Imajo K, Ozaki A, Nogami A, Taguri M, Yamanaka T, Kirikoshi H, Iwasaki T, Kurihashi T, Saito S, Nakajima A. Comparing the effects of tofogliflozin and pioglitazone in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus (ToPiND study): a randomized prospective open-label controlled trial. *BMJ Open Diabetes Res Care* 2021; **9** [PMID: 33593749 DOI: 10.1136/bmjdrc-2020-001990]

28 **Feng W**, Gao C, Bi Y, Wu M, Li P, Shen S, Chen W, Yin T, Zhu D. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. *J Diabetes* 2017; **9**: 800-809 [PMID: 28332301 DOI: 10.1111/1753-0407.12555]

29 **Omer Z**, Cetinkalp S, Akyildiz M, Yilmaz F, Batur Y, Yilmaz C, Akarca U. Efficacy of insulin-sensitizing agents in nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2010; **22**: 18-23 [PMID: 19667999 DOI: 10.1097/MEG.0b013e32832e2baf]

30 **Haukeland JW**, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjøro K, Haaland T, Løberg EM, Birkeland K. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol* 2009; **44**: 853-860 [PMID: 19811343 DOI: 10.1080/00365520902845268]

31 **Razavizade M**, Jamali R, Arj A, Matini SM, Moraveji A, Taherkhani E. The effect of pioglitazone and metformin on liver function tests, insulin resistance, and liver fat content in nonalcoholic Fatty liver disease: a randomized double blinded clinical trial. *Hepat Mon* 2013; **13**: e9270 [PMID: 23930133 DOI: 10.5812/hepatmon.9270]

32 **Tang W**, Xu Q, Hong T, Tong G, Feng W, Shen S, Bi Y, Zhu D. Comparative efficacy of anti-diabetic agents on nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized and non-randomized studies. *Diabetes Metab Res Rev* 2016; **32**: 200-216 [PMID: 26381272 DOI: 10.1002/dmrr.2713]

33 **Fan H**, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. *Arq Bras Endocrinol Metabol* 2013; **57**: 702-708 [PMID: 24402015 DOI: 10.1590/s0004-27302013000900005]

34 **Smits MM**, Tonneijck L, Muskiet MH, Kramer MH, Pouwels PJ, Pieters-van den Bos IC, Hoekstra T, Diamant M, van Raalte DH, Cahen DL. Twelve week liraglutide or sitagliptin does not affect hepatic fat in type 2 diabetes: a randomised placebo-controlled trial. *Diabetologia* 2016; **59**: 2588-2593 [PMID: 27627981 DOI: 10.1007/s00125-016-4100-7]

35 **Hajiaghamohammadi AA**, Ziaee A, Oveisi S, Masroor H. Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic Fatty liver disease: a randomized controlled pilot study. *Hepat Mon* 2012; **12**: e6099 [PMID: 23087748 DOI: 10.5812/hepatmon.6099]

36 **Shargorodsky M**, Omelchenko E, Matas Z, Boaz M, Gavish D. Relation between augmentation index and adiponectin during one-year metformin treatment for nonalcoholic steatohepatosis: effects beyond glucose lowering? *Cardiovasc Diabetol* 2012; **11**: 61 [PMID: 22676459 DOI: 10.1186/1475-2840-11-61]

37 **Kazemi R**, Aduli M, Sotoudeh M, Malekzadeh R, Seddighi N, Sepanlou SG, Merat S. Metformin in nonalcoholic steatohepatitis: a randomized controlled trial. *Middle East J Dig Dis* 2012; **4**: 16-22 [PMID: 24829630]

38 **Anushiravani A**, Haddadi N, Pourfarmanbar M, Mohammadkarimi V. Treatment options for nonalcoholic fatty liver disease: a double-blinded randomized placebo-controlled trial. *Eur J Gastroenterol Hepatol* 2019; **31**: 613-617 [PMID: 30920975 DOI: 10.1097/MEG.0000000000001369]

39 **Eriksson JW**, Lundkvist P, Jansson PA, Johansson L, Kvarnström M, Moris L, Miliotis T, Forsberg GB, Risérus U, Lind L, Oscarsson J. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia* 2018; **61**: 1923-1934 [PMID: 29971527 DOI: 10.1007/s00125-018-4675-2]

40 **Ratziu V**, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, Podevin P, Lacorte JM, Bernhardt C, Bruckert E, Grimaldi A, Poynard T; LIDO Study Group. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* 2008; **135**: 100-110 [PMID: 18503774 DOI: 10.1053/j.gastro.2008.03.078]

41 **Cusi K**, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med* 2016; **165**: 305-315 [PMID: 27322798 DOI: 10.7326/M15-1774]

42 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

43 **Aithal GP**, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1176-1184 [PMID: 18718471 DOI: 10.1053/j.gastro.2008.06.047]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com