ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2023 April 15; 14(4): 352-446

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 14 Number 4 April 15, 2023

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Observational Study

World Journal of Diabetes

Monthly Volume 14 Number 4 April 15, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, She-Yu Li, MD, Associate Professor, Department of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. lisheyu@gmail.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, PubMed Central, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 Edition of Journal Citation Reports® cites the 2021 impact factor (IF) for *WJD* as 4.560; IF without journal self cites: 4.450; 5-year IF: 5.370; Journal Citation Indicator: 0.62; Ranking: 62 among 146 journals in endocrinology and metabolism; and Quartile category: Q2.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Yu-Xi Chen*; **Production Department Director:** *Xu Guo*; **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

EDITORS-IN-CHIEF
Lu Cai, Md. Shahidul Islam, Michael Horowitz

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-9358/editorialboard.htm

PUBLICATION DATE
April 15, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 352-363

DOI: 10.4239/wjd.v14.i4.352

ISSN 1948-9358 (online)

REVIEW

Role of antidiabetic agents in type 2 diabetes patients with chronic kidney disease

Wei-Ren Lin, Kuan-Hung Liu, Tsai-Chieh Ling, Ming-Cheng Wang, Wei-Hung Lin

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

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

P-Reviewer: Balbaa ME, Egypt; Elfaki I, Saudi Arabia; Hojs R, Slovenia

Received: November 27, 2022
Peer-review started: November 27, 2022
First decision: December 26, 2022
Revised: January 10, 2023
Accepted: April 4, 2023
Article in press: April 4, 2023
Published online: April 15, 2023

Wei-Ren Lin, Kuan-Hung Liu, Tsai-Chieh Ling, Ming-Cheng Wang, Wei-Hung Lin, Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan 704, Taiwan

Corresponding author: Wei-Hung Lin, MD, PhD, Associate Professor, Doctor, Staff Physician, Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital, No. 138 Sheng-Li Road, Tainan 704, Taiwan. dindonwhlin@hotmail.com

Abstract

Insulin resistance is a condition in which the target tissues have a decreased response to insulin signaling, resulting in glucose uptake defect, and an increased blood sugar level. Pancreatic beta cells thus enhance insulin production to compensate. This situation may cause further beta cell dysfunction and failure, which can lead diabetes mellitus (DM). Insulin resistance is thus an important cause of the development of type 2 DM. Insulin resistance has also been found to have a strong relationship with cardiovascular disease and is common in chronic kidney disease (CKD) patients. The mechanisms of insulin resistance in CKD are complex and multifactorial. They include physical inactivity, inflammation and oxidative stress, metabolic acidosis, vitamin D deficiency, adipose tissue dysfunction, uremic toxins, and renin-angiotensin-aldosterone system activation. Currently, available anti-diabetic agents, such as biguanides, sulfonylureas, thiazolidinediones, alfa-glucosidase inhibitors, glucagon-like peptide-1-based agents, and sodium-glucose co-transporter-2 inhibitors, have different effects on insulin resistance. In this short review, we describe the potential mechanisms of insulin resistance in CKD patients. We also review the interaction of currently available anti-diabetic medications with insulin resistance.

Key Words: Insulin resistance; Chronic kidney disease; Cardiovascular events; Anti-diabetic agents

Core Tip: Insulin resistance is the main cause of type 2 diabetes mellitus and is associated with cardiovascular events. It is also common in chronic kidney disease patients. We discuss the mechanisms of insulin resistance in such patients and the interaction of currently available anti-diabetic medications with insulin resistance.

Citation: Lin WR, Liu KH, Ling TC, Wang MC, Lin WH. Role of antidiabetic agents in type 2 diabetes patients with chronic kidney disease. *World J Diabetes* 2023; 14(4): 352-363
URL: https://www.wjgnet.com/1948-9358/full/v14/i4/352.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i4.352

INTRODUCTION

Insulin resistance is a condition in which a tissue or organ has reduced sensitivity to insulin-initiated biological processes. To compensate for lower sensitivity, insulin secretion by pancreatic beta cells increases, causing hyperinsulinemia. Insulin resistance is thought to be an important contributor to beta cell dysfunction, which eventually leads to diabetes mellitus (DM). It is associated with risk factors for cardiovascular (CV) disease, such as inflammation, oxidative stress, and endothelial dysfunction[1]. Diabetes nephropathy is a common complication of DM. It causes albuminuria and renal function deterioration[2]. Insulin resistance is also common in chronic kidney disease (CKD) patients[3]. There are various anti-diabetes medications, including biguanides, sulfonylureas (SUs), thiazolidinediones (TZDs), alfa-glucosidase inhibitors (AGIs), glucagon-like peptide-1 (GLP-1)-based agents, and sodium-glucose co-transporter-2 inhibitors (SGLT2Is). In this article, we briefly summarize the mechanism of insulin resistance in CKD patients and the effect of currently available anti-diabetic medications on insulin resistance.

INSULIN RESISTANCE IN CKD PATIENTS

Insulin binds to its receptor and induces insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation. IRS-1 then phosphorylates phosphatidyl-inositol-3-kinase and produces phosphatidylinositol-triphosphate (PIP3). PIP3 activates the protein kinase B/Akt pathway[1] (Figure 1). This effect can induce glucose transporter 4 (GLUT4) translocation to the cell membrane and cause glucose uptake. Insulin resistance presents when this signaling pathway dysfunction occurs. There are many different effects on the insulin signaling pathway in CKD patients, which subsequentially cause insulin resistance.

Physical inactivity and insulin resistance

Insulin resistance may increase after several days of bed rest in a healthy population[4] and contributes to impaired microvascular function. Physical activity is decreased in CKD patients[1]. In the CKD mouse model, more physical activity can increase insulin sensitivity[5]. In patients with end-stage kidney disease (ESRD), moderate physical training can improve glucose tolerance and reduce the plasma insulin level[6].

Inflammation, oxidative stress, and insulin resistance

Serum proinflammatory cytokines, such as C-reactive protein, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), are elevated in CKD patients, indicating systemic inflammation and increased oxidative stress[7]. TNF-α can directly inhibit IRS-1 function[8,9] and cause free fatty acid (FFA) accumulation by activating lipolysis, which indirectly inhibits IRS-1[10]. IL-6 can also, directly and indirectly, inhibit IRS-1 by stimulating the suppressor of cytokine signaling-3 pathway[11,12]. Reactive oxygen species (ROS), generated by inflammatory cytokines, FFA oxidation, or mitochondria, not only inhibit IRS-1 phosphorylation but also induce GLUT4 degradation by activating the casein kinase-2 pathway[13]. The expression and function of nuclear factor-erythroid-2-related factor-2 (Nrf2), which can enhance antioxidant and anti-inflammatory activity genes, are reduced in CKD patients[14]. Nrf2 deficiency might be another cause of insulin resistance in CKD patients[1,15] (Figure 1).

Metabolic acidosis

Metabolic acidosis is a common metabolic abnormality due to an impairment of renal acid excretion in CKD patients. Metabolic acidosis is also a risk factor for insulin resistance due to impaired glucose metabolism and cellular insulin sensitivity[16]. Metabolic acidosis reduces insulin binding to its receptor IRS-1 and down-regulates the following intracellular signaling in adipocytes and myocytes[17,18]

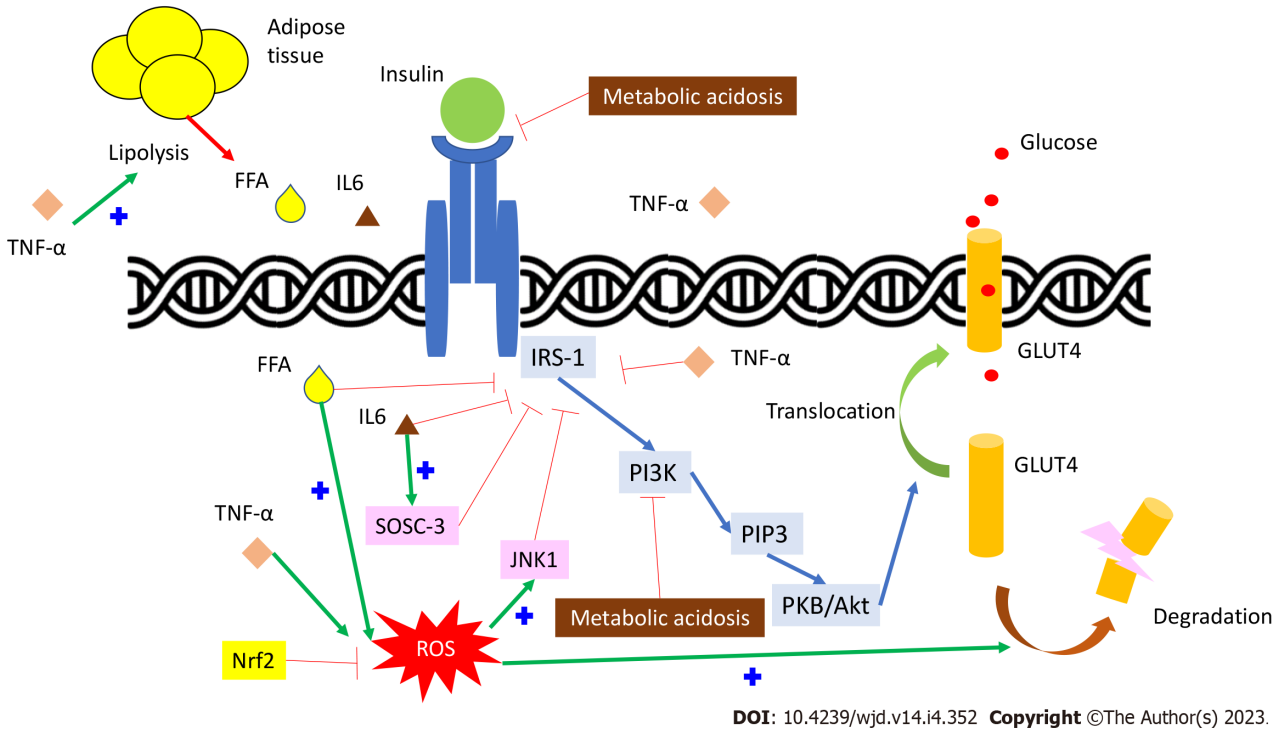


Figure 1 Insulin signaling pathway and inflammation and oxidative stress in insulin resistance. IRS-1: Insulin receptor substrate-1; PI3K: Phosphatidyl-inositol-3-kinase (PI3K); PIP3: Phosphatidylinositol-triphosphate; PKB/Akt: Protein kinase B/Akt pathway; GLUT4: Glucose transporter 4 (GLUT4): FFA: Free fatty acids; TNF-α: Tumor necrosis factor alfa; IL-6: Interleukin-6; SOSC-3: Suppressor of cytokine signaling-3 pathway; ROS: Reactive oxygen species; JNK1: C-Jun N-terminal kinase 1; Nrf2: Nuclear factor-erythroid-2-related factor-2.

(Figure 1). Furthermore, insulin resistance can be reduced by correcting metabolic acidosis in CKD patients[19].

Vitamin D deficiency

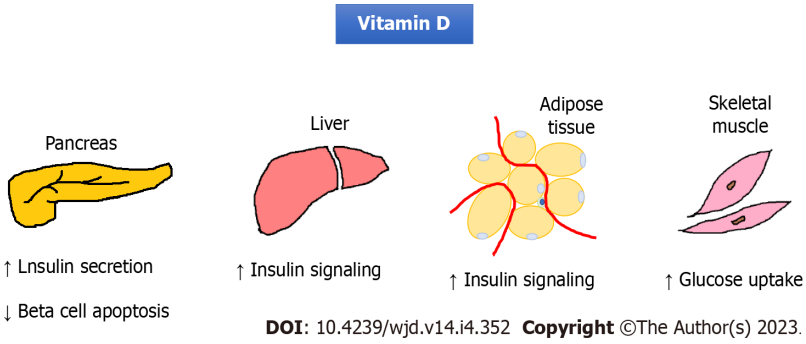
Vitamin D is a hormone that regulates calcium homeostasis. Vitamin D may have a role in insulin secretion because of the vitamin D receptor presenting in pancreatic beta cells[20]. Vitamin D can also reduce pancreatic islets apoptosis caused by systemic chronic inflammation[21]. Insulin secretion by pancreatic beta cells requires extracellular calcium infusion. The vitamin D level is important for the normal homeostasis of extracellular calcium levels[22]. Moreover, vitamin D can promote insulin-induced uptake from the liver, adipose tissue, and skeletal muscle tissue[23] (Figure 2). A large cross-sectional study revealed a strong association between the vitamin D levels and insulin resistance[24]. Some epidemiological studies also showed an association between vitamin D deficiency and risk for type 2 DM (T2DM)[25]. Therefore, many trials have tried to examine the therapeutic potential of vitamin D supplementation. However, a meta-analysis that included 28 randomized controlled trials in 2018 showed that vitamin D supplementation had no significant effect on controlling the fasting plasma glucose level, improving insulin resistance, or preventing T2DM[25]. In contrast, two recent studies showed that high-dose vitamin D supplementation could reduce insulin resistance and oxidative stress [26,27]. Further investigation is needed to clarify the effect of vitamin D on insulin resistance.

Adipose tissue dysfunction

Obesity enhances hepatic gluconeogenesis and increases circulating FFAs through lipolysis of adipose tissue. It has been hypothesized that hyperinsulinemia is the initial effect in obese patients[28]. Furthermore, adipose tissue can secret adipokines and inflammatory markers[29]. These conditions can subsequently induce insulin resistance. CKD patients suffer from adipose reduction. However, many metabolic abnormalities present in CKD patients are similar to those in the obese condition, such as a high circulating FFA level and a high level of inflammatory cytokines[30]. Adipose disarrangement and adipokine dysregulation may be the cause[31].

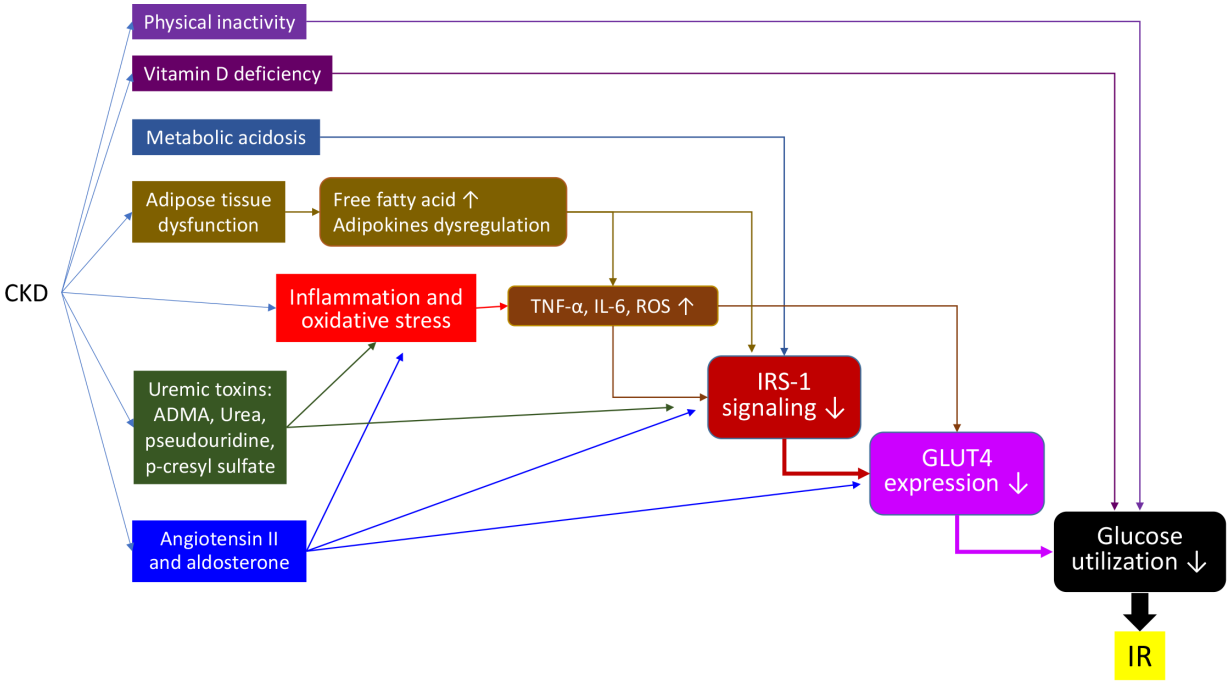
Uremic toxin

Uremic toxin accumulation may induce insulin resistance in CKD patients. Water-soluble toxins, such as asymmetric dimethylarginine (ADMA)[32] and pseudouridine[33], have been proven to cause insulin resistance. ADMA, an endogenous nitric oxide synthase inhibitor, can cause endothelial dysfunction. It could reduce IRS-1 and GLUT4 expression and induce IRS-1 degradation[34]. Pseudouridine inhibits



DOI: 10.4239/wjd.v14.i4.352 **Copyright** ©The Author(s) 2023.

Figure 2 Effect of vitamin D on insulin secretion and signaling.



DOI: 10.4239/wjd.v14.i4.352 **Copyright** ©The Author(s) 2023.

Figure 3 Summary of mechanisms of chronic kidney disease that induce insulin resistance. CKD: Chronic kidney disease; IRS-1: Insulin receptor substrate-1; GLUT4: Glucose transporter 4: TNF-α: Tumor necrosis factor alfa; IL-6: Interleukin-6; ROS: Reactive oxygen species; ADMA: Asymmetric dimethylarginine.

insulin signaling and glucose uptake in rat muscle cells. Urea can induce ROS generation and cause insulin resistance in the uremic mouse model[35]. Furthermore, a positive relationship between high blood urea nitrogen and incident DM has been reported[36]. The serum level of protein-bound toxins, such as p-cresyl sulfate, also increases in CKD patients. P-cresyl sulfate can cause an impaired insulin signaling pathway in animal models[37]. It also results in lipid redistribution to the liver and muscle, which increases ROS production and inflammation[37].

Renin angiotensin aldosterone system activation

A high level of angiotensin II (Ang II), which is a common condition in CKD patients, may cause insulin resistance. Ang II stimulates IL-6 production[38], which then causes insulin resistance. Aldosterone increases as renal function declines. Aldosterone can induce insulin resistance not only directly by impairing IRS-1 function and GLU4 translocation, but also indirectly by affecting the production of other circulating factors such as inflammatory cytokines[39]. A small study showed that the administration of a mineralocorticoid receptor antagonist (MRA) such as spironolactone can ameliorate insulin resistance in CKD patients and rats[40]. However, the Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity clinical study revealed that steroidal MRAs may increase the risk of new DM development[41]. A recent clinical study showed no improvement in insulin sensitivity in a T2DM patient after 8 wk of steroidal MRA eplerenone use[42]. The benefit of the use of steroidal MRAs in the management of insulin resistance is not clear. Moreover, for CKD patients, steroidal MRAs should

be used with caution because of the higher risks of hyperkalemia and gynecomastia (Figure 3).

EFFECT OF ANTI-DIABETIC AGENTS ON INSULIN RESISTANCE

It is well known that chronic high blood sugar itself can cause insulin resistance and pancreatic islet beta cell dysfunction, which is called glucotoxicity[43]. This effect is mainly through the activation of oxidative stress (as previously described). Therefore, lowering the blood sugar level can attenuate insulin resistance. All anti-diabetic medication decreases the blood sugar level and thus partially decreases insulin resistance. As previously described, high weight and obesity are other common causes of insulin resistance. Therefore, body weight loss is expected to decrease insulin resistance. Some anti-diabetic medications decrease body weight and thus could improve insulin resistance *via* this mechanism.

Biguanides: Metformin

Metformin is a first-line drug for treating T2DM[44]. Metformin reduces blood glucose mainly through the suppression of hepatic gluconeogenesis by activating liver AMP-activated protein kinase (AMPK) without causing hypoglycemia[45]. AMPK also reduces liver lipogenesis and increases FFA oxidation, thereby decreasing liver steatosis and increasing hepatic insulin sensitivity[45]. In addition to inhibiting hepatocyte glucose production, metformin enhances insulin-stimulated glucose utilization and FFA oxidation in peripheral tissue, including skeletal muscle and fat tissue[46]. Metformin also stimulates enterocytes to uptake and utilize glucose, which results in a net glucose absorption decrease[45], and induces GLP-1 secretion[45]. Polycystic ovary syndrome (PCOS) is a disease with insulin resistance and hyperinsulinemia. The administration of metformin can improve dyslipidemia and inflammation[47]. However, administration for CKD patients should be done carefully due to its side effect of lactic acidosis. Despite the scarcity of data on the reduction of insulin resistance by metformin in CKD patients, metformin has been shown to slow the progression of DM in high-risk population[48].

TZDs

TZDs are nuclear transcription factor peroxisome proliferator-activated receptor (PPAR) agonists. PPARγ is essential for new insulin-sensitive adipocyte differentiation and promotes FFA uptake and storage in subcutaneous adipose rather than visceral adipose tissue[49,50]. A reduction in the FFA level is associated with insulin resistance reduction. TZDs also increases glucose uptake by hepatocytes and skeletal muscle cells by increasing GLUT4 expression and translocation[49,50]. These effects decrease serum glucose without elevating insulin, which improves insulin resistance. Some clinical research showed that TZDs could also improve insulin resistance in hemodialysis patients[51,52]. However, side effects associated with subcutaneous adipose deposition (*e.g.*, weight gain) and fluid retention, which can lead to heart failure, limit their clinical use[50]. TZDs may be a good option for sugar control because of the limited choice for T2DM in CKD patients. TZDs have a beneficial effect on reducing insulin resistance but the side effect of fluid retention is of concern.

SUs and meglitinides

SUs can increase insulin secretion by altering the resting potential of islet beta cells *via* the inhibition of the adenosine triphosphate (ATP)-sensitive potassium channel (K-ATP channel). SUs are mainly removed from the liver and kidney, so the effect may be enhanced in CKD patients[53,54]. SUs can enhance peripheral glucose utilization directly by increasing GLUT4 expression *in vitro* and indirectly due to a reduction of glucotoxicity[55]. The improvement in insulin resistance by SUs may be a short-term effect[56]. Meglitinides can also stimulate insulin secretion *via* a similar pathway with a shorter onset time and duration. The effect of meglitinides on insulin resistance is still unclear[57]. Meglitinides can reduce glucotoxicity like SUs and temporally improve insulin resistance. However, body weight gain is a common side effect of SUs and meglitinides, which may increase insulin resistance. A recent study examined the effect of meglitinides in hemodialysis patients. Compared to the placebo group (which used only voglibose, an AGI), add-on meglitinide significantly decreased insulin resistance, fasting glucose, hemoglobin A1c (HbA1c) and glycated albumin levels[58]. The decrease in insulin resistance caused by meglitinide may be *via* a decrease in glucotoxicity.

Alpha-glucosidase inhibitors

AGIs can reversibly depress intestinal alpha-glucosidase activity, which delays sugar absorption[59]. They can reduce postprandial hyperglycemia without increasing the insulin level. Acarbose, an alpha-glucosidase inhibitor, was found to improve insulin sensitivity in fructose-fed rats[60]. In the STOP-NIDDM trial, acarbose administration in impaired-glucose-tolerance patients significantly increased the reversion of impaired glucose tolerance to normal glucose tolerance[61] and significantly reduced the risk of CV events and hypertension[62]. Acarbose has a potential side effect of hepatotoxicity that is possibly dose-dependent[63]. Due to accumulation in CKD, acarbose should be avoided in these patients[63]. However, a recent study showed that there was no relationship between acarbose use and

Table 1 Effects of anti-diabetic medications on insulin resistance and cardiovascular outcome with precautions for patients with chronic kidney disease

	Insulin resistance	CV effects[44]		CKD group
		ASCVD	HF	
Metformin	Liver gluconeogenesis (increased). Peripheral tissue glucose utilizing (increased). Net glucose absorption (decreased). Insulin resistance (markedly decreased) may benefit PCOS	Potential benefit	Neutral	eGFR < 30: Lactic acidosis
TZDs	Insulin signaling (increased). FFA (increased). Insulin resistance (markedly decreased)	Potential benefit	Increase risk	No dose adjustment required. Fluid retention
SUs and meglitinides	Glucotoxicity (decreased). GLUT4 expression (increased). Insulin resistance, mainly by decreasing glucotoxicity (decreased)	Neutral	Neutral	Low dose initiation to prevent hypoglycemia
Alpha-glucosidase inhibitors	Glucotoxicity (decreased). Insulin resistance, mainly by decreasing glucotoxicity (decreased)	Potential benefit	Neutral	Contraindication in CrCl < 25 (lack of data). Liver injury?
DPP-4 inhibitors	Improved islet beta cell function. Inflammation (decreased)? Insulin resistance (decreased)	Neutral	Potential risk: Saxagliptin and alogliptin	Generally safe. They can be used in CKD group (no dose adjustment for linagliptin)
GLP-1RAs	Oxidative stress, inflammation (decreased). GLUT4 expression (increased). Insulin signaling (increased). Body weight (decreased): Insulin resistance (markedly decreased)	Benefit	Neutral	No dose adjustment required
SGLT2Is	Peripheral tissue glucose utilization (increased). Energy expenditure (increased). Induce M2 macrophage polarization: Insulin resistance (markedly decreased)	Benefit	Benefit	Decrease sugar lowering effect in CKD group. Do not initiate when eGFR < 20

CKD: Chronic kidney disease; CV: Cardiovascular; ASCVD: Atherosclerotic cardiovascular disease; HF: Heart failure; PCOS: Polycystic ovary syndrome; eGFR: Estimated glomerular filtration rate; FFA: Free fatty acid; TZDs: Thiazolidinediones; SUs: Sulfonylureas; GLUT4: Glucose transporter 4; DPP-4: Dipeptidyl peptidase-4; GLP-1RAs: Glucagon-like peptide-1 receptor agonists; SGLT2Is: Sodium-glucose co-transporter-2 inhibitors.

liver injury in a severe renal insufficiency group[64]. So far, there is little evidence that acarbose improves the insulin signaling pathway. A study showed that voglibose monotherapy in hemodialysis patients could reduce HbA1c but not clinical insulin resistance[58].

Therapies based on GLP-1

GLP-1, which is secreted from the small intestine, can stimulate insulin secretion from pancreatic islet beta cells after food intake. It can also delay gastric emptying, inhibit inappropriate post-meal glucagon release, and decrease appetite *via* the central nervous system[65,66]. GLP-1 is rapidly switched to an inactive form by dipeptidyl peptidase-4 (DPP-4) enzyme[67]. Therefore, DPP-4 inhibitors and GLP-1 agonists can both decrease the post-prandial blood sugar level without obvious hypoglycemia.

DPP-4 inhibitors have a neutral effect on CV events, development or progression of renal function, and body weight[68-71]. Some studies showed that DPP-4 inhibitors, such as sitagliptin[72] and vildagliptin[73], can improve clinical insulin sensitivity. Some studies also revealed that DPP-4 inhibitors have an anti-inflammation effect[74,75]. A 2019 meta-analysis showed that DPP-4 inhibitors can improve both beta cell function and insulin resistance, although the effect was weak[76]. A new DPP-4 inhibitor omarigliptin was found to reduce insulin resistance and systemic inflammation in T2DM patients[77]. However, there is current no evidence that the use of DPP4-inhibitors reduces insulin resistance in the CKD group.

GLP-1 receptor agonists (GLP-1RAs) have been shown to have benefits for atherosclerotic CV disease (ASCVD) and have been suggested as a first-line therapy for patients with ASCVD or a high risk of ASCVD[44]. GLP-1RAs can attenuate oxidative stress, ameliorate inflammatory response, increase GLUT4 expression and translocation, amplify insulin signaling transduction, and improve the plasma lipid profile[78]. All of these effects can improve insulin sensitivity. GLP-1RAs also have an effect on body weight loss[79,80], which also decreases insulin resistance. The meta-analysis in 2019 also showed that GLP-1RAs significantly increase islet beta cell function and reduce insulin resistance and the fasting glucose level[76]. Furthermore, GLP-1RAs are a potential treatment choice for PCOS, which is highly correlated with insulin resistance[81]. GLP-1RAs such as liraglutide, dulaglutide and semaglutide, have been reported to have good efficacy and a good safety profile in the advanced CKD group, including hemodialysis patients[82]. Although there is currently no direct evidence of GLP-1RAs improving insulin resistance in the CKD group, it can be hypothesized theoretically.

Sodium-glucose cotransporter 2 inhibitors

SGLT2Is inhibit renal tubule glucose reabsorption, and thus increase urinary glucose excretion and improve hyperglycemia. SGLT2Is also have a diuretic effect by decreasing sodium reabsorption. SGLT2Is have favorable CV effects, especially in the heart failure group[44]. SGLT2Is can delay renal function deterioration with or without DM[83-85]. The effect may result from decreasing intraglomerular pressure *via* tubuloglomerular feedback. Some studies have recently shown that SGLT2Is also have potential for improving insulin sensitivity. Tofogliflozin, a class of SGLT2I, was found to improve insulin resistance in skeletal muscle by stimulating glucose uptake in obese mice[86]. It also accelerated lipolysis in adipose tissue and reduced adipose tissue mass. The reduction of hyperinsulinemia as a result of decreasing blood sugar level after the administration of SGLT2Is may be the mechanism. Another SGLT2I, empagliflozin, can reduce fat mass by increasing energy expenditure, promoting fat browning, and enhancing fatty acid oxidation in skeletal muscle in high-fat-diet-induced obese mice[87]. It was also found to induce M2 macrophage polarization in fat and the liver, which had an anti-inflammation effect[87]. In humans, dapagliflozin can reduce body weight and body fat mass and improve muscle insulin sensitivity[88]. In summary, SGLT2Is can stimulate glucose uptake in skeletal muscle tissue, decrease fat mass, promote fat browning, and reduce inflammation. These effects can attenuate insulin resistance. However, these effects are decreased in CKD patients. The effect of SGLT2Is on insulin resistance in the CKD group is unclear (Table 1).

OTHER MEDICATIONS USED FOR CKD: POTENTIAL EFFECT ON INSULIN RESISTANCE

AST-120 (Kremezin)

As mentioned, protein-bound uremic toxins such as p-cresyl sulfate can induce insulin resistance. AST-120 (Kremezin) is an oral carbonaceous adsorbent. It can absorb toxins generated by intestinal microbiota and decrease the systemic and local uremic toxin levels. A study compared AST-120-fed diabetic CKD (underwent two-third nephrectomy) rats to control diabetic CKD rats[89]. The mean blood glucose level and the mean dose of exogenous insulin used in the AST-120-fed group were significantly reduced[89].

Renin-Ang system blockades

As previously described, Ang II can induce insulin resistance. The main pathway for producing Ang II is *via* the renin-Ang system (RAS). The use of RAS blockades, including Ang-converting-enzyme inhibitor and Ang II receptor blocker, are standard care for CKD and diabetes nephropathy because of their beneficial effects on CV and kidney outcomes. Some studies showed that RAS blockades improve insulin resistance compared to other anti-hypertensive agents[90,91]. A large study that recruited 5269 impaired-glucose-tolerance patients showed that the administration of ramipril significantly reduced the post-load serum glucose level[92]. Another large trial showed that the use of valsartan decreased serum fasting glucose, the post-load glucose level, and even 5-year diabetes development[93].

Finerenone

Aldosterone overproduction may induce insulin resistance. The current evidence for the benefit of using steroidal MRAs to manage insulin resistance is still unclear. Finerenone, a novel non-steroidal MRA, has a higher affinity to the mineralocorticoid receptor and fewer side effects compared to those of steroidal MRAs. Recent studies indicated that finerenone has a beneficial effect on the heart and kidneys in CKD and T2DM patients[94,95]. A recent animal study found that finerenone can increase brown adipose tissue and thus improve insulin resistance[96]. Finerenone may be an attractive therapy choice for treating insulin resistance in CKD patients.

Endothelin-1

Endothelin-1 (ET-1) is a peptide secreted by endothelium. ET-1 is thought to induce vasoconstriction, cell proliferation, and inflammation. There is a positive correlation between the serum ET-1 level and insulin resistance[97]. The administration of an endothelin receptor antagonist, such as atrasentan, can improve hepatic insulin sensitivity in insulin resistance rats. A recent study revealed that atrasentan can improve peripheral glucose homeostasis, dyslipidemia, and liver triglycerides in high-fat-diet mice[98]. Therefore, ET-1 antagonists may be a therapeutic choice for improving insulin resistance. The SONAR trial showed better kidney outcomes with long-term atrasentan use in selected T2DM patients with CKD. However, fluid overload and anemia were significantly higher in the atrasentan group[99].

CONCLUSION

CKD patients have a higher risk for CV events. Insulin resistance is common in CKD patients and may cause CV disease in this group. Therefore, knowing the pathogenesis of insulin resistance in CKD is

crucial. It can provide a way to manage potential CV comorbidities or ESRD. Several kinds of anti-diabetic medication can not only decrease the blood sugar level but also improve insulin resistance. Some of them may have a beneficial effect on CKD. Moreover, recent novel medications for managing CKD can also reduce insulin resistance. MicroRNAs (miRNAs) are small non-coding RNAs that can regulate target mRNA expression[100]. They have been found to have an effect on glucose metabolism. For example, a recent study showed that miR-126 single nucleotide polymorphism was associated with T2DM[101]. MiRNAs may be a target therapeutic strategy in the future. Small-molecule insulin mimetics, which can stimulate the insulin-signaling pathway, have been recently introduced[102,103]; however, this research is still in the early stages. Further research on halting insulin resistance in CKD patients is required.

FOOTNOTES

Author contributions: Lin WR wrote the paper; Liu KH, Ling TC, Wang MC and Lin WH edited and revised manuscript; and all authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: Taiwan

ORCID number: Wei-Ren Lin 0000-0002-8763-4609; Wei-Hung Lin 0000-0003-3690-627X.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Zhao S

REFERENCES

1 **Spoto B**, Pisano A, Zoccali C. Insulin resistance in chronic kidney disease: a systematic review. *Am J Physiol Renal Physiol* 2016; **311**: F1087-F1108 [PMID: 27707707 DOI: 10.1152/ajprenal.00340.2016]
2 **Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group**. KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. *Kidney Int* 2022; **102**: S1-S127 [PMID: 36272764 DOI: 10.1016/j.kint.2022.06.008]
3 **Fliser D**, Pacini G, Engelleiter R, Kautzky-Willer A, Prager R, Franek E, Ritz E. Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. *Kidney Int* 1998; **53**: 1343-1347 [PMID: 9573550 DOI: 10.1046/j.1523-1755.1998.00898.x]
4 **Hamburg NM**, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, Gokce N, Ruderman NB, Keaney JF Jr, Vita JA. Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2650-2656 [PMID: 17932315 DOI: 10.1161/ATVBAHA.107.153288]
5 **Davis TA**, Klahr S, Karl IE. Glucose metabolism in muscle of sedentary and exercised rats with azotemia. *Am J Physiol* 1987; **252**: F138-F145 [PMID: 3544866 DOI: 10.1152/ajprenal.1987.252.1.F138]
6 **Goldberg AP**, Hagberg JM, Delmez JA, Haynes ME, Harter HR. Metabolic effects of exercise training in hemodialysis patients. *Kidney Int* 1980; **18**: 754-761 [PMID: 7206459 DOI: 10.1038/ki.1980.194]
7 **Liao MT**, Sung CC, Hung KC, Wu CC, Lo L, Lu KC. Insulin resistance in patients with chronic kidney disease. *J Biomed Biotechnol* 2012; **2012**: 691369 [PMID: 22919275 DOI: 10.1155/2012/691369]
8 **Lee DF**, Kuo HP, Chen CT, Wei Y, Chou CK, Hung JY, Yen CJ, Hung MC. IKKbeta suppression of TSC1 function links the mTOR pathway with insulin resistance. *Int J Mol Med* 2008; **22**: 633-638 [PMID: 18949383 DOI: 10.3892/ijmm_00000065]
9 **Plomgaard P**, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects *via* inhibition of Akt substrate 160 phosphorylation. *Diabetes* 2005; **54**: 2939-2945 [PMID: 16186396 DOI: 10.2337/diabetes.54.10.2939]
10 **Plomgaard P**, Fischer CP, Ibfelt T, Pedersen BK, van Hall G. Tumor necrosis factor-alpha modulates human *in vivo* lipolysis. *J Clin Endocrinol Metab* 2008; **93**: 543-549 [PMID: 18029463 DOI: 10.1210/jc.2007-1761]
11 **Senn JJ**, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 2002; **51**: 3391-3399 [PMID: 12453891 DOI: 10.2337/diabetes.51.12.3391]
12 **Rieusset J**, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H. Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes* 2004; **53**: 2232-2241 [PMID: 15331532 DOI: 10.2337/diabetes.53.9.2232]

13 **Hurrle S**, Hsu WH. The etiology of oxidative stress in insulin resistance. *Biomed J* 2017; **40**: 257-262 [PMID: 29179880 DOI: 10.1016/j.bj.2017.06.007]

14 **Aminzadeh MA**, Nicholas SB, Norris KC, Vaziri ND. Role of impaired Nrf2 activation in the pathogenesis of oxidative stress and inflammation in chronic tubulo-interstitial nephropathy. *Nephrol Dial Transplant* 2013; **28**: 2038-2045 [PMID: 23512109 DOI: 10.1093/ndt/gft022]

15 **Li S**, Eguchi N, Lau H, Ichii H. The Role of the Nrf2 Signaling in Obesity and Insulin Resistance. *Int J Mol Sci* 2020; **21** [PMID: 32971975 DOI: 10.3390/ijms21186973]

16 **DeFronzo RA**, Beckles AD. Glucose intolerance following chronic metabolic acidosis in man. *Am J Physiol* 1979; **236**: E328-E334 [PMID: 434194 DOI: 10.1152/ajpendo.1979.236.4.E328]

17 **Hayata H**, Miyazaki H, Niisato N, Yokoyama N, Marunaka Y. Lowered extracellular pH is involved in the pathogenesis of skeletal muscle insulin resistance. *Biochem Biophys Res Commun* 2014; **445**: 170-174 [PMID: 24502946 DOI: 10.1016/j.bbrc.2014.01.162]

18 **Franch HA**, Raissi S, Wang X, Zheng B, Bailey JL, Price SR. Acidosis impairs insulin receptor substrate-1-associated phosphoinositide 3-kinase signaling in muscle cells: consequences on proteolysis. *Am J Physiol Renal Physiol* 2004; **287**: F700-F706 [PMID: 15161606 DOI: 10.1152/ajprenal.00440.2003]

19 **Bellasi A**, Di Micco L, Santoro D, Marzocco S, De Simone E, Cozzolino M, Di Lullo L, Guastaferro P, Di Iorio B; UBI study investigators. Correction of metabolic acidosis improves insulin resistance in chronic kidney disease. *BMC Nephrol* 2016; **17**: 158 [PMID: 27770799 DOI: 10.1186/s12882-016-0372-x]

20 **Greco EA**, Lenzi A, Migliaccio S. Role of Hypovitaminosis D in the Pathogenesis of Obesity-Induced Insulin Resistance. *Nutrients* 2019; **11** [PMID: 31266190 DOI: 10.3390/nu11071506]

21 **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]

22 **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]

23 **Huang Y**, Ishizuka T, Miura A, Kajita K, Ishizawa M, Kimura M, Yamamoto Y, Kawai Y, Morita H, Uno Y, Yasuda K. Effect of 1 alpha,25-dihydroxy vitamin D3 and vitamin E on insulin-induced glucose uptake in rat adipocytes. *Diabetes Res Clin Pract* 2002; **55**: 175-183 [PMID: 11850093 DOI: 10.1016/s0168-8227(01)00324-2]

24 **Xu Z**, Gong R, Luo G, Wang M, Li D, Chen Y, Shen X, Wei X, Feng N, Wang S. Association between vitamin D3 levels and insulin resistance: a large sample cross-sectional study. *Sci Rep* 2022; **12**: 119 [PMID: 34997087 DOI: 10.1038/s41598-021-04109-7]

25 **He S**, Yu S, Zhou Z, Wang C, Wu Y, Li W. Effect of vitamin D supplementation on fasting plasma glucose, insulin resistance and prevention of type 2 diabetes mellitus in non-diabetics: A systematic review and meta-analysis. *Biomed Rep* 2018; **8**: 475-484 [PMID: 29725526 DOI: 10.3892/br.2018.1074]

26 **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]

27 **Wenclewska S**, Szymczak-Pajor I, Drzewoski J, Bunk M, Śliwińska A. Vitamin D Supplementation Reduces Both Oxidative DNA Damage and Insulin Resistance in the Elderly with Metabolic Disorders. *Int J Mol Sci* 2019; **20** [PMID: 31200560 DOI: 10.3390/ijms20122891]

28 **Czech MP**. Insulin action and resistance in obesity and type 2 diabetes. *Nat Med* 2017; **23**: 804-814 [PMID: 28697184 DOI: 10.1038/nm.4350]

29 **Ouchi N**, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011; **11**: 85-97 [PMID: 21252989 DOI: 10.1038/nri2921]

30 **Lee DM**, Knight-Gibson C, Samuelsson O, Attman PO, Wang CS, Alaupovic P. Lipoprotein particle abnormalities and the impaired lipolysis in renal insufficiency. *Kidney Int* 2002; **61**: 209-218 [PMID: 11786103 DOI: 10.1046/j.1523-1755.2002.00116.x]

31 **Koppe L**, Pelletier CC, Alix PM, Kalbacher E, Fouque D, Soulage CO, Guebre-Egziabher F. Insulin resistance in chronic kidney disease: new lessons from experimental models. *Nephrol Dial Transplant* 2014; **29**: 1666-1674 [PMID: 24286973 DOI: 10.1093/ndt/gft435]

32 **Kielstein JT**, Zoccali C. Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? *Am J Kidney Dis* 2005; **46**: 186-202 [PMID: 16112037 DOI: 10.1053/j.ajkd.2005.05.009]

33 **Dzúrik R**, Spustová V, Lajdová I. Inhibition of glucose utilization in isolated rat soleus muscle by pseudouridine: implications for renal failure. *Nephron* 1993; **65**: 108-110 [PMID: 8413768 DOI: 10.1159/000187450]

34 **Lee W**, Lee HJ, Jang HB, Kim HJ, Ban HJ, Kim KY, Nam MS, Choi JS, Lee KT, Cho SB, Park SI. Asymmetric dimethylarginine (ADMA) is identified as a potential biomarker of insulin resistance in skeletal muscle. *Sci Rep* 2018; **8**: 2133 [PMID: 29391561 DOI: 10.1038/s41598-018-20549-0]

35 **D'Apolito M**, Du X, Zong H, Catucci A, Maiuri L, Trivisano T, Pettoello-Mantovani M, Campanozzi A, Raia V, Pessin JE, Brownlee M, Giardino I. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. *J Clin Invest* 2010; **120**: 203-213 [PMID: 19955654 DOI: 10.1172/JCI37672]

36 **Xie Y**, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z. Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. *Kidney Int* 2018; **93**: 741-752 [PMID: 29241622 DOI: 10.1016/j.kint.2017.08.033]

37 **Koppe L**, Pillon NJ, Vella RE, Croze ML, Pelletier CC, Chambert S, Massy Z, Glorieux G, Vanholder R, Dugenet Y, Soula HA, Fouque D, Soulage CO. p-Cresyl sulfate promotes insulin resistance associated with CKD. *J Am Soc Nephrol* 2013; **24**: 88-99 [PMID: 23274953 DOI: 10.1681/ASN.2012050503]

38 **Zhang L**, Du J, Hu Z, Han G, Delafontaine P, Garcia G, Mitch WE. IL-6 and serum amyloid A synergy mediates angiotensin II-induced muscle wasting. *J Am Soc Nephrol* 2009; **20**: 604-612 [PMID: 19158350 DOI: 10.1681/ASN.2008060628]

39 **Luther JM**. Effects of aldosterone on insulin sensitivity and secretion. *Steroids* 2014; **91**: 54-60 [PMID: 25194457 DOI: 10.1016/j.steroids.2014.08.016]

40 **Hosoya K**, Minakuchi H, Wakino S, Fujimura K, Hasegawa K, Komatsu M, Yoshifuji A, Futatsugi K, Shinozuka K, Washida N, Kanda T, Tokuyama H, Hayashi K, Itoh H. Insulin resistance in chronic kidney disease is ameliorated by spironolactone in rats and humans. *Kidney Int* 2015; **87**: 749-760 [PMID: 25337775 DOI: 10.1038/ki.2014.348]

41 **Preiss D**, Zetterstrand S, McMurray JJ, Ostergren J, Michelson EL, Granger CB, Yusuf S, Swedberg K, Pfeffer MA, Gerstein HC, Sattar N; Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity Investigators. Predictors of development of diabetes in patients with chronic heart failure in the Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity (CHARM) program. *Diabetes Care* 2009; **32**: 915-920 [PMID: 19196892 DOI: 10.2337/dc08-1709]

42 **Finsen SH**, Hansen MR, Hoffmann-Petersen J, Højgaard HF, Mortensen SP. Eight weeks of mineralocorticoid blockade does not improve insulin sensitivity in type 2 diabetes. *Physiol Rep* 2021; **9**: e14971 [PMID: 34350730 DOI: 10.14814/phy2.14971]

43 **Kawahito S**, Kitahata H, Oshita S. Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress. *World J Gastroenterol* 2009; **15**: 4137-4142 [PMID: 19725147 DOI: 10.3748/wjg.15.4137]

44 **American Diabetes Association Professional Practice Committee**. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2022. *Diabetes Care* 2022; **45**: S125-S143 [PMID: 34964831 DOI: 10.2337/dc22-S009]

45 **Foretz M**, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat Rev Endocrinol* 2019; **15**: 569-589 [PMID: 31439934 DOI: 10.1038/s41574-019-0242-2]

46 . Diagnostic ultrasound. *Vet Clin North Am Equine Pract* 1986; **2**: 1-261 [PMID: 3516318 DOI: 10.3390/ijms23031264]

47 **Wild RA**, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, Lobo R, Norman RJ, Talbott E, Dumesic DA. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab* 2010; **95**: 2038-2049 [PMID: 20375205 DOI: 10.1210/jc.2009-2724]

48 **Diabetes Prevention Program Research Group**. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: the Diabetes Prevention Program Outcomes Study. *Lancet Diabetes Endocrinol* 2015; **3**: 866-875 [PMID: 26377054 DOI: 10.1016/S2213-8587(15)00291-0]

49 **Staels B**, Fruchart JC. Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes* 2005; **54**: 2460-2470 [PMID: 16046315 DOI: 10.2337/diabetes.54.8.2460]

50 **Yki-Järvinen H**. Thiazolidinediones. *N Engl J Med* 2004; **351**: 1106-1118 [PMID: 15356308 DOI: 10.1056/NEJMra041001]

51 **Abe M**, Okada K, Kikuchi F, Matsumoto K. Clinical investigation of the effects of pioglitazone on the improvement of insulin resistance and blood pressure in type 2-diabetic patients undergoing hemodialysis. *Clin Nephrol* 2008; **70**: 220-228 [PMID: 18793563 DOI: 10.5414/cnp70220]

52 **Abe M**, Okada K, Maruyama T, Maruyama N, Soma M, Matsumoto K. Clinical effectiveness and safety evaluation of long-term pioglitazone treatment for erythropoietin responsiveness and insulin resistance in type 2 diabetic patients on hemodialysis. *Expert Opin Pharmacother* 2010; **11**: 1611-1620 [PMID: 20540652 DOI: 10.1517/14656566.2010.495119]

53 **Cheng AY**, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* 2005; **172**: 213-226 [PMID: 15655244 DOI: 10.1001/jama.287.3.360]

54 **Mandarino LJ**, Gerich JE. Prolonged sulfonylurea administration decreases insulin resistance and increases insulin secretion in non-insulin-dependent diabetes mellitus: evidence for improved insulin action at a postreceptor site in hepatic as well as extrahepatic tissues. *Diabetes Care* 1984; **7** Suppl 1: 89-99 [PMID: 6376034]

55 **Müller G**. The molecular mechanism of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug Amaryl. *Mol Med* 2000; **6**: 907-933 [PMID: 11147570 DOI: 10.1007/bf03401827]

56 **Hanefeld M**. Pioglitazone and sulfonylureas: effectively treating type 2 diabetes. *Int J Clin Pract Suppl* 2007; **61**: 20-27 [PMID: 17594390 DOI: 10.1111/j.1742-1241.2007.01361.x]

57 **Bailey CJ**. Insulin resistance: Impact on therapeutic developments in diabetes. *Diab Vasc Dis Res* 2019; **16**: 128-132 [PMID: 31014100 DOI: 10.1177/1479164119827570]

58 **Abe M**, Okada K, Maruyama T, Maruyama N, Matsumoto K. Combination therapy with mitiglinide and voglibose improves glycemic control in type 2 diabetic patients on hemodialysis. *Expert Opin Pharmacother* 2010; **11**: 169-176 [PMID: 20025554 DOI: 10.1517/14656560903530683]

59 **van de Laar FA**. Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. *Vasc Health Risk Manag* 2008; **4**: 1189-1195 [PMID: 19337532 DOI: 10.2147/vhrm.s3119]

60 **Nakamura K**, Yamagishi S, Matsui T, Inoue H. Acarbose, an alpha-glucosidase inhibitor, improves insulin resistance in fructose-fed rats. *Drugs Exp Clin Res* 2005; **31**: 155-159 [PMID: 16223205 DOI: 10.1177/147323000603400510]

61 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trail Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* 2002; **359**: 2072-2077 [PMID: 12086760 DOI: 10.1016/s0140-6736(02)08905-5]

62 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003; **290**: 486-494 [PMID: 12876091 DOI: 10.1001/jama.290.4.486]

63 **Bayer HealthCare Pharmaceuticals Inc**. Product Information: PRECOSER (acarbose tablets). 2015. [cited 10 June 2022]. Available from: https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=c85bc02a-bf18-4690-84b8-2c256bce5f9f

64 **Lin WH**, Yang CY, Kuo S, Kuo TH, Roan JN, Li CY, Wang MC, Ou HT. Hepatic and cardiovascular safety of acarbose among type 2 diabetes patients with end-stage renal disease: A nationwide population-based longitudinal study. *Diabetes Res Clin Pract* 2021; **172**: 108489 [PMID: 33035600 DOI: 10.1016/j.diabres.2020.108489]

65 **Koliaki C**, Doupis J. Incretin-based therapy: a powerful and promising weapon in the treatment of type 2 diabetes

mellitus. *Diabetes Ther* 2011; **2**: 101-121 [PMID: 22127804 DOI: 10.1007/s13300-011-0002-3]

66 **Turton MD**, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996; **379**: 69-72 [PMID: 8538742 DOI: 10.1038/379069a0]

67 **Vella A**. Mechanism of action of DPP-4 inhibitors--new insights. *J Clin Endocrinol Metab* 2012; **97**: 2626-2628 [PMID: 22869847 DOI: 10.1210/jc.2012-2396]

68 **Rosenstock J**, Perkovic V, Johansen OE, Cooper ME, Kahn SE, Marx N, Alexander JH, Pencina M, Toto RD, Wanner C, Zinman B, Woerle HJ, Baanstra D, Pfarr E, Schnaidt S, Meinicke T, George JT, von Eynatten M, McGuire DK; CARMELINA Investigators. Effect of Linagliptin *vs* Placebo on Major Cardiovascular Events in Adults With Type 2 Diabetes and High Cardiovascular and Renal Risk: The CARMELINA Randomized Clinical Trial. *JAMA* 2019; **321**: 69-79 [PMID: 30418475 DOI: 10.1001/jama.2018.18269]

69 **Green JB**, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR; TECOS Study Group. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 232-242 [PMID: 26052984 DOI: 10.1056/NEJMoa1501352]

70 **Scirica BM**, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I; SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317-1326 [PMID: 23992601 DOI: 10.1056/NEJMoa1307684]

71 **Karagiannis T**, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. *BMJ* 2012; **344**: e1369 [PMID: 22411919 DOI: 10.1136/bmj.e1369]

72 **Herman GA**, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, Golor G, Schrodter A, Keymeulen B, Lasseter KC, Kipnes MS, Snyder K, Hilliard D, Tanen M, Cilissen C, De Smet M, de Lepeleire I, Van Dyck K, Wang AQ, Zeng W, Davies MJ, Tanaka W, Holst JJ, Deacon CF, Gottesdiener KM, Wagner JA. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2006; **91**: 4612-4619 [PMID: 16912128 DOI: 10.1210/jc.2006-1009]

73 **Azuma K**, Rádiková Z, Mancino J, Toledo FG, Thomas E, Kangani C, Dalla Man C, Cobelli C, Holst JJ, Deacon CF, He Y, Ligueros-Saylan M, Serra D, Foley JE, Kelley DE. Measurements of islet function and glucose metabolism with the dipeptidyl peptidase 4 inhibitor vildagliptin in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2008; **93**: 459-464 [PMID: 18042650 DOI: 10.1210/jc.2007-1369]

74 **Yang L**, Yuan J, Zhou Z. Emerging roles of dipeptidyl peptidase 4 inhibitors: anti-inflammatory and immunomodulatory effect and its application in diabetes mellitus. *Can J Diabetes* 2014; **38**: 473-479 [PMID: 25034244 DOI: 10.1016/j.jcjd.2014.01.008]

75 **Trzaskalski NA**, Fadzeyeva E, Mulvihill EE. Dipeptidyl Peptidase-4 at the Interface Between Inflammation and Metabolism. *Clin Med Insights Endocrinol Diabetes* 2020; **13**: 1179551420912972 [PMID: 32231442 DOI: 10.1177/1179551420912972]

76 **Wu S**, Gao L, Cipriani A, Huang Y, Yang Z, Yang J, Yu S, Zhang Y, Chai S, Zhang Z, Sun F, Zhan S. The effects of incretin-based therapies on β-cell function and insulin resistance in type 2 diabetes: A systematic review and network meta-analysis combining 360 trials. *Diabetes Obes Metab* 2019; **21**: 975-983 [PMID: 30536884 DOI: 10.1111/dom.13613]

77 **Hattori S**. Omarigliptin decreases inflammation and insulin resistance in a pleiotropic manner in patients with type 2 diabetes. *Diabetol Metab Syndr* 2020; **12**: 24 [PMID: 32211075 DOI: 10.1186/s13098-020-00533-3]

78 **Yaribeygi H**, Sathyapalan T, Sahebkar A. Molecular mechanisms by which GLP-1 RA and DPP-4i induce insulin sensitivity. *Life Sci* 2019; **234**: 116776 [PMID: 31425698 DOI: 10.1016/j.lfs.2019.116776]

79 **Nuffer WA**, Trujillo JM. Liraglutide: A New Option for the Treatment of Obesity. *Pharmacotherapy* 2015; **35**: 926-934 [PMID: 26497479 DOI: 10.1002/phar.1639]

80 **Capehorn MS**, Catarig AM, Furberg JK, Janez A, Price HC, Tadayon S, Vergès B, Marre M. Efficacy and safety of once-weekly semaglutide 1.0mg *vs* once-daily liraglutide 1.2mg as add-on to 1-3 oral antidiabetic drugs in subjects with type 2 diabetes (SUSTAIN 10). *Diabetes Metab* 2020; **46**: 100-109 [PMID: 31539622 DOI: 10.1016/j.diabet.2019.101117]

81 **Bednarz K**, Kowalczyk K, Cwynar M, Czapla D, Czarkowski W, Kmita D, Nowak A, Madej P. The Role of Glp-1 Receptor Agonists in Insulin Resistance with Concomitant Obesity Treatment in Polycystic Ovary Syndrome. *Int J Mol Sci* 2022; **23** [PMID: 35457152 DOI: 10.3390/ijms23084334]

82 **Górriz JL**, Soler MJ, Navarro-González JF, García-Carro C, Puchades MJ, D'Marco L, Martínez Castelao A, Fernández-Fernández B, Ortiz A, Górriz-Zambrano C, Navarro-Pérez J, Gorgojo-Martinez JJ. GLP-1 Receptor Agonists and Diabetic Kidney Disease: A Call of Attention to Nephrologists. *J Clin Med* 2020; **9** [PMID: 32235471 DOI: 10.3390/jcm9040947]

83 **Wanner C**, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, Johansen OE, Woerle HJ, Broedl UC, Zinman B; EMPA-REG OUTCOME Investigators. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. *N Engl J Med* 2016; **375**: 323-334 [PMID: 27299675 DOI: 10.1056/NEJMoa1515920]

84 **Perkovic V**, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, Edwards R, Agarwal R, Bakris G, Bull S, Cannon CP, Capuano G, Chu PL, de Zeeuw D, Greene T, Levin A, Pollock C, Wheeler DC, Yavin Y, Zhang H, Zinman B, Meininger G, Brenner BM, Mahaffey KW; CREDENCE Trial Investigators. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N Engl J Med* 2019; **380**: 2295-2306 [PMID: 30990260 DOI: 10.1056/NEJMoa1811744]

85 **Heerspink HJL**, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, Mann JFE, McMurray JJV, Lindberg M, Rossing P, Sjöström CD, Toto RD, Langkilde AM, Wheeler DC; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. *N Engl J Med* 2020; **383**: 1436-1446 [PMID: 32970396 DOI: 10.1056/NEJMoa2024816]

86 **Obata A**, Kubota N, Kubota T, Iwamoto M, Sato H, Sakurai Y, Takamoto I, Katsuyama H, Suzuki Y, Fukazawa M, Ikeda S, Iwayama K, Tokuyama K, Ueki K, Kadowaki T. Tofogliflozin Improves Insulin Resistance in Skeletal Muscle and

Accelerates Lipolysis in Adipose Tissue in Male Mice. *Endocrinology* 2016; **157**: 1029-1042 [PMID: 26713783 DOI: 10.1210/en.2015-1588]

87 **Xu L**, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, Mayoux E, Kaneko S, Ota T. SGLT2 Inhibition by Empagliflozin Promotes Fat Utilization and Browning and Attenuates Inflammation and Insulin Resistance by Polarizing M2 Macrophages in Diet-induced Obese Mice. *EBioMedicine* 2017; **20**: 137-149 [PMID: 28579299 DOI: 10.1016/j.ebiom.2017.05.028]

88 **Merovci A**, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D, Xiong J, Perez Z, Norton L, Abdul-Ghani MA, DeFronzo RA. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest* 2014; **124**: 509-514 [PMID: 24463448 DOI: 10.1172/JCI70704]

89 **Okada K**, Takahashi Y, Okawa E, Onishi Y, Hagi C, Aoki K, Shibahara H, Higuchi T, Nagura Y, Kanmatsuse K, Takahashi S. Relationship between insulin resistance and uremic toxins in the gastrointestinal tract. *Nephron* 2001; **88**: 384-386 [PMID: 11474237 DOI: 10.1159/000046026]

90 **Grassi G**, Seravalle G, Dell'Oro R, Trevano FQ, Bombelli M, Scopelliti F, Facchini A, Mancia G; CROSS Study. Comparative effects of candesartan and hydrochlorothiazide on blood pressure, insulin sensitivity, and sympathetic drive in obese hypertensive individuals: results of the CROSS study. *J Hypertens* 2003; **21**: 1761-1769 [PMID: 12923410 DOI: 10.1097/00004872-200309000-00027]

91 **Jin HM**, Pan Y. Angiotensin type-1 receptor blockade with losartan increases insulin sensitivity and improves glucose homeostasis in subjects with type 2 diabetes and nephropathy. *Nephrol Dial Transplant* 2007; **22**: 1943-1949 [PMID: 17308317 DOI: 10.1093/ndt/gfm049]

92 **DREAM Trial Investigators**, Bosch J, Yusuf S, Gerstein HC, Pogue J, Sheridan P, Dagenais G, Diaz R, Avezum A, Lanas F, Probstfield J, Fodor G, Holman RR. Effect of ramipril on the incidence of diabetes. *N Engl J Med* 2006; **355**: 1551-1562 [PMID: 16980380 DOI: 10.1056/NEJMoa065061]

93 **NAVIGATOR Study Group**, McMurray JJ, Holman RR, Haffner SM, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR, Chiang FT, Charbonnel B, Chow CC, Davies MJ, Deedwania P, Diem P, Einhorn D, Fonseca V, Fulcher GR, Gaciong Z, Gaztambide S, Giles T, Horton E, Ilkova H, Jenssen T, Kahn SE, Krum H, Laakso M, Leiter LA, Levitt NS, Mareev V, Martinez F, Masson C, Mazzone T, Meaney E, Nesto R, Pan C, Prager R, Raptis SA, Rutten GE, Sandstroem H, Schaper F, Scheen A, Schmitz O, Sinay I, Soska V, Stender S, Tamás G, Tognoni G, Tuomilehto J, Villamil AS, Vozár J, Califf RM. Effect of valsartan on the incidence of diabetes and cardiovascular events. *N Engl J Med* 2010; **362**: 1477-1490 [PMID: 20228403 DOI: 10.1056/NEJMoa1001121]

94 **Bakris GL**, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, Kolkhof P, Nowack C, Schloemer P, Joseph A, Filippatos G; FIDELIO-DKD Investigators. Effect of Finerenone on Chronic Kidney Disease Outcomes in Type 2 Diabetes. *N Engl J Med* 2020; **383**: 2219-2229 [PMID: 33264825 DOI: 10.1056/NEJMoa2025845]

95 **Pitt B**, Filippatos G, Agarwal R, Anker SD, Bakris GL, Rossing P, Joseph A, Kolkhof P, Nowack C, Schloemer P, Ruilope LM; FIGARO-DKD Investigators. Cardiovascular Events with Finerenone in Kidney Disease and Type 2 Diabetes. *N Engl J Med* 2021; **385**: 2252-2263 [PMID: 34449181 DOI: 10.1056/NEJMoa2110956]

96 **Marzolla V**, Feraco A, Limana F, Kolkhof P, Armani A, Caprio M. Class-specific responses of brown adipose tissue to steroidal and nonsteroidal mineralocorticoid receptor antagonists. *J Endocrinol Invest* 2022; **45**: 215-220 [PMID: 34272678 DOI: 10.1007/s40618-021-01635-z]

97 **Jenkins HN**, Rivera-Gonzalez O, Gibert Y, Speed JS. Endothelin-1 in the pathophysiology of obesity and insulin resistance. *Obes Rev* 2020; **21**: e13086 [PMID: 32627269 DOI: 10.1111/obr.13086]

98 **Rivera-Gonzalez O**, Wilson NA, Coats LE, Taylor EB, Speed JS. Endothelin receptor antagonism improves glucose handling, dyslipidemia, and adipose tissue inflammation in obese mice. *Clin Sci (Lond)* 2021; **135**: 1773-1789 [PMID: 34278410 DOI: 10.1042/CS20210549]

99 **Heerspink HJL**, Parving HH, Andress DL, Bakris G, Correa-Rotter R, Hou FF, Kitzman DW, Kohan D, Makino H, McMurray JJV, Melnick JZ, Miller MG, Pergola PE, Perkovic V, Tobe S, Yi T, Wigderson M, de Zeeuw D; SONAR Committees and Investigators. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial. *Lancet* 2019; **393**: 1937-1947 [PMID: 30995972 DOI: 10.1016/S0140-6736(19)30772-X]

100 **Li M**, Chi X, Wang Y, Setrerrahmane S, Xie W, Xu H. Trends in insulin resistance: insights into mechanisms and therapeutic strategy. *Signal Transduct Target Ther* 2022; **7**: 216 [PMID: 35794109 DOI: 10.1038/s41392-022-01073-0]

101 **Mir R**, Elfaki I, Duhier FMA, Alotaibi MA, AlAlawy AI, Barnawi J, Babakr AT, Mir MM, Mirghani H, Hamadi A, Dabla PK. Molecular Determination of mirRNA-126 rs4636297, Phosphoinositide-3-Kinase Regulatory Subunit 1-Gene Variability rs7713645, rs706713 (Tyr73Tyr), rs3730089 (Met326Ile) and Their Association with Susceptibility to T2D. *J Pers Med* 2021; **11** [PMID: 34575638 DOI: 10.3390/jpm11090861]

102 **Lin B**, Li Z, Park K, Deng L, Pai A, Zhong L, Pirrung MC, Webster NJ. Identification of novel orally available small molecule insulin mimetics. *J Pharmacol Exp Ther* 2007; **323**: 579-585 [PMID: 17687071 DOI: 10.1124/jpet.107.126102]

103 **Lan ZJ**, Lei Z, Yiannikouris A, Yerramreddy TR, Li X, Kincaid H, Eastridge K, Gadberry H, Power C, Xiao R, Lei L, Seale O, Dawson K, Power R. Non-peptidyl small molecule, adenosine, 5'-Se-methyl-5'-seleno-, 2',3'-diacetate, activates insulin receptor and attenuates hyperglycemia in type 2 diabetic Lepr(db/db) mice. *Cell Mol Life Sci* 2020; **77**: 1623-1643 [PMID: 31378829 DOI: 10.1007/s00018-019-03249-4]

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 364-395

DOI: 10.4239/wjd.v14.i4.364

ISSN 1948-9358 (online)

REVIEW

Therapeutic role of growth factors in treating diabetic wound

Shen-Yuan Zheng, Xin-Xing Wan, Piniel Alphayo Kambey, Yan Luo, Xi-Min Hu, Yi-Fan Liu, Jia-Qi Shan, Yu-Wei Chen, Kun Xiong

Specialty type: Dermatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

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

P-Reviewer: Khan NR, Pakistan; Li Y, China

Received: December 23, 2022
Peer-review started: December 23, 2022
First decision: January 9, 2023
Revised: January 16, 2023
Accepted: March 21, 2023
Article in press: March 21, 2023
Published online: April 15, 2023

Shen-Yuan Zheng, Xi-Min Hu, Department of Dermatology, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China

Shen-Yuan Zheng, Xi-Min Hu, Kun Xiong, Department of Anatomy and Neurobiology, School of Basic Medical Science, Central South University, Changsha 410013, Hunan Province, China

Xin-Xing Wan, Department of Endocrinology, Third Xiangya Hospital, Central South University, Changsha 410013, Hunan Province, China

Piniel Alphayo Kambey, Department of Neurobiology and Anatomy, Xuzhou Medical University, Xuzhou 221004, Jiangsu Province, China

Yan Luo, Yi-Fan Liu, Jia-Qi Shan, Yu-Wei Chen, Clinical Medicine Eight-Year Program, Xiangya School of Medicine, Central South University, Changsha 410013, Hunan Province, China

Kun Xiong, Key Laboratory of Emergency and Trauma, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, Hainan Province, China

Kun Xiong, Hunan Key Laboratory of Ophthalmology, Central South University, Changsha 410013, Hunan Province, China

Corresponding author: Kun Xiong, Doctor, MD, PhD, Professor, Department of Anatomy and Neurobiology, School of Basic Medical Science, Central South University, No. 172 Tongzipo Road, Changsha 410013, Hunan Province, China. xiongkun2001@163.com

Abstract

Wounds in diabetic patients, especially diabetic foot ulcers, are more difficult to heal compared with normal wounds and can easily deteriorate, leading to amputation. Common treatments cannot heal diabetic wounds or control their many complications. Growth factors are found to play important roles in regulating complex diabetic wound healing. Different growth factors such as transforming growth factor beta 1, insulin-like growth factor, and vascular endothelial growth factor play different roles in diabetic wound healing. This implies that a therapeutic modality modulating different growth factors to suit wound healing can significantly improve the treatment of diabetic wounds. Further, some current treatments have been shown to promote the healing of diabetic wounds by modulating specific growth factors. The purpose of this study was to discuss the role played by each growth factor in therapeutic approaches so as to stimulate further therapeutic thinking.

Key Words: Growth factor; Skin; Diabetic wound; Therapy; Biomaterial; Delivery system

Core Tip: This review summarizes the main causes of poor wound healing in diabetes and the role of various therapeutically available growth factors in wound healing. In terms of treatment, it summarizes the treatment methods and drug delivery systems of various growth factors, and discusses the therapeutic effects of different methods and the special properties of drug delivery systems. We hope these discussions will provide the basis for more effective treatments, advance growth factor research, and help more people with diabetes heal their wounds.

Citation: Zheng SY, Wan XX, Kambey PA, Luo Y, Hu XM, Liu YF, Shan JQ, Chen YW, Xiong K. Therapeutic role of growth factors in treating diabetic wound. *World J Diabetes* 2023; 14(4): 364-395
URL: https://www.wjgnet.com/1948-9358/full/v14/i4/364.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i4.364

INTRODUCTION

The prevalence of diabetes continues to increase at an alarming rate worldwide[1,2]. According to a recent analysis, the global prevalence of diabetes among adults aged 20-79 years is currently at 536.6 million and is projected to rise to 783.2 million by 2045[3]. As of 2015, diabetes was a direct cause of death for about 1.5 million people worldwide (WHO. Accessible at http://www.who.int/diabetes/en, accessed on 26 November 2022). Complications such as cardiovascular disease, nephropathy, retinopathy, neuropathy, and diabetic wounds occur in patients with diabetes. Diabetic wounds are one of the consequences having a lasting impact on patients with diabetes. Diabetic foot ulcer (DFU) is the most common type of diabetic wounds, which has a recurrence rate of 30%-50%[4,5]. Currently, no effective means of foreseeing the development of diabetic sores exist. Thus, the primary goals of treating diabetic wounds include identifying them early, performing a thorough examination, debriding and cleansing the wounds, and preventing or controlling the spread of infection.

One of the trickiest aspects of managing diabetes is dealing with wounds. Normal wound care is insufficient for diabetic wounds due to the differences in blood composition, vascular development, nerve survival, and inflammatory processes[6,7]. Many therapies have been developed for diabetic wounds in recent years[4,8], but those involving growth factors (GFs) have gained the maximum attention. GFs govern most of the processes involved in wound healing[9]. Although several GFs have been shown to be useful in treating diabetic wounds, only a few have been authorized for use in clinical practice. Among the three GF products available, only Regal Maltose has been approved by the Food and Drug Administration for treating neuropathic diabetic ulcers. The limitations in trial design, poor patient compliance, risk of immunogenicity, protein degradation, and variation in the responsiveness and healing support supplied by surrounding tissues are only a few of the many obstacles that stand in the way of the therapeutic application of GF products. It is essential, therefore, to have a firm grasp on the specifics of diabetic wounds, the healing effects of various GFs, and the provision of a dependable and efficient GF delivery system to propose a GF therapy.

In this review, we summarized the major factors contributing to impaired wound healing in patients with diabetes, and the significance of several GFs currently available for therapeutic use. We also conducted illustrative GF treatment experiments to explore various delivery mechanisms and facilitate an understanding about the therapeutic effects of various strategies.

WHY IS THE HEALING OF DIABETIC WOUNDS DIFFICULT?

Vascular complications

Intermittent claudication, ischemia-induced rest discomfort, skin ulcers, and avascular necrosis are all symptoms of peripheral arterial disease (PAD), a group of disorders caused by arterial stenosis distal to the aortic arch. A strong correlation exists between diabetes and PAD; however, determining the true frequency of PAD in patients with diabetes is challenging due to the many complicating factors[10]. One cross-sectional study indicated that 43.87% of patients with DFU also had peripheral artery disease[11]. The most immediate effect of PAD, whether it affects local micro or macro vessels, is a disruption in blood flow or even ischemia[12]. Further, a number of negative consequences occur due to microcirculatory dysfunction. The microcirculation of patients with diabetes differs from that of patients without

diabetes in several important ways, including increased vascular permeability, poor autoregulation, and unresponsiveness to vasodilatory stimuli[10,13]. The endothelial dysfunction due to hyperinsulinemia or hyperglycemia is the primary cause of these characteristics[14,15]. Capillary damage and oxidative stress are two complications of diabetes that can be exacerbated by microcirculatory perfusion problems [16].

Tissues surrounding diabetic wounds may suffer from hypoxia and anemia due to circulatory abnormalities triggered by PAD. The activity and gene expression in cells of injured tissues may be affected by hypoxia[17]. The combination of vascular damage and increased tissue oxygen consumption can lead to hypoxia in diabetic wounds, just as it does in regular wounds. However, hypoxia triggers hypoxia-inducible factor-1 (HIF-1) to enhance wound repair in healthy individuals, but hyperglycemia inhibits HIF-1 and hence slows wound healing in patients with diabetes[18-20]. Since hypoxia is detrimental to healing rather than serving as positive feedback that accelerates diabetic wound closure, it is no longer a factor in promoting wound closure. Lower oxygen levels in DFU were linked to slower wound healing in a study on flow-mediated skin fluorescence monitoring[21]. Furthermore, anemia can inhibit the healing process by inhibiting the metabolic pathways in injured tissues. Patients with diabetes often suffer from anemia, and those with severe foot ulcers are particularly at risk[22-24]. Patients with severe anemia have a higher likelihood of experiencing adverse malignant outcomes, according to a number of studies. These outcomes include a more severe disease-free interval, a more severe infection, and even death[23,25-27]. The impact of anemia on DFU is still debatable, as some studies have shown that it is not significantly linked to the severity or prognosis of DFU[28,29].

Moreover, diabetic wounds frequently exhibit impaired angiogenesis, which results in reduced vascularity and capillary density[30]. Inhibiting the death of important cells in damaged tissue, providing proliferative support activity, facilitating the remodeling phase of repair, and promoting healing growth are all facilitated by oxygen and nutrients provided by angiogenesis[31-33]. However, in diabetic wounds, many factors that promote angiogenesis are disrupted due to hyperglycemia and chronic inflammation, for example, the release of vascular endothelial growth factor (VEGF)[34] and platelet-derived growth factor (PDGF)[35] and the composition ratio of Ang1/Ang2/Tie2 complex[36, 37]. Moreover, the unique internal environment of diabetes may also contribute to the effects of various anti-angiogenic factors, such as the anti-angiogenic factor pigment epithelium-derived factor[38]. These factors interfere with inflammation-mediated angiogenesis and delay the transition from inflammation to proliferative remodeling in wound healing.

Hyperglycemia

Hyperglycemia can delay the healing of diabetic wounds and even exacerbate DFU through the impaired function of various skin cells and peripheral neuropathy. In patients with diabetes, hyperglycemia is an important factor causing dysfunction or reduction of endothelial cells[39-44], which are essential for the healing of diabetic wounds[45,46]. Further, hyperglycemia affects protein synthesis and migration and proliferation of keratinocytes and fibroblasts, which disrupts important processes of re-epithelialization[47-49], for instance, the altered expression of cytoskeletal keratin proteins (K2/K6/K10) and a laminin-5 α3 chain precursor protein (LM-3A32) in DFU keratinocytes[50]. Also, the fibroblasts from DFU exhibit morphological changes, GF energy, extracellular matrix (ECM) deposition, and reduced proliferation and migration of fibroblasts[51-54]. In the pathogenesis of neuropathy, hyperglycemia can damage nerves through the polyol pathway, hexosamine pathway, oxidative stress, advanced glycation end-products (AGEs) pathway, PARP pathway, NF-κB pathway, and so forth[55].

Hyperglycemia can also induce a delay in diabetic wound healing *via* free radicals or reactive oxygen species (ROS). In patients with diabetes, hyperglycemia can induce excessive ROS production through several pathways[56]: (1) Reactions in mitochondria[57,58]; (2) impairment of intracellular antioxidative defense systems[59,60]; (3) glycosylation and subsequent signal transduction[61,62]; (4) lipid peroxidation[55,63]; (5) activation of free radical generator enzymes[64,65]; (6) polyol pathway[66]; (7) protein kinase C pathway[67,68]; and (8) hexosamine pathway[69]. These pathways have been verified in patients with diabetes and are abnormally active and hence disrupt the metabolism of ROS in hyperglycemia. Although the presence of ROS can, sometimes, promote wound healing (*e.g.*, bacterially infected wounds)[70,71], excessive release of ROS can lead to cell and tissue damage and delayed wound healing in DFU[72].

Neuropathy

More than 90% of patients with DFU also have diabetic neuropathy[73]. The most common types of neuropathy are sensory, motor, and autonomic neuropathies of the periphery[74]. Diabetes can cause neuropathy in numerous ways, the most common of which are: (1) Autoimmunity; (2) microvascular dysfunction; and (3) various humoral variables (hyperglycemia, hyperinsulinemia, and so on)[75-79].

An important risk factor for wound formation in neuropathy is the deterioration of subjective sensation[80-82]. The selective targeting of C and Aδ fibers by neuropathy in diabetes can lead to neuropathic pain and/or sensory loss[83]. Studies have shown reduced cutaneous innervation in the biopsies of patients with diabetes based on reduced immunoreactivity of protein gene product 9.5 (PGP9.5) (detecting sensory neurons) and various neuropeptides, specifically calcitonin gene-related peptide, substance P (SP), and neuropeptide Y[84,85]. Reduced nerve density, a more fragmented distri-

bution across the dermis[86,87], and reduced nerve afferents in the epidermis[88-91] and dermal papillae[92] are found in the skin of patients with diabetes, even in the absence of clinically detectable sensory neuropathy[93]. Moreover, patients with diabetes may show a significant reduction in amplitude and nerve conduction velocity associated with nerve fiber loss[94]. Patients may have trouble deciphering the severity of their sores or ulcers in the limbs, especially if their pain threshold has been drastically lowered[95]. These variables increase the likelihood of diabetic wound development and may also contribute to the progression of existing wounds. The upper-body paralysis from autonomic neuropathy reduces perspiration production, leading to dry, cracked skin that can increase susceptibility to irritation and infection while slowing the healing process[72,78]. In dull pain, the increased pressure on the plantar surface of the foot caused by motor neuropathy can cause ischemia and possibly death of tissues in the affected area[78].

Skin innervation is important for normal wound healing and can impact wound healing processes such as keratinocyte proliferation[96], wound re-epithelialization[97], wound contraction[98], and production of granulation tissue[99]. However, in diabetic wounds, neuropathy can impede these steps, delaying the healing of wounds[100]. Further, SP stimulates leukocyte chemotaxis to promote wound healing during the inflammatory phase of a wound[101]. However, reduced SP levels in denervated tissues in patients with diabetes may lead to delayed wound healing[102,103]. Denervation leads to delayed protein extravasation and cell migration[104,105]. Animals exposed to capsaicin have no vasodilation and plasma protein extravasation at the time of injury[106]. A similar delay in inflammatory cell migration was observed in mice with diabetes[107-109]. Chemical and surgical denervation can reduce small nerve fibers in the skin by at least 70%, which leads to poor wound repair[110]. The reduction in skin sensory nerves by subcutaneous injection of capsaicin in mice and rats without diabetes delayed re-epithelialization, reduced epidermal stem cell migration, and inhibited angiogenesis and VEGF expression[111-113].

In fact, many types of neuropathy are complicated by diabetes, and not every neuropathy affects the efficiency of wound healing[78,114]. The neuropathy discussed in this study refers to the ubiquitous disorders of the cutaneous nerves and dysregulation of neuropeptide secretion.

Microbial infection

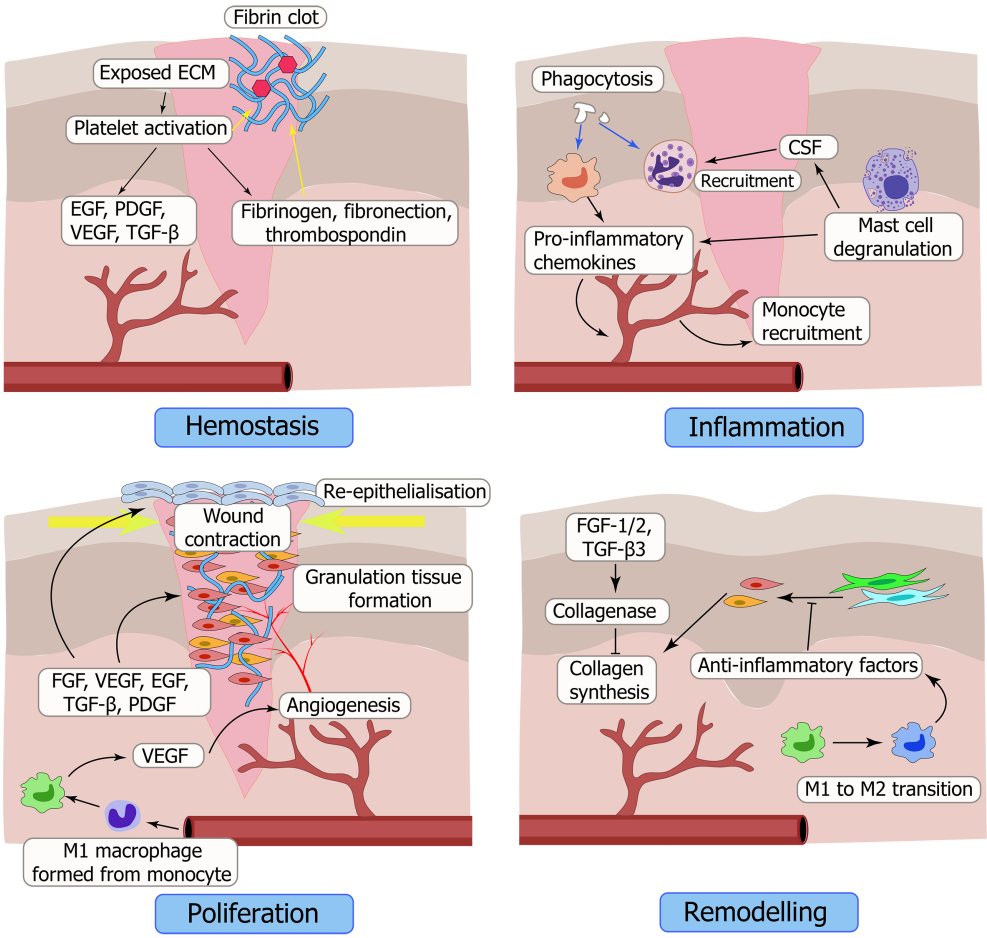
The wounds in patients with diabetes are highly susceptible to microbial invasion, often leading to life-threatening infections that delay wound closure. The damage of the skin barrier, such as increased trans epidermal water loss and decreased secretion of antimicrobial peptides[115,116], has been linked to an increased risk of infection. Neuropathy and chronic inflammation have been proposed as possible causes of this damage[72,117]. Furthermore, the microbial composition of patients with diabetes differs from that of healthy individuals[118-120]. *Staphylococcus aureus* and *S. epidermidis*, for example, are more likely to colonize the skin of patients with diabetes[119,120]. Diabetic wounds have a more restricted microbiome than healthy skin and are home to bacteria such as *Klebsiella* sp., *Abiotrophia* sp., *Escherichia coli*, and *Peptoniphilus* sp.[121-123]. Notably, *S. aureus* and *Streptococcus genera* predominate among the harmful microorganisms in infected wounds[124,125]. The increased release of pro-inflammatory cytokines and a prolonged inflammatory phase due to bacteria and endotoxins in an infected wound are two factors that prevent the wounds from healing[126]. Additionally, the pathogenic bacteria or their secretions continuously damage the wound's tissue and cells, slowing the healing process.

Inflammation

Unlike nondiabetic acute wounds, DFUs have a nonlytic inflammatory phase, with numerous neutrophils and macrophages identified in the wound[127-129] and persistent release of pro-inflammatory cytokines such as leukocyte interleukin (IL)-1, tumor necrosis factor-α (TNF-α), plasma C-reactive protein, and others[128,130,131]. Poor phagocytic activity and dysfunctional leukocytes are also common in patients with diabetes[132-134]. However, in DFU, M1 macrophages continue to dominate the wound milieu and perpetuate inflammation, whereas, in normal wounds, M2 macrophages (promote tissue repair) progressively replace M1 macrophages (promote inflammation)[135-137]. Because of the ongoing inflammatory response, neutrophils remain activated and secrete proteases, which indiscriminately destroy the wound microenvironment[138]. However, inflammation can stifle angiogenesis by limiting VEGF production[139,140]. Diabetic wounds cannot heal properly because chronic inflammation continues to cause harm to tissue cells even after the remodeling phase has begun [109].

Diabetic wound healing requires specific treatment

Normal wound healing can be divided into four stages: hemostasis, inflammation, hyperplasia and remodeling (Figure 1). But for diabetic wounds, the injuries, infections, and other consequences can all be exacerbated by diabetes, which slows recovery time[141] (Figure 2). Excessive formation of AGEs, insufficient neovascularization, insufficient concentration of GFs, imbalance between metabolism and nutrient delivery, abnormal regulation of gene expression, and impaired vascularization are just some of the factors making the healing of diabetic wounds difficult[142]. Therefore, common treatment measures cannot effectively improve the condition of diabetic wounds. Currently, many treatments exist for diabetic wounds, such as oxygen therapy, negative-pressure wound therapy, platelet-rich



DOI: 10.4239/wjd.v14.i4.364

Figure 1 The wound healing process is generally divided into four stages: Hemostasis, inflammation, hyperplasia and reconstruction. During the hemostatic stage, platelets will come into contact with collagen, resulting in platelet activation and aggregation; central thrombin triggers the formation of a fibrin network; the fibrin network strengthens platelet aggregation into a stable clot, effectively preventing bleeding. During the inflammatory phase, neutrophils and macrophages enter the wound to destroy bacteria and remove debris, and secrete growth factors, inflammatory factors, and chemokines that attract immune system cells to the wound to promote tissue repair. During the hyperplastic phase, granulation tissue grows and fills the wound, new blood vessels form, the wound shrinks, and epithelial cells gradually cover the surface of the wound. Finally, it enters the reconstruction stage, the fibrin of the granulation tissue gradually arranges regularly under the action of the pulling force, the epidermis gradually matures, M1 macrophages gradually transform into M2 macrophages, and inflammation and collagen synthesis are gradually inhibited.

plasma, stem cells, and cell- and tissue-based products[4,143-150]. Among these, GF therapy has been regarded as an important means to treat diabetic wounds due to its ability to participate in promoting various stages of healing.

ROLE OF GFS IN THE HEALING OF DIABETIC WOUNDS

GFs execute an important role in impaired wound healing, especially in diabetic wounds. They affect many processes, such as the growth and movement of different types of cells, endothelial cell stimulation, angiogenesis, fibroblast chemotaxis, and changes in inflammatory cells. GFs that accelerate and promote wound healing through their physiological effects mainly include VEGF, PDGF, epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor β (TGF-β), hepatocyte growth factor (HGF), and so forth (Table 1)[108,151-197].

VEGF

The VEGF family consists of a variety of GFs, among which VEGF-A and VEGF-C are mainly involved in wound healing[161]. VEGF-A is produced by endothelial cells, keratinocytes, fibroblasts, smooth muscle cells, platelets, neutrophils, and macrophages[198-200]. It binds to the tyrosine kinase surface receptors Flt-1 (VEGF receptor 1) and kinase insert region receptor (KDR) (VEGF receptor 2) located on the endothelial surface of blood vessels[201-203]. By acting on these receptors, VEGF-A can participate in the chemotaxis of endothelial cells and promote endothelial cell proliferation, differentiation, and

Table 1 Main growth factors in wound healing

Type of GF	Source cells	Target cells	Receptor/signaling protein	Involved wound healing process	Acute wound	Chronic wound	Ref.
VEGF	Keratinocytes, fibroblasts, macrophages, endothelial cells	Endothelial cells, macrophages	ICAM-1, VCAM-1, PLCγ/PKC/Ras/Raf/MEK/ERK	Inflammation, angiogenesis	Increased	Decreased	[151-158]
TGF-β	Fibroblasts, keratinocytes, macrophages, platelets	Fibroblasts, keratinocytes, macrophages, leukocytes, endothelial cells	TGF-βRI-II, Smad 2-4, α-SMA, MAPK, integrins	Inflammation, angiogenesis, granulation tissue formation, collagen synthesis, matrix formation and remodeling, leukocyte chemotactic function	Increased	Decreased	[157, 159-174]
PDGF	Platelets	Leukocytes, macrophages, fibroblasts	PDGFR, Ras/Erk1/2/MAPK, PI3K	Inflammation, re-epithelialization, collagen deposition, tissue remodelling	Increased	Decreased	[108, 157, 175, 176]
HGF	Fibroblasts	Endothelial cells, keratinocytes	c-Met, ERK1/2, Akt, PAK-1/2, Gab1	Suppression of inflammation, granulation tissue formation, angiogenesis, re-epithelialization	-	-	[155, 176-180]
bFGF	Keratinocytes, fibroblasts, endothelial cells	Keratinocytes, fibroblasts, endothelial cells	ERK2	Angiogenesis, granulation tissue formation	Increased	Decreased	[108, 175, 181-184]
FGF-7, FGF-10	Fibroblasts, keratinocytes	Keratinocytes	Peroxiredoxin-6, Nrf2, Nrf3	Re-epithelialization, detoxification of ROS	-	-	[185-188]
EGF, HB-EGF, TGF-α	Platelets, macrophages, keratinocytes	Fibroblasts, endothelial cells, keratinocytes	EGFR, STAT3, AP1, PI3K, ERK	Tissue formation, re-epithelialization	Increased	Decreased	[189-197]

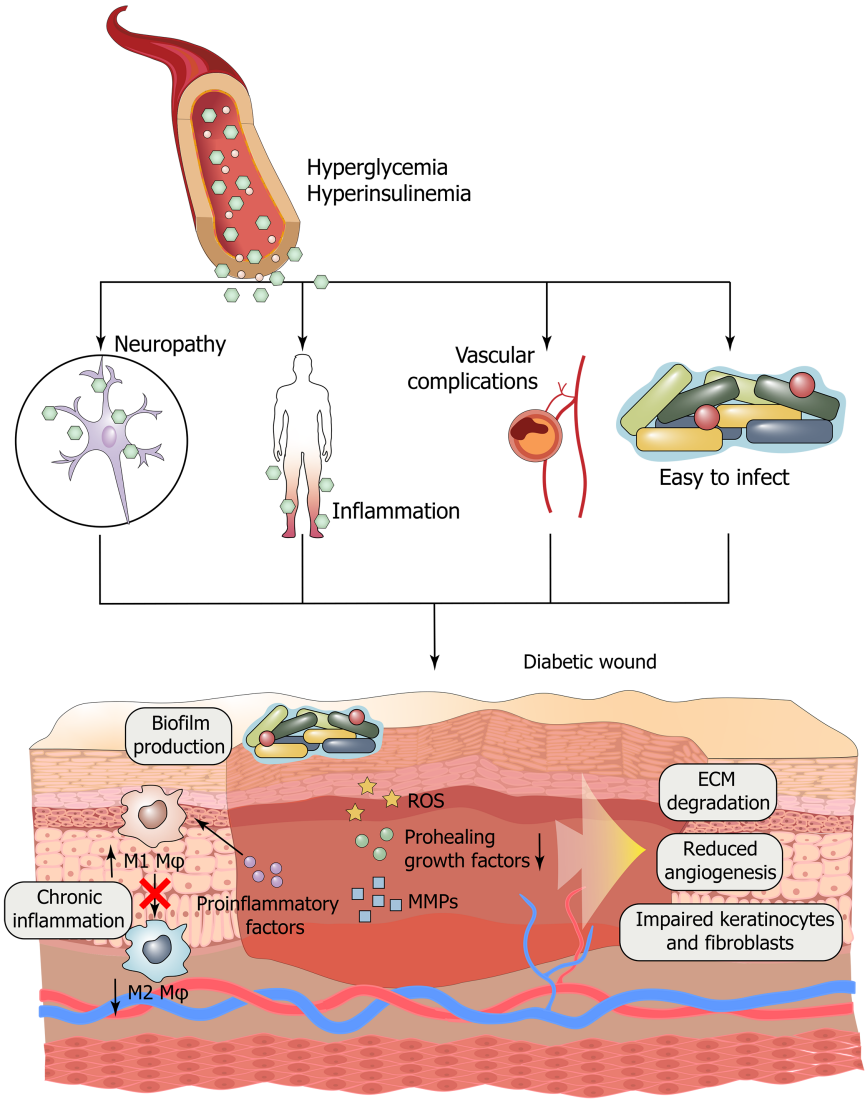
ICAM-1: Intercellular adhesion molecule 1; VCAM-1: Vascular cell adhesion molecule 1; PLC: Phospholipase C; PKC: Protein kinase C; α-SMA: α smooth muscle actin; MAPK: Mitogen-activated protein kinase; PI3K: Phosphoinositide 3-kinase; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; HGF: Hepatocyte growth factor; HB-EGF: heparin-binding epidermal growth factor.

regulation of vascular permeability[204-206]. VEGF-A levels are elevated in nondiabetic wounds[152, 207]. Other GFs that can enhance VEGF-A expression include TGF-β1, EGF, TGF-α, KGF, bFGF, and PDGF-BB[208,209]. VEGF can promote angiogenesis to restore tissue perfusion, re-establish microcirculation, and increase oxygen tension in the wound[153]. In diabetic wounds, VEGF-A can promote early angiogenesis, especially the migration of endothelial cells, and improve the re-epithelialization and granulation tissue formation of diabetic wounds[210,211]. *In vivo*, the wounds in mice with diabetes exhibited accelerated re-epithelialization and contraction of wound area after treatment with VEGF mRNA delivery[212,213]. Many drugs and stem cells promote diabetic wound healing through VEGF [214-216].

VEGF-C is released primarily by macrophages and acts through VEGF receptor 3, which is expressed on lymphatic endothelial cells, pore endothelial cells, and monocytes/macrophages[217-219]. The proteolytically processed mature form of VEGF-C can also bind to KDR in the vascular endothelium to increase vascular permeability[220]. The proteolytically processed mature form of VEGF-C can also bind to KDR in the vascular endothelium to increase vascular permeability[220,221]. The administration of VEGF-C *via* an adenoviral vector to diabetic wounds accelerated healing in animal models of diabetes [222].

PDGF

Many different homologous and heterodimeric GFs exist in the PDGF family. Platelets, macrophages, vascular endothelium, fibroblasts, and keratinocytes are the primary cell types responsible for PDGF production in wounds[223-225]. PDGF is required for the majority of wound healing processes. It is found in wound fluid and secreted from the degranulated plate following injury[176,226]. It promotes the proliferation and migration of inflammatory cells such as neutrophils, fibroblasts, macrophages, and smooth muscle cells[227-229]. Furthermore, it promotes tissue debridement and granulation tissue development *via* macrophages by increasing the production and secretion of GFs such as TGF-β[223]. Further, PDGF plays a crucial role in developing mature blood vessels[230]. It promotes myofibroblast differentiation to rescue delayed wound healing after a diabetic wound penetrates subcutaneously and causes muscle damage[231]. The combination of PDGF-BB, VEGF, and EGF has been shown to increase



DOI: 10.4239/wjd.v14.i4.364

Figure 2 Hyperglycemia and hyperinsulinemia are often the core causes of numerous problems in diabetic individuals. These consequences add to the wound's aberrant features, like prolonged inflammation, poor tissue regeneration, slowed angiogenesis, *etc*. Moreover, the persistent breakdown of the skin barrier makes such wounds extremely susceptible to infection, which further hinders the healing process. Mφ: Macrophages; ECM: Extracellular matrix; ROS: Reactive oxygen species.

cell composition and promote wound healing in diabetic wounds, and the use of PDGF-BB alone has been approved for treating diabetic wounds[232]. PDGF-D was also found to be highly effective when applied to wounds in patients with diabetes and ischemia[233]. PDGF is often used in combination with VEGF, which has a significant positive effect on angiogenesis and recovery in diabetic wounds[234-236]. *In vivo*, PDGF-BB can improve the healing quality of full-thickness excision wounds in rats with diabetes; promote angiogenesis, cell proliferation, and epithelialization; and led to thicker and more organized collagen fiber deposition[237]. For clinical trials, the application of PDGF can significantly reduce the healing rate of diabetic wounds and improve the probability of complete healing[238-241].

EGF

Many members of the EGF family aid in wound healing, such as heparin-binding EGF (HB-EGF) and TGF-α[161]. The binding of these ligands to the EGF receptor (EGFR) causes the receptor to dimerize and autophosphorylate, which in turn triggers the tyrosine phosphorylation of downstream proteins inside the cell[191]. Studies *in vitro* demonstrated that EGFR activation facilitated re-epithelialization by increasing keratinocyte proliferation and migration in wounds[242-246].

The paracrine action of EGF on keratinocytes is primarily mediated by its secretion from platelets, macrophages, and fibroblasts[247]. Wound re-epithelialization and tensile strength were both remarkably improved by post-injury EGF upregulation in nondiabetic patients[195]. However, EGF levels were found to be lower in diabetic wounds, and a majority of EGFRs were found to be trans-located to the cytoplasm rather than localized on the cell membrane[161,197]. The addition of topical

EGF to diabetic wounds has been shown in clinical trials to improve epithelialization and speed up healing[248,249]. Various attempts have been made to load EGF into various delivery systems for treating diabetic wounds[250,251]. *In vivo*, the application of EGF balances collagen distribution, increases granulation formation, and accelerates wound healing[251-253]. In a clinical trial of 68 patients treated with combined EGF and dressings, 52 had diabetic wounds, which healed completely within 2-14 wk with a low recurrence rate[254].

TGF-α is mainly secreted by platelets, keratinocytes, macrophages, fibroblasts, and lymphocytes[255-258]. It has been shown to increase keratinocyte migration and proliferation and induce the expression of K6 and K16[194,259,260]. In *in vivo* studies, TGF-α played a role in early stimulation and maintenance of wound epithelialization in partial-thickness wounds[261]. *In vivo*, TGF-α can be combined with PDGF-BB to make the wound healing speed in mice with diabetes close to that of nondiabetic mice [262]. However, TGF-α has not been applied to the clinical treatment of wounds so far.

HB-EGF is also upregulated in nondiabetic wounds and secreted by keratinocytes[199,263]. HB-EGF can promote re-epithelialization by binding to the EGFR subtypes HER1 and HER472[264,265]. *In vivo*, HB-EGF is thought to play a role in promoting keratinocyte migration, showing its importance in the early stages of re-epithelialization[189]. At present, HB-EGF has been widely regarded as one of the targets for treating skin wounds and carried in various delivery systems[266]. In a rodent diabetic wound model, HB-EGF improved re-epithelialization and increased collagen content and wound contraction *via* a heparin-based cohesive delivery system[267]. *In vivo*, HB-EGF can promote the proliferation and migration of epidermal keratinocytes in full-thickness excision wounds of mice with diabetes and accelerate epithelialization[267].

FGF

FGF family is a cell signaling protein family comprising 23 members. The members of this family mainly involved in skin wound healing are FGF2, FGF7 (or KGF1), and FGF10 (or KGF2). FGF2, the basic FGF, is mainly involved in granulation tissue formation, re-epithelialization, and matrix formation and remodeling in wounds[161,182]. FGF7 and FGF10 stimulate keratinocyte proliferation and migration, promote re-epithelialization, and increase the transcription of factors involved in ROS detoxification [268,269]. FGF2 is deficient in diabetic wounds, and wound closure is accelerated following the topical application of FGF2[183,184].

TGF-β

The TGF-β superfamily comprises many members playing essential roles in development and repair. TGF-β1 and TGF-β2 are significant players in wound repair, and can be potent stimulators of extracellular matrix protein and integrin expression[168,270,271]. TGF-β1 is abundantly released from platelets immediately after injury[272]. Latent TGF-βs in the wound matrix also allow the sustained release of proteolytic enzymes. This combination of different cell sources ensures a continuous supply of TGF-β throughout the repair process[273]. Additionally, some researchers have reported the presence of TGF-βs in wound fluid[274,275]. At the cellular regulatory level, TGF-β has many cellular regulatory functions, such as attracting macrophages and fibroblasts to the wound area to improve healing[276, 277]. Also, TGF-β can promote re-epithelialization mainly by enhancing keratinocyte migration *via* regulatory factor forkhead box-1 after binding to receptors on the cell surface[277,278]. Moreover, studies show the involvement of TGF-β in scar formation, later wound repair, angiogenesis, and granulation tissue formation[163,169,172,273,279]. In diabetic wounds, TGF-β also promotes wound healing[162]. Compared with other reduced GFs, TGF-β showed a lack of upregulation in diabetic wounds, which might be a factor delaying the healing[279,280]. *In vivo*, the TGF-β/Small mothers against decapentaplegic (Smad) pathway is often activated as an important factor in promoting diabetic wound healing, for example, WDR74 and Baicalin[281,282].

HGF

HGF is a GF capable of regulating the growth, motility, and morphogenesis of various types of cells [283-285]. In wounds, HGF is mainly derived from fibroblasts, acts on epithelial cells, keratinocytes, and endothelial cells, and participates in healing processes such as suppression of inflammation, granulation tissue formation, angiogenesis, and re-epithelialization[155,177,179,180]. Although no changes in HGF levels have been reported in diabetic wounds, the delayed healing process appears to be associated with an imbalance in the activation and inactivation of the HGF/c-Met pathway[9,178,286]. Moreover, HGF can assist other GFs in promoting healing in diabetic wounds[287].

Nerve growth factor

Nerve growth factor (NGF) is a neurotrophic factor essential for the development and survival of some sympathetic and sensory neurons in the central and peripheral nervous systems[288]. NGF levels increase when wounds appear. NGF mRNA is detected in newly formed epithelial cells and granulation tissue fibroblasts at the wound edge[289], with exceptionally high expression in granulation tissue myofibroblasts[290]. Additionally, NGF in wounds can also originate from salivary gland secretion and be transported *via* serum[289]. In wound healing, NGF mainly involves keratinocyte proliferation;

proliferation, differentiation, and migration of epidermal stem cells; angiogenesis; fibroplasia; and peripheral nerve regeneration[291-297]. NGF levels are much lower in diabetic wounds and surrounding tissue than in normal skin wounds[298,299]. When NGF was applied explicitly to diabetic wounds, the healing and efficacy rate significantly improved[300,301].

Insulin-like growth factor

Insulin-like growth factors (IGFs) are anti-catabolic and anabolic drugs having two isoforms: IGF-1 and IGF-2[302]. They can regulate the growth and differentiation of cells throughout the body[303]. In normal skin, only a few cells express this protein. However, all epidermal cells and some inflammatory cells were found to produce IGF in the initial 1-3 d after injury[304]. IGF-1 may be involved in granulation formation in wounds, inhibit apoptotic pathways, and attenuate pro-inflammatory cytokine production[142]. In diabetic wounds, the expression of IGFs is markedly decreased and is absent in the basal layer of the epidermis and fibroblasts[305-307].

Connective tissue growth factor

The connective tissue growth factor (CTGF) is a member of the cellular communication network family [308], also known as CCN2. It can stimulate the proliferation and differentiation of fibroblasts in the skin [309]. Further, CTGF is involved in promoting cell adhesion, inflammatory cell chemotaxis, and cell differentiation[310,311]. The specific role of CTGF in nondiabetic wound healing has yet to be definitively concluded. However, the application of recombinant human CTGF in diabetic wounds did show better collagen IV accumulation and macrophage infiltration. Also, it increased α-smooth muscle actin level and healing rate in diabetic wounds compared with nontreated diabetic wounds[312]. In rats with diabetes, individuals treated with CTGF exhibited increased aggregation of type IV collagen, α-smooth muscle actin level, and macrophage infiltration; the rate of diabetic wound healing was also significantly accelerated in these individuals[312].

Colony-stimulating factor

Colony-stimulating factor (CSF) family has many isoforms, but the CSFs involved in wound healing are mainly granulocyte-macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF). GM-CSF primarily stimulates cell proliferation and differentiation in wound healing and stimulates stem cells to produce granulocytes and monocytes[7]. However, the effectiveness of GM-CSF in promoting wound healing appears to be only somewhat recognized at present[313]. G-CSF is mainly involved in the inflammatory process of wounds and is related to the formation of neutrophils[314]. The ability of G-CSF to promote healing and resist infection has been verified in randomized clinical trials of diabetic wounds[315,316].

Prolonged healing results from GF deficiency in diabetic wounds

GFs are essential for healing diabetic wounds, as outlined earlier, because of their pro-healing effects. However, it is challenging to observe normal GF-regulated healing events in nontreated diabetic wounds due to the absence of GFs[157,161,192]. This prevents the healing process of hemostasis, inflammation, granulation tissue formation, wound contraction and re-epithelialization, and remodeling from functioning correctly. Moreover, it further leads to prolonged inflammation, tissue hypoxia, wound infection, and chronic healing[141]. Although this does not imply that the missing GF is the source of the lack of healing function, supplementing the wound with the required GFs has an excellent healing-promoting effect.

GF-RELATED THERAPY FOR DIABETIC WOUND HEALING

The application of exogenous GFs is considered a promising approach for treating diabetic wounds. The reason for using GFs is their ability to stimulate and regulate complex cellular and molecular events to alleviate the specific adverse effects of vascular complications, neuropathy, and inflammation in diabetic wounds, which are essential for good and rapid diabetic wound healing[142,317]. So far, a series of GFs, including PDGF, VEGF, EGF, FGF, TGF-β, KGF, and IGF, has shown the potential to accelerate diabetic wound healing[225,257]. Therefore, introducing appropriate GFs into diabetic wounds effectively promotes chronic healing (Table 2)[237,262,280,318-348].

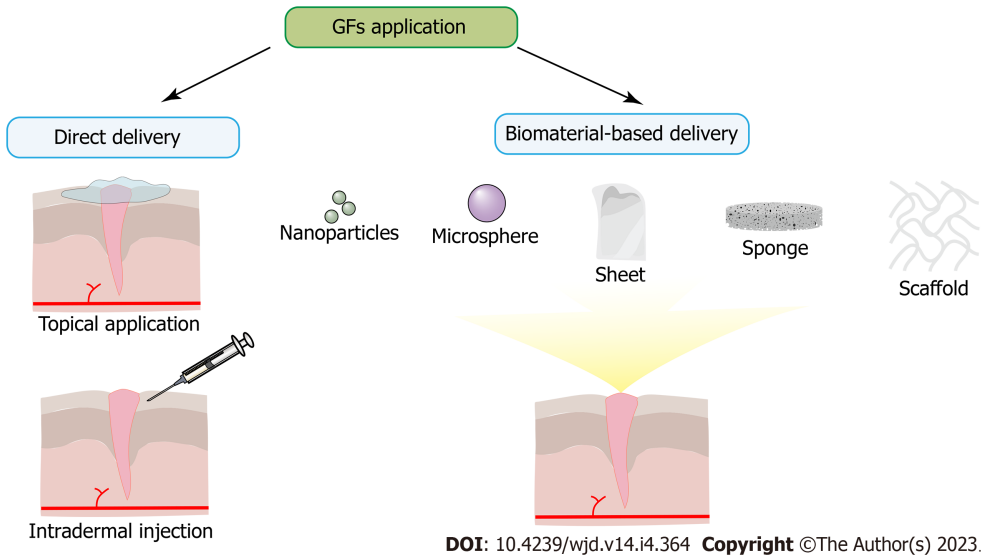
The standard methods of introducing GFs can be divided into direct or biomaterial-based delivery (Figure 3). Direct delivery is achieved *via* topical application or intradermal injection but with only short-term bioactivity due to proteolysis and destabilizing support. For example, a large injection of multiple GFs is insufficient to maintain angiogenesis[349]. Biomaterial-based delivery is achieved by incorporating GFs into ECM-like hydrogels, scaffolds, or particles, which can provide proteolytic protection and structural support to maintain the bioactivity of GFs[141]. Furthermore, the increase in the levels of GFs in diabetic wounds can also be achieved by gene-mediated delivery methods, which can be divided into plasmid DNA delivery, transfection with nonintegrating viral vectors, chemical carrier delivery, and gene-eluting biomaterial constructs[317].

Table 2 Biomaterial systems applied for the delivery of growth factors in diabetic wounds

Therapeutic agents	Delivery system and route	Animal type	Wound size	Response on wound closure	Ref.
PDGF/TGF-α	Gel/topical spraying to wound bed	C57BL/KsJ-db/db mouse	Full-thickness wound measuring 1.5 cm × 1.5 cm	Accelerated wound closure at 15-21 d	[262]
bFGF	Chitosan film/topical using	C57BL/KsJ-db/db mice	Full-thickness wound (1.6 cm diameter)	Reduced wound area and increased ECM formation	[318]
bFGF	Chitosan/hydrogels implant	C57BL/KsJ-db/db mice	Full-thickness wounds (about 100 mm^2)	80% wound closure by 12 d	[319]
pDNA TGF-β1	PEG-PLGA-PEG/hydrogels implant	C57BKS.Cg-m +/+ Leprdb female mice	Full-thickness wounds (7 mm × 7 mm)	Accelerated wound closure at 5 d	[280]
bFGF	Chitosan, hydrogel/topical using	C57BL/KsJ-db/db mice	Full-thickness circular wounds (about 100 mm^2)	Accelerated tissue filling rate of wounds and increased number of CD-34-positive vessels	[320]
PDGF-BB	Carboxymethyl cellulose hydrogel/topical using	C57/Bl6 wild-type mice and lep/r db/db homozygous diabetic mice	Either a 0.6 cm^2, 1.0 cm^2, or 1.5 cm^2 full-thickness area of skin	Accelerated healing by enhanced granulation tissue formationand angiogenesis	[321]
bFGF	Collagen, PGA/porous scaffolds implant	C57BLKS/J Iar- + Leprdb/ + Leprdb	Full-thickness wounds (6 mm diameter	NA	[322]
rhPDGF	Gel/topical spraying to wound bed	Wistar diabetic rats	Full-thickness dermal wounds of 2.54 cm^2 (1.8 cm diameter)	Outstanding re-epithelialization within the first 7 d	[323]
bFGF	Chondroitin-6-sulfate, heparin/hydrogels implant	C57BLKs/J-m1/db, db/db mice, hetero-zygous (m1/db)	Full-thickness wounds (1.6 cm diameter)	89% wound closure by 2 wk	[324]
rhEGF	PCL, PCL-PEG/non-woven mesh (electrospun) implant		Ful-thickness wounds (0.8 cm diameter)	Accelerated wound closure at 7 d	[325]
PDGF	5% polyethylene glycol gel/intradermal injection	Wistar rats	Full-thickness wounds (1.8 cm diameter)	Significant wound improvement within 14 d	[237]
aFGF	Collagen, chitosan/porous scaffolds implant	SD rats	Whole skin layer round wounds (1.8 cm diameter)	Complete healing at 14 d	[326]
rhEGF	PLGA microspheres	SD rats	Full-thickness dermal wounds (2.54 cm^2, 1.9 cm diameter)	90% healing rate on the 14^{th} day	[327]
Collagen-binding domain (CBD)-VEGF	Collagen domain/praye onto the traumatic surface	SD rats	Full-thickness wounds (2 cm × 2.5 cm)	95% healing rate basically reached after 21 d	[328]
rhEGF	PLGA nanoparticles/topical spraying to wound bed	SD rats	Full-thickness dermal wounds (1.8 cm in diameter)	Complete wound closure by 21 d	[329]
bFGF	PELA/non-woven mesh (electrospun) implant	SD rats	Full-thickness circular wounds (about 250 mm^2 each)	Complete wound closure by 3 wk	[330]
rhEGF	Dextrin conjugated/topical using	BKS.Cg-m a/a +/+ Leprdb/J db/db mice	Full-thickness wounds (10 mm × 10 mm)	Accelerated wound closure, neo-dermal tissue formation, increased granulation tissue deposition and angiogenesis	[331]
EGF	Collagen, hyaluronic acid/porous scaffolds implant	BKS.Cg-+Leprdb/+Leprdb (db/db) mice	Full-thickness wounds (15 mm × 20 mm)	N/A	[332]
pDNA bFGF	PELA/electrospun mesh implant	Male SD rats	Full-thickness wounds (about 250 mm^2)	Complete wound closure by 3 wk	[333]
bFGF	Collagen, gelatine/porous scaffolds implant	BKS.Cg- + Leprdb/+ Leprdb/Jcl	8 mm diameter and 3 mm thickness	NA	[334]
VEGF/bFGF	PLGA nps, fibrin/porous scaffolds implant	BKS.Cg-m+/+ Lepr, db/db	Full-thickness dermal wound (0.8 cm in diameter)	85% wound closure at 15 d	[335]
rhEGF	PLGA-alginate microspheres/intralesional	Wistar rats	Full-thickness dermal wound (1 cm in diameter)	90% wound closure at 11 d	[336]

	injection				
rhEGF	Lipid nanocarriers/topical application to wound bed	BKS.Cg-m+/+Lepr 286 db/J	Full-thickness wounds 0.8 cm in diameter	95% wound closure at 15 d	[337]
VEGF, bFGF, EGF, PDGF	Collagen, hyaluronic acid, gelatine nps/non-woven mesh (electrospun) implant	SD rats	Full thickness wound (diameter of 15 mm)	Complete wound closure by 4 wk	[338]
pDNA VEGF	Hyaluronic acid/hydrogels implant	db/db mice	Full-thickness wounds were then generated using a 6 mm biopsy punch (4 mm for wounds on smaller balb/c mice)	Induction of wound closure by day 8-10	[339]
VEGF	PLGA nanoparticles/intradermal injection	db/db mice	Full thickness excisional wounds, two (8 mm diameter) and four (6 mm diameter)	Complete wound closure by 19 d	[340]
VEGF, PDGF	Poly (β-amino esters), poly (acrylic acid), heparan sulfate/woven nylon mesh implant	db/db mice	Full-thickness skin wound	Accelerated wound closure at 14 d	[341]
rhEGF	NaCMCh-rhEGF/hydrogels implant	SD rats	Full-thickness wounds (2 cm diameter, 3.14 cm^2 circular area)	Wound healed in day 15	[342]
rhEGF	PU/porous scaffolds implant	SD rats	Full-thickness wounds (dimensions of 2 cm × 2 cm)	97% wound closure at 21 d	[343]
VEGF	PEG, heparin/hydrogels implant	Cg-m +/+ Leprdb/J (db/db) mice	Full-thickness punch biopsy wound	-	[344]
rhPDGF	PLGA/Non-woven mesh (electrospun) implant	SD rats	Full-thickness excision (8.0 mm in diameter)	Complete wound closure by 14 d	[345]
bFGF	Chitosan, hydrogel + heparin/topical using	C57BL/KsJ-db/db mice	-	Significant angiogenesis and collateral circulation construction	[346]
bFGF	Gelatin hydrogel microspheres/topical injection	C57BL/KsJ-db/db mice	Full-thickness wounds (10 mm in diameter)	Accelerated diabetic skin wound healing and reduced scarring	[347]
bFGF	Acidic gelatin sheet/topical coverage	C57BL/KsJ-db/db mice	Full-thickness wound (1.5 cm × 1.5 cm)	Promoted neoepithelialization, granulation, neovascularization, and wound healing	[348]

VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; rhEGF: Recombinant human epidermal growth factor.



DOI: 10.4239/wjd.v14.i4.364

Figure 3 In the direct delivery system, growth factors are fully exposed to the wound environment and are easily proteolyzed. In the delivery system built by biomaterials, growth factors can persist in the wound to promote healing. GFs: Growth factors.

GF-loaded delivery therapy

This treatment method requires suitable biological materials that maintain the structure and biological activity of GFs, high encapsulation efficiency, and bioavailability, ensuring the complete release of GFs. Moreover, it is necessary to consider the biocompatibility, degradation, and absorption characteristics of the delivery system. Typically, synthetic polymers of the polyester family, such as polyglycolic acid, poly (lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL), or polymers of natural origins, such as collagen, gelatin, fibrin, hyaluronic acid, dextran, alginate, and chitosan, are common delivery materials [141] (Table 3)[318,342,346-348,350-370]. Attempts to improve the feasibility of clinical treatment through innovative delivery systems have attracted much attention (Table 4)[240,241,371-378].

Biodegradable polymer particles are site-specific controlled-release therapeutic systems. Chu *et al* [329] used a double-emulsion method to develop recombinant human EGF (rhEGF)-loaded PLGA nanoparticles and applied them to diabetic wound management. The results showed that the group treated with rhEGF PLGA nanoparticles had the best sustained GF release and the fastest wound healing compared with the group treated with rhEGF or PLGA alone[329]. Another study that used the intradermal route to deliver VEGF-loaded PLGA nanoparticles found that PLGA nanoparticles could sustain VEGF release for 30 d and showed a promoted healing response[340]. Further, another study pointed out that using PLGA-alginate microspheres as the GF carriers could significantly reduce the frequency of administration while maintaining the therapeutic effect[336].

Therapeutic polymer nanofiber mats are new carriers for GF-loaded dressings. This material has the characteristics of high porosity and large surface area to facilitate the penetration of GFs and the circulation of body fluids. rhPDGF-mixed PLGA nanofiber could release rhPDGF for 21 d and significantly induce the complete closure of diabetic wounds in rats with diabetes[345]. The PCL or PCL-polyethylene glycol (PEG) nanofibers implanted with rhEGF also exhibited effective promotion of wound re-epithelialization *via* increasing keratinocyte proliferation and phenotypic expression in diabetic wounds[325]. Hence, the production of carrier materials and loading of various GFs can be adjusted to achieve better wound healing ability[338]. Moreover, a research group experimented with a polymeric fiber mat different from electrospinning, namely a hydrolytically degradable four-layer structure consisting of polyacrylic acid, poly-β-amino acid ester, VEGF or PDGF, and heparan sulfate. In this structure, GFs could be stacked between each layer of materials to promote the complementary effects on wound healing[341].

The three-dimensional biomaterial is a typical structure used to make dressings with GFs. Both sponges and foams are used as standard wound dressings due to their high absorbency and permeability to moisture and oxygen[379]. Collagen is a new material that can be used to develop biomimetic scaffolds. The collagen base can act as a scaffold and bind other natural polymers, such as gelatin, hyaluronic acid, and chitosan, or other synthetic materials[322,36,332,334,380]. Collagen combined with other polymers to generate composite scaffolds can provide resistance to collagenase digestion and sustained slow release of GFs[326]. Further, a study showed that hydrophilic polyurethane (PU) formed by the copolymerization of polyethylene glycol can serve as a dressing material with good moisture conditions in the wound bed [water vapor transmission rate of approximately 3000 g/(m^2 × day)]. The PU dressing loaded with rhEGF sustained the release of rhEGF for 7 d and eventually promoted re-epithelialization and complete recovery of diabetic wounds in rats[343].

GF gene-targeted therapeutic delivery

Gene-mediated therapeutic delivery at diabetic wounds is primarily the local transfection of therapeutic transgenes or complementary DNA into cells to increase the transcription of GF messenger RNAs and maintain high concentrations of local GFs[381] (Table 5)[162,222,382-393].

Naked plasmids are the most basic vector form that can accommodate large amounts of genomic DNA. Early research on diabetic wound therapy focused on forcing pDNA into cells by intradermal injection, with higher pDNA infusions achievable with the aid of electroporation[162,382-384,394]. Furthermore, Yoon *et al*[385] used an ultrasonic microbubble agent (SonoVue) to assist in the ultrasonic puncture delivery of VEGF165-encoded microplastics in diabetic wounds of mice. Their results showed significantly increased cutaneous blood perfusion, accelerated wound closure, and complete recovery of normal wound tissue in mice undergoing ultrasonic portion[385].

Viral vector transfection has excellent freedom of improvement and can efficiently integrate *GF* genes into wound cells for expression, thus having significant advantages in delivering therapeutic genes. The most commonly used viral vectors during diabetic wound care include lentivirus (LV), adenovirus (AV), and adeno-associated virus (AAV). The transfection of the *VEGF*^{165} gene with replication-defective AV has been reported to induce and accelerate early wound healing responses, including angiogenesis and granulation tissue formation, in mice with diabetes[387,386]. Furthermore, Galeano *et al*[390] found that viral transfection could release VEGF in diabetic wounds for 4 mo (even after wound healing). Furthermore, de Felipe proposed that a single viral vector capable of transfecting multiple genes could be used for treatment to address the issue that the transfection of a single GF or GF isoform gene only activated a single corresponding signaling pathway rather than promoting multiple stages of wound healing[395]. Jazwa *et al*[391] demonstrated the simultaneous delivery of *VEGF-A* and *FGF4* genes *via* bicistronic AAV, and the results showed that the therapeutic effect of multiple gene delivery was better

Table 3 Summary of evaluation of the effectiveness of some biomaterial delivery systems

Biomaterial	Forms	GFs	Effects	Ref.
Chitosan	Film	rhEGF	Sustained release *in vitro* for 24 h and extended therapeutic effect	[350]
Chitosan	Film	bFGF	The activity of bFGF remained stable for 21 d at 5 °C, and 86.2% of the activity was maintained at 25 °C	[318]
Chitosan	Hydrogel	EGF	97.3% release after 24 h *in* an *in vitro* study and sustained therapeutic effect	[351]
Chitosan	Hydrogel	bFGF	Significant angiogenesis and collateral circulation construction after addition of heparin in chitosan-bFGF system	[346]
CMC-Chitosan	Hydrogel (as the carrier of NaCMCh-rhEGF nanoparticle)	rhEGF	*In vitro* results indicated that the conjugated form exhibited greater stability to proteolysis and also retained EGF therapeutic activity	[342]
CNC-HA-chitosan	Nanoparticle + scaffold	GM-CSF	Proper mechanical properties, high swelling capacity (swelling ratio: 2622.1% ± 35.2%) and controlled release of GM-CSF up to 48 h	[352]
PVA-gelatin-chitosan	Hydrogel	bFGF	*In vitro* release-cumulative over 25 d, non-toxic to fibroblasts	[353]
Chitosan-nanodiamond	Hydrogel	VEGF	3-d sustained release, improved hydrogel mechanical properties and better biocompatibility	[354]
N-carboxymethyl chitosan-alginate	Hydrogel	EGF	12 h sustained release, non-toxic	[355]
CMCS-poly (vinyl alcohol) (PVA)-alginate microspheres	Hydrogel	bFGF	48 h sustained release, high activity for two weeks	[356]
Hyaluronic acid-sulfated glycosaminoglycan-heparin	Hydrogel	bFGF	Remain highly active for 14 d	[357]
Heparin + PEGDA	Hydrogel	bFGF	Remain active over 35 d	[358]
Collagen-transglutaminase	Hydrogel	bFGF	Suitable mechanical properties and biocompatibility, sustained release up to 48 h	[359]
CBD	Collagen membrane	PDGF	Maintain a higher concentration and stronger biological activity of PDGF	[360]
Collagen	Scaffold	VEGF-A	Cross-linking slows the degradation rate of collagen scaffolds and improves the persistent activity of VEGF	[361]
Extracellular matrix protein (INSUREGRAF®)	Scaffold	EGF	8 h sustained release and active	[362]
GTA-collagen sponge	Sponge	rhEGF	Sustained release and activity for about 10 d, no cytotoxicity	[363]
TFA-denatured collagen	Sponge	bFGF	Sustained release for 18 d and remains largely active	[364]
PCL nanofibers (surface coating with collagen type I)	Hydrogel	G-CSF	Accumulative *in vitro* release for 15 d, no cytotoxicity	[365]
Gelatin	Microspheres	VEGF	Sustained release over 14 d	[347]
Gelatin	Sheet	bFGF	Sustained release for about 14 d	[348]
Gelatin	Sponge	EGF	Increased tensile strength	[366]
Gelatin	TEECM + Gelatin hydrogel microspheres	EGF	Cumulative *in vitro* release over 14 d	[367]
EUP polysaccharide, gelatin	Electrospun hydrogel sponge	PDGF-BB	*In vitro* release lasts 48 h	[368]
Fibrin	Hydrogel	VEGF	*In vitro* release lasts 7 d	[369]
Fibrin	Hydrogel	VEGF	Sustained release of VEGF for 15 d	[370]

CBD: Collagen-binding domain; CNC: Cellulose nanocrystal; CMC: Carboxymethyl cellulose; GTA: Glutaraldehyde; TFA: Trifluoroacetic acid; TEECM: Tissue-engineered extracellular matrix; CMCS: Carboxymethyl chitosan; PEGDA: Poly (ethylene glycol) diacrylate; PCL: Chitosan nanoparticles-Poly (ε-

caprolactone); NaCMCh-rhEGF: Sodium carboxymethyl chitosan-recombinant human epidermal growth factor conjugate; VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; rhEGF: Recombinant human epidermal growth factor.

Table 4 Clinical trials of growth factors in diabetic wounds

Therapeutic agents	Delivery system and route	Response on wound closure	Ref.
EGF	Cream	Significantly improve wound healing rates and reduced the risk of amputation	[371]
bFGF	CGS/suture to surrounding skin	Significant wound improvement within 14 d	[372]
PDGF	Topical gel wound dressing	Reduce healing time by 30%	[373]
PDGF	Topical becaplermin gel	Improve wound healing by 35%	[240]
bFGF	0.0005% benzalkonium chloride in saline/spray on the wound	Significantly reduce wound area	[374]
rhVEGF	Methylcellulose gel/apply evenly to wounds and edges	Significantly increase incidence of complete wound healing	[375]
PDGF	Becaplermin gel/topical apply	The incidence of complete closure was significantly increased by 43%	[241]
EGF	Intralesional injection	Reduced wound area and increased re-epithelialization rate	[376]
EGF	Topical spray	Faster healing velocity and higher complete healing rate	[377]
EGF	Topical hydrogel	78% of wounds healed after 30 d	[378]

CGS: Collagen/gelatin spong; rhVEGF: Recombinant human vascular endothelial growth factor; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; FGF: Fibroblast growth factor.

Table 5 Gene delivery vectors applied for the growth factor treatment of diabetic wounds

Therapeutic agents	Delivery system and route	Response on wound closure	Ref.
Plasmid KGF-1	Intradermal injection	Enhanced wound closure at day 9	[382]
Plasmid TGF-β1	Intradermal injection	Complete wound closure by 7 d	[162]
Plasmid TGF-β1	Intradermal injection, Electroporation	Early induction of closure by day 5	[383]
Plasmid KGF-1	Intradermal injection, Electroporation	Enhanced wound closure at day 12	[384]
Minicircle-VEGF	Subcutaneous injection, Sonoporation	Complete wound closure by 12 d	[385]
Adenovirus encoding VEGF	Topical application to wound bed	Complete wound closure by 13 d	[386]
Adenovirus encoding VEGF	Intradermal injection	Complete wound closure by 27 d	[387]
Adenovirus encoding PDGF	Intralesional injection	Residual epithelial gap of 3 mm at day 7	[388]
Adenovirus encoding VEGF□C	Intradermal injection	Complete wound closure by 21 d	[222]
Lentivirus encoding PDGF	Injected into base and wound margin	No effect	[389]
Adeno-associated virus encoding VEGF	Intradermal injection	Complete re-epithelialization at 28 d	[390]
Bicistronic Adeno-associated virus encoding VEGF-A and FGF4	Intradermal injection	Complete wound closure by 17 d	[391]
RGDK□lipopeptide:rhPDGF-B lipoplex	Subcutaneous injection	Complete wound closure by 12 d	[392]
Minicircle VEGF	Subcutaneous injection	Complete wound closure by 12 d	[393]

VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; FGF: Fibroblast growth factor.

than that of single GF.

Substances such as cationic polymers and lipids are emerging chemical carriers due to their ability to form electrostatic complexes with anionic biomolecules such as pDNA[396]. The advantage of this type of chemical carrier is that it can avoid the use of potentially immunogenic viruses, improve the biosta-

bility of pDNA, and facilitate cellular uptake[397]. For example, a single subcutaneous injection of rhPDGF-B loaded with complexing integrin receptor ligand-conjugated lipopeptide or a complex consisting of arginine-grafted dendrimers loaded with minicyclic VEGF can accelerate the induction of complete wound closure in mice with diabetes[392,393].

Gene-eluting biomaterial scaffolds are similar to GF-loaded scaffolds and focus on improving the stability of the vector. Lee *et al*[280] developed a thermosensitive hydrogel synthesized from PEG, PLGA, and PEG that enabled the controlled release of encapsulated plasmids (containing the *TGF-β1* gene) and the acquisition of accelerated re-epithelialization. The customizable properties of hydrogels bridge the gap between conventional gel-based systems and solid scaffolds, and the porosity of hydrogels provides a large area for released polymers to come into contact with infiltrating cells. However, the contribution of angiogenesis in transfected cells to wound closure is insignificant as the release of polymers is thought to be extremely slow[339]. Nevertheless, this characteristic based on the electrostatic interaction of positively charged polymers in an anionic hyaluronic acid hydrogel matrix provides the basis for developing more controllable polymer delivery systems. For instance, Yang *et al*[333] showed in 2012 that the molecular weight and content of PEG in the copolymer matrix could be changed to regulate the release of polymers (plasmid bFGF/polyethyleneimine) from the core of core-sheath emulsion electrospun fibers. However, such an approach appears to be flawed in diabetic wounds. The problem of low cell availability at the wound edge and reduced cell migration may increase the difficulty of regulating transfection efficiency *in vivo* with this system[398-400].

CONCLUSION

Diabetic wounds are encompassed by various factors (*e.g.*, vascular system abnormalities, neuropathy, and inflammatory process stagnation) induced by the underlying disease and various concomitant diseases that impede normal wound healing. Furthermore, GFs that govern numerous healing processes are rarely detected in diabetic wounds compared with normal healing. The effects of GFs are particularly specific and have been shown to be beneficial in addressing the discussed diabetic wound features. As a result, GFs can be regarded as a direct and effective agent in managing and treating diabetic wounds. Nonetheless, it is disheartening that only a handful of products have entered clinical trials thus far[141]. We discussed the peculiarity of diabetic wounds and provided a theoretical basis and potential of GFs in treating diabetic wounds and optimizing therapeutic techniques. Combining GF with other therapies such as stem cell transplant, cytokine therapy, and anti-inflammatory drugs can be a promising treatment for diabetic wounds, albeit extensive studies are warranted to further examine the efficacy of this combination treatment strategy.

FOOTNOTES

Author contributions: Zheng SY were the major contributors to literature reviewing, manuscript writing, and descriptive figures creating; Zheng SY, Luo Y, Liu YF, Shan JQ and Chen YW were the major contributors to screen the literature; Zheng SY and Xiong K were the major contributors to design the study; Hu XM assisted in the literature review and tables editing; Xiong K and Wan XX were major contributors to manuscript revision; Kambey PA was responsible for the content flow and language editing; The final manuscript was read and approved by all authors.

Supported by the National Natural Science Foundation of China, No. 81971891 and No. 82172196; Key Laboratory of Emergency and Trauma (Hainan Medical University) of Ministry of Education, No. KLET-202108; and the College Students' Innovation and Entrepreneurship Project, No. S20210026020013.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: China

ORCID number: Shen-Yuan Zheng 0000-0003-2424-6040; Xin-Xing Wan 0000-0002-6617-0331; Piniel Alphayo Kambey 0000-0003-2436-0943; Yan Luo 0000-0002-5830-1936; Xi-Min Hu 0000-0003-0119-6394; Jia-Qi Shan 0000-0002-8772-3564; Yu-Wei Chen 0000-0002-8721-3687; Kun Xiong 0000-0002-3103-6028.

S-Editor: Gong ZM
L-Editor: A

P-Editor: Gong ZM

REFERENCES

1 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]

2 **Thomas CC**, Philipson LH. Update on diabetes classification. *Med Clin North Am* 2015; **99**: 1-16 [PMID: 25456640 DOI: 10.1016/j.mcna.2014.08.015]

3 **Sun H**, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022; **183**: 109119 [PMID: 34879977 DOI: 10.1016/j.diabres.2021.109119]

4 **Burgess JL**, Wyant WA, Abdo Abujamra B, Kirsner RS, Jozic I. Diabetic Wound-Healing Science. *Medicina (Kaunas)* 2021; **57** [PMID: 34684109 DOI: 10.3390/medicina57101072]

5 **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]

6 **Patel S**, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomed Pharmacother* 2019; **112**: 108615 [PMID: 30784919 DOI: 10.1016/j.biopha.2019.108615]

7 **Sharma P**, Kumar A, Dey AD, Behl T, Chadha S. Stem cells and growth factors-based delivery approaches for chronic wound repair and regeneration: A promise to heal from within. *Life Sci* 2021; **268**: 118932 [PMID: 33400933 DOI: 10.1016/j.lfs.2020.118932]

8 **Okur ME**, Bülbül EÖ, Mutlu G, Eleftheriadou K, Karantas ID, Okur NÜ, Siafaka PI. An Updated Review for the Diabetic Wound Healing Systems. *Curr Drug Targets* 2022; **23**: 393-419 [PMID: 34521324 DOI: 10.2174/1389450122666210914104428]

9 **Behm B**, Babilas P, Landthaler M, Schreml S. Cytokines, chemokines and growth factors in wound healing. *J Eur Acad Dermatol Venereol* 2012; **26**: 812-820 [PMID: 22211801 DOI: 10.1111/j.1468-3083.2011.04415.x]

10 **Yang SL**, Zhu LY, Han R, Sun LL, Li JX, Dou JT. Pathophysiology of peripheral arterial disease in diabetes mellitus. *J Diabetes* 2017; **9**: 133-140 [PMID: 27556728 DOI: 10.1111/1753-0407.12474]

11 **Azhar A**, Basheer M, Abdelgawad MS, Roshdi H, Kamel MF. Prevalence of Peripheral Arterial Disease in Diabetic Foot Ulcer Patients and its Impact in Limb Salvage. *Int J Low Extrem Wounds* 2021; 15347346211027063 [PMID: 34142882 DOI: 10.1177/15347346211027063]

12 **Forsythe RO**, Brownrigg J, Hinchliffe RJ. Peripheral arterial disease and revascularization of the diabetic foot. *Diabetes Obes Metab* 2015; **17**: 435-444 [PMID: 25469642 DOI: 10.1111/dom.12422]

13 **Wyss CR**, Matsen FA 3rd, Simmons CW, Burgess EM. Transcutaneous oxygen tension measurements on limbs of diabetic and nondiabetic patients with peripheral vascular disease. *Surgery* 1984; **95**: 339-346 [PMID: 6701790]

14 **Bakker W**, Eringa EC, Sipkema P, van Hinsbergh VW. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell Tissue Res* 2009; **335**: 165-189 [PMID: 18941783 DOI: 10.1007/s00441-008-0685-6]

15 **Hayden MR**. Endothelial activation and dysfunction in metabolic syndrome, type 2 diabetes and coronavirus disease 2019. *J Int Med Res* 2020; **48**: 300060520939746 [PMID: 32722979 DOI: 10.1177/0300060520939746]

16 **Dos Santos JM**, Tewari S, Mendes RH. The Role of Oxidative Stress in the Development of Diabetes Mellitus and Its Complications. *J Diabetes Res* 2019; **2019**: 4189813 [PMID: 31192263 DOI: 10.1155/2019/4189813]

17 **D'Alessandro S**, Magnavacca A, Perego F, Fumagalli M, Sangiovanni E, Prato M, Dell'Agli M, Basilico N. Effect of

Hypoxia on Gene Expression in Cell Populations Involved in Wound Healing. *Biomed Res Int* 2019; **2019**: 2626374 [PMID: 31534956 DOI: 10.1155/2019/2626374]

18 **Catrina SB**, Okamoto K, Pereira T, Brismar K, Poellinger L. Hyperglycemia regulates hypoxia-inducible factor-1alpha protein stability and function. *Diabetes* 2004; **53**: 3226-3232 [PMID: 15561954 DOI: 10.2337/diabetes.53.12.3226]

19 **Gao W**, Ferguson G, Connell P, Walshe T, Murphy R, Birney YA, O'Brien C, Cahill PA. High glucose concentrations alter hypoxia-induced control of vascular smooth muscle cell growth *via* a HIF-1alpha-dependent pathway. *J Mol Cell Cardiol* 2007; **42**: 609-619 [PMID: 17321542 DOI: 10.1016/j.yjmcc.2006.12.006]

20 **Ruthenborg RJ**, Ban JJ, Wazir A, Takeda N, Kim JW. Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1. *Mol Cells* 2014; **37**: 637-643 [PMID: 24957212 DOI: 10.14348/molcells.2014.0150]

21 **Los-Stegienta A**, Katarzynska J, Borkowska A, Marcinek A, Cypryk K, Gebicki J. Differentiation of Diabetic Foot Ulcers Based on Stimulation of Myogenic Oscillations by Transient Ischemia. *Vasc Health Risk Manag* 2021; **17**: 145-152 [PMID: 33907408 DOI: 10.2147/VHRM.S307366]

22 **Aborajooh E**, Alqaisi TM, Yassin M, Alqpelat E, Abofaraj A, Alrawajih T, Alzoubi H, Abu Lubad M. Diabetic foot ulcer in Southern Jordan: A cross-sectional Study of Clinical and Microbiological Aspects. *Ann Med Surg (Lond)* 2022; **76**: 103552 [PMID: 35495384 DOI: 10.1016/j.amsu.2022.103552]

23 **Gezawa ID**, Ugwu ET, Ezeani I, Adeleye O, Okpe I, Enamino M. Anemia in patients with diabetic foot ulcer and its impact on disease outcome among Nigerians: Results from the MEDFUN study. *PLoS One* 2019; **14**: e0226226 [PMID: 31846473 DOI: 10.1371/journal.pone.0226226]

24 **Shareef AM**, Ahmedani MY, Waris N. Strong association of anemia in people with diabetic foot ulcers (DFUs): Study from a specialist foot care center. *Pak J Med Sci* 2019; **35**: 1216-1220 [PMID: 31488981 DOI: 10.12669/pjms.35.5.1421]

25 **Costa RHR**, Cardoso NA, Procópio RJ, Navarro TP, Dardik A, de Loiola Cisneros L. Diabetic foot ulcer carries high amputation and mortality rates, particularly in the presence of advanced age, peripheral artery disease and anemia. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S583-S587 [PMID: 28465149 DOI: 10.1016/j.dsx.2017.04.008]

26 **Ezeani IU**, Ugwu ET, Adeleye FO, Gezawa ID, Okpe IO, Enamino MI. Determinants of wound healing in patients hospitalized for diabetic foot ulcer: results from the MEDFUN study. *Endocr Regul* 2020; **54**: 207-216 [PMID: 32857716 DOI: 10.2478/enr-2020-0023]

27 **Yammine K**, Hayek F, Assi C. Is there an association between anemia and diabetic foot ulcers? *Wound Repair Regen* 2021; **29**: 432-442 [PMID: 33591644 DOI: 10.1111/wrr.12902]

28 **Kow RY**, Low CL, Ruben JK, Zaharul-Azri MZ, Lim BC. Predictive Factors of Major Lower Extremity Amputations in Diabetic Foot Infections: A Cross-sectional Study at District Hospital in Malaysia. *Malays Orthop J* 2019; **13**: 45-52 [PMID: 31890110 DOI: 10.5704/MOJ.1911.008]

29 **Shabhay A**, Horumpende P, Shabhay Z, Mganga A, Van Baal J, Msuya D, Chilonga K, Chugulu S. Clinical profiles of diabetic foot ulcer patients undergoing major limb amputation at a tertiary care center in North-eastern Tanzania. *BMC Surg* 2021; **21**: 34 [PMID: 33435942 DOI: 10.1186/s12893-021-01051-3]

30 **Dinh T**, Veves A. Microcirculation of the diabetic foot. *Curr Pharm Des* 2005; **11**: 2301-2309 [PMID: 16022669 DOI: 10.2174/1381612054367328]

31 **Li J**, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. *Microsc Res Tech* 2003; **60**: 107-114 [PMID: 12500267 DOI: 10.1002/jemt.10249]

32 **Okonkwo UA**, DiPietro LA. Diabetes and Wound Angiogenesis. *Int J Mol Sci* 2017; **18** [PMID: 28671607 DOI: 10.3390/ijms18071419]

33 **Velnar T**, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009; **37**: 1528-1542 [PMID: 19930861 DOI: 10.1177/147323000903700531]

34 **Seitz O**, Schürmann C, Hermes N, Müller E, Pfeilschifter J, Frank S, Goren I. Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. *Exp Diabetes Res* 2010; **2010**: 476969 [PMID: 21318183 DOI: 10.1155/2010/476969]

35 **Beer HD**, Longaker MT, Werner S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. *J Invest Dermatol* 1997; **109**: 132-138 [PMID: 9242497 DOI: 10.1111/1523-1747.ep12319188]

36 **Isidori AM**, Venneri MA, Fiore D. Angiopoietin-1 and Angiopoietin-2 in metabolic disorders: therapeutic strategies to restore the highs and lows of angiogenesis in diabetes. *J Endocrinol Invest* 2016; **39**: 1235-1246 [PMID: 27344309 DOI: 10.1007/s40618-016-0502-0]

37 **Kämpfer H**, Pfeilschifter J, Frank S. Expressional regulation of angiopoietin-1 and -2 and the tie-1 and -2 receptor tyrosine kinases during cutaneous wound healing: a comparative study of normal and impaired repair. *Lab Invest* 2001; **81**: 361-373 [PMID: 11310829 DOI: 10.1038/Labinvest.3780244]

38 **Qi W**, Yang C, Dai Z, Che D, Feng J, Mao Y, Cheng R, Wang Z, He X, Zhou T, Gu X, Yan L, Yang X, Ma JX, Gao G. High levels of pigment epithelium-derived factor in diabetes impair wound healing through suppression of Wnt signaling. *Diabetes* 2015; **64**: 1407-1419 [PMID: 25368097 DOI: 10.2337/db14-1111]

39 **Chen YH**, Lin SJ, Lin FY, Wu TC, Tsao CR, Huang PH, Liu PL, Chen YL, Chen JW. High glucose impairs early and late endothelial progenitor cells by modifying nitric oxide-related but not oxidative stress-mediated mechanisms. *Diabetes* 2007; **56**: 1559-1568 [PMID: 17389326 DOI: 10.2337/db06-1103]

40 **Hu L**, Dai SC, Luan X, Chen J, Cannavicci A. Dysfunction and Therapeutic Potential of Endothelial Progenitor Cells in Diabetes Mellitus. *J Clin Med Res* 2018; **10**: 752-757 [PMID: 30214646 DOI: 10.14740/jocmr3581w]

41 **Kränkel N**, Adams V, Linke A, Gielen S, Erbs S, Lenk K, Schuler G, Hambrecht R. Hyperglycemia reduces survival and impairs function of circulating blood-derived progenitor cells. *Arterioscler Thromb Vasc Biol* 2005; **25**: 698-703 [PMID: 15662022 DOI: 10.1161/01.ATV.0000156401.04325.8f]

42 **Loomans CJ**, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 2004; **53**: 195-199 [PMID: 14693715 DOI: 10.2337/diabetes.53.1.195]

43 **Tepper OM**, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial

progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002; **106**: 2781-2786 [PMID: 12451003 DOI: 10.1161/01.cir.0000039526.42991.93]

44 **van Ark J**, Moser J, Lexis CP, Bekkema F, Pop I, van der Horst IC, Zeebregts CJ, van Goor H, Wolffenbuttel BH, Hillebrands JL. Type 2 diabetes mellitus is associated with an imbalance in circulating endothelial and smooth muscle progenitor cell numbers. *Diabetologia* 2012; **55**: 2501-2512 [PMID: 22648662 DOI: 10.1007/s00125-012-2590-5]

45 **Pyšná A**, Bém R, Němcová A, Fejfarová V, Jirkovská A, Hazdrová J, Jude EB, Dubský M. Endothelial Progenitor Cells Biology in Diabetes Mellitus and Peripheral Arterial Disease and their Therapeutic Potential. *Stem Cell Rev Rep* 2019; **15**: 157-165 [PMID: 30413930 DOI: 10.1007/s12015-018-9863-4]

46 **Yu JQ**, Liu XF, Chin LK, Liu AQ, Luo KQ. Study of endothelial cell apoptosis using fluorescence resonance energy transfer (FRET) biosensor cell line with hemodynamic microfluidic chip system. *Lab Chip* 2013; **13**: 2693-2700 [PMID: 23620256 DOI: 10.1039/c3lc50105a]

47 **Andrade TAM**, Masson-Meyers DS, Caetano GF, Terra VA, Ovidio PP, Jordão-Júnior AA, Frade MAC. Skin changes in streptozotocin-induced diabetic rats. *Biochem Biophys Res Commun* 2017; **490**: 1154-1161 [PMID: 28668393 DOI: 10.1016/j.bbrc.2017.06.166]

48 **Kim JH**, Yoon NY, Kim DH, Jung M, Jun M, Park HY, Chung CH, Lee K, Kim S, Park CS, Liu KH, Choi EH. Impaired permeability and antimicrobial barriers in type 2 diabetes skin are linked to increased serum levels of advanced glycation end-product. *Exp Dermatol* 2018; **27**: 815-823 [PMID: 29151267 DOI: 10.1111/exd.13466]

49 **Lima AL**, Illing T, Schliemann S, Elsner P. Cutaneous Manifestations of Diabetes Mellitus: A Review. *Am J Clin Dermatol* 2017; **18**: 541-553 [PMID: 28374407 DOI: 10.1007/s40257-017-0275-z]

50 **Blakytny R**, Jude EB. Altered molecular mechanisms of diabetic foot ulcers. *Int J Low Extrem Wounds* 2009; **8**: 95-104 [PMID: 19443898 DOI: 10.1177/1534734609337151]

51 **Berlanga-Acosta J**, Mendoza-Mari Y, Martínez MD, Valdés-Perez C, Ojalvo AG, Armstrong DG. Expression of cell proliferation cycle negative regulators in fibroblasts of an ischemic diabetic foot ulcer. A clinical case report. *Int Wound J* 2013; **10**: 232-236 [PMID: 23194110 DOI: 10.1111/j.1742-481X.2012.12000.x]

52 **Liang L**, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, Smith A, Yanez V, Veves A, Kirsner RS, Garlick JA, Tomic-Canic M. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. *Wound Repair Regen* 2016; **24**: 943-953 [PMID: 27607190 DOI: 10.1111/wrr.12470]

53 **Maione AG**, Brudno Y, Stojadinovic O, Park LK, Smith A, Tellechea A, Leal EC, Kearney CJ, Veves A, Tomic-Canic M, Mooney DJ, Garlick JA. Three-dimensional human tissue models that incorporate diabetic foot ulcer-derived fibroblasts mimic *in vivo* features of chronic wounds. *Tissue Eng Part C Methods* 2015; **21**: 499-508 [PMID: 25343343 DOI: 10.1089/ten.TEC.2014.0414]

54 **Maione AG**, Smith A, Kashpur O, Yanez V, Knight E, Mooney DJ, Veves A, Tomic-Canic M, Garlick JA. Altered ECM deposition by diabetic foot ulcer-derived fibroblasts implicates fibronectin in chronic wound repair. *Wound Repair Regen* 2016; **24**: 630-643 [PMID: 27102877 DOI: 10.1111/wrr.12437]

55 **Dewanjee S**, Das S, Das AK, Bhattacharjee N, Dihingia A, Dua TK, Kalita J, Manna P. Molecular mechanism of diabetic neuropathy and its pharmacotherapeutic targets. *Eur J Pharmacol* 2018; **833**: 472-523 [PMID: 29966615 DOI: 10.1016/j.ejphar.2018.06.034]

56 **Yaribeygi H**, Atkin SL, Sahebkar A. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. *J Cell Physiol* 2019; **234**: 1300-1312 [PMID: 30146696 DOI: 10.1002/jcp.27164]

57 **Fakhruddin S**, Alanazi W, Jackson KE. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. *J Diabetes Res* 2017; **2017**: 8379327 [PMID: 28164134 DOI: 10.1155/2017/8379327]

58 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723 DOI: 10.1161/CIRCRESAHA.110.223545]

59 **Shailey S**, Basir SF. Strengthening of antioxidant defense by Azadirachta indica in alloxan-diabetic rat tissues. *J Ayurveda Integr Med* 2012; **3**: 130-135 [PMID: 23125509 DOI: 10.4103/0975-9476.100174]

60 **Sindhu RK**, Koo JR, Roberts CK, Vaziri ND. Dysregulation of hepatic superoxide dismutase, catalase and glutathione peroxidase in diabetes: response to insulin and antioxidant therapies. *Clin Exp Hypertens* 2004; **26**: 43-53 [PMID: 15000296 DOI: 10.1081/ceh-120027330]

61 **Goldin A**, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; **114**: 597-605 [PMID: 16894049 DOI: 10.1161/CIRCULATIONAHA.106.621854]

62 **Park HY**, Kim JH, Jung M, Chung CH, Hasham R, Park CS, Choi EH. A long-standing hyperglycaemic condition impairs skin barrier by accelerating skin ageing process. *Exp Dermatol* 2011; **20**: 969-974 [PMID: 22017743 DOI: 10.1111/j.1600-0625.2011.01364.x]

63 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI21625]

64 **Lee HJ**, Lee DY, Mariappan MM, Feliers D, Ghosh-Choudhury G, Abboud HE, Gorin Y, Kasinath BS. Hydrogen sulfide inhibits high glucose-induced NADPH oxidase 4 expression and matrix increase by recruiting inducible nitric oxide synthase in kidney proximal tubular epithelial cells. *J Biol Chem* 2017; **292**: 5665-5675 [PMID: 28188286 DOI: 10.1074/jbc.M116.766758]

65 **Liu J**, Wang C, Liu F, Lu Y, Cheng J. Metabonomics revealed xanthine oxidase-induced oxidative stress and inflammation in the pathogenesis of diabetic nephropathy. *Anal Bioanal Chem* 2015; **407**: 2569-2579 [PMID: 25636229 DOI: 10.1007/s00216-015-8481-0]

66 **Chung SS**, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. *J Am Soc Nephrol* 2003; **14**: S233-S236 [PMID: 12874437 DOI: 10.1097/01.asn.0000077408.15865.06]

67 **Chen F**, Yu Y, Haigh S, Johnson J, Lucas R, Stepp DW, Fulton DJ. Regulation of NADPH oxidase 5 by protein kinase C isoforms. *PLoS One* 2014; **9**: e88405 [PMID: 24505490 DOI: 10.1371/journal.pone.0088405]

68 **Xu S**, Zhao Y, Jin C, Yu L, Ding F, Fu G, Zhu J. PKC/NADPH oxidase are involved in the protective effect of pioglitazone in high homocysteine-induced paracrine dyfunction in endothelial progenitor cells. *Am J Transl Res* 2017; **9**: 1037-1048 [PMID: 28386331]

69 **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]

70 **Rodriguez PG**, Felix FN, Woodley DT, Shim EK. The role of oxygen in wound healing: a review of the literature. *Dermatol Surg* 2008; **34**: 1159-1169 [PMID: 18513296 DOI: 10.1111/j.1524-4725.2008.34254.x]

71 **Xu S**, Chisholm AD. C. elegans epidermal wounding induces a mitochondrial ROS burst that promotes wound repair. *Dev Cell* 2014; **31**: 48-60 [PMID: 25313960 DOI: 10.1016/j.devcel.2014.08.002]

72 **Deng L**, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. *Oxid Med Cell Longev* 2021; **2021**: 8852759 [PMID: 33628388 DOI: 10.1155/2021/8852759]

73 **Kumar S**, Ashe HA, Parnell LN, Fernando DJ, Tsigos C, Young RJ, Ward JD, Boulton AJ. The prevalence of foot ulceration and its correlates in type 2 diabetic patients: a population-based study. *Diabet Med* 1994; **11**: 480-484 [PMID: 8088127 DOI: 10.1111/j.1464-5491.1994.tb00310.x]

74 **Gilbey SG**. Neuropathy and foot problems in diabetes. *Clin Med (Lond)* 2004; **4**: 318-323 [PMID: 15372890 DOI: 10.7861/clinmedicine.4-4-318]

75 **Baum P**, Toyka KV, Blüher M, Kosacka J, Nowicki M. Inflammatory Mechanisms in the Pathophysiology of Diabetic Peripheral Neuropathy (DN)-New Aspects. *Int J Mol Sci* 2021; **22** [PMID: 34639176 DOI: 10.3390/ijms221910835]

76 **Mengstie MA**, Chekol Abebe E, Behaile Teklemariam A, Tilahun Mulu A, Agidew MM, Teshome Azezew M, Zewde EA, Agegnehu Teshome A. Endogenous advanced glycation end products in the pathogenesis of chronic diabetic complications. *Front Mol Biosci* 2022; **9**: 1002710 [PMID: 36188225 DOI: 10.3389/fmolb.2022.1002710]

77 **Sandireddy R**, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014; **2014**: 674987 [PMID: 24883061 DOI: 10.1155/2014/674987]

78 **Volmer-Thole M**, Lobmann R. Neuropathy and Diabetic Foot Syndrome. *Int J Mol Sci* 2016; **17** [PMID: 27294922 DOI: 10.3390/ijms17060917]

79 **Zenker J**, Ziegler D, Chrast R. Novel pathogenic pathways in diabetic neuropathy. *Trends Neurosci* 2013; **36**: 439-449 [PMID: 23725712 DOI: 10.1016/j.tins.2013.04.008]

80 **Boulton AJ**, Kirsner RS, Vileikyte L. Clinical practice. Neuropathic diabetic foot ulcers. *N Engl J Med* 2004; **351**: 48-55 [PMID: 15229307 DOI: 10.1056/NEJMcp032966]

81 **Peltier A**, Goutman SA, Callaghan BC. Painful diabetic neuropathy. *BMJ* 2014; **348**: g1799 [PMID: 24803311 DOI: 10.1136/bmj.g1799]

82 **Walczak A**, Szczepankiewicz AA, Ruszczycki B, Magalska A, Zamlynska K, Dzwonek J, Wilczek E, Zybura-Broda K, Rylski M, Malinowska M, Dabrowski M, Szczepinska T, Pawlowski K, Pyskaty M, Wlodarczyk J, Szczerbal I, Switonski M, Cremer M, Wilczynski GM. Novel higher-order epigenetic regulation of the Bdnf gene upon seizures. *J Neurosci* 2013; **33**: 2507-2511 [PMID: 23392678 DOI: 10.1523/JNEUROSCI.1085-12.2013]

83 **Barker AR**, Rosson GD, Dellon AL. Wound healing in denervated tissue. *Ann Plast Surg* 2006; **57**: 339-342 [PMID: 16929207 DOI: 10.1097/01.sap.0000221465.69826.b7]

84 **Levy DM**, Karanth SS, Springall DR, Polak JM. Depletion of cutaneous nerves and neuropeptides in diabetes mellitus: an immunocytochemical study. *Diabetologia* 1989; **32**: 427-433 [PMID: 2478407 DOI: 10.1007/BF00271262]

85 **Lindberger M**, Schröder HD, Schultzberg M, Kristensson K, Persson A, Ostman J, Link H. Nerve fibre studies in skin biopsies in peripheral neuropathies. I. Immunohistochemical analysis of neuropeptides in diabetes mellitus. *J Neurol Sci* 1989; **93**: 289-296 [PMID: 2480400 DOI: 10.1016/0022-510x(89)90198-6]

86 **Hsieh ST**, Chiang HY, Lin WM. Pathology of nerve terminal degeneration in the skin. *J Neuropathol Exp Neurol* 2000; **59**: 297-307 [PMID: 10759185 DOI: 10.1093/jnen/59.4.297]

87 **Krishnan ST**, Quattrini C, Jeziorska M, Malik RA, Rayman G. Neurovascular factors in wound healing in the foot skin of type 2 diabetic subjects. *Diabetes Care* 2007; **30**: 3058-3062 [PMID: 17898089 DOI: 10.2337/dc07-1421]

88 **Bönhof GJ**, Strom A, Püttgen S, Ringel B, Brüggemann J, Bódis K, Müssig K, Szendroedi J, Roden M, Ziegler D. Patterns of cutaneous nerve fibre loss and regeneration in type 2 diabetes with painful and painless polyneuropathy. *Diabetologia* 2017; **60**: 2495-2503 [PMID: 28914336 DOI: 10.1007/s00125-017-4438-5]

89 **Luo KR**, Chao CC, Chen YT, Huang CM, Yang NC, Kan HW, Wang SH, Yang WS, Hsieh ST. Quantitation of sudomotor innervation in skin biopsies of patients with diabetic neuropathy. *J Neuropathol Exp Neurol* 2011; **70**: 930-938 [PMID: 21937916 DOI: 10.1097/NEN.0b013e318230b0f4]

90 **Luo KR**, Chao CC, Hsieh PC, Lue JH, Hsieh ST. Effect of glycemic control on sudomotor denervation in type 2 diabetes. *Diabetes Care* 2012; **35**: 612-616 [PMID: 22301122 DOI: 10.2337/dc11-1607]

91 **Ziegler D**, Papanas N, Zhivov A, Allgeier S, Winter K, Ziegler I, Brüggemann J, Strom A, Peschel S, Köhler B, Stachs O, Guthoff RF, Roden M; German Diabetes Study (GDS) Group. Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. *Diabetes* 2014; **63**: 2454-2463 [PMID: 24574045 DOI: 10.2337/db13-1819]

92 **Gibran NS**, Jang YC, Isik FF, Greenhalgh DG, Muffley LA, Underwood RA, Usui ML, Larsen J, Smith DG, Bunnett N, Ansel JC, Olerud JE. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *J Surg Res* 2002; **108**: 122-128 [PMID: 12443724 DOI: 10.1006/jsre.2002.6525]

93 **Galkowska H**, Olszewski WL, Wojewodzka U, Rosinski G, Karnafel W. Neurogenic factors in the impaired healing of diabetic foot ulcers. *J Surg Res* 2006; **134**: 252-258 [PMID: 16580687 DOI: 10.1016/j.jss.2006.02.006]

94 **Shun CT**, Chang YC, Wu HP, Hsieh SC, Lin WM, Lin YH, Tai TY, Hsieh ST. Skin denervation in type 2 diabetes: correlations with diabetic duration and functional impairments. *Brain* 2004; **127**: 1593-1605 [PMID: 15128619 DOI: 10.1093/brain/awh180]

95 **Boulton AJ**. What you can't feel can hurt you. *J Vasc Surg* 2010; **52**: 28S-30S [PMID: 20804930 DOI:

10.1016/j.jvs.2010.06.005]
96 **Hsieh ST**, Choi S, Lin WM, Chang YC, Mcarthur JC, Griffin JW. Epidermal denervation and its effects on keratinocytes and Langerhans cells. *J Neurocytol* 1996; **25**: 513-524 [PMID: 8910797 DOI: 10.1007/BF02284819]
97 **Buckley G**, Wong J, Metcalfe AD, Ferguson MW. Denervation affects regenerative responses in MRL/MpJ and repair in C57BL/6 ear wounds. *J Anat* 2012; **220**: 3-12 [PMID: 22066944 DOI: 10.1111/j.1469-7580.2011.01452.x]
98 **Engin C**, Demirkan F, Ayhan S, Atabay K, Baran NK. Delayed effect of denervation on wound contraction in rat skin. *Plast Reconstr Surg* 1996; **98**: 1063-1067 [PMID: 8911477 DOI: 10.1097/00006534-199611000-00021]
99 **Alapure BV**, Lu Y, Peng H, Hong S. Surgical Denervation of Specific Cutaneous Nerves Impedes Excisional Wound Healing of Small Animal Ear Pinnae. *Mol Neurobiol* 2018; **55**: 1236-1243 [PMID: 28110472 DOI: 10.1007/s12035-017-0390-0]
100 **Ebenezer GJ**, O'Donnell R, Hauer P, Cimino NP, McArthur JC, Polydefkis M. Impaired neurovascular repair in subjects with diabetes following experimental intracutaneous axotomy. *Brain* 2011; **134**: 1853-1863 [PMID: 21616974 DOI: 10.1093/brain/awr086]
101 **Richards AM**, Floyd DC, Terenghi G, McGrouther DA. Cellular changes in denervated tissue during wound healing in a rat model. *Br J Dermatol* 1999; **140**: 1093-1099 [PMID: 10354076 DOI: 10.1046/j.1365-2133.1999.02908.x]
102 **Chiang HY**, Chen CT, Chien HF, Hsieh ST. Skin denervation, neuropathology, and neuropathic pain in a laser-induced focal neuropathy. *Neurobiol Dis* 2005; **18**: 40-53 [PMID: 15649695 DOI: 10.1016/j.nbd.2004.09.006]
103 **Fitzgerald M**. Capsaicin and sensory neurones--a review. *Pain* 1983; **15**: 109-130 [PMID: 6189047 DOI: 10.1016/0304-3959(83)90012-x]
104 **Gamse R**, Holzer P, Lembeck F. Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br J Pharmacol* 1980; **68**: 207-213 [PMID: 6153545 DOI: 10.1111/j.1476-5381.1980.tb10409.x]
105 **Helme RD**, Andrews PV. The effect of nerve lesions on the inflammatory response to injury. *J Neurosci Res* 1985; **13**: 453-459 [PMID: 3921721 DOI: 10.1002/jnr.490130311]
106 **Jancsó N**, Jancsó-Gábor A, Szolcsányi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br J Pharmacol Chemother* 1967; **31**: 138-151 [PMID: 6055248 DOI: 10.1111/j.1476-5381.1967.tb01984.x]
107 **Fahey TJ 3rd**, Sadaty A, Jones WG 2nd, Barber A, Smoller B, Shires GT. Diabetes impairs the late inflammatory response to wound healing. *J Surg Res* 1991; **50**: 308-313 [PMID: 2020184 DOI: 10.1016/0022-4804(91)90196-s]
108 **Greenhalgh DG**, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am J Pathol* 1990; **136**: 1235-1246 [PMID: 2356856]
109 **Wetzler C**, Kämpfer H, Stallmeyer B, Pfeilschifter J, Frank S. Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. *J Invest Dermatol* 2000; **115**: 245-253 [PMID: 10951242 DOI: 10.1046/j.1523-1747.2000.00029.x]
110 **Wallengren J**, Chen D, Sundler F. Neuropeptide-containing C-fibres and wound healing in rat skin. Neither capsaicin nor peripheral neurotomy affect the rate of healing. *Br J Dermatol* 1999; **140**: 400-408 [PMID: 10233257 DOI: 10.1046/j.1365-2133.1999.02699.x]
111 **Martínez-Martínez E**, Galván-Hernández CI, Toscano-Márquez B, Gutiérrez-Ospina G. Modulatory role of sensory innervation on hair follicle stem cell progeny during wound healing of the rat skin. *PLoS One* 2012; **7**: e36421 [PMID: 22574159 DOI: 10.1371/journal.pone.0036421]
112 **Smith PG**, Liu M. Impaired cutaneous wound healing after sensory denervation in developing rats: effects on cell proliferation and apoptosis. *Cell Tissue Res* 2002; **307**: 281-291 [PMID: 11904764 DOI: 10.1007/s00441-001-0477-8]
113 **Toda M**, Suzuki T, Hosono K, Kurihara Y, Kurihara H, Hayashi I, Kitasato H, Hoka S, Majima M. Roles of calcitonin gene-related peptide in facilitation of wound healing and angiogenesis. *Biomed Pharmacother* 2008; **62**: 352-359 [PMID: 18430544 DOI: 10.1016/j.biopha.2008.02.003]
114 **Feldman EL**, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, Bril V, Russell JW, Viswanathan V. Diabetic neuropathy. *Nat Rev Dis Primers* 2019; **5**: 42 [PMID: 31197183 DOI: 10.1038/s41572-019-0097-9]
115 **Ibuki A**, Kuriyama S, Toyosaki Y, Aiba M, Hidaka M, Horie Y, Fujimoto C, Isami F, Shibata E, Terauchi Y, Akase T. Aging-like physiological changes in the skin of Japanese obese diabetic patients. *SAGE Open Med* 2018; **6**: 2050312118756662 [PMID: 29449943 DOI: 10.1177/2050312118756662]
116 **Rivas-Santiago B**, Trujillo V, Montoya A, Gonzalez-Curiel I, Castañeda-Delgado J, Cardenas A, Rincon K, Hernandez ML, Hernández-Pando R. Expression of antimicrobial peptides in diabetic foot ulcer. *J Dermatol Sci* 2012; **65**: 19-26 [PMID: 22047630 DOI: 10.1016/j.jdermsci.2011.09.013]
117 **Smith FJ**, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-Jones S, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WH. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006; **38**: 337-342 [PMID: 16444271 DOI: 10.1038/ng1743]
118 **Gardiner M**, Vicaretti M, Sparks J, Bansal S, Bush S, Liu M, Darling A, Harry E, Burke CM. A longitudinal study of the diabetic skin and wound microbiome. *PeerJ* 2017; **5**: e3543 [PMID: 28740749 DOI: 10.7717/peerj.3543]
119 **Redel H**, Gao Z, Li H, Alekseyenko AV, Zhou Y, Perez-Perez GI, Weinstock G, Sodergren E, Blaser MJ. Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. *J Infect Dis* 2013; **207**: 1105-1114 [PMID: 23300163 DOI: 10.1093/infdis/jit005]
120 **Thimmappaiah Jagadeesh A**, Prakash PY, Karthik Rao N, Ramya V. Culture characterization of the skin microbiome in Type 2 diabetes mellitus: A focus on the role of innate immunity. *Diabetes Res Clin Pract* 2017; **134**: 1-7 [PMID: 28951341 DOI: 10.1016/j.diabres.2017.09.007]
121 **Gontcharova V**, Youn E, Sun Y, Wolcott RD, Dowd SE. A comparison of bacterial composition in diabetic ulcers and contralateral intact skin. *Open Microbiol J* 2010; **4**: 8-19 [PMID: 20461221 DOI: 10.2174/1874285801004010008]
122 **Oates A**, Bowling FL, Boulton AJ, McBain AJ. Molecular and culture-based assessment of the microbial diversity of

diabetic chronic foot wounds and contralateral skin sites. *J Clin Microbiol* 2012; **50**: 2263-2271 [PMID: 22553231 DOI: 10.1128/JCM.06599-11]

123 **Park JU**, Oh B, Lee JP, Choi MH, Lee MJ, Kim BS. Influence of Microbiota on Diabetic Foot Wound in Comparison with Adjacent Normal Skin Based on the Clinical Features. *Biomed Res Int* 2019; **2019**: 7459236 [PMID: 31531366 DOI: 10.1155/2019/7459236]

124 **Messad N**, Landraud L, Canivet B, Lina G, Richard JL, Sotto A, Lavigne JP, Lemichez E; French Study Group on the Diabetic Foot. Distribution of edin in Staphylococcus aureus isolated from diabetic foot ulcers. *Clin Microbiol Infect* 2013; **19**: 875-880 [PMID: 23176291 DOI: 10.1111/1469-0691.12084]

125 **Radzieta M**, Sadeghpour-Heravi F, Peters TJ, Hu H, Vickery K, Jeffries T, Dickson HG, Schwarzer S, Jensen SO, Malone M. A multiomics approach to identify host-microbe alterations associated with infection severity in diabetic foot infections: a pilot study. *NPJ Biofilms Microbiomes* 2021; **7**: 29 [PMID: 33753735 DOI: 10.1038/s41522-021-00202-x]

126 **Guo S**, Dipietro LA. Factors affecting wound healing. *J Dent Res* 2010; **89**: 219-229 [PMID: 20139336 DOI: 10.1177/0022034509359125]

127 **Eming SA**, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci Transl Med* 2014; **6**: 265sr6 [PMID: 25473038 DOI: 10.1126/scitranslmed.3009337]

128 **MacLeod AS**, Mansbridge JN. The Innate Immune System in Acute and Chronic Wounds. *Adv Wound Care (New Rochelle)* 2016; **5**: 65-78 [PMID: 26862464 DOI: 10.1089/wound.2014.0608]

129 **Sindrilaru A**, Peters T, Wieschalka S, Baican C, Baican A, Peter H, Hainzl A, Schatz S, Qi Y, Schlecht A, Weiss JM, Wlaschek M, Sunderkötter C, Scharffetter-Kochanek K. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest* 2011; **121**: 985-997 [PMID: 21317534 DOI: 10.1172/JCI44490]

130 **Beidler SK**, Douillet CD, Berndt DF, Keagy BA, Rich PB, Marston WA. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. *J Vasc Surg* 2009; **49**: 1013-1020 [PMID: 19341889 DOI: 10.1016/j.jvs.2008.11.049]

131 **Doupis J**, Lyons TE, Wu S, Gnardellis C, Dinh T, Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. *J Clin Endocrinol Metab* 2009; **94**: 2157-2163 [PMID: 19276232 DOI: 10.1210/jc.2008-2385]

132 **Bagdade JD**, Root RK, Bulger RJ. Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes* 1974; **23**: 9-15 [PMID: 4809622 DOI: 10.2337/diab.23.1.9]

133 **Bybee JD**, Rogers DE. The Phagocytic Activity of Polymorphonuclear Leukocytes Obtained from Patients with Diabetes Mellitus. *J Lab Clin Med* 1964; **64**: 1-13 [PMID: 14192564]

134 **Richard C**, Wadowski M, Goruk S, Cameron L, Sharma AM, Field CJ. Individuals with obesity and type 2 diabetes have additional immune dysfunction compared with obese individuals who are metabolically healthy. *BMJ Open Diabetes Res Care* 2017; **5**: e000379 [PMID: 28761653 DOI: 10.1136/bmjdrc-2016-000379]

135 **Montanaro M**, Meloni M, Anemona L, Giurato L, Scimeca M, Izzo V, Servadei F, Smirnov A, Candi E, Mauriello A, Uccioli L. Macrophage Activation and M2 Polarization in Wound Bed of Diabetic Patients Treated by Dermal/Epidermal Substitute Nevelia. *Int J Low Extrem Wounds* 2022; **21**: 377-383 [PMID: 32815405 DOI: 10.1177/1534734620945559]

136 **Morey M**, O'Gaora P, Pandit A, Hélary C. Hyperglycemia acts in synergy with hypoxia to maintain the pro-inflammatory phenotype of macrophages. *PLoS One* 2019; **14**: e0220577 [PMID: 31415598 DOI: 10.1371/journal.pone.0220577]

137 **Sindrilaru A**, Scharffetter-Kochanek K. Disclosure of the Culprits: Macrophages-Versatile Regulators of Wound Healing. *Adv Wound Care (New Rochelle)* 2013; **2**: 357-368 [PMID: 24587973 DOI: 10.1089/wound.2012.0407]

138 **Khanna S**, Biswas S, Shang Y, Collard E, Azad A, Kauh C, Bhasker V, Gordillo GM, Sen CK, Roy S. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. *PLoS One* 2010; **5**: e9539 [PMID: 20209061 DOI: 10.1371/journal.pone.0009539]

139 **Jayasuriya R**, Dhamodharan U, Karan AN, Anandharaj A, Rajesh K, Ramkumar KM. Role of Nrf2 in MALAT1/ HIF-1α loop on the regulation of angiogenesis in diabetic foot ulcer. *Free Radic Biol Med* 2020; **156**: 168-175 [PMID: 32473205 DOI: 10.1016/j.freeradbiomed.2020.05.018]

140 **Zhu L**, Zhong Q, Yang T, Xiao X. Improved therapeutic effects on diabetic foot by human mesenchymal stem cells expressing MALAT1 as a sponge for microRNA-205-5p. *Aging (Albany NY)* 2019; **11**: 12236-12245 [PMID: 31866580 DOI: 10.18632/aging.102562]

141 **Berry-Kilgour C**, Cabral J, Wise L. Advancements in the Delivery of Growth Factors and Cytokines for the Treatment of Cutaneous Wound Indications. *Adv Wound Care (New Rochelle)* 2021; **10**: 596-622 [PMID: 33086946 DOI: 10.1089/wound.2020.1183]

142 **Zubair M**, Ahmad J. Role of growth factors and cytokines in diabetic foot ulcer healing: A detailed review. *Rev Endocr Metab Disord* 2019; **20**: 207-217 [PMID: 30937614 DOI: 10.1007/s11154-019-09492-1]

143 **Armstrong DG**, Orgill DP, Galiano RD, Glat PM, Kaufman JP, Carter MJ, Zelen CM. An observational pilot study using a purified reconstituted bilayer matrix to treat non-healing diabetic foot ulcers. *Int Wound J* 2020; **17**: 966-973 [PMID: 32266774 DOI: 10.1111/iwj.13353]

144 **Bini Antunes M**, Costa L, Carneiro M, Santos F, Oliveira R, Ferreira A, Sampaio M, Guimarães R, Pereira J, Neto H, Amil M, Coutinho J, Carvalho R. Topic platelet gel application in chronic diabetic foot ulcers. *Diabetes Metab Syndr* 2019; **13**: 644-647 [PMID: 30641782 DOI: 10.1016/j.dsx.2018.11.032]

145 **Erdoğan A**, Düzgün AP, Erdoğan K, Özkan MB, Coşkun F. Efficacy of Hyperbaric Oxygen Therapy in Diabetic Foot Ulcers Based on Wagner Classification. *J Foot Ankle Surg* 2018; **57**: 1115-1119 [PMID: 30368425 DOI: 10.1053/j.jfas.2018.05.011]

146 **Gonzalez IG**, Angel MA, Baez MV, Ruiz Flores B, de Los Angeles Martinez Ferretiz M, Woolf SV, López I, Sandoval-Jurado L, Pat-Espadas FG, Cruz AA, Delgado AT. Handcrafted Vacuum-Assisted Device for Skin Ulcers Treatment Versus Traditional Therapy, Randomized Controlled Trial. *World J Surg* 2017; **41**: 386-393 [PMID: 27822727 DOI: 10.1007/s00268-016-3782-9]

147 **He M**, Guo X, Li T, Jiang X, Chen Y, Yuan Y, Chen B, Yang G, Fan Y, Liang Z, Armstrong DG, Deng W. Comparison of

Allogeneic Platelet-rich Plasma With Autologous Platelet-rich Plasma for the Treatment of Diabetic Lower Extremity Ulcers. *Cell Transplant* 2020; **29**: 963689720931428 [PMID: 32510240 DOI: 10.1177/0963689720931428]

148 **Irawan H**, Semadi IN, Widiana IGR. A Pilot Study of Short-Duration Hyperbaric Oxygen Therapy to Improve HbA1c, Leukocyte, and Serum Creatinine in Patients with Diabetic Foot Ulcer Wagner 3-4. *ScientificWorldJournal* 2018; **2018**: 6425857 [PMID: 30158840 DOI: 10.1155/2018/6425857]

149 **Shi R**, Lian W, Jin Y, Cao C, Han S, Yang X, Zhao S, Li M, Zhao H. Role and effect of vein-transplanted human umbilical cord mesenchymal stem cells in the repair of diabetic foot ulcers in rats. *Acta Biochim Biophys Sin (Shanghai)* 2020; **52**: 620-630 [PMID: 32484226 DOI: 10.1093/abbs/gmaa039]

150 **Viezzer C**, Mazzuca R, Machado DC, de Camargo Forte MM, Gómez Ribelles JL. A new waterborne chitosan-based polyurethane hydrogel as a vehicle to transplant bone marrow mesenchymal cells improved wound healing of ulcers in a diabetic rat model. *Carbohydr Polym* 2020; **231**: 115734 [PMID: 31888801 DOI: 10.1016/j.carbpol.2019.115734]

151 **Chen CC**, Mo FE, Lau LF. The angiogenic factor Cyr61 activates a genetic program for wound healing in human skin fibroblasts. *J Biol Chem* 2001; **276**: 47329-47337 [PMID: 11584015 DOI: 10.1074/jbc.M107666200]

152 **Frank S**, Hübner G, Breier G, Longaker MT, Greenhalgh DG, Werner S. Regulation of vascular endothelial growth factor expression in cultured keratinocytes. Implications for normal and impaired wound healing. *J Biol Chem* 1995; **270**: 12607-12613 [PMID: 7759509 DOI: 10.1074/jbc.270.21.12607]

153 **Johnson KE**, Wilgus TA. Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair. *Adv Wound Care (New Rochelle)* 2014; **3**: 647-661 [PMID: 25302139 DOI: 10.1089/wound.2013.0517]

154 **Macedo L**, Pinhal-Enfield G, Alshits V, Elson G, Cronstein BN, Leibovich SJ. Wound healing is impaired in MyD88-deficient mice: a role for MyD88 in the regulation of wound healing by adenosine A2A receptors. *Am J Pathol* 2007; **171**: 1774-1788 [PMID: 17974599 DOI: 10.2353/ajpath.2007.061048]

155 **Min JK**, Lee YM, Kim JH, Kim YM, Kim SW, Lee SY, Gho YS, Oh GT, Kwon YG. Hepatocyte growth factor suppresses vascular endothelial growth factor-induced expression of endothelial ICAM-1 and VCAM-1 by inhibiting the nuclear factor-kappaB pathway. *Circ Res* 2005; **96**: 300-307 [PMID: 15637298 DOI: 10.1161/01.RES.0000155330.07887.EE]

156 **Pola R**, Ling LE, Silver M, Corbley MJ, Kearney M, Blake Pepinsky R, Shapiro R, Taylor FR, Baker DP, Asahara T, Isner JM. The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. *Nat Med* 2001; **7**: 706-711 [PMID: 11385508 DOI: 10.1038/89083]

157 **Robson MC**. The role of growth factors in the healing of chronic wounds. *Wound Repair Regen* 1997; **5**: 12-17 [PMID: 16984452 DOI: 10.1046/j.1524-475X.1997.50106.x]

158 **Zhang Z**, Schluesener HJ. Mammalian toll-like receptors: from endogenous ligands to tissue regeneration. *Cell Mol Life Sci* 2006; **63**: 2901-2907 [PMID: 17072502 DOI: 10.1007/s00018-006-6189-1]

159 **Amendt C**, Mann A, Schirmacher P, Blessing M. Resistance of keratinocytes to TGFbeta-mediated growth restriction and apoptosis induction accelerates re-epithelialization in skin wounds. *J Cell Sci* 2002; **115**: 2189-2198 [PMID: 11973359 DOI: 10.1242/jcs.115.10.2189]

160 **Ashcroft GS**, Yang X, Glick AB, Weinstein M, Letterio JL, Mizel DE, Anzano M, Greenwell-Wild T, Wahl SM, Deng C, Roberts AB. Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol* 1999; **1**: 260-266 [PMID: 10559937 DOI: 10.1038/12971]

161 **Barrientos S**, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. *Wound Repair Regen* 2008; **16**: 585-601 [PMID: 19128254 DOI: 10.1111/j.1524-475X.2008.00410.x]

162 **Chesnoy S**, Lee PY, Huang L. Intradermal injection of transforming growth factor-beta1 gene enhances wound healing in genetically diabetic mice. *Pharm Res* 2003; **20**: 345-350 [PMID: 12669952 DOI: 10.1023/a:1022635600479]

163 **Desmoulière A**, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 1993; **122**: 103-111 [PMID: 8314838 DOI: 10.1083/jcb.122.1.103]

164 **Gailit J**, Welch MP, Clark RA. TGF-beta 1 stimulates expression of keratinocyte integrins during re-epithelialization of cutaneous wounds. *J Invest Dermatol* 1994; **103**: 221-227 [PMID: 8040614 DOI: 10.1111/1523-1747.ep12393176]

165 **Klass BR**, Grobbelaar AO, Rolfe KJ. Transforming growth factor beta1 signalling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance. *Postgrad Med J* 2009; **85**: 9-14 [PMID: 19240282 DOI: 10.1136/pgmj.2008.069831]

166 **Kopecki Z**, Luchetti MM, Adams DH, Strudwick X, Mantamadiotis T, Stoppacciaro A, Gabrielli A, Ramsay RG, Cowin AJ. Collagen loss and impaired wound healing is associated with c-Myb deficiency. *J Pathol* 2007; **211**: 351-361 [PMID: 17152050 DOI: 10.1002/path.2113]

167 **Lamar JM**, Iyer V, DiPersio CM. Integrin alpha3beta1 potentiates TGFbeta-mediated induction of MMP-9 in immortalized keratinocytes. *J Invest Dermatol* 2008; **128**: 575-586 [PMID: 17762853 DOI: 10.1038/sj.jid.5701042]

168 **Munger JS**, Sheppard D. Cross talk among TGF-β signaling pathways, integrins, and the extracellular matrix. *Cold Spring Harb Perspect Biol* 2011; **3**: a005017 [PMID: 21900405 DOI: 10.1101/cshperspect.a005017]

169 **Pastar I**, Stojadinovic O, Krzyzanowska A, Barrientos S, Stuelten C, Zimmerman K, Blumenberg M, Brem H, Tomic-Canic M. Attenuation of the transforming growth factor beta-signaling pathway in chronic venous ulcers. *Mol Med* 2010; **16**: 92-101 [PMID: 20069132 DOI: 10.2119/molmed.2009.00149]

170 **Philipp K**, Riedel F, Germann G, Hörmann K, Sauerbier M. TGF-beta antisense oligonucleotides reduce mRNA expression of matrix metalloproteinases in cultured wound-healing-related cells. *Int J Mol Med* 2005; **15**: 299-303 [PMID: 15647847]

171 **Postlethwaite AE**, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J Exp Med* 1987; **165**: 251-256 [PMID: 3491869 DOI: 10.1084/jem.165.1.251]

172 **Roberts AB**, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 1986; **83**: 4167-4171 [PMID: 2424019 DOI: 10.1073/pnas.83.12.4167]

173 **Stuelten CH**, DaCosta Byfield S, Arany PR, Karpova TS, Stetler-Stevenson WG, Roberts AB. Breast cancer cells induce stromal fibroblasts to express MMP-9 *via* secretion of TNF-alpha and TGF-beta. *J Cell Sci* 2005; **118**: 2143-2153 [PMID: 15855236 DOI: 10.1242/jcs.02334]

174 **Yuan W**, Varga J. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. *J Biol Chem* 2001; **276**: 38502-38510 [PMID: 11502752 DOI: 10.1074/jbc.M107081200]

175 **Pierce GF**, Tarpley JE, Yanagihara D, Mustoe TA, Fox GM, Thomason A. Platelet-derived growth factor (BB homodimer), transforming growth factor-beta 1, and basic fibroblast growth factor in dermal wound healing. Neovessel and matrix formation and cessation of repair. *Am J Pathol* 1992; **140**: 1375-1388 [PMID: 1376557]

176 **Trengove NJ**, Bielefeldt-Ohmann H, Stacey MC. Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers. *Wound Repair Regen* 2000; **8**: 13-25 [PMID: 10760211 DOI: 10.1046/j.1524-475x.2000.00013.x]

177 **Buchstein N**, Hoffmann D, Smola H, Lang S, Paulsson M, Niemann C, Krieg T, Eming SA. Alternative proteolytic processing of hepatocyte growth factor during wound repair. *Am J Pathol* 2009; **174**: 2116-2128 [PMID: 19389925 DOI: 10.2353/ajpath.2009.080597]

178 **Chmielowiec J**, Borowiak M, Morkel M, Stradal T, Munz B, Werner S, Wehland J, Birchmeier C, Birchmeier W. c-Met is essential for wound healing in the skin. *J Cell Biol* 2007; **177**: 151-162 [PMID: 17403932 DOI: 10.1083/jcb.200701086]

179 **Toyoda M**, Takayama H, Horiguchi N, Otsuka T, Fukusato T, Merlino G, Takagi H, Mori M. Overexpression of hepatocyte growth factor/scatter factor promotes vascularization and granulation tissue formation in vivo. *FEBS Lett* 2001; **509**: 95-100 [PMID: 11734213 DOI: 10.1016/s0014-5793(01)03126-x]

180 **Yoshida S**, Yamaguchi Y, Itami S, Yoshikawa K, Tabata Y, Matsumoto K, Nakamura T. Neutralization of hepatocyte growth factor leads to retarded cutaneous wound healing associated with decreased neovascularization and granulation tissue formation. *J Invest Dermatol* 2003; **120**: 335-343 [PMID: 12542542 DOI: 10.1046/j.1523-1747.2003.12039.x]

181 **Bikfalvi A**, Klein S, Pintucci G, Rifkin DB. Biological roles of fibroblast growth factor-2. *Endocr Rev* 1997; **18**: 26-45 [PMID: 9034785 DOI: 10.1210/edrv.18.1.0292]

182 **Powers CJ**, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. *Endocr Relat Cancer* 2000; **7**: 165-197 [PMID: 11021964 DOI: 10.1677/erc.0.0070165]

183 **Robson MC**, Hill DP, Smith PD, Wang X, Meyer-Siegler K, Ko F, VandeBerg JS, Payne WG, Ochs D, Robson LE. Sequential cytokine therapy for pressure ulcers: clinical and mechanistic response. *Ann Surg* 2000; **231**: 600-611 [PMID: 10749622 DOI: 10.1097/00000658-200004000-00020]

184 **Sogabe Y**, Abe M, Yokoyama Y, Ishikawa O. Basic fibroblast growth factor stimulates human keratinocyte motility by Rac activation. *Wound Repair Regen* 2006; **14**: 457-462 [PMID: 16939574 DOI: 10.1111/j.1743-6109.2006.00143.x]

185 **Braun S**, auf dem Keller U, Steiling H, Werner S. Fibroblast growth factors in epithelial repair and cytoprotection. *Philos Trans R Soc Lond B Biol Sci* 2004; **359**: 753-757 [PMID: 15293802 DOI: 10.1098/rstb.2004.1464]

186 **Davidson JM**. First-class delivery: getting growth factors to their destination. *J Invest Dermatol* 2008; **128**: 1360-1362 [PMID: 18478013 DOI: 10.1038/jid.2008.128]

187 **Marti GP**, Mohebi P, Liu L, Wang J, Miyashita T, Harmon JW. KGF-1 for wound healing in animal models. *Methods Mol Biol* 2008; **423**: 383-391 [PMID: 18370216 DOI: 10.1007/978-1-59745-194-9_30]

188 **Werner S**, Smola H, Liao X, Longaker MT, Krieg T, Hofschneider PH, Williams LT. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science* 1994; **266**: 819-822 [PMID: 7973639 DOI: 10.1126/science.7973639]

189 **Shirakata Y**, Kimura R, Nanba D, Iwamoto R, Tokumaru S, Morimoto C, Yokota K, Nakamura M, Sayama K, Mekada E, Higashiyama S, Hashimoto K. Heparin-binding EGF-like growth factor accelerates keratinocyte migration and skin wound healing. *J Cell Sci* 2005; **118**: 2363-2370 [PMID: 15923649 DOI: 10.1242/jcs.02346]

190 **Sano S**, Itami S, Takeda K, Tarutani M, Yamaguchi Y, Miura H, Yoshikawa K, Akira S, Takeda J. Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J* 1999; **18**: 4657-4668 [PMID: 10469645 DOI: 10.1093/emboj/18.17.4657]

191 **Oda K**, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Syst Biol* 2005; **1**: 2005.0010 [PMID: 16729045 DOI: 10.1038/msb4100014]

192 **Mast BA**, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Repair Regen* 1996; **4**: 411-420 [PMID: 17309691 DOI: 10.1046/j.1524-475X.1996.40404.x]

193 **Li G**, Gustafson-Brown C, Hanks SK, Nason K, Arbeit JM, Pogliano K, Wisdom RM, Johnson RS. c-Jun is essential for organization of the epidermal leading edge. *Dev Cell* 2003; **4**: 865-877 [PMID: 12791271 DOI: 10.1016/s1534-5807(03)00159-x]

194 **Jiang CK**, Magnaldo T, Ohtsuki M, Freedberg IM, Bernerd F, Blumenberg M. Epidermal growth factor and transforming growth factor alpha specifically induce the activation- and hyperproliferation-associated keratins 6 and 16. *Proc Natl Acad Sci USA* 1993; **90**: 6786-6790 [PMID: 7688128 DOI: 10.1073/pnas.90.14.6786]

195 **Brown GL**, Curtsinger LJ, White M, Mitchell RO, Pietsch J, Nordquist R, von Fraunhofer A, Schultz GS. Acceleration of tensile strength of incisions treated with EGF and TGF-beta. *Ann Surg* 1988; **208**: 788-794 [PMID: 3264140 DOI: 10.1097/00000658-198812000-00019]

196 **Brown GL**, Curtsinger L 3rd, Brightwell JR, Ackerman DM, Tobin GR, Polk HC Jr, George-Nascimento C, Valenzuela P, Schultz GS. Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. *J Exp Med* 1986; **163**: 1319-1324 [PMID: 3486247 DOI: 10.1084/jem.163.5.1319]

197 **Brem H**, Stojadinovic O, Diegelmann RF, Entero H, Lee B, Pastar I, Golinko M, Rosenberg H, Tomic-Canic M. Molecular markers in patients with chronic wounds to guide surgical debridement. *Mol Med* 2007; **13**: 30-39 [PMID: 17515955 DOI: 10.2119/2006-00054.Brem]

198 **Nissen NN**, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol* 1998; **152**: 1445-1452 [PMID: 9626049]

199 **Namiki A**, Brogi E, Kearney M, Kim EA, Wu T, Couffinhal T, Varticovski L, Isner JM. Hypoxia induces vascular endothelial growth factor in cultured human endothelial cells. *J Biol Chem* 1995; **270**: 31189-31195 [PMID: 8537383 DOI: 10.1074/jbc.270.52.31189]

200 **Banks RE**, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer* 1998; **77**: 956-964 [PMID: 9528841 DOI: 10.1038/bjc.1998.158]

201 **Terman BI**, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Böhlen P. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem Biophys Res Commun* 1992; **187**: 1579-1586 [PMID: 1417831 DOI: 10.1016/0006-291x(92)90483-2]

202 **de Vries C**, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science* 1992; **255**: 989-991 [PMID: 1312256 DOI: 10.1126/science.1312256]

203 **Breier G**, Damert A, Plate KH, Risau W. Angiogenesis in embryos and ischemic diseases. *Thromb Haemost* 1997; **78**: 678-683 [PMID: 9198238]

204 **Waltenberger J**, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem* 1994; **269**: 26988-26995 [PMID: 7929439]

205 **Malavaud B**, Tack I, Jonca F, Praddaude F, Moro F, Ader JL, Plouët J. Activation of Flk-1/KDR mediates angiogenesis but not hypotension. *Cardiovasc Res* 1997; **36**: 276-281 [PMID: 9463639 DOI: 10.1016/s0008-6363(97)00177-6]

206 **Clauss M**. Functions of the VEGF receptor-1 (FLT-1) in the vasculature. *Trends Cardiovasc Med* 1998; **8**: 241-245 [PMID: 14987558 DOI: 10.1016/s1050-1738(98)00015-2]

207 **Shukla A**, Dubey MP, Srivastava R, Srivastava BS. Differential expression of proteins during healing of cutaneous wounds in experimental normal and chronic models. *Biochem Biophys Res Commun* 1998; **244**: 434-439 [PMID: 9514941 DOI: 10.1006/bbrc.1998.8286]

208 **Stavri GT**, Zachary IC, Baskerville PA, Martin JF, Erusalimsky JD. Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells. Synergistic interaction with hypoxia. *Circulation* 1995; **92**: 11-14 [PMID: 7788904 DOI: 10.1161/01.cir.92.1.11]

209 **Brogi E**, Wu T, Namiki A, Isner JM. Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only. *Circulation* 1994; **90**: 649-652 [PMID: 8044933 DOI: 10.1161/01.cir.90.2.649]

210 **Song SH**, Lee MO, Lee JS, Jeong HC, Kim HG, Kim WS, Hur M, Cha HJ. Genetic modification of human adipose-derived stem cells for promoting wound healing. *J Dermatol Sci* 2012; **66**: 98-107 [PMID: 22472356 DOI: 10.1016/j.jdermsci.2012.02.010]

211 **Huang SP**, Hsu CC, Chang SC, Wang CH, Deng SC, Dai NT, Chen TM, Chan JY, Chen SG, Huang SM. Adipose-derived stem cells seeded on acellular dermal matrix grafts enhance wound healing in a murine model of a full-thickness defect. *Ann Plast Surg* 2012; **69**: 656-662 [PMID: 23154338 DOI: 10.1097/SAP.0b013e318273f909]

212 **Sun N**, Ning B, Hansson KM, Bruce AC, Seaman SA, Zhang C, Rikard M, DeRosa CA, Fraser CL, Wågberg M, Fritsche-Danielson R, Wikström J, Chien KR, Lundahl A, Hölttä M, Carlsson LG, Peirce SM, Hu S. Modified VEGF-A mRNA induces sustained multifaceted microvascular response and accelerates diabetic wound healing. *Sci Rep* 2018; **8**: 17509 [PMID: 30504800 DOI: 10.1038/s41598-018-35570-6]

213 **Zha W**, Wang J, Guo Z, Zhang Y, Wang Y, Dong S, Liu C, Xing H, Li X. Efficient delivery of VEGF-A mRNA for promoting diabetic wound healing *via* ionizable lipid nanoparticles. *Int J Pharm* 2022; **632**: 122565 [PMID: 36586634 DOI: 10.1016/j.ijpharm.2022.122565]

214 **Chen L**, Zheng Q, Liu Y, Li L, Chen X, Wang L. Adipose-derived stem cells promote diabetic wound healing *via* the recruitment and differentiation of endothelial progenitor cells into endothelial cells mediated by the VEGF-PLCγ-ERK pathway. *Arch Biochem Biophys* 2020; **692**: 108531 [PMID: 32745464 DOI: 10.1016/j.abb.2020.108531]

215 **Zafari F**, Shirian S, Sadeghi M, Teimourian S, Bakhtiyari M. CD93 hematopoietic stem cells improve diabetic wound healing by VEGF activation and downregulation of DAPK-1. *J Cell Physiol* 2020; **235**: 2366-2376 [PMID: 31549396 DOI: 10.1002/jcp.29142]

216 **Zhou J**, Ni M, Liu X, Ren Z, Zheng Z. Curcumol Promotes Vascular Endothelial Growth Factor (VEGF)-Mediated Diabetic Wound Healing in Streptozotocin-Induced Hyperglycemic Rats. *Med Sci Monit* 2017; **23**: 555-562 [PMID: 28138126 DOI: 10.12659/msm.902859]

217 **Skobe M**, Hamberg LM, Hawighorst T, Schirner M, Wolf GL, Alitalo K, Detmar M. Concurrent induction of lymphangiogenesis, angiogenesis, and macrophage recruitment by vascular endothelial growth factor-C in melanoma. *Am J Pathol* 2001; **159**: 893-903 [PMID: 11549582 DOI: 10.1016/S0002-9440(10)61765-8]

218 **Schoppmann SF**, Fenzl A, Nagy K, Unger S, Bayer G, Geleff S, Gnant M, Horvat R, Jakesz R, Birner P. VEGF-C expressing tumor-associated macrophages in lymph node positive breast cancer: impact on lymphangiogenesis and survival. *Surgery* 2006; **139**: 839-846 [PMID: 16782443 DOI: 10.1016/j.surg.2005.12.008]

219 **Partanen TA**, Arola J, Saaristo A, Jussila L, Ora A, Miettinen M, Stacker SA, Achen MG, Alitalo K. VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues. *FASEB J* 2000; **14**: 2087-2096 [PMID: 11023993 DOI: 10.1096/fj.99-1049com]

220 **McColl BK**, Baldwin ME, Roufail S, Freeman C, Moritz RL, Simpson RJ, Alitalo K, Stacker SA, Achen MG. Plasmin activates the lymphangiogenic growth factors VEGF-C and VEGF-D. *J Exp Med* 2003; **198**: 863-868 [PMID: 12963694 DOI: 10.1084/jem.20030361]

221 **Güç E**, Briquez PS, Foretay D, Fankhauser MA, Hubbell JA, Kilarski WW, Swartz MA. Local induction of lymphangiogenesis with engineered fibrin-binding VEGF-C promotes wound healing by increasing immune cell trafficking and matrix remodeling. *Biomaterials* 2017; **131**: 160-175 [PMID: 28410495 DOI: 10.1016/j.biomaterials.2017.03.033]

222 **Saaristo A**, Tammela T, Farkkilā A, Kärkkäinen M, Suominen E, Yla-Herttuala S, Alitalo K. Vascular endothelial growth factor-C accelerates diabetic wound healing. *Am J Pathol* 2006; **169**: 1080-1087 [PMID: 16936280 DOI: 10.2353/ajpath.2006.051251]

223 **Uutela M**, Wirzenius M, Paavonen K, Rajantie I, He Y, Karpanen T, Lohela M, Wiig H, Salven P, Pajusola K, Eriksson U, Alitalo K. PDGF-D induces macrophage recruitment, increased interstitial pressure, and blood vessel maturation during angiogenesis. *Blood* 2004; **104**: 3198-3204 [PMID: 15271796 DOI: 10.1182/blood-2004-04-1485]

224 **Niessen FB**, Andriessen MP, Schalkwijk J, Visser L, Timens W. Keratinocyte-derived growth factors play a role in the formation of hypertrophic scars. *J Pathol* 2001; **194**: 207-216 [PMID: 11400150 DOI: 10.1002/path.853]

225 **Bennett SP**, Griffiths GD, Schor AM, Leese GP, Schor SL. Growth factors in the treatment of diabetic foot ulcers. *Br J Surg* 2003; **90**: 133-146 [PMID: 12555288 DOI: 10.1002/bjs.4019]

226 **Vogt PM**, Lehnhardt M, Wagner D, Jansen V, Krieg M, Steinau HU. Determination of endogenous growth factors in human wound fluid: temporal presence and profiles of secretion. *Plast Reconstr Surg* 1998; **102**: 117-123 [PMID: 9655416 DOI: 10.1097/00006534-199807000-00018]

227 **Pontén A**, Folestad EB, Pietras K, Eriksson U. Platelet-derived growth factor D induces cardiac fibrosis and proliferation of vascular smooth muscle cells in heart-specific transgenic mice. *Circ Res* 2005; **97**: 1036-1045 [PMID: 16224065 DOI: 10.1161/01.RES.0000190590.31545.d4]

228 **Heldin CH**, Westermark B. Mechanism of action and *in vivo* role of platelet-derived growth factor. *Physiol Rev* 1999; **79**: 1283-1316 [PMID: 10508235 DOI: 10.1152/physrev.1999.79.4.1283]

229 **Bowen-Pope DF**, Raines EW. History of discovery: platelet-derived growth factor. *Arterioscler Thromb Vasc Biol* 2011; **31**: 2397-2401 [PMID: 22011752 DOI: 10.1161/ATVBAHA.108.179556]

230 **Folestad E**, Kunath A, Wågsäter D. PDGF-C and PDGF-D signaling in vascular diseases and animal models. *Mol Aspects Med* 2018; **62**: 1-11 [PMID: 29410092 DOI: 10.1016/j.mam.2018.01.005]

231 **Demaria M**, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dollé ME, Hoeijmakers JH, de Bruin A, Hara E, Campisi J. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell* 2014; **31**: 722-733 [PMID: 25499914 DOI: 10.1016/j.devcel.2014.11.012]

232 **White MJV**, Briquez PS, White DAV, Hubbell JA. VEGF-A, PDGF-BB and HB-EGF engineered for promiscuous super affinity to the extracellular matrix improve wound healing in a model of type 1 diabetes. *NPJ Regen Med* 2021; **6**: 76 [PMID: 34795305 DOI: 10.1038/s41536-021-00189-1]

233 **Moriya J**, Wu X, Zavala-Solorio J, Ross J, Liang XH, Ferrara N. Platelet-derived growth factor C promotes revascularization in ischemic limbs of diabetic mice. *J Vasc Surg* 2014; **59**: 1402-9.e1 [PMID: 23856609 DOI: 10.1016/j.jvs.2013.04.053]

234 **Drela E**, Kulwas A, Jundziłł W, Góralczyk B, Boinska J, Drewniak W, Gadomska G, Rość D. VEGF-A and PDGF-BB--angiogenic factors and the stage of diabetic foot syndrome advancement. *Endokrynol Pol* 2014; **65**: 306-312 [PMID: 25185854 DOI: 10.5603/EP.2014.0042]

235 **Kartika RW**, Alwi I, Suyatna FD, Yunir E, Waspadji S, Immanuel S, Silalahi T, Sungkar S, Rachmat J, Reksodiputro MH, Bardosono S. The role of VEGF, PDGF and IL-6 on diabetic foot ulcer after Platelet Rich Fibrin + hyaluronic therapy. *Heliyon* 2021; **7**: e07934 [PMID: 34585000 DOI: 10.1016/j.heliyon.2021.e07934]

236 **Shi R**, Lian W, Han S, Cao C, Jin Y, Yuan Y, Zhao H, Li M. Nanosphere-mediated co-delivery of VEGF-A and PDGF-B genes for accelerating diabetic foot ulcers healing in rats. *Gene Ther* 2018; **25**: 425-438 [PMID: 29955127 DOI: 10.1038/s41434-018-0027-6]

237 **Li H**, Fu X, Zhang L, Huang Q, Wu Z, Sun T. Research of PDGF-BB gel on the wound healing of diabetic rats and its pharmacodynamics. *J Surg Res* 2008; **145**: 41-48 [PMID: 18082770 DOI: 10.1016/j.jss.2007.02.044]

238 **Embil JM**, Papp K, Sibbald G, Tousignant J, Smiell JM, Wong B, Lau CY. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. *Wound Repair Regen* 2000; **8**: 162-168 [PMID: 10886806 DOI: 10.1046/j.1524-475x.2000.00162.x]

239 **Ma C**, Hernandez MA, Kirkpatrick VE, Liang LJ, Nouvong AL, Gordon II. Topical platelet-derived growth factor *vs* placebo therapy of diabetic foot ulcers offloaded with windowed casts: a randomized, controlled trial. *Wounds* 2015; **27**: 83-91 [PMID: 25855851]

240 **Smiell JM**, Wieman TJ, Steed DL, Perry BH, Sampson AR, Schwab BH. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. *Wound Repair Regen* 1999; **7**: 335-346 [PMID: 10564562 DOI: 10.1046/j.1524-475x.1999.00335.x]

241 **Wieman TJ**, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care* 1998; **21**: 822-827 [PMID: 9589248 DOI: 10.2337/diacare.21.5.822]

242 **Rheinwald JG**, Green H. Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature* 1977; **265**: 421-424 [PMID: 299924 DOI: 10.1038/265421a0]

243 **McCawley LJ**, O'Brien P, Hudson LG. Epidermal growth factor (EGF)- and scatter factor/hepatocyte growth factor (SF/HGF)- mediated keratinocyte migration is coincident with induction of matrix metalloproteinase (MMP)-9. *J Cell Physiol* 1998; **176**: 255-265 [PMID: 9648913 DOI: 10.1002/(SICI)1097-4652(199808)176:2<255::AID-JCP4>3.0.CO;2-N]

244 **Massagué J**, Pandiella A. Membrane-anchored growth factors. *Annu Rev Biochem* 1993; **62**: 515-541 [PMID: 8394682 DOI: 10.1146/annurev.bi.62.070193.002503]

245 **Martin P**. Wound healing--aiming for perfect skin regeneration. *Science* 1997; **276**: 75-81 [PMID: 9082989 DOI: 10.1126/science.276.5309.75]

246 **Hudson LG**, McCawley LJ. Contributions of the epidermal growth factor receptor to keratinocyte motility. *Microsc Res Tech* 1998; **43**: 444-455 [PMID: 9858341 DOI: 10.1002/(SICI)1097-0029(19981201)43:5<444::AID-JEMT10>3.0.CO;2-C]

247 **Schultz G**, Rotatori DS, Clark W. EGF and TGF-alpha in wound healing and repair. *J Cell Biochem* 1991; **45**: 346-352 [PMID: 2045428 DOI: 10.1002/jcb.240450407]

248 **Wei Y**, Li J, Huang Y, Lei X, Zhang L, Yin M, Deng J, Wang X, Fu X, Wu J. The clinical effectiveness and safety of using epidermal growth factor, fibroblast growth factor and granulocyte-macrophage colony stimulating factor as therapeutics in acute skin wound healing: a systematic review and meta-analysis. *Burns Trauma* 2022; **10**: tkac002 [PMID: 35265723 DOI: 10.1093/burnst/tkac002]

249 **Brown GL**, Nanney LB, Griffen J, Cramer AB, Yancey JM, Curtsinger LJ 3rd, Holtzin L, Schultz GS, Jurkiewicz MJ, Lynch JB. Enhancement of wound healing by topical treatment with epidermal growth factor. *N Engl J Med* 1989; **321**:

76-79 [PMID: 2659995 DOI: 10.1056/NEJM198907133210203]
250 **Zhu J**, Jiang G, Hong W, Zhang Y, Xu B, Song G, Liu T, Hong C, Ruan L. Rapid gelation of oxidized hyaluronic acid and succinyl chitosan for integration with insulin-loaded micelles and epidermal growth factor on diabetic wound healing. *Mater Sci Eng C Mater Biol Appl* 2020; **117**: 111273 [PMID: 32919637 DOI: 10.1016/j.msec.2020.111273]
251 **Jeong S**, Kim B, Park M, Ban E, Lee SH, Kim A. Improved Diabetic Wound Healing by EGF Encapsulation in Gelatin-Alginate Coacervates. *Pharmaceutics* 2020; **12** [PMID: 32276508 DOI: 10.3390/pharmaceutics12040334]
252 **Hu P**, Lei Q, Duan S, Fu Y, Pan H, Chang C, Zheng Z, Wu Y, Zhang Z, Li R, Li YY, Ao N. In-situ formable dextran/chitosan-based hydrogels functionalized with collagen and EGF for diabetic wounds healing. *Biomater Adv* 2022; **136**: 212773 [PMID: 35929312 DOI: 10.1016/j.bioadv.2022.212773]
253 **Lee HJ**, Jeong M, Na YG, Kim SJ, Lee HK, Cho CW. An EGF- and Curcumin-Co-Encapsulated Nanostructured Lipid Carrier Accelerates Chronic-Wound Healing in Diabetic Rats. *Molecules* 2020; **25** [PMID: 33050393 DOI: 10.3390/molecules25204610]
254 **Hong JP**, Jung HD, Kim YW. Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. *Ann Plast Surg* 2006; **56**: 394-8; discussion 399 [PMID: 16557070 DOI: 10.1097/01.sap.0000198731.12407.0c]
255 **Rappolee DA**, Mark D, Banda MJ, Werb Z. Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping. *Science* 1988; **241**: 708-712 [PMID: 3041594 DOI: 10.1126/science.3041594]
256 **Pittelkow MR**, Cook PW, Shipley GD, Derynck R, Coffey RJ Jr. Autonomous growth of human keratinocytes requires epidermal growth factor receptor occupancy. *Cell Growth Differ* 1993; **4**: 513-521 [PMID: 8373735]
257 **Papanas N**, Maltezos E. Growth factors in the treatment of diabetic foot ulcers: new technologies, any promises? *Int J Low Extrem Wounds* 2007; **6**: 37-53 [PMID: 17344201 DOI: 10.1177/1534734606298416]
258 **Coffey RJ Jr**, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR. Production and auto-induction of transforming growth factor-alpha in human keratinocytes. *Nature* 1987; **328**: 817-820 [PMID: 2442615 DOI: 10.1038/328817a0]
259 **Li Y**, Fan J, Chen M, Li W, Woodley DT. Transforming growth factor-alpha: a major human serum factor that promotes human keratinocyte migration. *J Invest Dermatol* 2006; **126**: 2096-2105 [PMID: 16691197 DOI: 10.1038/sj.jid.5700350]
260 **Cha D**, O'Brien P, O'Toole EA, Woodley DT, Hudson LG. Enhanced modulation of keratinocyte motility by transforming growth factor-alpha (TGF-alpha) relative to epidermal growth factor (EGF). *J Invest Dermatol* 1996; **106**: 590-597 [PMID: 8617990 DOI: 10.1111/1523-1747.ep12345083]
261 **Kim I**, Mogford JE, Chao JD, Mustoe TA. Wound epithelialization deficits in the transforming growth factor-alpha knockout mouse. *Wound Repair Regen* 2001; **9**: 386-390 [PMID: 11896982 DOI: 10.1046/j.1524-475x.2001.00386.x]
262 **Brown RL**, Breeden MP, Greenhalgh DG. PDGF and TGF-alpha act synergistically to improve wound healing in the genetically diabetic mouse. *J Surg Res* 1994; **56**: 562-570 [PMID: 8015312 DOI: 10.1006/jsre.1994.1090]
263 **Marikovsky M**, Breuing K, Liu PY, Eriksson E, Higashiyama S, Farber P, Abraham J, Klagsbrun M. Appearance of heparin-binding EGF-like growth factor in wound fluid as a response to injury. *Proc Natl Acad Sci USA* 1993; **90**: 3889-3893 [PMID: 8483908 DOI: 10.1073/pnas.90.9.3889]
264 **Taylor SR**, Markesbery MG, Harding PA. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) and proteolytic processing by a disintegrin and metalloproteinases (ADAM): a regulator of several pathways. *Semin Cell Dev Biol* 2014; **28**: 22-30 [PMID: 24680771 DOI: 10.1016/j.semcdb.2014.03.004]
265 **Hashimoto K**, Higashiyama S, Asada H, Hashimura E, Kobayashi T, Sudo K, Nakagawa T, Damm D, Yoshikawa K, Taniguchi N. Heparin-binding epidermal growth factor-like growth factor is an autocrine growth factor for human keratinocytes. *J Biol Chem* 1994; **269**: 20060-20066 [PMID: 8051092]
266 **Dao DT**, Anez-Bustillos L, Adam RM, Puder M, Bielenberg DR. Heparin-Binding Epidermal Growth Factor-Like Growth Factor as a Critical Mediator of Tissue Repair and Regeneration. *Am J Pathol* 2018; **188**: 2446-2456 [PMID: 30142332 DOI: 10.1016/j.ajpath.2018.07.016]
267 **Johnson NR**, Wang Y. Coacervate delivery of HB-EGF accelerates healing of type 2 diabetic wounds. *Wound Repair Regen* 2015; **23**: 591-600 [PMID: 26032846 DOI: 10.1111/wrr.12319]
268 **Zhang X**, Ibrahimi OA, Olsen SK, Umemori H, Mohammadi M, Ornitz DM. Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. *J Biol Chem* 2006; **281**: 15694-15700 [PMID: 16597617 DOI: 10.1074/jbc.M601252200]
269 **Raja**, Sivamani K, Garcia MS, Isseroff RR. Wound re-epithelialization: modulating keratinocyte migration in wound healing. *Front Biosci* 2007; **12**: 2849-2868 [PMID: 17485264 DOI: 10.2741/2277]
270 **Santoro MM**, Gaudino G. Cellular and molecular facets of keratinocyte reepithelization during wound healing. *Exp Cell Res* 2005; **304**: 274-286 [PMID: 15707592 DOI: 10.1016/j.yexcr.2004.10.033]
271 **Massagué J**. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990; **6**: 597-641 [PMID: 2177343 DOI: 10.1146/annurev.cb.06.110190.003121]
272 **Assoian RK**, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. *J Biol Chem* 1983; **258**: 7155-7160 [PMID: 6602130]
273 **Werner S**, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 2003; **83**: 835-870 [PMID: 12843410 DOI: 10.1152/physrev.2003.83.3.835]
274 **Watters JM**, Blakslee JM, March RJ, Redmond ML. The influence of age on the severity of peritonitis. *Can J Surg* 1996; **39**: 142-146 [PMID: 8769925]
275 **Breuing K**, Andree C, Helo G, Slama J, Liu PY, Eriksson E. Growth factors in the repair of partial thickness porcine skin wounds. *Plast Reconstr Surg* 1997; **100**: 657-664 [PMID: 9283564 DOI: 10.1097/00006534-199709000-00018]
276 **Roberts AB**. Transforming growth factor-beta: activity and efficacy in animal models of wound healing. *Wound Repair Regen* 1995; **3**: 408-418 [PMID: 17147652 DOI: 10.1046/j.1524-475X.1995.30405.x]
277 **Martinez-Ferrer M**, Afshar-Sherif AR, Uwamariya C, de Crombrugghe B, Davidson JM, Bhowmick NA. Dermal transforming growth factor-beta responsiveness mediates wound contraction and epithelial closure. *Am J Pathol* 2010; **176**: 98-107 [PMID: 19959810 DOI: 10.2353/ajpath.2010.090283]

278 **Peplow PV**, Chatterjee MP. A review of the influence of growth factors and cytokines in *in vitro* human keratinocyte migration. *Cytokine* 2013; **62**: 1-21 [PMID: 23490414 DOI: 10.1016/j.cyto.2013.02.015]

279 **Jude EB**, Blakytny R, Bulmer J, Boulton AJ, Ferguson MW. Transforming growth factor-beta 1, 2, 3 and receptor type I and II in diabetic foot ulcers. *Diabet Med* 2002; **19**: 440-447 [PMID: 12060054 DOI: 10.1046/j.1464-5491.2002.00692.x]

280 **Lee PY**, Li Z, Huang L. Thermosensitive hydrogel as a Tgf-beta1 gene delivery vehicle enhances diabetic wound healing. *Pharm Res* 2003; **20**: 1995-2000 [PMID: 14725365 DOI: 10.1023/b:pham.0000008048.58777.da]

281 **Geng K**, Ma X, Jiang Z, Gu J, Huang W, Wang W, Xu Y. WDR74 facilitates TGF-β/Smad pathway activation to promote M2 macrophage polarization and diabetic foot ulcer wound healing in mice. *Cell Biol Toxicol* 2022 [PMID: 35982296 DOI: 10.1007/s10565-022-09748-8]

282 **Mao X**, Li Z, Li B, Wang H. Baicalin regulates mRNA expression of VEGF-c, Ang-1/Tie2, TGF-β and Smad2/3 to inhibit wound healing in streptozotocin-induced diabetic foot ulcer rats. *J Biochem Mol Toxicol* 2021; **35**: e22893 [PMID: 34414639 DOI: 10.1002/jbt.22893]

283 **Nakanishi K**, Uenoyama M, Tomita N, Morishita R, Kaneda Y, Ogihara T, Matsumoto K, Nakamura T, Maruta A, Matsuyama S, Kawai T, Aurues T, Hayashi T, Ikeda T. Gene transfer of human hepatocyte growth factor into rat skin wounds mediated by liposomes coated with the sendai virus (hemagglutinating virus of Japan). *Am J Pathol* 2002; **161**: 1761-1772 [PMID: 12414523 DOI: 10.1016/S0002-9440(10)64453-7]

284 **Matsumoto K**, Nakamura T. Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration. *Biochem Biophys Res Commun* 1997; **239**: 639-644 [PMID: 9367820 DOI: 10.1006/bbrc.1997.7517]

285 **Conway K**, Price P, Harding KG, Jiang WG. The molecular and clinical impact of hepatocyte growth factor, its receptor, activators, and inhibitors in wound healing. *Wound Repair Regen* 2006; **14**: 2-10 [PMID: 16476066 DOI: 10.1111/j.1743-6109.2005.00081.x]

286 **Conway K**, Ruge F, Price P, Harding KG, Jiang WG. Hepatocyte growth factor regulation: an integral part of why wounds become chronic. *Wound Repair Regen* 2007; **15**: 683-692 [PMID: 17971014 DOI: 10.1111/j.1524-475X.2007.00296.x]

287 **Barć P**, Antkiewicz M, Frączkowska-Sioma K, Kupczyńska D, Lubieniecki P, Witkiewicz W, Małodobra-Mazur M, Baczyńska D, Janczak D, Skóra JP. Two-Stage Gene Therapy (VEGF, HGF and ANG1 Plasmids) as Adjunctive Therapy in the Treatment of Critical Lower Limb Ischemia in Diabetic Foot Syndrome. *Int J Environ Res Public Health* 2022; **19** [PMID: 36232122 DOI: 10.3390/ijerph191912818]

288 **Wise BL**, Seidel MF, Lane NE. The evolution of nerve growth factor inhibition in clinical medicine. *Nat Rev Rheumatol* 2021; **17**: 34-46 [PMID: 33219344 DOI: 10.1038/s41584-020-00528-4]

289 **Matsuda H**, Koyama H, Sato H, Sawada J, Itakura A, Tanaka A, Matsumoto M, Konno K, Ushio H, Matsuda K. Role of nerve growth factor in cutaneous wound healing: accelerating effects in normal and healing-impaired diabetic mice. *J Exp Med* 1998; **187**: 297-306 [PMID: 9449710 DOI: 10.1084/jem.187.3.297]

290 **Hasan W**, Zhang R, Liu M, Warn JD, Smith PG. Coordinate expression of NGF and alpha-smooth muscle actin mRNA and protein in cutaneous wound tissue of developing and adult rats. *Cell Tissue Res* 2000; **300**: 97-109 [PMID: 10805079 DOI: 10.1007/s004410000175]

291 **Tanigawa T**, Ahluwalia A, Watanabe T, Arakawa T, Tarnawski AS. Nerve growth factor injected into the gastric ulcer base incorporates into endothelial, neuronal, glial and epithelial cells: implications for angiogenesis, mucosal regeneration and ulcer healing. *J Physiol Pharmacol* 2015; **66**: 617-621 [PMID: 26348086]

292 **Marconi A**, Terracina M, Fila C, Franchi J, Bonté F, Romagnoli G, Maurelli R, Failla CM, Dumas M, Pincelli C. Expression and function of neurotrophins and their receptors in cultured human keratinocytes. *J Invest Dermatol* 2003; **121**: 1515-1521 [PMID: 14675204 DOI: 10.1111/j.1523-1747.2003.12624.x]

293 **Landi F**, Aloe L, Russo A, Cesari M, Onder G, Bonini S, Carbonin PU, Bernabei R. Topical treatment of pressure ulcers with nerve growth factor: a randomized clinical trial. *Ann Intern Med* 2003; **139**: 635-641 [PMID: 14568851 DOI: 10.7326/0003-4819-139-8-200310210-00006]

294 **Gibran NS**, Tamura R, Tsou R, Isik FF. Human dermal microvascular endothelial cells produce nerve growth factor: implications for wound repair. *Shock* 2003; **19**: 127-130 [PMID: 12578120 DOI: 10.1097/00024382-200302000-00007]

295 **Dechant G**, Barde YA. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci* 2002; **5**: 1131-1136 [PMID: 12404007 DOI: 10.1038/nn1102-1131]

296 **Bernabei R**, Landi F, Bonini S, Onder G, Lambiase A, Pola R, Aloe L. Effect of topical application of nerve-growth factor on pressure ulcers. *Lancet* 1999; **354**: 307 [PMID: 10440316 DOI: 10.1016/S0140-6736(99)02784-1]

297 **Ashrafi M**, Baguneid M, Bayat A. The Role of Neuromediators and Innervation in Cutaneous Wound Healing. *Acta Derm Venereol* 2016; **96**: 587-594 [PMID: 26676806 DOI: 10.2340/00015555-2321]

298 **Shi CM**, Qu JF, Cheng TM. Effects of the nerve growth factor on the survival and wound healing in mice with combined radiation and wound injury. *J Radiat Res* 2003; **44**: 223-228 [PMID: 14646225 DOI: 10.1269/jrr.44.223]

299 **Anand P**, Terenghi G, Warner G, Kopelman P, Williams-Chestnut RE, Sinicropi DV. The role of endogenous nerve growth factor in human diabetic neuropathy. *Nat Med* 1996; **2**: 703-707 [PMID: 8640566 DOI: 10.1038/nm0696-703]

300 **Rocco ML**, Soligo M, Manni L, Aloe L. Nerve Growth Factor: Early Studies and Recent Clinical Trials. *Curr Neuropharmacol* 2018; **16**: 1455-1465 [PMID: 29651949 DOI: 10.2174/1570159X16666180412092859]

301 **Generini S**, Tuveri MA, Matucci Cerinic M, Mastinu F, Manni L, Aloe L. Topical application of nerve growth factor in human diabetic foot ulcers. A study of three cases. *Exp Clin Endocrinol Diabetes* 2004; **112**: 542-544 [PMID: 15505764 DOI: 10.1055/s-2004-821313]

302 **Stuard WL**, Titone R, Robertson DM. The IGF/Insulin-IGFBP Axis in Corneal Development, Wound Healing, and Disease. *Front Endocrinol (Lausanne)* 2020; **11**: 24 [PMID: 32194500 DOI: 10.3389/fendo.2020.00024]

303 **Baxter RC**, Martin JL. Radioimmunoassay of growth hormone-dependent insulinlike growth factor binding protein in human plasma. *J Clin Invest* 1986; **78**: 1504-1512 [PMID: 2431001 DOI: 10.1172/JCI112742]

304 **Gartner MH**, Benson JD, Caldwell MD. Insulin-like growth factors I and II expression in the healing wound. *J Surg Res* 1992; **52**: 389-394 [PMID: 1350650 DOI: 10.1016/0022-4804(92)90121-f]

305 **Brown DL**, Kane CD, Chernausek SD, Greenhalgh DG. Differential expression and localization of insulin-like growth

factors I and II in cutaneous wounds of diabetic and nondiabetic mice. *Am J Pathol* 1997; **151**: 715-724 [PMID: 9284820]

306 **Blakytny R**, Jude EB, Martin Gibson J, Boulton AJ, Ferguson MW. Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. *J Pathol* 2000; **190**: 589-594 [PMID: 10727985 DOI: 10.1002/(SICI)1096-9896(200004)190:5<589::AID-PATH553>3.0.CO;2-T]

307 **Bitar MS**, Labbad ZN. Transforming growth factor-beta and insulin-like growth factor-I in relation to diabetes-induced impairment of wound healing. *J Surg Res* 1996; **61**: 113-119 [PMID: 8769952 DOI: 10.1006/jsre.1996.0090]

308 **Bork P**. The modular architecture of a new family of growth regulators related to connective tissue growth factor. *FEBS Lett* 1993; **327**: 125-130 [PMID: 7687569 DOI: 10.1016/0014-5793(93)80155-n]

309 **Daniels A**, van Bilsen M, Goldschmeding R, van der Vusse GJ, van Nieuwenhoven FA. Connective tissue growth factor and cardiac fibrosis. *Acta Physiol (Oxf)* 2009; **195**: 321-338 [PMID: 19040711 DOI: 10.1111/j.1748-1716.2008.01936.x]

310 **Yosimichi G**, Nakanishi T, Nishida T, Hattori T, Takano-Yamamoto T, Takigawa M. CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42 MAPK/extracellular-signal regulated kinase (ERK). *Eur J Biochem* 2001; **268**: 6058-6065 [PMID: 11732999 DOI: 10.1046/j.0014-2956.2001.02553.x]

311 **Bradham DM**, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J Cell Biol* 1991; **114**: 1285-1294 [PMID: 1654338 DOI: 10.1083/jcb.114.6.1285]

312 **Henshaw FR**, Boughton P, Lo L, McLennan SV, Twigg SM. Topically applied connective tissue growth factor/CCN2 improves diabetic preclinical cutaneous wound healing: potential role for CTGF in human diabetic foot ulcer healing. *J Diabetes Res* 2015; **2015**: 236238 [PMID: 25789327 DOI: 10.1155/2015/236238]

313 **Hu X**, Sun H, Han C, Wang X, Yu W. Topically applied rhGM-CSF for the wound healing: a systematic review. *Burns* 2011; **37**: 729-741 [PMID: 20926197 DOI: 10.1016/j.burns.2010.08.016]

314 **Zarei F**, Negahdari B, Eatemadi A. Diabetic ulcer regeneration: stem cells, biomaterials, growth factors. *Artif Cells Nanomed Biotechnol* 2018; **46**: 26-32 [PMID: 28355923 DOI: 10.1080/21691401.2017.1304407]

315 **Gough A**, Clapperton M, Rolando N, Foster AV, Philpott-Howard J, Edmonds ME. Randomised placebo-controlled trial of granulocyte-colony stimulating factor in diabetic foot infection. *Lancet* 1997; **350**: 855-859 [PMID: 9310604 DOI: 10.1016/S0140-6736(97)04495-4]

316 **de Lalla F**, Pellizzer G, Strazzabosco M, Martini Z, Du Jardin G, Lora L, Fabris P, Benedetti P, Erle G. Randomized prospective controlled trial of recombinant granulocyte colony-stimulating factor as adjunctive therapy for limb-threatening diabetic foot infection. *Antimicrob Agents Chemother* 2001; **45**: 1094-1098 [PMID: 11257020 DOI: 10.1128/AAC.45.4.1094-1098.2001]

317 **Laiva AL**, O'Brien FJ, Keogh MB. Innovations in gene and growth factor delivery systems for diabetic wound healing. *J Tissue Eng Regen Med* 2018; **12**: e296-e312 [PMID: 28482114 DOI: 10.1002/term.2443]

318 **Mizuno K**, Yamamura K, Yano K, Osada T, Saeki S, Takimoto N, Sakurai T, Nimura Y. Effect of chitosan film containing basic fibroblast growth factor on wound healing in genetically diabetic mice. *J Biomed Mater Res A* 2003; **64**: 177-181 [PMID: 12483711 DOI: 10.1002/jbm.a.10396]

319 **Obara K**, Ishihara M, Ishizuka T, Fujita M, Ozeki Y, Maehara T, Saito Y, Yura H, Matsui T, Hattori H, Kikuchi M, Kurita A. Photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired db/db mice. *Biomaterials* 2003; **24**: 3437-3444 [PMID: 12809772 DOI: 10.1016/s0142-9612(03)00220-5]

320 **Obara K**, Ishihara M, Fujita M, Kanatani Y, Hattori H, Matsui T, Takase B, Ozeki Y, Nakamura S, Ishizuka T, Tominaga S, Hiroi S, Kawai T, Maehara T. Acceleration of wound healing in healing-impaired db/db mice with a photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2. *Wound Repair Regen* 2005; **13**: 390-397 [PMID: 16008728 DOI: 10.1111/j.1067-1927.2005.130406.x]

321 **Chan RK**, Liu PH, Pietramaggiori G, Ibrahim SI, Hechtman HB, Orgill DP. Effect of recombinant platelet-derived growth factor (Regranex) on wound closure in genetically diabetic mice. *J Burn Care Res* 2006; **27**: 202-205 [PMID: 16566566 DOI: 10.1097/01.BCR.0000202898.11277.58]

322 **Nagato H**, Umebayashi Y, Wako M, Tabata Y, Manabe M. Collagen-poly glycolic acid hybrid matrix with basic fibroblast growth factor accelerated angiogenesis and granulation tissue formation in diabetic mice. *J Dermatol* 2006; **33**: 670-675 [PMID: 17040495 DOI: 10.1111/j.1346-8138.2006.00157.x]

323 **Cheng B**, Liu HW, Fu XB, Sun TZ, Sheng ZY. Recombinant human platelet-derived growth factor enhanced dermal wound healing by a pathway involving ERK and c-fos in diabetic rats. *J Dermatol Sci* 2007; **45**: 193-201 [PMID: 17270401 DOI: 10.1016/j.jdermsci.2006.11.014]

324 **Liu Y**, Cai S, Shu XZ, Shelby J, Prestwich GD. Release of basic fibroblast growth factor from a crosslinked glycosaminoglycan hydrogel promotes wound healing. *Wound Repair Regen* 2007; **15**: 245-251 [PMID: 17352757 DOI: 10.1111/j.1524-475X.2007.00211.x]

325 **Choi JS**, Leong KW, Yoo HS. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). *Biomaterials* 2008; **29**: 587-596 [PMID: 17997153 DOI: 10.1016/j.biomaterials.2007.10.012]

326 **Wang W**, Lin S, Xiao Y, Huang Y, Tan Y, Cai L, Li X. Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblast growth factor in healing-impaired STZ diabetic rats. *Life Sci* 2008; **82**: 190-204 [PMID: 18164317 DOI: 10.1016/j.lfs.2007.11.009]

327 **Dong X**, Xu J, Wang W, Luo H, Liang X, Zhang L, Wang H, Wang P, Chang J. Repair effect of diabetic ulcers with recombinant human epidermal growth factor loaded by sustained-release microspheres. *Sci China C Life Sci* 2008; **51**: 1039-1044 [PMID: 18989647 DOI: 10.1007/s11427-008-0126-5]

328 **Yan X**, Chen B, Lin Y, Li Y, Xiao Z, Hou X, Tan Q, Dai J. Acceleration of diabetic wound healing by collagen-binding vascular endothelial growth factor in diabetic rat model. *Diabetes Res Clin Pract* 2010; **90**: 66-72 [PMID: 20667614 DOI: 10.1016/j.diabres.2010.07.001]

329 **Chu Y**, Yu D, Wang P, Xu J, Li D, Ding M. Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats. *Wound Repair Regen* 2010; **18**: 499-505 [PMID: 20840519

DOI: 10.1111/j.1524-475X.2010.00612.x]

330 **Yang Y**, Xia T, Zhi W, Wei L, Weng J, Zhang C, Li X. Promotion of skin regeneration in diabetic rats by electrospun core-sheath fibers loaded with basic fibroblast growth factor. *Biomaterials* 2011; **32**: 4243-4254 [PMID: 21402405 DOI: 10.1016/j.biomaterials.2011.02.042]

331 **Hardwicke JT**, Hart J, Bell A, Duncan R, Thomas DW, Moseley R. The effect of dextrin-rhEGF on the healing of full-thickness, excisional wounds in the (db/db) diabetic mouse. *J Control Release* 2011; **152**: 411-417 [PMID: 21435363 DOI: 10.1016/j.jconrel.2011.03.016]

332 **Kondo S**, Niiyama H, Yu A, Kuroyanagi Y. Evaluation of a wound dressing composed of hyaluronic acid and collagen sponge containing epidermal growth factor in diabetic mice. *J Biomater Sci Polym Ed* 2012; **23**: 1729-1740 [PMID: 21943516 DOI: 10.1163/092050611X597799]

333 **Yang Y**, Xia T, Chen F, Wei W, Liu C, He S, Li X. Electrospun fibers with plasmid bFGF polyplex loadings promote skin wound healing in diabetic rats. *Mol Pharm* 2012; **9**: 48-58 [PMID: 22091745 DOI: 10.1021/mp200246b]

334 **Kanda N**, Morimoto N, Ayvazyan AA, Takemoto S, Kawai K, Nakamura Y, Sakamoto Y, Taira T, Suzuki S. Evaluation of a novel collagen-gelatin scaffold for achieving the sustained release of basic fibroblast growth factor in a diabetic mouse model. *J Tissue Eng Regen Med* 2014; **8**: 29-40 [PMID: 22628359 DOI: 10.1002/term.1492]

335 **Losi P**, Briganti E, Errico C, Lisella A, Sanguinetti E, Chiellini F, Soldani G. Fibrin-based scaffold incorporating VEGF- and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice. *Acta Biomater* 2013; **9**: 7814-7821 [PMID: 23603001 DOI: 10.1016/j.actbio.2013.04.019]

336 **Gainza G**, Aguirre JJ, Pedraz JL, Hernández RM, Igartua M. rhEGF-loaded PLGA-Alginate microspheres enhance the healing of full-thickness excisional wounds in diabetised Wistar rats. *Eur J Pharm Sci* 2013; **50**: 243-252 [PMID: 23872142 DOI: 10.1016/j.ejps.2013.07.003]

337 **Gainza G**, Pastor M, Aguirre JJ, Villullas S, Pedraz JL, Hernandez RM, Igartua M. A novel strategy for the treatment of chronic wounds based on the topical administration of rhEGF-loaded lipid nanoparticles: In vitro bioactivity and *in vivo* effectiveness in healing-impaired db/db mice. *J Control Release* 2014; **185**: 51-61 [PMID: 24794895 DOI: 10.1016/j.jconrel.2014.04.032]

338 **Lai HJ**, Kuan CH, Wu HC, Tsai JC, Chen TM, Hsieh DJ, Wang TW. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. *Acta Biomater* 2014; **10**: 4156-4166 [PMID: 24814882 DOI: 10.1016/j.actbio.2014.05.001]

339 **Tokatlian T**, Cam C, Segura T. Porous hyaluronic acid hydrogels for localized nonviral DNA delivery in a diabetic wound healing model. *Adv Healthc Mater* 2015; **4**: 1084-1091 [PMID: 25694196 DOI: 10.1002/adhm.201400783]

340 **Chereddy KK**, Lopes A, Koussoroplis S, Payen V, Moia C, Zhu H, Sonveaux P, Carmeliet P, des Rieux A, Vandermeulen G, Préat V. Combined effects of PLGA and vascular endothelial growth factor promote the healing of non-diabetic and diabetic wounds. *Nanomedicine* 2015; **11**: 1975-1984 [PMID: 26238081 DOI: 10.1016/j.nano.2015.07.006]

341 **Almquist BD**, Castleberry SA, Sun JB, Lu AY, Hammond PT. Combination Growth Factor Therapy *via* Electrostatically Assembled Wound Dressings Improves Diabetic Ulcer Healing In Vivo. *Adv Healthc Mater* 2015; **4**: 2090-2099 [PMID: 26270898 DOI: 10.1002/adhm.201500403]

342 **Hajimiri M**, Shahverdi S, Esfandiari MA, Larijani B, Atyabi F, Rajabiani A, Dehpour AR, Amini M, Dinarvand R. Preparation of hydrogel embedded polymer-growth factor conjugated nanoparticles as a diabetic wound dressing. *Drug Dev Ind Pharm* 2016; **42**: 707-719 [PMID: 26289000 DOI: 10.3109/03639045.2015.1075030]

343 **Pyun DG**, Choi HJ, Yoon HS, Thambi T, Lee DS. Polyurethane foam containing rhEGF as a dressing material for healing diabetic wounds: Synthesis, characterization, *in vitro* and *in vivo* studies. *Colloids Surf B Biointerfaces* 2015; **135**: 699-706 [PMID: 26340359 DOI: 10.1016/j.colsurfb.2015.08.029]

344 **Freudenberg U**, Zieris A, Chwalek K, Tsurkan MV, Maitz MF, Atallah P, Levental KR, Eming SA, Werner C. Heparin desulfation modulates VEGF release and angiogenesis in diabetic wounds. *J Control Release* 2015; **220**: 79-88 [PMID: 26478015 DOI: 10.1016/j.jconrel.2015.10.028]

345 **Lee CH**, Liu KS, Chang SH, Chen WJ, Hung KC, Liu SJ, Pang JS, Juang JH, Chou CC, Chang PC, Chen YT, Wang FS. Promoting Diabetic Wound Therapy Using Biodegradable rhPDGF-Loaded Nanofibrous Membranes: CONSORT-Compliant Article. *Medicine (Baltimore)* 2015; **94**: e1873 [PMID: 26632682 DOI: 10.1097/MD.0000000000001873]

346 **Fujita M**, Ishihara M, Shimizu M, Obara K, Nakamura S, Kanatani Y, Morimoto Y, Takase B, Matsui T, Kikuchi M, Maehara T. Therapeutic angiogenesis induced by controlled release of fibroblast growth factor-2 from injectable chitosan/non-anticoagulant heparin hydrogel in a rat hindlimb ischemia model. *Wound Repair Regen* 2007; **15**: 58-65 [PMID: 17244320 DOI: 10.1111/j.1524-475X.2006.00185.x]

347 **Huang C**, Orbay H, Tobita M, Miyamoto M, Tabata Y, Hyakusoku H, Mizuno H. Proapoptotic effect of control-released basic fibroblast growth factor on skin wound healing in a diabetic mouse model. *Wound Repair Regen* 2016; **24**: 65-74 [PMID: 26488443 DOI: 10.1111/wrr.12375]

348 **Miyoshi M**, Kawazoe T, Igawa HH, Tabata Y, Ikada Y, Suzuki S. Effects of bFGF incorporated into a gelatin sheet on wound healing. *J Biomater Sci Polym Ed* 2005; **16**: 893-907 [PMID: 16128295 DOI: 10.1163/1568562054255709]

349 **Richardson TP**, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol* 2001; **19**: 1029-1034 [PMID: 11689847 DOI: 10.1038/nbt1101-1029]

350 **Hong JP**, Kim YW, Lee SK, Kim SH, Min KH. The effect of continuous release of recombinant human epidermal growth factor (rh-EGF) in chitosan film on full thickness excisional porcine wounds. *Ann Plast Surg* 2008; **61**: 457-462 [PMID: 18812721 DOI: 10.1097/SAP.0b013e31815bfeac]

351 **Alemdaroğlu C**, Değim Z, Celebi N, Zor F, Oztürk S, Erdoğan D. An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. *Burns* 2006; **32**: 319-327 [PMID: 16527411 DOI: 10.1016/j.burns.2005.10.015]

352 **Karimi Dehkordi N**, Minaiyan M, Talebi A, Akbari V, Taheri A. Nanocrystalline cellulose-hyaluronic acid composite enriched with GM-CSF loaded chitosan nanoparticles for enhanced wound healing. *Biomed Mater* 2019; **14**: 035003 [PMID: 30690433 DOI: 10.1088/1748-605X/ab026c]

353 **Shamloo A**, Sarmadi M, Aghababaie Z, Vossoughi M. Accelerated full-thickness wound healing *via* sustained bFGF

delivery based on a PVA/chitosan/gelatin hydrogel incorporating PCL microspheres. *Int J Pharm* 2018; **537**: 278-289 [PMID: 29288809 DOI: 10.1016/j.ijpharm.2017.12.045]

354 **Pacelli S**, Acosta F, Chakravarti AR, Samanta SG, Whitlow J, Modaresi S, Ahmed RPH, Rajasingh J, Paul A. Nanodiamond-based injectable hydrogel for sustained growth factor release: Preparation, characterization and *in vitro* analysis. *Acta Biomater* 2017; **58**: 479-491 [PMID: 28532899 DOI: 10.1016/j.actbio.2017.05.026]

355 **Hu Y**, Zhang Z, Li Y, Ding X, Li D, Shen C, Xu FJ. Dual-Crosslinked Amorphous Polysaccharide Hydrogels Based on Chitosan/Alginate for Wound Healing Applications. *Macromol Rapid Commun* 2018; **39**: e1800069 [PMID: 29855096 DOI: 10.1002/marc.201800069]

356 **Liu Q**, Huang Y, Lan Y, Zuo Q, Li C, Zhang Y, Guo R, Xue W. Acceleration of skin regeneration in full-thickness burns by incorporation of bFGF-loaded alginate microspheres into a CMCS-PVA hydrogel. *J Tissue Eng Regen Med* 2017; **11**: 1562-1573 [PMID: 26118827 DOI: 10.1002/term.2057]

357 **Liu LS**, Ng CK, Thompson AY, Poser JW, Spiro RC. Hyaluronate-heparin conjugate gels for the delivery of basic fibroblast growth factor (FGF-2). *J Biomed Mater Res* 2002; **62**: 128-135 [PMID: 12124794 DOI: 10.1002/jbm.10238]

358 **Cai S**, Liu Y, Zheng Shu X, Prestwich GD. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials* 2005; **26**: 6054-6067 [PMID: 15958243 DOI: 10.1016/j.biomaterials.2005.03.012]

359 **Guo Y**, Xu B, Wang Y, Li Y, Si H, Zheng X, Chen Z, Chen F, Fan D. Dramatic promotion of wound healing using a recombinant human-like collagen and bFGF cross-linked hydrogel by transglutaminase. *J Biomater Sci Polym Ed* 2019; **30**: 1591-1603 [PMID: 31411556 DOI: 10.1080/09205063.2019.1652416]

360 **Sun W**, Lin H, Xie H, Chen B, Zhao W, Han Q, Zhao Y, Xiao Z, Dai J. Collagen membranes loaded with collagen-binding human PDGF-BB accelerate wound healing in a rabbit dermal ischemic ulcer model. *Growth Factors* 2007; **25**: 309-318 [PMID: 18236209 DOI: 10.1080/08977190701803885]

361 **He Q**, Zhao Y, Chen B, Xiao Z, Zhang J, Chen L, Chen W, Deng F, Dai J. Improved cellularization and angiogenesis using collagen scaffolds chemically conjugated with vascular endothelial growth factor. *Acta Biomater* 2011; **7**: 1084-1093 [PMID: 20977949 DOI: 10.1016/j.actbio.2010.10.022]

362 **Yoon D**, Yoon D, Cha HJ, Lee JS, Chun W. Enhancement of wound healing efficiency mediated by artificial dermis functionalized with EGF or NRG1. *Biomed Mater* 2018; **13**: 045007 [PMID: 29386409 DOI: 10.1088/1748-605X/aaac37]

363 **Yang CH**. Evaluation of the release rate of bioactive recombinant human epidermal growth factor from crosslinking collagen sponges. *J Mater Sci Mater Med* 2008; **19**: 1433-1440 [PMID: 17914624 DOI: 10.1007/s10856-007-3249-5]

364 **Côté MF**, Laroche G, Gagnon E, Chevallier P, Doillon CJ. Denatured collagen as support for a FGF-2 delivery system: physicochemical characterizations and *in vitro* release kinetics and bioactivity. *Biomaterials* 2004; **25**: 3761-3772 [PMID: 15020152 DOI: 10.1016/j.biomaterials.2003.10.026]

365 **Tanha S**, Rafiee-Tehrani M, Abdollahi M, Vakilian S, Esmaili Z, Naraghi ZS, Seyedjafari E, Javar HA. G-CSF loaded nanofiber/nanoparticle composite coated with collagen promotes wound healing in vivo. *J Biomed Mater Res A* 2017; **105**: 2830-2842 [PMID: 28589686 DOI: 10.1002/jbm.a.36135]

366 **Dogan S**, Demirer S, Kepenekci I, Erkek B, Kiziltay A, Hasirci N, Müftüoglu S, Nazikoglu A, Renda N, Dincer UD, Elhan A, Kuterdem E. Epidermal growth factor-containing wound closure enhances wound healing in non-diabetic and diabetic rats. *Int Wound J* 2009; **6**: 107-115 [PMID: 19432660 DOI: 10.1111/j.1742-481X.2009.00584.x]

367 **Huang S**, Zhang Y, Tang L, Deng Z, Lu W, Feng F, Xu X, Jin Y. Functional bilayered skin substitute constructed by tissue-engineered extracellular matrix and microsphere-incorporated gelatin hydrogel for wound repair. *Tissue Eng Part A* 2009; **15**: 2617-2624 [PMID: 19199780 DOI: 10.1089/ten.TEA.2008.0505]

368 **Li Q**, Niu Y, Diao H, Wang L, Chen X, Wang Y, Dong L, Wang C. In situ sequestration of endogenous PDGF-BB with an ECM-mimetic sponge for accelerated wound healing. *Biomaterials* 2017; **148**: 54-68 [PMID: 28964982 DOI: 10.1016/j.biomaterials.2017.09.028]

369 **Sacchi V**, Mittermayr R, Hartinger J, Martino MM, Lorentz KM, Wolbank S, Hofmann A, Largo RA, Marschall JS, Groppa E, Gianni-Barrera R, Ehrbar M, Hubbell JA, Redl H, Banfi A. Long-lasting fibrin matrices ensure stable and functional angiogenesis by highly tunable, sustained delivery of recombinant VEGF164. *Proc Natl Acad Sci USA* 2014; **111**: 6952-6957 [PMID: 24778233 DOI: 10.1073/pnas.1404605111]

370 **Zhao N**, Coyne J, Xu M, Zhang X, Suzuki A, Shi P, Lai J, Fong GH, Xiong N, Wang Y. Assembly of Bifunctional Aptamer-Fibrinogen Macromer for VEGF Delivery and Skin Wound Healing. *Chem Mater* 2019; **31**: 1006-1015 [PMID: 31558852 DOI: 10.1021/acs.chemmater.8b04486]

371 **Tsang MW**, Wong WK, Hung CS, Lai KM, Tang W, Cheung EY, Kam G, Leung L, Chan CW, Chu CM, Lam EK. Human epidermal growth factor enhances healing of diabetic foot ulcers. *Diabetes Care* 2003; **26**: 1856-1861 [PMID: 12766123 DOI: 10.2337/diacare.26.6.1856]

372 **Morimoto N**, Yoshimura K, Niimi M, Ito T, Aya R, Fujitaka J, Tada H, Teramukai S, Murayama T, Toyooka C, Miura K, Takemoto S, Kanda N, Kawai K, Yokode M, Shimizu A, Suzuki S. Novel collagen/gelatin scaffold with sustained release of basic fibroblast growth factor: clinical trial for chronic skin ulcers. *Tissue Eng Part A* 2013; **19**: 1931-1940 [PMID: 23541061 DOI: 10.1089/ten.tea.2012.0634]

373 **Steed DL**. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. *Plast Reconstr Surg* 2006; **117**: 143S-149S; discussion 150S [PMID: 16799381 DOI: 10.1097/01.prs.0000222526.21512.4c]

374 **UCHI H**, IGARASHI A, URABE K, KOGA T, NAKAYAMA J, KAWAMORI R, TAMAKI K, HIRAKATA H, OHURA T, FURUE M. Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer. *Eur J Dermatol* 2009; **19**: 461-468 [PMID: 19638336 DOI: 10.1684/ejd.2009.0750]

375 **Hanft JR**, Pollak RA, Barbul A, van Gils C, Kwon PS, Gray SM, Lynch CJ, Semba CP, Breen TJ. Phase I trial on the safety of topical rhVEGF on chronic neuropathic diabetic foot ulcers. *J Wound Care* 2008; **17**: 30-32, 34 [PMID: 18210954 DOI: 10.12968/jowc.2008.17.1.27917]

376 **Gomez-Villa R**, Aguilar-Rebolledo F, Lozano-Platonoff A, Teran-Soto JM, Fabian-Victoriano MR, Kresch-Tronik NS, Garrido-Espíndola X, Garcia-Solis A, Bondani-Guasti A, Bierzwinsky-Sneider G, Contreras-Ruiz J. Efficacy of

intralesional recombinant human epidermal growth factor in diabetic foot ulcers in Mexican patients: a randomized double-blinded controlled trial. *Wound Repair Regen* 2014; **22**: 497-503 [PMID: 25041620 DOI: 10.1111/wrr.12187]

377 **Park KH**, Han SH, Hong JP, Han SK, Lee DH, Kim BS, Ahn JH, Lee JW. Topical epidermal growth factor spray for the treatment of chronic diabetic foot ulcers: A phase III multicenter, double-blind, randomized, placebo-controlled trial. *Diabetes Res Clin Pract* 2018; **142**: 335-344 [PMID: 29902542 DOI: 10.1016/j.diabres.2018.06.002]

378 **Viswanathan V**, Juttada U, Babu M. Efficacy of Recombinant Human Epidermal Growth Factor (Regen-D 150) in Healing Diabetic Foot Ulcers: A Hospital-Based Randomized Controlled Trial. *Int J Low Extrem Wounds* 2020; **19**: 158-164 [PMID: 31878810 DOI: 10.1177/1534734619892791]

379 **Moura LI**, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. *Acta Biomater* 2013; **9**: 7093-7114 [PMID: 23542233 DOI: 10.1016/j.actbio.2013.03.033]

380 **Moura LI**, Dias AM, Suesca E, Casadiegos S, Leal EC, Fontanilla MR, Carvalho L, de Sousa HC, Carvalho E. Neurotensin-loaded collagen dressings reduce inflammation and improve wound healing in diabetic mice. *Biochim Biophys Acta* 2014; **1842**: 32-43 [PMID: 24161538 DOI: 10.1016/j.bbadis.2013.10.009]

381 **Raftery RM**, Walsh DP, Castaño IM, Heise A, Duffy GP, Cryan SA, O'Brien FJ. Delivering Nucleic-Acid Based Nanomedicines on Biomaterial Scaffolds for Orthopedic Tissue Repair: Challenges, Progress and Future Perspectives. *Adv Mater* 2016; **28**: 5447-5469 [PMID: 26840618 DOI: 10.1002/adma.201505088]

382 **Byrnes CK**, Khan FH, Nass PH, Hatoum C, Duncan MD, Harmon JW. Success and limitations of a naked plasmid transfection protocol for keratinocyte growth factor-1 to enhance cutaneous wound healing. *Wound Repair Regen* 2001; **9**: 341-346 [PMID: 11896976 DOI: 10.1046/j.1524-475x.2001.00341.x]

383 **Lee PY**, Chesnoy S, Huang L. Electroporatic delivery of TGF-beta1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice. *J Invest Dermatol* 2004; **123**: 791-798 [PMID: 15373787 DOI: 10.1111/j.0022-202X.2004.23309.x]

384 **Marti G**, Ferguson M, Wang J, Byrnes C, Dieb R, Qaiser R, Bonde P, Duncan MD, Harmon JW. Electroporative transfection with KGF-1 DNA improves wound healing in a diabetic mouse model. *Gene Ther* 2004; **11**: 1780-1785 [PMID: 15470477 DOI: 10.1038/sj.gt.3302383]

385 **Yoon CS**, Jung HS, Kwon MJ, Lee SH, Kim CW, Kim MK, Lee M, Park JH. Sonoporation of the minicircle-VEGF(165) for wound healing of diabetic mice. *Pharm Res* 2009; **26**: 794-801 [PMID: 18998201 DOI: 10.1007/s11095-008-9778-x]

386 **Romano Di Peppe S**, Mangoni A, Zambruno G, Spinetti G, Melillo G, Napolitano M, Capogrossi MC. Adenovirus-mediated VEGF(165) gene transfer enhances wound healing by promoting angiogenesis in CD1 diabetic mice. *Gene Ther* 2002; **9**: 1271-1277 [PMID: 12224009 DOI: 10.1038/sj.gt.3301798]

387 **Brem H**, Kodra A, Golinko MS, Entero H, Stojadinovic O, Wang VM, Sheahan CM, Weinberg AD, Woo SL, Ehrlich HP, Tomic-Canic M. Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing. *J Invest Dermatol* 2009; **129**: 2275-2287 [PMID: 19282838 DOI: 10.1038/jid.2009.26]

388 **Keswani SG**, Katz AB, Lim FY, Zoltick P, Radu A, Alaee D, Herlyn M, Crombleholme TM. Adenoviral mediated gene transfer of PDGF-B enhances wound healing in type I and type II diabetic wounds. *Wound Repair Regen* 2004; **12**: 497-504 [PMID: 15453831 DOI: 10.1111/j.1067-1927.2004.12501.x]

389 **Lee JA**, Conejero JA, Mason JM, Parrett BM, Wear-Maggitti KD, Grant RT, Breitbart AS. Lentiviral transfection with the PDGF-B gene improves diabetic wound healing. *Plast Reconstr Surg* 2005; **116**: 532-538 [PMID: 16079687 DOI: 10.1097/01.prs.0000172892.78964.49]

390 **Galeano M**, Deodato B, Altavilla D, Cucinotta D, Arsic N, Marini H, Torre V, Giacca M, Squadrito F. Adeno-associated viral vector-mediated human vascular endothelial growth factor gene transfer stimulates angiogenesis and wound healing in the genetically diabetic mouse. *Diabetologia* 2003; **46**: 546-555 [PMID: 12677400 DOI: 10.1007/s00125-003-1064-1]

391 **Jazwa A**, Kucharzewska P, Leja J, Zagorska A, Sierpniowska A, Stepniewski J, Kozakowska M, Taha H, Ochiya T, Derlacz R, Vahakangas E, Yla-Herttuala S, Jozkowicz A, Dulak J. Combined vascular endothelial growth factor-A and fibroblast growth factor 4 gene transfer improves wound healing in diabetic mice. *Genet Vaccines Ther* 2010; **8**: 6 [PMID: 20804557 DOI: 10.1186/1479-0556-8-6]

392 **Bhattacharyya J**, Mondal G, Madhusudana K, Agawane SB, Ramakrishna S, Gangireddy SR, Madhavi RD, Reddy PK, Konda VR, Rao SR, Udaykumar P, Chaudhuri A. Single subcutaneous administration of RGDK-lipopeptide:rhPDGF-B gene complex heals wounds in streptozotocin-induced diabetic rats. *Mol Pharm* 2009; **6**: 918-927 [PMID: 19388683 DOI: 10.1021/mp800231z]

393 **Kwon MJ**, An S, Choi S, Nam K, Jung HS, Yoon CS, Ko JH, Jun HJ, Kim TK, Jung SJ, Park JH, Lee Y, Park JS. Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer. *J Gene Med* 2012; **14**: 272-278 [PMID: 22407991 DOI: 10.1002/jgm.2618]

394 **Liu L**, Marti GP, Wei X, Zhang X, Zhang H, Liu YV, Nastai M, Semenza GL, Harmon JW. Age-dependent impairment of HIF-1alpha expression in diabetic mice: Correction with electroporation-facilitated gene therapy increases wound healing, angiogenesis, and circulating angiogenic cells. *J Cell Physiol* 2008; **217**: 319-327 [PMID: 18506785 DOI: 10.1002/jcp.21503]

395 **de Felipe P**. Polycistronic viral vectors. *Curr Gene Ther* 2002; **2**: 355-378 [PMID: 12189721 DOI: 10.2174/1566523023347742]

396 **Lv H**, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. *J Control Release* 2006; **114**: 100-109 [PMID: 16831482 DOI: 10.1016/j.jconrel.2006.04.014]

397 **Samal SK**, Dash M, Van Vlierberghe S, Kaplan DL, Chiellini E, van Blitterswijk C, Moroni L, Dubruel P. Cationic polymers and their therapeutic potential. *Chem Soc Rev* 2012; **41**: 7147-7194 [PMID: 22885409 DOI: 10.1039/c2cs35094g]

398 **Brem H**, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest* 2007; **117**: 1219-1222 [PMID: 17476353 DOI: 10.1172/JCI32169]

399 **Lerman OZ**, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *Am J Pathol* 2003; **162**: 303-312 [PMID: 12507913 DOI: 10.1016/S0002-9440(10)63821-7]

400 **Xuan YH**, Huang BB, Tian HS, Chi LS, Duan YM, Wang X, Zhu ZX, Cai WH, Zhu YT, Wei TM, Ye HB, Cong WT, Jin LT. High-glucose inhibits human fibroblast cell migration in wound healing *via* repression of bFGF-regulating JNK phosphorylation. *PLoS One* 2014; **9**: e108182 [PMID: 25244316 DOI: 10.1371/journal.pone.0108182]

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 396-411

DOI: 10.4239/wjd.v14.i4.396

ISSN 1948-9358 (online)

REVIEW

Management of diabesity: Current concepts

Maria Michaelidou, Joseph M Pappachan, Mohammad Sadiq Jeeyavudeen

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Emran TB, Bangladesh; Millman JF, Japan; Peng XC, China

Received: December 25, 2022
Peer-review started: December 25, 2022
First decision: January 9, 2023
Revised: January 15, 2023
Accepted: March 20, 2023
Article in press: March 20, 2023
Published online: April 15, 2023

Maria Michaelidou, Joseph M Pappachan, Department of Endocrinology and Metabolism, Lancashire Teaching Hospitals NHS Trust, Preston PR2 9HT, United Kingdom

Joseph M Pappachan, Faculty of Science, Manchester Metropolitan University, Manchester M15 6BH, United Kingdom

Joseph M Pappachan, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester M13 9PL, United Kingdom

Mohammad Sadiq Jeeyavudeen, Department of Endocrinology & Metabolism, University Hospitals of Edinburgh, Edinburgh EH16 4SA, United Kingdom

Corresponding author: Joseph M Pappachan, FRCP, MD, Academic Editor, Consultant in Endocrinology, Professor, Senior Researcher, Department of Endocrinology and Metabolism, Lancashire Teaching Hospitals NHS Trust, Sharoe Green Lane, Preston PR2 9HT, United Kingdom. drpappachan@yahoo.co.in

Abstract

The global prevalence of obesity is increasing rapidly with an exponential rise in incidence of type 2 diabetes mellitus in recent years. 'Diabesity', the term coined to show the strong interlink between obesity and diabetes, is the direct consequence of the obesity pandemic, and poses significant challenges in the management of the disease. Without addressing the clinical and mechanistic complications of obesity such as metabolic-associated fatty liver disease and obstructive sleep apnoea, a rational management algorithm for diabesity cannot be developed. Several classes of anti-diabetic medications including insulins, sulphonylureas, thiazolidinediones and meglitinides are associated with the risk of weight gain and may potentially worsen diabesity. Therefore, appropriate selection of antidiabetic drug regimen is crucial in the medical management of diabesity. The role of non-pharmacological measures such as dietary adjustments, exercise interventions and bariatric procedures should also be emphasised. Unfortunately, the importance of appropriate and optimal management of diabesity is often overlooked by medical professionals when achieving adequate glycemic control which results in inappropriate management of the disease and its complications. This review provides a narrative clinical update on the evidence behind the management of diabesity.

Key Words: Obesity; Diabesity; Metabolic-associated fatty liver disease; Antidiabetic medications; Glycemic control; Metabolic surgery

Core Tip: The worldwide prevalence of diabesity is increasing exponentially because of the global obesity pandemic in the past few decades. This is also expected to increase the prevalence of other associated chronic disorders such as hypertension, dyslipidemia, metabolic-associated fatty liver disease (MAFLD), obstructive sleep apnoea (OSA), and cardiovascular disease. Rational management of diabesity involves prompt control of diabetes while also optimally addressing other comorbidities such as obesity, hypertension, dyslipidemia, MAFLD, OSA and cardiovascular risk. Appropriate selection of the antidiabetic therapeutic agents and early planning of metabolic surgery when indicated are crucial to scientifically approach patients with diabesity. This evidence-based review addresses the key issues regarding management of diabesity.

Citation: Michaelidou M, Pappachan JM, Jeeyavudeen MS. Management of diabesity: Current concepts. *World J Diabetes* 2023; 14(4): 396-411
URL: https://www.wjgnet.com/1948-9358/full/v14/i4/396.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i4.396

INTRODUCTION

Obesity has become a global pandemic over the past three decades because of the increase in prevalence of adverse lifestyle behaviours such as over-consumption of processed food and physical inactivity in the latter half of twentieth century. Consequently, a tsunami of more than fifty obesity-related problems has emerged which threatens human health and wellbeing on a global level. 800 million people around the world at present live with obesity, with an estimated health expenditure of one trillion United States dollars budgeted for managing obesity-related conditions in the year 2025[1]. There is also an expected increase in childhood obesity to 250 million by the year 2030. Unless there is a massive global action plan from health agencies and administrators to curtail the obesity pandemic, this is likely to continue to significantly impact individuals and healthcare systems.

The worst metabolic consequence of obesity is type 2 diabetes mellitus (T2DM), which substantially increases cardiovascular disease-related morbidity and mortality. Other obesity associated comorbidities such as dyslipidemia, hypertension, metabolic-associated fatty liver disease (MAFLD), obstructive sleep apnoea (OSA), heart failure, ischaemic heart disease, cancers and osteoarthritis further increase the morbidity, mortality, and health-related quality of life, especially when these patients additionally have T2DM. The term "diabesity" was coined by Sims *et al*[2] to describe the very strong pathophysiological link between diabetes and excess body weight in 1970s. Visceral adiposity, which leads to insulin resistance, is the putative mechanism in the development of diabesity. Therefore, an ideal treatment strategy for the disease should also include optimal management of obesity[3-5]. The real-world approach to the management of diabetes often ignores these crucial issues by simply focusing on glycaemic control. This manuscript provides an up-to-date overview of diabesity, in terms of pathophysiology and management. Particularly, we have reviewed and summarised newly published data from clinical trials that have shown the significant impact of glucagon-like peptide-1 (GLP-1) and sodium glucose cotransporter-2 (SGLT-2) inhibitors on diabesity. Therefore, we elaborate the conceptual, pathobiological, mechanistic, and therapeutic aspects of diabesity in this comprehensive review.

PATHOPHYSIOLOGY OF DIABESITY

The link between obesity and diabetes has been described in numerous studies to date. The key pathophysiological process is thought to be the development of insulin resistance, which, in turn, is associated with significant cardiovascular and metabolic morbidities, such as the development of T2DM [6]. Insulin resistance is described as inefficient glucose metabolism despite adequate insulin secretion [7]. In the presence of insulin resistance, a variety of insulin-sensitive tissues including the liver, skeletal muscle, as well as adipose tissue exhibit altered biochemical and cellular pathways[8].

There is a plethora of proposed pathophysiological processes for the development of diabetes in obesity. Perhaps the most described pathophysiological theory is that of chronic inflammation, with adipocytes shown to secrete a variety of pro-inflammatory substances including interleukin-6, tumor necrosis factor alpha and inflammatory cytokines, which, in turn, result in local and systemic inflammatory processes[9]. The resulting oxidative stress in adipocytes has been associated with lower adiponectin secretion, a protein that has been linked to improved glucose regulation and reduced

incidence of metabolic syndrome[10].

Another proposed mechanism is that of excess circulating lipid substrates, such as fatty acids, due to excessive consumption through a high fat diet, which also results in chronic inflammation and oxidative stress[11,12]. A long-term high fat diet may result in lipid deposition in tissues that would not normally be targeted by insulin. Triglyceride accumulation in the liver and skeletal muscle has also been shown to promote insulin resistance through impaired insulin signaling[13]. More recent literature describes the potential association between mitochondrial dysfunction, particularly in skeletal muscle, and diabetes. Mitochondrial dysfunction may be a consequence of pro-inflammatory stress and oxidation, affecting the organelle function leading to insulin resistance, at least in skeletal muscle tissues[14]. Further research is required to characterise the interplay between energy metabolism and the development of diabetes at mitochondrial level.

Recent publications highlight the impact of gut bacteria on obesity and insulin resistance. The gut microbiome has been shown to have a significant impact on nutrient metabolism. Individuals with reduced heterogeneity in their gut microbiome have been found to be at a greater risk of obesity and insulin resistance[15]. Linking to excess fat consumption, research suggests that excess lipid intake may result in bacterial production of short chain fatty acids, which in turn affect energy balance and metabolism[16,17] (Figure 1).

MAJOR COMORBIDITIES TO BE ADDRESSED IN MANAGING DIABESITY

MAFLD

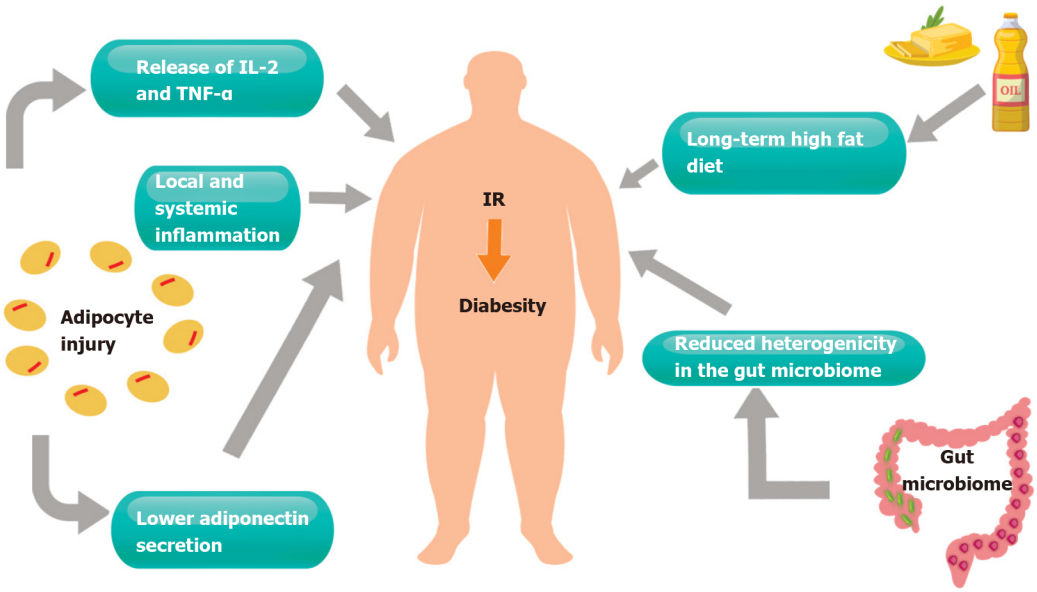
Metabolic dysfunction, and the inflammatory processes in obesity and T2DM, have been closely associated with non-alcoholic fatty liver disease. This has prompted the use of the new term, MAFLD, given the significant link between fatty liver disease and insulin resistance[18,19]. A variety of pathophysiological processes, as described in the previous section, result in excess adipose tissue accumulation in the liver. As a result, hepatic cellular damage, oxidative stress, and insulin resistance develop in such cases. A study investigating the presence of fatty liver disease *vs* simple hepatic steatosis in patients with T2DM or metabolic syndrome, revealed that almost all diabetic patients showed evidence of steatohepatitis on liver biopsy, without necessarily showing derangement in their liver function (98.6% of patients with T2DM *vs* 58.5% of patients with metabolic syndrome, $P < 0.0001$). This suggests that MAFLD may be one of the early end-organ complications of T2DM and metabolic syndrome, with early occult onset and progression without any clinical signs[20]. Hence early identification and management may have a significant impact on mortality and morbidity. MAFLD has also been directly associated with increased cardiovascular morbidity and both micro- and macrovascular complications in diabetic patients, further solidifying the need for early identification and management [21].

OSA and sleep disturbances

OSA involves partial or complete obstruction of the airway during sleep, which results in transient hypoxaemia, sleep restriction and reduction in intrathoracic pressures[22]. These have been linked to the activation of the hypothalamic pituitary adrenal axis resulting in excess stress hormone release and increased cardiovascular morbidity. OSA and hypoxia have also been linked to alterations in adipokine levels as well as oxidative stress. All, in turn, lead to the accumulation of excess adipose tissue and the development insulin resistance[23]. The reverse has also been reported, *i.e.,* OSA could be aggravated by T2DM and obesity[23,24]. One could argue that a patient with both conditions would be at risk of significant morbidity, with diabetes that would be challenging to control without optimising their sleep apnoea. Sleep apnoea and T2DM have both been associated with increased cardiovascular morbidity and mortality, hence a combination of the two may prove more detrimental[25]. Evidence suggests that the prolonged use of continuous positive airway pressure ventilation (CPAP), other than improving sleep quality and reducing hypoxaemia, may also improve glucose tolerance and insulin sensitivity in obese individuals[26-28]. A study of 40 T2DM patients treated with CPAP for 3 mo, assessed the effect of CPAP therapy on insulin resistance using hyperinsulinaemic euglycaemic clamp. Results revealed significant improvement in insulin sensitivity, as early as 2 d after commencing treatment with CPAP (5.75 ± 4.20 *vs* 6.79 ± 4.91 micromol/kg/min at 2 d; $P = 0.003$). The improvement persisted after 3 mo of CPAP therapy[28]. Hence, early identification and treatment of OSA in diabesity patients with CPAP may help to reduce cardiovascular complications and improve glycaemic control.

Dyslipidemia and cardiovascular risk

It has been proposed that diabesity significantly contributes to endothelial damage and heart failure, which are both exacerbated by dyslipidaemia. Through the mechanism of altered mitochondrial energy expenditure as previously described, diabesity may have a direct effect on mitochondrial function in cardiac muscle. This may result in inefficient energy production and expenditure, causing cardiac stress [29]. Additionally, it has been reported that patients with diabetes exhibit cardiac oxidative stress and poor utilisation of energy substrates, in turn causing dysfunction in cardiac muscle contraction[29,30].



DOI: 10.4239/wjd.v14.i4.396

Figure 1 Pathophysiology of diabesity as evidenced by recent literature. IL-2: Interleukin-2; TNF-α: Tumor necrosis alpha; IR: Insulin resistance.

Increased consumption of fats, which results in increased circulatory fatty acids, may also contribute to cardiac steatosis which will also impair cardiac function. Studies using cardiac magnetic resonance (MR) spectroscopy have demonstrated cardiac steatosis, both in patients with diabetes, as well as in those with obesity[31]. Hence diabesity may have a direct link to structural alterations and stress of cardiac muscle, leading to heart failure. This is further supported by the Framingham study, which also demonstrated that an elevated glycated haemoglobin (HbA1c) level was associated with more severe heart failure[32].

Hypertension and coronary artery disease

The association between diabetes, obesity and macrovascular disease has been well established through the years[33,34]. Burke *et al*[34] demonstrated that obese individuals without metabolic complications had a significantly higher coronary calcium score when compared to non-obese patients (17% increase in risk of higher coronary artery calcium scores). This suggests that obesity alone, even without the presence of diabetes, would result in increased coronary artery disease risk. Kronmal *et al*[35] investigated the association between coronary artery calcium progression and a multitude of risk factors which included T2DM in 5756 individuals. Results demonstrated that T2DM, particularly of longer duration, was associated with progression of coronary artery calcium. This suggests that patients with diabetes are at significantly increased risk of myocardial events[35]. Therefore, in the diabesity epidemic, it would be of paramount importance that the patient's cardiovascular risk is stratified and addressed early on, in order to prevent significant cardiovascular events, perhaps with coronary artery calcium assessment. Recent advances in cardiac imaging modalities, including cardiac MR and computed tomography coronary angiography, may allow for early identification and management of coronary artery disease in diabesity patients as shown in recent trials[29,36].

Diabetes-related chronic kidney disease

It has been suggested that the development of diabesity-related kidney disease may be mediated through insulin resistance. Insulin is essential for normal glomerular function and podocyte biology, and dysfunctional insulin signaling has been shown to dysregulate vascular endothelial growth factor A signaling pathway and affects glucose transport[37]. Studies demonstrated podocyte damage, glomerulosclerosis, and albuminuria occur in patients with insulin resistance[38]. In more recent years, studies have also suggested that proximal tubule cells have a role in nutrient regulation in the kidney. Excessive amounts of glucose, fatty acids, and amino acids present in the proximal tubule due to obesity or diabetes, will cause dysregulation in the relevant pathways normally protecting kidney, and consequently result in tubular injury, fibrosis, and inflammation[37-39]. Therefore, medications such as SGLT-2 inhibitors may help to prevent the presence of excessive nutrients in proximal tubule cells, due to induction of glycosuria, and therefore help to minimise tubular damage through this mechanism[40].

MANAGEMENT OF DIABESITY - LIFESTYLE MEASURES

Dietary modifications

As mentioned earlier, T2DM and obesity are inter-linked and share common pathophysiological mechanisms of disease origin, especially adverse lifestyle behaviours. Hence, the first step in the management of diabesity, prior to medical intervention would be to attempt weight loss *via* lifestyle modifications such as changes in diet and exercise. Multiple studies have shown that weight loss can induce remission of T2DM. It has been suggested that weight loss of 15 kg can induce remission of T2DM in around 70% of individuals[41,42]. Achieving weight loss through dietary restriction can be difficult and challenging, and ultimately, it aims to reverse the mechanisms induced by nutrient overconsumption. Therefore, setting realistic stepwise targets may aid patients in achieving sustained weight loss. Smaller degrees of weight loss such as 5 kg have been shown to decrease fasting blood glucose and improve the insulin sensing ability of adipose, skeletal muscle and liver tissues. Stepwise incremental weight loss has been shown to result in a gradual improvement in HbA1c[43].

Larger randomised controlled trials have been conducted in this field, such as the look-AHEAD trial, which demonstrated that remission of diabetes correlated with the degree of weight loss and was inversely correlated with the duration of T2DM[44]. A similar primary care study in the United Kingdom, DiRECT, randomised patients in a control group (standard diabetes care) or a structured weight management program. The weight management program group showed greater weight loss [10 kg *vs* 1 kg, adjusted difference -8.8 kg, 95% confidence interval (CI): -10.3 to -7.3; $P < 0.0001$], greater incidence of diabetes remission (46% *vs* 4%, odds ratio = 19.7, 95%CI: 7.8 to 49.8; $P < 0.0001$), with the degree of weight loss correlating to the improvement in diabetes management[45]. However, large scale trials mainly involved short-term high intensity weight loss programmes. We could argue that a long-term, permanent change in diet would be desired to achieve sustainable benefits in our patient cohort. Ajala *et al*[40] has reviewed the beneficial effects of possible dietary modifications in overweight patients with diabetes. Their study demonstrated that a lower carbohydrate, lower glycaemic index, Mediterranean style diet and high protein diet led to significant improvement in glycaemic control [HbA1c change of -0.12% ($P = 0.04$), -0.14% ($P = 0.008$), -0.47% ($P < 0.00001$), and -0.28% ($P < 0.00001$), respectively][46]. Mediterranean and high protein diets have also been shown to reduce cardiovascular risk in diabetes patients and should be considered[46,47]. More recent research (Ozsoy *et al*[48], 2022) investigated the possibility of modulating gut microbiota through diet, hence altering neurotransmitter pathways of satiety and insulin sensitivity. It was suggested that perhaps the Mediterranean diet would be successful in achieving a varied balance in the gut microbiome.

Physical activity

Regular physical activity, regardless of the associated weight loss, has been shown to improve HbA1c and insulin sensitivity. Exercise comes with a variety of benefits including reduction in visceral adipose tissue, improvement in lipids and blood pressure and hence a reduction in the cardiometabolic risk[49,50]. Effects seem to be proportional to energy expenditure, *i.e.*, total calories burnt, rather than the type of exercise itself[50,51]. Therefore, exercise programmes should be personalised and tailored to patient preference and co-morbidities. Despite the improvement in biochemical parameters, exercise alone does not seem to lead to the remission of diabetes[49-52], therefore a combined diet and exercise programme is recommended in order to achieve the best possible outcome. Results from the Malmö study further support this, where patients on a combined diet and exercise modification programme demonstrated long-term sustainable benefits, with half the T2DM patients in remission at 5 years. Improvement in glycaemic control was proportional to the degree of weight loss ($r = 0.19$, $P < 0.02$) and also to change in fitness levels ($r = 0.22$, $P < 0.02$)[53].

DRUG THERAPY

Metformin

Metformin is one of the first-line medications for the management of T2DM and is known for its good safety profile and low cost. Metformin works by inhibiting hepatic gluconeogenesis, improving insulin sensitivity in skeletal muscle and reduction of appetite, although its mechanism of action is not yet fully understood[54,55]. It is one of the antidiabetic medications recommended globally as first-line therapy for T2DM[56]. Previous studies have demonstrated significant weight loss benefits, ranging between 0.6 to 2.9 kg[5]. Wu *et al*[57] has proposed that, in addition to the known mechanisms of action of metformin, this medication may also positively affect the composition of the gut microbiome and may potentially lead to improved glucose metabolism in the gut, resulting in weight loss. Day *et al*[58] has suggested that metformin also increases the expression of growth differentiating factor 15 (GDF-15), which results in appetite suppression and weight loss. However, more *in vivo* studies are required to further investigate this association. Multiple health benefits have been demonstrated with metformin use, which include reversibility of hepatic steatosis and a lipid lowering effect. It has been suggested

that these are indirect effects of weight loss associated with metformin, rather than a direct effect on hepatic lipid metabolism and storage[59]. A recent review by Lv *et al*[60] looking into the clinical benefits of metformin, demonstrated significant benefits in a variety of health issues including chronic kidney disease, cardiovascular disease and several cancers by preventing growth and metastasis of tumor cells and the effect on the gut microbiome[60-62].

Insulin

Insulin therapy is recommended in patients with significantly elevated HbA1c, where the aim is rapid and effective optimisation of glycaemic control. This may be the case in patients with significant diabetic complications or patients requiring urgent surgical procedures[5]. However, insulin therapy has been associated with significant weight gain, especially when compared to other hypoglycaemic agents. Weight gain ranging between 1.56-5.75 kg has been reported with insulin use, which may make clinicians reluctant to prescribe it in overweight/obese diabetic patients[63]. It has been previously demonstrated that the dose as well as the type of insulin may influence the degree of weight gain in diabetic patients. Greater doses of insulin have been associated with greater weight gain[64]. Basal insulin has been shown to have a lesser effect on weight than pre-mixed insulin[65]. Freemantle *et al*[66] suggested that the use of glargine insulin resulted in significantly less weight change when compared to pre-mixed insulin. Results when using glargine were comparable with detemir, degludec, and neutral protamine Hagedorn insulin. Therefore, careful consideration, with joint decision making between patients and specialists, is recommended when prescribing insulin in patients with diabesity, to minimise the risk of weight gain while achieving the benefits of rapid optimisation of glycaemic control.

Sulphonylureas

Sulfonylureas are generally not recommended in patients with obesity, due to significant risk of hypoglycaemia, weight gain, reduced efficacy, and the need for careful dose titration in patients with renal disease[67]. Hypoglycaemia risk has been reported to vary between sulfonylurea agents, with gliclazide having the lowest reported incidence among this class of medications[68]. Usually prescribed in combination with other agents, sulfonylureas have been reported to result in weight gain of approximately 2.01-2.3 kg[63,69]. Therefore, sulfonylureas appear to pose significant risk of hypoglycaemia, as well as weight gain, and poorer outcomes in patients with diabesity. Furthermore, recent large randomised controlled trials did not show any significant change in cardiovascular risk, in patients treated with sulfonylureas *vs* other oral antihyperglycaemic agents such as pioglitazone or linagliptin [70-72].

Thiazolidinediones

Thiazolidinediones are the only oral antihyperglycaemic agents that specifically target and improve the phenomenon of insulin resistance. Through the activation of the peroxisome proliferator-activated receptor gamma system, they increase glucose utilisation, decrease hepatic steatosis, and decrease circulating free fatty acids[73]. These oral agents, despite having a low risk of hypoglycaemia, and relatively favourable efficacy in HbA1c reduction, have been also associated with significant weight gain between 2.3-4.25 kg[63]. Weight gain in patients on this class of medication is not entirely attributed to the deposition of adipose tissue, as patients may experience fluid retention as a side effect of thiazolidinedione use, which also leads to apparent weight gain[74]. A recent review (Lebovitz *et al* [73]) summarised the different trials looking into the effects of pioglitazone and rosiglitazone on cardiovascular risk. It has been suggested that both medications have been superior to sulfonylureas, with pioglitazone showing a more favourable efficacy and safety profile.

Hesitation to prescribe these agents has also risen from previously reported risk of bladder malignancy, osteoporosis, and heart failure. More recent literature (Lewis *et al*[75]) suggests that these risks may not be as grave as previously thought. Recent retrospective follow-up studies of patients diagnosed with bladder cancer, did not find a significant association between pioglitazone use and the development of bladder malignancy[75,76]. However, the literature relating to thiazolidinedione use and fracture risk confirms increased risk of fractures in female patients, regardless of menopausal status. The TOSCA-IT study did not find a significantly increased risk of fractures when comparing patients on pioglitazone *vs* sulfonylurea therapy[70]. Although it has been proven that thiazolidinediones increase the incidence of heart failure, there is no causal link to progression, severity, or death from heart failure in such patients[73]. Therefore, overall data may suggest superiority of this class of medication over sulfonylureas in patients diabesity especially when they have co-existing MAFLD.

Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4) agents are considered as "weight neutral" agents, with minimal effect on overall body weight[63]. This class of medications works by inhibiting the breakdown of GLP-1 and glucose dependent insulinotropic peptide (GIP) in the gut, maximising the duration of action of these incretin hormones[5]. They have been reported to positively impact HbA1c control, with about an overall 0.75% reduction when prescribed alongside other antihyperglycaemics[77]. This class of medications is not generally used as monotherapy, rather than in combination with other oral

antihyperglycaemic agents. The addition of DPP-4 inhibitors to metformin therapy, showed favourable weight outcomes when compared to the addition of sulfonylureas or thiazolidinedione in recent trials [78]. However, caution needs to be exercised when prescribing this class of medications to patients with cardiovascular disease, as the use of DPP-4 inhibitors has been shown to increase the incidence of hospitalisation from heart failure. Saxagliptin, vidagliptin and alogliptin have all been associated with an increased incidence of hospitalisation for heart failure in diabetic patients[79]. It is thought that clinical deterioration is evident shortly after treatment is initiated, and this requires careful titration and clinical monitoring.

GLP-1 receptor agonists

GLP-1 receptor agonists work by the stimulation of insulin secretion and suppression of glucagon production. Furthermore, they modulate receptors in the gut to delay gastric emptying, as well as neurons involved in appetite regulation. These mechanisms lead to reduced appetite and improved feeling of satiety, hence contributing to weight loss[63]. Current preparations include subcutaneous injections and oral tablets. Injections vary in frequency, from daily (exenatide, lixisenatide, and liraglutide) to once weekly (exenatide-extended release, dulaglutide, albiglutide, and semaglutide). A recently approved oral form of semaglutide is also licensed for use, which has shown comparable effects to the subcutaneous semaglutide preparation[80].

There have been multiple research trials with significant data, surrounding the use of GLP-1 inhibitors in diabetes, as well as obesity alone, in the recent years, as summarised in Table 1. Data has shown an association with significant weight loss, ranging between 1.14-6.9 kg, and an improvement in HbA1c between 1%-2%[63]. High dose liraglutide has been approved for use in the management of obesity. Semaglutide, has also recently been shown to be beneficial in the management obese non-diabetic patients. A recent study (Wilding *et al*[81]) showed that the use of 2.4 mg semaglutide alongside lifestyle intervention, resulted in sustainable weight loss after 68 wk (-14.9% for semaglutide *vs* -2.4% for placebo). However, a significant percentage of patients in the semaglutide group had to discontinue therapy due to side effects (4.5% for semaglutide *vs* 0.8% for placebo). Research has also demonstrated that the improvement in glycaemic control achieved by GLP-1 agonists was comparable to that of insulin[82]. Therefore, GLP-1 agonists should be the first-line injectable therapy for consideration in diabesity patients, with remarkable benefits on body weight while also resulting in improved glycaemic control.

Additionally, GLP-1 agonists have been shown to have significant systemic benefits, including reduction in cardiovascular risk in high-risk patients[83], and reduction in urinary albumin excretion. The 2019 REWIND trial investigated the effect of dulaglutide on renal outcomes, and reported improved morbidity, particularly a reduction in the incidence of new macroalbuminuria, and slower renal function decline[84]. Another trial conducted by Mann *et al*[85], comparing liraglutide with placebo, showed reduced rates of development of renal disease (hazard ratio = 0.78; 95%CI: 0.67 to 0.92; $P = 0.003$), particularly macroalbuminuria (hazard ratio = 0.74; 95%CI: 0.60 to 0.91; $P = 0.004$).

Figure 2 shows the mechanisms of actions of GLP-1 agonists in improving diabesity. A concern with the use of GLP-1 agonists is the high rates of discontinuation by patients, often due to pronounced gastrointestinal side effects of nausea, diarrhoea or vomiting which are commonly reported. Mostly, these are self-limiting presentations that resolve with time[80]. Furthermore, the use of GLP-1 agonists was previously thought to be associated with pancreatitis, and the development of certain types of cancer, such as pancreatic and thyroid cancer[86]. However, a meta-analysis by Abd El Aziz *et al*[87] argues that there is no significant risk of developing pancreatitis or malignancy secondary to GLP-1 agonist therapy. It is also thought that GLP-1 agonists are expected to cause a modest rise in lipase and amylase, which occasionally led to the misdiagnosis of pancreatitis in the previously reported data.

SGLT-2 inhibitors

Use of SGLT-2 inhibitors have been an important area of research in the last few years in many clinical trials, with great developments and changes in diabetes, cardiovascular and renal guidelines recently (Table 2). Their main mechanism of action is the reduction of glucose reabsorption in the renal tubules, resulting in increased glucose excretion in the urine[88]. They have been associated with a reduction in HbA1c of about 0.69% and a weight loss between 0.9-2.5 kg[63]. Weight loss is thought to occur due to caloric deficit from impaired glucose reabsorption from the kidney. Studies have shown improvement in waist circumference, central obesity, and visceral adiposity, all of which are features of diabesity as well as metabolic syndrome[89].

There has been extensive research into the role of SGLT-2 inhibitors in improving diabetes, cardiovascular and renal morbidity. A recent meta-analysis by Giugliano *et al*[90] revealed a reduction in hospitalisation or death from heart failure by 23%, with significantly reduced risk in every trial included. Results were comparable in patients with and without diabetes, indicating that SGLT-2 inhibitors have significant cardiovascular benefits in both groups of patients. Therefore, significant cardiovascular risk may be observed in diabesity patients.

Another meta-analysis of 8 trials, demonstrated the benefits on renal mortality and morbidity through the use of SGLT-2 inhibitors, with an overall reduced risk by 35%[91]. Data was strongest for canagliflozin, with significantly positive results in every trial[90,91]. The EMPEROR-R trial further

Table 1 The benefits of different glucagon-like peptide-1 agonist molecules in the management of diabesity based on recent landmark clinical trials[103]

Drug molecule	Mean weight reduction in kg (95%CI)	Mean % HbA1c reduction (95%CI)	Follow-up period (yr)
Exenatide (weekly)	-1.27 (-1.4 to -1.13)	-0.53 (-0.57 to -0.50)	3.2
Liraglutide	-2.3 (-2.5 to -2.0)	-0.40 (-0.45 to -0.34)	3.8
Lixisenatide	-0.7 (-0.9 to -0.5)	-0.27 (-0.31 to -0.22)	2.1
Albiglutide	-0.83 (-1.06 to -0.60)	-0.52 (-0.58 to -0.45)	1.5
Dulaglutide	-1.46 (-1.67 to -1.25)	-0.61 (-0.65 to -0.58)	5.4
Semaglutide	-2.87 (-3.47 to –2.28) for 0.5 mg	-0.66 (-0.80 to – 0.52) for 0.5 mg	2.0
	-4.5 (-4.94 to -3.75) for 1 mg	-1.05 (-1.19 to -0.91) for 1 mg	

HbA1C: Haemoglobin; CI: Confidence interval.

Table 2 The beneficial effects of different sodium glucose cotransporter-2 inhibitors in the management of diabesity based on recent landmark clinical trials[104-108]

Ref.	Drug molecule	Mean weight reduction in kg (SE/95%CI)	Mean % haemoglobin reduction (95%CI)	Follow-up
Neal *et al*[104], 2017 (CANVAS)	Canagliflozin	-1.60 (- 1.70 to -1.51)	-0.58 (-0.61 to -0.56)	3.5 yr
Wiviott *et al*[105], 2019 (DECLARE)	Dapagliflozin	-1.80 (-2.00 to -1.70)	-0.42 (-0.45 to -0.40)	4.2 yr
Häring *et al*[106], 2014 (EMPA-REG)	Empagliflozin	-1.63 (-2.11 to -1.5) for 10 mg	-0.57 (-0.70 to -0.43) for 10 mg	24 wk
		-2.01 (-2.49 to -1.53) for 25 mg	-0.64 (-0.77 to -0.50) for 25 mg	
Rosenstock *et al*[107], 2018 (VERTIS-MET)	Ertugliflozin	-3.00 (-3.30 to -2.70) for 5 mg	-0.70 (-0.90 to -0.50) for 5 mg	24 wk
		-2.90 (-3.20 to -2.60) for 15 mg	-0.90 (-1.00 to -0.70) for 15 mg	
Dagogo-Jack *et al*[108], 2018 (VERTIS-SITA)	Ertugliflozin	-2.0 (-2.7 to -1.4) for 5 mg	-0.70 (-0.90 to -0.50) for 5 mg	26 wk
		-1.7 (-2.4 to -1.1) for 10 mg	-0.80 (-0.90 to -0.60) for 10 mg	

HbA1C: Haemoglobin; CI: Confidence interval.

demonstrated that empagliflozin improved renal outcomes in patients with reduced ejection fraction or other types of heart failure[92].

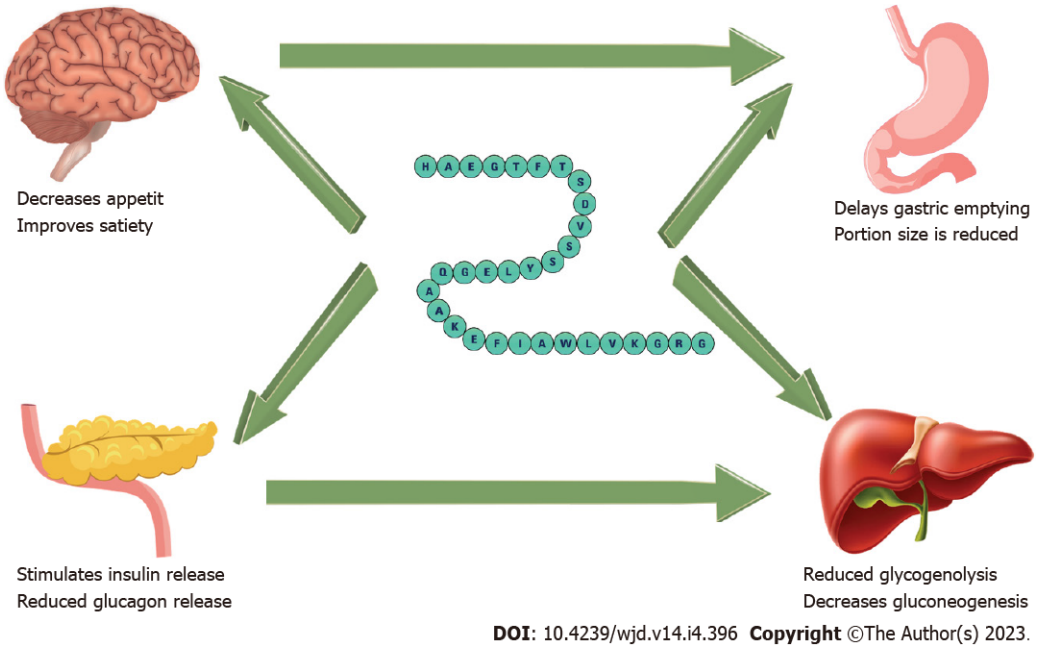
The main risks associated with the use of SGLT-2 inhibitors include increased incidence of urinary tract infections and genital thrush, due to glycosuria, as well as euglycaemic ketoacidosis. However, significant benefits have been reported in obesity, renal and cardiovascular health, making this class of medication one of the most important in the management of diabetes and obesity, whether they are in co-existence or not. Patient counselling will help to identify and manage side effects promptly and efficiently (Figure 3).

Amylin analogues

Amylin is a pancreatic hormone secreted in response to the presence of nutrients in the gut. It modulates calcitonin and amylin receptors, and thereby regulates energy utilisation as well as body weight. This is achieved through the enhancement of satiety and delay in gastric emptying. Treatment with amylin analogues is not currently licensed in England but licensed and used abroad. It involves mealtime injections, similar to insulin[93]. Pramlintide, a type of amylin analogue has been associated with a reduction in HbA1c and also a weight loss of 1.5-2.5 kg[63]. Several studies have shown a significant reduction in body weight, with or without the presence of diabetes. However, most of these trials are pre-clinical and further research is needed to further evaluate the beneficial effects and safety profile of amylin analogues in day-to-day clinical practice[93].

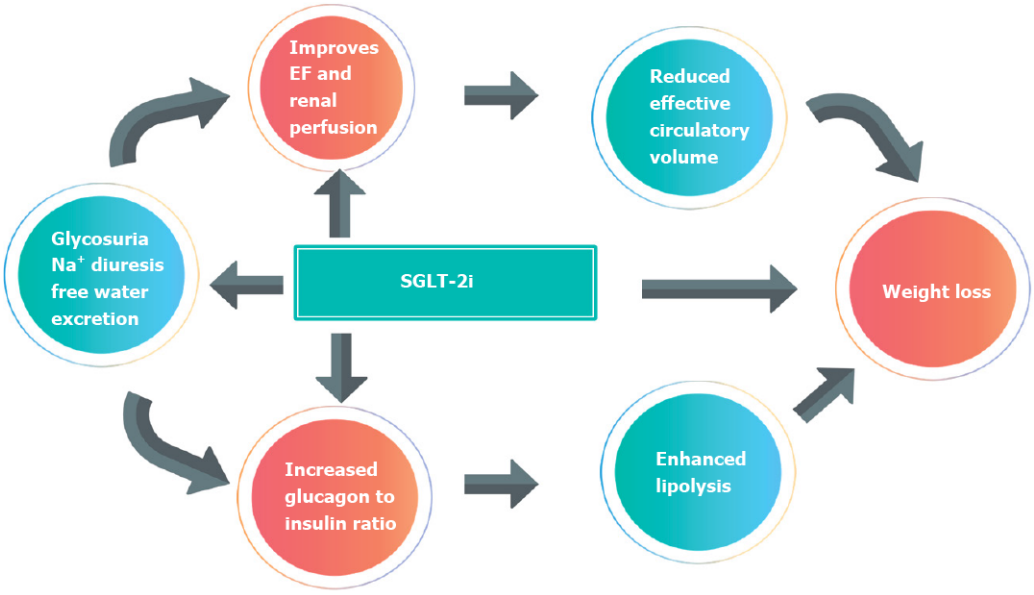
Other anti-obesity drugs

In recent years, diabetic medications have been used more and more in the management of obesity. As discussed above, metformin, GLP-1 analogues and SGLT-2 inhibitors show significant weight loss effects. Amylin analogues, although not globally licensed, also demonstrate a beneficial weight loss



DOI: 10.4239/wjd.v14.i4.396 **Copyright** ©The Author(s) 2023.

Figure 2 The mechanisms of actions of glucagon-like insulinotropic peptide-1 agonists in improving diabesity.



DOI: 10.4239/wjd.v14.i4.396 **Copyright** ©The Author(s) 2023.

Figure 3 Mechanisms of actions of sodium glucose cotransporter-2 inhibitors in improving diabesity and their potential effects on organ protection such as kidney and heart. EF: Ejection fraction; SGLT-2i: Sodium glucose cotransporter-2 inhibitor.

effect. The recent literature mentions the discovery of GLP/GIP/glucagon receptor poly-agonists, with affinity to more than one receptor[94]. While most medications in this category are still in the experimental phase, a GLP1/GIP poly-agonist, Tirzepatide, demonstrated to have significant effects on weight loss in a recent phase 3 trial. This is a medication administered *via* weekly subcutaneous injection. This medication has recently been licensed for use in obese patients with T2DM[95].

Current developments in anti-obesity drugs seem to target leptin, ghrelin, GDF-15 pathways. Leptin sensitisers show promising results in patients with genetic obesity, although there is no significant weight reduction in obese patients without genetic cause, such as most diabesity patients[94]. Combination therapy with amylin analogues (pramlintide) has shown greater effects in weight reduction in obese rats (12.7%, $P < 0.01$)[96]. Hence the role of leptin sensitisers in managing diabesity is currently very limited and requires further investigation. Melanocortin 4 receptors (MC4R), stimulated by leptin to regulate energy expenditure, are other potential targets of novel anti-obesity medications. MC4R agonists, particularly setmelanotide, were licensed in the treatment of genetic obesity, but again,

their effect on polygenic obesity is not clear at this stage[94]. Treatments involving ghrelin modulation, a hormone that stimulates appetite, adipose tissue deposition and insulin secretion, are still under trials with no significant benefits demonstrated so far[94]. GDF-15 has been linked to metabolic disease, cardiovascular disease, and cancer. As mentioned earlier, metformin modulation of GDF-15 levels is a proposed mechanism of weight loss[58]. Exogenous administration of GDF15 has been shown to reduce body weight, however evidence comes from animal studies, therefore further research is required in this area[94].

BARIATRIC PROCEDURES

Bariatric endoscopy

Endoscopic bariatric surgery is a rapidly developing intervention that has revolutionised bariatric surgery. Endoscopic methods target the stomach, and through a variety of techniques aim to restrict gastric capacity, emptying or food absorption[97,98]. Intragastric balloon treatment (IGB) is the most widely used device since its introduction in 1982. IGB is generally recommended for patients with a body mass index (BMI) between 35-40 kg/m^2, or as an interim measure, with an aim for more definitive bariatric surgery, in patients with BMI > 40 kg/m^2. Studies have shown a BMI reduction of 5.7 kg/m^2 and improvement in diabetes and cardiovascular parameters with IGB[99]. Further techniques have been developed more recently, such as endoscopic sleeve surgery, where the capacity of the stomach is reduced. In this procedure, sutures are applied along the gastric wall, with the help of endoscopic equipment. Studies showed a 18% total body weight loss at 24 mo, with relatively low adverse effect profile[100].

Metabolic surgery

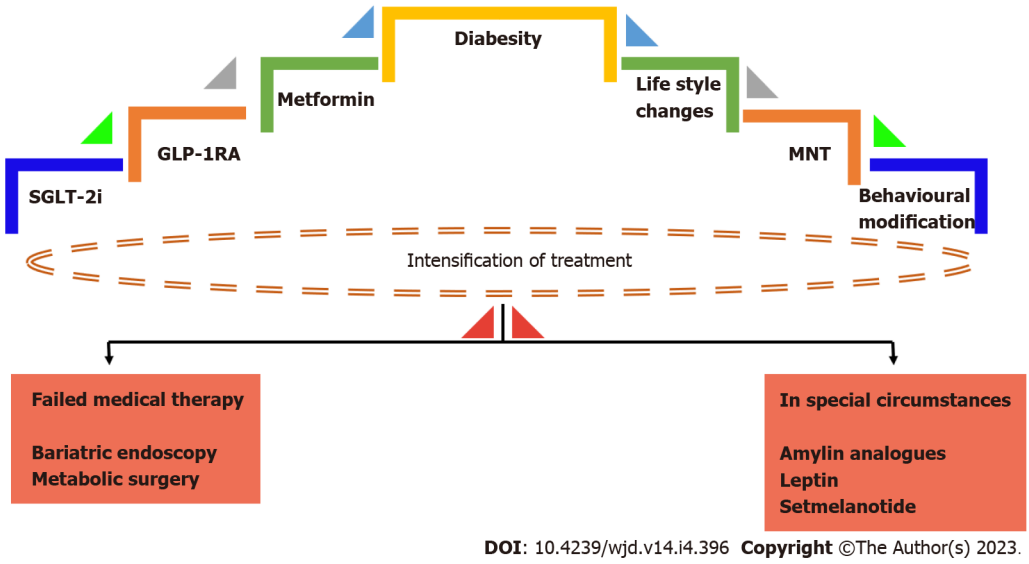
Bariatric surgery continues to be the most effective mode of definitive treatment for marked weight loss and diabesity. A recent review by Roth *et al*[101] reviewed the current literature around weight loss and the side effect profile depending on the type of metabolic surgery. Duodenal switch was associated with the most weight loss, of 70%-80% at 2 years, and resolution of obesity and diabetes related co-morbidities. Roux-en-Y bypass and sleeve gastrectomy showed similar reduction in BMI, ranging between 60%-70%, with studies failing to demonstrate superiority of one over the other. Lower rates of diabetes remission were observed in insulin dependent diabetics following bariatric surgery compared to patients on oral agents, with rates of 16% *vs* 56% at 12 years. In terms of cardiovascular disease, metabolic surgery was shown to improve 5-year survival from coronary artery disease, in diabetic patients who are insulin treated[101]. Robotic assisted surgery is becoming increasingly more prominent, with data so far showing similar outcomes in robotic and laparoscopic bariatric procedures [102].

FUTURE CONSIDERATIONS

The last few years have seen significant developments in the management of obesity and diabetes. Numerous trials revealed the benefits of SGLT-2 inhibitors and GLP-1 agonists in both achieving good glycaemic control as well as weight loss as summarised earlier. Ongoing research and developments in anti-obesity pharmacopeia show promising results, with anti-obesity medication targeting various hormones involved in the pathophysiological process of appetite, satiety, and adipose tissue deposition. This area of research is still in its infancy, with most trials still in the early stages. Studies in human subjects are yet to be developed. Modulation of leptin and ghrelin shows promising results in animal studies and shall be a future topic of research. The new concept of the gut microbiome and its effects on energy expenditure and metabolism is another area where further research is required, in order to further understand its contribution to obesity. With rapidly evolving technology, it is expected that less invasive endoscopic and robotic bariatric techniques will soon be developed for bariatric surgery. An algorithm for management of diabesity is shown in Figure 4.

CONCLUSION

With the obesity epidemic, diabetes, and the evident link between the two, the diabesity, is a widely recognised emerging public health issue. Understanding the pathophysiological mechanisms involved in the interplay between diabetes and obesity is paramount, both for primary as well as secondary care physicians, in order to guide the clinical management of such patients. Our paper offers a summary of the current concepts in the pathophysiology and management of diabesity. The emerging theories around the impact of the gut microbiome on diabetes and obesity are still in their infancy but show promising results so far. The potential role of metformin in modulating the gut microbiome is an area



DOI: 10.4239/wjd.v14.i4.396

Figure 4 An evidence-based management algorithm for the management of diabesity. GLP-1RA: Glucagon-like insulinotropic peptide; MNT: Medical nutrition therapy; SGLT-2i: Sodium glucose cotransporter-2 inhibitor.

that requires further investigation. Future research and better understanding of the involvement of gut bacteria in energy expenditure and the development of diabesity may guide the development of modulatory treatments and reduce risk in affected individuals.

Diabesity is associated with a host of complications, including MAFLD. Our review of literature suggests that the majority of patients with T2DM exhibit hepatic steatosis in the initial stages of their diabetes, without derangement of liver function. Occult liver disease may be difficult to diagnose unless liver biopsy is carried out, and understandably this would not be indicated as a baseline investigation in all obese diabetic patients. Clinicians should be mindful of the likelihood of MAFLD in obese diabetics, and offer management to improve weight and glycaemic control, which will in turn aid to reduce the long-term consequences of hepatic steatosis. Early identification and management of OSA is also important, as intervention in the form of CPAP has been shown to improve insulin resistance. Therefore, early diagnosis and management of OSA may aid glycaemic control, whereas missing this diagnosis may make glycaemic management more challenging, leading to further complications. Additionally, monitoring for cardiovascular disease, potentially with coronary artery calcium score, as well as early cardiology referral where indicated, would be important in order to address and minimize cardiovascular mortality and morbidity.

Lifestyle modification remains paramount, but it is important to set realistic achievable targets which are agreed between the patient and the multi-disciplinary team. From our review, we could infer that exercise alone is not expected to result in significant improvement in diabetic control, unless accompanied by weight loss. On reviewing the recent literature, the common theme appears to be that a Mediterranean diet may be beneficial in achieving weight loss and better glycaemic control in diabesity patients. The Mediterranean diet has been shown to have a greater impact on overall HbA1c when compared to low carbohydrate, high protein, and low fat dietary modifications. When combined with exercise, dietary modification appears to be more effective, with a significant chance of inducing T2DM remission if the patient is motivated and adheres to the interventions offered.

Rapid advances in pharmacotherapy have revealed the dual action of medications such as GLP-1 agonists and SGLT-2 inhibitors in improving diabetes and achieving weight loss. The most recent relevant trials as summarised in our review show promising results, with improvement in both weight and HbA1c with these agents. GLP-1 agonists and SGLT-2 inhibitors are expected to play a pivotal role in the management of diabesity in the coming years, with an increasing number of patients being prescribed these medications.

Newly proposed mechanisms of action of metformin to improve weight and glycaemic control have been published, and these include a potential effect on the gut microbiome, and an association with increased GDF15 levels. Further research is required to confirm these modulatory effects, but metformin remains one of the first line medications for the treatment of diabesity. Rapid advances in technology show a promising future for bariatric surgery, which can be very effective in achieving weight loss and reversing diabetes. Therefore, an early referral to weight management services for patients that fulfil the relevant criteria, and timely intervention could lead to early resolution of the disease.

FOOTNOTES

Author contributions: Michaelidou M substantially contributed to literature search, interpretation of relevant literature, article drafting, and revision; Pappachan JM conceived the idea, contributed to the interpretation of relevant literature, and revision of the paper; Jeeyavudeen MS contributed to the conception and design of the article, literature search and revision of the article created the figures; and all authors have read and approved the final version of the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: United Kingdom

ORCID number: Maria Michaelidou 0000-0002-7657-6626; Joseph M Pappachan 0000-0003-0886-5255; Mohammad Sadiq Jeeyavudeen 0000-0002-1461-0534.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 Obesity fact sheet. World Obesity Day 2022. [cited 3 October 2022]. Available from: https://www.worldobesityday.org/assets/downloads/Obesity_factsheet_one_pager.pdf
2 **Sims EA**, Danforth E Jr, Horton ES, Bray GA, Glennon JA, Salans LB. Endocrine and metabolic effects of experimental obesity in man. *Recent Prog Horm Res* 1973; **29**: 457-496 [PMID: 4750591 DOI: 10.1016/b978-0-12-571129-6.50016-6]
3 **Pappachan JM**, Viswanath AK. Medical Management of Diabesity: Do We Have Realistic Targets? *Curr Diab Rep* 2017; **17**: 4 [PMID: 28101792 DOI: 10.1007/s11892-017-0828-9]
4 **El Khoury L**, Chouillard E, Chahine E, Saikaly E, Debs T, Kassir R. Metabolic Surgery and Diabesity: a Systematic Review. *Obes Surg* 2018; **28**: 2069-2077 [PMID: 29679334 DOI: 10.1007/s11695-018-3252-6]
5 **Pappachan JM**, Fernandez CJ, Chacko EC. Diabesity and antidiabetic drugs. *Mol Aspects Med* 2019; **66**: 3-12 [PMID: 30391234 DOI: 10.1016/j.mam.2018.10.004]
6 **Barazzoni R**, Gortan Cappellari G, Ragni M, Nisoli E. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord* 2018; **23**: 149-157 [PMID: 29397563 DOI: 10.1007/s40519-018-0481-6]
7 **Luzi L**, Petrides AS, De Fronzo RA. Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. *Diabetes* 1993; **42**: 1868-1877 [PMID: 8243833 DOI: 10.2337/diab.42.12.1868]
8 **Wilcox G**. Insulin and insulin resistance. *Clin Biochem Rev* 2005; **26**: 19-39 [PMID: 16278749]
9 **Kern PA**, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995; **95**: 2111-2119 [PMID: 7738178 DOI: 10.1172/JCI117899]
10 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI21625]
11 **Boden G**, Chen X. Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. *J Clin Invest* 1995; **96**: 1261-1268 [PMID: 7657800 DOI: 10.1172/JCI118160]
12 **Boden G**. Obesity, insulin resistance and free fatty acids. *Curr Opin Endocrinol Diabetes Obes* 2011; **18**: 139-143 [PMID: 21297467 DOI: 10.1097/MED.0b013e3283444b09]
13 **Ritter O**, Jelenik T, Roden M. Lipid-mediated muscle insulin resistance: different fat, different pathways? *J Mol Med (Berl)* 2015; **93**: 831-843 [PMID: 26108617 DOI: 10.1007/s00109-015-1310-2]
14 **Barazzoni R**. Skeletal muscle mitochondrial protein metabolism and function in ageing and type 2 diabetes. *Curr Opin Clin Nutr Metab Care* 2004; **7**: 97-102 [PMID: 15090909 DOI: 10.1097/00075197-200401000-00015]
15 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]
16 **Geurts L**, Neyrinck AM, Delzenne NM, Knauf C, Cani PD. Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics. *Benef Microbes* 2014; **5**: 3-17 [PMID: 23886976 DOI: 10.3920/BM2012.0065]
17 **Kimura I**, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi

T, Miyauchi S, Shioi G, Inoue H, Tsujimoto G. The gut microbiota suppresses insulin-mediated fat accumulation *via* the short-chain fatty acid receptor GPR43. *Nat Commun* 2013; **4**: 1829 [PMID: 23652017 DOI: 10.1038/ncomms2852]

18 **Sakurai Y**, Kubota N, Yamauchi T, Kadowaki T. Role of Insulin Resistance in MAFLD. *Int J Mol Sci* 2021; **22** [PMID: 33923817 DOI: 10.3390/ijms22084156]

19 **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]

20 **Masarone M**, Rosato V, Aglitti A, Bucci T, Caruso R, Salvatore T, Sasso FC, Tripodi MF, Persico M. Liver biopsy in type 2 diabetes mellitus: Steatohepatitis represents the sole feature of liver damage. *PLoS One* 2017; **12**: e0178473 [PMID: 28570615 DOI: 10.1371/journal.pone.0178473]

21 **Targher G**, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nat Rev Endocrinol* 2018; **14**: 99-114 [PMID: 29286050 DOI: 10.1038/nrendo.2017.173]

22 **Muraki I**, Wada H, Tanigawa T. Sleep apnea and type 2 diabetes. *J Diabetes Investig* 2018; **9**: 991-997 [PMID: 29453905 DOI: 10.1111/jdi.12823]

23 **Zhang SX**, Khalyfa A, Wang Y, Carreras A, Hakim F, Neel BA, Brady MJ, Qiao Z, Hirotsu C, Gozal D. Sleep fragmentation promotes NADPH oxidase 2-mediated adipose tissue inflammation leading to insulin resistance in mice. *Int J Obes (Lond)* 2014; **38**: 619-624 [PMID: 23897221 DOI: 10.1038/ijo.2013.139]

24 **Resnick HE**, Redline S, Shahar E, Gilpin A, Newman A, Walter R, Ewy GA, Howard BV, Punjabi NM; Sleep Heart Health Study. Diabetes and sleep disturbances: findings from the Sleep Heart Health Study. *Diabetes Care* 2003; **26**: 702-709 [PMID: 12610025 DOI: 10.2337/diacare.26.3.702]

25 **Strand LB**, Carnethon M, Biggs ML, Djoussé L, Kaplan RC, Siscovick DS, Robbins JA, Redline S, Patel SR, Janszky I, Mukamal KJ. Sleep Disturbances and Glucose Metabolism in Older Adults: The Cardiovascular Health Study. *Diabetes Care* 2015; **38**: 2050-2058 [PMID: 26384390 DOI: 10.2337/dc15-0137]

26 **Garcia JM**, Sharafkhaneh H, Hirshkowitz M, Elkhatib R, Sharafkhaneh A. Weight and metabolic effects of CPAP in obstructive sleep apnea patients with obesity. *Respir Res* 2011; **12**: 80 [PMID: 21676224 DOI: 10.1186/1465-9921-12-80]

27 **Trenell MI**, Ward JA, Yee BJ, Phillips CL, Kemp GJ, Grunstein RR, Thompson CH. Influence of constant positive airway pressure therapy on lipid storage, muscle metabolism and insulin action in obese patients with severe obstructive sleep apnoea syndrome. *Diabetes Obes Metab* 2007; **9**: 679-687 [PMID: 17697060 DOI: 10.1111/j.1463-1326.2006.00649.x]

28 **Harsch IA**, Schahin SP, Radespiel-Tröger M, Weintz O, Jahreiss H, Fuchs FS, Wiest GH, Hahn EG, Lohmann T, Konturek PC, Ficker JH. Continuous positive airway pressure treatment rapidly improves insulin sensitivity in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2004; **169**: 156-162 [PMID: 14512265 DOI: 10.1164/rccm.200302-206OC]

29 **Ng ACT**, Delgado V, Borlaug BA, Bax JJ. Diabesity: the combined burden of obesity and diabetes on heart disease and the role of imaging. *Nat Rev Cardiol* 2021; **18**: 291-304 [PMID: 33188304 DOI: 10.1038/s41569-020-00465-5]

30 **Montaigne D**, Marechal X, Coisne A, Debry N, Modine T, Fayad G, Potelle C, El Arid JM, Mouton S, Sebti Y, Duez H, Preau S, Remy-Jouet I, Zerimech F, Koussa M, Richard V, Neviere R, Edme JL, Lefebvre P, Staels B. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation* 2014; **130**: 554-564 [PMID: 24928681 DOI: 10.1161/CIRCULATIONAHA.113.008476]

31 **McGavock JM**, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007; **116**: 1170-1175 [PMID: 17698735 DOI: 10.1161/CIRCULATIONAHA.106.645614]

32 **Kannel WB**, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol* 1974; **34**: 29-34 [PMID: 4835750 DOI: 10.1016/0002-9149(74)90089-7]

33 **Echouffo-Tcheugui JB**, Short MI, Xanthakis V, Field P, Sponholtz TR, Larson MG, Vasan RS. Natural History of Obesity Subphenotypes: Dynamic Changes Over Two Decades and Prognosis in the Framingham Heart Study. *J Clin Endocrinol Metab* 2019; **104**: 738-752 [PMID: 30339231 DOI: 10.1210/jc.2018-01321]

34 **Burke GL**, Bertoni AG, Shea S, Tracy R, Watson KE, Blumenthal RS, Chung H, Carnethon MR. The impact of obesity on cardiovascular disease risk factors and subclinical vascular disease: the Multi-Ethnic Study of Atherosclerosis. *Arch Intern Med* 2008; **168**: 928-935 [PMID: 18474756 DOI: 10.1001/archinte.168.9.928]

35 **Kronmal RA**, McClelland RL, Detrano R, Shea S, Lima JA, Cushman M, Bild DE, Burke GL. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation* 2007; **115**: 2722-2730 [PMID: 17502571 DOI: 10.1161/CIRCULATIONAHA.106.674143]

36 **SCOT-HEART Investigators**, Newby DE, Adamson PD, Berry C, Boon NA, Dweck MR, Flather M, Forbes J, Hunter A, Lewis S, MacLean S, Mills NL, Norrie J, Roditi G, Shah ASV, Timmis AD, van Beek EJR, Williams MC. Coronary CT Angiography and 5-Year Risk of Myocardial Infarction. *N Engl J Med* 2018; **379**: 924-933 [PMID: 30145934 DOI: 10.1056/NEJMoa1805971]

37 **Hinden L**, Kogot-Levin A, Tam J, Leibowitz G. Pathogenesis of diabesity-induced kidney disease: role of kidney nutrient sensing. *FEBS J* 2022; **289**: 901-921 [PMID: 33630415 DOI: 10.1111/febs.15790]

38 **Lavin DP**, White MF, Brazil DP. IRS proteins and diabetic complications. *Diabetologia* 2016; **59**: 2280-2291 [PMID: 27514532 DOI: 10.1007/s00125-016-4072-7]

39 **Cai T**, Ke Q, Fang Y, Wen P, Chen H, Yuan Q, Luo J, Zhang Y, Sun Q, Lv Y, Zen K, Jiang L, Zhou Y, Yang J. Sodium-glucose cotransporter 2 inhibition suppresses HIF-1α-mediated metabolic switch from lipid oxidation to glycolysis in kidney tubule cells of diabetic mice. *Cell Death Dis* 2020; **11**: 390 [PMID: 32444604 DOI: 10.1038/s41419-020-2544-7]

40 **Vallon V**, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, Cunard R, Sharma K, Thomson SC, Rieg T. Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. *Am J Physiol Renal Physiol* 2013; **304**: F156-F167 [PMID: 23152292 DOI: 10.1152/ajprenal.00409.2012]

41 **Hughes TA**, Gwynne JT, Switzer BR, Herbst C, White G. Effects of caloric restriction and weight loss on glycemic

control, insulin release and resistance, and atherosclerotic risk in obese patients with type II diabetes mellitus. *Am J Med* 1984; **77**: 7-17 [PMID: 6377892 DOI: 10.1016/0002-9343(84)90429-7]

42 **Steven S**, Taylor R. Restoring normoglycaemia by use of a very low calorie diet in long- and short-duration Type 2 diabetes. *Diabet Med* 2015; **32**: 1149-1155 [PMID: 25683066 DOI: 10.1111/dme.12722]

43 **Wing RR**, Koeske R, Epstein LH, Nowalk MP, Gooding W, Becker D. Long-term effects of modest weight loss in type II diabetic patients. *Arch Intern Med* 1987; **147**: 1749-1753 [PMID: 3310940]

44 **Gregg EW**, Chen H, Wagenknecht LE, Clark JM, Delahanty LM, Bantle J, Pownall HJ, Johnson KC, Safford MM, Kitabchi AE, Pi-Sunyer FX, Wing RR, Bertoni AG; Look AHEAD Research Group. Association of an intensive lifestyle intervention with remission of type 2 diabetes. *JAMA* 2012; **308**: 2489-2496 [PMID: 23288372 DOI: 10.1001/jama.2012.67929]

45 **Lean ME**, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, Peters C, Zhyzhneuskaya S, Al-Mrabeh A, Hollingsworth KG, Rodrigues AM, Rehackova L, Adamson AJ, Sniehotta FF, Mathers JC, Ross HM, McIlvenna Y, Stefanetti R, Trenell M, Welsh P, Kean S, Ford I, McConnachie A, Sattar N, Taylor R. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet* 2018; **391**: 541-551 [PMID: 29221645 DOI: 10.1016/S0140-6736(17)33102-1]

46 **Ajala O**, English P, Pinkney J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *Am J Clin Nutr* 2013; **97**: 505-516 [PMID: 23364002 DOI: 10.3945/ajcn.112.042457]

47 **Magkos F**, Hjorth MF, Astrup A. Diet and exercise in the prevention and treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* 2020; **16**: 545-555 [PMID: 32690918 DOI: 10.1038/s41574-020-0381-5]

48 **Ozsoy S**, Sultanoglu N, Sanlidag T. The role of Mediterranean diet and gut microbiota in type-2 diabetes mellitus associated with obesity (diabesity). *J Prev Med Hyg* 2022; **63**: E87-E92 [PMID: 36479504 DOI: 10.15167/2421-4248/jpmh2022.63.2S3.2751]

49 **Boulé NG**, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA* 2001; **286**: 1218-1227 [PMID: 11559268 DOI: 10.1001/jama.286.10.1218]

50 **Di Loreto C**, Fanelli C, Lucidi P, Murdolo G, De Cicco A, Parlanti N, Ranchelli A, Fatone C, Taglioni C, Santeusanio F, De Feo P. Make your diabetic patients walk: long-term impact of different amounts of physical activity on type 2 diabetes. *Diabetes Care* 2005; **28**: 1295-1302 [PMID: 15920042 DOI: 10.2337/diacare.28.6.1295]

51 **Balducci S**, Zanuso S, Cardelli P, Salvi L, Bazuro A, Pugliese L, Maccora C, Iacobini C, Conti FG, Nicolucci A, Pugliese G; Italian Diabetes Exercise Study (IDES) Investigators. Effect of high- versus low-intensity supervised aerobic and resistance training on modifiable cardiovascular risk factors in type 2 diabetes; the Italian Diabetes and Exercise Study (IDES). *PLoS One* 2012; **7**: e49297 [PMID: 23185314 DOI: 10.1371/journal.pone.0049297]

52 **Balducci S**, Sacchetti M, Haxhi J, Orlando G, D'Errico V, Fallucca S, Menini S, Pugliese G. Physical exercise as therapy for type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2014; **30** Suppl 1: 13-23 [PMID: 24353273 DOI: 10.1002/dmrr.2514]

53 **Eriksson KF**, Lindgärde F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmö feasibility study. *Diabetologia* 1991; **34**: 891-898 [PMID: 1778354 DOI: 10.1007/BF00400196]

54 **LaMoia TE**, Shulman GI. Cellular and Molecular Mechanisms of Metformin Action. *Endocr Rev* 2021; **42**: 77-96 [PMID: 32897388 DOI: 10.1210/endrev/bnaa023]

55 **Hostalek U**, Gwilt M, Hildemann S. Therapeutic Use of Metformin in Prediabetes and Diabetes Prevention. *Drugs* 2015; **75**: 1071-1094 [PMID: 26059289 DOI: 10.1007/s40265-015-0416-8]

56 **Lazzaroni E**, Ben Nasr M, Loretelli C, Pastore I, Plebani L, Lunati ME, Vallone L, Bolla AM, Rossi A, Montefusco L, Ippolito E, Berra C, D'Addio F, Zuccotti GV, Fiorina P. Anti-diabetic drugs and weight loss in patients with type 2 diabetes. *Pharmacol Res* 2021; **171**: 105782 [PMID: 34302978 DOI: 10.1016/j.phrs.2021.105782]

57 **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]

58 **Day EA**, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow MR, Gutgesell RM, Lu R, Raphenya AR, Kabiri M, McArthur AG, McInnes N, Hess S, Paré G, Gerstein HC, Steinberg GR. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. *Nat Metab* 2019; **1**: 1202-1208 [PMID: 32694673 DOI: 10.1038/s42255-019-0146-4]

59 **Petrie JR**, Chaturvedi N, Ford I, Brouwers MCGJ, Greenlaw N, Tillin T, Hramiak I, Hughes AD, Jenkins AJ, Klein BEK, Klein R, Ooi TC, Rossing P, Stehouwer CDA, Sattar N, Colhoun HM; REMOVAL Study Group. Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind, randomised, placebo-controlled trial. *Lancet Diabetes Endocrinol* 2017; **5**: 597-609 [PMID: 28615149 DOI: 10.1016/S2213-8587(17)30194-8]

60 **Lv Z**, Guo Y. Metformin and Its Benefits for Various Diseases. *Front Endocrinol (Lausanne)* 2020; **11**: 191 [PMID: 32425881 DOI: 10.3389/fendo.2020.00191]

61 **Bell S**, Farran B, McGurnaghan S, McCrimmon RJ, Leese GP, Petrie JR, McKeigue P, Sattar N, Wild S, McKnight J, Lindsay R, Colhoun HM, Looker H. Risk of acute kidney injury and survival in patients treated with Metformin: an observational cohort study. *BMC Nephrol* 2017; **18**: 163 [PMID: 28526011 DOI: 10.1186/s12882-017-0579-5]

62 **Mohan M**, Al-Talabany S, McKinnie A, Mordi IR, Singh JSS, Gandy SJ, Baig F, Hussain MS, Bhalraam U, Khan F, Choy AM, Matthew S, Houston JG, Struthers AD, George J, Lang CC. A randomized controlled trial of metformin on left ventricular hypertrophy in patients with coronary artery disease without diabetes: the MET-REMODEL trial. *Eur Heart J* 2019; **40**: 3409-3417 [PMID: 30993313 DOI: 10.1093/eurheartj/ehz203]

63 **Apovian CM**, Okemah J, O'Neil PM. Body Weight Considerations in the Management of Type 2 Diabetes. *Adv Ther* 2019; **36**: 44-58 [PMID: 30465123 DOI: 10.1007/s12325-018-0824-8]

64 **Lasserson DS**, Glasziou P, Perera R, Holman RR, Farmer AJ. Optimal insulin regimens in type 2 diabetes mellitus: systematic review and meta-analyses. *Diabetologia* 2009; **52**: 1990-2000 [PMID: 19644668 DOI:

10.1007/s00125-009-1468-7]
65 **Valentine V**, Goldman J, Shubrook JH. Rationale for, Initiation and Titration of the Basal Insulin/GLP-1RA Fixed-Ratio Combination Products, IDegLira and IGlarLixi, for the Management of Type 2 Diabetes. *Diabetes Ther* 2017; **8**: 739-752 [PMID: 28721686 DOI: 10.1007/s13300-017-0287-y]
66 **Freemantle N**, Chou E, Frois C, Zhuo D, Lehmacher W, Vlajnic A, Wang H, Chung HW, Zhang Q, Wu E, Gerrits C. Safety and efficacy of insulin glargine 300 u/mL compared with other basal insulin therapies in patients with type 2 diabetes mellitus: a network meta-analysis. *BMJ Open* 2016; **6**: e009421 [PMID: 26880669 DOI: 10.1136/bmjopen-2015-009421]
67 **Schwartz S**, Herman M. Revisiting weight reduction and management in the diabetic patient: Novel therapies provide new strategies. *Postgrad Med* 2015; **127**: 480-493 [PMID: 25913393 DOI: 10.1080/00325481.2015.1043182]
68 **Leiter LA**. Latest Evidence on Sulfonylureas: What's New? *Diabetes Ther* 2020; **11**: 15-22 [PMID: 32323156 DOI: 10.1007/s13300-020-00812-2]
69 **McIntosh B**, Cameron C, Singh SR, Yu C, Ahuja T, Welton NJ, Dahl M. Second-line therapy in patients with type 2 diabetes inadequately controlled with metformin monotherapy: a systematic review and mixed-treatment comparison meta-analysis. *Open Med* 2011; **5**: e35-e48 [PMID: 22046219]
70 **Vaccaro O**, Masulli M, Nicolucci A, Bonora E, Del Prato S, Maggioni AP, Rivellese AA, Squatrito S, Giorda CB, Sesti G, Mocarelli P, Lucisano G, Sacco M, Signorini S, Cappellini F, Perriello G, Babini AC, Lapolla A, Gregori G, Giordano C, Corsi L, Buzzetti R, Clemente G, Di Cianni G, Iannarelli R, Cordera R, La Macchia O, Zamboni C, Scaranna C, Boemi M, Iovine C, Lauro D, Leotta S, Dall'Aglio E, Cannarsa E, Tonutti L, Pugliese G, Bossi AC, Anichini R, Dotta F, Di Benedetto A, Citro G, Antenucci D, Ricci L, Giorgino F, Santini C, Gnasso A, De Cosmo S, Zavaroni D, Vedovato M, Consoli A, Calabrese M, di Bartolo P, Fornengo P, Riccardi G; Thiazolidinediones Or Sulfonylureas Cardiovascular Accidents Intervention Trial (TOSCA. IT) study group; Italian Diabetes Society. Effects on the incidence of cardiovascular events of the addition of pioglitazone versus sulfonylureas in patients with type 2 diabetes inadequately controlled with metformin (TOSCA.IT): a randomised, multicentre trial. *Lancet Diabetes Endocrinol* 2017; **5**: 887-897 [PMID: 28917544 DOI: 10.1016/S2213-8587(17)30317-0]
71 **ADVANCE Collaborative Group**, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; **358**: 2560-2572 [PMID: 18539916 DOI: 10.1056/NEJMoa0802987]
72 **Nath B**, Suvarna VR. CARdiovascular Outcome study of LINAgliptin versus glimepiride in patients with T2D trial. *Perspect Clin Res* 2020; **11**: 44-46 [PMID: 32154149 DOI: 10.4103/picr.PICR_157_19]
73 **Lebovitz HE**. Thiazolidinediones: the Forgotten Diabetes Medications. *Curr Diab Rep* 2019; **19**: 151 [PMID: 31776781 DOI: 10.1007/s11892-019-1270-y]
74 **Fonseca V**. Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am J Med* 2003; **115** Suppl 8A: 42S-48S [PMID: 14678865 DOI: 10.1016/j.amjmed.2003.09.005]
75 **Lewis JD**, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, Ehrlich SF, Mamtani R, Bilker W, Vaughn DJ, Nessel L, Van Den Eeden SK, Ferrara A. Pioglitazone Use and Risk of Bladder Cancer and Other Common Cancers in Persons With Diabetes. *JAMA* 2015; **314**: 265-277 [PMID: 26197187 DOI: 10.1001/jama.2015.7996]
76 **Tuccori M**, Filion KB, Yin H, Yu OH, Platt RW, Azoulay L. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ* 2016; **352**: i1541 [PMID: 27029385 DOI: 10.1136/bmj.i1541]
77 **Sherifali D**, Nerenberg K, Pullenayegum E, Cheng JE, Gerstein HC. The effect of oral antidiabetic agents on A1C levels: a systematic review and meta-analysis. *Diabetes Care* 2010; **33**: 1859-1864 [PMID: 20484130 DOI: 10.2337/dc09-1727]
78 **McIntosh B**, Cameron C, Singh SR, Yu C, Dolovich L, Houlden R. Choice of therapy in patients with type 2 diabetes inadequately controlled with metformin and a sulphonylurea: a systematic review and mixed-treatment comparison meta-analysis. *Open Med* 2012; **6**: e62-e74 [PMID: 23696771]
79 **Packer M**. Do DPP-4 Inhibitors Cause Heart Failure Events by Promoting Adrenergically Mediated Cardiotoxicity? *Circ Res* 2018; **122**: 928-932 [PMID: 29436388 DOI: 10.1161/CIRCRESAHA.118.312673]
80 **Nauck MA**, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. *Mol Metab* 2021; **46**: 101102 [PMID: 33068776 DOI: 10.1016/j.molmet.2020.101102]
81 **Wilding JPH**, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, McGowan BM, Rosenstock J, Tran MTD, Wadden TA, Wharton S, Yokote K, Zeuthen N, Kushner RF; STEP 1 Study Group. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med* 2021; **384**: 989-1002 [PMID: 33567185 DOI: 10.1056/NEJMoa2032183]
82 **Abd El Aziz MS**, Kahle M, Meier JJ, Nauck MA. A meta-analysis comparing clinical effects of short- or long-acting GLP-1 receptor agonists versus insulin treatment from head-to-head studies in type 2 diabetic patients. *Diabetes Obes Metab* 2017; **19**: 216-227 [PMID: 27717195 DOI: 10.1111/dom.12804]
83 **Kristensen SL**, Rørth R, Jhund PS, Docherty KF, Sattar N, Preiss D, Køber L, Petrie MC, McMurray JJV. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol* 2019; **7**: 776-785 [PMID: 31422062 DOI: 10.1016/S2213-8587(19)30249-9]
84 **Gerstein HC**, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, Probstfield J, Botros FT, Riddle MC, Rydén L, Xavier D, Atisso CM, Dyal L, Hall S, Rao-Melacini P, Wong G, Avezum A, Basile J, Chung N, Conget I, Cushman WC, Franek E, Hancu N, Hanefeld M, Holt S, Jansky P, Keltai M, Lanas F, Leiter LA, Lopez-Jaramillo P, Cardona Munoz EG, Pirags V, Pogosova N, Raubenheimer PJ, Shaw JE, Sheu WH, Temelkova-Kurktschiev T; REWIND Investigators. Dulaglutide and renal outcomes in type 2 diabetes: an exploratory analysis of the REWIND randomised, placebo-controlled trial. *Lancet* 2019; **394**: 131-138 [PMID: 31189509 DOI: 10.1016/S0140-6736(19)31150-X]
85 **Mann JFE**, Ørsted DD, Brown-Frandsen K, Marso SP, Poulter NR, Rasmussen S, Tornøe K, Zinman B, Buse JB; LEADER Steering Committee and Investigators. Liraglutide and Renal Outcomes in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 839-848 [PMID: 28854085 DOI: 10.1056/NEJMoa1616011]
86 **Nauck MA**, Friedrich N. Do GLP-1-based therapies increase cancer risk? *Diabetes Care* 2013; **36**: S245-S252 [PMID:

23882053 DOI: 10.2337/dcS13-2004]
87 **Abd El Aziz M**, Cahyadi O, Meier JJ, Schmidt WE, Nauck MA. Incretin-based glucose-lowering medications and the risk of acute pancreatitis and malignancies: a meta-analysis based on cardiovascular outcomes trials. *Diabetes Obes Metab* 2020; **22**: 699-704 [PMID: 31750601 DOI: 10.1111/dom.13924]
88 **Pfister M**, Whaley JM, Zhang L, List JF. Inhibition of SGLT2: a novel strategy for treatment of type 2 diabetes mellitus. *Clin Pharmacol Ther* 2011; **89**: 621-625 [PMID: 21346749 DOI: 10.1038/clpt.2011.16]
89 **Li J**, Gong Y, Li C, Lu Y, Liu Y, Shao Y. Long-term efficacy and safety of sodium-glucose cotransporter-2 inhibitors as add-on to metformin treatment in the management of type 2 diabetes mellitus: A meta-analysis. *Medicine (Baltimore)* 2017; **96**: e7201 [PMID: 28682870 DOI: 10.1097/MD.0000000000007201]
90 **Giugliano D**, Longo M, Scappaticcio L, Bellastella G, Maiorino MI, Esposito K. SGLT-2 inhibitors and cardiorenal outcomes in patients with or without type 2 diabetes: a meta-analysis of 11 CVOTs. *Cardiovasc Diabetol* 2021; **20**: 236 [PMID: 34915880 DOI: 10.1186/s12933-021-01430-3]
91 **Giugliano D**, Longo M, Caruso P, Maiorino MI, Bellastella G, Esposito K. Sodium-glucose co-transporter-2 inhibitors for the prevention of cardiorenal outcomes in type 2 diabetes: An updated meta-analysis. *Diabetes Obes Metab* 2021; **23**: 1672-1676 [PMID: 33710721 DOI: 10.1111/dom.14374]
92 **Packer M**, Butler J, Zannad F, Pocock SJ, Filippatos G, Ferreira JP, Brueckmann M, Jamal W, Zeller C, Wanner C, Anker SD; EMPEROR Study Group. Empagliflozin and Major Renal Outcomes in Heart Failure. *N Engl J Med* 2021; **385**: 1531-1533 [PMID: 34449179 DOI: 10.1056/NEJMc2112411]
93 **Mathiesen DS**, Bagger JI, Knop FK. Long-acting amylin analogues for the management of obesity. *Curr Opin Endocrinol Diabetes Obes* 2022; **29**: 183-190 [PMID: 35066542 DOI: 10.1097/MED.0000000000000716]
94 **Müller TD**, Blüher M, Tschöp MH, DiMarchi RD. Anti-obesity drug discovery: advances and challenges. *Nat Rev Drug Discov* 2022; **21**: 201-223 [PMID: 34815532 DOI: 10.1038/s41573-021-00337-8]
95 **Jastreboff AM**, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, Kiyosue A, Zhang S, Liu B, Bunck MC, Stefanski A; SURMOUNT-1 Investigators. Tirzepatide Once Weekly for the Treatment of Obesity. *N Engl J Med* 2022; **387**: 205-216 [PMID: 35658024 DOI: 10.1056/NEJMoa2206038]
96 **Roth JD**, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, Baron AD. Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. *Proc Natl Acad Sci U S A* 2008; **105**: 7257-7262 [PMID: 18458326 DOI: 10.1073/pnas.0706473105]
97 **Rothstein RI**. Bariatric and Metabolic Endoscopy. *Gastrointest Endosc Clin N Am* 2017; **27**: xv-xvi [PMID: 28292413 DOI: 10.1016/j.giec.2017.01.006]
98 **Li SH**, Wang YJ, Zhang ST. Development of Bariatric and Metabolic Endoscopy. *Chin Med J (Engl)* 2018; **131**: 88-94 [PMID: 29271386 DOI: 10.4103/0366-6999.221283]
99 **Imaz I**, Martínez-Cervell C, García-Alvarez EE, Sendra-Gutiérrez JM, González-Enríquez J. Safety and effectiveness of the intragastric balloon for obesity. A meta-analysis. *Obes Surg* 2008; **18**: 841-846 [PMID: 18459025 DOI: 10.1007/s11695-007-9331-8]
100 **Lopez-Nava G**, Sharaiha RZ, Vargas EJ, Bazerbachi F, Manoel GN, Bautista-Castaño I, Acosta A, Topazian MD, Mundi MS, Kumta N, Kahaleh M, Herr AM, Shukla A, Aronne L, Gostout CJ, Abu Dayyeh BK. Endoscopic Sleeve Gastroplasty for Obesity: a Multicenter Study of 248 Patients with 24 Months Follow-Up. *Obes Surg* 2017; **27**: 2649-2655 [PMID: 28451929 DOI: 10.1007/s11695-017-2693-7]
101 **Roth AE**, Thornley CJ, Blackstone RP. Outcomes in Bariatric and Metabolic Surgery: an Updated 5-Year Review. *Curr Obes Rep* 2020; **9**: 380-389 [PMID: 32607822 DOI: 10.1007/s13679-020-00389-8]
102 **Ecker BL**, Maduka R, Ramdon A, Dempsey DT, Dumon KR, Williams NN. Resident education in robotic-assisted vertical sleeve gastrectomy: outcomes and cost-analysis of 411 consecutive cases. *Surg Obes Relat Dis* 2016; **12**: 313-320 [PMID: 26363712 DOI: 10.1016/j.soard.2015.05.011]
103 **Qin J**, Song L. Glucagon-like peptide-1 (GLP-1) receptor agonists and cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of double-blind, randomized, placebo-controlled clinical trials. *BMC Endocr Disord* 2022; **22**: 125 [PMID: 35546664 DOI: 10.1186/s12902-022-01036-0]
104 **Neal B**, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 644-657 [PMID: 28605608 DOI: 10.1056/NEJMoa1611925]
105 **Wiviott SD**, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, Silverman MG, Zelniker TA, Kuder JF, Murphy SA, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Ruff CT, Gause-Nilsson IAM, Fredriksson M, Johansson PA, Langkilde AM, Sabatine MS; DECLARE–TIMI 58 Investigators. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2019; **380**: 347-357 [PMID: 30415602 DOI: 10.1056/NEJMoa1812389]
106 **Häring HU**, Merker L, Seewaldt-Becker E, Weimer M, Meinicke T, Broedl UC, Woerle HJ; EMPA-REG MET Trial Investigators. Empagliflozin as add-on to metformin in patients with type 2 diabetes: a 24-week, randomized, double-blind, placebo-controlled trial. *Diabetes Care* 2014; **37**: 1650-1659 [PMID: 24722494 DOI: 10.2337/dc13-2105]
107 **Rosenstock J**, Frias J, Páll D, Charbonnel B, Pascu R, Saur D, Darekar A, Huyck S, Shi H, Lauring B, Terra SG. Effect of ertugliflozin on glucose control, body weight, blood pressure and bone density in type 2 diabetes mellitus inadequately controlled on metformin monotherapy (VERTIS MET). *Diabetes Obes Metab* 2018; **20**: 520-529 [PMID: 28857451 DOI: 10.1111/dom.13103]
108 **Dagogo-Jack S**, Liu J, Eldor R, Amorin G, Johnson J, Hille D, Liao Y, Huyck S, Golm G, Terra SG, Mancuso JP, Engel SS, Lauring B. Efficacy and safety of the addition of ertugliflozin in patients with type 2 diabetes mellitus inadequately controlled with metformin and sitagliptin: The VERTIS SITA2 placebo-controlled randomized study. *Diabetes Obes Metab* 2018; **20**: 530-540 [PMID: 28921862 DOI: 10.1111/dom.13116]

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 412-423

DOI: 10.4239/wjd.v14.i4.412

ISSN 1948-9358 (online)

MINIREVIEWS

Treatment on Nature's lap: Use of herbal products in the management of hyperglycemia

Somdatta Giri, Jayaprakash Sahoo, Ayan Roy, Sadishkumar Kamalanathan, Dukhabandhu Naik

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Ariyachet C, Thailand; Zeng Y, China

Received: September 19, 2022
Peer-review started: September 19, 2022
First decision: December 12, 2022
Revised: December 20, 2022
Accepted: January 9, 2023
Article in press: January 9, 2023
Published online: April 15, 2023

Somdatta Giri, Jayaprakash Sahoo, Sadishkumar Kamalanathan, Dukhabandhu Naik, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

Ayan Roy, Department of Endocrinology, All India Institute of Medical Sciences, Kalyani 741245, West Bengal, India

Corresponding author: Jayaprakash Sahoo, MBBS, MD, DM, Additional Professor & Head, 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

Diabetes mellitus (DM) is characterized by persistently elevated blood glucose concentration that lead to multisystem complications. There are about 400 medicinal plants cited to have a beneficial effect on DM. We must choose products wisely based on data derived from scientific studies. However, a major obstacle in the amalgamation of herbal medicine in modern medical practices is the lack of clinical data on its safety, efficacy and drug interaction. Trials of these herbal products often underreport the side effects and other crucial intervention steps deviating from the standards set by Consolidated Standards of Reporting Trials. Due to a lack of knowledge of the active compounds present in most herbal medicines, product standardization is difficult. Cost-effectiveness is another issue that needs to be kept in mind. In this mini-review, we focus on the anti-hyperglycemic effect of herbal products that are commonly used, along with the concerns stated above.

Key Words: Herbal product; HbA1C; Diabetes mellitus; Active compound; Natural therapy; Cost-effectiveness

Core Tip: Diabetes mellitus is an age-old disease. The journey for its remedy came down from nature's lap. Even in today's world, half of diabetes patients have used herbal medicines once in their lifetime. It is important to know the active molecule and its interaction with other drugs, which will help to predict therapeutic efficacy and also to standardize the products. A major hindrance is the lack of clinical data providing its safety and efficacy. This review focuses on the dose and efficacy of herbal products that are commonly used, along with the concerns stated above.

Citation: Giri S, Sahoo J, Roy A, Kamalanathan S, Naik D. Treatment on Nature's lap: Use of herbal products in the management of hyperglycemia. *World J Diabetes* 2023; 14(4): 412-423
URL: https://www.wjgnet.com/1948-9358/full/v14/i4/412.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i4.412

INTRODUCTION

Diabetes mellitus (DM) is an age-old disease. It is characterized by persistently elevated blood glucose concentration that manifests as passing large quantities of sweet-tasting urine. Its existence was found in 1550 BC in an Egyptian papyrus. In India, Charak and Susruta also reported the sweet taste of urine in polyuric states, which attracted ants and other insects in 400-500 BC[1]. Before the advent of allopathic medicine, plants and plant products derived from nature were the only place of trust for people for years. Then modern medicine came in the realm. Interestingly, many allopathic drugs have an herbal background.

Most prescribed drug metformin's origin is linked to *Galega officinalis* which was used as herbal medicine in the 19th century in Europe[2]. This plant is rich in guanidine. In the 1930s, guanidine derivatives were in day to day practice, but because of toxicity, they were removed from the market. Metformin which is a dimethyl-biguanide, re-entered into the clinical practice as an antimalarial agent and presently, after 65 years of its discovery, this drug holds the first place in the management of DM. Similarly, alpha-glucosidase inhibitors like voglibose, acarbose and miglitol are derived from microbes [3]. Phlorizin is a compound found in the bark of the apple tree. In 1835, its glucosuric effect was documented. Many years later, from this observation, sodium-glucose cotransporter-2 (SGLT-2) inhibitors were invented[4]. So the study of natural products is expected to open the door for the development of novel drugs in the modern management of diabetes in the future.

Nevertheless, some areas of concern need to be addressed while using herbal products. These are the potential side effects, drug interactions, lack of product standardization and increasing cost. A major obstacle in the amalgamation of herbal medicine in modern medical practices is the lack of clinical data on its safety and efficacy. There is a need to conduct randomized control trials (RCTs) comparing herbal drugs with existing oral hypoglycemic drugs (OHAs). There are about 400 medicinal plants claimed to have beneficial effect in DM. Many of those products have overall health benefits but minimal effect on hyperglycemia. Here in this mini-review, we focus on the anti-hyperglycemic effect of herbal products that are commonly used, along with the concerns stated above.

SEARCH STRATEGY

Two authors (Giri S and Roy A) conducted the initial search in both 'PubMed database', and 'Google Scholar' for relevant articles. The references of these articles were also searched for additional relevant studies. The keywords used in the search were: 'Herbal Products'; 'HbA1C'; 'Natural treatment'; 'phytochemicals'; 'Fasting blood glucose'; 'HOMA-IR'. Only publications in English language were included. Kamalanathan S, Naik D and Sahoo J selected the relevant articles to be included.

COMMONLY USED HERBAL PRODUCTS

The prevalence of herbal medicine use among diabetes patients is reported to be as high as 58.5%[5]. Plant products have been used as herbal remedies individually or in formulations. GlycaCare-II is an herbal formulation supplement for subjects with DM that contains cinnamon, bitter melon, vijayasar, gurmar, jamun extract and a bioavailability enhancer piperine. Majeed *et al*[6] showed that this formulation has similar potential as metformin in the reduction of glycated hemoglobin (HbA1c) and fasting blood sugar (FBS). Diabecon, diasulin, and pancreatic tonics are similar formulated herbal drugs commonly available in market. However, one of the fundamental issues with these herbal formulations is that active ingredients are not precisely defined. But it is essential to define the active compounds to

determine the therapeutic efficacy and standardization of a product.

Most commonly used plants in the formulated herbal drugs are cinnamon gurmar, cumin, black cumin, psyllium, sesame, barberry, aloe, vijayasar, bael, fenugreek, jamun, ginger, little gourd, neem, sweet potato, amla, bitter melons, garlic, turmeric, guduchi. Dose, efficacy, active molecules, cost of these herbal products are described in Table 1.

MECHANISM OF ACTION

Active compound of great interest are quercetin, palmatin, berberin, gymnemic, gurmarin, phlorizin, kaempferol, rosmarinic acid, cyanidin, rutin, catechin and ellagic acid[7]. Various natural products of plant origin have been reported to target on multiple sites (Figure 1).

NATURAL PRODUCTS WITH STRONG EVIDENCE AS ANTI-HYPERGLYCEMIC REMEDY

These are discussed in a decreasing order of their efficacy.

Cinnamon (cinnamomum)

Cinnamon's dried bark is used as a spice for many recipes. Since time immemorial, it has been an essential traditional medicine against diabetes. Its bark powder is sold in the hard gelatine capsule formulation. Sharma *et al*[8] from India, in a double blind RCT, studied the effect of 3 g and 6 g of cinnamon powder in 150 newly diagnosed diabetes individuals who were OHA naïve. Over 3 mo, significant glycemic control status (mean FBS fall 111 mg/dL and mean HbA1c fall 1.2%) was achieved in the cinnamon group. Similarly, a study from Iran compared the effect of cinnamon powder with probiotics (*Lactobacillus acidophilus*) on blood glucose in 136 patients[9]. A significant drop in FBS (33 mg/dL) was observed in them who consumed both cinnamon powder and probiotics. It is possible that cinnamon brings out the effect by modification of gut microbiome because its active ingredient cinnamaldehyde has anti-microbial properties.

On the contrary, a significant effect on blood glucose (fall by 11 mg/dL) was not observed in another Iranian RCT where 3 g of cinnamon powder was used over a period of 2 mo[10]. Similarly, Hasanzade *et al*[11] concluded that 1 g of cinnamon had no effect compared to placebo on glycemic control over one month. The heterogeneity of these results might be due to the variable duration of follow-up or due to differences in ethnicity and body composition of study participants. A study was designed to evaluate the glucose lowering efficacy of cinnamon with different body mass index (BMI) where 140 diabetes patients were randomly assigned to 4 groups: Cinnamon (BMI ≥ 27, BMI < 27) and placebo (BMI ≥ 27, BMI < 27)[12]. At the end of 3 mo, glycemic outcomes were significantly more prominent (FBS fall by 20 mg/dL *vs* 6 mg/dL, $P < 0.001$) in patients with higher BMI in cinnamon group. When adjusted for BMI, visceral fat and homeostatic model assessment for insulin resistance (HOMA-IR) index by univariate model analysis, it was evident that glycemic outcomes were mediated through the decrement in insulin resistance. Therefore, it can be concluded that cinnamon has an antidiabetic effect. It acts though modification of gut microbiota and more of use in patients with higher BMI.

Gurmar (gymnema)

It is a perennial climber found in India and Africa. Various studies used 0.4-10 g/d gymnema leaf powder. It is available in tablet or capsule form. Its active compound, gymnemic acid is believed to block glucose absorption in the small intestine[13]. Gurmarin, another molecule in this product, has anti-sweetener activity. Li *et al*[14] from Pakistan demonstrated the effect of 1g powder on 32 middle aged diabetic patients in a non-randomized trial. After one month, mean FBS decreased by 81 mg/dL. Fall in HbA1C ranges from 0.32% to 1.57% in different studies[15,16].

Cumin (cuminum)

The effect of 100-500 mg of cumin on FBS reduction ranges from 3 mg/dL to 56 mg/dL[17,18]. The formulation used are oil or powder form. On the other hand, 2-3 g of black cumin powder can reduce FBS by 17-23 mg/dL and HbA1C by 0.3%-0.6%[19,20]. Both types of cumin have thymoquinone as their active component which acts as an antioxidant.

Psyllium (plantago)

Psyllium husk 6.8-10.5 g/d consumption reduces FBS from 20-53 mg/dL and HbA1C from 1%-1.6% with a significant decrement of insulin resistance (HOMA-IR reduction of 5.5)[21,22]. In a meta-analysis by Xiao *et al*[23], there were no major side effects on psyllium consumption. Water soluble fiber makes a barrier in the intestine for the absorption of glucose and other reducing sugars. It also influences release of various gut peptide, especially ghrelin and peptide YY and insulin. Alteration of gut flora is another possible mechanism that helps to control hyperglycemia. As in most of the studies, psyllium is found to

Table 1 Description of plant products

Herbal product	Active molecule	Part of plant	Dose	FBS fall (mg/dL)	HbA1C fall (%)	Cost (Rs.)	Ref.
Cinnamon	Procyanidin type A polimer	Bark	0.5-6 g	2-111	0-1.2	811/400 g	[8,11]
Gurmar	Gymnemic acid	Leaf	0.4-10 g	6-81	0.3-1.5	799/60 cap	[15,16]
Cumin	Thymoquinone	Seed	0.1-0.5 g	3-56	1-1.8	99/100 g	[17,18]
Psyllium	NA	Husk	6.8-10.5 g	20-53	1-1.6	105/100 g	[21,22]
Sesame	Sesamine	Seed	0.2 g or 30 mL	34-52	0.7-1.1	399/1 kg	[24,25]
Barberry	Berberine	Root, stem, bark	0.9-1.5 g	57-68	1.4-1.9	810/60 cap	[28]
Aloe	Acemannan	Leaf	600-1000 g	13-44	0.4-0.7	121/100 g	[29]
Vijayasar	Epicatechin	Bark	1-4 g	32-43	0.4	365/1 kg	[30]
Fenugreek	4-OH isoleucin	Seed	1-100 g	15-41	0.2-1.5	199/200 g	[31,32]
Bael	Aegeline	Seed, leaf, fruit pulp	5-20 g	34-41	1.9	325/400 g	[34,35]
Jamun	Gallic acid	Seed	10 g	18-33	0.4-0.6	265/250 g	[37]
Ginger	Gingerol, shogaol	Rhizome	1.6-2 g	10-29	0.04-1.1	400/300 g	[38]
Little gourd	Pectin	Leaf, fruit	0.5-1 g	20-25	0.6-0.7	NA	[42]
Neem	Nimbidiol	Root, bark	1 g	23	1.5	119/100 g	[44]
Sweet potato	Anthocyanins	Leaf, tuber	4 g	10-19	0.2-0.3	NA	[45]
Amla	Gallic acid	Fruit	0.5-10 g	13-15	0.4-0.5	209/200 g	[46,47]
Bitter melon	Momordin, charantin	Whole plant	0.8-4 g	5-15	0.3	399/500 g	[51]
Garlic	Allicin	Bulb	0.9-1.5 g	4-10	0.2-0.8	485/400 g	[54]
Turmeric	Curcumin	Rhizome	0.5-2.1 g	2-9	0.02-0.9	599/500 g	[55]
Guduchi	Palmatine	Stem	0.5-1.5 g	5-8	0.2-0.5	150/60 cap	[57]
Jack fruit	Proanthocyanidin, flavonoids	Leaf	30 g	29	0.25	266/400 g	[62]

FBS: Fasting blood sugar; NA: Not available.

have a consistent effect on glycemic control, which can be considered a good alternative for diabetes management.

Sesame (sesamum)

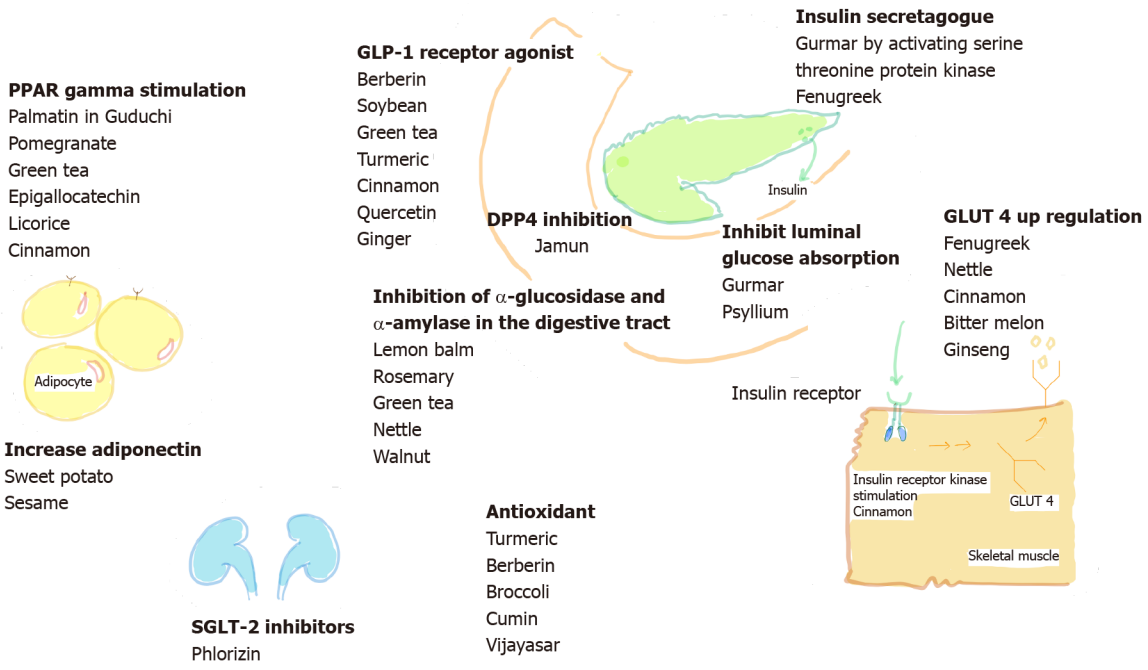
The active compound is sesamine. FBS reduction ranges from 34 mg/dL to 52 mg/dL[24]. HbA1C reduction ranges from 0.7%-1.1%[25]. Dose of sesame used in various studies is 200 mg/d. Sesame oil consumption favorably changes insulin resistance, despite its modest effect on FBS. Although canola oil consumption increases FBS, when both oils are mixed for cooking, the beneficial effects of sesame oil is retained[26]. A blend of 20% cold-pressed un-refined sesame oil and 80% physically refined rice bran oil as cooking oil also lowered hyperglycemia and improved the lipid profile in a study by Devarajan *et al* [27]. Moreover, the combination of sesame oil blend and glibenclamide treatment in these patients significantly improved hyperglycemia.

Barberry (berberis)

The hypoglycemic effect of berberine was reported in 1988 when it was used to treat diarrhoea in diabetes patients in China. It is found in the roots, rhizomes, stems and bark of berberis. In a study on 36 diabetes patients, Yin *et al*[28] showed that the hypoglycemic effect of berberine (HbA1C fall 1.4%, FBS fall 68 mg/dL, PPBS fall 158 mg/dL) was similar to that of metformin (HbA1C fall 1.9, FBS fall 57 mg/dL, PPBS fall 138 mg/dL) over 3 mo of treatment. Usual dose of berberine 500 mg TID sometimes causes abdominal discomfort but, 300 mg TID is a well-tolerated dose.

Aloe (aloe vera)

It is a houseplant. Aloe vera gel is prepared from the leaf pulp. Aloe juice is an exudate from the outer skin of the leaves. And 600 to 1000 g of gel powder brings about 6-44 mg/dL fall in FBS and 0.4% to 0.7% fall in HbA1C[29]. The active compound acemannan is a mucopolysaccharide.



DOI: 10.4239/wjd.v14.i4.412

Figure 1 Potential mechanism of action of herbal products. PPAR: Peroxisome proliferator-activated receptors; GLP-1: Glucagon-like peptide 1; DPP4: Dipeptidyl-peptidase 4; SGLT-2: Sodium-glucose cotransporter-2; GLUT4: Glucose transporter type 4.

Vijayasar (pterocarpus)

It is found as a component of many herbal products. The dose is 1-4 g of bark powder. A flexible dose open trial by Indian Council of Medical Research[30], 2-4 g/d Vijayasar, brought down blood glucose to the target range in 67 (69%) of 97 patients by 3-mo treatment. FBS, PPBS and HbA1c were decreased by 32 mg/dL, 45 mg/dL and 0.4%, respectively. No side effects were reported. In an RCT by Maurya *et al* [29], efficacy of aloe vera, vijayasar and their combination was compared to 1 mg of glimepiride. It was found that aloe (FBS reduction by 43 mg/dL) and vijayasar (by 43 mg/dL) showed synergism (by 54 mg/dL) for blood glucose control, but they were inferior to glimepiride (by 72 mg/dL). Its active principle epicatechin augments insulin release by increasing the cyclic adenosine monophosphate level. Flatulence has been reported; otherwise it is safe. It can be concluded that supplementation of 4g powder can be considered for the treatment diabetes.

Fenugreek (trigonella)

It is a short lived plant and used for texture, flavor and color of food. Its health-promoting effect is outlined in Ayurvedic medicine. Usual dose is 1-100 g of seed extract powder. Its active compound amino acid 4-hydroxy isoleucine stimulates glucose induced insulin secretion from the perfused pancreas *in vitro*. The human trial showed a decrease in FBS range from 15 to 41 mg/dL, a decrease in HbA1C from 0.2% to 1.46% and HOMA-IR from 0.44 to 0.68. Singh *et al*[31] from India compared 500 mg fenugreek with 5 mg glipizide in 60 diabetes patients. The patients were randomized into three groups. They received either fenugreek seed extract 500 mg twice a day, glipizide 5 mg once daily, or a combination of glipizide 2.5 mg and fenugreek seed extract 500 mg once daily for 3 mo. It was found that both had significant effect on glycemic control. Glipizide monotherapy was more efficacious (mean FBS fall 58 mg/dL) in controlling hyperglycema than fenugreek monotherapy (mean FBS fall 41 mg/dL). However, it is fenugreek which reduced glycemia and dyslipidemia simultaneously and so this plant deserves a place in the management of metabolic syndrome. Another RCT from China[32] showed that 2 g of fenugreek powder when added to patients with uncontrolled blood glucose who were already on sulfonylurea for more than one year can further reduce the mean FBS by 33 mg/dL and HbA1C by 1.46%. Similarly, when added to metformin, it can further reduceblood glucose by 10 mg/dL [33]. Indeed it can be an add-on remedy for DM.

Bael (aegle)

In an RCT by Yaheya and Ismail[34], fenugreek seed powder 20 g (FG) and dried bael leave powder 5 g (BL) were compared with OHA. Four groups were set up. Groups 1 to 4 received FG, BL, FG+BL and OHA, respectively. After 4 mo, PPBS was compared to baseline. The highest reduction in glucose was observed in those patients who received a combination of FG and BL, suggesting that these two products may have a synergistic effect. BL showed a comparable reduction in PPBS to standard OHA.

Similarly, an RCT from India showed that 20 g of bael leaf juice can reduce glucose by 34 mg/dL when added to OHA treatment over 2 mo[35]. Its active molecule, aegeline is proposed to upregulate the translocation of glucose transporter type 4 (GLUT 4). These results clearly indicate that bael is an inexpensive dietary supplement that has potential to improve glycemic control.

Jamun (syzygium)

In India, its kernel is used as household remedy for diabetes. Gallic acid and other phenolic compounds in Jamun are responsible for its anti-glycemic effect. Its seed, leaf, stem bark and fruit are used as herbal medicine. In an open labeled RCT by Acharya *et al*[36], 10 g of jamun seed powder was compared with 500 mg metformin in 30 newly diagnosed type 2 DM patients over 6 mo. Jamun reduced mean FBS by 18 mg/dL and HbA1C by 0.4%, whereas metformin reduced them by 41 mg/dL and 1.4%, respectively. Fall in PPBS was not significant. Another RCT from India showed that jamun, when added to the existing OHA, brought about a significant reduction in both FBS (33 mg/dL) and PPBS (43 mg/dL) over 3 mo[37]. In most of the trials, leaves and seeds of jamun were used for the treatment of diabetes. In contrast, tea extracts prepared from the leaves did not show a hypoglycaemic effect.

Ginger (zingiber)

Its active compound gingerol and shogaol is thought to have antiglycemic property. It is claimed to have three significant properties-immunomodulatory, anticancer, and anti-inflammatory. A number of RCTs were performed to show its effect on blood glucose, but most of them show a modest reduction in glycemic parameters. Carvalho *et al*[38] showed that consumption of ginger powder 1.2 g/d over 3 mo can reduce mean FBS by 29 mg/dL. Similarly, an RCT by Makhdoomi Arzati *et al*[39] showed mean FBS reduction by 26 mg/dL and HbA1C reduction by 0.4% by 2 g of ginger powder in 10 wk. When added to standard treatment, 3 g powder of ginger decreased FBS by a further 19 mg/dL and HbA1C by 0.7% over 3 mo[40]. Although these results are encouraging, Mahluji *et al*[41] found that 2 g of ginger did not produce any effect on FBS and HbA1C but reduced serum insulin resistance over 2 mo.

Little gourd (coccinia)

It is an edible perennial vegetable. It has been widely used as an ingredient in traditional herbal medicine for the treatment of several diseases, including DM. Its leaf and fruit extract is formulated as a natural therapy. Several trials have shown that 500 mg to 1000 mg of coccinia powder can have a moderate glucose lowering effect. Wasana *et al*[42] found a reduction in insulin resistance along with a reduction of glycemic parameters (mean FBS by 25 mg/dL, mean HbA1C by 0.6%). Active compound pectin, triterpenes are responsible for its effect on blood glucose.

Neem (azadirachta)

Neem is considered a miraculous tree for its medicinal effects. It is rich in alkaloids, flavonoids and terpenoids. Its root and stem bark contain nimbidiol, which is a diterpenoid that causes intestinal disaccharidase and glucoamylase inhibition[43]. Pingali *et al*[44] included 80 diabetes patients who were on metformin. Neem extract was prepared from its leaves and twigs and patients were randomly assigned to take capsules of 250 mg, 500 mg and 1000 mg of this extract. After 3 mo, significant reduction in FBS (23 mg/dL), HbA1C (1.5%) and HOMA-IR (2.6%) was noticed in the 1000 mg group. It can certainly be a weapon to combat DM as a herbal product.

Sweet potato (ipomoea)

This plant is native to the tropical and subtropical belts. Apart from cooking, it is also used in traditional medicine for DM. Its active compounds are isolated from leaves and tuber. It is rich in anthocyanins, polyphenols and flavonoids. Ludvik *et al*[45] have shown that 4 g powder of sweet potato can reduce FBS from 10 to 19 mg/dL and reduce HbA1C by 0.21% - 0.3%.

Amla (emblica)

Various studies used 1-10 g of amla powder. The average fall in FBS is 13 mg/dL and fall in HbA1C is 0.5%[46,47]. Active compounds are gallic acid, ellagic acid, quercetin, and chebulinic acid[48]. Triphala, a combination of amla, haritaki and bibhitaki, is considered to have a pleiotropic effect in ayurvedic medicine. Nevertheless, a systematic review by Phimarn *et al*[49] fails to establish any effect on blood glucose.

Bitter melon (momordica)

It is commonly used as an alternative therapy for the treatment of diabetes. Different doses (from 800 mg to 6 g/d) and different parts of this plant such as leaves, fruit pulp, seeds and whole plant have been studied for antidiabetic effect. Momordin and charantin are active compounds. Kim *et al*[50] conducted an RCT with 2.3 g of bitter melon powder on 96 diabetic subjects. At the end of 3 mo, the mean FBS reduction was only 5 mg/dL without any significant impact on other glycemic parameters. An open-label, randomized, active-controlled, multicentric, phase III study from India[51] compared 800 mg bitter melon fruit extract with metformin. Eighty-three subjects received bitter melon and 40 subjects

received metformin. After 15 wk, mean fall in FBS and HbA1C in the bitter melon group was 14 mg/dL and 0.28% and in the metformin group 25 mg/dL and 0.62%, respectively. This study showed non-inferiority of bitter melon to metformin, although its effects were modest. Similarly, a weaker antidiabetic effect has been exhibited in comparison to glibenclamide 5 mg in a trial by Rahman *et al*[52].

Garlic (allium sativum)

Since antiquity, garlic has been part and parcel of many Indian dishes and herbal medicine. Garlic powder is formulated in capsule form. It has allicin, a sulfur-containing amino acid that can combine with cysteine and other sulphydryl group containing amino acids and spares insulin from inactivation [53]. Kumar *et al*[54] demonstrated that garlic, when added to metformin treatment, helps in further blood glucose reduction, although the effect is insignificant. Reduction of glycemia is modest in various studies.

Turmeric (curcumin)

Turmeric is used in food preparation as a spice. It has versatile pharmacological effects described by both *in vitro* and *in vivo* studies. Its bioactive molecule is curcumin which is present in the rhizome of this plant. Its active peroxisome proliferator-activated receptor gamma (PPAR-γ) is similar to thiazolidinedione. Despite its efficacy in *in vitro* studies, human trials fail to document any significant effect. Its bioavailability is a major hindrance to its efficacy. FBS reduction ranges from 2 to 9 mg/dL. The commonly used dose is 450-2100 mg/d[55].

Guduchi (tinospora)

A study on water extract or powder form of a mature stem of tinospora by Roy[56] and Mishra *et al*[57] demonstrated that 500-1500 mg capsule can reduce FBS by 5-8 mg/dL and HbA1C by 0.22%-0.54%. Its active compound, palmatine, is known to stimulate PPAR-γ).

Plant with SGLT-2 inhibitory effects

The discovery of novel SGLT-2 inhibitors dates back to 1835 when phlorizin was isolated for the first time from the barks of an apple tree by French chemists[58]. It is mainly found in the young shoots, roots, leaves and barks of the apple tree, while in fruit, it is most abundant in the seeds. Nevertheless, seeds of ripe fruit contain a toxic cyanogenic glycoside[59], so seeds of unripe fruit seeds are to be used. Apple, as an intact fruit, does not have glucose-lowering properties. Moreover, the bioavailability of natural phlorizin is less as it is hydrolyzed by lactase-phlorizin hydrolase in the brush border of the small intestine. Apart from the apple tree, quercetin, strawberry, rose hip and pear have phenolic components with SGLT-2 inhibitory effects. When consumed as food item, these may not bring about a reasonable effect due to the presence of carbohydrates within these fruits.

NATURAL PRODUCTS WITH WEAK EVIDENCE AS ANTI-HYPERGLYCEMIC REMEDY

Licorice

Its root extract has been used widely in Chinese medicine as antidiabetic therapy. However, evidence is not sound in human studies[60]. The active compound is glycyrrhizic acid. Due to its 11-beta hydroxysteroid dehydrogenase-2 enzyme inhibitory property, it can cause hypokalemia, hypertension and rhabdomyolysis[61].

Jack fruit

Rao *et al*[62] from India demonstrated the effect of jack fruit leaf flour on 40 diabetes patients in a RCT. They found that replacing an equal volume of wheat flour in daily meals with jackfruit flour could significantly lower the blood glucose level (mean FBS by 29 mg/dL, and mean HbA1C by 0.25%)[62]. However this finding needs confirmation by further RCTs.

Ayush-82

An ayurvedic hypoglycemic formulation consisting of seeds of mango, seeds of bitter melon, seeds of jamun, and leaves of gurmar were tried in fairly large sample size (n = 350), which revealed a statistically significant reduction in blood glucose levels in DM[63]. A polyherbal formulation named BGR-34 was developed by the Council of Scientific and Industrial Research, Government of India.

Similarly, nettle, pomegranate, shilajit, beans, tea, ginkgo biloba, saffron lack sufficient clinical data in diabetes management although these are cited as antihyperglycemic medicine in Ayurveda. Central Council for Research in Ayurvedic Sciences recommends certain fruits and vegetables for diabetes patients. Those are fenugreek, bitter melon, garlic, unripe banana, jamun, amla, pomegranate, bael, guava, apple, and orange.

SAFETY OF HERBAL MEDICINES

In common belief, herbal products are regarded to be very safe to consume. This is because most previous trials did not monitor side effects. This is a myth. We know about Chinese herb nephropathy due to aristolochia used for obesity. Among antidiabetic drugs, ginseng can cause insomnia, anxiety, and hypertension. Due to its estrogenic effects, it can cause breast tenderness and vaginal bleeding. Garlic, fenugreek, cinnamon and ginger can cause increased bleeding tendency due to the presence of a coumarin like compound. Aloe can also prolong bleeding time[64]. So these products must be stopped if the patient is on anticoagulants or patient plans an operation. Gastrointestinal side effects are quite common with ingestion of these natural products. Apart from these, fenugreek can also cause facial swelling and itching. As it contains mucilage, absorption of other drugs can be compromised. Both fenugreek and bitter melon are contraindicated in pregnancy. Alfa and beta momorcharin in bitter melon have abortifacient effect[65]. On the other hand, neem products have contraceptive property[66]. Bitter melon has been reported to cause hemolytic anemia in glucose 6 phosphate dehydrogenase deficient individuals (due to presence of vicine) and convulsion in children[65]. Side effects of amla are mild headache, fever, gastritis and increased bleeding tendency[67]. Aloe vera, turmeric, and ginseng have Cytochrome P450 (CYP) enzyme-inducing or inhibitory properties. Particularly, aloe vera can inhibit CYP3A4 and CYP2D6; thereby, it has the potential to alter levels of pioglitazone and repaglinide [68]. Similarly, consumption of neem can reduce the bioavailability of glipizide. Turmeric can inhibit P-glycoprotein 1, which is an efflux pump, and thereby increases glyburide concentration[69]. So intake of these products may lead to significant drug interaction.

Product standardization of these herbal formulations is not tightly regulated. Unlike allopathic medicines, herbal products are obtained from nature. The chemical composition of these herbs may alter based on the season and growing conditions. For example, ginsenoid, the active moiety in ginseng, varies from 36% to 112%[70]. Herbal medicine sold in India as antidiabetic remedy was found to contain glibenclamide as an adulterant[71]. Bioassays need to be developed for biological standardization and toxicological assessment. Many a time, heavy metals are found in various ayurvedic products much are above their acceptable range[72]. All these need to be considered before using those.

COST-EFFECTIVENESS OF HERBAL MEDICINES

Even though these products are readily available, processed plant products sold in the market in the proper formulation are costly. Cost of the products based on the price mentioned in Amazon online shopping app. at the time of writing of the article are mentioned in Table 1. The most popular drug metformin is a cheap drug. On head-to-head comparison studies with a low dose of metformin, it is evident that most of these products are inferior to metformin. Therefore cost-effectiveness has to be considered before consuming those.

CONCLUSION

Although many OHAs in modern medicine have a natural origin, consuming them in their original form may not bring out much benefit as we have discussed with the example of metformin. On the other hand, we have seen isolation of phlorizin paved the way to the discovery of modern SGLT 2 inhibitors. It is worth to find out the active molecule and their mechanisms of action not only for a better understanding of their effects but also for product standardization.

DM is a chronic disease and it is associated with multiple comorbidities. So, its remedies should be chosen wisely based on the data on their safety, efficacy and drug interactions. In this article, we have given an overview of herbal products with anti-hyperglycaemic effects based on various RCTs. We often use them as food items but in sub-optimal doses. We have also mentioned doses and proper formulation from the right part of the plant used in various clinical trials. Nonetheless, these trials of natural products often underreport the important steps of the interventions, thereby deviate from the standards set by Consolidated Standards of Reporting Trials. These need to be brought under a regulatory framework which will eventually generate faith in these herbal medicines. Last but not the least, cost-effectiveness should also be kept in mind before using them.

FOOTNOTES

Author contributions: Giri S and Roy A did the literature search; Giri S wrote the first draft; Sahoo J, Roy A, Kamalanathan S and Naik D supervised the writing, gave intellectual inputs, and critically revised the manuscript; all of them approved the final version of the manuscript to be published.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: India

ORCID number: Somdatta Giri 0000-0002-3464-7860; Jayaprakash Sahoo 0000-0002-8805-143X; Ayan Roy 0000-0003-4419-9376; Sadishkumar Kamalanathan 0000-0002-2371-0625; Dukhabandhu Naik 0000-0003-4568-877X.

S-Editor: Gao CC
L-Editor: Ma JY-MedE
P-Editor: Gao CC

REFERENCES

1 **Holt RIG**, Cockram CS, Flyvbjerg A, Goldstein BJ. Textbook of Diabetes. 5th ed. John Wiley & Sons, Inc., 2017
2 **Bailey CJ**. Metformin: historical overview. *Diabetologia* 2017; **60**: 1566-1576 [PMID: 28776081 DOI: 10.1007/s00125-017-4318-z]
3 **Matsuo T**, Odaka H, Ikeda H. Effect of an intestinal disaccharidase inhibitor (AO-128) on obesity and diabetes. *Am J Clin Nutr* 1992; **55**: 314S-317S [PMID: 1728846 DOI: 10.1093/ajcn/55.1.314s]
4 **Rieg T**, Vallon V. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia* 2018; **61**: 2079-2086 [PMID: 30132033 DOI: 10.1007/s00125-018-4654-7]
5 **Kifle ZD**, Bayleyegn B, Yimer Tadesse T, Woldeyohanins AE. Prevalence and associated factors of herbal medicine use among adult diabetes mellitus patients at government hospital, Ethiopia: An institutional-based cross-sectional study. *Metabol Open* 2021; **11**: 100120 [PMID: 34485891 DOI: 10.1016/j.metop.2021.100120]
6 **Majeed M**, Majeed A, Nagabhusahnam K, Mundkur L, Paulose S. A randomized, double-blind clinical trial of a herbal formulation (GlycaCare-II) for the management of type 2 diabetes in comparison with metformin. *Diabetol Metab Syndr* 2021; **13**: 132 [PMID: 34789340 DOI: 10.1186/s13098-021-00746-0]
7 **Egbuna C**, Awuchi CG, Kushwaha G, Rudrapal M, Patrick-Iwuanyanwu KC, Singh O, Odoh UE, Khan J, Jeevanandam J, Kumarasamy S, Chukwube VO, Narayanan M, Palai S, Găman MA, Uche CZ, Ogaji DS, Ezeofor NJ, Mtewa AG, Patrick-Iwuanyanwu CC, Kesh SS, Shivamallu C, Saravanan K, Tijjani H, Akram M, Ifemeje JC, Olisah MC, Chikwendu CJ. Bioactive Compounds Effective Against Type 2 Diabetes Mellitus: A Systematic Review. *Curr Top Med Chem* 2021; **21**: 1067-1095 [PMID: 33966619 DOI: 10.2174/1568026621666210509161059]
8 **Sharma P**, Sharma S, Agrawal R, Agarwal V, Singhal S. A randomised double blind placebo control trial of cinnamon supplementation on glycemic control and lipid profile in type 2 diabetes mellitus. *Australian J Herbal Med* 2012; **24**: 108-119
9 **Mirmiranpour H**, Huseini HF, Derakhshanian H, Khodaii Z, Tavakoli-Far B. Effects of probiotic, cinnamon, and synbiotic supplementation on glycemic control and antioxidant status in people with type 2 diabetes; a randomized, double-blind, placebo-controlled study. *J Diabetes Metab Disord* 2020; **19**: 53-60 [PMID: 32550156 DOI: 10.1007/s40200-019-00474-3]
10 **Talaei B**, Amouzegar A, Sahranavard S, Hedayati M, Mirmiran P, Azizi F. Effects of Cinnamon Consumption on Glycemic Indicators, Advanced Glycation End Products, and Antioxidant Status in Type 2 Diabetic Patients. *Nutrients* 2017; **9** [PMID: 28885566 DOI: 10.3390/nu9090991]
11 **Hasanzade F**, Toliat M, Emami SA, Emamimoghaadam Z. The Effect of Cinnamon on Glucose of Type II Diabetes Patients. *J Tradit Complement Med* 2013; **3**: 171-174 [PMID: 24716174 DOI: 10.4103/2225-4110.114900]
12 **Zare R**, Nadjarzadeh A, Zarshenas MM, Shams M, Heydari M. Efficacy of cinnamon in patients with type II diabetes mellitus: A randomized controlled clinical trial. *Clin Nutr* 2019; **38**: 549-556 [PMID: 29605574 DOI: 10.1016/j.clnu.2018.03.003]
13 **Kanetkar P**, Singhal R, Kamat M. Gymnema sylvestre: A Memoir. *J Clin Biochem Nutr* 2007; **41**: 77-81 [PMID: 18193099 DOI: 10.3164/jcbn.2007010]
14 **Li Y**, Zheng M, Zhai X, Huang Y, Khalid A, Malik A, Shah P, Karim S, Azhar S, Hou X. Effect of-gymnema sylvestre, citrullus colocynthis and artemisia absinthium on blood glucose and lipid profile in diabetic human. *Acta Pol Pharm* 2015; **72**: 981-985 [PMID: 26665406]
15 **Baskaran K**, Kizar Ahamath B, Radha Shanmugasundaram K, Shanmugasundaram ER. Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin-dependent diabetes mellitus patients. *J Ethnopharmacol* 1990; **30**: 295-300 [PMID: 2259217 DOI: 10.1016/0378-8741(90)90108-6]
16 **Shanmugasundaram ER**, Rajeswari G, Baskaran K, Rajesh Kumar BR, Radha Shanmugasundaram K, Kizar Ahmath B. Use of Gymnema sylvestre leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. *J Ethnopharmacol* 1990; **30**: 281-294 [PMID: 2259216 DOI: 10.1016/0378-8741(90)90107-5]
17 **Hendre A**, Sontakke A, Phatak R, Patil S, Jadhav S. Role of Cumin in Management of Type 2 Diabetes Mellitus with respect to its Antidiabetic and Antioxidant Property. *Int J Res Pharm Sci* 2020; **11**: 4157-4161 [DOI: 10.26452/ijrps.v11i3.2622]
18 **Jafari S**, Sattari R, Ghavamzadeh S. Evaluation the effect of 50 and 100 mg doses of Cuminum cyminum essential oil on

glycemic indices, insulin resistance and serum inflammatory factors on patients with diabetes type II: A double-blind randomized placebo-controlled clinical trial. *J Tradit Complement Med* 2017; **7**: 332-338 [PMID: 28725629 DOI: 10.1016/j.jtcme.2016.08.004]

19 **Kaatabi H**, Bamosa AO, Badar A, Al-Elq A, Abou-Hozaifa B, Lebda F, Al-Khadra A, Al-Almaie S. Nigella sativa improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo controlled participant blinded clinical trial. *PLoS One* 2015; **10**: e0113486 [PMID: 25706772 DOI: 10.1371/journal.pone.0113486]

20 **Najmi A**, Nasiruddin M, Khan R, Haque SF. Therapeutic effect of Nigella sativa in patients of poor glycemic control. *Asian J Pharm Clin Res* 2012; **5**: 224-228 [DOI: 10.22159/ajpcr.2019.v12i10.34985]

21 **Ziai SA**, Larijani B, Akhoondzadeh S, Fakhrzadeh H, Dastpak A, Bandarian F, Rezai A, Badi HN, Emami T. Psyllium decreased serum glucose and glycosylated hemoglobin significantly in diabetic outpatients. *J Ethnopharmacol* 2005; **102**: 202-207 [PMID: 16154305 DOI: 10.1016/j.jep.2005.06.042]

22 **Abutair AS**, Naser IA, Hamed AT. Soluble fibers from psyllium improve glycemic response and body weight among diabetes type 2 patients (randomized control trial). *Nutr J* 2016; **15**: 86 [PMID: 27733151 DOI: 10.1186/s12937-016-0207-4]

23 **Xiao Z**, Chen H, Zhang Y, Deng H, Wang K, Bhagavathula AS, Almuhairi SJ, Ryan PM, Rahmani J, Dang M, Kontogiannis V, Vick A, Wei Y. The effect of psyllium consumption on weight, body mass index, lipid profile, and glucose metabolism in diabetic patients: A systematic review and dose-response meta-analysis of randomized controlled trials. *Phytother Res* 2020; **34**: 1237-1247 [PMID: 31919936 DOI: 10.1002/ptr.6609]

24 **Aslam F**, Iqbal S, Nasir M, Anjum AA. White Sesame Seed Oil Mitigates Blood Glucose Level, Reduces Oxidative Stress, and Improves Biomarkers of Hepatic and Renal Function in Participants with Type 2 Diabetes Mellitus. *J Am Coll Nutr* 2019; **38**: 235-246 [PMID: 30260748 DOI: 10.1080/07315724.2018.1500183]

25 **Mohammad Shahi M**, Zakerzadeh M, Zakerkish M, Zarei M, Saki A. Effect of Sesamin Supplementation on Glycemic Status, Inflammatory Markers, and Adiponectin Levels in Patients with Type 2 Diabetes Mellitus. *J Diet Suppl* 2017; **14**: 65-75 [PMID: 27450646 DOI: 10.1080/19390211.2016.1204404]

26 **Raeisi-Dehkordi H**, Amiri M, Zimorovat A, Moghtaderi F, Zarei S, Forbes SC, Salehi-Abargouei A. Canola oil compared with sesame and sesame-canola oil on glycaemic control and liver function in patients with type 2 diabetes: A three-way randomized triple-blind cross-over trial. *Diabetes Metab Res Rev* 2021; **37**: e3399 [PMID: 32860716 DOI: 10.1002/dmrr.3399]

27 **Devarajan S**, Chatterjee B, Urata H, Zhang B, Ali A, Singh R, Ganapathy S. A Blend of Sesame and Rice Bran Oils Lowers Hyperglycemia and Improves the Lipids. *Am J Med* 2016; **129**: 731-739 [PMID: 27046245 DOI: 10.1016/j.amjmed.2016.02.044]

28 **Yin J**, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism* 2008; **57**: 712-717 [PMID: 18442638 DOI: 10.1016/j.metabol.2008.01.013]

29 **Maurya AK**, Jain A, Pathak A, Chaudhary P, Rajdan N. Efficacy of vijaysar, aloevera alone and their combination in the treatment of newly diagnosed cases of type 2 diabetes mellitus: a randomized single blind prospective study. *Int J Basic Clin Pharmacol* 2017; **6**: 962-967 [DOI: 10.18203/2319-2003.ijbcp20171112]

30 Flexible dose open trial of Vijayasar in cases of newly-diagnosed non-insulin-dependent diabetes mellitus. Indian Council of Medical Research (ICMR), Collaborating Centres, New Delhi. *Indian J Med Res* 1998; **108**: 24-29 [PMID: 9745215]

31 **Singh A**, Rai J, Mahajan DS. Comparative evaluation of glipizide and fenugreek (Trigonella foenum-graecum) seeds as monotherapy and combination therapy on glycaemic control and lipid profile in patients with type 2 diabetes mellitus. *Int J Basic Clin Pharmacol* 2016; **5**: 942-950 [DOI: 10.18203/2319-2003.ijbcp20161549]

32 **Lu FR**, Shen L, Qin Y, Gao L, Li H, Dai Y. Clinical observation on trigonella foenum-graecum L. total saponins in combination with sulfonylureas in the treatment of type 2 diabetes mellitus. *Chin J Integr Med* 2008; **14**: 56-60 [PMID: 18219452 DOI: 10.1007/s11655-007-9005-3]

33 **Kaur M**, Singh N, Sharma G, Singh D. To study the efficacy and tolerability of fenugreek seed powder as add-on therapy with metformin in patients of type-2 diabetes mellitus. *Int J Basic Clin Pharmacol* 2016; **5**: 378-383 [DOI: 10.18203/2319-2003.ijbcp20160748]

34 **Yaheya M**, Ismail M. Clinical Evaluation of Antidiabetic Activity of Trigonella Seeds and Aegle marmelos Leaves. *World Appl Sci J* 2009; **7**: 1231-1234 [DOI: 10.21276/ijprhs.2016.04.13]

35 **Nigam V**, Nambiar VS. Aegle marmelos leaf juice as a complementary therapy to control type 2 diabetes – Randomised controlled trial in Gujarat, India. *Adv Integr Med* 2019; **6**: 11-22 [DOI: 10.1016/j.aimed.2018.03.002]

36 **Acharya S**, Shivaprakash G, Baliga R, Adhikari P, Jyothi G, Pai MRSM. Effect of Eugenia jambolana on plasma glucose, insulin sensitivity and HDL-C levels: preliminary results of a randomized clinical trial. *J Pharm Res* 2010; **3**: 1268-1270 [DOI: 10.1001/jama.1992.03480060057030]

37 **Sidana S**, Singh VB, Meena BL, Beniwal S, Singh K, Kumar D, Singla R. Effect of Syzygium cumini (jamun) seed powder on glycemic control: A double-blind randomized controlled trial. *J Med Soc* 2017; **31**: 185-189 [DOI: 10.4103/jms.jms_62_16]

38 **Carvalho GCN**, Lira-Neto JCG, Araújo MFM, Freitas RWJF, Zanetti ML, Damasceno MMC. Effectiveness of ginger in reducing metabolic levels in people with diabetes: a randomized clinical trial. *Rev Lat Am Enfermagem* 2020; **28**: e3369 [PMID: 33053078 DOI: 10.1590/1518-8345.3870.3369]

39 **Makhdoomi Arzati M**, Mohammadzadeh Honarvar N, Saedisomeolia A, Anvari S, Effatpanah M, Makhdoomi Arzati R, Yekaninejad MS, Hashemi R, Djalali M. The Effects of Ginger on Fasting Blood Sugar, Hemoglobin A1c, and Lipid Profiles in Patients with Type 2 Diabetes. *Int J Endocrinol Metab* 2017; **15**: e57927 [PMID: 29344037 DOI: 10.5812/ijem.57927]

40 **Shidfar F**, Rajab A, Rahideh T, Khandouzi N, Hosseini S, Shidfar S. The effect of ginger (Zingiber officinale) on glycemic markers in patients with type 2 diabetes. *J Complement Integr Med* 2015; **12**: 165-170 [PMID: 25719344 DOI: 10.1515/jcim-2014-0021]

41 **Mahluji S**, Attari VE, Mobasseri M, Payahoo L, Ostadrahimi A, Golzari SE. Effects of ginger (Zingiber officinale) on plasma glucose level, HbA1c and insulin sensitivity in type 2 diabetic patients. *Int J Food Sci Nutr* 2013; **64**: 682-686

[PMID: 23496212 DOI: 10.3109/09637486.2013.775223]
42 **Wasana KGP**, Attanayake AP, Weerarathna TP, Jayatilaka KAPW. Efficacy and safety of a herbal drug of Coccinia grandis (Linn.) Voigt in patients with type 2 diabetes mellitus: A double blind randomized placebo controlled clinical trial. *Phytomedicine* 2021; **81**: 153431 [PMID: 33352495 DOI: 10.1016/j.phymed.2020.153431]
43 **Mukherjee A**, Sengupta S. Characterization of nimbidiol as a potent intestinal disaccharidase and glucoamylase inhibitor present in Azadirachta indica (neem) useful for the treatment of diabetes. *J Enzyme Inhib Med Chem* 2013; **28**: 900-910 [PMID: 22803678 DOI: 10.3109/14756366.2012.694877]
44 **Pingali U**, Ali MA, Gundagani S, Nutalapati C. Evaluation of the Effect of an Aqueous Extract of Azadirachta indica (Neem) Leaves and Twigs on Glycemic Control, Endothelial Dysfunction and Systemic Inflammation in Subjects with Type 2 Diabetes Mellitus - A Randomized, Double-Blind, Placebo-Controlled Clinical Study. *Diabetes Metab Syndr Obes* 2020; **13**: 4401-4412 [PMID: 33244247 DOI: 10.2147/DMSO.S274378]
45 **Ludvik B**, Hanefeld M, Pacini G. Improved metabolic control by Ipomoea batatas (Caiapo) is associated with increased adiponectin and decreased fibrinogen levels in type 2 diabetic subjects. *Diabetes Obes Metab* 2008; **10**: 586-592 [PMID: 17645559 DOI: 10.1111/j.1463-1326.2007.00752.x]
46 **Akhtar MS**, Ramzan A, Ali A, Ahmad M. Effect of Amla fruit (Emblica officinalis Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. *Int J Food Sci Nutr* 2011; **62**: 609-616 [PMID: 21495900 DOI: 10.3109/09637486.2011.560565]
47 **Usharani P**, Fatima N, Muralidhar N. Effects of Phyllanthus emblica extract on endothelial dysfunction and biomarkers of oxidative stress in patients with type 2 diabetes mellitus: a randomized, double-blind, controlled study. *Diabetes Metab Syndr Obes* 2013; **6**: 275-284 [PMID: 23935377 DOI: 10.2147/DMSO.S46341]
48 **Huang HZ**, Qiu M, Lin JZ, Li MQ, Ma XT, Ran F, Luo CH, Wei XC, Xu RC, Tan P, Fan SH, Yang M, Han L, Zhang DK. Potential effect of tropical fruits Phyllanthus emblica L. for the prevention and management of type 2 diabetic complications: a systematic review of recent advances. *Eur J Nutr* 2021; **60**: 3525-3542 [PMID: 33439332 DOI: 10.1007/s00394-020-02471-2]
49 **Phimarn W**, Sungthong B, Itabe H. Effects of Triphala on Lipid and Glucose Profiles and Anthropometric Parameters: A Systematic Review. *J Evid Based Integr Med* 2021; **26**: 2515690X211011038 [PMID: 33886393 DOI: 10.1177/2515690X211011038]
50 **Kim SK**, Jung J, Jung JH, Yoon N, Kang SS, Roh GS, Hahm JR. Hypoglycemic efficacy and safety of Momordica charantia (bitter melon) in patients with type 2 diabetes mellitus. *Complement Ther Med* 2020; **52**: 102524 [PMID: 32951763 DOI: 10.1016/j.ctim.2020.102524]
51 **Suthar AC**, Pai VG, Kadam Y, Tongaonkar A, Kale S, Deshpande AB, Kolke S, Tanna S, Deshpande SV, Chawla P, Biswas D, Sharma S. Efficacy and Safety of PDM011011 Capsules as Compared to Metformin in Subjects with Type-2 Diabetes Mellitus: An Open-Label, Randomized, Active-Controlled, Multicentric, Phase III Study. *J Diabetes Mellitus* 2016; **6**: 38-48 [DOI: 10.4236/jdm.2016.61005]
52 **Rahman IU**, Khan RU, Rahman KU, Bashir M. Lower hypoglycemic but higher antiatherogenic effects of bitter melon than glibenclamide in type 2 diabetic patients. *Nutr J* 2015; **14**: 13 [PMID: 25623883 DOI: 10.1186/1475-2891-14-13]
53 **Khan R**. Effects of garlic on blood glucose levels and HbA1c in patients with type 2 diabetes mellitus. *J Med Plant Res* 2011; **5**: 2922-2928
54 **Kumar R**, Chhatwal S, Arora S, Sharma S, Singh J, Singh N, Bhandari V, Khurana A. Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase- lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. *Diabetes Metab Syndr Obes* 2013; **6**: 49-56 [PMID: 23378779 DOI: 10.2147/DMSO.S38888]
55 **Adab Z**, Eghtesadi S, Vafa MR, Heydari I, Shojaii A, Haqqani H, Arablou T, Eghtesadi M. Effect of turmeric on glycemic status, lipid profile, hs-CRP, and total antioxidant capacity in hyperlipidemic type 2 diabetes mellitus patients. *Phytother Res* 2019; **33**: 1173-1181 [PMID: 30859660 DOI: 10.1002/ptr.6312]
56 **Roy K**. Tinospora cordifolia stem supplementation in diabetic dyslipidemia: an open labelled randomized controlled trial. *Funct Foods Health Dis* 2015; **5**: 265-274 [DOI: 10.31989/ffhd.v5i8.208]
57 **Mishra S**, Verma N, Bhattacharya S, Usman K, Himanshu D, Singh P, Anjum B. Effect of Tinospora cordifolia as an add - on therapy on the blood glucose levels of patients with Type 2 diabetes. *Int J Basic Clin Pharmacol* 2017; **4**: 537-541 [DOI: 10.18203/2319-2003.ijbcp20150035]
58 **Ehrenkranz JR**, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. *Diabetes Metab Res Rev* 2005; **21**: 31-38 [PMID: 15624123 DOI: 10.1002/dmrr.532]
59 **Lu Y**, Yeap Foo L. Constitution of some chemical components of apple seed. *Food Chem* 1998; **61**: 29-33 [DOI: 10.1016/s0308-8146(97)00123-4]
60 **Alizadeh M**, Namazi N, Mirtaheri E, Sargheini N, Kheirouri S. Changes of Insulin Resistance and Adipokines Following Supplementation with Glycyrrhiza Glabra L. Extract in Combination with a Low-Calorie Diet in Overweight and Obese Subjects: a Randomized Double Blind Clinical Trial. *Adv Pharm Bull* 2018; **8**: 123-130 [PMID: 29670847 DOI: 10.15171/apb.2018.015]
61 **Yang LY**, Yin JH, Yang J, Ren Y, Xiang CY, Wang CY. Liquorice-induced severe hypokalemic rhabdomyolysis with Gitelman syndrome and diabetes: A case report. *World J Clin Cases* 2019; **7**: 1200-1205 [PMID: 31183353 DOI: 10.12998/wjcc.v7.i10.1200]
62 **Rao AG**, Naik KS, Unnikrishnan AG, Joseph J. Efficacy of green jackfruit flour as a medical nutrition therapy replacing rice or wheat in patients with type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled study. *Nutr Diabetes* 2021; **11**: 18 [PMID: 34127645 DOI: 10.1038/s41387-021-00161-4]
63 **Panda AK**. Efficacy of Ayurveda Formulation Ayush-82 (IME-9) in Newly Diagnosed Type 2 Diabetics: Retrospective Analysis of Individual Data. *J Tradit Med Clin Natur* 2017; **6**: 250 [DOI: 10.4172/2573-4555.1000250]
64 **Lee A**, Chui PT, Aun CS, Gin T, Lau AS. Possible interaction between sevoflurane and Aloe vera. *Ann Pharmacother* 2004; **38**: 1651-1654 [PMID: 15292490 DOI: 10.1345/aph.1E098]
65 **Basch E**, Gabardi S, Ulbricht C. Bitter melon (Momordica charantia): a review of efficacy and safety. *Am J Health Syst Pharm* 2003; **60**: 356-359 [PMID: 12625217 DOI: 10.1093/ajhp/60.4.356]

66 **Patil SM**, Shirahatti PS, V B CK, Ramu R, M N NP. Azadirachta indica A. Juss (neem) as a contraceptive: An evidence-based review on its pharmacological efficiency. *Phytomedicine* 2021; **88**: 153596 [PMID: 34092456 DOI: 10.1016/j.phymed.2021.153596]

67 **Hashem-Dabaghian F**, Ziaee M, Ghaffari S, Nabati F, Kianbakht S. A systematic review on the cardiovascular pharmacology of Emblica officinalis Gaertn. *J Cardiovasc Thorac Res* 2018; **10**: 118-128 [PMID: 30386531 DOI: 10.15171/jcvtr.2018.20]

68 **Djuv A**, Nilsen OG. Aloe vera juice: IC_{50} and dual mechanistic inhibition of CYP3A4 and CYP2D6. *Phytother Res* 2012; **26**: 445-451 [PMID: 21842479 DOI: 10.1002/ptr.3564]

69 **Neerati P**, Devde R, Gangi AK. Evaluation of the effect of curcumin capsules on glyburide therapy in patients with type-2 diabetes mellitus. *Phytother Res* 2014; **28**: 1796-1800 [PMID: 25044423 DOI: 10.1002/ptr.5201]

70 **Vuksan V**, Sievenpiper JL. Herbal remedies in the management of diabetes: lessons learned from the study of ginseng. *Nutr Metab Cardiovasc Dis* 2005; **15**: 149-160 [PMID: 15955462 DOI: 10.1016/j.numecd.2005.05.001]

71 **Kulambil Padinjakara RN**, Ashawesh K, Butt S, Nair R, Patel V. Herbal remedy for diabetes: two case reports. *Exp Clin Endocrinol Diabetes* 2009; **117**: 3-5 [PMID: 19140093 DOI: 10.1055/s-0028-1085426]

72 **Saper RB**, Phillips RS, Sehgal A, Khouri N, Davis RB, Paquin J, Thuppil V, Kales SN. Lead, mercury, and arsenic in US- and Indian-manufactured Ayurvedic medicines sold *via* the Internet. *JAMA* 2008; **300**: 915-923 [PMID: 18728265 DOI: 10.1001/jama.300.8.915]

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 424-434

DOI: 10.4239/wjd.v14.i4.424

ISSN 1948-9358 (online)

MINIREVIEWS

Semaglutide-eye-catching results

Maja Cigrovski Berkovic, Felice Strollo

Specialty type: Endocrinology and metabolism

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Alsaidan A, Saudi Arabia; Morya AK, India

Received: December 15, 2022
Peer-review started: December 15, 2022
First decision: January 20, 2023
Revised: February 8, 2023
Accepted: March 20, 2023
Article in press: March 20, 2023
Published online: April 15, 2023

Maja Cigrovski Berkovic, Faculty of Kinesiology, University of Zagreb, Zagreb 10000, Croatia

Felice Strollo, Department of Endocrinology and Metabolism, IRCCS San Raffaele Pisana, Rome 00163, Italy

Corresponding author: Maja Cigrovski Berkovic, MD, PhD, Adjunct Associate Professor, Faculty of Kinesiology, University of Zagreb, 15 Horvacanski zavoj, Zagreb 10000, Croatia. maja.cigrovskiberkovic@gmail.com

Abstract

Semaglutide is a glucagon-like peptide-1 receptor agonist used either orally every day or subcutaneously once a week for the treatment of type 2 diabetes mellitus and, more recently, at higher doses, for the treatment of obesity. Both diseases are reaching epidemic proportions and often coexist, posing patients with a high risk for cardiovascular disease and death. Therefore, an agent such as semaglutide, which offers clinically significant weight loss and cardiovascular benefits, is essential and will be increasingly used in high-risk patients. However, during the SUSTAIN clinical trial program (Semaglutide Unabated Sustainability in treatment of type 2 diabetes), a safety issue concerning the progression and worsening of diabetic retinopathy emerged. The existing explanation so far mainly supports the role of the magnitude and speed of HbA1c reduction, a phenomenon also associated with insulin treatment and bariatric surgery. Whether and to which extent the effect is direct is still a matter of debate and an intriguing topic to investigate for suitable preventative and rehabilitative purposes. In this minireview, we will summarize the available data and suggest guidelines for a comprehensive semaglutide clinical utilization until new evidence becomes available.

Key Words: Glucagon-like peptide-1 receptor agonists; Semaglutide; Diabetic retinopathy; Microvascular complications; Cardiovascular benefit; Rehabilitation

Core Tip: Glucagon-like peptide-1 receptor agonists (GLP-1RAs) benefit patients with type 2 diabetes mellitus in terms of glucose management, weight control, oxidative damage, and prevention of adverse renal and cardiovascular events without increasing the of risk of hypoglycemia. After reviewing the literature to investigate upon such a clinically relevant issue, the authors found that: Semaglutide per se seems to cause no direct damage to the retina, and the reported adverse effects might even be ascribed to a bias in the trial design. Special attention to the retinopathy status should be paid at present when using semaglutide in older patients with longstanding diabetes.

Citation: Cigrovski Berkovic M, Strollo F. Semaglutide-eye-catching results. *World J Diabetes* 2023; 14(4): 424-434
URL: https://www.wjgnet.com/1948-9358/full/v14/i4/424.htm
DOI: https://dx.doi.org/10.4239/wjd.v14.i4.424

INTRODUCTION

Glucagon-like peptide-1 receptor agonists (GLP-1RA) are a class of drugs increasingly used for the treatment of patients with type 2 diabetes mellitus (T2DM), enhancing glucose management while promoting weight loss and exposing patients to no significant hypoglycemic risk[1]. In addition to the abovementioned advantages, most agents within the class also provide cardiovascular benefits to diabetic patients, who are well known to be at high risk for cardiovascular atherosclerotic disease. Specifically, seven dedicated cardiovascular outcome trials (CVOTs), *i.e.*, LEADER, SUSTAIN-6, EXSCEL, HARMONY, REWIND, ELIXA, and PIONEER-6, included patients on liraglutide, once weekly sc semaglutide, exenatide, albiglutide, dulaglutide, lixisenatide, and daily oral semaglutide respectively. Data obtained from those CVOTs suggested benefits for T2DM patients with a longstanding history of diabetes, obesity, or overweight in terms of primary and secondary prevention of adverse cardiovascular (CV) events for liraglutide, semaglutide, albiglutide, and dulaglutide, while neutrality was proven in the ELIXA trial[2-8]. Other benefits were also noticed, including reduced albuminuria and improved kidney function in terms of hindered diabetic nephropathy progression and lower need for kidney replacement therapy[9]. However, conflicting data were reported concerning another relevant microvascular complication, *i.e.*, diabetic retinopathy (DR)[10].

DR-PATHOPHYSIOLOGY

DR is the main microvascular complication of diabetes, currently affecting approximately 100 million people worldwide. It increases the likelihood of visual impairment and blindness by 64% and 27%, respectively. Data from the US suggests that almost a third of patients with diabetes over 40 years of age have DR[11,12]. According to the meta-analysis performed for patients with type 1 diabetes, the long-term risk of developing DR can be reduced by intensive glucose control, granting the achievement of near-normal glucose levels[13]. Despite United Kingdom Prospective Diabetes Study results showing that each 1% reduction in HbA1c was associated with a 37% reduction in the development of retinopathy in patients with T2DM[14], conflicting results were also reported concerning the association of tight glucose control with the worsening of previously identified DR signs[15]. However, along with persistent hyperglycemia, risk factors for DR onset include the patient's age, diabetes duration, and high blood pressure, together with genetic predisposition, smoking habits, anemia, non-Caucasian ethnicity, and hyperlipidemia[16-19]. Indeed, pre-existing DR progression can also be related to the magnitude of HbA1c reduction, where data support the evidence of temporary, paradoxical DR worsening during the first three to 36 mo of initiation of intensive glucose lowering before the long-term benefits of glucose optimization become apparent[13,20,21]. The abovementioned deterioration in DR has been described in patients with T1DM and T2DM and during pregnancy. It was initially noticed in patients intensively treated with continuous subcutaneous insulin infusion, but afterward with various diabetic pharmacotherapy, as well as bariatric surgery and pancreatic transplantation[22].

The pathophysiology of DR includes persistent hyperglycemia-related retinal microvessel dilatation, abnormal permeability, and ischemic occlusion with subsequent neovascularization resulting from up-regulation of vascular endothelial growth factor (VEGF) expression[14,23-25]. The evidence available from *in vivo* and *in vitro* models suggests a significant role of longstanding and generalized low-grade chronic inflammation inducing neurodegeneration and oxidative stress at the retinal level[26-29]. Also, according to Zhou *et al*[30], visual function maintenance requires special attention to any available methods, including GLP-1 analogs, to protect retinal ganglion cells from their high intrinsic vulnerability to any sources of damage.

In addition, what is often forgotten is that the metabolic memory, widely recognized as a significant factor in diabetes complications onset and progression, involves the whole body, including the eye, where it acts by increasing the cytosolic protein Drp1 translocation inside the mitochondria with enhanced GTPase activity in response to various stimuli, including hyperglycemia[31,32]. Such changes consistently impair mitochondrial dynamics even after getting back to normoglycemia. Because of that, retinal capillary cells undergo accelerated apoptosis due to cytochrome C leakage into the cytosol due to functional and structural mitochondrial changes, including the DNA. This phenomenon impairs perfusion of acellular capillaries with subsequent neovascularization[33,34].

THEORIES EXPLAINING EARLY WORSENING OF DR

Although various theories exist trying to explain changes in retinal blood flow and hemodynamics associated with early worsening of DR, the mechanism has not been fully elucidated, while optimal *in vitro* conditions and animal models are still lacking[35]. For example, the osmotic theory suggests blood glucose changes to alter lens hydration through altered osmotic pressure, thus causing hyperopic/myopic changes[36,37]. Indeed, insulin has been mostly associated with early DR worsening so far. The mechanisms behind that phenomenon seem to be blood-retinal barrier disruption in the presence of hyperglycemia, consequent to microvascular changes induced by enhanced VEGF production and expression through increased endothelial cell reactive oxygen species (ROS) concentrations, and the often associated hypoglycemic events, which also trigger oxidative stress thus further building up ROS concentrations over time[35,38-40]. In addition, persistently increased IGF-1 levels accompanying fast-improved glucose control might also have a role in initiating or worsening DR[41]. The classification and reporting of this phenomenon are further complicated by the highly variable definition of "DR worsening" by different authors, including cotton-wool exudates, hemorrhages, microaneurysms, intraretinal microvascular abnormalities, and capillary-free areas[42]. Nonetheless, we feel reassured enough by some reports concerning improved DR in the follow-up of patients maintaining tight glucose control over time[43].

GLP-1RA EFFICACY IN GLUCOSE LOWERING AND VASCULAR EFFECTS

As already pointed out, among GLP-1RAs, semaglutide, dulaglutide, and liraglutide proved to prevent major cardiovascular events in the large CVOTs published so far[44]. However, the mechanism behind this observation is still unclear but might include the already mentioned antihyperglycemic and weight loss effects, the potential to decrease systolic and diastolic blood pressure, and more direct endothelial protective mechanisms[45]. In addition to that, GLP-1RAs offer benefits in terms of lowering unfavorable renal outcomes[9]. Such relevant effects also depend on their ability to rapidly and consistently grant quite good glycemic control with significant reductions in HbA1c levels[46]. Their effect on DR is poorly understood and somewhat conflicting, as experimental data even suggests for GLP-1RAs protective effects through hindered blood-retinal barrier disruption and retinal neuron apoptosis[47].

On the other hand, one of the published retrospective studies with exenatide showed a significant proportion of treated patients with fast-improved HbA1c to be at risk of development and progression of DR, in addition to other potentially identified risk factors such as duration of diabetes and presence of preexistent DR[48]. However, the follow-up study published by the same authors reported improved DR in 62% of patients, with no documented DR status changes in a further 18% of patients and DR progression in the remaining 20% (n = 8). Moreover, maculopathy verified in the initial study either regressed or kept stable. In the case of patients with new-onset DR occurring after exenatide initiation, there was evidence of some improvement in 71% (n = 10), and stabilization in three more patients (21%) [49]. An additional interventional case study with exenatide showed complete regression of diabetes-related macular edema and improved visual acuity within one month[50]. On the other hand, multivariate analysis in the AngioSafe study, which included a cohort of 3154 patients with T2DM, found no association between the exposure to GLP-1RAs (liraglutide and exendin-4) and severe DR (P = 0.47)[51]. Similarly, cohort studies from registries did not show any additional risk of DR complications with GLP-1RAs as compared to oral incretin therapy with dipeptidyl peptidase-4 (DPP-4) inhibitors [52], while the results of Brooks and Lissett's investigation suggested a dramatic deterioration of DR be associated with significantly improved HbA1c levels achieved within four months of exenatide treatment[53].

Further on, the LEADER, SUSTAIN-6, and REWIND CVOTs, including liraglutide, subcutaneous semaglutide, and dulaglutide and having retinopathy as a secondary outcome measure (quite broadly pre-specified as vitreous hemorrhage, diabetes-related blindness defined as Snellen visual acuity equal or less than 20/200 or a visual field lower than 20 degrees, or requirement for retinal photocoagulation, intra-vitreal anti- VEGF treatment, or vitrectomy) reported no decrease in DR rate[3,4,7]. Instead, DR complications tended to increase in LEADER and REWIND [hazard ratios 1.15 (95%CI: 0.87-1.52) and

1.24 (95%CI: 0.92-1.68), and significantly increased in the SUSTAIN-6 trial [50 events/1648 people in the semaglutide group *vs* 29 events/1649 people in the placebo group; hazard ratio 1.76 (95%CI: 1.11-2.78)]. In the meta-analysis by Bethel and coauthors[54] including patients treated with semaglutide (oral and subcutaneous), dulaglutide, exenatide, and albiglutide, with similar age (range 62-66 years), and BMI (32-33 kg/m^2), 31%-46% were women, and the mean duration of diabetes ranged from 10 to 15 years. Mean HbA1c ranged from 7.3% to 8.7% (56.3-71.6 mmol/mol), and previous CVD was established in all patients included in the HARMONY trial (albiglutide), while 70% of patients in the REWIND trial (dulaglutide) only had CV risk factors. The prevalence of a differently defined among trials DR ranged from 9.0% to 28.2% at baseline in the REWIND, HARMONY, and PIONEER-6 trials. Moreover, PIONEER-6 excluded patients with already established DR, while only SUSTAIN-6 and PIONEER-6 evaluated retinopathy outcomes with fundus photography or fundoscopy as a scheduled study protocol. The authors did not report a significant association between DR risk and overall GLP-1RA use. However, the data suggested higher differences in the HbA1c levels during the first three months of GLP-1RA initiation in SUSTAIN-6 to represent the most significant risk for DR, while trials EXSCEL, HARMONY, and PIONEER-6, which were associated with negligible impact on HbA1c during the first three months of treatment initiation, did not point out any significant DR risk. Moreover, in line with other evidence, DR risk was not related to blood pressure or weight changes[55]. So, the most critical limitation in DR retinopathy data interpretation is the non-homogeneity of all GLP-1RAs CVOTs in the method of adjudication of DR events and the assessment of DR through widely used scores[10,56,57].

IS SEMAGLUTIDE TO BLAME?

Semaglutide had an extensive efficacy and safety program (SUSTAIN). In studies SUSTAIN 1-5 and SUSTAIN 7, the DR status was annotated within the medical history as not present, present, or unknown, and if present, designated as proliferative, nonproliferative, or unknown and considered an adverse event; patients with proliferative DR and maculopathy requiring acute treatment or HbA1c > 10% and > 10.5%, respectively, did not enter the study. In the SUSTAIN-6, instead, patient exclusion criteria did not take into consideration upper HbA1c limits or the presence of DR, which was indeed adjudicated as a composite endpoint as already described[58,59]. When compared to the overall SUSTAIN-6 study population, those patients developing eye complications had the more severe disease as defined by longer diabetes duration (17.5 years *vs* 13.9 years), higher initial HbA1c (9.4% *vs* 8.7%), and higher insulin-treatment rate (75.9% *vs* 58.0%). Moreover, those patients had a higher proportion of more advanced DR complications at baseline and achieved a more relevant HbA1c reduction during the first 16 wk of semaglutide treatment[58].

On the other hand, a recent meta-analysis of GLP-1RA CVOTs failed to find any association between the drug class with retinopathy. However, after stressing that the abovementioned trials had a median follow-up of 3.4 years (*i.e.*, much shorter than needed for retinopathy onset), used different diagnostic criteria, and were not even powered enough to assess the incidence of retinopathy, the authors reported on a significant association of such complication with HbA1c downward slope (*i.e.*, 0.77, $P < 0.01$), quantifiable as a 6%, 14%, or 8% increased Ln (OR) at 3-mo, 1-year, and overall follow-up, respectively, every 0.1% (1.09 mmol/mol) increase in HbA1c reduction[54].

On the whole, according to a post hoc analysis, the degree of HbA1c decrease and pre-existing retinopathy stood out as the main retinopathy worsening factors[58], yet SUSTAIN-7, *i.e.*, a head-to-head dulaglutide-semaglutide comparison study, showed DR onset or worsening in only two patients (1%) on semaglutide 0.5 mg, two (1%) on dulaglutide 0.75 mg, two (1%) on semaglutide 1.0 mg, and three (1%) on dulaglutide 1.5 mg[59]. Indeed, as no one expects any drugs to develop advanced DR signs in such a short period as the one provided by SUSTAIN-6, a randomization bias might have occurred regarding DR severity assessment at study entry due to the focus on cardiovascular disease monitoring. Once again, the above consideration and the dramatic drop in blood glucose levels might better explain DR results[60].

DATA FROM OBESITY TRIALS INVOLVING GLP-1RAS

Contrary to what we reported above, when turning to GLP1-RAs utilization in obesity, retinopathy was not reported as a complication in long-duration trials assessing weekly semaglutide 2.4 mg injection *vs* placebo for weight loss in obese patients[61-63], and based on a recent revision of the literature, no definite conclusions can be drawn on the role of semaglutide in the incidence or worsening of retinopathy[64], especially when taking into account a systematic review and meta-analysis of all trials, which ruled out any increased DR risk compared to placebo (RR: 1.14, 95%CI: 0.98-1.33), despite suggesting caution in the case of older patients with the long-standing disease (age ≥ 60 years and diabetes duration ≥ 10 years) due to a higher DR risk (RR: 1.27, 95%CI: 1.02-1.59; RR: 1.28, 95%CI: 1.04-1.58, respectively)[65].

EXPERIMENTAL DATA ON MECHANISMS UNDERLYING THE GLP-1RAS/RETINOPATHY ASSOCIATION

The Consortium for the Early Treatment of DR (EUROCONDOR) study failed to find any signs of neurodegeneration in a significant percentage of patients with microangiopathic retinal lesions[66,67]. However, as already mentioned before, neurodegeneration has been proposed as a hallmark of DR[68-70]. Indeed, the American Diabetes Association (ADA) has classified DR as a microvascular and neurovascular complication[29]. In Figure 1 we present the schematics of development of DR. So, at present, drugs like GLP-1RAs, expected to protect both neurons and microvessels, are suggested for the management of early DR stages[71,72].

In such a perspective, a protocol recently set up to rule out a direct role of the drug in retinal damage evaluated the effect of a semaglutide eye-drop solution on retinal neurodegeneration, neuroinflammation, and early vascular leakage in mice. Study results suggested that the drug prevents exactly those three features of diabetes-related retinal damage. The mechanism behind this phenomenon seemed to rely on the decisive anti-inflammatory action linked to decreased expression of NF-kB, proinflammatory cytokines (IL-1, IL-6, and IL-18), and ICAM-1, as well as on the prevention of neuroretinal cell apoptosis promoted by the activation of Akt pathway, which is essential for neuron survival[70]. Moreover, as blood glucose levels were not affected by topical administration, no confounding factors were present due to eventually occurring drops in glucose concentrations. Interestingly, recent results from mice experiments also suggested that semaglutide is unable to cross the blood-brain barrier[73] and has beneficial rather than deleterious effects, as already reported with other GLP-1RAs in the experimental animals[71,72,74,75], possibly due to improved blood-retina barrier function and neuronal apoptosis [47], reduced glutamate levels obtained through upregulated glutamate-aspartate transporter (GLAST) [75], and decreased placental growth factor and intercellular adhesion molecule-1 expression[71,72].

THE FUTURE OF SEMAGLUTIDE REGARDING RETINOPATHY

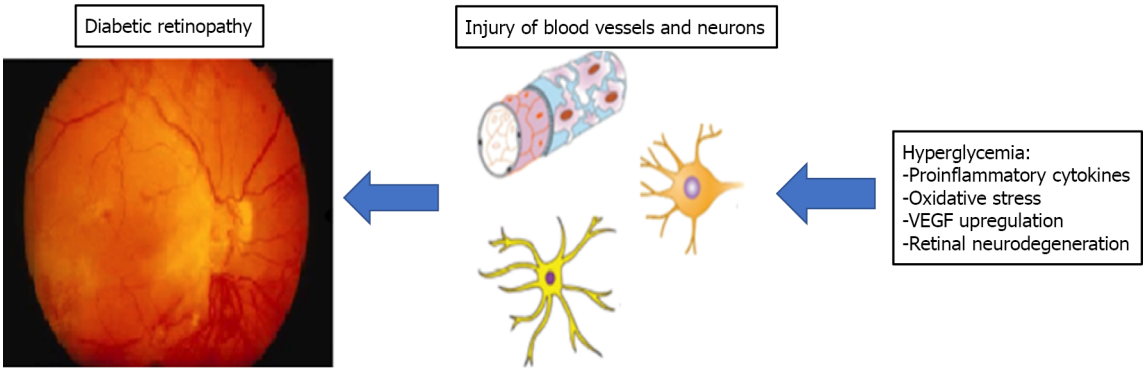
The potential direct mechanism of action of GLP-1RA on the retinal cells is still elusive and debated. Although the GLP-1 receptor is present in the human eye, no GLP-1R expression was found in the eyes of people with long-standing proliferative DR[76].

However, the effects of a newly identified confounding factor, *i.e.*, subtle differences in individual gut microbiota composition, cannot be easily ruled out. Indeed, despite the relationship between intestinal microbiota and host still requiring complete elucidation, gut dysbiosis, *i.e.*, the chronic disequilibrium within the many different microbial colonies, seems associated with several inflammatory/metabolic diseases and central nervous system disorders, including retinopathy as an expression of the emerging concept of the so-called "microbiota-retina axis". Indeed, as longstanding diabetes is associated not only with retinopathy but also with significant intestinal dysbiosis[77,78], relevant changes in the bacterial population might trigger the onset of retinopathy *via* their influence on the lipid content of both retinal and CNS tissues, eventually responsible for decreased intraocular succinate concentrations or increased trimethylamine-N-oxide (TMAO) plasma levels[79-86].

Retinopathy is a disabling disease, so rehabilitation should start as soon as possible when prevention fails. Based on the abovementioned neuroinflammatory mechanisms, several anti-inflammatory substances may help prevent DR progression, including nutritional supplements like resveratrol. However, the latter, despite looking promising enough, has a too low level of bioavailability and is contraindicated in the frequently occurring case of iron-deficiency-dependent anemia[87].

DETAILS CONCERNING SEMAGLUTIDE'S PHARMACOLOGIC PROPERTIES FAVORING DR SAFETY

Semaglutide has a 94% amino acid sequence homology to native GLP-1. However, structural modifications from endogenous GLP-1, *i.e.*, alanine residue substitution at the 8th position with Aib, make it less susceptible to degradation by DPP-4 while acylation of Lysine residue at the 26th position and attachment of a C18 fatty-diacid increase its binding affinity to albumin. The above changes result in a half-life of approximately one week, making it appropriate for once-weekly use in clinical practice. In the phase-3 SUSTAIN trials semaglutide showed superiority to different comparators and during different stages of diabetes in reducing HbA1c and body weight. In a SUSTAIN-6 trial investigating cardiovascular outcomes, semaglutide led to a 26% reduction in risk of the primary 3-point MACE (a composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke) when compared to placebo. The pharmacokinetic properties of semaglutide are not significantly affected by impaired hepatic or renal function. Therefore, no dose adjustments are required in that case. It achieves a steady state concentration in 4 wk to 5 wk (both subcutaneous and oral form). Indeed, in the case of subcutaneous semaglutide, 1 d to 3 d are needed to achieve the maximum concentration, and in the case



DOI: 10.4239/wjd.v14.i4.424

Figure 1 Main mechanisms involved in blood vessels injury and neuroinflammation in the diabetic retina. VEGF: Vascular endothelial growth factor.

of oral semaglutide, one hour is needed following intake. Moreover, during clinical pharmacology studies, no relevant impact of semaglutide on concomitant orally administered medications was observed, making its use safe in a broad population[88]. Semaglutide improves the efficiency of incretin function by activating GLP-1 receptors and enhancing GLP-1 to supraphysiological levels. It reduces fasting and postprandial glucose levels by promoting insulin secretion in a glucose-dependent manner and suppressing hepatic gluconeogenesis through blunted glucagon release. Moreover, it improves both proinsulin to insulin ratio, which suggests improved β-cell function and insulin sensitivity through body weight and fat loss consequent to reduced energy intake and gastric motility[89].

A cardiovascular risk (3-P MACE) reduction effect compared to placebo was shown for injectable and oral semaglutide in SUSTAIN-6 and PIONEER-6 trials, respectively. Animal studies have shown antiatherosclerotic effects mediated by regulating multiple inflammatory pathways and antiapoptotic effects in cardiac cells[90]. In addition, three ongoing trials, *i.e.*, SOUL (NCT03914326), SELECT (Semaglutide Effects on Heart Disease and Stroke in Patients with Overweight or Obesity; NCT03574597), and STRIDE (NCT04560998), will give further insight into different cardiovascular effects of both oral and subcutaneous semaglutide in patients with and without T2DM, and overweight/obesity[91-93].

The semaglutide mechanism of action is glucose-dependent and, therefore, associated per se with a shallow risk of hypoglycemia. Nevertheless, semaglutide cannot avoid the risk of hypoglycemia due to add-on sulfonylureas or insulin. Such drug association might initially cause acute glucose level drops and probably transient worsening of DR, as observed in the first four weeks of treatment[58].

CONCLUSION

Nowadays, the most robust relationship between semaglutide treatment and early worsening of DR might find its sizable initial impact on HbA1c as a suitable explanation. Indeed, both semaglutide formulations (subcutaneous and oral) have been investigated in two phase-3 clinical programs, *i.e.*, the SUSTAIN and PIONEER program. Combining all individual studies within these two programs, over 21500 patients participated in the studies with a treatment duration of at least 26 wk, giving a plethora of safety evidence[94]. Data insinuating a connection between semaglutide and DR emerged during the phase-3 trials. However, the answer to whether that phenomenon depended on the drug itself or the magnitude of fast-occurring glucose lowering will come from the presently underway FOCUS trial on approximately 1500 patients with T2DM, a bilateral 10 to 75 Early Treatment DR Study (ETDRS) score and no need for ophthalmologic therapy. Such a study will analyze once-weekly subcutaneous semaglutide 1.0 mg compared with placebo for up to 5 years, with the primary endpoint of at least three-digit ETDRS score progression[95]. Until FOCUS results become available, caution is mandatory in patients with DR. It may be wise to perform fundoscopy prior to semaglutide therapy, and existing DR should be treated concomitantly. In addition, given the strong effects of semaglutide on glucose levels, down-titrating basal insulin therapy or stopping sulphonylurea will prevent rapid decreases in glucose concentrations, thereby reducing the risk of acute DR worsening. Guidelines on DR management do not differ for patients receiving other antidiabetic agents. Therefore, the same treatment, including anti-VEGF agents, should be applied.

Both current and future research on GLP-1RA is quite exciting and targets different metabolic conditions and health aspects associated with both T2DM and obesity, such as cognitive health and dementia, PCO, and NAFLD.

However, the scientific community must shed light on the topic and draw definite conclusions concerning a direct mechanism of one (or more) GLP-1RA class drug(s) in retinopathy development and worsening.

Care required by T2DM patients initiating GLP-1RA treatment does not differ from that expected with other types of intensive glucose-lowering medication, *i.e.*, early detection of DR onset/progression and, eventually, specific treatment. Screening for DR is recommended for patients at the time of T2DM diagnosis and then annually while also taking into account individual metabolic control and baseline DR conditions[96]. In the case of severe, proliferative DR, treatment of retinopathy should start before or with intensive glucose-lowering therapy, expecting a typically transient worsening though[97]. While increasingly more overweight and obese T2DM patients at high CV risk start on GLP-1RAs, clinicians should consider their DR status upon initiation and adopt the best available treatment for DR when present.

When choosing semaglutide in the intensification of T2DM treatment, a cautious attitude is mandatory, at the moment, especially in the case of coexisting insulin treatment and advanced disease stages.

FOOTNOTES

Author contributions: Cigrovski Berkovic M conceived and wrote the original draft; Strollo F was involved in data collection and analysis and writing the manuscript; both authors approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest.

Country/Territory of origin: Croatia

ORCID number: Maja Cigrovski Berkovic 0000-0003-0750-9785.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Giugliano D**, Scappaticcio L, Longo M, Caruso P, Maiorino MI, Bellastella G, Ceriello A, Chiodini P, Esposito K. GLP-1 receptor agonists and cardiorenal outcomes in type 2 diabetes: an updated meta-analysis of eight CVOTs. *Cardiovasc Diabetol* 2021; **20**: 189 [PMID: 34526024 DOI: 10.1186/s12933-021-01366-8]

2 **Pfeffer MA**, Claggett B, Diaz R, Dickstein K, Gerstein HC, Køber LV, Lawson FC, Ping L, Wei X, Lewis EF, Maggioni AP, McMurray JJ, Probstfield JL, Riddle MC, Solomon SD, Tardif JC; ELIXA Investigators. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med* 2015; **373**: 2247-2257 [PMID: 26630143 DOI: 10.1056/NEJMoa1509225]

3 **Marso SP**, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; **375**: 311-322 [PMID: 27295427 DOI: 10.1056/NEJMoa1603827]

4 **Marso SP**, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T; SUSTAIN-6 Investigators. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016; **375**: 1834-1844 [PMID: 27633186 DOI: 10.1056/NEJMoa1607141]

5 **Holman RR**, Bethel MA, Mentz RJ, Thompson VP, Lokhnygina Y, Buse JB, Chan JC, Choi J, Gustavson SM, Iqbal N, Maggioni AP, Marso SP, Öhman P, Pagidipati NJ, Poulter N, Ramachandran A, Zinman B, Hernandez AF; EXSCEL Study Group. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 1228-1239 [PMID: 28910237 DOI: 10.1056/NEJMoa1612917]

6 **Green JB**, Hernandez AF, D'Agostino RB, Granger CB, Janmohamed S, Jones NP, Leiter LA, Noronha D, Russell R, Sigmon K, Del Prato S, McMurray JJV. Harmony Outcomes: A randomized, double-blind, placebo-controlled trial of the effect of albiglutide on major cardiovascular events in patients with type 2 diabetes mellitus-Rationale, design, and baseline characteristics. *Am Heart J* 2018; **203**: 30-38 [PMID: 30015066 DOI: 10.1016/j.ahj.2018.03.030]

7 **Gerstein HC**, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, Probstfield J, Riesmeyer JS, Riddle MC, Rydén L, Xavier D, Atisso CM, Dyal L, Hall S, Rao-Melacini P, Wong G, Avezum A, Basile J, Chung N, Conget I,

Cushman WC, Franek E, Hancu N, Hanefeld M, Holt S, Jansky P, Keltai M, Lanas F, Leiter LA, Lopez-Jaramillo P, Cardona Munoz EG, Pirags V, Pogosova N, Raubenheimer PJ, Shaw JE, Sheu WH, Temelkova-Kurktschiev T; REWIND Investigators. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. *Lancet* 2019; **394**: 121-130 [PMID: 31189511 DOI: 10.1016/S0140-6736(19)31149-3]

8 **Husain M**, Birkenfeld AL, Donsmark M, Dungan K, Eliaschewitz FG, Franco DR, Jeppesen OK, Lingvay I, Mosenzon O, Pedersen SD, Tack CJ, Thomsen M, Vilsbøll T, Warren ML, Bain SC; PIONEER 6 Investigators. Oral Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2019; **381**: 841-851 [PMID: 31185157 DOI: 10.1056/NEJMoa1901118]

9 **Kristensen SL**, Rørth R, Jhund PS, Docherty KF, Sattar N, Preiss D, Køber L, Petrie MC, McMurray JJV. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol* 2019; **7**: 776-785 [PMID: 31422062 DOI: 10.1016/S2213-8587(19)30249-9]

10 **Saw M**, Wong VW, Ho IV, Liew G. New anti-hyperglycaemic agents for type 2 diabetes and their effects on diabetic retinopathy. *Eye (Lond)* 2019; **33**: 1842-1851 [PMID: 31227789 DOI: 10.1038/s41433-019-0494-z]

11 **Leasher JL**, Bourne RR, Flaxman SR, Jonas JB, Keeffe J, Naidoo K, Pesudovs K, Price H, White RA, Wong TY, Resnikoff S, Taylor HR; Vision Loss Expert Group of the Global Burden of Disease Study. Global Estimates on the Number of People Blind or Visually Impaired by Diabetic Retinopathy: A Meta-analysis From 1990 to 2010. *Diabetes Care* 2016; **39**: 1643-1649 [PMID: 27555623 DOI: 10.2337/dc15-2171]

12 **Zhang X**, Saaddine JB, Chou CF, Cotch MF, Cheng YJ, Geiss LS, Gregg EW, Albright AL, Klein BE, Klein R. Prevalence of diabetic retinopathy in the United States, 2005-2008. *JAMA* 2010; **304**: 649-656 [PMID: 20699456 DOI: 10.1001/jama.2010.1111]

13 **Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group**, Lachin JM, White NH, Hainsworth DP, Sun W, Cleary PA, Nathan DM. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes* 2015; **64**: 631-642 [PMID: 25204977 DOI: 10.2337/db14-0930]

14 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976]

15 **Fullerton B**, Jeitler K, Seitz M, Horvath K, Berghold A, Siebenhofer A. Intensive glucose control versus conventional glucose control for type 1 diabetes mellitus. *Cochrane Database Syst Rev* 2014; **2014**: CD009122 [PMID: 24526393 DOI: 10.1002/14651858.CD009122.pub2]

16 **Scanlon PH**. Improving the screening of risk factors in diabetic retinopathy. *Expert Rev Endocrinol Metab* 2022; **17**: 235-243 [PMID: 35730170 DOI: 10.1080/17446651.2022.2078305]

17 **Bryl A**, Mrugacz M, Falkowski M, Zorena K. The Effect of Hyperlipidemia on the Course of Diabetic Retinopathy-Literature Review. *J Clin Med* 2022; **11** [PMID: 35628887 DOI: 10.3390/jcm11102761]

18 **Bryl A**, Mrugacz M, Falkowski M, Zorena K. The Effect of Diet and Lifestyle on the Course of Diabetic Retinopathy-A Review of the Literature. *Nutrients* 2022; **14** [PMID: 35334909 DOI: 10.3390/nu14061252]

19 **Milluzzo A**, Barchitta M, Maugeri A, Magnano San Lio R, Favara G, Mazzone MG, Sciacca L, Agodi A. Do Nutrients and Nutraceuticals Play a Role in Diabetic Retinopathy? *Nutrients* 2022; **14** [PMID: 36297113 DOI: 10.3390/nu14204430]

20 Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial. *Arch Ophthalmol* 1998; **116**: 874-886 [PMID: 9682700 DOI: 10.1001/archopht.116.7.874]

21 **Bain SC**, Klufas MA, Ho A, Matthews DR. Worsening of diabetic retinopathy with rapid improvement in systemic glucose control: A review. *Diabetes Obes Metab* 2019; **21**: 454-466 [PMID: 30226298 DOI: 10.1111/dom.13538]

22 **Feldman-Billard S**, Larger É, Massin P; Standards for screeningand surveillance of ocular complications in people with diabetes SFD study group. Early worsening of diabetic retinopathy after rapid improvement of blood glucose control in patients with diabetes. *Diabetes Metab* 2018; **44**: 4-14 [PMID: 29217386 DOI: 10.1016/j.diabet.2017.10.014]

23 **Beltramo E**, Porta M. Pericyte loss in diabetic retinopathy: mechanisms and consequences. *Curr Med Chem* 2013; **20**: 3218-3225 [PMID: 23745544 DOI: 10.2174/09298673113209990022]

24 **Lupo G**, Motta C, Giurdanella G, Anfuso CD, Alberghina M, Drago F, Salomone S, Bucolo C. Role of phospholipases A2 in diabetic retinopathy: in vitro and in vivo studies. *Biochem Pharmacol* 2013; **86**: 1603-1613 [PMID: 24076420 DOI: 10.1016/j.bcp.2013.09.008]

25 **Diabetes Control and Complications Trial Research Group**, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

26 **Abcouwer SF**. Müller Cell-Microglia Cross Talk Drives Neuroinflammation in Diabetic Retinopathy. *Diabetes* 2017; **66**: 261-263 [PMID: 28108606 DOI: 10.2337/dbi16-0047]

27 **Sasaki M**, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, Kobayashi S, Ishida S, Tsubota K. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia* 2010; **53**: 971-979 [PMID: 20162412 DOI: 10.1007/s00125-009-1655-6]

28 **Boss JD**, Singh PK, Pandya HK, Tosi J, Kim C, Tewari A, Juzych MS, Abrams GW, Kumar A. Assessment of Neurotrophins and Inflammatory Mediators in Vitreous of Patients With Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2017; **58**: 5594-5603 [PMID: 29084332 DOI: 10.1167/iovs.17-21973]

29 **Solomon SD**, Chew E, Duh EJ, Sobrin L, Sun JK, VanderBeek BL, Wykoff CC, Gardner TW. Diabetic Retinopathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 412-418 [PMID: 28223445 DOI: 10.2337/dc16-2641]

30 **Zhou HR**, Ma XF, Lin WJ, Hao M, Yu XY, Li HX, Xu CY, Kuang HY. Neuroprotective Role of GLP-1 Analog for Retinal Ganglion Cells *via* PINK1/Parkin-Mediated Mitophagy in Diabetic Retinopathy. *Front Pharmacol* 2020; **11**: 589114 [PMID: 33679385 DOI: 10.3389/fphar.2020.589114]

31 **Mohammad G**, Kowluru RA. Mitochondrial Dynamics in the Metabolic Memory of Diabetic Retinopathy. *J Diabetes Res* 2022; **2022**: 3555889 [PMID: 35399705 DOI: 10.1155/2022/3555889]

32 **Duraisamy AJ**, Mohammad G, Kowluru RA. Mitochondrial fusion and maintenance of mitochondrial homeostasis in diabetic retinopathy. *Biochim Biophys Acta Mol Basis Dis* 2019; **1865**: 1617-1626 [PMID: 30922813 DOI: 10.1016/j.bbadis.2019.03.013]

33 **Kanwar M**, Chan PS, Kern TS, Kowluru RA. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci* 2007; **48**: 3805-3811 [PMID: 17652755 DOI: 10.1167/iovs.06-1280]

34 **Kowluru RA**. Mitochondrial Stability in Diabetic Retinopathy: Lessons Learned From Epigenetics. *Diabetes* 2019; **68**: 241-247 [PMID: 30665952 DOI: 10.2337/dbi18-0016]

35 **Jingi AM**, Tankeu AT, Ateba NA, Noubiap JJ. Mechanism of worsening diabetic retinopathy with rapid lowering of blood glucose: the synergistic hypothesis. *BMC Endocr Disord* 2017; **17**: 63 [PMID: 29017477 DOI: 10.1186/s12902-017-0213-3]

36 **Okamoto F**, Sone H, Nonoyama T, Hommura S. Refractive changes in diabetic patients during intensive glycaemic control. *Br J Ophthalmol* 2000; **84**: 1097-1102 [PMID: 11004091 DOI: 10.1136/bjo.84.10.1097]

37 **Casson RJ**, Wood JP, Osborne NN. Hypoglycaemia exacerbates ischaemic retinal injury in rats. *Br J Ophthalmol* 2004; **88**: 816-820 [PMID: 15148218 DOI: 10.1136/bjo.2003.024661]

38 **Wu H**, Jiang C, Gan D, Liao Y, Ren H, Sun Z, Zhang M, Xu G. Different effects of low- and high-dose insulin on ROS production and VEGF expression in bovine retinal microvascular endothelial cells in the presence of high glucose. *Graefes Arch Clin Exp Ophthalmol* 2011; **249**: 1303-1310 [PMID: 21494874 DOI: 10.1007/s00417-011-1677-x]

39 **Poulaki V**, Qin W, Joussen AM, Hurlbut P, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP. Acute intensive insulin therapy exacerbates diabetic blood-retinal barrier breakdown *via* hypoxia-inducible factor-1alpha and VEGF. *J Clin Invest* 2002; **109**: 805-815 [PMID: 11901189 DOI: 10.1172/JCI13776]

40 **Kennedy A**, Frank RN. The influence of glucose concentration and hypoxia on VEGF secretion by cultured retinal cells. *Curr Eye Res* 2011; **36**: 168-177 [PMID: 21158590 DOI: 10.3109/02713683.2010.521968]

41 **Henricsson M**, Berntorp K, Fernlund P, Sundkvist G. Progression of retinopathy in insulin-treated type 2 diabetic patients. *Diabetes Care* 2002; **25**: 381-385 [PMID: 11815514 DOI: 10.2337/diacare.25.2.381]

42 **Wong TY**, Cheung CM, Larsen M, Sharma S, Simó R. Diabetic retinopathy. *Nat Rev Dis Primers* 2016; **2**: 16012 [PMID: 27159554 DOI: 10.1038/nrdp.2016.12]

43 **Kohner EM**, Sleightholm M. Does microaneurysm count reflect severity of early diabetic retinopathy? *Ophthalmology* 1986; **93**: 586-589 [PMID: 3725317 DOI: 10.1016/s0161-6420(86)33692-3]

44 **American Diabetes Association**. 10. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2021. *Diabetes Care* 2021; **44**: S125-S150 [PMID: 33298421 DOI: 10.2337/dc21-S010]

45 **Ma X**, Liu Z, Ilyas I, Little PJ, Kamato D, Sahebka A, Chen Z, Luo S, Zheng X, Weng J, Xu S. GLP-1 receptor agonists (GLP-1RAs): cardiovascular actions and therapeutic potential. *Int J Biol Sci* 2021; **17**: 2050-2068 [PMID: 34131405 DOI: 10.7150/ijbs.59965]

46 **Dungan KM**, Povedano ST, Forst T, González JG, Atisso C, Sealls W, Fahrbach JL. Once-weekly dulaglutide versus once-daily liraglutide in metformin-treated patients with type 2 diabetes (AWARD-6): a randomised, open-label, phase 3, non-inferiority trial. *Lancet* 2014; **384**: 1349-1357 [PMID: 25018121 DOI: 10.1016/S0140-6736(14)60976-4]

47 **Pang B**, Zhou H, Kuang H. The potential benefits of glucagon-like peptide-1 receptor agonists for diabetic retinopathy. *Peptides* 2018; **100**: 123-126 [PMID: 28807775 DOI: 10.1016/j.peptides.2017.08.003]

48 **Varadhan L**, Humphreys T, Hariman C, Walker AB, Varughese GI. GLP-1 agonist treatment: implications for diabetic retinopathy screening. *Diabetes Res Clin Pract* 2011; **94**: e68-e71 [PMID: 21906831 DOI: 10.1016/j.diabres.2011.08.017]

49 **Varadhan L**, Humphreys T, Walker AB, Varughese GI. The impact of improved glycaemic control with GLP-1 receptor agonist therapy on diabetic retinopathy. *Diabetes Res Clin Pract* 2014; **103**: e37-e39 [PMID: 24456992 DOI: 10.1016/j.diabres.2013.12.041]

50 **Sarao V**, Veritti D, Lanzetta P. Regression of diabetic macular edema after subcutaneous exenatide. *Acta Diabetol* 2014; **51**: 505-508 [PMID: 23925692 DOI: 10.1007/s00592-013-0506-6]

51 **Gaborit B**, Julla JB, Besbes S, Proust M, Vincentelli C, Alos B, Ancel P, Alzaid F, Garcia R, Mailly P, Sabatier F, Righini M, Gascon P, Matonti F, Houssays M, Goumidi L, Vignaud L, Guillonneau X, Erginay A, Dupas B, Marie-Louise J, Autié M, Vidal-Trecan T, Riveline JP, Venteclef N, Massin P, Muller L, Dutour A, Gautier JF, Germain S. Glucagon-like Peptide 1 Receptor Agonists, Diabetic Retinopathy and Angiogenesis: The AngioSafe Type 2 Diabetes Study. *J Clin Endocrinol Metab* 2020; **105** [PMID: 31589290 DOI: 10.1210/clinem/dgz069]

52 **Ueda P**, Pasternak B, Eliasson B, Svensson AM, Franzén S, Gudbjörnsdottir S, Hveem K, Jonasson C, Melbye M, Svanström H. Glucagon-Like Peptide 1 Receptor Agonists and Risk of Diabetic Retinopathy Complications: Cohort Study in Nationwide Registers From Two Countries. *Diabetes Care* 2019; **42**: e92-e94 [PMID: 31010872 DOI: 10.2337/dc18-2532]

53 **Brooks AM**, Lissett CA. A dramatic deterioration in diabetic retinopathy with improvement in glycated haemoglobin (HbA(1c)) on exenatide treatment. *Diabet Med* 2009; **26**: 190 [PMID: 19236627 DOI: 10.1111/j.1464-5491.2008.02650.x]

54 **Bethel MA**, Diaz R, Castellana N, Bhattacharya I, Gerstein HC, Lakshmanan MC. HbA(1c) Change and Diabetic Retinopathy During GLP-1 Receptor Agonist Cardiovascular Outcome Trials: A Meta-analysis and Meta-regression. *Diabetes Care* 2021; **44**: 290-296 [PMID: 33444163 DOI: 10.2337/dc20-1815]

55 **Zhu W**, Wu Y, Meng YF, Xing Q, Tao JJ, Lu J. Association of obesity and risk of diabetic retinopathy in diabetes patients: A meta-analysis of prospective cohort studies. *Medicine (Baltimore)* 2018; **97**: e11807 [PMID: 30095648 DOI: 10.1097/MD.0000000000011807]

56 **Do DV**, Wang X, Vedula SS, Marrone M, Sleilati G, Hawkins BS, Frank RN. Blood pressure control for diabetic retinopathy. *Cochrane Database Syst Rev* 2015; **1**: CD006127 [PMID: 25637717 DOI: 10.1002/14651858.CD006127.pub2]

57 **Wilkinson CP**, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R,

Verdaguer JT; Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology* 2003; **110**: 1677-1682 [PMID: 13129861 DOI: 10.1016/S0161-6420(03)00475-5]

58 **Vilsbøll T**, Bain SC, Leiter LA, Lingvay I, Matthews D, Simó R, Helmark IC, Wijayasinghe N, Larsen M. Semaglutide, reduction in glycated haemoglobin and the risk of diabetic retinopathy. *Diabetes Obes Metab* 2018; **20**: 889-897 [PMID: 29178519 DOI: 10.1111/dom.13172]

59 **Pratley RE**, Aroda VR, Lingvay I, Lüdemann J, Andreassen C, Navarria A, Viljoen A; SUSTAIN 7 investigators. Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. *Lancet Diabetes Endocrinol* 2018; **6**: 275-286 [PMID: 29397376 DOI: 10.1016/S2213-8587(18)30024-X]

60 **Simó R**, Bogdanov P, Ramos H, Huerta J, Simó-Servat O, Hernández C. Effects of the Topical Administration of Semaglutide on Retinal Neuroinflammation and Vascular Leakage in Experimental Diabetes. *Biomedicines* 2021; **9** [PMID: 34440130 DOI: 10.3390/biomedicines9080926]

61 **Wadden TA**, Bailey TS, Billings LK, Davies M, Frias JP, Koroleva A, Lingvay I, O'Neil PM, Rubino DM, Skovgaard D, Wallenstein SOR, Garvey WT; STEP 3 Investigators. Effect of Subcutaneous Semaglutide vs Placebo as an Adjunct to Intensive Behavioral Therapy on Body Weight in Adults With Overweight or Obesity: The STEP 3 Randomized Clinical Trial. *JAMA* 2021; **325**: 1403-1413 [PMID: 33625476 DOI: 10.1001/jama.2021.1831]

62 **Wilding JPH**, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, McGowan BM, Rosenstock J, Tran MTD, Wadden TA, Wharton S, Yokote K, Zeuthen N, Kushner RF; STEP 1 Study Group. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med* 2021; **384**: 989-1002 [PMID: 33567185 DOI: 10.1056/NEJMoa2032183]

63 **Rubino D**, Abrahamsson N, Davies M, Hesse D, Greenway FL, Jensen C, Lingvay I, Mosenzon O, Rosenstock J, Rubio MA, Rudofsky G, Tadayon S, Wadden TA, Dicker D; STEP 4 Investigators. Effect of Continued Weekly Subcutaneous Semaglutide vs Placebo on Weight Loss Maintenance in Adults With Overweight or Obesity: The STEP 4 Randomized Clinical Trial. *JAMA* 2021; **325**: 1414-1425 [PMID: 33755728 DOI: 10.1001/jama.2021.3224]

64 **Gallwitz B**, Giorgino F. Clinical Perspectives on the Use of Subcutaneous and Oral Formulations of Semaglutide. *Front Endocrinol (Lausanne)* 2021; **12**: 645507 [PMID: 34267725 DOI: 10.3389/fendo.2021.645507]

65 **Wang F**, Mao Y, Wang H, Liu Y, Huang P. Semaglutide and Diabetic Retinopathy Risk in Patients with Type 2 Diabetes Mellitus: A Meta-Analysis of Randomized Controlled Trials. *Clin Drug Investig* 2022; **42**: 17-28 [PMID: 34894326 DOI: 10.1007/s40261-021-01110-w]

66 **Santos AR**, Ribeiro L, Bandello F, Lattanzio R, Egan C, Frydkjaer-Olsen U, García-Arumí J, Gibson J, Grauslund J, Harding SP, Lang GE, Massin P, Midena E, Scanlon P, Aldington SJ, Simão S, Schwartz C, Ponsati B, Porta M, Costa MÂ, Hernández C, Cunha-Vaz J, Simó R; European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR). Functional and Structural Findings of Neurodegeneration in Early Stages of Diabetic Retinopathy: Cross-sectional Analyses of Baseline Data of the EUROCONDOR Project. *Diabetes* 2017; **66**: 2503-2510 [PMID: 28663190 DOI: 10.2337/db16-1453]

67 **Hernández C**, Porta M, Bandello F, Grauslund J, Harding SP, Aldington SJ, Egan C, Frydkjaer-Olsen U, García-Arumí J, Gibson J, Lang GE, Lattanzio R, Massin P, Midena E, Ponsati B, Ribeiro L, Scanlon P, Cunha-Vaz J, Simó R. The Usefulness of Serum Biomarkers in the Early Stages of Diabetic Retinopathy: Results of the EUROCONDOR Clinical Trial. *J Clin Med* 2020; **9** [PMID: 32344735 DOI: 10.3390/jcm9041233]

68 **Simó R**, Hernández C; European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR). Neurodegeneration is an early event in diabetic retinopathy: therapeutic implications. *Br J Ophthalmol* 2012; **96**: 1285-1290 [PMID: 22887976 DOI: 10.1136/bjophthalmol-2012-302005]

69 **Simó R**, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia* 2018; **61**: 1902-1912 [PMID: 30030554 DOI: 10.1007/s00125-018-4692-1]

70 **Simó R**, Simó-Servat O, Bogdanov P, Hernández C. Diabetic Retinopathy: Role of Neurodegeneration and Therapeutic Perspectives. *Asia Pac J Ophthalmol (Phila)* 2022; **11**: 160-167 [PMID: 35533335 DOI: 10.1097/APO.0000000000000510]

71 **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]

72 **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]

73 **Salameh TS**, Rhea EM, Talbot K, Banks WA. Brain uptake pharmacokinetics of incretin receptor agonists showing promise as Alzheimer's and Parkinson's disease therapeutics. *Biochem Pharmacol* 2020; **180**: 114187 [PMID: 32755557 DOI: 10.1016/j.bcp.2020.114187]

74 **Zhang Y**, Wang Q, Zhang J, Lei X, Xu GT, Ye W. Protection of exendin-4 analogue in early experimental diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2009; **247**: 699-706 [PMID: 19084986 DOI: 10.1007/s00417-008-1004-3]

75 **Zhang Y**, Zhang J, Wang Q, Lei X, Chu Q, Xu GT, Ye W. Intravitreal injection of exendin-4 analogue protects retinal cells in early diabetic rats. *Invest Ophthalmol Vis Sci* 2011; **52**: 278-285 [PMID: 20688733 DOI: 10.1167/iovs.09-4727]

76 **Hebsgaard JB**, Pyke C, Yildirim E, Knudsen LB, Heegaard S, Kvist PH. Glucagon-like peptide-1 receptor expression in the human eye. *Diabetes Obes Metab* 2018; **20**: 2304-2308 [PMID: 29707863 DOI: 10.1111/dom.13339]

77 **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]

78 **Liu K**, Zou J, Fan H, Hu H, You Z. Causal effects of gut microbiota on diabetic retinopathy: A Mendelian randomization study. *Front Immunol* 2022; **13**: 930318 [PMID: 36159877 DOI: 10.3389/fimmu.2022.930318]

79 **Oresic M**, Seppänen-Laakso T, Yetukuri L, Bäckhed F, Hänninen V. Gut microbiota affects lens and retinal lipid composition. *Exp Eye Res* 2009; **89**: 604-607 [PMID: 19591827 DOI: 10.1016/j.exer.2009.06.018]

80 **Albouery M**, Buteau B, Grégoire S, Cherbuy C, Pais de Barros JP, Martine L, Chain F, Cabaret S, Berdeaux O, Bron AM, Acar N, Langella P, Bringer MA. Age-Related Changes in the Gut Microbiota Modify Brain Lipid Composition. *Front*

Cell Infect Microbiol 2019; **9**: 444 [PMID: 31993375 DOI: 10.3389/fcimb.2019.00444]

81 **Barba I**, Garcia-Ramírez M, Hernández C, Alonso MA, Masmiquel L, García-Dorado D, Simó R. Metabolic fingerprints of proliferative diabetic retinopathy: an 1H-NMR-based metabonomic approach using vitreous humor. *Invest Ophthalmol Vis Sci* 2010; **51**: 4416-4421 [PMID: 20375324 DOI: 10.1167/iovs.10-5348]

82 **Jin H**, Zhu B, Liu X, Jin J, Zou H. Metabolic characterization of diabetic retinopathy: An (1)H-NMR-based metabolomic approach using human aqueous humor. *J Pharm Biomed Anal* 2019; **174**: 414-421 [PMID: 31212142 DOI: 10.1016/j.jpba.2019.06.013]

83 **Liu W**, Wang C, Xia Y, Xia W, Liu G, Ren C, Gu Y, Li X, Lu P. Elevated plasma trimethylamine-N-oxide levels are associated with diabetic retinopathy. *Acta Diabetol* 2021; **58**: 221-229 [PMID: 33064205 DOI: 10.1007/s00592-020-01610-9]

84 **Nowiński A**, Ufnal M. Trimethylamine N-oxide: A harmful, protective or diagnostic marker in lifestyle diseases? *Nutrition* 2018; **46**: 7-12 [PMID: 29290360 DOI: 10.1016/j.nut.2017.08.001]

85 **Shan Z**, Sun T, Huang H, Chen S, Chen L, Luo C, Yang W, Yang X, Yao P, Cheng J, Hu FB, Liu L. Association between microbiota-dependent metabolite trimethylamine-N-oxide and type 2 diabetes. *Am J Clin Nutr* 2017; **106**: 888-894 [PMID: 28724646 DOI: 10.3945/ajcn.117.157107]

86 **Bringer MA**, Gabrielle PH, Bron AM, Creuzot-Garcher C, Acar N. The gut microbiota in retinal diseases. *Exp Eye Res* 2022; **214**: 108867 [PMID: 34856206 DOI: 10.1016/j.exer.2021.108867]

87 **Bryl A**, Falkowski M, Zorena K, Mrugacz M. The Role of Resveratrol in Eye Diseases-A Review of the Literature. *Nutrients* 2022; **14** [PMID: 35889930 DOI: 10.3390/nu14142974]

88 **Ikushima I**, Jensen L, Flint A, Nishida T, Zacho J, Irie S. A Randomized Trial Investigating the Pharmacokinetics, Pharmacodynamics, and Safety of Subcutaneous Semaglutide Once-Weekly in Healthy Male Japanese and Caucasian Subjects. *Adv Ther* 2018; **35**: 531-544 [PMID: 29536338 DOI: 10.1007/s12325-018-0677-1]

89 **Mahapatra MK**, Karuppasamy M, Sahoo BM. Semaglutide, a glucagon like peptide-1 receptor agonist with cardiovascular benefits for management of type 2 diabetes. *Rev Endocr Metab Disord* 2022; **23**: 521-539 [PMID: 34993760 DOI: 10.1007/s11154-021-09699-1]

90 **Iorga RA**, Bacalbasa N, Carsote M, Bratu OG, Stanescu AMA, Bungau S, Pantis C, Diaconu CC. Metabolic and cardiovascular benefits of GLP-1 agonists, besides the hypoglycemic effect (Review). *Exp Ther Med* 2020; **20**: 2396-2400 [PMID: 32765722 DOI: 10.3892/etm.2020.8714]

91 **ClinicalTrials**. A Heart Disease Study of Semaglutide in Patients With Type 2 Diabetes (SOUL). 2019. [cited 4 December 2022]. Available from: https://clinicaltrials.gov/ct2/show/NCT03914326

92 **ClinicalTrials**. Semaglutide Effects on Heart Disease and Stroke in Patients With Overweight or Obesity (SELECT). 2018. [cited 4 December 2022]. Available from: https://clinicaltrials.gov/ct2/show/NCT03574597

93 **ClinicalTrials**. A Research Study to Compare a Medicine Called Semaglutide Against Placebo in People with Peripheral Arterial Disease and Type 2 Diabetes (STRIDE). 2020. [cited 4 December 2022]. Available from: https://www.clinicaltrials.gov/ct2/show/NCT04560998

94 **Smits MM**, Van Raalte DH. Safety of Semaglutide. *Front Endocrinol (Lausanne)* 2021; **12**: 645563 [PMID: 34305810 DOI: 10.3389/fendo.2021.645563]

95 **ClinicalTrials**. US National Library of Medicine. A research study to look at how semaglutide compared to placebo affects diabetic eye disease in people with type 2 diabetes (FOCUS). [cited 4 December 2022]. Available from: https://clinicaltrials.gov/ct2/show/NCT03811561

96 Introduction: Standards of Medical Care in Diabetes-2020. *Diabetes Care* 2020; **43**: S1-S2 [PMID: 31862741 DOI: 10.2337/dc20-Sint]

97 **Aiello LP**; DCCT/EDIC Research Group. Diabetic retinopathy and other ocular findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care* 2014; **37**: 17-23 [PMID: 24356593 DOI: 10.2337/dc13-2251]

World Journal of Diabetes

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

World J Diabetes 2023 April 15; 14(4): 435-446

DOI: 10.4239/wjd.v14.i4.435

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Validity and reliability of the Polish version of the Michigan Neuropathy Screening Instrument

Edyta Sutkowska, Dominik Marciniak, Magdalena Koszewicz, Edyta Dziadkowiak, Slawomir Budrewicz, Karolina Biernat, Natalia Kuciel, Justyna Mazurek, Katarzyna Hap

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

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

P-Reviewer: Fan Z, China; Messias LHD, Slovakia; Ozden F, Turkey; Wan H, China

Received: November 8, 2022
Peer-review started: November 8, 2022
First decision: November 27, 2022
Revised: December 11, 2022
Accepted: January 9, 2023
Article in press: January 9, 2023
Published online: April 15, 2023

Edyta Sutkowska, Karolina Biernat, Natalia Kuciel, Justyna Mazurek, Katarzyna Hap, University Rehabilitation Centre, Wroclaw Medical University, Wroclaw 50-556, Poland

Dominik Marciniak, Department of Drugs Form Technology, Wroclaw Medical University, Wroclaw 50-556, Poland

Magdalena Koszewicz, Edyta Dziadkowiak, Slawomir Budrewicz, Department of Neurology, Wroclaw Medical University, Wroclaw 50-556, Poland

Corresponding author: Katarzyna Hap, Doctor, University Rehabilitation Centre, Wroclaw Medical University, Borowska Street 213, Wroclaw 50-556, Poland.
katarzyna.hap@umw.edu.pl

Abstract

BACKGROUND

Diabetic sensorimotor polyneuropathy is an important risk factor for foot ulceration and amputation. Thus, patients with diabetes should be screened for this disorder according to local guidelines. An obstacle to the diagnosis of this disease may be the lack of unified diagnostic criteria due to the lack of properly validated scales used for assessment.

AIM

To validate both sections (A and B) of the Michigan Neuropathy Screening Instrument (MNSI) in Polish (PL) patients with diabetes.

METHODS

A cross-sectional study using a test (A1, B1) and re-test (A2, B2) formula was performed in 80 patients with diabetes. The gold standard used for neuropathy detection was a nerve conduction study (NCS) which was performed in all participants. Reliability of the MNSI-PL was assessed using the Cronbach's alpha, Kuder-Richardson formula 20 (KR-20), split-half reliability, the Gottman split-half tests, and correlation between first and second half was accessed. Stability was assessed using an intraclass correlation coefficient (ICC). For external validation, we used simple linear correlation, binomial regression, and agreement between two different tools using a Bland-Altman plot analysis.

RESULTS

The scale was internally consistent (Cronbach's alpha for the full scale: 0.81 for A and 0.87 for B). MNSI-PL scores in test/retest showed high stability (ICC = 0.73 for A and ICC = 0.97 for B). The statistically important correlations between MNSI-PL and NCS were found for B1, B2, and A1 (P < 0.005). The cut-off points of ≥ 3 for section A (sensitivity of 90%-100%; specificity of 33%-40%) and ≥ 2 for section B (sensitivity of 81%-84%; specificity of 60%-70%) were obtained during neuropathy detection.

CONCLUSION

The MNSI-PL is a reliable and valid instrument in screening for diabetic neuropathy.

Key Words: Michigan Neuropathy Screening Instrument; Validity; Reliability; Diabetic neuropathy; Chronic complications

Core Tip: The Michigan Neuropathy Screening Instrument (MNSI) is a collective tool for assessing the peripheral nervous system in patients with diabetes mellitus and is widely used to evaluate patients in many countries. In Poland, the MNSI has not yet been validated, thus is a problem when using it in daily practice and for research purposes. We concluded that the Polish MNSI is a reliable and accurate screening tool for peripheral neuropathy, and we hope that it can be used by our colleagues in their studies. We also proposed cutoff points of both scales for patients and for physicians, hoping that MNSI can be used by other authors from different countries

Citation: Sutkowska E, Marciniak D, Koszewicz M, Dziadkowiak E, Budrewicz S, Biernat K, Kuciel N, Mazurek J, Hap K. Validity and reliability of the Polish version of the Michigan Neuropathy Screening Instrument. *World J Diabetes* 2023; 14(4): 435-446

URL: https://www.wjgnet.com/1948-9358/full/v14/i4/435.htm

DOI: https://dx.doi.org/10.4239/wjd.v14.i4.435

INTRODUCTION

A peripheral, symmetric sensorimotor polyneuropathy is the most common type of neuropathy in patients with diabetes mellitus (DM)[1]. Epidemiological data show that it occurs in approximately 28%-50% of patients with DM[2,3] depending on the population studied. It may begin developing in prediabetes[4], as carbohydrate disturbances start a few years before the diagnosis of type 2 DM (T2DM). According to guidelines, patients with T2DM should be screened for complications at the time of diagnosis. An examination in patients with type 1 DM (T1DM) should be performed approximately 5 years after the beginning of the disease. To minimize the risk of severe complications and to monitor neuropathy severity, regular inspections and examinations are recommended to be performed at least annually according to the International Working Group on the Diabetic Foot (2019)[5] as well as many local guidelines including those from the Polish Diabetes Association[6].

Peripheral neuropathy can be bothersome and significantly reduce a patient's quality of life. It is difficult to effectively treat and up to 50% of diabetic peripheral neuropathies can be asymptomatic. As a result of the deformation and loss of protective sensation caused by peripheral neuropathy, ulceration and finally amputation can occur[7-9] , leading to the patient's disability. Therefore, the early detection of this characteristic disorder of the peripheral nervous system is important using proven and readily available tools. The examiner should ask patients about their subjective feeling (symptoms) and perform tests to assess the function of small and large fibers[10]. All necessary tests are included in the Michigan Neuropathy Screening Instrument (MNSI)[11], which is a collective tool used to assess the peripheral nervous system in patients with DM and has been validated for patients in many countries. So far, this tool has not been validated to assess neuropathy in Poland. The use of MNSI may improve the diagnostic process of peripheral neuropathy and could be helpful in future studies. The reliability and precision of this tool was validated in previous studies[11-13]. The first section of the MNSI is subjective and consists of 15 self-administered questions, while the second section is a physical assessment that should be completed by health professionals.

MATERIALS AND METHODS

According to the information from the dedicated website (https://eprovide.mapi-trust.org/instruments/michigan-neuropathy-screening-instrument), the MNSI has been translated into Chinese, Czech, Finnish, French, German, Hungarian, Portuguese, Romanian, Spanish, and Turkish languages and validated. After confirming that the scale has not been validated in Polish (PL), the investigators obtained consent to validate the MNSI. The original English version is freely available for download from the Michigan Diabetes Research Center's (MDRC's) website. The project was financially supported by the Ministry of Health Subvention according to the number SUBZ.C310.22.075 from the IT Simple System of the Wroclaw Medical University.

Our cross-sectional study was conducted at the University Rehabilitation Centre at Wroclaw Medical University in collaboration with the Department of Neurology. The study was approved by the Wroclaw Medical University's ethics committee (approval no: 1007/2021). The patients were recruited from NZOZ Nowy Dwor in Wroclaw, Poland, and in accordance with the Declaration of Helsinki. All patients included in the study provided written informed consent to participate in the study.

Between January and April 2022, 89 patients met the inclusion criteria and were eligible for participation in the study. However, 8 of them did not come to the clinic within the stipulated time frame. Thus, a total of 81 patients signed the consent and participated in the study. One of the patients recalled during the course of the project that she had received chemotherapy for cancer treatment and was excluded from the analysis, this left a population of 80 participants.

Translation and cross-cultural adaptation

For the patient's section of the MNSI, the only change proposed by both the patients and the committee was to replace the wording "legs" with "legs and feet". This is because for many people the word "legs" is commonly used to refer to the area from the groin to the ankles. For this reason, the question about feeling in the legs may not take into account problems with the feet. The same problem was reported by the team from Portugal[14]. Patients were also asked what exactly the authors meant in point number 13: "Are you able to sense your feet when you walk?" (original version). In PL, it is hard to translate this into a simple and short form. This was also identified in the retranslation because after retranslation the sentence was: "Do you feel the right sensation in your feet when walking?" and in the committee's opinion, it may be misleading. Thus, we suggested that even though this part of the scale is self-administered, the practitioner could help patients to understand the question. For the examiner's section, we stressed that each of the abnormalities should be recorded if present. This was not clear in the translated version according to the diabetologist.

In the instruction "How to Use the Michigan Neuropathy Screening Instrument", the following elements were changed after translation into PL to better reflect what was done: (1) In the part entitled "vibration sensation": We stressed in PL the word "bilaterally"; and (2) In the part entitled "ankle reflexes": In "(…) foot dorsiflexed slightly" we also used the form dorsiflexed (in medical PL: "zgięcie grzbietowe") because the medical form is understandable, and there is no need to use the colloquial wording ("bend up") as proposed by the translator as this part of the MNSI is done by professionals.

The same layout as in the document from the University of Michigan was kept, which is similar in the original version but is enriched with a monofilament test. Despite the problem with question 13 in the self-administered part of the MNSI, the differences between the retranslation and original text were related to addressing the patient. For example, in question 1 of the original version, the question was stated as, "Are your legs and/or feet numb?", whereas the retranslation stated, "Do you experience numbness in your legs and/or feet?" , these differences were deemed to be of little importance.

Population

A study population composed of patients with a diagnosis of DM, using the PL Diabetes Association criteria[6], who attended the NZOZ Nowy Dwor, where the principal researcher works as a diabetologist were identified. The inclusion criteria were: (1) DM diagnosis except gestational; (2) Age ≥ 18; and (3) Native language is PL. Exclusion criteria were: (1) Chronic renal failure requiring renal replacement therapy; (2) Liver cirrhosis; (3) Symptomatic cerebrovascular disease; (4) Active cancer; (5) History of chemotherapy or radiotherapy; (6) Autoimmune disease; (7) Chronic infectious disease; (8) Alcohol or drug abuse; (9) Clinical radicular neuropathy; and (10) Mental or physical conditions that could compromise the evaluation of clinical symptoms. Exclusion criteria were established with a neurologist and were based on prior Portuguese and Turkish validation.

The type of DM, DM duration, body mass, height, body mass index, and glycated hemoglobin no more than 3 mo before the study were collected to characterize the study population. The information necessary to confirm the above-mentioned inclusion and exclusion criteria was obtained from the patient's records or based on a short interview performed just before the study started if no records were available.

The MNSI with its 2 sections (A and B) was administered to all of the patients twice within an interval of 1-2 wk. A nerve conduction study (NCS) was performed on the same day as the first MNSI. Both specialists who performed the MNSI and NCS were blind to the NCS result and MNSI score for the

patient, respectively.

MNSI

The first part of the MNSI (part A) consists of 15 self-administered "yes/no" questions on foot sensation including pain, numbness, and temperature sensitivity. The scoring is awarded according to a special key. Because items 4 (a circulation measurement) and 10 (a general status measurement) were excluded from the original scale, the highest score for this part of the MNSI is 13 points. The patient should answer all of these questions by him/herself. The higher the points, the more severe the neuropathic symptoms[12].

The next part of the MNSI (part B) is a physical examination involving the assessment of the foot's appearance (deformities, dry skin, nail abnormalities, callous, infection), a semi-quantitative assessment of vibration sensation at the dorsum of the great toe using a 128-Hz tuning fork, ankle reflexes, and monofilament testing using a 10-g Semmes-Weinstein monofilament. In the original version of the MNSI[11], testing with a monofilament was not included but since cross-sectional studies found it to be highly sensitive for predicting the risk of foot ulceration, it was incorporated into the scale from the University of Michigan[15]. In part B, the results were scored with points assigned separately to positive tests of the right/left foot. The maximum possible score in the second section after adding the monofilament test was 10 points. The information on how to perform tests was incorporated as part of instruction for practitioners and was translated into PL to standardize the examination in Supplementary material (No 1 "Instruction of MNSI-PL for practitioners").

After permission to validate, we used the English version which is freely available on the MDRC's website. This MNSI document was translated into PL independently by a diabetologist (being aware of the concept, T1) and a professional translator (not informed of the concept, T2) from GROY in Supplementary material (No 2 "MNSI-PL"). These translations were then compared by a panel of four investigators to identify potential questions about the meaning of items in both sections. Additionally, four randomly selected patients assessed whether the text from the first section was understandable and helped select the more understandable wording among the versions from the translator and the diabetologist where the translation differed. Finally, the translated document was improved (final version – T1/2) and was given to a different professional translator from GROY (BT1) and one professional (BT2) to translate back into English. These individuals were blinded to the original version of the MNSI. Both English versions (retranslated and T1/2) were then compared and discrepancies were identified, reviewed, and confirmed to be acceptable (no conceptual errors were found) by the investigators.

NCS

The Viking Quest version 10.0 device connected to a Thermal Sensory Analyzer II 2001 (TSA II), a VSA – 3000 Vibratory Sensory Analyzer (Medoc, Israel), Viking Select version 7.1.1c., and a Nicolet Biomedical device with the Multi-Mode Program (MMP Plus) software were used during the electrophysiological studies. The standard electrophysiological examination consisted of sensory and motor conduction velocity tests with the F-wave estimation. The tests were performed in the left peroneal and tibial nerves and both sural nerves. We assessed distal latency or the shortest latency of F-wave (in milliseconds – ms), potential amplitude (in microvolts – uV for sensory testing, and in millivolts – mV for motor testing), and conduction velocity (in meters per second – m/s). A standard distance between electrodes and distal points of stimulations was preserved for all tests. We used an antidromic technique for sensory conduction velocity estimations. The same conditions were kept during the studies including a room temperature between 21-23 °C and a foot temperature not less than 32 °C[16,17]. Additionally, a conduction velocity distribution test using the collision technique was performed on the peroneal nerve [18]. Vibratory, temperature, and warm- and cold-induced pain thresholds were assessed over the S1 dermatome using quantitative sensory testing[18].

A qualified neurologist obtained the results, if there was an abnormality in any evaluated parameter (latency, amplitude, conduction velocity) in individual nerves in the test, the patient received 1 point. Points 4 meant severe polyneuropathy, 0 normal, 1 minimal, 2 mild, and 3 moderate polyneuropathy.

The scoring for the detection of neuropathy based on NCS for statistical analysis (dichotomous data) was defined as follows: (1) No neuropathy – if no disturbances in nerve conduction were found; and (2) neuropathy – if pathology was found within at least one test. We did not score the severity of neuropathy for statistical analysis.

Statistical analysis

Statistical analyses were carried out using the computer program Statistics version 14.0 and R version 4.0.3. To statistically evaluate the degree of correspondence between the MNSI and NCS (gold standard) scores, summed values obtained from MNSI parts A and B at both time points, the summed NCS and NCS presented in dichotomous form were used (vide Methodology, NCS). The basis for the estimation of the minimum sample size was the analysis of the reliability of the test with Cronbach's alpha coefficient[19]. The basis for its estimation was the following accepted assumptions: Minimum acceptable Cronbach's alpha (H0) = 0.64, expected Cronbach's alpha (H1) = 0.77, significance level (α) = 0.05; power (1 – β) = 0.8, number of items (k) = 15-20. Based on the accepted assumptions and literature

information, the minimum sample size was set at 80.

The continuous data were expressed as means with minimum-maximum values and standard deviations. The qualitative data were expressed as percentages. Reliability was measured by Cronbach's alpha using Kuder-Richardson Formula 20 (KR-20) coefficient. The consistency values for KR-20 were considered enough when 0.6-0.7; good when 0.7-0.8, and very good when > 0.8. KR-20 values over 0.6 indicated that items had integrity and the test was homogenous[20-22]. Also, the split-half reliability, the Gottman split-half and the correlation between the first and second half were assessed. Intraclass correlation coefficient (ICC) values for consistency (ICC consistency) and agreement (ICC agreement) were calculated for a two-way random effects model to evaluate the stability[20]. An ICC above 0.60 was considered a good fit[21].

To evaluate the statistical significance of the correlation between the summary results obtained using the compared tools (MNSI *vs* NCS), we used a simple linear correlation, multinomial regression, and a Bland-Altman plot to evaluate the agreement between the two different instruments. The next step was to assess the correlation between the results of the NCS (expressed on a dichotomous scale) and the sum values obtained from parts A and B of the MNSI at the two time points. For this purpose, a multinomial logistic regression model was used. The area under the receiver-operating characteristic (ROC) curves were estimated to measure the discriminatory power of the test. The area under the ROC curve (AUC) represented the performance of a test in predicting the outcome.

RESULTS

Patients

A total of 80 patients participated in our validation study (39 women and 41 men; 48.75% and 51.25%, respectively). The sample characteristics are presented in Table 1. Most of the patients were diagnosed with T2DM (n = 77; 96.25%), and only 3 (3.75%) of them had T1DM. The weight and height were the only parameters that differentiated women and men in the studied population. All the patients were tested using the MNSI scale twice (A1B1 and A2B2) and agreed for NCS.

Reliability and stability results

The consistency values for KR-20 were considered enough when 0.6-0.7; good when 0.7-0.8, and very good when > 0.8. In our study, the reliability was measured for both parts (A and B) of the MNSI together and separately for A and B. These results showed very good consistency with a Cronbach's alpha for the full scale of 0.81 for part A and 0.87 for part B (Table 2). The analyses of separated items (A and B) are presented in the Supplementary materials (Supplementary Tables 1 and 2 for items from parts A and B, respectively). KR-20 values over 0.6 indicated that items had integrity, and the test was homogenous which was achieved in all the analyses performed in our study[21,22] (Table 2).

Regarding stability, all patients were retested within 7-14 d, and the MNSI scores between the test and re-test showed an ICC 0.73 for part A and an ICC 0.97 for part B. For both agreement and consistency, a high level of statistical significance was seen (Table 3). An ICC above 0.60 was considered a good fit[21]. Detailed factor analysis is shown in the Supplementary Tables 3 and 4.

External validation

The simple linear correlation between the sum of the NCS scores and B scores on the MNSI was statistically significant when comparing the test and re-test (P < 0.001; P < 0.001 respectively). For part A of the MNSI, a statistically significant correlation was confirmed for part A1 (P < 0.001) but not A2 (P > 0.001). Statistically significant bivariate regression models were established for the two time points, confirming a statistically significant correlation of part B and no statistically significant correlation for part A of the MNSI (Supplementary Table 5).

The model of multinomial logistic regression of both parameters (A and B) at time point one [odds ratio (OR) = 2.4, P = 0.022 and OR = 2.3, P = 0.009 for A1 and B1, respectively] and statistical significance for B2 (OR = 1.8, P = 0.009) and its absence for A2 (OR = 1, P = 1) (Supplementary Table 6) were confirmed, thereby also underlining the greater influence of the B-MNSI variable on the occurrence of an event (here neuropathy) assessed using the gold standard NCS. Analysis of the Bland-Altman plots showed high mutual compatibility between the results of the compared tools (MNSI *vs* NCS, Figure 1).

The ROC area between the NCS and MNSI (section A and/or B) scores helped to validate the scale. A value of 0.70-0.79 indicated reasonable test performance, 0.80-0.89 indicated good performance, and ≥ 0.9 indicated very good performance. The results of AUC for the NCS and both parts of the MNSI (Table 4) reached statistical significance for B1, B2, and A1 (P < 0.001) but not for A2. We determined the cut-off points for the patient's and practitioner's part regarding the sensitivity and specificity of each item to predict neuropathy as confirmed by NCS.

We obtained the same values for optimal cut-off points during both time points for the MNSI's patient version (part A) as well as the practitioner version (part B), which could be found as 3 and 2 respectively (Table 5). The sensitivity and specificity cut-off points for A were 33%-40% and 90%-100%, respectively. The sensitivity and specificity cut-off points for B were 81%-84% and 60%-70%, res-

Table 1 General sample characteristics

Variable, *n* = 80	Sex	Descriptive statistics					Mann-Whitney *U* test
		Valid (*n*)	Mean	Minimum	Maximum	SD	*P* value
Age (yr)	Female	39	69.00	45.00	80.00	6.27	0.151516
	Male	41	65.32	37.00	76.00	9.56	
Weight (kg)	Female	39	77.36	54.00	108.00	12.24	2.55E-03
	Male	41	88.66	63.00	150.00	16.52	
Height (cm)	Female	39	161.38	150.00	174.00	5.97	1.78E-11
	Male	41	175.15	164.00	190.00	6.90	
BMI (kg/m^2)	Female	39	29.68	21.90	40.20	4.52	2.64E-01
	Male	41	28.84	21.50	44.30	4.90	
HbA1c (%)	Female	38	6.42	5.20	8.50	0.79	4.87E-01
	Male	40	6.58	5.00	8.80	0.97	
DM[1] duration (yr)	Female	39	12.10	0.00	37.00	8.13	2.90E-01

[1]Diabetes mellitus duration for less than 6 mo = 0, between 6-12 mo = 1 year.
BMI: Body mass index; DM: Diabetes mellitus; HbA1c: Glycated hemoglobin; SD: Standard deviation.

Table 2 Reliability results for the patient and practitioner sections of the Michigan Neuropathy Screening Instrument-Polish version

Part A of MNSI			Part B of MNSI	
***n* = 80**	Cronbach alpha, full scale: 0.81790		Cronbach alpha, full scale: 0.87095	
	Corr 1st & 2nd half: 0.745791		Corr 1st & 2nd half: 0.972206	
	Split-half reliability: 0.854388		Split-half reliability: 0.985907	
	Guttman split-half: 0.840785		Guttman split-half: 0.985838	
	Summary for A1	Summary for A2	Summary for B1	Summary for B2
Mean	4.250000	4.150000	6.212500	6.175000
Sum	340.0000	332.0000	497.0000	494.0000
SD	3.403275	4.313946	3.925553	3.860790
Variance	11.58228	18.61013	15.40997	14.90570
Cronbach alpha	0.6499672	0.7060083	0.7337023	0.7346509

A1: Patient version; A2: Patient version (after 1-2 wk); B1: Patient examination; B2: Patient examination (after 1-2 wk); MNSI-PL: Michigan Neuropathy Screening Instrument-Polish version; SD: Standard deviation.

pectively.

DISCUSSION

Dedicated to different populations (language, culture differences), the existing instruments in medicine should not only be simply translated but also validated[23]. The validation process should be completed using a representative sample to demonstrate adequate reliability and validity. Peripheral neuropathy is the most common form of diabetic neuropathy, affecting the nerves of the limbs, particularly those of the feet. The MNSI is a low-cost, rapid tool with a user-friendly format that helps detect pathology of the peripheral nervous system in patients with DM and has been validated using NCS (gold standard) for neuropathy detection[15]. After publication, the MNSI has been translated into several languages. A list of available translations that have been validated is available for distribution on the Mapi Research Trust website. However, a PL version has not been added to this list as it has not been validated. Therefore, in our study, we evaluated both the reliability and validity of a PL version of the MNSI

Table 3 Stability of the patient and practitioner sections of the Michigan Neuropathy Screening Instrument-Polish version

Part of MNSI	Mean (SD)		ICC for agreement		ICC for consistency	
	Test score	Re-test score	$ICC_{agreement}$	*P* value	$ICC_{consistency}$	*P* value
A	2.125 (1.70)	2.075 (2.16)	0.728	9.05E-15	0.725	9.47E-15
B	3.106 (1.96)	3.088 (1.93)	0.972	6.19E-52	0.972	8.53E-52

Part A: Patient version of Michigan Neuropathy Screening Instrument; Part B: Patient examination. ICC: Intraclass correlation coefficient; MNSI-PL: Michigan Neuropathy Screening Instrument-Polish version.

Table 4 The area under the curve values for Nerve Conduction Study and Michigan Neuropathy Screening Instrument Polish version (version A and B) at both time points

Variable	AUC	*P* value
A1	0.749	0.0003
B1	0.796	0.0000
A2	0.617	0.2296
B2	0.770	0.0001

A1: Patient version; A2: Patient version (after 1-2 wk); AUC: Area under the curve; B1: Patient examination; B2: Patient examination (after 1-2 wk).

Table 5 The cut-off points for Michigan Neuropathy Screening Instrument Polish version at the two time points and their sensitivity and specificity, and their positive and negative predictive values based on receiver operating characteristic curves

Variable	True positives, *n*, %	False positives, *n*, %	False negatives, *n*, %	True negatives, *n*, %	Sensitivity	Specificity	Optimal cut-off point
A1	28/35	0/0	42/52.5	10/12.5	0.400	1.000	3
B1	57/71.25	3/3.75	13/16.25	7/8.75	0.814	0.700	2
A2	23/28.75	1/1.25	47/58.75	9/11.25	0.329	0.900	3
B2	59/73.75	4/5	11/13.75	6/7.5	0.843	0.600	2

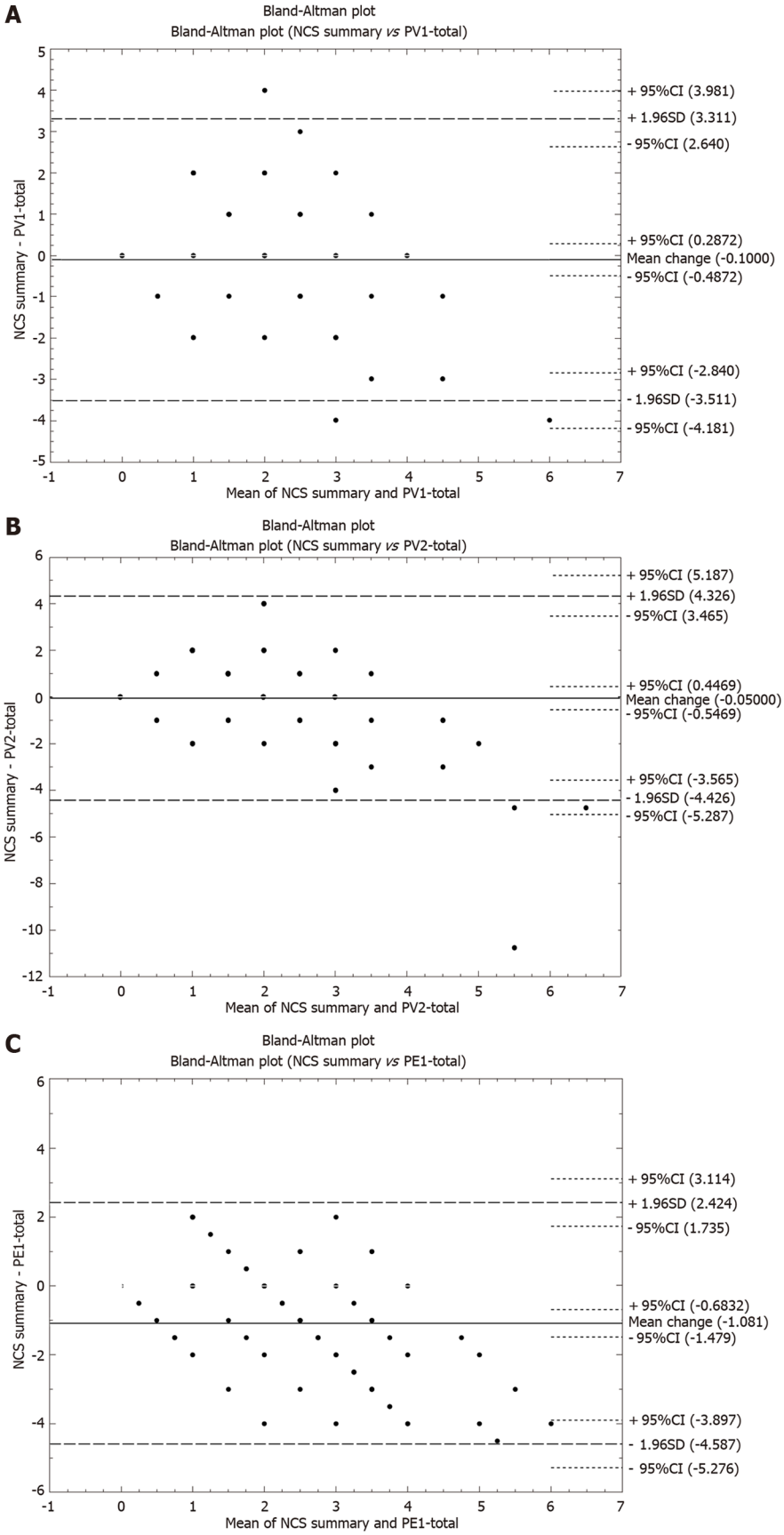
A1: Patient version; A2: Patient version (after 1-2 wk); B1: Patient examination; B2: Patient examination (after 1-2 wk).

following the principles of proper validation[23].

Based on the KR-20, split-half analysis, and ICC, we found that the MNSI-PL is a reliable and stable tool with high internal consistency using a cut-off value of 3 for the questionnaire (part A) and 2 for the examination (part B). The models of multinomial regression and multinomial logistic regression confirmed the greater influence of the B-MNSI variable on the diagnosis of an event (neuropathy) when NCS was used as the gold standard for this diagnosis. The ability to predict neuropathy based on section B was found to be high in other studies[15,24] and even higher compared to section A alone if this part was used together with B[24]. This is understandable as the MNSI consists of two parts, section A which is rather subjective because it is self-administered by the patient, and section B which includes tests to be performed by professionals.

The evaluation of a patient's peripheral nerve function can be done directly, without subjective bias, by electromyography or biopsy[25-27]. However, because of their invasiveness, persistent discomfort, sensory loss, risk of non-healing, and need to be completed by a specialist, these tools are not first-line diagnostic procedures. The analysis of the Bland-Altman plots in our study unequivocally demonstrates the high concordance between MNSI and NCS test results. This makes the MNSI-PL a reliable screening tool for the diagnosis of peripheral neuropathy in diabetic patients.

For each of the MNSI parts, the cutoff point to confirm neuropathy has been discussed in the literature. However, a cut-off point of 7 for the first section (A in our study) has been previously accepted to be abnormal[11]. Later studies have proposed a cut-off point of ≥ 4 to improve the performance of the MNSI and achieve acceptable levels of sensitivity and specificity. The study that revised the previously existed threshold of ≥ 7 for part A of the MNSI by setting it at ≥ 4 achieved a sensitivity of 40% and specificity of 92%[28]. In our study, a threshold of 3 for part A was found to be


A
Bland-Altman plot
Bland-Altman plot (NCS summary *vs* PV1-total)
+ 95%CI (3.981)
+ 1.96SD (3.311)
- 95%CI (2.640)
+ 95%CI (0.2872)
Mean change (-0.1000)
- 95%CI (-0.4872)
+ 95%CI (-2.840)
- 1.96SD (-3.511)
- 95%CI (-4.181)
NCS summary - PV1-total
Mean of NCS summary and PV1-total
B
Bland-Altman plot
Bland-Altman plot (NCS summary *vs* PV2-total)
+ 95%CI (5.187)
+ 1.96SD (4.326)
- 95%CI (3.465)
+ 95%CI (0.4469)
Mean change (-0.05000)
- 95%CI (-0.5469)
+ 95%CI (-3.565)
- 1.96SD (-4.426)
- 95%CI (-5.287)
NCS summary - PV2-total
Mean of NCS summary and PV2-total
C
Bland-Altman plot
Bland-Altman plot (NCS summary *vs* PE1-total)
+ 95%CI (3.114)
+ 1.96SD (2.424)
- 95%CI (1.735)
+ 95%CI (-0.6832)
Mean change (-1.081)
- 95%CI (-1.479)
+ 95%CI (-3.897)
- 1.96SD (-4.587)
- 95%CI (-5.276)
NCS summary - PE1-total
Mean of NCS summary and PE1-total

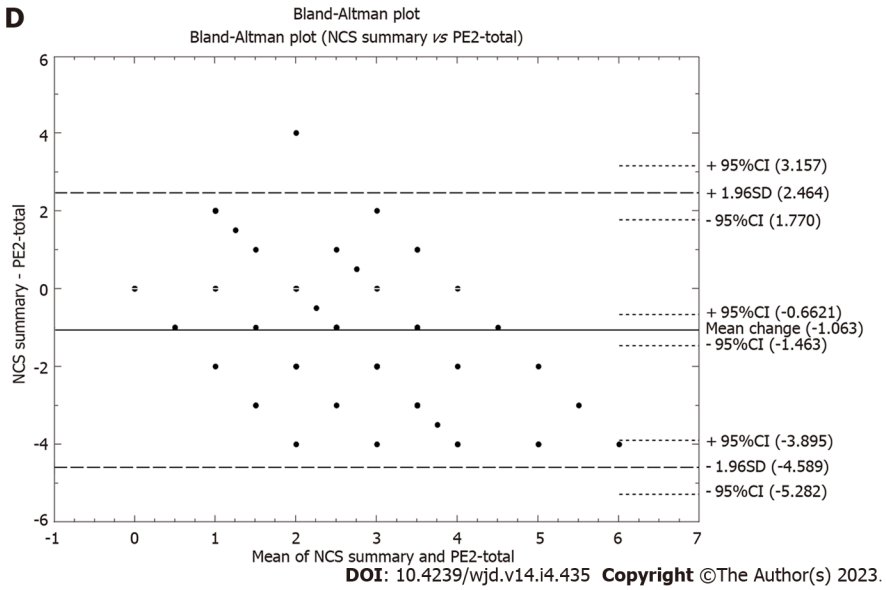


Figure 1 Bland-Altman plots: evaluation of the agreement between the two different instruments (nerve conduction study and Michigan Neuropathy Screening Instrument). A: Agreement between nerve conduction study (NCS) and patient's version (PV)1; B: Agreement between NCS and PV2; C: Agreement between NCS and patient examination (PE)1; D: Agreement between NCS and PE2. CI: Confidence interval; MNSI: Michigan Neuropathy Screening Instrument; NCS: Nerve conduction study; PE1 or 2: Patient examination 1 or 2 (part B1 or B2 of the Michigan Neuropathy Screening Instrument); PV1 or 2: Patient's version 1 or 2 (part A1 or A2 of the Michigan Neuropathy Screening Instrument); SD: Standard deviation.

the optimal cut-off point and had a specificity of 33%-40%, which shows the probability of having a normal MNSI result in the absence of confirmed neuropathy. The sensitivity reached 90%-100%, which shows the probability of having a positive result in the presence of confirmed neuropathy. Compared to the study by Herman *et al*[28], we achieved a lower specificity but higher sensitivity and the sensitivity and specificity from our validation study were closer to the results found in the Portuguese[14], Arabic [29] and Turkish[24] analyses. We also proposed a similar cut-off point for A as used in these two studies[14,24]. A high sensitivity gives the test a high ability to correctly identify patients with the disease. Based on our observations during the application of the test to patients, the self-administered part should be filled out with an accompanied professional (doctor, nurse, physiotherapist) to explain any doubts if necessary. It would also be useful when illiterate individuals are completing the questionnaire[24].

For part B (examination) of the MNSI, a score greater than 2 was considered abnormal according to the original scoring algorithm[11]. A threshold of ≥ 2.5 was shown to be 61% sensitive and 79% specific in confirming clinical neuropathy in Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications[27]. However, in the study by Moghtaderi *et al*[15], the sensitivity and specificity of the examination with a cut-off point of 2 was found to be 65% and 83%. In our study, we found the optimal cut-off point of 2 for section B with a sensitivity of 81%-84% and specificity of 60%-70%, which was similar to the Portuguese study (86% sensitivity and 61% specificity using the same cut-off point)[14].

To summarize both parts of the MNSI, the same cut-off points used in our study for sections A and B were proposed by the authors of the Portuguese version[14] with similar levels of sensitivity and specificity and in one of the Turkish validation studies[30]. As we mentioned, the results from our validation process stressed the importance of part B in detecting neuropathy compared to part A, which was similar to the results of the Turkish validation study[24].

The strengths of our study include a sufficient number of individuals based on the described calculation model, the completion of NCS on the whole group of studied patients, a wide panel of statistical analysis to confirm good reliability and stability of the tool, and confirming the cut-off points for parts A and B seen in previous studies with similar sensitivities and specificities[15,21,28,30,31].

There are limitations to our study. The questionnaire and examination process were limited in which items could not rule out subclinical neuropathy and an autonomic component[32]. Although a small percentage of patients with T1DM (less than 4%) can be considered a limitation because it does not reflect the proportion of different DM types in the population[33], this does not affect the usefulness of the MNSI. Patients with T1DM are usually younger, so we expect that it is easier for them to understand and answer the questions in part A. Additionally, the type of DM has no impact on part B of the MNSI as this is done by a professional.

The recommendations of the PL Diabetes Association do not propose the use of appropriate scales for assessing peripheral neuropathy[6]. This is due to the lack of adaptation of scales such as the MNSI to the PL system, which contributes to a lack of uniformity in assessing a patient's condition. As a result,

some centers create informal evaluation criteria and rules (even though they use the same tools and tests). Similar problems also arise during the design of clinical trials, for which only the English-language scale can be used. Therefore, the introduction of a validated scale would help to standardize screening for neuropathy in diabetic patients.

CONCLUSION

The MNSI-PL is a reliable screening tool for diabetic peripheral sensorimotor neuropathy when using a cut-off of ≥ 3 for section A and ≥ 2 for section B. Part B is stronger in detecting neuropathy as it is more objective. Additionally, the whole test is useful in clinical practice as well as in our country.

ARTICLE HIGHLIGHTS

Research background

The Michigan Neuropathy Screening Instrument (MNSI) is a collective tool for assessing the peripheral nervous system in patients with diabetes mellitus and is widely used to evaluate patients in many countries.

Research motivation

In Poland, the MNSI has not yet been validated, thus this is a problem when using it in daily practice and for research purposes.

Research objectives

This study aimed to validate both sections (A and B) of the MNSI in Polish (PL) patients with diabetes.

Research methods

A cross-sectional study using a test (A1, B1) and re-test (A2, B2) formula was performed in 80 patients with diabetes. The gold standard used for neuropathy detection was a nerve conduction study (NCS) which was performed on all participants. Reliability of the MNSI-PL was assessed using the Cronbach's alpha, Kuder-Richardson formula 20, split-half reliability, the Gottman split-half tests and correlation between first and second half was accessed. Stability was assessed using an intraclass correlation coefficient (ICC). For external validation, we used simple linear correlation, binomial regression, and agreement between two different tools using a Bland-Altman plot analysis.

Research results

The scale was internally consistent (Cronbach's alpha for the full scale: 0.81 for A and 0.87 for B). MNSI-PL scores between test/retest showed high stability (ICC = 0.73 for A and ICC = 0.97 for B). The statistically important correlations between MNSI-PL and NCS were found for B1, B2, and A1 ($P < 0.005$). The cut-off points of ≥ 3 for section A (sensitivity of 90%-100%; specificity of 33%-40%) and ≥ 2 for section B (sensitivity of 81%-84%; specificity of 60%-70%) were obtained during neuropathy detection.

Research conclusions

The MNSI-PL is a reliable and valid instrument in screening for diabetic neuropathy.

Research perspectives

PL MNSI is a reliable and accurate screening tool for peripheral neuropathy. We also proposed cutoff points for both scales (for patients and for physicians), and hope that this can also be used by other authors from different countries.

ACKNOWLEDGEMENTS

We would like to thank NZOZ Nowy Dwor for agreeing to recruit patients in their clinic.

FOOTNOTES

Author contributions: Sutkowska E designed the study, prepared the article and was major in substantial contributions to conception and design of the work; Sutkowska E, Marciniak D, and Hap K contributed to the data analysis and interpretation; Sutkowska E, Koszewicz M, Dziadkowiak E, Budrewicz S, Biernat K, Kuciel N, and

Mazurek J were involved in the acquisition and analysis of data for the work investigation; Koszewicz M and Dziadkowiak E participated in the interpretation of data for the work investigation; Marciniak D, Koszewicz M, Dziadkowiak E, Budrewicz S, Biernat K, Kuciel N, and Mazurek J revised the work critically for important intellectual content; Sutkowska E and Hap K drafted the work; and all authors approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Supported by the Ministry of Health Subvention from the IT Simple System of the Wroclaw Medical University by Wroclaw Medical University, No. SUBZ.C310.22.075; and the MCDTR grant from the National Institute of Diabetes and Digestive and Kidney Diseases, No. P30DK092926.

Institutional review board statement: Our cross-sectional study was conducted at the University Rehabilitation Centre at Wroclaw Medical University in collaboration with the Department of Neurology. The study was approved by the Wroclaw Medical University's ethics committee (Approval No: 1007/2021).

Informed consent statement: All patients included in the study were provided written informed consent to participate in the study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Country/Territory of origin: Poland

ORCID number: Edyta Sutkowska 0000-0002-1602-8978; Dominik Marciniak 0000-0002-3326-0840; Magdalena Koszewicz 0000-0002-8057-1617; Edyta Dziadkowiak 0000-0002-9618-9308; Slawomir Budrewicz 0000-0002-2044-6347; Karolina Biernat 0000-0003-1556-0177; Natalia Kuciel 0000-0002-1817-9550; Justyna Mazurek 0000-0001-8983-0286; Katarzyna Hap 0000-0001-9603-3613.

S-Editor: Wang JJ
L-Editor: Ma JY-MedE
P-Editor: Wang JJ

REFERENCES

1 **Bakker K**, Schaper NC; International Working Group on Diabetic Foot Editorial Board. The development of global consensus guidelines on the management and prevention of the diabetic foot 2011. *Diabetes Metab Res Rev* 2012; **28** Suppl 1: 116-118 [PMID: 22271736 DOI: 10.1002/dmrr.2254]
2 **Faselis C**, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular Complications of Type 2 Diabetes Mellitus. *Curr Vasc Pharmacol* 2020; **18**: 117-124 [PMID: 31057114 DOI: 10.2174/1570161117666190502103733]
3 **Hicks CW**, Selvin E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. *Curr Diab Rep* 2019; **19**: 86 [PMID: 31456118 DOI: 10.1007/s11892-019-1212-8]
4 **Stino AM**, Smith AG. Peripheral neuropathy in prediabetes and the metabolic syndrome. *J Diabetes Investig* 2017; **8**: 646-655 [PMID: 28267267 DOI: 10.1111/jdi.12650]
5 **Ibrahim A**. IDF Clinical Practice Recommendation on the Diabetic Foot: A guide for healthcare professionals. *Diabetes Res Clin Pract* 2017; **127**: 285-287 [PMID: 28495183 DOI: 10.1016/j.diabres.2017.04.013]
6 **Araszkiewicz A**, Badurska-Stankiewicz E, Borys S, Budzyński A, Cyganek K, Cypryk K, Czech A, Czupryniak L, Drzewoski J, Dzida G, Dziedzic T, Franek E, Gajewska D, Gawrecki A, Górska M, Grzeszczak W, Gumprecht J, Idzior-Waluś B, Jarosz-Chobot P, Kalarus Z, Karczewska-Kupczewska M, Klupa T, Koblik T, Kokoszka A, Korzon-Burakowska A, Kowalska I, Krętowski A, Majkowska L, Malecki M, Mamcarz A, Mirkiewicz-Sieradzka B, Młynarski W, Moczulski D. 2021 Guidelines on the management of patients with diabetes. A position of Diabetes Poland. *Clin Diabetol* 2021; **10**: 1-113 [DOI: 10.5603/DK.2021.0001]
7 **Wood WA**, Wood MA, Werter SA, Menn JJ, Hamilton SA, Jacoby R, Dellon AL. Testing for loss of protective sensation in patients with foot ulceration: a cross-sectional study. *J Am Podiatr Med Assoc* 2005; **95**: 469-474 [PMID: 16166466 DOI: 10.7547/0950469]

8 **Frykberg RG**, Lavery LA, Pham H, Harvey C, Harkless L, Veves A. Role of neuropathy and high foot pressures in diabetic foot ulceration. *Diabetes Care* 1998; **21**: 1714-1719 [PMID: 9773736 DOI: 10.2337/diacare.21.10.1714]

9 **Armstrong David G**. Detection of diabetic peripheral neuropathy: strategies for screening and diagnosis. In: Advanced Studies in Medicine. Somerville: Galen Publishing, 2005; 5: S1033-S1037

10 **American Diabetes Association Professional Practice Committee**, Draznin B, Aroda VR, Bakris G, Benson G, Brown FM, Freeman R, Green J, Huang E, Isaacs D, Kahan S, Leon J, Lyons SK, Peters AL, Prahalad P, Reusch JEB, Young-Hyman D. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Medical Care in Diabetes-2022. *Diabetes Care* 2022; **45**: S185-S194 [PMID: 34964887 DOI: 10.2337/dc22-S012]

11 **Feldman EL**, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care* 1994; **17**: 1281-1289 [PMID: 7821168 DOI: 10.2337/diacare.17.11.1281]

12 **Lunetta M**, Le Moli R, Grasso G, Sangiorgio L. A simplified diagnostic test for ambulatory screening of peripheral diabetic neuropathy. *Diabetes Res Clin Pract* 1998; **39**: 165-172 [PMID: 9649948 DOI: 10.1016/s0168-8227(98)00005-9]

13 **Dyck PJ**, Karnes J, O'Brien PC, Swanson CJ. Neuropathy Symptom Profile in health, motor neuron disease, diabetic neuropathy, and amyloidosis. *Neurology* 1986; **36**: 1300-1308 [PMID: 3762934 DOI: 10.1212/wnl.36.10.1300]

14 **Barbosa M**, Saavedra A, Severo M, Maier C, Carvalho D. Validation and Reliability of the Portuguese Version of the Michigan Neuropathy Screening Instrument. *Pain Pract* 2017; **17**: 514-521 [PMID: 27538385 DOI: 10.1111/papr.12479]

15 **Moghtaderi A**, Bakhshipour A, Rashidi H. Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy. *Clin Neurol Neurosurg* 2006; **108**: 477-481 [PMID: 16150538 DOI: 10.1016/j.clineuro.2005.08.003]

16 **OH Shin J**. Clinical electromyography: nerve conduction studies. Baltimore: Lippincott Williams & Wilkins, 2003

17 **Mallik A**, Weir AI. Nerve conduction studies: essentials and pitfalls in practice. *J Neurol Neurosurg Psychiatry* 2005; **76** Suppl 2: ii23-ii31 [PMID: 15961865 DOI: 10.1136/jnnp.2005.069138]

18 **Sander HW**. Collision testing. In: Kimura J, Handbook of clinical neurophysiology. United States: Elsevier, 2006; 7: 359-380

19 **Siao P**, Cros DP. Quantitative sensory testing. *Phys Med Rehabil Clin N Am* 2003; **14**: 261-286 [PMID: 12795516 DOI: 10.1016/s1047-9651(02)00122-5]

20 **Bonett DG**. Sample size requirements for testing and estimating coefficient alpha. *J Educ Behav Stat* 2002; **27**: 335-340 [DOI: 10.3102/10769986027004335]

21 **Terwee CB**, Bot SD, de Boer MR, van der Windt DA, Knol DL, Dekker J, Bouter LM, de Vet HC. Quality criteria were proposed for measurement properties of health status questionnaires. *J Clin Epidemiol* 2007; **60**: 34-42 [PMID: 17161752 DOI: 10.1016/j.jclinepi.2006.03.012]

22 **Cam MO**, Baysan-Arabacı L. Qualitative and quantitative steps on attitude scale construction. *Turkish J Res Devel in Nurs* 2010; **12**: 59-71

23 **Beaton DE**, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine (Phila Pa 1976)* 2000; **25**: 3186-3191 [PMID: 11124735 DOI: 10.1097/00007632-200012150-00014]

24 **Aktar Reyhanioğlu D**, Adiyaman SC, Bektaş M, Bulut O, Özgen Saydam B, Bayraktar F, Kara B. Validity and reliability of the Turkish version of the Michigan Neuropathy Screening Instrument. *Turk J Med Sci* 2020; **50**: 789-797 [PMID: 32178509 DOI: 10.3906/sag-1906-63]

25 **Theriault M**, Dort J, Sutherland G, Zochodne DW. A prospective quantitative study of sensory deficits after whole sural nerve biopsies in diabetic and nondiabetic patients. Surgical approach and the role of collateral sprouting. *Neurology* 1998; **50**: 480-484 [PMID: 9484376 DOI: 10.1212/wnl.50.2.480]

26 **Kennedy WR**, Nolano M, Wendelschafer-Crabb G, Johnson TL, Tamura E. A skin blister method to study epidermal nerves in peripheral nerve disease. *Muscle Nerve* 1999; **22**: 360-371 [PMID: 10086897 DOI: 10.1002/(sici)1097-4598(199903)22:3<360::aid-mus9>3.0.co;2-j]

27 **Kennedy WR**, Wendelschafer-Crabb G, Johnson T. Quantitation of epidermal nerves in diabetic neuropathy. *Neurology* 1996; **47**: 1042-1048 [PMID: 8857742 DOI: 10.1212/wnl.47.4.1042]

28 **Herman WH**, Pop-Busui R, Braffett BH, Martin CL, Cleary PA, Albers JW, Feldman EL; DCCT/EDIC Research Group. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. *Diabet Med* 2012; **29**: 937-944 [PMID: 22417277 DOI: 10.1111/j.1464-5491.2012.03644.x]

29 **Abuzinadah AR**, Alkully HS, Alanazy MH, Alrawaili MS, Milyani HA, AlAmri B, AlShareef AA, Bamaga AK. Translation, validation, and diagnostic accuracy of the Arabic version of the Michigan neuropathy screening instrument. *Medicine (Baltimore)* 2021; **100**: e27627 [PMID: 34871227 DOI: 10.1097/MD.0000000000027627]

30 **Kaymaz S**, Alkan H, Karasu U, Çobankara V. Turkish version of the Michigan Neuropathy Screening Instrument in the assessment of diabetic peripheral neuropathy: a validity and reliability study. *Diabetol Int* 2020; **11**: 283-292 [PMID: 32802710 DOI: 10.1007/s13340-020-00427-9]

31 **Xiong Q**, Lu B, Ye H, Wu X, Zhang T, Li Y. The Diagnostic Value of Neuropathy Symptom and Change Score, Neuropathy Impairment Score and Michigan Neuropathy Screening Instrument for Diabetic Peripheral Neuropathy. *Eur Neurol* 2015; **74**: 323-327 [PMID: 26684759 DOI: 10.1159/000441449]

32 **Ewing DJ**, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)* 1982; **285**: 916-918 [PMID: 6811067 DOI: 10.1136/bmj.285.6346.916]

33 **International Diabetes Federation**. Type 1 diabetes. [cited 3 July 2022]. Available form: https://www.idf.org/aboutdiabetes/type-1-diabetes.html

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