ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2022 July 15; 13(7): 471-586

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 13 Number 7 July 15, 2022

World Journal of Diabetes

Monthly Volume 13 Number 7 July 15, 2022

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Diabetes*, Agostino Di Ciaula, MD, Academic Fellow, Academic Research, Doctor, Medical Assistant, Clinica Medica "A. Murri", Department of Biomedical Sciences & Human Oncology, University of Bari Medical School, Bari 70124, Italy. agodiciaula@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

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
June 15, 2010

FREQUENCY
Monthly

PUBLICATION DATE
July 15, 2022

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 471-481

DOI: 10.4239/wjd.v13.i7.471

ISSN 1948-9358 (online)

EDITORIAL

Renin-angiotensin system blockers-SGLT2 inhibitors-mineralocorticoid receptor antagonists in diabetic kidney disease: A tale of the past two decades!

Awadhesh Kumar Singh, Ritu Singh

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

P-Reviewer: Techane T; Wang QY, China

Received: March 11, 2022
Peer-review started: March 11, 2022
First decision: April 18, 2022
Revised: April 19, 2022
Accepted: June 18, 2022
Article in press: June 18, 2022
Published online: July 15, 2022

Awadhesh Kumar Singh, Ritu Singh, Department of Diabetes & Endocrinology, G.D Hospital & Diabetes Institute, Kolkata 700013, West Bengal, India

Corresponding author: Awadhesh Kumar Singh, MBBS, MD, DM, Consultant Physician-Scientist, Intermediate Editor, Senior Postdoctoral Fellow, Senior Researcher, Department of Diabetes & Endocrinology, G.D Hospital & Diabetes Institute, 133A, Lenin Sarani, Kolkata 700013, West Bengal, India. draksingh_2001@yahoo.com

Abstract

Several pharmacological agents to prevent the progression of diabetic kidney disease (DKD) have been tested in patients with type 2 diabetes mellitus (T2DM) in the past two decades. With the exception of renin-angiotensin system blockers that have shown a significant reduction in the progression of DKD in 2001, no other pharmacological agent tested in the past two decades have shown any clinically meaningful result. Recently, the sodium-glucose cotransporter-2 inhibitor (SGLT-2i), canagliflozin, has shown a significant reduction in the composite of hard renal and cardiovascular (CV) endpoints including progression of end-stage kidney disease in patients with DKD with T2DM at the top of renin-angiotensin system blocker use. Another SGLT-2i, dapagliflozin, has also shown a significant reduction in the composite of renal and CV endpoints including death in patients with chronic kidney disease (CKD), regardless of T2DM status. Similar positive findings on renal outcomes were recently reported as a top-line result of the empagliflozin trial in patients with CKD regardless of T2DM. However, the full results of this trial have not yet been published. While the use of older steroidal mineralocorticoid receptor antagonists (MRAs) such as spironolactone in DKD is associated with a significant reduction in albuminuria outcomes, a novel non-steroidal MRA finerenone has additionally shown a significant reduction in the composite of hard renal and CV endpoints in patients with DKD and T2DM, with reasonably acceptable side effects.

Key Words: Renin-angiotensin system blockers; SGLT-2 inhibitors; Mineralocorticoid receptor antagonist; Diabetic kidney disease; Chronic kidney disease; Renal outcomes; Cardiovascular outcomes

Core Tip: Angiotensin receptor blockers were the first drug class to show a conclusive benefit in preventing diabetic kidney disease (DKD) progression through two randomized trials IDNT and RENAAL in 2001. Several newer pharmacological agents have been tested in DKD in the past 20 years without much success. Notably, recently conducted renal outcome trials of sodium-glucose cotransporter-2 inhibitors in patients with DKD such as CREDENCE, DAPA-CKD, and EMPA-KIDNEY have shown significant improvement in disease progression. Similarly, recent trials of the non-steroidal mineralocorticoid receptor antagonist finerenone (FIDELIO-DKD and FIGARO-DKD) have shown significant improvement in both renal and cardiac endpoints in DKD.

Citation: Singh AK, Singh R. Renin-angiotensin system blockers-SGLT2 inhibitors-mineralocorticoid receptor antagonists in diabetic kidney disease: A tale of the past two decades!. *World J Diabetes* 2022; 13(7): 471-481
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/471.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.471

INTRODUCTION

Type 2 diabetes mellitus (T2DM) remains the leading cause of both chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide[1]. The exact incidence and prevalence of CKD and ESKD from T2DM is difficult to assess due to infrequently performed invasive procedure of kidney biopsies (the gold standard for diagnosis of diabetic kidney disease [DKD]); and because most patients with DKD die before requiring renal replacement therapy. However, DKD affects nearly 20% of patients with T2DM[2-4]. Several factors that may lead to DKD include: the formation of advanced glycation end-products; generation of reactive oxygen species; activation of intercellular signals for proinflammatory and profibrotic gene expression causing cellular inflammation, injury, and fibrosis; alterations in glomerular hemodynamics; and associated hyperinsulinemia and insulin resistance further activating these pathogenic mechanisms[5]. Although the time to development of DKD in T2DM depends on multiple risk factors, its incidence is about 2% of patients per year and affects nearly 25% of patients within 10 years of diagnosis[6]. Classically, DKD progresses from three stages of albuminuria based on urinary albumin excretion: normal to mildly increased (< 30 mg/d or albumin/creatinine ratio [ACR] of < 30 mg/g), moderately increased (formerly called microalbuminuria-30 to 300 mg per day or ACR 30-300 mg/g), and severely increased (formerly called macroalbuminuria-> 300 mg per day or ACR > 300 mg/g) albuminuria. Importantly, the presence of severe albuminuria increases the annual risk of mortality by 4.6% compared with the risk of progression to ESKD (by 2.3%)[6]. These findings necessitate the role of pharmacological agents other than glycemic control in the management of DKD in patients with T2DM.

MANAGEMENT OF DKD IN T2DM

The general approach to managing DKD is similar to that in all patients with T2DM, which includes smoking cessation, weight loss, regular exercise, individualized glycemic targets, and statins. However, certain specific considerations are additionally needed in DKD which include: more intensive blood pressure lowering to prevent ESKD and cardiovascular (CV) morbidity in patients with severe albuminuria and to reduce mortality; and mandatory use of renin-angiotensin system blockers (RASBs), either angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), but not both. Since most individuals with DKD and hypertension require combination therapy, either a combination of an ACEI or ARB plus a dihydropyridine calcium channel blocker is the preferred regimen, except in patients with severe albuminuria where either a non-dihydropyridine CCB or a diuretic may be more suitable with RASB[7].

RASB era

Although there are several randomized controlled trials (*e.g.*, landmark studies: MICRO-HOPE, IRMA-2, and ADVANCE), which showed that RASB prevented progression from normal to microalbuminuria and micro- to macro-albuminuria in T2DM, reduction of albuminuria has generally been considered only a soft renal surrogate endpoint[8-10]. The first convincing evidence suggesting that RASB can significantly reduce hard renal endpoints and prevent the progression of CKD to ESKD in patients with T2DM with severe albuminuria dates back to 2001. The Irbesartan Diabetic Nephropathy Trial (IDNT) randomized 1715 T2DM patients (having urine protein excretion ≥ 0.9 g/d and mean serum creatinine of 1.7 mg/dL) to either irbesartan or amlodipine or placebo. At 2.6 years, the primary composite renal outcome (doubling of serum creatinine, development of ESKD or death from any cause) with irbesartan

was 20% lower than placebo (hazard ratio [HR], 0.80; 95% confidence interval [CI]: 0.66-0.97; P = 0.02) and 23% lower than amlodipine (HR: 0.77; 95%CI: 0.63-0.93; P = 0.006). However, neither any significant reduction in secondary CV endpoint (CV death, non-fatal myocardial infarction [MI], non-fatal stroke, heart failure hospitalization [HHF], or lower limb amputation) nor any reduction in all-cause death was noted with irbesartan, compared to either placebo or amlodipine[11]. The Reduction of Endpoints in Non-Insulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) trial randomized 1513 T2DM patients (having albuminuria > 300 mg/d and mean serum creatinine of 1.9 mg/dL) to either losartan or placebo or both, in addition to conventional antihypertensive drugs (but not ACEI). At 3.4 years, the primary outcome (doubling of serum creatinine, development of ESKD, or death from any cause) was reduced by 16% (HR: 0.84; 95%CI: 0.72-0.98; P = 0.020) in losartan *vs* the placebo group. However, no reduction in all-cause death was noted between losartan *vs* placebo[12]. Importantly, despite the positive renal outcomes with ARBs, a substantial residual risk did remain in both IDNT (residual risk-32.6%) and RENAAL trials (residual risk-43.5%). These findings necessitate additional safe pharmacological agents along with RASBs to further reduce the remaining residual risks in patients with DKD.

Experimental combination therapy and novel drug era

From 2001 until 2018, several combinations of RASB (ACEI plus ARB such as lisinopril plus losartan [VA NEPHRON-D trial] or telmisartan plus ramipril [ONTARGET trial]) were tried without any success. Few older agents such as atorvastatin (4D trial) and several newer novel pharmacological agents (*e.g.*, protein kinase C β [PKC-β] inhibitor: ruboxistaurin; darbepoetin-alfa; non-selective endothelin A receptor antagonist: avosentan; tumor growth factor-β [TGF-β] inhibitor: pirfenidine; pyridoxamine; a mixture of natural glycosaminoglycans polysaccharide: sulodexide; direct renin inhibitor: aliskiren; nuclear factor erythroid 2-related factor 2 [NRF-2] activator: bardoxolone methyl; and pentoxyphylline) were also tried in DKD with T2DM, without much success. Indeed, some of these studies showed harm and were stopped prematurely (Avosentan [ASCEND trial], Aliskiren [ALTITUDE trial], VA NEPHRON-D trial, and Bardoxolone [BEACON trial])[13-25].

Nevertheless, after failure of any favorable outcomes for nearly two decades, the year 2019 ushered a new hope for the management of DKD. A series of recent trials have shown a positive renal outcome including a reduction of death in patients with CKD and T2DM, at the top of RASB use. The SONAR (Study of Diabetic Nephropathy with Atrasentan [a selective endothelin A receptor antagonist]), randomized 2648 patients of CKD (eGFR 25-75 mL/min/1.73 m^2 and urinary ACR of 300-5000 mg/g) with T2DM who were receiving a maximum tolerated dose of RASB to either atrasentan 0.75 mg daily or placebo. At a median follow-up of 2.2 years, the primary composite renal endpoint (doubling of serum creatinine or ESKD) was reduced by 35% (HR: 0.65; 95%CI: 0.49-0.88; P = 0.005) in atrasentan *vs* placebo. However, a higher frequency of HHF (33%) and death (9%) was also noted with atrasentan compared to the placebo[26]. Meanwhile, several cardiovascular outcome trials (CVOTs) conducted with SGLT-2 inhibitors (SGLT-2i) in patients with T2DM, with or without DKD (EMPA-REG, CANVAS Program, and DECLARE-TIMI conducted with empagliflozin, canagliflozin, and dapagliflozin, respectively), have also shown a significant reduction in prespecified renal composite endpoints including progression to ESKD, albeit the renal outcomes were exploratory in nature in all these studies [27-29]. Similarly, studies conducted with non-selective steroidal mineralocorticoid receptor antagonists (MRAs) such as spironolactone and eplerenone have shown a significant reduction in proteinuria in patients with CKD although no conclusive evidence is yet available suggesting that these drugs prevent the progression of DKD. While a meta-analysis of 16 RCTs conducted with spironolactone in CKD at the top of RASB showed a significant reduction in proteinuria (although at the increased risk of hyperkalemia[30], a recent (2020) proteomic prediction and renin-angiotensin-aldosterone system inhibition prevention of early diabetic nephropathy in type 2 diabetic patients with normoalbuminuria study failed to show prevention of progression to microalbuminuria with spironolactone, at the end of 2.5 years of follow-up[31]. Another recently updated (2020) Cochrane meta-analysis involving 44 trials of steroidal MRA (spironolactone and eplerenone) in early stage-CKD (mild-to-moderate proteinuria) showed a significant reduction in proteinuria but an increased risk of hyperkalemia (2.17-fold), acute kidney injury (2.04-fold) and gynecomastia (5.14-fold) was noted with spironolactone[32]. Moreover, the latest (2021) Cochrane meta-analysis of 16 trials of steroidal MRA in late-stage CKD requiring dialysis showed a significant reduction in CV- and all-cause mortality but with a significant 6-fold increased risk of gynecomastia and 1.4-fold increased trend of hyperkalemia[33]. However, the major limitations of these meta-analyses include smaller numbers, shorter duration of studies, and potential risk of bias. Indeed, one RCT of spironolactone (Mineralocorticoid Receptor Antagonists in End-Stage Renal Disease trial, commonly known as MiREnDa) that assessed the safety and CV outcomes with spironolactone and another RCT (Spironolactone in Dialysis-Dependent ESRD, commonly known as SPin-D)-both failed to show any benefit on the left ventricular mass index (LVMI) over 40 wk, or diastolic function or LVMI over 36-wk, respectively along with a dose-dependent increased risk of hyperkalemia[34,35]. Similarly, an eplerenone pilot trial PHASE (Hemodialysis patients undergoing Aldosterone Antagonism with Eplerenone) failed to show any CV benefit and had a 4.5-fold increased risk of hyperkalemia against placebo[36].

SGLT-2i era

While SGLT-2i indicated improved renal outcomes in CVOTs of empagliflozin, canagliflozin, and dapagliflozin (EMPA-REG, CANVAS Program, and DECLARE-TIMI, respectively), the results of the first dedicated renal outcome study CREDENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation) in patients with DKD became available in the year 2019. CREDENCE trial randomized 4402 patients with CKD (eGFR 30 to < 90 mL/min/1.75 m^2 and urinary ACR 300-5000 mg/g) and T2DM already receiving RASB, to either canagliflozin 100 mg daily or placebo. At a median follow up of 2.62 years, the relative risk reduction of primary composite outcome (composite of ESKD, a doubling of the serum creatinine level, or death from renal or CV causes) was 30% (HR: 0.70; 95%CI: 0.59-0.82; P = 0.00001) lower with canagliflozin compared to placebo. ESKD reduced by 31% (HR 0.68; 95%CI: 0.54-0.86; P = 0.002) with canagliflozin compared to placebo. The secondary CV outcome, a composite of 3P-MACE (CV death, non-fatal MI and non-fatal stroke) was found to reduce by 20% (HR: 0.80; 95%CI: 0.67-0.95; P = 0.01), while HHF reduced by 39% (HR: 0.61; 95%CI: 0.47-0.80; P < 0.001) with canagliflozin when compared to placebo[37]. The results of the second kidney outcome trial (Dapagliflozin and Prevention of Adverse Outcomes in Chronic Kidney Disease [DAPA-CKD]) was published in the year 2020. DAPA-CKD randomized 4304 patients with CKD (eGFR 25 to 75 mL/min/1.73 m^2 and urinary ACR of 200 to 5000 mg/g) having 2906 patients with T2DM, to either dapagliflozin 10 mg or placebo. Over a median of 2.4 years, the primary outcome (composite of the sustained decline of eGFR of at least 50%, ESKD, or death from renal or cardiovascular cause) was 39% (HR: 0.61; 95%CI: 0.51-0.72; P < 0.001) lower with dapagliflozin compared to placebo. Reduction in primary renal composite was similar in patients both with (HR: 0.64; 95%CI: 0.52-0.79) or without (HR: 0.50; 95%CI: 0.35-0.72) T2DM with dapagliflozin *vs* placebo. The secondary CV endpoints (composite of CV death or HHF) were reduced by 29% (HR: 0.71; 95%CI: 0.55-0.92; P = 0.009), while all-cause death was reduced by 31% (HR: 0.69; 95%CI: 0.53-0.88; P = 0.004) with dapagliflozin compared to placebo[38]. Ongoing empagliflozin renal outcome trial (EMPA-KIDNEY) in patients with CKD due to either T2DM or non-diabetic cause has been recently (March 16, 2022) stopped owing to the positive results which met the prespecified threshold for early termination against placebo[39]. It should be noted however that the residual risk of CKD progression or kidney failure was still evident in CREDENCE and DAPA-CKD in about 10% of patients despite a full dose of concomitant RASB use after a median follow-up of nearly 2.5 years[37,38]. This necessitates further strategies to combat the progression of DKD in patients with T2DM.

MRA era

While several studies of steroidal MRA (spironolactone and eplerenone) have shown a significant reduction in soft surrogates of proteinuria in patients with DKD albeit, at increased risk of hyperkalemia and gynecomastia (spironolactone), no conclusive evidence of benefit is yet available with these MRAs concerning prevention of ESKD progression. Two ongoing phase 3b RCTs of spironolactone are currently evaluating the CV effect in patients with CKD on dialysis. While the ALdosterone Antagonist Chronic HEModialysis Interventional Survival Trial (commonly known as ALCHEMIST; NCT01848639) is evaluating the primary composite endpoint of non-fatal MI, acute coronary syndrome, HHF, nonfatal stroke, or CV death; the Aldosterone bloCkade for Health Improvement EValuation in End-stage Renal Disease (commonly known as ACHIEVE; NCT03020303) trial is evaluating the composite of CV death or HHF, in patients on maintenance dialysis. The results of both studies are expected in 2023[40].

Meanwhile, several newer, selective, non-steroidal MRA such as finerenone, esaxerenone, and apararenone have also been tried in DKD. The Mineralocorticoid Receptor Antagonist Tolerability Study in Diabetic Nephropathy (ARTS-DN) study, which evaluated various doses of finerenone, showed a dose-dependent significant reduction in UACR (24% and 38% reduction with 10 and 20 mg, respectively) in patients with T2DM having albuminuria (UACR ≥ 30 mg/g) and eGFR of > 30 mL/min/1.73 m^2 at the top of RASB use, although no difference in ≥ 30% decline in eGFR (secondary outcome) was noted against placebo[41]. Significant reduction in proteinuria was also exhibited by esaxerenone in the Esaxerenone in Patients with Type 2 Diabetes and Microalbuminuria (ESAX-DN) study and apararenone study in patients with DKD and T2DM[42,43]. Nevertheless, the conclusive evidence to prevent progression of DKD with MRA was first noted only with finerenone in The Finerenone in Reducing Kidney Failure and Disease Progression in DKD (FIDELIO-DKD) trial that become available in the year 2020. FIDELIO-DKD randomized 5734 patients with CKD (eGFR 25 to < 60 mL/min/1.73 m^2, urinary ACR of 30 to < 300 mg/g and diabetic retinopathy, or urinary ACR 300-5000 mg/g and eGFR 25 to < 75 mL/min/1.73 m^2) and T2DM on maximum licensed dose of RASB, to either finerenone 10 mg (< 60 mL/min/1.73 m^2) or 20 mg (≥ 60 mL/min/1.73 m^2) once daily, or placebo. At the median follow-up of 2.6 years, FIDELIO-DKD showed an 18% reduction (HR: 0.82; 95%CI: 0.73-0.93; P = 0.001) in primary renal outcome (composite of kidney failure, a sustained decrease of at least 40% in the eGFR from baseline, or death from renal causes) with finerenone compared to placebo. A significant reduction of 14% (HR: 0.86; 95%CI: 0.75-0.99; P = 0.03) in secondary CV outcome (composite of CV death, nonfatal MI, and nonfatal stroke, or HHF) was also shown with finerenone compared to placebo. Although adverse events were similar in both arms, hyperkalemia-related drug discontinuation was 2.5 times higher with finerenone (2.3%) compared to placebo (0.9%)[44]. Another study conducted with

Table 1 Studies (in chronological order) that evaluated hard renal or cardiovascular composites in patients having diabetic kidney disease and type 2 diabetes mellitus with various pharmacological agents

Ref.	*n*	Comparator	Duration (mean/median)	Primary endpoints	Results	Remarks
Lewis *et al* [11], 2001, IDNT	1715	Irbesartan 75-300 mg *vs* Amlodipine 2.5-10 mg *vs* PBO	2.6 yr	Composite of doubling of serum Cr, development of ESKD or death from any cause	The primary outcome with IRBE was 20% lower than PBO and 23% lower than AMLO. Doubling of Cr was significantly 33% lower in IRBE *vs* PBO ($P = 0.003$) and 37% lower with IRBE *vs* AMLO ($P < 0.001$). The risk of ESKD was non-significantly 23% lower *vs* both PBO and AMLO ($P = 0.07$, for both comparisons). No difference in CV- or all-cause death was noted	Similar BP control with IRBE and AMLO. Protection is independent of reduction in BP
Brenner *et al* [12], 2001, RENAAL	1513	Losartan 50-100 mg *vs* PBO	3.4 yr	Composite of doubling of serum Cr, development of ESKD or death from any cause	Primary outcome reduced by 16% risk in LOSA *vs* PBO ($P = 0.020$). LOSA reduced the doubling of Cr by 25% ($P = 0.006$) and ESKD by 28% ($P = 0.002$) *vs* PBO but no effect on death was noted. HHF was reduced by 32% in LOSA ($P = 0.005$) while proteinuria was reduced by 35% ($P < 0.001$) *vs* PBO	There was no active comparator, and the mean blood pressure throughout the study was lower among those assigned losartan
Wanner *et al* [13], 2005, 4D	1255	Atorvastatin 20 mg *vs* PBO (receiving hemodialysis)	4.0 yr	Composite of 3P-MACE (death from CV causes, nonfatal MI, and stroke)	No benefit in 3P-MACE (RR: 0.92; 95%CI: 0.77-1.10; $P = 0.37$) but significant increase in fatal stroke (RR: 2.03; $P = 0.04$)	An increase in stroke could be a chance finding, given the data from the CARDS trial that showed atorvastatin lowers the incidence of stroke (HR: 0.52; 95%CI: 0.31-0.89)
Tuttle *et al* [14], 2007, PKC-DRS, PKC-DMES and PKC-DRS 2 study	1157	Ruboxistaurin *vs* PBO	33-39 mo	Composite of doubling of serum Cr, development of advanced chronic kidney disease (stages 4 to 5), and death	No difference between the two group	-
Pfeffer *et al* [15], 2009, TREAT	4038	Darbepoetin alfa *vs* PBO	4.0 yr	Composite outcomes of death or a CV event (nonfatal MI, CHF, stroke, or hospitalization for myocardial ischemia) and of death or ESKD	No difference in the composite of death or ESKD (HR: 1.06; 95%CI: 0.95-1.19; $P = 0.29$) or ESKD (HR: 1.02; 95%CI: 0.87-1.18; $P = 0.83$) between darbepoetin alfa and PBO. An increase in stroke (fatal or nonfatal stroke) occurred in darbepoetin alfa compared with PBO (HR: 1.92; 95%CI: 1.38-2.68; $P < 0.001$)	-
Mann *et al*[16], 2010, ASCEND	1392	Avosentan 25/50 mg *vs* PBO	4 mo	Composite of doubling of serum Cr, ESKD, or death	No difference in primary outcome (25 mg 8.1% *vs* PBO 9.6%; $P = 0.46$; 50 mg 8.6% *vs* PBO 9.6%; $P = 0.79$) but a significant increase in CHF with avosentan (25 mg 5.9% *vs* PBO 2.2%; $P = 0.008$; 50 mg 6.1% *vs* PBO 2.2%; $P = 0.05$) compared with PBO	The trial terminated prematurely after a median follow-up of 4 mo (maximum 16 mo) because of an excess of CV events with avosentan
Sharma *et al* [17], 2011	77	Pirfenidone 1200/2400 mg *vs* PBO	1 yr	Change in eGFR	Mean eGFR significantly increased the pirfenidone 1200-mg/d group *vs* PBO (+3.3 *vs* -2.2 mL/min; $P = 0.03$) but no improvement in 2400-mg/d group	-
Pergola *et al* [18], 2011, BEAM	227	Bardoxolone 25/75/150 mg OD *vs* PBO	12 mo	Change in eGFR at 6 mo	Significant increase in mean eGFR both at 6-mo (8.2-11.4 mL/min; $P < 0.001$) and 12-mo (5.8-10.5 mL/min) against PBO	Muscle spasms were the MC observed S/E with BDX
Lewis *et al* [19], 2012	317	Pyridoxamine 150/300 mg BID *vs* PBO	52-wk	Change in serum Cr	No difference in outcome observed	-

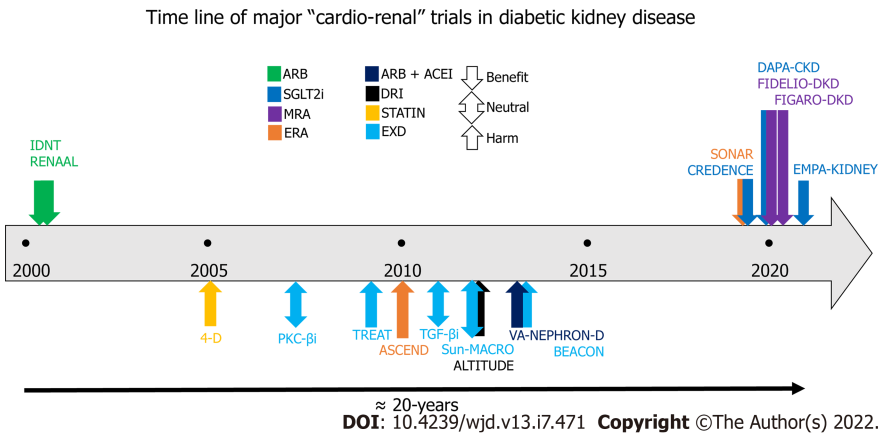
Packham *et al* [20], 2012, Sun-MACRO	1248	Sulodexide *vs* PBO	11 mo	Composite of a doubling of serum Cr, development of ESKD, or serum Cr ≥6.0 mg/dl	No difference in the outcome	The trial was stopped prematurely due to futility
Parving *et al* [21], 2012, ALTITUDE	8561	Aliskiren *vs* PBO	32.9 mo	Composite of CV death or the first occurrence of cardiac arrest with resuscitation; nonfatal MI; nonfatal stroke; unplanned HHF; ESKD, death attributable to kidney failure, or the need for RRT with no dialysis or transplantation available or initiated; or doubling of Cr level	Results of the primary endpoint were no different between the two arms (HR: 1.08; 95% 0.98-1.20; $P = 0.12$)	The trial was stopped prematurely after the second interim efficacy analysis because of significantly higher (11.2% *vs* 7.2%; $P < 0.001$) hyperkalemia (Serum K level ≥ 6 mmol/L) and hypotension (12.1% *vs* 8.3%; $P < 0.001$ in the aliskiren group *vs* PBO
Fried *et al*[22], 2013, VA NEPHRON-D	1448	Losartan plus lisinopril *vs* losartan plus PBO	2.2 yr	Composite of change in the eGFR, ESKD, or death	No difference in outcome (HR: 0.88; 95%CI: 0.70 to 1.12; $P = 0.30$). Combination therapy increased the risk of hyperkalemia ($P < 0.001$) and acute kidney injury ($P < 0.001$) compared to monotherapy	The trial was stopped prematurely
Mann *et al*[23], 2013, ONTARGET	3163	Ramipril 10 mg *vs* telmisartan 80 mg *vs* ramipril plus telmisartan (10 + 80)	56-mo	Composite of dialysis, doubling of serum Cr, and death	Combination therapy was associated with a non-significantly higher ESKD or doubling of serum creatinine (5.3% *vs* 4.8 %), but a similar death rate (2.3% *vs* 2.2 %) *vs* monotherapy. Combination therapy had higher rates of acute kidney injury requiring dialysis (1.4% *vs* 0.8 %)	This is the data of 3163 people having DKD from a total of 9628 patients with diabetes
de Zeeuw *et al* [24], 2013, BEACON	2185	Bardoxolone 20 mg OD *vs* PBO	9.0 mo	Composite of ESKD or CV death	No difference (HR: 0.98; 95%CI: 0.70-1.37; $P = 0.92$). Significant increase in HHF and death due to HHF with bardoxolone (HR: 1.83; 95%CI: 1.32-2.55; $P < 0.001$) *vs* PBO	The trial was stopped prematurely
Navarro-Gonzalez *et al* [25], 2015, PREDIAN	169	Pentoxyphylline 600 mg BID *vs* PBO	2-yr	Change in eGFR	Significant less decrease in eGFR in PTF *vs* PBO (-2.1 *vs* -6.5 mL/min; Group difference -4.3 mL/min; $P < 0.001$)	Open-label design and envelope (rather than computer-generated) randomization could have biased the results
Heerspink *et al* [26], 2019, SONAR	2648	Atrasentan 0.75 mg *vs* PBO	2.2 yr (Median)	Composite of doubling of serum Cr or ESKD or death from kidney failure	35% reduction in primary composite renal endpoint event (HR: 0.65; 95%CI: 0.49-0.88; $P = 0.005$)	HHF was insignificantly higher in atrasentan (HR: 1.33; 95%CI: 0.85-2.07; $P = 0.208$) *vs* PBO
Perkovic *et al* [37], 2019, CREDENCE	4401	Canagliflozin 100 mg *vs* PBO	2.6 yr	Composite of ESKD, doubling of serum Cr, or death from renal or CV causes	30% reduction in primary composite (HR: 0.70; 95%CI: 0.59-82; $P = 0.00001$), 34% reduction (HR: 0.66; 95%CI: 0.53-0.81; $P < 0.001$) in renal-specific composite (of ESKD, doubling of Cr, renal death) and 32% reduction in ESKD (HR: 0.68; 95%CI: 0.54-0.86; $P = 0.002$) in CANA *vs* PBO. Reduction in composite of 3P-MACE was 20% (HR: 0.80; 95%CI: 0.67-0.95; $P = 0.01$) while HHF reduced by 39% (HR: 0.61; 95%CI: 0.47-0.80; $P < 0.001$) in CANA arm *vs* PBO	The trial was stopped prematurely due to efficacy
Heerspink *et al* [38], 2020, DAPA-CKD	4304	Dapagliflozin 10 mg *vs* PBO	2.4 yr	Composite of ESKD, sustained decline in eGFR of at least 50%, or death from renal or CV causes	39% reduction in primary composite (HR: 0.61; 95%CI: 0.51-0.72; $P < 0.001$), 44% reduction (HR: 0.56; 95%CI: 0.45-0.68; $P < 0.001$) in renal-specific composite (of ESKD, decline in eGFR of at least 50%, or renal death), 29% reduction (HR: 0.71; 95%CI: 0.55-0.92; $P = 0.009$) in composite of CV death of HHF and 31% reduction in death (HR: 0.69;	The trial stopped prematurely due to efficacy

					95%CI: 0.53-0.88; *P* = 0.004) in DAPA arm *vs* PBO	
Bakris *et al* [44], 2020, FIDELIO-DKD	5734	Finerenone 10/20 mg *vs* PBO	2.6 yr	Composite of kidney failure, sustained decrease of at least 40% in the eGFR from baseline, or death from renal causes	18% reduction (HR: 0.82; 95%CI: 0.73-0.93; *P* = 0.001) in primary composite in FINE *vs* PBO arm. 14% reduction (HR: 0.86; 95%CI: 0.75-0.99; *P* = 0.03) in secondary outcome composite (CV death, non-fatal MI, non-fatal stroke, or HHF) in FINE *vs* PBO arm	Hyperkalemia-related discontinuation of the drug was higher in the FINE *vs* PBO (2.3% *vs* 0.9%) arm
Pitt *et al*[45], 2021, *FIGARO*-DKD	7437	Finerenone 10/20 mg *vs* PBO	3.4 yr	Composite of CV death, nonfatal MI, nonfatal stroke, or HHF. The secondary outcome was a composite of a decrease of eGFR by at least 40%, ESKD, or death from renal causes	13% reduction (HR: 0.87; 95%CI: 0.76-0.98; *P* = 0.03) in primary cardiac composite primarily driven due to 29% reduction (HR: 0.71; 95%CI: 0.56-0.90) in HHF with FINE *vs* PBO. Non-significant 13% reduction (HR: 0.87; 95%CI: 0.76-1.01) in secondary renal composite with FINE *vs* PBO	Hyperkalemia-related discontinuation of the drug was higher in the FINE *vs* PBO (1.2% *vs* 0.4%) arm

3P-MACE: 3-point major adverse cardiac events; AMLO: Amlodipine; BDX: Bardoxolone; BID: Twice daily; BP: Blood pressure; CANA; Canagliflozin; CHF: Congestive heart failure; CI: Confidence interval; Cr: Creatinine; CV: Cardiovascular; DAPA; Dapagliflozin; DKD: Diabetic kidney disease; eGFR: Estimated glomerular filtration rate; ESKD: End-stage kidney disease; FINE: Finerenone; HHF: Heart failure hospitalization; HR; Hazard ratio; IRBE: Irbesartan; LOSA: Losartan; MC: Most common; MI: Myocardial infarction; OD: Once daily; PBO: Placebo; PTF; Pentoxyphylline; RR: Relative risk; RRT: Renal replacement therapy; S/E: Side effects.

Finerenone FIGARO-DKD (Reducing Cardiovascular Mortality and Morbidity in DKD) has been published recently in 2021. FIGARO-DKD trial randomized 7437 patients with CKD (eGFR 25 to 90 mL/min/1.73 m^2 and urinary ACR of 30 to < 300 mg/g, or urinary ACR 300 to 500 mg/g and eGFR ≥ 60 mL/min/1.73 m^2) and T2DM to either finerenone 10 mg (25 to < 60 mL/min/1.73 m^2) or 20 mg (≥ 60 mL/min/1.73 m^2) once daily, or placebo on the maximum licensed dose of RASB. On a median follow-up of 3.4 years, the primary CV outcome (composite of CV death, nonfatal MI, and nonfatal stroke, or HHF) was significantly reduced by 13% (HR: 0.87; 95%CI: 0.76-0.98; *P* = 0.03) primarily driven by 29% reduction (HR: 0.71; 95%CI: 0.56-0.90) in HHF with finerenone compared to placebo. Interestingly, no significant difference (HR: 0.87; 95%CI: 0.76-1.01) was noted in secondary renal outcome (composite of kidney failure, a sustained decrease of at least 40% in the eGFR from baseline, or death from renal cause) with finerenone compared to placebo. Overall, no difference in the adverse events was noted in the two arms, however hyperkalemia-related drug discontinuation was 3-times higher with finerenone (1.2%) compared to placebo (0.4%)[45]. Table 1 summarizes the results from all these studies (in chronological order) which have been conducted in patients with T2DM having CKD that evaluated hard renal or cardiovascular composite endpoint as the primary objective[11-26,37,38,44,45]. Figure 1 is a schematic representation of timelines and outcomes from all these cardio-renal outcome trials.

In summary, several agents have been tried in the past two decades in patients with DKD and T2DM, but only three drug classes (RASB, SGLT-2i, and MRA especially finerenone) have conclusively shown both ≥ 30% reduction in albuminuria and a significant lowering in renal disease progression. It should be recalled that a cut-off of 30% geometric mean albuminuria reduction within 6 mo or an eGFR slope reduction of 0.5-1.0 mL/min/1.73 m^2/year over 2-3 years has been adopted as a surrogate renal endpoint for CKD progression for clinical trials by National Kidney Foundation, European Medicines Agency, and US Food and Drug Administration in the year 2020[46]. This cut-off seems to have primarily originated from at least two meta-analyses[47,48]. While the Reducing Albuminuria as Surrogate Endpoint (REASSURE) Consortium showed each 30% reduction in albuminuria lowered the risk of ESKD by 24%, a meta-analysis of observational studies involving nearly 700000 individuals found that a 30% reduction of albuminuria over 2 years lowered ESKD by 22%, regardless of drug class tested[47,48]. However, the pressing question which remains unanswered conclusively is whether the addition of MRA including finerenone to the patients who are already receiving SGLT-2i and RASB would help prevent further progression of kidney disease[49]. Mechanistically, the action of both SGLT-2i and MRA including finerenone appears to be complementary due to the following: (1) The differential mechanism of action. While SGLT-2i reduces glomerular hyperfiltration and could have direct beneficial cellular and metabolic effect, finerenone reduces inflammation and fibrosis by inhibiting mineralocorticoid receptor pathway; and (2) Hyperkalemia induced by finerenone (the commonest reason for drug discontinuation) can be counterbalanced by SGLT-2i. A recent meta-analysis from the pooled data of five RCTs (*n* = 8296) in patients with reduced ejection fraction showed SGLT-2i plus MRA to significantly reduce both cardiovascular composite of CV death or HHF (HR: 0.73; 95%CI: 0.66-0.80; *P* < 0.00001) and composite renal endpoints (HR 0.56; 95%CI: 0.39-0.81; *P* = 0.002) but with a significantly lower risk of hyperkalemia (HR 0.60; 95%CI: 0.42-0.87; *P* = 0.007), compared to MRA alone [50]. However, renal outcomes were exploratory endpoints in these RCTs included in this meta-analysis.



DOI: 10.4239/wjd.v13.i7.471

Figure 1 Major cardio-renal outcome trials in patients with diabetic kidney disease and type 2 diabetes mellitus. ARB: Angiotensin-receptor blocker; ACEI: Angiotensin converting enzyme inhibitors; DRI: Direct renin inhibitors; ERA: Endothelin A receptor antagonists; EXD: Experimental drugs; MRA: Mineralocorticoid receptor antagonists; PKC-βi: Protein-kinase C β inhibitor; SGLT-2i: Sodium-glucose co-transporter 2 inhibitors; TGF-βi: Tumor growth factor β inhibitor.

In FIDELIO-DKD, 4.6% (259/5674) patients were receiving SGLT-2i at the baseline and reduction in primary renal composite was similar ($P_{\text{Interaction}}$ = 0.21), regardless of the SGLT-2i use (SGLT-2i users: HR, 1.38; 95%CI: 0.61-3.10; SGLT-2i non-users: HR, 0.82; 95%CI: 0.72-0.92). Similarly, in FIGARO-DKD, 8.4% patients (618/7352) were receiving SGLT-2i at baseline and benefit in primary CV composite was similar, regardless of SGLT-2i use (SGLT-2i users: HR, 0.49; 95%CI: 0.28-0.86; SGLT-2i non-users: HR, 0.89; 95%CI: 0.78-1.01). Importantly, a recent subgroup analysis of FIDELIO-DKD found that finerenone caused a 25% reduction in UACR in patients receiving SGLT-2i at the baseline, and patients on SGLT-2i also had fewer hyperkalemia events. Indeed, this subgroup analysis stratified on the baseline SGLT-2i use reported a lesser episode of treatment-emergent hyperkalemia of both moderate (> 5.5 mmol/L) and severe (> 6.0 mmol/L) nature in combined SGLT-2i plus finerenone users (7% and 0%, respectively), compared with finerenone alone (22% and 5%, respectively)[51]. Notably, a recent meta-analysis of six cardio-renal trials involving 49875 individuals has found a 16% lower risk (HR: 0.84; 95%CI: 0.76-0.93) of serious hyperkalemia (> 6.0 mmol/L) with SGLT-2i without any higher risk of hypokalemia[52]. Collectively, these finding hints that combination therapy of SGLT-2i and finerenone would likely reduce the risk of hyperkalemia. Whether combining MRA to SGLT-2i would enhance the CV or renal outcome is not clearly known due to: (1) Low number of events in a small population of baseline SGLT-2i users in both FIDELIO-DKD and FIGARO-DKD trial (number of events 24 and 61, respectively); and (2) Absence of any dedicated RCT that has assessed the renal or CV outcome with the combination therapy in patients with CKD and T2DM. Efficacy and safety of finerenone plus empagliflozin compared with either finerenone or empagliflozin in 807 participants with CKD and T2DM (CONFIDENCE Trial, NCT05254002) is currently planned and expected to be complete by end of 2023[53].

CONCLUSION

While optimal glucose control, intensive blood pressure control, and use of RASB have been the traditional foundation of treatment in slowing the progression of kidney disease in patients with albuminuria and T2DM for the past two decades, the addition of SGLT-2i to this foundational treatment has further shown to reduce the disease progression including death (DAPA-CKD). Finerenone would be a welcome addition to the list of novel drugs that have been able to reduce the progression of CKD successfully in patients with T2DM along with RASB. It is also possible that finerenone plus SGLT-2i combination can further prevent the progression of DKD in T2DM but that has to be proved through dedicated RCTs.

FOOTNOTES

Author contributions: Singh AK designed the research; Singh R performed the research, Singh AK and Singh R analyzed the data; Singh AK wrote the editorial; Singh R revised the manuscript.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Country/Territory of origin: India

ORCID number: Awadhesh Kumar Singh 0000-0002-8374-4536; Ritu Singh 0000-0003-1779-0638.

S-Editor: Chang KL
L-Editor: Filipodia
P-Editor: Chang KL

REFERENCES

1 **International Diabetes Federation**. IDF Diabetes Atlas, 8th end. Brussels: International Diabetes Federation, 2017
2 **Afkarian M**, Sachs MC, Kestenbaum B, Hirsch IB, Tuttle KR, Himmelfarb J, de Boer IH. Kidney disease and increased mortality risk in type 2 diabetes. *J Am Soc Nephrol* 2013; **24**: 302-308 [PMID: 23362314 DOI: 10.1681/ASN.2012070718]
3 **Murphy D**, McCulloch CE, Lin F, Banerjee T, Bragg-Gresham JL, Eberhardt MS, Morgenstern H, Pavkov ME, Saran R, Powe NR, Hsu CY; Centers for Disease Control and Prevention Chronic Kidney Disease Surveillance Team. Trends in Prevalence of Chronic Kidney Disease in the United States. *Ann Intern Med* 2016; **165**: 473-481 [PMID: 27479614 DOI: 10.7326/M16-0273]
4 **Tuttle KR**, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, Hirsch IB, Kalantar-Zadeh K, Narva AS, Navaneethan SD, Neumiller JJ, Patel UD, Ratner RE, Whaley-Connell AT, Molitch ME. Diabetic kidney disease: a report from an ADA Consensus Conference. *Am J Kidney Dis* 2014; **64**: 510-533 [PMID: 25257325 DOI: 10.1053/j.ajkd.2014.08.001]
5 **Pichler R**, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. *Am J Physiol Renal Physiol* 2017; **312**: F716-F731 [PMID: 27558558 DOI: 10.1152/ajprenal.00314.2016]
6 **Adler AI**, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR; UKPDS GROUP. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int* 2003; **63**: 225-232 [PMID: 12472787 DOI: 10.1046/j.1523-1755.2003.00712.x]
7 **Jamerson K**, Weber MA, Bakris GL, Dahlöf B, Pitt B, Shi V, Hester A, Gupte J, Gatlin M, Velazquez EJ; ACCOMPLISH Trial Investigators. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med* 2008; **359**: 2417-2428 [PMID: 19052124 DOI: 10.1056/NEJMoa0806182]
8 Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. *Lancet* 2000; **355**: 253-259 [PMID: 10675071]
9 **Patel A**; ADVANCE Collaborative Group, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, Harrap S, Poulter N, Marre M, Cooper M, Glasziou P, Grobbee DE, Hamet P, Heller S, Liu LS, Mancia G, Mogensen CE, Pan CY, Rodgers A, Williams B. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 2007; **370**: 829-840 [PMID: 17765963 DOI: 10.1016/S0140-6736(07)61303-8]
10 **Parving HH**, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P; Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 2001; **345**: 870-878 [PMID: 11565519 DOI: 10.1056/NEJMoa011489]
11 **Lewis EJ**, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I; Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 2001; **345**: 851-860 [PMID: 11565517 DOI: 10.1056/NEJMoa011303]
12 **Brenner BM**, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S; RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861-869 [PMID: 11565518 DOI: 10.1056/NEJMoa011161]
13 **Wanner C**, Krane V, März W, Olschewski M, Mann JF, Ruf G, Ritz E; German Diabetes and Dialysis Study Investigators. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005; **353**: 238-248 [PMID: 16034009 DOI: 10.1056/NEJMoa043545]
14 **Tuttle KR**, McGill JB, Haney DJ, Lin TE, Anderson PW; PKC-DRS, PKC-DMES, and PKC-DRS 2 Study Groups. Kidney outcomes in long-term studies of ruboxistaurin for diabetic eye disease. *Clin J Am Soc Nephrol* 2007; **2**: 631-636 [PMID: 17699475 DOI: 10.2215/CJN.00840207]
15 **Pfeffer MA**, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill JB, McMurray JJ, Parfrey P, Parving HH, Remuzzi G, Singh AK, Solomon SD, Toto R; TREAT Investigators. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med* 2009; **361**: 2019-2032 [PMID: 19880844 DOI: 10.1056/NEJMoa0907845]
16 **Mann JF**, Green D, Jamerson K, Ruilope LM, Kuranoff SJ, Littke T, Viberti G; ASCEND Study Group. Avosentan for overt diabetic nephropathy. *J Am Soc Nephrol* 2010; **21**: 527-535 [PMID: 20167702 DOI: 10.1681/ASN.2009060593]

17 **Sharma K**, Ix JH, Mathew AV, Cho M, Pflueger A, Dunn SR, Francos B, Sharma S, Falkner B, McGowan TA, Donohue M, Ramachandrarao S, Xu R, Fervenza FC, Kopp JB. Pirfenidone for diabetic nephropathy. *J Am Soc Nephrol* 2011; **22**: 1144-1151 [PMID: 21511828 DOI: 10.1681/ASN.2010101049]

18 **Pergola PE**, Raskin P, Toto RD, Meyer CJ, Huff JW, Grossman EB, Krauth M, Ruiz S, Audhya P, Christ-Schmidt H, Wittes J, Warnock DG; BEAM Study Investigators. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N Engl J Med* 2011; **365**: 327-336 [PMID: 21699484 DOI: 10.1056/NEJMoa1105351]

19 **Lewis EJ**, Greene T, Spitalewiz S, Blumenthal S, Berl T, Hunsicker LG, Pohl MA, Rohde RD, Raz I, Yerushalmy Y, Yagil Y, Herskovits T, Atkins RC, Reutens AT, Packham DK, Lewis JB; Collaborative Study Group. Pyridorin in type 2 diabetic nephropathy. *J Am Soc Nephrol* 2012; **23**: 131-136 [PMID: 22034637 DOI: 10.1681/ASN.2011030272]

20 **Packham DK**, Wolfe R, Reutens AT, Berl T, Heerspink HL, Rohde R, Ivory S, Lewis J, Raz I, Wiegmann TB, Chan JC, de Zeeuw D, Lewis EJ, Atkins RC; Collaborative Study Group. Sulodexide fails to demonstrate renoprotection in overt type 2 diabetic nephropathy. *J Am Soc Nephrol* 2012; **23**: 123-130 [PMID: 22034636 DOI: 10.1681/ASN.2011040378]

21 **Parving HH**, Brenner BM, McMurray JJ, de Zeeuw D, Haffner SM, Solomon SD, Chaturvedi N, Persson F, Desai AS, Nicolaides M, Richard A, Xiang Z, Brunel P, Pfeffer MA; ALTITUDE Investigators. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N Engl J Med* 2012; **367**: 2204-2213 [PMID: 23121378 DOI: 10.1056/NEJMoa1208799]

22 **Fried LF**, Emanuele N, Zhang JH, Brophy M, Conner TA, Duckworth W, Leehey DJ, McCullough PA, O'Connor T, Palevsky PM, Reilly RF, Seliger SL, Warren SR, Watnick S, Peduzzi P, Guarino P; VA NEPHRON-D Investigators. Combined angiotensin inhibition for the treatment of diabetic nephropathy. *N Engl J Med* 2013; **369**: 1892-1903 [PMID: 24206457 DOI: 10.1056/NEJMoa1303154]

23 **Mann JF**, Anderson C, Gao P, Gerstein HC, Boehm M, Rydén L, Sleight P, Teo KK, Yusuf S; ONTARGET investigators. Dual inhibition of the renin-angiotensin system in high-risk diabetes and risk for stroke and other outcomes: results of the ONTARGET trial. *J Hypertens* 2013; **31**: 414-421 [PMID: 23249829 DOI: 10.1097/HJH.0b013e32835bf7b0]

24 **de Zeeuw D**, Akizawa T, Audhya P, Bakris GL, Chin M, Christ-Schmidt H, Goldsberry A, Houser M, Krauth M, Lambers Heerspink HJ, McMurray JJ, Meyer CJ, Parving HH, Remuzzi G, Toto RD, Vaziri ND, Wanner C, Wittes J, Wrolstad D, Chertow GM; BEACON Trial Investigators. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *N Engl J Med* 2013; **369**: 2492-2503 [PMID: 24206459 DOI: 10.1056/NEJMoa1306033]

25 **Navarro-González JF**, Mora-Fernández C, Muros de Fuentes M, Chahin J, Méndez ML, Gallego E, Macía M, del Castillo N, Rivero A, Getino MA, García P, Jarque A, García J. Effect of pentoxifylline on renal function and urinary albumin excretion in patients with diabetic kidney disease: the PREDIAN trial. *J Am Soc Nephrol* 2015; **26**: 220-229 [PMID: 24970885 DOI: 10.1681/ASN.2014010012]

26 **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]

27 **Zinman B**, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 2117-2128 [PMID: 26378978 DOI: 10.1056/NEJMoa1504720]

28 **Neal B**, Perkovic V, Matthews DR. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *N Engl J Med* 2017; **377**: 2099 [PMID: 29166232 DOI: 10.1056/NEJMc1712572]

29 **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]

30 **Hou J**, Xiong W, Cao L, Wen X, Li A. Spironolactone Add-on for Preventing or Slowing the Progression of Diabetic Nephropathy: A Meta-analysis. *Clin Ther* 2015; **37**: 2086-2103.e10 [PMID: 26254276 DOI: 10.1016/j.clinthera.2015.05.508]

31 **Tofte N**, Lindhardt M, Adamova K, Bakker SJL, Beige J, Beulens JWJ, Birkenfeld AL, Currie G, Delles C, Dimos I, Francová L, Frimodt-Møller M, Girman P, Göke R, Havrdova T, Heerspink HJL, Kooy A, Laverman GD, Mischak H, Navis G, Nijpels G, Noutsou M, Ortiz A, Parvanova A, Persson F, Petrie JR, Ruggenenti PL, Rutters F, Rychlík I, Siwy J, Spasovski G, Speeckaert M, Trillini M, Zürbig P, von der Leyen H, Rossing P; PRIORITY investigators. Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): a prospective observational study and embedded randomised placebo-controlled trial. *Lancet Diabetes Endocrinol* 2020; **8**: 301-312 [PMID: 32135136 DOI: 10.1016/S2213-8587(20)30026-7]

32 **Chung EY**, Ruospo M, Natale P, Bolignano D, Navaneethan SD, Palmer SC, Strippoli GF. Aldosterone antagonists in addition to renin angiotensin system antagonists for preventing the progression of chronic kidney disease. *Cochrane Database Syst Rev* 2020; **10**: CD007004 [PMID: 33107592 DOI: 10.1002/14651858.CD007004.pub4]

33 **Hasegawa T**, Nishiwaki H, Ota E, Levack WM, Noma H. Aldosterone antagonists for people with chronic kidney disease requiring dialysis. *Cochrane Database Syst Rev* 2021; **2**: CD013109 [PMID: 33586138 DOI: 10.1002/14651858.CD013109.pub2]

34 **Hammer F**, Malzahn U, Donhauser J, Betz C, Schneider MP, Grupp C, Pollak N, Störk S, Wanner C, Krane V; MiREnDa Study Group. A randomized controlled trial of the effect of spironolactone on left ventricular mass in hemodialysis patients. *Kidney Int* 2019; **95**: 983-991 [PMID: 30712923 DOI: 10.1016/j.kint.2018.11.025]

35 **Charytan DM**, Himmelfarb J, Ikizler TA, Raj DS, Hsu JY, Landis JR, Anderson AH, Hung AM, Mehrotra R, Sharma S, Weiner DE, Williams M, DiCarli M, Skali H, Kimmel PL, Kliger AS, Dember LM; Hemodialysis Novel Therapies Consortium. Safety and cardiovascular efficacy of spironolactone in dialysis-dependent ESRD (SPin-D): a randomized, placebo-controlled, multiple dosage trial. *Kidney Int* 2019; **95**: 973-982 [PMID: 30473139 DOI: 10.1016/j.kint.2018.08.034]

36 **Walsh M**, Manns B, Garg AX, Bueti J, Rabbat C, Smyth A, Tyrwhitt J, Bosch J, Gao P, Devereaux PJ, Wald R. The Safety

of Eplerenone in Hemodialysis Patients: A Noninferiority Randomized Controlled Trial. *Clin J Am Soc Nephrol* 2015; **10**: 1602-1608 [PMID: 26138259 DOI: 10.2215/CJN.12371214]

37 **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]

38 **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]

39 **Zoler ML**. Empagliflozin Scores Topline Win in EMPA-KIDNEY Trial. Mar 17, 2022. [cited 17 March 2022]. Available from: https://www.medscape.com/viewarticle/970434

40 **Georgianos PI**, Vaios V, Eleftheriadis T, Zebekakis P, Liakopoulos V. Mineralocorticoid Antagonists in ESRD: An Overview of Clinical Trial Evidence. *Curr Vasc Pharmacol* 2017; **15**: 599-606 [PMID: 28155610 DOI: 10.2174/1570161115666170201113817]

41 **Bakris GL**, Agarwal R, Chan JC, Cooper ME, Gansevoort RT, Haller H, Remuzzi G, Rossing P, Schmieder RE, Nowack C, Kolkhof P, Joseph A, Pieper A, Kimmeskamp-Kirschbaum N, Ruilope LM; Mineralocorticoid Receptor Antagonist Tolerability Study–Diabetic Nephropathy (ARTS-DN) Study Group. Effect of Finerenone on Albuminuria in Patients With Diabetic Nephropathy: A Randomized Clinical Trial. *JAMA* 2015; **314**: 884-894 [PMID: 26325557 DOI: 10.1001/jama.2015.10081]

42 **Ito S**, Kashihara N, Shikata K, Nangaku M, Wada T, Okuda Y, Sawanobori T. Esaxerenone (CS-3150) in Patients with Type 2 Diabetes and Microalbuminuria (ESAX-DN): Phase 3 Randomized Controlled Clinical Trial. *Clin J Am Soc Nephrol* 2020; **15**: 1715-1727 [PMID: 33239409 DOI: 10.2215/CJN.06870520]

43 **Wada T**, Inagaki M, Yoshinari T, Terata R, Totsuka N, Gotou M, Hashimoto G. Apararenone in patients with diabetic nephropathy: results of a randomized, double-blind, placebo-controlled phase 2 dose-response study and open-label extension study. *Clin Exp Nephrol* 2021; **25**: 120-130 [PMID: 32974732 DOI: 10.1007/s10157-020-01963-z]

44 **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]

45 **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]

46 **Levey AS**, Gansevoort RT, Coresh J, Inker LA, Heerspink HL, Grams ME, Greene T, Tighiouart H, Matsushita K, Ballew SH, Sang Y, Vonesh E, Ying J, Manley T, de Zeeuw D, Eckardt KU, Levin A, Perkovic V, Zhang L, Willis K. Change in Albuminuria and GFR as End Points for Clinical Trials in Early Stages of CKD: A Scientific Workshop Sponsored by the National Kidney Foundation in Collaboration With the US Food and Drug Administration and European Medicines Agency. *Am J Kidney Dis* 2020; **75**: 84-104 [PMID: 31473020 DOI: 10.1053/j.ajkd.2019.06.009]

47 **Heerspink HJ**, Kröpelin TF, Hoekman J, de Zeeuw D; Reducing Albuminuria as Surrogate Endpoint (REASSURE) Consortium. Drug-Induced Reduction in Albuminuria Is Associated with Subsequent Renoprotection: A Meta-Analysis. *J Am Soc Nephrol* 2015; **26**: 2055-2064 [PMID: 25421558 DOI: 10.1681/ASN.2014070688]

48 **Coresh J**, Heerspink HJL, Sang Y, Matsushita K, Arnlov J, Astor BC, Black C, Brunskill NJ, Carrero JJ, Feldman HI, Fox CS, Inker LA, Ishani A, Ito S, Jassal S, Konta T, Polkinghorne K, Romundstad S, Solbu MD, Stempniewicz N, Stengel B, Tonelli M, Umesawa M, Waikar SS, Wen CP, Wetzels JFM, Woodward M, Grams ME, Kovesdy CP, Levey AS, Gansevoort RT; Chronic Kidney Disease Prognosis Consortium and Chronic Kidney Disease Epidemiology Collaboration. Change in albuminuria and subsequent risk of end-stage kidney disease: an individual participant-level consortium meta-analysis of observational studies. *Lancet Diabetes Endocrinol* 2019; **7**: 115-127 [PMID: 30635225 DOI: 10.1016/S2213-8587(18)30313-9]

49 **Neuen BL**, Jardine MJ. SGLT2 inhibitors and finerenone: one or the other or both? *Nephrol Dial Transplant* 2022 [PMID: 35212745 DOI: 10.1093/ndt/gfac046]

50 **Cordovez RA**, Rivera K, Denila RA, Patricio M. Cardiorenal Effects of Sodium-Glucose Co-Transporter-2 Inhibitors in Combination with Mineralocorticoid Receptor Antagonist for Heart Failure with Reduced Ejection Fraction: A Systematic Review and Meta-Analysis. *JACC* 2022; **79**: 340 [DOI: 10.1016/s0735-1097(22)01331-6]

51 **Rossing P**, Filippatos G, Agarwal R, Anker SD, Pitt B, Ruilope LM, Chan JCN, Kooy A, McCafferty K, Schernthaner G, Wanner C, Joseph A, Scheerer MF, Scott C, Bakris GL; FIDELIO-DKD Investigators. Finerenone in Predominantly Advanced CKD and Type 2 Diabetes With or Without Sodium-Glucose Cotransporter-2 Inhibitor Therapy. *Kidney Int Rep* 2022; **7**: 36-45 [PMID: 35005312 DOI: 10.1016/j.ekir.2021.10.008]

52 **Neuen BL**, Oshima M, Agarwal R, Arnott C, Cherney DZ, Edwards R, Langkilde AM, Mahaffey KW, McGuire DK, Neal B, Perkovic V, Pong A, Sabatine MS, Raz I, Toyama T, Wanner C, Wheeler DC, Wiviott SD, Zinman B, Heerspink HJL. Sodium-Glucose Cotransporter 2 Inhibitors and Risk of Hyperkalemia in People With Type 2 Diabetes: A Meta-Analysis of Individual Participant Data From Randomized, Controlled Trials. *Circulation* 2022; **145**: 1460-1470 [PMID: 35394821 DOI: 10.1161/CIRCULATIONAHA.121.057736]

53 A Study to Learn How Well the Treatment Combination of Finerenone and Empagliflozin Works and How Safe it is Compared to Each Treatment Alone in Adult Participants With Long-term Kidney Disease (Chronic Kidney Disease) and Type 2 Diabetes. (Accessed on April 11, 2022). In: ClinicalTrials.gov [Internet]. Bethesda (MD): US. National Library of Medicine. Available from: http://clinicaltrials.gov/ct2/show/NCT05254002. ClinicalTrials.gov Identifier: NCT05254002

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 482-497

DOI: 10.4239/wjd.v13.i7.482

ISSN 1948-9358 (online)

REVIEW

Fetal programming of obesity and type 2 diabetes

Sumudu Nimali Seneviratne, Shaman Rajindrajith

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
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Islam SMRU, Bangladesh; Shuang WB, China; Ulasoglu C, Turkey
A-Editor: Guo L, China

Received: July 9, 2021
Peer-review started: July 9, 2021
First decision: August 8, 2021
Revised: August 18, 2021
Accepted: June 3, 2022
Article in press: June 3, 2022
Published online: July 15, 2022

Sumudu Nimali Seneviratne, Shaman Rajindrajith, Department of Paediatrics, Faculty of Medicine, University of Colombo, Colombo 08, Sri Lanka

Corresponding author: Sumudu Nimali Seneviratne, MD, PhD, Senior Lecturer, Department of Paediatrics, Faculty of Medicine, University of Colombo, 25, Kynsey Road, Colombo 08, Sri Lanka. sumudu@pdt.cmb.ac.lk

Abstract

The prevalence of obesity and type 2 diabetes mellitus has increased rapidly over the past few decades, and prevention efforts have not been successful. Fetal programming involves the earliest stage of obesity development, and provides a novel concept to complement other strategies for lifelong prevention of obesity and type 2 diabetes mellitus. The World Health Organization now advocates a life-course approach to prevent/control obesity, starting with pre-conceptional and antenatal maternal health. Maternal overnutrition, gestational diabetes mellitus and excessive gestational weight gain lead to fetal overgrowth, and "programs" the offspring with an increased risk of obesity and type 2 diabetes mellitus in childhood and adulthood. This review summarizes current data on fetal programming of obesity and type 2 diabetes mellitus including potential causative factors, mechanisms and interventions to reduce its impact.

Key Words: Developmental programming; Metabolic syndrome; Intergenerational obesity cycle; Insulin resistance; *In utero* environment

Core Tip: Fetal programming targets the earliest stages in the development of obesity and type 2 diabetes. It provides a novel paradigm to complement other strategies for lifelong prevention of obesity and type 2 diabetes. Maternal undernutrition/overnutrition, maternal diabetes, excessive gestational weight gain and certain paternal factors are now recognized as factors associated with adverse fetal programming of obesity and type 2 diabetes in the offspring. This review provides up-to date evidence on fetal programming of obesity and type 2 diabetes including potential causative factors and mechanisms as well as potential interventions to minimize its impact on future generations.

Citation: Seneviratne SN, Rajindrajith S. Fetal programming of obesity and type 2 diabetes. *World J Diabetes* 2022; 13(7): 482-497
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/482.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.482

INTRODUCTION

Obesity and type 2 diabetes mellitus rates are rising globally. Obesity is the commonest form of malnutrition in the developed world, and is rapidly increasing in developing countries[1-3]. Obesity is strongly associated with insulin resistance and the development of type 2 diabetes. By 2050, it is predicted that half a billion men, women, and children will have type 2 diabetes, of whom three quarters will be from low and middle income countries (LMIC)[4]. Diabetes and its complications including kidney disease, heart disease, stroke, retinopathy and neuropathy, lead to premature mortality, morbidity, disability and reduced quality of life in affected individuals. At present, someone dies due to diabetes-related complications every 7 s[5]. Furthermore, it also leads to decreased work-force productivity, increased healthcare utilization and escalating healthcare costs[6]. Ten percent of the global health expenditure is spent on diabetes-related care[4].

Obesity is the main driver of type 2 diabetes. Obesity refers to the excess accumulation of body fat to an extent that it is harmful to an individual's health. The fundamental cause of obesity is an imbalance between energy intake and expenditure, with excess energy being stored as fat in adipose tissue. This predisposes adipocytes to secrete more pro-inflammatory adipocytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), causing a state of low-grade inflammation and insulin resistance. An increase in insulin resistance necessitates a compensatory increase in insulin secretion from pancreatic β cells, and failure to achieve this demand results in diabetes. This, however, is a gradual process, and it may take years for diabetes to manifest clinically. Thus, early identification and modification of risk factors at the onset of the above trajectory could help prevent type 2 diabetes[5].

The etiology of obesity and type 2 diabetes is multifactorial and involves complex interactions between genetic, environmental and behavioral factors[3,7]. The rapid rise in obesity is mostly attributed to the unhealthy lifestyle associated with urbanization and technical advancement over the last three to four decades[8]. The present generations live within an obesogenic environment, with energy imbalance arising from excessive energy intake due to high fat, high-sugar, energy-dense processed foods, and a reduction in occupational, household and leisure-time physical activity[3,7,9]. However, there is evidence of an additional factor leading to increases in obesity and type 2 diabetes. This is the impact of the prenatal and early-life environment on long-term health *via* fetal programming.

The Developmental Origins of Health and Disease (DOHaD) concept states that early-life environmental influences at sensitive periods of development lead to lifelong effects on health and chronic disease risk[10]. There is evidence that exposure to an abnormal *in utero* environment disturbs the metabolic programming of the growing fetus, increasing the lifelong risk of chronic diseases including type 2 diabetes[11-15] This process is described as fetal or developmental programming[16]. Fetal programming is now recognized as a key factor contributing to the rapid rise in obesity and type 2 diabetes mellitus rates worldwide. Research in humans and animals over the past two decades has provided considerable evidence supporting 'developmental programming' by the intrauterine environment[17].

Fetal programming helps explain certain aspects of the obesity epidemic that cannot be fully explained by genetic and environmental factors. The relatively short time over which obesity and type 2 rates have escalated precludes genetic change as a major attributor[15,18]. Furthermore, energy homeostasis and body weight are regulated by biological systems established in early life. Thus, it is difficult to explain how lifestyle changes alone, can override these biological homeostatic mechanisms to bring about obesity[15,18]. Fetal programming is the most plausible reason for this phenomenon. Dysregulation of biological mechanisms maintaining body weight by early life fetal programming also helps explain why reversal of obesity is difficult[19].

FETAL PROGRAMMING OF OBESITY AND TYPE 2 DIABETES

Epidemiological, clinical, and basic sciences research suggest that the foundations of an individual's lifelong health, including predisposition towards obesity and type 2 diabetes is largely established during the 'first 1000 days of life' from day of conception to completion of the 2nd year of life. This is a highly sensitive period of growth and development in humans, where biological systems are formed and developed[10,20,21].

It is difficult to separate out effects of *in utero* exposure from genetic and nurturing influences in humans. However, studies in small mammals and other animal models have shown that prenatal

exposure to an adverse *in utero* environment associated with maternal overnutrition results in developmental programming of obesity and other disorders in offspring[22-25]. For example, in genetically-modified obesity-prone rats, greater postnatal adiposity was observed in offspring born to over-nourished dams, compared to normally nourished dams[22]. Furthermore, offspring of over-nourished dams developed greater body weight and body fat compared to offspring of lean dams, even when both groups were fostered by lean dams after birth[23]. These studies indicate that *in utero* exposure to maternal obesity *per se* increases susceptibility to obesity in later life, beyond genetics or nurturing practices.

The prenatal environment in humans appears to be influenced by maternal body composition, metabolism, stress and diet from conception and throughout pregnancy. Paternal influences are also being recognized. Thus, parental lifestyle appears to influence the health of the offspring prior to birth, *via* fetal programming. From the maturation of gametes through to early embryonic development, parental lifestyle can adversely influence long-term risks of offspring metabolic, cardiovascular, immune, and neurological morbidities[26].

FACTORS PREDISPOSING TO DEVELOPMENTAL PROGRAMMING OF OBESITY/TYPE 2 DIABETES AND POTENTIAL MECHANISTIC PATHWAYS

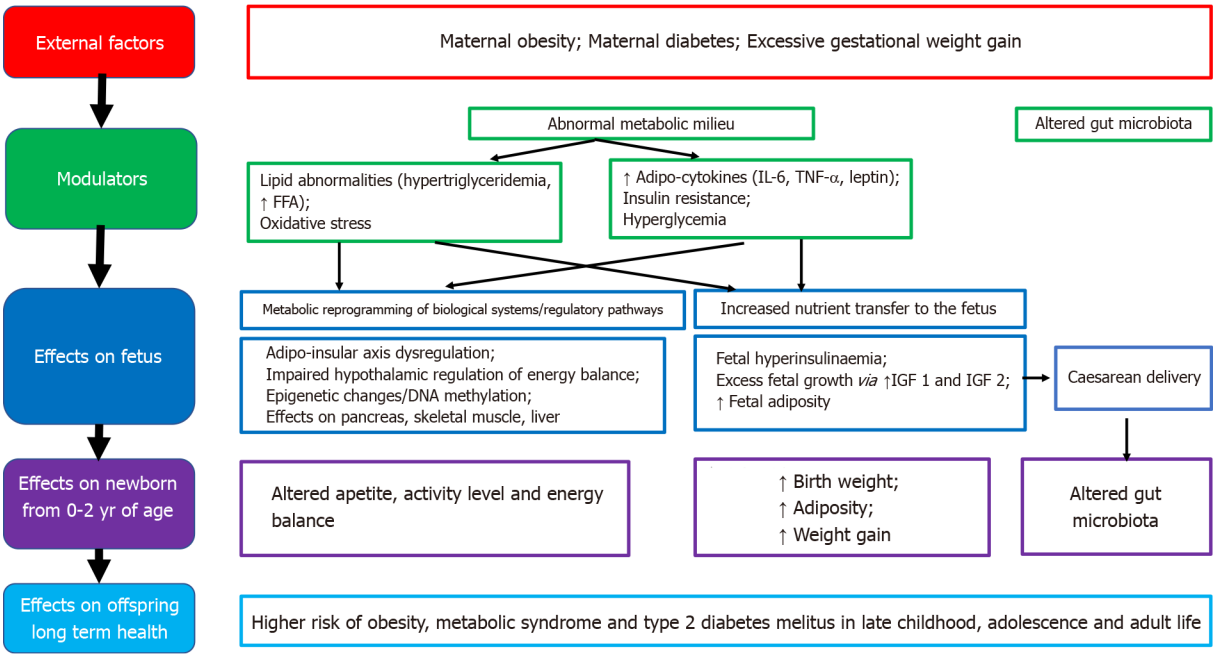
The field of developmental programming has begun to move beyond associations to potential causal mechanisms for developmental programming. Studies in humans and animal models are helping unravel underlying biological mechanisms underpinning fetal programming, including epigenetic, cellular, physiological, and metabolic processes[26]. We have however, still not gained a complete understanding of the complex ways in which the maternal genome, metabolome, and microbiome relate throughout pregnancy and lactation to increase the offspring's disease risk across the life span[25]. Determining mechanisms of fetal programming has been complicated by rapid changes in the social environment and human behavior. Thus, more studies are needed to help better delineate the pathophysiological mechanisms underpinning fetal programming[25].

Epigenetics, and mechanisms of epigenetic modification have led to increased understanding of developmental programming, and how environmental, genetic and epigenetic factors inter-relate to cause lasting effects on offspring size, adiposity and future metabolic outcomes. Neonatal methylation markers associated with birth weight from several gene loci, have shown significant associations with the prenatal environment, as well as longitudinal associations with offspring size and/or adiposity in early childhood, providing evidence that developmental pathways to adiposity begin before birth and are influenced by environmental, genetic and epigenetic factors[16]. Disruption of the gut microbiome observed in maternal obesity, antibiotic use in pregnancy, delivery and early infancy, and cesarean section have also been implicated in increased childhood obesity risk. Disruption of microbiome colonization during critical periods of early development can predispose offspring to obesity, asthma, allergy and diabetes. This may occur due to cesarean delivery, and the use of prophylactic antibiotics during cesarean section, as well as maternal exposure to antibiotics in the second and third trimesters of pregnancy, and use of antibiotics in the offspring in early infancy. Furthermore, increased maternal body mass index (BMI) *per se* is associated with altered intestinal microbial community structure of infants' stool up to 2 years of age[25]. Future research in epigenetics and the gut microbiome could yield greater insights into the mechanistic pathways as well as potential methods of modulating fetal programming.

Good maternal nutrition prior to and during pregnancy is important for optimizing offspring long-term health. Fetal programming, initially described in relation to fetal undernutrition, was associated with a higher risk of central obesity, diabetes, hypertension, coronary heart disease and stroke in adult life[12]. Fetal growth is influenced by the *in utero* environment, and there is trouble at both ends of the birthweight spectrum, with a 'J' shaped relationship between birth weight and future obesity risk[27]. It is proposed that the fetus 'senses' its future nutritional status *via in utero* signals from the mother, and responds in ways which establish lasting influences on weight and appetite control[28]. Many umbilical cord blood metabolites and hormones are associated with birth weight and adiposity in human infants [25]. Paradoxically, both a nutritionally limited or nutritionally excessive *in utero* environment can lead to later obesity and associated co-morbidities[29]. More recent evidence emphasizes the adverse developmental programming effects of fetal overnutrition, and its association with increased risk of obesity in childhood and adulthood[29,30]. In addition, paternal factors are also now being recognized to play a role in fetal programming. The effects of maternal overnutrition, maternal undernutrition/stress and paternal factors on fetal programming of obesity/type 2 diabetes including potential modulatory pathways and effects on the offspring are shown in Figures 1-3.

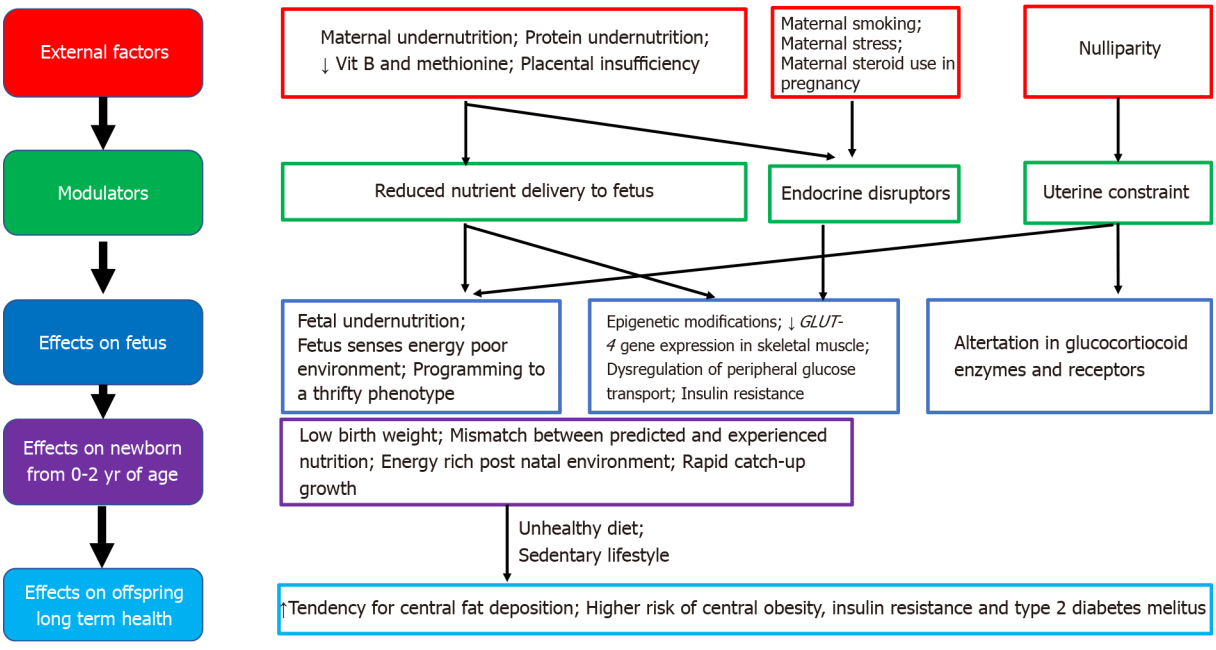
Maternal overnutrition

Concurrent with the global epidemic of obesity, the prevalence of overweight and obesity in women of reproductive age has risen rapidly over the last three decades[7,31,32]. There is now compelling evidence from human as well as animal studies that maternal obesity, diabetes and increased gestational



DOI: 10.4239/wjd.v13.i7.482

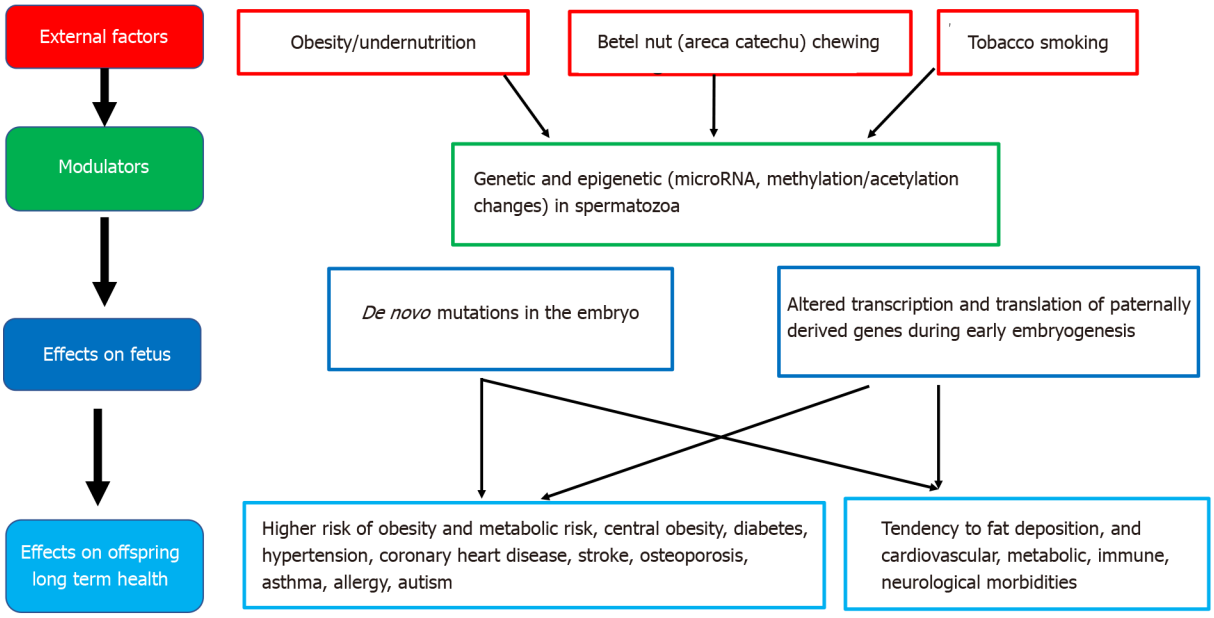
Figure 1 Associations between maternal overnutrition and fetal programming of obesity/type 2 diabetes mellitus including potential modulating factors and effects on offspring health. FFA: Free fatty acid; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin-6; IGF: Insulin-like growth factor.



DOI: 10.4239/wjd.v13.i7.482

Figure 2 Associations between maternal undernutrition and fetal programming of obesity/type 2 diabetes mellitus including potential modulating factors and effects on offspring health.

weight gain all increase offspring birth weight and lead to fetal programming of obesity in the offspring [33]. It is thought that offspring obesity is programmed by the 'obesogenic' maternal metabolic environment the fetus is exposed to *in utero* during development, setting in an 'obesity cycle', where maternal obesity leads to neonatal obesity which continues to childhood and adulthood, propagating obesity in the next generation[34,35]. Thus, an increase in overweight and obesity among women of reproductive age should be considered an important modulator of the global obesity epidemic, which is further propagating obesity in future generations.



DOI: 10.4239/wjd.v13.i7.482

Figure 3 Associations between paternal health factors and fetal programming of obesity/type 2 diabetes mellitus including potential modulating factors and effects on offspring health.

Children born to overweight/obese women have increased birth weight and an increased risk of obesity and metabolic dysregulation throughout life[34,36-38]. At birth, these babies have increased birth weight and adiposity[39], and thus, an increased risk of assisted delivery as well[40,41]. Exposure to maternal obesity and diabetes accelerates adipogenesis and impairs energy sensing, affecting neurodevelopment, liver, pancreas, and skeletal muscle development in the offspring, creating a lifelong impact on multiple systems[25]. The influence of maternal obesity on the risk of offspring obesity starts manifesting from early life[36,42]. These children show increased weight for age and length, in comparison to offspring of normal weight women, as early as six months of age[42], and their risk of obesity is increased two-fold as preschoolers, even after controlling for birth weight and other confounding factors[36]. They also have an increased risk of metabolic syndrome by late childhood[43]. Furthermore, high maternal BMI in pregnancy is an independent predictor of obesity in the adult offspring, at 30 years of age[37].

If the mother is obese during pregnancy, there is excess transfer of nutrients to the fetus, stimulating increased fetal insulin secretion, fetal overgrowth and increased adiposity. It is hypothesized that this tendency for fat accrual then tends to persist during childhood and adulthood. Furthermore, the metabolic milieu of overweight/obese mothers differs from normal weight mothers, with obese pregnancy being associated with higher insulin resistance, pro-inflammatory adipokines (leptin, IL-6, TNF-α) and lipid abnormalities. *In utero* exposure to this abnormal metabolic milieu is also implicated in fetal programming[31,35,44]. Factors associated with developmental programming of obesity in offspring of mothers who have obesity/diabetes in pregnancy include high glucose levels, triglycerides, free fatty acids, adiponectin, leptin, hypoxia, oxidative stress, inflammation, and the microbiome[25]. It is proposed that fetal exposure to this abnormal metabolic milieu leads to dysregulation of the offspring adipo-insular axis (leptin and insulin) causing alterations in the central nervous system regulation of appetite, activity level, energy balance and in adipocyte metabolism[14,34,44,45].

Maternal diabetes

Maternal diabetes during pregnancy is also strongly associated with fetal programming of obesity in the offspring. At present, approximately 20 million live births are affected by hyperglycemia in pregnancy, globally[4]. There is evidence that offspring of mothers with gestational diabetes mellitus have an increased risk of developing obesity, insulin resistance, type 2 diabetes, hypertension and cardiovascular complications at a relatively young age[46]. In the follow-up of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, 4160 children aged between 10-14 years, whose mothers had a 75-g oral glucose tolerance test at 28 wk of gestation demonstrated that exposure to higher maternal glucose spectrum levels *in utero* was significantly associated with childhood glucose levels and insulin resistance, independent of maternal and childhood BMI and family history of diabetes[47]. Studies have also shown associations between the sex of the fetus and maternal blood glucose concentrations during pregnancy, suggesting that fetal programming could be influenced by offspring gender as well[48,49].

Maternal undernutrition

Maternal undernutrition during critical periods of fetal development has been linked with fetal programming of central obesity, insulin resistance, metabolic syndrome and type 2 diabetes in later life, especially when exposed to an energy-rich diet postnatally. Adipogenesis, which begins *in utero* and accelerates in neonatal life, is a major candidate for developmental programming. According to the thrifty phenotype hypothesis, maternal undernutrition during critical periods leads to compensatory changes in the fetus, including tendency to store fat, which causes central obesity in later life, when there is a mismatch between the predicted and experienced postnatal nutritional environment[50,51].

Epigenetic pathways in fetal programming from *in utero* undernutrition described include histone modifications in skeletal muscle that directly decrease *GLUT-4* gene expression, which leads to metabolic dysregulation of peripheral glucose transport and insulin resistance, which can contribute to the development of type 2 diabetes in later life. These fetal programming changes, combined with the effects of obesity, ageing and physical inactivity, are the most important factors in determining type 2 diabetes in those born with low birthweight[51]. Furthermore, specific maternal nutrient deficiencies during pregnancy, including low maternal protein consumption, and poor vitamin B and methionine status are also associated with an increased risk of metabolic derangements and type 2 diabetes in later life. Evidence from animal studies show that a protein-restricted diet *in utero* programs susceptibility to obesity, when exposed to overnutrition in postnatal life[50,52].

Prenatal stress could also be a modulating factor for fetal programming of obesity in severe maternal malnutrition[53]. During fetal development, the hypothalamic-pituitary-adrenal axis is extremely susceptible to programming, and alterations in the expression and function of glucocorticoid receptors and major glucocorticoid regulatory enzymes are observed in those exposed to undernourishment in early life[54]. Other factors associated with fetal programming include maternal exposure to endocrine disruptors, maternal infection and smoking and nulliparity[17,55]. Nulliparity is potentially associated with subtle adverse metabolic outcomes in overweight/obese mothers and their offspring, through uterine constraint effects[55].

Paternal factors

Epidemiological and animal studies suggest that many factors, including paternal under- and over-nutrition, exposure to environmental toxins, father's health conditions such as diabetes, and even grandfather's nutritional status can program diseases in the offspring *via* germ cell-mediated transmission[56]. High paternal BMI has been linked with newborn adiposity[57]. Furthermore, paternal overweight/obesity appears to induces paternal programming of offspring phenotypes, through genetic and epigenetic changes in spermatozoa. Both human and rodent models have established that paternal obesity impairs sex hormones, basic sperm function, and molecular composition, which can result in perturbed embryo development and increase subsequent offspring disease burden[57]. Theories for the origin of male obesity-induced paternal programming include the accumulation of sperm DNA damage resulting in de novo mutations in the embryo and changes in sperm epigenetic marks (microRNA, methylation, or acetylation) altering the access, transcription, and translation of paternally derived genes during early embryogenesis[57].

Postnatal factors

In keeping with the concept of "the first 1000 days of life", postnatal factors from the time of birth to the second birthday of a child, could also contribute towards adverse programming increasing the risk of obesity and type 2 diabetes in later life. Our present state of knowledge includes mainly early life nutritional practices, including breastfeeding duration, timing of introducing complementary feeding, and protein rich foods[58]. The underlying mechanisms are yet unclear, but there is emerging evidence that it is associated with altered neuro-endocrine programming, and modified by breastfeeding duration and maternal pre-pregnancy overweight[58,59]. Breastfeeding including longer duration of exclusive breastfeeding and longer duration of partial breastfeeding have been associated with a reduced risk of later life obesity and obesity-related complications. Breastfeeding for greater than 40 wk has been associated with lower weight gain by 1 year, and longer duration of breastfeeding with lower odds of developing hypercholesterolemia, hypertension, obesity and type 2 diabetes in later life[60]. Furthermore, mothers who are overweight and obese appear to breastfeed their babies for a shorter duration and introduce complementary foods earlier than mothers of normal weight, which could play a role in their offspring having increased weight and BMI from early childhood[59]. Exclusive breast-feeding for 6 mo or longer, and delaying the introduction of complementary feeding until 5th month of age, are also associated with lower risk of overweight at 5-6 years of age[61]. In addition, social factors including poor nurturing practices and role modeling by parents, early introduction of highly processed high fat, high sugar snacks/meals and exposure to unhealthy food advertising, are early life factors associated with increased offspring obesity.

PREVENTION OF FETAL PROGRAMMING

Primary and secondary prevention of obesity are at the foundation of diabetes prevention programs. While several medical and lifestyle strategies have shown promising effects in slowing progression to and minimizing complications of type 2 diabetes, implementing community measures to prevent obesity/type 2 diabetes are bound to be more cost-effective and beneficial to the community at large, compared to the cost of screening, treating and managing complications of established obesity/type 2 diabetes.

There is now increasing focus on primary prevention of obesity/type 2 diabetes targeting the first 1000-d of life[5]. The first 1000-d of life offers a unique and critical window of opportunity to shape long-term health at the population level, which can have a lasting effect on a country's health and prosperity. Firstly, however, it is prudent to consider the important fundamental question of whether fetal programming of obesity can be minimized by interventions which improve the *in utero* environment of the fetus, in humans. The most promising research findings on preventing adverse fetal programming have come from animal models under experimental conditions. Whether these interventions could be applied in clinical practice, and their effectiveness remain uncertain. However, there are emerging data that improvement in fetal overnutrition and risk of obesity can be achieved *via* maternal interventions. Perhaps the best evidence available to date, is improvement in long-term health outcomes observed in offspring born to severely obese women, after maternal weight loss following bariatric surgery[62]. Studies comparing offspring pairs born to morbidly obese women conceived before and after substantial weight loss following bariatric surgery found that children conceived after surgery had a lower risk of macrosomia (birth weight > 4 kg) at birth, and continued to have better health outcomes in childhood and adolescence including a 50% lower risk of obesity, three-fold lower risk of severe obesity, and better insulin sensitivity and lipid profile, compared to their older siblings[62-64]. These findings confirm that pre-conception weight loss in severely obese mothers can lower fetal overnutrition and reduce the risk of obesity and metabolic complications in the offspring. However, weight reduction by bariatric surgery prior to conception is not an easily available or feasible option for most overweight and obese women of reproductive age, making it necessary to consider alternative interventions which could potentially improve offspring health outcomes.

The World Health Organization, having recognized and acknowledged the potential impact of fetal programming on the obesity epidemic, now advocates a life-course approach for the prevention and control of non-communicable diseases including obesity. This life cycle approach starts with maternal health including preconception, antenatal and postnatal care, and maternal nutrition[65,66]. Potential measures that can be taken at various stages of the life cycle to reduce adverse effects of fetal programming of obesity and type 2 diabetes in future generations are shown in Figure 4.

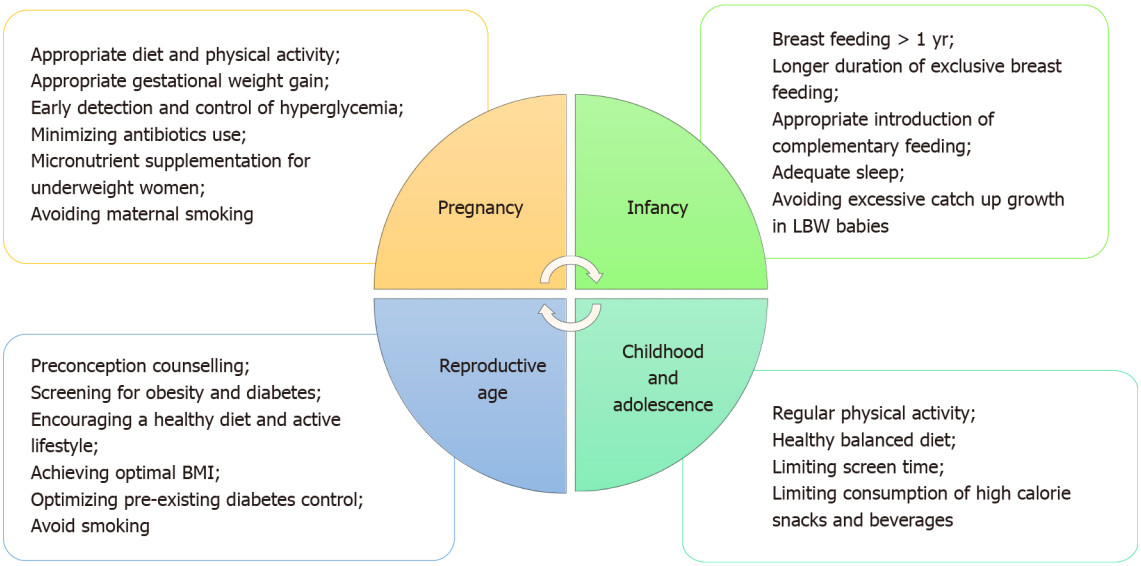
Lifestyle interventions during pregnancy

When considering the impact of intrauterine overnutrition and macrosomia on obesity risk in the next generation, public health measures for healthy maternal weight throughout the reproductive years is justified. Ideally body weight should be optimized to a healthy BMI in all women planning a pregnancy, but this is easier said than done. Pregnancy itself, however, can be an opportune period to commence healthy lifestyle changes, if heath care providers consider it as a "teachable moment" to educate pregnant women on the potential benefits to the baby as well as the mother, and utilize regular and frequent contact with heath care services during this time to provide encouragement and guidance to institute lifestyle interventions[67].

Lifestyle interventions during pregnancy and postpartum appear to reduce gestational weight gain, pregnancy-induced hypertension, need for cesarean section and neonatal respiratory distress syndrome, without any risk of harm to the mother or neonate, across all maternal BMI categories[68]. Thus, a healthy diet and regular exercise for all healthy women during pregnancy and postpartum is a low-cost and feasible intervention which has been advocated as a global health policy[68,69].

Antenatal lifestyle intervention in maternal obesity

Given the rising rates of obesity in women of reproductive age, first in developed, and subsequently in developing countries over the past few decades, there is an urgent need for effective interventions to reduce adverse fetal programming due to maternal obesity[70]. There is expert consensus, that antenatal lifestyle interventions in overweight and obese pregnant women could alter adverse fetal programming and improve offspring health[71,72]. It is postulated that modifying the obesogenic *in utero* environment by lifestyle changes such as increased antenatal physical activity or improved dietary intake during pregnancy could reduce harmful programming effects in the offspring[72]. Antenatal nutritional/lifestyle interventions in overweight/obese pregnant women could potentially be effective by preventing excessive maternal gestational weight gain, and by reducing the risk of developing gestational diabetes, and improving the unhealthy maternal metabolic milieu (insulin resistance, hyperinsulinemia, hyperglycemia, hyperlipidemia, and increased inflammatory markers) which lead to adverse fetal programming[73,74].



DOI: 10.4239/wjd.v13.i7.482

Figure 4 Potential measures that can be taken at various stages of the lifecycle to reduce adverse effects of fetal programming of obesity and type 2 diabetes mellitus in future generations. LBW: Low birth weight; BMI: Body mass index.

Several studies have investigated if lifestyle interventions in overweight and obese mothers during pregnancy can attenuate offspring programming of obesity. In overweight/obese women, multi-component interventions with both a diet and physical activity component have shown some promise, with reduction in gestational weight gain, pregnancy-induced hypertension, macrosomia and neonatal respiratory distress syndrome[68]. Diet was associated with greater reductions in the risk of gestational diabetes mellitus, pregnancy-induced hypertension and preterm birth, compared with any other intervention[68]. However, the effects of these interventions on long-term offspring health are unclear. Studies such as the LIMIT study in Australia reported that providing pregnant women who were overweight or obese with an antenatal dietary and lifestyle intervention improved maternal diet and physical activity during pregnancy, but did not alter 6-mo infant growth and adiposity, or childhood dietary intake, growth and adiposity at 3-5 years of age.

Prenatal exercise has been considered a potential intervention to reduce adverse fetal programming, especially in pregnancies complicated by obesity and/or diabetes[70,75]. Previously, there were concerns regarding the safety of exercise during pregnancy, due to fears regarding teratogenicity from exercise-induced hyperthermia, and fetal hypoxia and intrauterine growth retardation from redistribution of blood flow and nutrients away from the utero-placental circulation during exercise[76]. However, studies on maternal antenatal exercise over the past 20 years have demonstrated that mild-to-moderate intensity antenatal exercise in healthy well-nourished women does not cause observable harm to the fetus[77-80]. There has since been a gradual change of opinion that moderate antenatal exercise is not only safe, but may also be beneficial to offspring health[71,78]. Detailed small scale studies have shown that offspring of physically active lean women who engaged in regular vigorous exercise during pregnancy had lower birthweight and subcutaneous fat at birth, and continued to have lower weight and subcutaneous fat in childhood[81].

However, the effects of antenatal exercise during pregnancy on offspring health appear to vary depending on exercise intensity and frequency, as well as its timing in relation to the period of gestation [82-85]. Commencing exercise in early pregnancy appeared to stimulate feto-placental growth, and increase birth weight, while exercising in the second half of pregnancy appeared to reduce birth weight [83,86]. Furthermore, while it is postulated that regular antenatal exercise during the second half of pregnancy may lead to a reduction in birth weight and adiposity in the offspring, which may be protective against obesity in later life[87], it does not appear to be effective in practice, especially in overweight and obese women[88]. The results of clinical trials targeting antenatal exercise in overweight and obese women have led to varying/inconclusive findings on birthweight and other markers of fetal programming[89,90]. Many trials on supervised antenatal exercise interventions in overweight and obese women have reported a lack of effect on birth weight, or other markers of fetal programming[88, 89,91,92]. One explanation for this could be that obese women, who are generally less physically active, tend to further reduce activity levels during pregnancy[70].

Thus, at present, lifestyle interventions during pregnancy in women with obesity/diabetes have not shown much effect on infant or childhood outcomes. However, many such clinical trials started later in pregnancy, and it is possible that developmental programming occurs much earlier and interventions focusing on healthy lifestyle interventions in pregnant humans are missing the crucial time period for

effectiveness[25].

Interventions for pregnancies complicated by gestational diabetes/pre-existing maternal diabetes

In woman with diabetes in pregnancy, tight glycemic control will help minimize adverse fetal programming of obesity and diabetes in the offspring. For women with both pre-existing and gestational diabetes, offspring outcomes can be optimized by ensuring appropriate gestational weight gain, and optimal glycemic control *via* close monitoring of blood glucose levels, and appropriate medical and nutritional therapy and exercise, throughout the pregnancy[93].

For women with pre-existing type 2 diabetes, insulin has long been considered the gold standard managing diabetes during pregnancy[93]. Careful blood glucose monitoring and titration of insulin doses are important as total daily insulin requirement increases linearly with advancing pregnancy[93]. For women with pre-existing diabetes, it is also important to provide preconception counseling, to achieve optimal pre-conceptional body weight and glycemic control, prior to pregnancy whenever possible. The onus is on health care providers to educate and counsel women with diabetes, particularly on the importance of these aspects not only for their own health status but also to protect their unborn baby from the risks of fetal programming.

For women with gestational diabetes, both insulin and metformin can be used to maintain blood glucose levels if lifestyle interventions are inadequate to achieve adequate glycemic control. Furthermore, for women with a previous history of gestational diabetes, post-partum weight reduction prior to pregnancy could potentially help reduce gestational diabetes mellitus and its associated complications in subsequent pregnancies[94]. The MiG TOFU study, a prospective longitudinal follow-up study in Australia and New Zealand which randomized pregnant women with gestational diabetes mellitus to either metformin or insulin therapy, found that mothers on metformin, had higher glycemia in pregnancy and higher rates of babies with birth weight > 90th percentile, compared to those on insulin therapy, while offspring had similar adiposity at 2 years of age, and similar total and abdominal percentage of body fat and metabolic measures at 7-9 years of age[95].

Interventions for maternal undernutrition

Due to a paucity of evidence from long-term follow-up studies, current recommendations to reduce adverse fetal programming effects of maternal undernutrition, presume that interventions helping to optimize pregnancy outcomes and promote healthy infant growth and development will also help improve the long-term risk of chronic diseases such as central obesity and type 2 diabetes. These recommendations include optimizing maternal nutrition prior to pregnancy, ensuring adequate micronutrient intake in the preconception period and throughout pregnancy before birth, and encouraging breastfeeding and high quality complementary foods to the offspring after birth[96]. Maternal multiple micronutrient supplementation including vitamin and mineral supplementation during the preconception period and early pregnancy have shown some benefit in reducing fetal undernutrition and other adverse fetal programming effects in undernourished mothers[21].

Balanced protein-energy supplementation also appears to be an effective intervention to reduce the prevalence of low birthweight and small-for-gestational-age births, especially in undernourished women[97]. Thus, ensuring appropriate and adequate intake of micronutrients, essential fats and protein supplementation in mothers with undernutrition during pregnancy, could improve the nutritional condition of the mother, and confer a protective benefit to the offspring by reducing fetal growth restriction and low birth weight in developing countries with high rates of maternal undernutrition[96].

Interventions in offspring in infancy and childhood

Intervention strategies to reduce adverse effects of fetal programming of later life obesity may be more effective if they target multiple modifiable factors, focusing on the first 1000-d of life.

Breastfeeding appears to protect against obesity in childhood, and could be a modifying factor to mitigate the adverse effects of fetal programming *in utero*. Promoting longer duration of full breastfeeding and partial breastfeeding, and delaying the introduction of complementary feeding could protect the offspring from obesity. Exclusive breastfeeding for 6 mo or longer, and delaying the introduction of complementary feeding until 5th month of age, has been associated with a lower risk of overweight at 5-6 years of age[61]. The protective effects of breastfeeding on the offspring of diabetic mothers in very early life appears somewhat conflicting, with one study suggesting a potential negative long-term influence on the risk of becoming overweight in offspring exposed to breast milk from mothers with diabetes (type 1 or gestational diabetes) during the first week of life[60]. However, overall, the benefits of breastfeeding appear to be beneficial, and protect infants from the adverse effects of fetal programming of obesity and type 2 diabetes. Women who were overweight or obese before pregnancy, appear to breastfeed their offspring for a shorter time and introduce complementary feeding earlier than normal weight women, which could contribute towards their children being heavier and having a higher BMI by end of infancy[59]. Thus, it is especially important to take measures to encourage and support longer duration of breastfeeding in women who are overweight or obese.

Further protective measures that could be helpful in optimizing long-term health during infancy include ensuring adequate sleep and minimizing antibiotic use. Early antibiotic use before 2 years of age has been associated with disruption of the gut microbiota, and a higher risk of childhood overweight and obesity[98]. Recent evidence on the associations with gut microbiota and infant weight gain or child weight status, suggest that dietary manipulation with human milk and pre/probiotic formulations holds promise for preventing obesity[99].

Furthermore, as short sleep duration increased the risk of childhood obesity, public health efforts that encourage children to have sufficient sleep time are also important in combating obesity[100]. Project Viva prospectively studied the cumulative number of modifiable early-life risk factors associated with programming of obesity/type 2 diabetes in mother-offspring pairs including: maternal smoking and consumption of high sugar-sweetened beverages during pregnancy, excessive gestational weight gain; breastfeeding for less than 1 year; complementary food introduction before 4 mo; and infant sleep duration less than 12 h daily. When reassessed in early adolescence, they found that offspring with 5-6 risk factors had a 2.5 higher rate of obesity and metabolic syndrome, compared to those with 0-1 risk factors[101]. Thus, it appears that promoting exclusive breastfeeding for at least the first 4 mo of life, and continuation of breastfeeding beyond the first year of life, as well as ensuring adequate sleep for infants, could potentially reduce the risk of further life obesity in infants who have already been exposed to risk factors for adverse fetal programming *in utero*.

Other potential strategies to reduce the adverse impact of fetal programming include identifying and targeting young children at higher risk of fetal programming of obesity/diabetes such as offspring of mothers with obesity/diabetes/undernutrition during pregnancy, especially those being reared in highly urbanized obesogenic environments, for healthy lifestyle interventions during early childhood to encourage them towards a healthier lifestyle, and prevent adverse metabolic health outcomes in later life[46]. Pairing breastfeeding with healthy weaning foods is likely to promote healthy weight trajectories.

A recent review reported that several multicomponent trials promoting breastfeeding, responsive feeding, and a healthy diet (increased fruit and vegetables, and limiting sugar sweetened beverages and unhealthy snacks) through home visits or education at baby health clinics over 1-2 years duration, showed relative reductions in BMI in offspring at the end of the intervention, although early benefits were not maintained in the two trials reporting follow-up 1 year to 3 years later[102]. Thus, there is some evidence that nutrition or feeding interventions in the first two years of life can have a positive impact on a child's BMI, but maintaining this benefit may require continued intervention and sustainable environmental change[102].

Observational studies suggest that rapid weight gain in infancy also increases the long-term risk of obesity and type 2 diabetes in infants from both low-and high-income countries, among infants born preterm or at term, with normal or low birth weight for gestation[103]. Furthermore, it has been hypothesized that the increased risk of adverse long-term outcomes including central obesity and type 2 diabetes in low birth weight infants may be driven by accelerated postnatal catch-up growth. While some studies on health outcomes in babies with low birth weight have reported that increased catch up growth was associated with higher BMI or higher serum cholesterol levels in early adolescence, the quality and quantity of the evidence is limited[102]. Thus, it is prudent to recommend "striking a healthy balance", especially for low and middle income countries, until more information on underlying mechanisms and suitable interventions on minimizing adverse effects of catch up growth in low birth weight infants become available[104].

Beyond infancy, promising interventional approaches for pre-school age children include age appropriate health and nutrition education for preschoolers, combined with teaching parents behavioral change strategies and increasing parenting skills[105]. For school children, school-based interventions have been shown to be effective in reducing excessive weight gain in children[106]. Programs involving both school and family and lasting ≤ 1 year were the most efficacious for primary school children aged between 6 and 12 years; while family-based interventions have been effective in children < 6 years old [107].

Preconception care

Healthy lifestyle behaviors during the preconception period are important to optimize maternal and child outcomes. Community nurses and midwifery professions who are active across both preconception and pregnancy could play an important role in such interventions[108]. Many women of reproductive age do not appear to have optimal preconception lifestyle behaviors, and a recent systematic review identified the absence of knowledge on healthy behaviors as the most common barrier[109]. The need for further studies on how to best improve preconception women's capability, opportunity, and motivation to modify their lifestyle behaviors has been emphasized[109]. At present, there is a lack of international consensus guidelines on weight management preconception, and its impact on fertility, pregnancy and subsequent maternal and infant outcomes.

The reversibility of obesity-induced parental programming has only recently received attention. These programmed changes to offspring health may be partially restored *via* diet/exercise interventions in obese fathers, prior to conception, *via* improvement in sperm DNA integrity. Promising results in animal models utilizing diet and exercise interventions have shown improvements in sperm function

and molecular composition, resulting in restorations of both embryo and fetal health and subsequent male offspring fertility[57]. However, it is noteworthy, that most data surrounding paternal obesity and offspring phenotypes have come from rodent models, and implications for clinical practice warrants further research[57].

CHALLENGES AND THE WAY FORWARD

Maintaining a healthy maternal BMI and lifestyle from preconception and throughout pregnancy will help minimize the risk of future obesity in the offspring. A balanced diet with low glycemic load, and light-to-moderate intensity physical activity for 30-60 min daily, at least for 3-5 d per week is recommended[68]. Maternal pre-pregnancy and early pregnancy metabolic conditions often programs early placental function and gene expression in the first trimester of pregnancy, prior to when most intervention trials are initiated[110]. Interventions commenced during pregnancy have met with limited success in preventing adverse fetal programming effects. This could be because most interventions were instituted after the first trimester, where it may have been too late to have a positive impact on fetus programming.

Given the widespread and long-lasting impact of adverse fetal programming, a population-based life-course approach is warranted, until more focused and specific ways to prevent adverse fetal programming are discovered. As the evidence on the peri-conceptional environment on offspring long-term health is compelling, updated guidelines and guidance for parental preparation for pregnancy, prior to conception to protect the health of offspring is required[26,110]. This should be followed by proper guidance for parents regarding appropriate nutrition, physical activity, and screen time in early childhood. Furthermore, school based interventions with family involvement could be effective in improving dietary habits and lifestyle in primary school children[111]. Whole community interventions addressing both policy and behavior change are needed[112]. Wider dissemination of health messages advocating healthy lifestyle as a means of providing a better chance of a healthier life for future generations is recommended[112].

CONCLUSION

Fetal programming is an important contributor to the global obesity epidemic. Risk factors for adverse fetal programming include maternal obesity, diabetes, undernutrition, smoking, stress, operative delivery and use of antibiotics in pregnancy, as well as paternal factors including over/undernutrition. These factors lead to fetal programming *via* multiple complex pathways including alterations in organ formation and homeostatic pathways, epigenetic changes, and changes in gut microbiota. Specific mechanistic pathways are still being unraveled. Using this knowledge to find effective and feasible methods of preventing adverse fetal programming is an issue of global importance. The current state of knowledge dictates that future research should be directed towards earlier interventions starting in the pre-conceptional period. Until such time, a multi-pronged life-course approach, focusing on maternal health, antenatal and postnatal care, as well as healthy lifestyle interventions for preschoolers, school children, and young adults of reproductive age is advocated.

ACKNOWLEDGEMENTS

We wish to acknowledge Dr. Jayakody C and Dr. Hewakuruppu S for their assistance with development of the figures for this manuscript. We are grateful to Dr. Perera BJC, Joint Editor of The Sri Lanka Journal of Child Health, for editing the final manuscript.

FOOTNOTES

Author contributions: Seneviratne SN and Rajindrajith S conceptualized and designed this manuscript; Seneviratne SN reviewed the literature and wrote the manuscript; both authors 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: Sri Lanka

ORCID number: Sumudu Nimali Seneviratne 0000-0003-2960-9269; Shaman Rajindrajith 0000-0003-1379-5052.

S-Editor: Gao CC
L-Editor: Webster JR
P-Editor: Gao CC

REFERENCES

1 **Stevens GA**, Singh GM, Lu Y, Danaei G, Lin JK, Finucane MM, Bahalim AN, McIntire RK, Gutierrez HR, Cowan M, Paciorek CJ, Farzadfar F, Riley L, Ezzati M; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in adult overweight and obesity prevalences. *Popul Health Metr* 2012; **10**: 22 [PMID: 23167948 DOI: 10.1186/1478-7954-10-22]
2 **Gupta N**, Goel K, Shah P, Misra A. Childhood obesity in developing countries: epidemiology, determinants, and prevention. *Endocr Rev* 2012; **33**: 48-70 [PMID: 22240243 DOI: 10.1210/er.2010-0028]
3 **Maggi S**, Busetto L, Noale M, Limongi F, Crepaldi G. Obesity: Definition and Epidemiology. In: Lenzi A, Migliaccio S, Donini LM. Multidisciplinary Approach to Obesity. Cham: Springer, 2015: 31-39
4 **International Diabetes Federation**. IDF Diabetes Atlas. Brussels, Belgium 2019. [cited 6 May 2021]. Available from: https://www.diabetesatlas.org/
5 **Estampador AC**, Franks PW. Precision Medicine in Obesity and Type 2 Diabetes: The Relevance of Early-Life Exposures. *Clin Chem* 2018; **64**: 130-141 [PMID: 29097511 DOI: 10.1373/clinchem.2017.273540]
6 **Lehnert T**, Sonntag D, Konnopka A, Riedel-Heller S, König HH. Economic costs of overweight and obesity. *Best Pract Res Clin Endocrinol Metab* 2013; **27**: 105-115 [PMID: 23731873 DOI: 10.1016/j.beem.2013.01.002]
7 Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000; **894**: i-xii, 1 [PMID: 11234459]
8 **World Health Organization**. Obesity and overweight 2012. [cited 28 April 2021]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/inwordex.html
9 **Eby JG**, Colditz GA. Obesity/Overweight: Prevention and Weight Management. In: Kris H. International Encyclopedia of Public Health. Oxford: Academic Press, 2008: 602-609
10 **Gillman MW**. Developmental origins of health and disease. *N Engl J Med* 2005; **353**: 1848-1850 [PMID: 16251542 DOI: 10.1056/NEJMe058187]
11 **Gluckman PD**, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008; **359**: 61-73 [PMID: 18596274 DOI: 10.1056/NEJMra0708473]
12 **Barker DJ**. In utero programming of chronic disease. *Clin Sci (Lond)* 1998; **95**: 115-128 [PMID: 9680492]
13 **Barker DJ**. The fetal and infant origins of adult disease. *BMJ* 1990; **301**: 1111 [PMID: 2252919 DOI: 10.1136/bmj.301.6761.1111]
14 **Oken E**, Gillman MW. Fetal origins of obesity. *Obes Res* 2003; **11**: 496-506 [PMID: 12690076 DOI: 10.1038/oby.2003.69]
15 **Gluckman PD**, Hanson M, Zimmet P, Forrester T. Losing the war against obesity: the need for a developmental perspective. *Sci Transl Med* 2011; **3**: 93cm19 [PMID: 21795585 DOI: 10.1126/scitranslmed.3002554]
16 **Lin X**, Lim IY, Wu Y, Teh AL, Chen L, Aris IM, Soh SE, Tint MT, MacIsaac JL, Morin AM, Yap F, Tan KH, Saw SM, Kobor MS, Meaney MJ, Godfrey KM, Chong YS, Holbrook JD, Lee YS, Gluckman PD, Karnani N; GUSTO study group. Developmental pathways to adiposity begin before birth and are influenced by genotype, prenatal environment and epigenome. *BMC Med* 2017; **15**: 50 [PMID: 28264723 DOI: 10.1186/s12916-017-0800-1]
17 **Fernandez-Twinn DS**, Hjort L, Novakovic B, Ozanne SE, Saffery R. Intrauterine programming of obesity and type 2 diabetes. *Diabetologia* 2019; **62**: 1789-1801 [PMID: 31451874 DOI: 10.1007/s00125-019-4951-9]
18 **Chung WK**, Leibel RL. Considerations regarding the genetics of obesity. *Obesity (Silver Spring)* 2008; **16** Suppl 3: S33-S39 [PMID: 19037210 DOI: 10.1038/oby.2008.514]
19 **Leibel RL**, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 1995; **332**: 621-628 [PMID: 7632212 DOI: 10.1056/NEJM199503093321001]
20 **Hanson MA**, Gluckman PD, Ma RC, Matzen P, Biesma RG. Early life opportunities for prevention of diabetes in low and middle income countries. *BMC Public Health* 2012; **12**: 1025 [PMID: 23176627 DOI: 10.1186/1471-2458-12-1025]
21 **Kinshella MW**, Moore SE, Elango R. The missing focus on women's health in the First 1,000 days approach to nutrition. *Public Health Nutr* 2021; **24**: 1526-1530 [PMID: 33023698 DOI: 10.1017/S1368980020003894]
22 **Levin BE**, Govek E. Gestational obesity accentuates obesity in obesity-prone progeny. *Am J Physiol* 1998; **275**: R1374-R1379 [PMID: 9756571 DOI: 10.1152/ajpregu.1998.275.4.R1374]
23 **Shankar K**, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**: R528-R538 [PMID: 18032473 DOI: 10.1152/ajpregu.00316.2007]
24 **Muhlhausler BS**, Adam CL, Findlay PA, Duffield JA, McMillen IC. Increased maternal nutrition alters development of the appetite-regulating network in the brain. *FASEB J* 2006; **20**: 1257-1259 [PMID: 16684802 DOI: 10.1096/fj.05-5241fje]
25 **Friedman JE**. Developmental Programming of Obesity and Diabetes in Mouse, Monkey, and Man in 2018: Where Are We Headed? *Diabetes* 2018; **67**: 2137-2151 [PMID: 30348820 DOI: 10.2337/dbi17-0011]
26 **Fleming TP**, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, Barker M, Saffery R, Yajnik CS,

Eckert JJ, Hanson MA, Forrester T, Gluckman PD, Godfrey KM. Origins of lifetime health around the time of conception: causes and consequences. *Lancet* 2018; **391**: 1842-1852 [PMID: 29673874 DOI: 10.1016/S0140-6736(18)30312-X]

27 **Parsons TJ**, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ* 2001; **323**: 1331-1335 [PMID: 11739217 DOI: 10.1136/bmj.323.7325.1331]

28 **Gluckman PD**, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol* 2007; **19**: 1-19 [PMID: 17160980 DOI: 10.1002/ajhb.20590]

29 **Dabelea D**, Crume T. Maternal environment and the transgenerational cycle of obesity and diabetes. *Diabetes* 2011; **60**: 1849-1855 [PMID: 21709280 DOI: 10.2337/db11-0400]

30 **Gillman MW**, Barker D, Bier D, Cagampang F, Challis J, Fall C, Godfrey K, Gluckman P, Hanson M, Kuh D, Nathanielsz P, Nestel P, Thornburg KL. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Pediatr Res* 2007; **61**: 625-629 [PMID: 17413866 DOI: 10.1203/pdr.0b013e3180459fcd]

31 **Catalano PM**. Obesity, insulin resistance, and pregnancy outcome. *Reproduction* 2010; **140**: 365-371 [PMID: 20457594 DOI: 10.1530/REP-10-0088]

32 **Finucane MM**, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, Singh GM, Gutierrez HR, Lu Y, Bahalim AN, Farzadfar F, Riley LM, Ezzati M; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *Lancet* 2011; **377**: 557-567 [PMID: 21295846 DOI: 10.1016/S0140-6736(10)62037-5]

33 **Whitaker RC**, Dietz WH. Role of the prenatal environment in the development of obesity. *J Pediatr* 1998; **132**: 768-776 [PMID: 9602184 DOI: 10.1016/s0022-3476(98)70302-6]

34 **Oken E**. Maternal and child obesity: the causal link. *Obstet Gynecol Clin North Am* 2009; **36**: 361-377, ix [PMID: 19501319 DOI: 10.1016/j.ogc.2009.03.007]

35 **Nelson SM**, Matthews P, Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. *Hum Reprod Update* 2010; **16**: 255-275 [PMID: 19966268 DOI: 10.1093/humupd/dmp050]

36 **Whitaker RC**. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 2004; **114**: e29-e36 [PMID: 15231970 DOI: 10.1542/peds.114.1.e29]

37 **Reynolds RM**, Osmond C, Phillips DI, Godfrey KM. Maternal BMI, parity, and pregnancy weight gain: influences on offspring adiposity in young adulthood. *J Clin Endocrinol Metab* 2010; **95**: 5365-5369 [PMID: 20702520 DOI: 10.1210/jc.2010-0697]

38 **Drake AJ**, Reynolds RM. Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction* 2010; **140**: 387-398 [PMID: 20562299 DOI: 10.1530/REP-10-0077]

39 **Guihard-Costa AM**, Papiernik E, Kolb S. Maternal predictors of subcutaneous fat in the term newborn. *Acta Paediatr* 2004; **93**: 346-349 [PMID: 15124837 DOI: 10.1080/08035250410023007]

40 **Oral E**, Cağdaş A, Gezer A, Kaleli S, Aydinli K, Oçer F. Perinatal and maternal outcomes of fetal macrosomia. *Eur J Obstet Gynecol Reprod Biol* 2001; **99**: 167-171 [PMID: 11788165 DOI: 10.1016/s0301-2115(01)00416-x]

41 **Wikström I**, Axelsson O, Bergström R, Meirik O. Traumatic injury in large-for-date infants. *Acta Obstet Gynecol Scand* 1988; **67**: 259-264 [PMID: 3176946 DOI: 10.3109/00016348809004216]

42 **Deierlein AL**, Siega-Riz AM, Adair LS, Herring AH. Effects of pre-pregnancy body mass index and gestational weight gain on infant anthropometric outcomes. *J Pediatr* 2011; **158**: 221-226 [PMID: 20863516 DOI: 10.1016/j.jpeds.2010.08.008]

43 **Boney CM**, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005; **115**: e290-e296 [PMID: 15741354 DOI: 10.1542/peds.2004-1808]

44 **Catalano PM**, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care* 2009; **32**: 1076-1080 [PMID: 19460915 DOI: 10.2337/dc08-2077]

45 **McMillen IC**, Edwards LJ, Duffield J, Muhlhausler BS. Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity. *Reproduction* 2006; **131**: 415-427 [PMID: 16514185 DOI: 10.1530/rep.1.00303]

46 **Bianco ME**, Josefson JL. Hyperglycemia During Pregnancy and Long-Term Offspring Outcomes. *Curr Diab Rep* 2019; **19**: 143 [PMID: 31754898 DOI: 10.1007/s11892-019-1267-6]

47 **Scholtens DM**, Kuang A, Lowe LP, Hamilton J, Lawrence JM, Lebenthal Y, Brickman WJ, Clayton P, Ma RC, McCance D, Tam WH, Catalano PM, Linder B, Dyer AR, Lowe WL Jr, Metzger BE; HAPO Follow-up Study Cooperative Research Group; HAPO Follow-Up Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal Glycemia and Childhood Glucose Metabolism. *Diabetes Care* 2019; **42**: 381-392 [PMID: 30617141 DOI: 10.2337/dc18-2021]

48 **Seneviratne SN**, Derraik JGB, Jiang Y, McCowan LME, Gusso S, Cutfield WS, Hofman PL. The sex of the foetus affects maternal blood glucose concentrations in overweight and obese pregnant women. *J Obstet Gynaecol* 2017; **37**: 667-669 [PMID: 28019134 DOI: 10.1080/01443615.2016.1256970]

49 **Retnakaran R**, Kramer CK, Ye C, Kew S, Hanley AJ, Connelly PW, Sermer M, Zinman B. Fetal sex and maternal risk of gestational diabetes mellitus: the impact of having a boy. *Diabetes Care* 2015; **38**: 844-851 [PMID: 25693837 DOI: 10.2337/dc14-2551]

50 **Taylor PD**, Poston L. Developmental programming of obesity in mammals. *Exp Physiol* 2007; **92**: 287-298 [PMID: 17170060 DOI: 10.1113/expphysiol.2005.032854]

51 **Hales CN**, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001; **60**: 5-20 [PMID: 11809615 DOI: 10.1093/bmb/60.1.5]

52 **Ozanne SE**, Hales CN. Lifespan: catch-up growth and obesity in male mice. *Nature* 2004; **427**: 411-412 [PMID: 14749819 DOI: 10.1038/427411b]

53 **Entringer S**, Buss C, Swanson JM, Cooper DM, Wing DA, Waffarn F, Wadhwa PD. Fetal programming of body composition, obesity, and metabolic function: the role of intrauterine stress and stress biology. *J Nutr Metab* 2012; **2012**:

632548 [PMID: 22655178 DOI: 10.1155/2012/632548]
54 **Correia-Branco A**, Keating E, Martel F. Maternal undernutrition and fetal developmental programming of obesity: the glucocorticoid connection. *Reprod Sci* 2015; **22**: 138-145 [PMID: 25001018 DOI: 10.1177/1933719114542012]
55 **Seneviratne SN**, Derraik JGB, Jiang Y, McCowan LME, Gusso S, Biggs JB, Parry GK, Chiavaroli V, Cutfield WS, Hofman PL. Nulliparity is associated with subtle adverse metabolic outcomes in overweight/obese mothers and their offspring. *Clin Endocrinol (Oxf)* 2017; **87**: 545-551 [PMID: 28727231 DOI: 10.1111/cen.13426]
56 **Li J**, Tsuprykov O, Yang X, Hocher B. Paternal programming of offspring cardiometabolic diseases in later life. *J Hypertens* 2016; **34**: 2111-2126 [PMID: 27457668 DOI: 10.1097/HJH.0000000000001051]
57 **McPherson NO**, Fullston T, Aitken RJ, Lane M. Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. *Ann Nutr Metab* 2014; **64**: 231-238 [PMID: 25300265 DOI: 10.1159/000365026]
58 **Marseglia L**, Manti S, D'Angelo G, Cuppari C, Salpietro V, Filippelli M, Trovato A, Gitto E, Salpietro C, Arrigo T. Obesity and breastfeeding: The strength of association. *Women Birth* 2015; **28**: 81-86 [PMID: 25595034 DOI: 10.1016/j.wombi.2014.12.007]
59 **Mäkelä J**, Vaarno J, Kaljonen A, Niinikoski H, Lagström H. Maternal overweight impacts infant feeding patterns--the STEPS Study. *Eur J Clin Nutr* 2014; **68**: 43-49 [PMID: 24219892 DOI: 10.1038/ejcn.2013.229]
60 **Plagemann A**, Harder T. Breast feeding and the risk of obesity and related metabolic diseases in the child. *Metab Syndr Relat Disord* 2005; **3**: 222-232 [PMID: 18370791 DOI: 10.1089/met.2005.3.222]
61 **Sirkka O**, Vrijkotte T, Halberstadt J, Abrahamse-Berkeveld M, Hoekstra T, Seidell J, Olthof M. Prospective associations of age at complementary feeding and exclusive breastfeeding duration with body mass index at 5-6 years within different risk groups. *Pediatr Obes* 2018; **13**: 522-529 [PMID: 29695025 DOI: 10.1111/ijpo.12289]
62 **Kral JG**, Biron S, Simard S, Hould FS, Lebel S, Marceau S, Marceau P. Large maternal weight loss from obesity surgery prevents transmission of obesity to children who were followed for 2 to 18 years. *Pediatrics* 2006; **118**: e1644-e1649 [PMID: 17142494 DOI: 10.1542/peds.2006-1379]
63 **Richards DS**, Miller DK, Goodman GN. Pregnancy after gastric bypass for morbid obesity. *J Reprod Med* 1987; **32**: 172-176 [PMID: 3572896]
64 **Ducarme G**, Revaux A, Rodrigues A, Aissaoui F, Pharisien I, Uzan M. Obstetric outcome following laparoscopic adjustable gastric banding. *Int J Gynaecol Obstet* 2007; **98**: 244-247 [PMID: 17433814 DOI: 10.1016/j.ijgo.2007.02.020]
65 **World Health Organization**. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. [cited 12 May 2021]. Available from: https://apps.who.int/iris/bitstream/handle/10665/94384/9789241506236_eng.pdf?sequence=1
66 **Hanson MA**, Gluckman PD. Developmental origins of health and disease--global public health implications. *Best Pract Res Clin Obstet Gynaecol* 2015; **29**: 24-31 [PMID: 25225058 DOI: 10.1016/j.bpobgyn.2014.06.007]
67 **Phelan S**. Pregnancy: a "teachable moment" for weight control and obesity prevention. *Am J Obstet Gynecol* 2010; **202**: 135.e1-135.e8 [PMID: 19683692 DOI: 10.1016/j.ajog.2009.06.008]
68 **Farpour-Lambert NJ**, Ells LJ, Martinez de Tejada B, Scott C. Obesity and Weight Gain in Pregnancy and Postpartum: an Evidence Review of Lifestyle Interventions to Inform Maternal and Child Health Policies. *Front Endocrinol (Lausanne)* 2018; **9**: 546 [PMID: 30319539 DOI: 10.3389/fendo.2018.00546]
69 **Armengaud JB**, Simeoni U. Offspring of Mothers with Hyperglycemia in Pregnancy: Short-Term Consequences for Newborns and Infants. *Front Diabetes* 2020; **28**: 194-200 [DOI: 10.1159/000480175]
70 **Seneviratne SN**, McCowan LM, Cutfield WS, Derraik JG, Hofman PL. Exercise in pregnancies complicated by obesity: achieving benefits and overcoming barriers. *Am J Obstet Gynecol* 2015; **212**: 442-449 [PMID: 24909342 DOI: 10.1016/j.ajog.2014.06.009]
71 Impact of physical activity during pregnancy and postpartum on chronic disease risk. *Med Sci Sports Exerc* 2006; **38**: 989-1006 [PMID: 16672855 DOI: 10.1249/01.mss.0000218147.51025.8a]
72 **Adamo KB**, Ferraro ZM, Brett KE. Can we modify the intrauterine environment to halt the intergenerational cycle of obesity? *Int J Environ Res Public Health* 2012; **9**: 1263-1307 [PMID: 22690193 DOI: 10.3390/ijerph9041263]
73 Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press (US); 2009– [PMID: 20669500]
74 **Gillman MW**, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. *Pediatrics* 2003; **111**: e221-e226 [PMID: 12612275 DOI: 10.1542/peds.111.3.e221]
75 **Davenport MH**, Meah VL, Ruchat SM, Davies GA, Skow RJ, Barrowman N, Adamo KB, Poitras VJ, Gray CE, Jaramillo Garcia A, Sobierajski F, Riske L, James M, Kathol AJ, Nuspl M, Marchand AA, Nagpal TS, Slater LG, Weeks A, Barakat R, Mottola MF. Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. *Br J Sports Med* 2018; **52**: 1386-1396 [PMID: 30337465 DOI: 10.1136/bjsports-2018-099836]
76 **McMurray RG**, Mottola MF, Wolfe LA, Artal R, Millar L, Pivarnik JM. Recent advances in understanding maternal and fetal responses to exercise. *Med Sci Sports Exerc* 1993; **25**: 1305-1321 [PMID: 8107536]
77 **Kramer MS**, McDonald SW. Aerobic exercise for women during pregnancy. *Cochrane Database Syst Rev* 2006; CD000180 [PMID: 16855953 DOI: 10.1002/14651858.CD000180.pub2]
78 **Gavard JA**, Artal R. Effect of exercise on pregnancy outcome. *Clin Obstet Gynecol* 2008; **51**: 467-480 [PMID: 18463475 DOI: 10.1097/GRF.0b013e31816feb1d]
79 **Barakat R**, Ruiz JR, Rodríguez-Romo G, Montejo-Rodríguez R, Lucia A. Does exercise training during pregnancy influence fetal cardiovascular responses to an exercise stimulus? *Br J Sports Med* 2010; **44**: 762-764 [PMID: 19752154 DOI: 10.1136/bjsm.2009.062547]
80 **Szymanski LM**, Satin AJ. Exercise during pregnancy: fetal responses to current public health guidelines. *Obstet Gynecol* 2012; **119**: 603-610 [PMID: 22314872 DOI: 10.1097/AOG.0b013e31824760b5]
81 **Clapp JF 3rd**. Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy. *J Pediatr* 1996; **129**: 856-863 [PMID: 8969727 DOI: 10.1016/s0022-3476(96)70029-x]

82 **Clapp JF 3rd**, Kim H, Burciu B, Schmidt S, Petry K, Lopez B. Continuing regular exercise during pregnancy: effect of exercise volume on fetoplacental growth. *Am J Obstet Gynecol* 2002; **186**: 142-147 [PMID: 11810100 DOI: 10.1067/mob.2002.119109]

83 **Hopkins SA**, Cutfield WS. Exercise in pregnancy: weighing up the long-term impact on the next generation. *Exerc Sport Sci Rev* 2011; **39**: 120-127 [PMID: 21519301 DOI: 10.1097/JES.0b013e31821a5527]

84 **Bell RJ**, Palma SM, Lumley JM. The effect of vigorous exercise during pregnancy on birth-weight. *Aust N Z J Obstet Gynaecol* 1995; **35**: 46-51 [PMID: 7771999 DOI: 10.1111/j.1479-828x.1995.tb01829.x]

85 **Leet T**, Flick L. Effect of exercise on birthweight. *Clin Obstet Gynecol* 2003; **46**: 423-431 [PMID: 12808392 DOI: 10.1097/00003081-200306000-00021]

86 **Atapattu N**, Mohsin F, Zabeen B, Seneviratne SN. Pediatric diabetes care in Sri Lanka and Bangladesh: Reaching the community. *Pediatr Diabetes* 2021; **22**: 112-115 [PMID: 33232549 DOI: 10.1111/pedi.13102]

87 **Seneviratne SN**, Parry GK, McCowan LM, Ekeroma A, Jiang Y, Gusso S, Peres G, Rodrigues RO, Craigie S, Cutfield WS, Hofman PL. Antenatal exercise in overweight and obese women and its effects on offspring and maternal health: design and rationale of the IMPROVE (Improving Maternal and Progeny Obesity Via Exercise) randomised controlled trial. *BMC Pregnancy Childbirth* 2014; **14**: 148 [PMID: 24767604 DOI: 10.1186/1471-2393-14-148]

88 **Seneviratne SN**, Jiang Y, Derraik J, McCowan L, Parry GK, Biggs JB, Craigie S, Gusso S, Peres G, Rodrigues RO, Ekeroma A, Cutfield WS, Hofman PL. Effects of antenatal exercise in overweight and obese pregnant women on maternal and perinatal outcomes: a randomised controlled trial. *BJOG* 2016; **123**: 588-597 [PMID: 26542419 DOI: 10.1111/1471-0528.13738]

89 **Sui Z**, Grivell RM, Dodd JM. Antenatal exercise to improve outcomes in overweight or obese women: A systematic review. *Acta Obstet Gynecol Scand* 2012; **91**: 538-545 [PMID: 22229625 DOI: 10.1111/j.1600-0412.2012.01357.x]

90 **Choi J**, Fukuoka Y, Lee JH. The effects of physical activity and physical activity plus diet interventions on body weight in overweight or obese women who are pregnant or in postpartum: a systematic review and meta-analysis of randomized controlled trials. *Prev Med* 2013; **56**: 351-364 [PMID: 23480971 DOI: 10.1016/j.ypmed.2013.02.021]

91 **Santos IA**, Stein R, Fuchs SC, Duncan BB, Ribeiro JP, Kroeff LR, Carballo MT, Schmidt MI. Aerobic exercise and submaximal functional capacity in overweight pregnant women: a randomized trial. *Obstet Gynecol* 2005; **106**: 243-249 [PMID: 16055571 DOI: 10.1097/01.AOG.0000171113.36624.86]

92 **Nascimento SL**, Surita FG, Parpinelli MÂ, Siani S, Pinto e Silva JL. The effect of an antenatal physical exercise programme on maternal/perinatal outcomes and quality of life in overweight and obese pregnant women: a randomised clinical trial. *BJOG* 2011; **118**: 1455-1463 [PMID: 21895947 DOI: 10.1111/j.1471-0528.2011.03084.x]

93 **Berry DC**, Boggess K, Johnson QB. Management of Pregnant Women with Type 2 Diabetes Mellitus and the Consequences of Fetal Programming in Their Offspring. *Curr Diab Rep* 2016; **16**: 36 [PMID: 26983624 DOI: 10.1007/s11892-016-0733-7]

94 **Kim SY**, Sharma AJ, Callaghan WM. Gestational diabetes and childhood obesity: what is the link? *Curr Opin Obstet Gynecol* 2012; **24**: 376-381 [PMID: 23000698 DOI: 10.1097/GCO.0b013e328359f0f4]

95 **Rowan JA**, Rush EC, Plank LD, Lu J, Obolonkin V, Coat S, Hague WM. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition and metabolic outcomes at 7-9 years of age. *BMJ Open Diabetes Res Care* 2018; **6**: e000456 [PMID: 29682291 DOI: 10.1136/bmjdrc-2017-000456]

96 **Triunfo S**, Lanzone A. Impact of maternal under nutrition on obstetric outcomes. *J Endocrinol Invest* 2015; **38**: 31-38 [PMID: 25194427 DOI: 10.1007/s40618-014-0168-4]

97 **Imdad A**, Bhutta ZA. Maternal nutrition and birth outcomes: effect of balanced protein-energy supplementation. *Paediatr Perinat Epidemiol* 2012; **26** Suppl 1: 178-190 [PMID: 22742610 DOI: 10.1111/j.1365-3016.2012.01308.x]

98 **Miller SA**, Wu RKS, Oremus M. The association between antibiotic use in infancy and childhood overweight or obesity: a systematic review and meta-analysis. *Obes Rev* 2018; **19**: 1463-1475 [PMID: 30035851 DOI: 10.1111/obr.12717]

99 **Koleva PT**, Bridgman SL, Kozyrskyj AL. The infant gut microbiome: evidence for obesity risk and dietary intervention. *Nutrients* 2015; **7**: 2237-2260 [PMID: 25835047 DOI: 10.3390/nu7042237]

100 **Li L**, Zhang S, Huang Y, Chen K. Sleep duration and obesity in children: A systematic review and meta-analysis of prospective cohort studies. *J Paediatr Child Health* 2017; **53**: 378-385 [PMID: 28073179 DOI: 10.1111/jpc.13434]

101 **Hu J**, Aris IM, Lin PD, Rifas-Shiman SL, Perng W, Woo Baidal JA, Wen D, Oken E. Longitudinal associations of modifiable risk factors in the first 1000 days with weight status and metabolic risk in early adolescence. *Am J Clin Nutr* 2020 [PMID: 33184628 DOI: 10.1093/ajcn/nqaa297]

102 **Koplin JJ**, Kerr JA, Lodge C, Garner C, Dharmage SC, Wake M, Allen KJ. Infant and young child feeding interventions targeting overweight and obesity: A narrative review. *Obes Rev* 2019; **20** Suppl 1: 31-44 [PMID: 31419047 DOI: 10.1111/obr.12798]

103 **Singhal A**. Long-Term Adverse Effects of Early Growth Acceleration or Catch-Up Growth. *Ann Nutr Metab* 2017; **70**: 236-240 [PMID: 28301849 DOI: 10.1159/000464302]

104 **Jain V**, Singhal A. Catch up growth in low birth weight infants: striking a healthy balance. *Rev Endocr Metab Disord* 2012; **13**: 141-147 [PMID: 22415299 DOI: 10.1007/s11154-012-9216-6]

105 **Ling J**, Robbins LB, Wen F, Zhang N. Lifestyle Interventions in Preschool Children: A Meta-analysis of Effectiveness. *Am J Prev Med* 2017; **53**: 102-112 [PMID: 28237633 DOI: 10.1016/j.amepre.2017.01.018]

106 **Liu Z**, Xu HM, Wen LM, Peng YZ, Lin LZ, Zhou S, Li WH, Wang HJ. A systematic review and meta-analysis of the overall effects of school-based obesity prevention interventions and effect differences by intervention components. *Int J Behav Nutr Phys Act* 2019; **16**: 95 [PMID: 31665040 DOI: 10.1186/s12966-019-0848-8]

107 **Gori D**, Guaraldi F, Cinocca S, Moser G, Rucci P, Fantini MP. Effectiveness of educational and lifestyle interventions to prevent paediatric obesity: systematic review and meta-analyses of randomized and non-randomized controlled trials. *Obes Sci Pract* 2017; **3**: 235-248 [PMID: 29071100 DOI: 10.1002/osp4.111]

108 **Walker R**, Morris H, Lang S, Hampton K, Boyle J, Skouteris H. Co-designing preconception and pregnancy care for healthy maternal lifestyles and obesity prevention. *Women Birth* 2020; **33**: 473-478 [PMID: 31812498 DOI: 10.1016/j.wombi.2019.11.005]

109 **Kandel P**, Lim S, Pirotta S, Skouteris H, Moran LJ, Hill B. Enablers and barriers to women's lifestyle behavior change during the preconception period: A systematic review. *Obes Rev* 2021; **22**: e13235 [PMID: 33754474 DOI: 10.1111/obr.13235]

110 **Catalano P**, deMouzon SH. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. *Int J Obes (Lond)* 2015; **39**: 642-649 [PMID: 25777180 DOI: 10.1038/ijo.2015.15]

111 **Seneviratne SN**, Sachchithananthan S, Gamage PSA, Peiris R, Wickramasinghe VP, Somasundaram N. Effectiveness and acceptability of a novel school-based healthy eating program among primary school children in urban Sri Lanka. *BMC Public Health* 2021; **21**: 2083 [PMID: 34774025 DOI: 10.1186/s12889-021-12041-8]

112 **Nader PR**, Huang TT, Gahagan S, Kumanyika S, Hammond RA, Christoffel KK. Next steps in obesity prevention: altering early life systems to support healthy parents, infants, and toddlers. *Child Obes* 2012; **8**: 195-204 [PMID: 22799545 DOI: 10.1089/chi.2012.0004]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 498-520

DOI: 10.4239/wjd.v13.i7.498

ISSN 1948-9358 (online)

REVIEW

Biochemical composition of the glomerular extracellular matrix in patients with diabetic kidney disease

María M Adeva-Andany, Natalia Carneiro-Freire

Specialty type: Medicine, general and internal

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: Bayoumi RAL, United Arab Emirates; Javor E, Croatia

Received: February 1, 2022
Peer-review started: February 1, 2022
First decision: April 18, 2022
Revised: April 19, 2022
Accepted: June 24, 2022
Article in press: June 24, 2022
Published online: July 15, 2022

María M Adeva-Andany, Natalia Carneiro-Freire, Nephrology, Hospital Juan Cardona, Ferrol 15406, Spain

Corresponding author: María M Adeva-Andany, MD, PhD, Consultant Physician-Scientist, Nephrology, Hospital Juan Cardona, Pardo Bazán x/n, Ferrol 15406, Spain.
madevaa@yahoo.com

Abstract

In the glomeruli, mesangial cells produce mesangial matrix while podocytes wrap glomerular capillaries with cellular extensions named foot processes and tether the glomerular basement membrane (GBM). The turnover of the mature GBM and the ability of adult podocytes to repair injured GBM are unclear. The actin cytoskeleton is a major cytoplasmic component of podocyte foot processes and links the cell to the GBM. Predominant components of the normal glomerular extracellular matrix (ECM) include glycosaminoglycans, proteoglycans, laminins, fibronectin-1, and several types of collagen. In patients with diabetes, multiorgan composition of extracellular tissues is anomalous, including the kidney, so that the constitution and arrangement of glomerular ECM is profoundly altered. In patients with diabetic kidney disease (DKD), the global quantity of glomerular ECM is increased. The level of sulfated proteoglycans is reduced while hyaluronic acid is augmented, compared to control subjects. The concentration of mesangial fibronectin-1 varies depending on the stage of DKD. Mesangial type III collagen is abundant in patients with DKD, unlike normal kidneys. The amount of type V and type VI collagens is higher in DKD and increases with the progression of the disease. The GBM contains lower amount of type IV collagen in DKD compared to normal tissue. Further, genetic variants in the α3 chain of type IV collagen may modulate susceptibility to DKD and end-stage kidney disease. Human cellular models of glomerular cells, analyses of human glomerular proteome, and improved microscopy procedures have been developed to investigate the molecular composition and organization of the human glomerular ECM.

Key Words: Diabetes; Kidney disease; Glycosaminoglycans; Factor H; Sialic acid; Laminin; Collagen; Fibronectin-1; Extracellular matrix

Core Tip: Diabetic kidney disease is associated with profound disturbance in glomerular extracellular matrix (ECM). Understanding the mechanisms that regulate glomerular ECM synthesis and repair may contribute to design therapeutic strategies that improve clinical outcomes. The cytoskeleton inside the foot processes of podocytes is connected to the glomerular basement membrane (GBM) *via* associated proteins. There is a reciprocal interaction between the cellular cytoskeleton and the extracellular tissue that contribute to regulate ECM composition. Loss of anchor points in the GBM may lead to podocyte detachment. Likewise, alterations in the podocyte cytoskeleton may unfasten the cell and impair the filtration barrier.

Citation: Adeva-Andany MM, Carneiro-Freire N. Biochemical composition of the glomerular extracellular matrix in patients with diabetic kidney disease. *World J Diabetes* 2022; 13(7): 498-520
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/498.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.498

INTRODUCTION

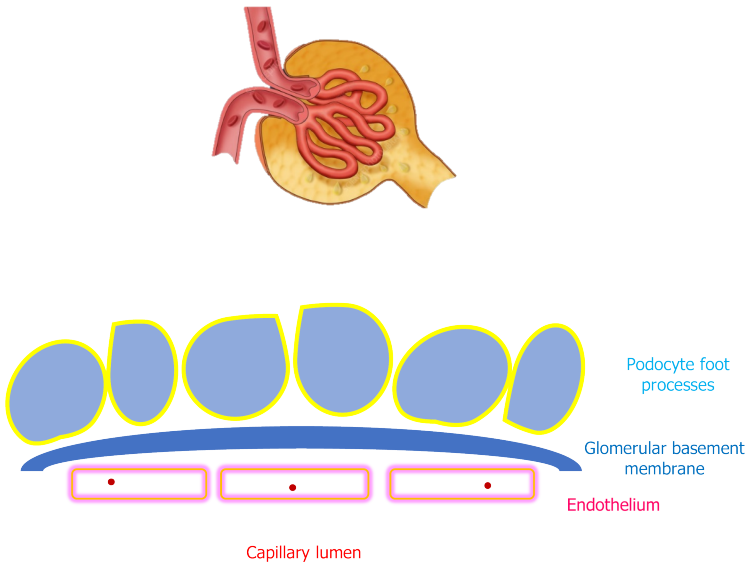
In human glomeruli, mesangial cells (which are thought to be akin to vascular smooth muscle cells) produce mesangial matrix while podocytes wrap glomerular capillaries with cellular extensions named foot processes and tether underneath glomerular basement membrane (GBM) (Figure 1). The turnover of the GBM present at birth and the ability of adult podocytes to restore damaged GBM are unclear. These cells have limited proliferation capacity, but they can undergo hypertrophy to compensate for the detachment and loss of contiguous podocytes, thus avoiding uncovered GBM areas to preserve the filtration barrier. Podocyte detachment may be caused by an altered composition of the GBM with deficiency of anchor points or by anomalies in the connection apparatus that links the foot processes to the GBM. The actin cytoskeleton is a major cytoplasmic component of the podocyte foot processes and connects the cell to the GBM. Actin-associated proteins such as α-actinin-4 and inverted formin-2 attach the actin cytoskeleton to plasma membrane components (such as integrins, syndecans and dystroglycans), which in turn bind to their ligands in the GBM, including laminin and fibronectin-1[1-4]. The integrity of the GBM is crucial to maintain the filtration barrier, as highlighted by the clinical consequences of disorders that alter GBM components, such as laminin or collagen. Diabetes and other conditions associated with insulin resistance (such as Alström syndrome) are associated with a systemic and pronounced alteration in the composition of extracellular matrix (ECM), including the kidney and the blood vessels, that leads to multi-organ interstitial fibrosis[5]. Pathogenic mechanisms underlying this disturbance are unclear. Understanding the pathways of ECM assembly and remodeling and the cell-ECM interactions is crucial for designing therapeutic strategies and tissue engineering. A growing number of procedures have been improved and developed to investigate the biochemical composition and architecture of the ECM and its mutual interaction with the contiguous cells. Among them are biochemical assays to identify and quantify ECM components, genetic methods to investigate gene expression, imaging procedures, human cell cultures, and *in vitro* pharmacological evaluations to assess metabolic pathways.

Nuclear magnetic resonance spectroscopy and soft-ionization mass spectrometry represent complimentary techniques for ECM research. Mass spectrometry techniques (such as matrix-assisted laser desorption and ionization) are useful for compositional analysis whereas nuclear magnetic resonance spectroscopy evaluates the molecular architecture of the ECM and its dynamics[6]. Raman spectroscopy is a label-free vibrational technique that contributes to characterize the molecular ECM structure and composition[7,8].

Histological methods for ECM analysis with conventional microscopy include immunohistochemistry and zymography. The former can be utilized to determine the localization of various ECM proteins while the latter may be used to evaluate proteinase activity in the ECM. In addition, imaging methods have been designed to characterize the human ECM and the adjacent cells at the molecular and cellular level. Scanning electron microscopy and multi-harmonic generation microscopy can be used to visualize ECM components and assess their structural properties[9]. Multiphoton imaging has been described to analyze the human structural organization of elastin and collagen during mechanical loading[10].

The construction of flat and tubular collagen gel-based scaffolds cellularized with vascular smooth muscle cells have enriched vascular tissue engineering[11]. Microgel assembly, a macroscopic aggregate formed by assembly of microgels, can be applied to tissue engineering and cell cultures[12].

A variety of genetic techniques are useful on ECM research. MicroRNAs are noncoding RNAs that regulate gene expression and participate in ECM pathophysiology. Microarrays can be used to determine microRNA profiles[13]. Weighted gene co-expression network analysis enables the identification of clusters of related genes that can be associated with specific clinical phenotypes. This technique has been used to assess differentially expressed ECM genes in patients with diabetic kidney



DOI: 10.4239/wjd.v13.i7.498

Figure 1 Glomerular capillaries and glomerular basement membrane.

disease (DKD) and other glomerular diseases[14,15]. The Matrisome Project has been developed to characterize genes encoding structural and associated ECM proteins (http://matrisomeproject.mit.edu).

Human cell culture technology and pharmacological investigations on cultured cells are instrumental tools on ECM research. Human pluripotent stem cells in culture have been used to generate models of various tissues. The presence of ECM in the cultures provides cues to the cells that modify their behavior and improves similarity to the native human tissue, including the kidney[16]. Stem cells reside in "niches" within the ECM and the composition of the ECM contributes to define the degree of quiescence and turnover of these cells[17]. Manufacture of a suitable ECM is crucial to alter growth, differentiation, and proliferation of stem cells and use them for tissue engineering[18]. Three-dimensional decellularized ECM derived from mesenchymal stem cell cultures has been attained by application of macromolecular crowding[19]. Three-dimensional tissue constructs that recapitulate human fibrous connective tissue have been achieved by using cultures of primary human fibroblasts, enabling the quantification of cell-derived changes in ECM synthesis in response to several stimuli, such as nutrient composition or pharmacological compounds[20]. Treatment of human bone marrow-derived mesenchymal cells with high molecular weight hyaluronic acid increases fibronectin production and ECM deposition, suggesting that hyaluronic acid-based biomaterials may be useful to promote ECM formation[21]. A pulsatile perfusion culture of progenitor cells has been developed as an *in vitro* system to construct vascular tissue[22]. Cell-matrix interactions may be investigated by micro-electro-mechanical systems and Organ-on-a-Chip technology[23].

In the kidney, human cellular models of glomerular epithelial cells have been developed that can be used to evaluate podocyte pathophysiology and investigate therapeutic strategies[24]. Investigations of the glomerular proteome have provided information on the proteins expressed in the glomerular ECM of adult normal human kidney. A database has been created that may be used for clinical research on the pathophysiology of kidney diseases[25-27]. Proteomic analysis of human glomerular ECM may be conducted from sections retrieved by kidney biopsy samples[28]. The sub-diffraction resolution stochastic optical reconstruction microscopy (STORM) facilitates the investigation of the molecular organization within the human GBM[29].

NORMAL COMPOSITION OF THE HUMAN GBM AND MESANGIAL MATRIX

The analysis of the specific composition of the GBM and mesangial matrix is hindered by the technical obstacle of adequately separate these two compartments of glomerular ECM, as procedures that isolate glomerular ECM achieve samples that contain both GBM and mesangial matrix. However, immunohistochemical analyses contribute to determine the differential constitution of the two structures (Table 1)[27,30-32]. Major components of the normal glomerular ECM are laminin and collagen. In addition, glycosaminoglycans, proteoglycans, sialic acid, and fibronectin-1 are important constituents of the kidney ECM in humans.

Table 1 Major components of the glomerular basement membrane and the mesangial matrix in normal human glomeruli

	Glomerular basement membrane	Mesangial matrix
Heparan sulfate proteoglycan	Abundant	Abundant
Laminin	Major component	Minor component
Fibronectin	Minor component	Major component
Type I collagen	Absent in most studies	Absent in most studies
Type III collagen	Absent in most studies	Absent in most studies
Type IV collagen	Major component	Present (inconsistent amounts)
Type V collagen	Present	Present
Type VI collagen	Present	Present
Type XVII collagen	Present	Unknown
Type XVIII collagen	Present	Present
Tubulointerstitial nephritis antigen-like-1	Low abundance	High abundance
Nidogen / Entactin	Present	Low abundance
Fibulin-1	Present	Present
Fibrillin-1	Present	Present
Nephronectin	Present	Present
Vitronectin	Absent	Present
Microfibril-associated proteins	Absent	Present

Glycosaminoglycans, proteoglycans, and sialic acid in the normal glomerulus

Proteoglycans consist of a core protein attached to one or more glycosaminoglycan chains, which are formed by linear polysaccharides. Sulfate groups are usually bound to the unbranched polysaccharide chains, creating a high negative charge[33,34].

In kidney specimens from healthy humans, heparan sulfate is the predominant glycosaminoglycan present in the glomerular ECM, followed by hyaluronate, dermatan sulfate, and chondroitin sulfate isomers 4-sulfate and 6-sulfate[35-37].

Immunohistochemical studies show that both GBM and mesangial matrix contain heparan sulfate proteoglycans[33,38-40]. Among them, mass spectrometry-based analyses of normal human kidney samples reveal that agrin and perlecan are present in the glomerular proteome[27]. Agrin is a major heparan sulfate proteoglycan present in human GBM. Immunoelectron microscopy shows a linear distribution of agrin throughout the width of the normal GBM. In addition to the GBM, agrin mRNA and protein are detected in normal lungs[34,41]. Localization of agrin to the human GBM has been confirmed by STORM. Using this procedure, agrin is predominantly detected at the epithelial surface compared to the endothelial aspect of the GBM[29]. The precise function of agrin in the human kidney has not been defined, but it may contribute to the adhesion of the GBM to the podocyte by tethering laminins to cell surface receptors such as integrins or α-dystroglycan[34,41]. In normal human kidney specimens, perlecan stained the GBM only slightly, in contrast to the strong staining of the mesangium, the Bowman's capsule, and the tubular basement membrane. The function of this heparan sulfate proteoglycan in the normal kidney remains to be clarified[34,42,43]. Unlike agrin, immunoelectron microscopy shows that perlecan is distributed only on the endothelial side of the GBM[34].

Sialic acid (neuraminic acid) is a nine-carbon carbohydrate that may exist as several derivatives. In humans, the most common sialic acid byproduct is the acetylated compound N-acetyl-neuraminic acid. Sialic acid typically occupies the terminal domain of oligosaccharide chains of some glycolipids and glycoproteins and usually protrudes from the cell surface. Sialidases (neuraminidases) are enzymes that remove sialic acid residues from glycosaminoglycans attached to proteins or lipids on the cell surface (desialylation). Sialyltransferases catalyze the addition of sialic acid residues to glycosaminoglycans (sialylation). Sialylated conjugates are identified by specific binding to lectins or by cationic dyes such as alcian blue[44,45]. In normal human kidney specimens, sialic acid stains strongly the podocytes, unlike glomerular capillaries and Bowman's capsule[44].

Both sulfated glycosaminoglycans and sialic acid are polyanions that have an essential role in the identification of "self" structures to avoid complement activation and subsequent complement-mediated injury in host tissues[46-49]. Sulfation can occur at various positions within the glycosaminoglycan structure creating the potential for high molecular variability. The unique position of sulfate groups in the glycosaminoglycan molecule is named sulfation code and defines functional character-

istics of the sulfated glycosaminoglycan, such as its interaction with proteins. Hyaluronic acid is a glycosaminoglycan that lacks sulfate groups and is not attached to a protein core to form proteoglycans [50]. Factor H is a glycoprotein that inhibits the alternative pathway of complement in "self" structures (as opposed to foreign elements such as pathogens), by recognizing sialic acid or sulfated glycosaminoglycans present on "self" biological surfaces. The interaction between factor H and sulfated glycosaminoglycans is highly specific and depends upon the sulfation code. Little or no binding occurs with hyaluronic acid. The binding of factor H to sialic acid or sulfated glycosaminoglycans on biological surfaces protects the host from autolytic complement attack (Figure 2). Deficit of binding sites for factor H due to loss of sulfated glycosaminoglycans or sialic acid (or alteration of the sulfation code or the sialylation pattern) impairs factor H binding to "self" structures and may result in complement-mediated damage due to unrestrained activation of the alternative pathway of complement[46,48,49,51-54].

Laminin in the normal glomerulus

Protein quantification of the glomerular ECM proteome by mass spectrometry reveals that laminin isoforms and type IV collagen are the most abundant proteins in the glomerular ECM[27]. Laminins are heterotrimeric proteins composed of α, β, and γ glycoprotein chains. Different α, β, and γ chains create diverse isoforms of laminin heterotrimers, such as laminin α5/β2/γ1 (laminin 521). Laminin heterotrimers polymerize in the extracellular space to form a network. Laminin polymerization is required for initiation of basement membrane formation. The actin cytoskeleton plays an important role in extracellular laminin polymerization. *In vitro* studies using myotubes reveal that the organization of extracellular laminin into networks is abnormal when the actin cytoskeleton is disrupted with cytochalasin (an agent that prevents actin polymerization) compared to control myotubes free of this compound. Cytochalasin-treated myotubes show no arrangement of surface laminin into complex networks, unlike control myotubes that show normal laminin array. However, no detrimental effect on laminin network formation was observed with wortmannin, an inhibitor of phosphatidylinositol 3-kinase[55].

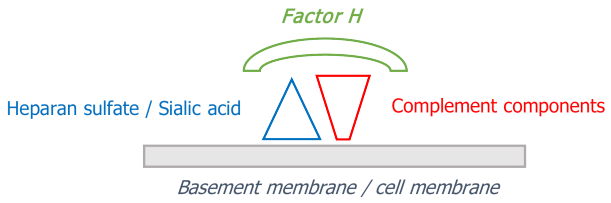
Laminin isoforms has been identified in the mesangial matrix of normal human kidneys, although this protein is predominantly detected in the GBM. Immunohistochemical studies show a continuous staining for laminin in the normal GBM. Immunogold electron microscopy reveals that laminin is distributed throughout the entire thickness of the GBM[37-40,56]. The organization of laminin 521 in normal human GBM has been investigated with STORM. Laminin 521 (and agrin) have their N-terminal domains facing the interior of the GBM while their C-terminal domains are oriented towards the surface of endothelial cells and podocytes[29].

The important functional role of laminin in the glomeruli is underlined by the clinical consequences of genetic mutations that alter the protein. Mutations in the gene that codes the β2 chain of laminin cause an autosomal recessive clinical spectrum of disorders that ranges from isolated congenital nephrotic syndrome (type 5) to Pierson syndrome, which consists of a combination of ocular abnormalities, neurological manifestations due to defects of the neuromuscular junction, and congenital nephrotic syndrome with diffuse mesangial sclerosis progressing rapidly to end-stage kidney disease (ESKD)[57,58].

Fibronectin-1 in the normal glomerulus

Fibronectin-1 is a dimeric glycoprotein circulating in normal plasma and present in the healthy human kidney. Immunohistochemical studies show that fibronectin-1 is mainly present in the mesangium and to a far less degree in the GBM. Staining for fibronectin-1 also occurs in the Bowman's capsule and the peritubular interstitium[27,37,39,40,59,60]. The function of fibronectin-1 in the kidney is largely unknown. *In vitro* studies using cultured fibronectin-null cell lines find that fibronectin-1 polymerization in the ECM is involved in the deposition of other ECM components, such fibulin, type III collagen, and type I collagen[61]. Fibronectin-1 possesses several domains that may function as binding sites for other molecules, including collagen and cell surface proteins such as integrins. Fibronectin-1 may connect to plasma membrane proteins which in turn are linked to the intracellular actin cytoskeleton. There is a reciprocal relationship between fibronectin-1 and the actin cytoskeleton. Agents that disrupt actin polymerization block the extracellular organization of fibronectin-1 into a network. In turn, inhibition of fibronectin-1 polymerization in the ECM induces changes in the actin cytoskeleton [62]. *In vitro* studies using cultured human podocytes show that fibronectin-1 is essential for the attachment of podocytes to the GBM during mechanical stress. Mechanical stretch induces a marked upregulation of fibronectin-1 in normal podocytes. Accordingly, in podocyte cell lines lacking fibronectin-1, a loss of podocytes greater than 80% is observed following mechanical stress[4].

An abnormal glomerular accumulation of fibronectin-1 may occur in acquired disorders, such as DKD and other diseases that feature mesangial expansion, such as lupus nephritis, IgA nephropathy, and membranoproliferative glomerulonephritis[62-65]. In addition, glomerulopathy with fibronectin-1 deposits (fibronectin nephropathy) is an autosomal dominant disease characterized by deposits of fibronectin-1 in the mesangial matrix and subendothelial space. Mutations in the *FN1* locus (that encodes fibronectin-1) at 2q32 have been identified as the genetic cause of the disease[63]. Clinical manifestations include proteinuria, hematuria, hypertension, and kidney failure that may progress to



DOI: 10.4239/wjd.v13.i7.498

Figure 2 Binding of complement factor H to components of the alternative pathway of complement (C3b) and heparan sulfate/sialic acid on "self" structures (cell membranes or basement membranes).

ESKD. Asymptomatic patients harboring *FN1* mutations have been documented. Light microscopy reveals enlarged glomeruli with deposits of eosinophilic material in the mesangium and subendothelial space that shows reactivity with periodic acid Schiff (PAS) and trichrome stains while methenamine silver and Congo red stain negative. No immunoglobulin or complement factors are detectable by immunofluorescence studies. Electron microscopy reveals a normal GBM and large electron-dense deposits in the mesangium extending to the subendothelial space. Diagnosis can be established by specific immunohistochemical analysis, as the glomerular deposits stain intensely with anti-fibronectin-1 antibodies (Table 2)[62-65].

Collagen in the normal glomerulus

Initial studies suggested the presence of collagen in normal human glomerular ECM by the high amount of glycine, hydroxyproline, and hydroxylysine in glomerular extracts[30,66-68]. Relative protein quantification confirms an abundant amount of type I, type IV, and type VI collagen in human glomerular ECM. As mentioned, type IV collagen and laminin are the most abundant proteins in the normal glomerular ECM[27].

Type I collagen in the normal glomerulus: Some studies fail to find type I collagen in normal human glomerular ECM, either the GBM or the mesangium[39,40,69]. However, mass spectrometry performed in adult kidney samples identifies abundant type I collagen in the glomerular ECM, although its localization to a specific glomerular ECM sector (GBM, mesangial matrix, or other) is undefined[27].

Type III collagen in the normal glomerulus: Type III collagen mRNA or protein have not been detected in healthy human glomeruli. Neither the mesangial matrix nor the GBM normally possess type III collagen[33,38-40,69-71]. However, type III collagen has been observed in sclerotic glomeruli, suggesting that production of this collagen type is linked to the progression of glomerular sclerosis[69]. In addition, glomerular type III collagen has been demonstrated in human kidney diseases, such as DKD, LIM homeodomain transcription factor-1β (LMX1β)-associated nephropathy (LAN) and type III collagen glomerulopathy.

Heterozygous loss of function mutations in the *LMXIβ* gene (located on chromosome 9q34) cause LAN. Patients with LAN may present with isolated nephropathy or may exhibit additional extrarenal clinical manifestations composing the nail-patella syndrome[72,73]. The LMXIβ protein is a transcription factor that possesses two LIM domains (cysteine rich sequences that usually mediate protein-protein interactions) and a homeodomain that regulates target gene transcription. The precise role of the LIM-homeodomain protein LMXIβ in humans remains unknown[74,75]. Nail-patella syndrome or onycho-osteodysplasia is characterized by the association of nail hypoplasia or dysplasia, bone abnormalities that affect the knees, elbows, and pelvis, glaucoma, sensorineural hearing impairment, and nephropathy. Renal manifestations include hematuria, proteinuria, and kidney failure that may evolve to ESKD. *LMX1β* mutations may also cause isolated autosomal dominant kidney involvement with no extrarenal manifestations[72,73,75-78]. LAN is characterized by deposition of type III collagen within the GBM on electron microscopy examination. Fibrillar type III collagen bundles may be seen occasionally in the mesangial matrix as well. The GBM may demonstrate focal irregular thickening, thinning, splitting, or wrinkling and may contain patchy electron-lucent ("moth-eaten") areas. Hyperplasia and effacement of podocyte foot processes is usually observed. The basement membrane of kidney tubules appears markedly thickened and demonstrates type III collagen deposition[78,79]. Light microscopy examination may reveal focal segmental glomerulosclerosis (FSGS) or unremarkable findings, such as mild interstitial fibrosis or mesangial proliferation. Immunofluorescence microscopy yields negative or non-specific findings, such as slight granular deposits of C3 in the mesangium. Specific immunohistochemical analyses show that the fibrillar material present within the GBM (and occasionally the mesangial matrix) is type III collagen[78-82]. The histological phenotype of LAN is expanding, as heterozygous mutations in the *LMXIβ* gene have been reported in patients with autosomal dominant FSGS without ultrastructural abnormalities of the GBM and in families with FSGS and myelin figures and zebra bodies (electron-dense multilamellar inclusions) in podocytes, mesangial

Table 2 Staining characteristics of the mesangial deposits in diabetic kidney diseases, fibronectin-1 nephropathy, and type III collagen glomerulopathy

	Periodic acid Schiff	Methenamine silver	Congo red	Specific analysis
Diabetic kidney disease	Positive	Positive	Negative	Unknown material
Fibronectin-1 nephropathy	Positive	Negative	Negative	Fibronectin-1
Type III collagen nephropathy	Negative	Negative	Negative	Type III collagen

cells, and tubular epithelium. Patients affected with LAN and myelin figures and zebra bodies are free of Fabry's disease, which is the typical cause of these inclusions. Therefore, *LMX1β* pathogenic variants should be ruled out as a potential cause of autosomal dominant kidney disease, sporadic and hereditary forms of FSGS, and steroid-resistant nephrotic syndrome, regardless of extrarenal manifestations. In addition, the presence of myelin figures or zebra bodies may hint toward LAN diagnosis in patients free of lysosomal storage disorders or drug-induced phospholipidosis, although the mechanism underlying the appearance of these structures in LAN is unclear[80,82,83].

Type III collagen glomerulopathy (collagenofibrotic glomerulopathy) is characterized by deposition of type III collagen fibrils within the mesangial matrix and along the subendothelial aspect of a normal GBM. The cause of the excessive production and deposition of type III collagen in the glomeruli is unknown. The diagnosis of the disease is confirmed by electron microscopy and specific immunohistochemistry demonstrating the presence of mesangial type III collagen. Light microscopy reveals diffuse mesangial expansion that cause glomerular enlargement. In the advanced stage, the expanded mesangium shows a lobular appearance reminiscent of Kimmelstiel-Wilson nodules of DKD. In addition, the subendothelial accumulation of this material causes double contour or "reduplication" of the GBM. Unlike DKD, the amorphous material present in the mesangium stains negative with PAS. Masson's trichrome stain identifies the blue-colored collagen within the mesangium. Immunofluorescence microscopy studies are negative for immunoglobulins and complement components. Immunohistochemistry reveals strong staining for antibodies to type III collagen in the widened mesangial and subendothelial areas. Electron microscopy reveals a normal GBM and confirms the accumulation of electron-dense fibrillar material consistent with dense collagen bundles in mesangial and subendothelial zones. The fibrils exhibit a transverse band structure with distinctive periodicity suggesting type III collagen fibers. The mesangium and subendothelium acquire a mottled appearance due to the presence of collagen fibrils[84-86]. Accumulation of mesangial type III collagen has been reported in one patient with inherited factor H deficiency[87].

Type IV collagen in the normal glomerulus: Type IV collagen is an abundant protein of the glomerular ECM and may be observed in the GBM and the mesangium. In the normal GBM, a distinct continuous staining for type IV collagen indicates that this collagen type is a predominant component[27,31,37,39,71,88].

The molecule of type IV collagen consists of three α chains. Six genes (*COL4A1-6*) encode six different α chains that create several isoforms of type IV collagen. The α3, α4, and α5 chains of type IV collagen contain more cysteine than the chains α1 and α2. Therefore, α1α1α2 (α112) trimers possess fewer disulfide bonds than α3α4α5 (α345) heterotrimers. The relative protein abundance of the α1 and α2 chains in normal adult glomerular ECM has been reported higher compared to the richness of the α3, α4, and α5 chains[27].

Ultrastructural examination with immunogold technique reveals that type IV collagen is concentrated in the endothelial zone and decreases towards the epithelial third of the GBM in normal human glomeruli. In addition, the α1 chain is distributed primarily along the endothelial side of GBM whereas the α3 and α4 chains are seen throughout the thickness of the GBM[56,89]. Kidney assessment with STORM reveals that type IV collagen α345 trimers are localized at the center of the GBM while type IV collagen α112 trimers are located to the endothelial side. Co-labeling for both trimers of type IV collagen (α345 and α112) suggest the α112 network occupies the space between the central α345 Layer and the endothelial surface of the GBM[29].

In addition to the GBM, type IV collagen is detectable in the mesangial matrix of normal human glomeruli[38-40,43,71]. Investigations using quantitative immunogold electron microscopy show that mesangial type IV collagen labeling appears uniform throughout the mesangial matrix and extends to the subendothelial side of the GBM[56,89]. Electron microscopy examination with immunogold technique shows that the α1 chain of type IV collagen is distributed primarily along the mesangial matrix and the endothelial side of GBM whereas the α3 chain of type IV collagen is not detected in normal human mesangial matrix[32].

The relevance of type IV collagen to kidney structure and function is highlighted by the clinical consequences of mutations in genes that code the α chains of this collagen type. Mutations in the *COL4A3-5* genes cause type IV collagen-related kidney disease (Table 3). The *COL4A5* gene encodes the α5 chain and maps to the X chromosome. Mutations in this gene account for X-linked Alport syndrome.

Table 3 Type IV collagen-related kidney disease

	Gene/location	Protein	Mutation	Risk of progression to end-stage kidney disease
X-linked Alport syndrome	*COL4A5*/X chromosome	α5 chain of type IV collagen	Hemizygous (males) or heterozygous (females) mutations	Hemizygous: 100%; Heterozygous: 25%
Autosomal recessive Alport syndrome	*COL4A4* or *COL4A3* /2q36-37	α4 and α3 chains of type IV collagen	Biallelic (homozygous or compound heterozygous) mutations	100%
Autosomal dominant Alport syndrome	*COL4A4* or/*COL4A3* 2q36-37	α4 and α3 chains of type IV collagen	Heterozygous mutations in the α4 or α 3 chains	20% in patients with risk factors for progression
Digenic Alport syndrome	Two of the *COL4A3-5* genes	Two of the α3-5 chains		

Males harbor hemizygous mutations whereas females carry heterozygous mutations. The *COL4A4* and *COL4A3* genes encode respectively the α4 and α3 chains of type IV collagen and are located on chromosome locus 2q36-37. Mutations in these genes cause autosomal Alport syndrome. Biallelic (homozygous or compound heterozygous) mutations in either one of them result in autosomal recessive Alport syndrome whereas autosomal dominant Alport syndrome is due to heterozygous mutations in either the *COL4A4* or *COL4A3* genes. Mutations in two of the *COL4A5*, *COL4A4*, or *COL4A3* genes cause digenic Alport syndrome[90-94].

Mutations in type IV collagen are highly prevalent. Genome-wide association studies show that 1 in 600 subjects from the Icelandic population carry a variant in the *COL4A3* gene associated with hematuria and albuminuria. In the UK population, the *COL4A4* variant rs35138315 (Ser969X) has a carrier frequency of 0.13% and is also associated with hematuria and albuminuria[95]. Among 24 Greek families with familial microscopic hematuria, next generation sequencing identifies pathogenic mutations in the *COL4A3-5* genes in 17 (71%) of them[96]. Mutations in the *COL4A3-5* genes are also frequently found in patients with sporadic and familial FSGS[94,95,97-99]. Pathogenic variants in any of the *COL4A3-5* genes are found in up to 10% of patients with renal failure of unknown cause and in some families with IgA nephropathy[94]. Therefore, indications for screening for pathogenic variants in the *COL4A5*, *COL4A4*, or *COL4A3* genes have been extended beyond the classical Alport syndrome phenotype (hematuria, renal failure, family history of hematuria or renal failure) to include FSGS, persistent proteinuria, steroid-resistant nephrotic syndrome, familial IgA nephropathy, and ESKD without an obvious cause[94].

The phenotypical expression of mutations in type IV collagen (*COL4A3-5* genes) is heterogeneous. Patients with type IV collagen-related nephropathy may exhibit isolated microscopic hematuria, proteinuria, or kidney failure that evolves to ESKD. In addition, patients with type IV collagen mutations may experience extrarenal manifestations such as sensorineural hearing loss, lenticonus, and retinopathy[90-93,97]. In patients with mutations in the *COL4A5* gene (X-linked Alport syndrome), hemizygous males have a 100% risk of progression to ESKD while heterozygous females (formerly called carriers) have substantial risk associated with proteinuria, progressive renal disease, and sensorineural hearing loss. Their lifetime risk of progression to ESKD is approximately 25%. Patients with autosomal recessive Alport syndrome (due to biallelic mutations in *COL4A4* or *COL4A3* genes) have a 100% risk of ESKD. Patients with heterozygous mutations in *COL4A4* or *COL4A3* genes (autosomal dominant Alport syndrome) may be asymptomatic or may exhibit hematuria or proteinuria and include patients previously diagnosed with thin basement membrane nephropathy. Risk factors for progression to ESKD in these subjects include proteinuria, sensorineural deafness, family history of progression to ESKD and renal biopsy findings of FSGS or GBM thickening and disarray. The risk of ESKD is up to 20% among those with risk factors. Patients with heterozygous mutations in *COL4A4* or *COL4A3* genes without kidney manifestations (hematuria or proteinuria) generally have a good prognosis but should be screened in a yearly basis[93].

The kidney histological phenotype of mutations in type IV collagen is characterized by GBM alterations, effacement of podocyte foot processes, and FSGS. Light microscopy examination of kidney samples from patients with Alport syndrome may reveal normal glomeruli or only minor mesangial widening. Immunofluorescent staining generally renders negative or nonspecific results. Electron microscopy usually provides the diagnosis, revealing changes in the GBM that may include areas of thinning, thickening, lamellation, and splitting. Initially, the GBM exhibits segmental thinning followed by progressive thickening and disorganization. In addition, diffuse podocyte foot process effacement occurs very frequently[74,97,99-101]. Patients with pathogenic variants affecting the α chains of type IV collagen may display FSGS with or without GBM changes[91,97-99,102,103].

Type V collagen in the normal glomerulus: In normal human glomeruli, type V collagen shows a distribution similar to type IV, being detectable in the GBM and the mesangium[38-40].

Type VI collagen in the normal glomerulus: In human adult glomerular tissue, mass spectrometry quantitative analyses show that type VI collagen is highly abundant[27]. In normal kidney samples, immunogold electron microscopy and immunohistochemical analyses show that the glomerular distribution of type VI collagen is comparable to that of the α1 chain of type IV collagen, namely along the mesangial matrix and the endothelial aspect of the GBM mainly[27,31,32,38,40].

Other types of collagen (type XV, type XVII and type XVIII collagen) in the normal glomerulus: Type XV collagen (α1 chain) has been found among ECM proteins in the glomerular proteome although its biological significance is uncertain[27].

Type XVII collagen is a transmembrane molecule involved in epithelial adhesion that has been identified as an autoantigen in bullous pemphigoid, a blistering skin disease of autoimmune origin. The association of bullous pemphigoid and a glomerular disease with characteristics of anti-GBM disease and membranous nephropathy has been reported in a 75-year-old man that also had circulating IgG against BP180, the 180-kDa bullous pemphigoid antigen (type XVII collagen). The kidney biopsy exhibited endocapillary inflammation without crescents. Direct immunofluorescence showed strong IgG and C3 staining in a combined granular and linear pattern along the GBM. Electron microscopy revealed subepithelial deposits[104]. In a kidney biopsy sample collected from a 4-year-old girl with hematuria, immunoelectron microscopy reveals that type XVII collagen is expressed in the foot processes of podocytes. In addition, type XVII collagen can be seen in the adjacent lamina rara externa of the GBM[105].

Type XVIII collagen has been identified among the ECM proteins in the glomerular proteome, being present in the GBM and the mesangium. Its expression pattern is similar to that of the α1 and α2 chains of type IV collagen[27,106].

Other components and factors that may modulate the normal composition of the glomerular ECM

The tubulointerstitial nephritis antigen-like-1 (TINAGL1) is highly abundant in normal glomerular ECM, being predominantly localized to the mesangial matrix. TINAGL1 is a glycoprotein structurally related to the tubulointerstitial nephritis antigen, a protein of the tubular basement membrane that is the antigenic target in autoimmune anti-tubular basement membrane disease. Nephronectin, vitronectin, fibulin-1, and fibrillin-1 have been identified as components of glomerular proteome using mass spectrometry. Nephronectin is present both in the GBM and mesangial matrix while vitronectin is localized in the mesangial matrix alone[27].

Matrix metalloproteinases and their inhibitors: Matrix metalloproteinases (MMPs) and their inhibitors are present in the ECM, but the particular isoforms distributed to the human kidney and their specific pathophysiologic role remain largely unknown. Disruption of the balance between MMPs and their inhibitors in the extracellular space has been implicated in the development of kidney fibrosis. Plasma concentration of MMPs and their inhibitors have been correlated with insulin resistance and kidney disease in clinical studies, suggesting that the composition of the ECM is altered in these conditions[107-109]. In the Renal Iohexol Clearance Survey, higher MMP-7 Levels were independently associated with increased risk of accelerated glomerular filtration rate (GFR) decline and incident chronic kidney disease among 1324 adults from the general population free of baseline diabetes, kidney disease or cardiovascular disease, over a median observation period of 5.6 years. In contrast, MMP2 and tissue inhibitor of metalloproteinase-1 (TIMP-1) showed no association with kidney disease[107]. Patients with insulin resistance display increased plasma TIMP-1 Level compared with healthy subjects. Accordingly, elevated plasma TIMP-1 concentration may be a marker of interstitial fibrosis due to excessive collagen deposition[108,109].

In vitro studies show that the expression of MMPs in the kidney ECM may be regulated at least in part by growth factors and ECM components[110,111]. In human kidney tubular cells, transforming growth factor-β1 (TGF-β1) induces MMP-2 expression *via* up-regulation of integrin-linked kinase[110], while elevated glucose concentration decreases MMP-9 and MMP-2 expression and increases TIMP-1 expression[112]. In cultured human glomerular epithelial cells, the expression of MMP-2 and MMP-9 is down-regulated by the presence of the α3 chain of type IV collagen[111], while high glucose concentration reduces MMP-2 expression and up-regulates TIMP-2[113].

Growth factors: The ECM composition is modulated by growth factors such as TGF-β1, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). TGF-β1 and PDGFs may promote ECM fibrosis in the kidney at least in part *via* integrins and integrin-associated proteins[114,115]. *In vitro* investigations using human mesangial cells show that TGF-β1 induces mesangial matrix expansion [116]. Accordingly, up-regulation of TGF-β1 is observed in the areas of interstitial and fibrosis in human fibrotic kidneys, compared with control kidneys[117]. Likewise, the expression of TGF-β1 and type IV collagen is increased in kidney allografts with interstitial fibrosis compared to normal kidney tissue [118]. VEGF and its receptors are expressed in normal human kidney, particularly in podocytes and mesangial cells. In normal podocytes, transmission electron microscopy examination reveals that VEGF may be detected in the intracellular compartment (36%) and associated with the cell membrane (63%) [119]. *In vitro* studies show that VEGF induces a proliferative effect on human mesangial cells[120,121].

The role of VEGF in glomerular pathophysiology is largely unknown, but neutralizing VEGF activity may increase the risk of kidney disease, as bevacizumab (a monoclonal antibody against human VEGF-A) therapy has been associated with elevated risk of proteinuria and hypertension among cancer patients in a systematic review and meta-analysis of clinical trials[122]. Rapamycin therapy has been associated with reduced VEGF expression in the human kidney that might contribute to explain the renal side-effects of this drug[123].

Integrins and integrin-associated proteins: Growth factors may interact with integrins to initiate signaling cascades. Integrins are plasma membrane proteins that link structurally and functionally the cell cytoskeleton with the extracellular space (Figure 3). Inside the cell, the cytoplasmic domain of integrins connects with the cytoskeleton *via* integrin-associated proteins, including integrin-linked kinase, particularly interesting new cysteine-histidine-rich protein (PINCH1), parvin proteins, and calponin homology domain-containing integrin-linked kinase-binding protein (CH-ILKBP)[114,124-128]. PINCH1 is an adaptor protein that comprises five LIM domains and interacts with integrin-linked kinase[129]. The parvins are partner proteins to integrin-linked kinase and PINCH1[130]. CH-ILKBP interacts with integrin-linked kinase, PINCH1, and the cytoskeleton. The interaction with integrin-linked kinase mediates the plasma membrane localization of CH-ILKBP. Northern blot analyses show widespread CH-ILKBP expression in human tissues, particularly in the heart, skeletal muscle, and kidney[131]. *In vitro* studies using human cell lines (HeLa cells) show that depletion of CH-ILKBP prevents the membrane translocation and the phosphorylation of protein kinase B (AKT), suggesting that CH-ILKBP facilitates the activation of this kinase in response to extracellular signals[132]. Integrins and integrin-associated proteins convey cues from growth factors and ECM components to intracellular pathways, although specific signaling cascades are not fully elucidated in humans[110,117,118]. Integrin signaling *via* integrin-associated proteins has been implicated in the regulation of ECM deposition and may be involved in the development of kidney fibrosis, both in native kidneys and kidney allografts, although underlying mechanisms remain largely unsolved[118]. An up-regulation of β1 integrin and integrin-linked kinase has been observed in areas of interstitial fibrosis in human fibrotic kidneys, compared with control kidneys[117]. *In vitro* experiments using cultured human proximal tubular cells reveal that overexpression of integrin-linked kinase and PINCH1 increases fibronectin-1 expression and its extracellular assembly, whereas PINCH1 knockdown reduces TGFβ1-mediated fibronectin-1 expression[110,124]. *In vitro* studies show that α3β1 integrin largely mediates the adhesion of human glomerular epithelial cells to type IV collagen[133]. Glucose concentration in the medium may alter integrin expression and the binding to type IV collagen in human glomerular epithelial cells[113], and human proximal tubular epithelial cells[112].

COMPOSITION OF THE GLOMERULAR ECM IN DKD

Diabetes is associated with a profound alteration in the composition of extracellular tissues throughout the body, including the kidney and the blood vessels. Patients with diabetes demonstrate increased interstitial collagen production and deposition that leads to fibrosis. Alström syndrome is an autosomal recessive disease due to mutations in the ALMS1 protein, characterized by the presence of early childhood insulin resistance. Like diabetes, patients with Alström syndrome typically show systemic fibrosis of extracellular tissues[5,36,134-136]. In patients with DKD, the amount and biochemical composition of the GBM and mesangial matrix are markedly anomalous. The global amount of glomerular ECM is increased, the level of heparan sulfate proteoglycans is reduced, and the collagen content is augmented compared to normal kidneys (Table 4)[36,134]. Furthermore, the abnormal composition of the glomerular ECM becomes more pronounced with the progression of DKD. Advanced sclerotic lesions show increased type III collagen and reduced amount of heparan sulfate proteoglycan and fibronectin-1 compared to earlier stages of DKD (Figure 4)[39,137,138].

Glomerular glycosaminoglycans, proteoglycans, and sialic acid in DKD

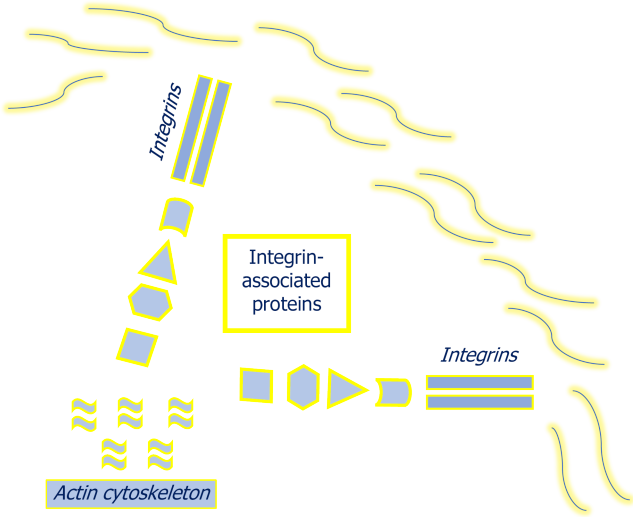
In patients with diabetes, the content of heparan sulfate in the glomerular ECM is prominently reduced while the global amount of extracellular tissue is increased. A quantitative assessment conducted by immunochemical procedures reveals that the abundance of heparan sulfate proteoglycan in the GBM of patients with diabetes is 30% lower than that of control subjects. The decrease in glomerular heparan sulfate has been also observed in other diseases, such as C3 glomerulopathy, membranous nephropathy, minimal change disease, and lupus nephritis[33,36,37,139].

The reduction in glomerular heparan sulfate proteoglycan associated with DKD starts to occur early and becomes more severe with the advance of the disorder. In patients with mild diffuse glomerulosclerosis, the staining pattern of heparan sulfate proteoglycan is reduced in the thickened mesangial matrix while in more pronounced diffuse glomerulosclerosis and mesangial nodules the enlarged matrix lacks heparan sulfate proteoglycan completely[33,39]. In contrast, the amount of hyaluronic acid is increased in the glomerular ECM of patients with DKD compared to control subjects[140]. As mentioned, heparan sulfate is a major ligand for factor H, an inhibitor of the alternative pathway of

Table 4 Different composition of glomerular extracellular matrix (glomerular basement membrane and mesangial matrix) in normal subjects and patients with diabetes

	Normal glomeruli	*Diabetic kidney disease*
Heparan sulfate proteoglycans	GBM and mesangial matrix	Decreased amount
Laminin	Predominantly in the GBM	Inconsistent
Fibronectin-1	Mainly in the mesangial matrix	It varies according to DKD stage
Type I collagen	Inconsistent	No detectable
Type III collagen	Absent	Abundant
Type IV collagen	Abundant in the GBM	Reduced GBM amount
Type V collagen	Similar to type IV collagen	Increased mesangial amount
Type VI collagen	GBM and mesangial matrix	Increased mesangial amount

GBM: Glomerular basement membrane; DKD: Diabetic kidney disease.

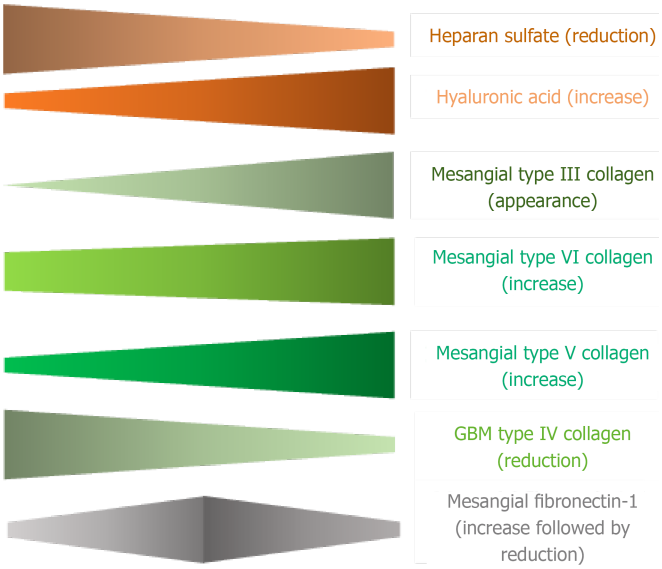


DOI: 10.4239/wjd.v13.i7.498

Figure 3 Integrins and integrin-associated proteins.

complement on "self" biological surfaces. Loss of heparan sulfate (or altered sulfation pattern) may result in reduced factor H attachment to "self" structures, subsequent activation of the alternative pathway and complement-mediated injury, like occurs in the presence of mutated factor H (C3 glomerulopathy). Complement activation *via* the alternative pathway may contribute to the progression of renal and vascular complications in human diabetes. In patients with biopsy-proven DKD, a higher level of factor H in the urine has been independently associated with worse kidney outcomes, including onset of ESKD and faster kidney function decline, compared to control subjects[141]. Further, clinical studies have shown an association between single nucleotide polymorphisms in factor H and adverse clinical outcomes in different population groups of non-diabetic and diabetic patients[142,143]. In African American patients, genetic changes in factor H gene, such as the intronic variant rs379489, have been associated with ESKD in both non-diabetic and type 2 diabetes (T2D), compared to controls[142]. In 1158 T2D patients prospectively followed in the randomized controlled trial Bergamo Nephrologic Complications of T2D (BENEDICT), the single nucleotide polymorphism in the factor H gene c.2808G>T (p.Glu936Asp) is independently associated with increased risk of microalbuminuria and cardiovascular complications (Asp/Asp homozygotes, recessive model). T2D patients Asp/Asp homozygotes are at increased risk of microalbuminuria and cardiovascular events compared to carriers of one or two wild type Glu alleles[143].

Among patients with diabetes, the reported amount of glomerular sialic acid has been inconsistent. A decline in the content of sialic acid has been detected in the glomerular ECM of patients with diabetes, compared to normal kidney samples[68,144]. However, an increased expression of sialic acid on podocytes has been observed in patients with DKD and other kidney diseases without differences among them[44].



DOI: 10.4239/wjd.v13.i7.498

Figure 4 Schematic variation in some components of the glomerular extracellular matrix according to the progression of diabetic kidney disease. GBM: Glomerular basement membrane.

A study that applied weighted gene co-expression network analysis to 179 human glomeruli reveals that two small leucine-rich proteoglycans (lumican and fibromodulin) are more abundant in the ECM of patients with DKD compared to controls and other glomerular diseases, such as IgA nephropathy or membranous nephropathy. Further, the expression level of lumican and fibromodulin is negatively correlated with kidney function. The specificity of lumican and fibromodulin in kidney samples from patients with DKD in comparison to normal specimens and patients with other glomerular diseases suggests that these ECM components may become potential diagnostic biomarkers for DKD[14].

Glomerular laminin in DKD

The laminin content of the glomerular ECM in patients with diabetes has been barely reported and the values are variable[37,39]. In human kidneys obtained at autopsy, there is a marked reduction in laminin content in the diabetic GBM compared to non-diabetic control subjects. Radioimmunoassays indicated that GBM from patients with diabetes contains average values of laminin that were 60% of control subjects[37]. However, immunohistochemical studies show an increased glomerular deposition of laminin in kidney biopsy samples from type 1 diabetes (T1D) and T2D patients with diffuse and nodular glomerulosclerosis[39].

Glomerular fibronectin-1 in DKD

Immunohistochemical studies reveal that the amount of mesangial fibronectin-1 is abnormal in patients with diabetes and varies with the advance of DKD. Antibodies to fibronectin-1 normally stain the mesangium and the subendothelial aspect of the GBM. Early lesions of mesangial expansion are associated with increased staining for fibronectin-1. However, a marked diminution in fluorescent intensity for fibronectin-1 is documented in more advanced mesangial enlargement (nodular lesions). Compared with normal tissues and early lesions of DKD, the progression of the disease is associated with a noticeable reduction in the amount of glomerular fibronectin-1[39,40,137]. The increase in mesangial fibronectin-1 that occurs in the early stage of DKD also takes place in other glomerular diseases characterized by mesangial expansion, such as mesangiocapillary glomerulonephritis[59,60]. An up-regulation of fibronectin-1 expression in the glomeruli has been also observed in patients with hypertension compared to normal kidneys[4]. Likewise, the amount of fibronectin-1 in vascular tissue is increased in patients with diabetes before the development of atherosclerosis lesions[59,145]. The content of fibronectin-1 in the intima-media of normal aorta specimens is more elevated in patients with diabetes (T1D and T2D) compared to control subjects, suggesting that diabetic patients develop structural alterations in the connective tissue of their arteries before the appearance of vascular disease [145]. In patients with DKD, the thickened capillary walls also contain a markedly elevated amount of fibronectin-1[59].

Glomerular collagen in DKD

Earlier studies found elevated glomerular ECM levels of glycine, hydroxyproline, hydroxylysine, and hexoses in patients with diabetes compared to normal kidney samples, suggesting an increase in the amount of collagen in the glomerular ECM from diabetic patients[30,36,66-68]. Radioimmunoassays

confirmed collagen enrichment in the glomerular ECM of patients with diabetes[37]. Accordingly, electron microscopy examination shows accumulation of collagen fibrils in the mesangium of patients with DKD[146].

Glomerular type I collagen in DKD: No glomerular type I collagen has been detected in DKD at any stage of the disorder[33,39,40,137,147].

Glomerular type III collagen in DKD: Unlike normal glomeruli, type III collagen is identified in the mesangium of patients with DKD and its amount increases gradually with the progression of the disease. Early DKD lesions (diffuse glomerulosclerosis) show positive staining for type III collagen that increases in more advanced mesangial nodular lesions. In the late stage of global sclerosis, type III collagen is diffusely present in the sclerotic mesangial matrix. Therefore, *de novo* synthesis of type III collagen in glomeruli occurs in patients with DKD[33,39,40,70,71]. A patient with T1D and collagenofibrotic glomerulopathy has been reported[148].

Glomerular type IV collagen in DKD: In patients with diabetes, immunohistochemical estimates of type IV collagen in the GBM reveal reduced staining compared to normal tissue. Accordingly, the density of gold particles for type IV collagen is decreased in the GBM of T1D patients on quantitative immunogold electron microscopy examination. Like in normal subjects, the labeling of antibody against type IV collagen in the GBM is concentrated in the endothelial zone and decreases towards the epithelial aspect of the GMB in diabetic patients[33,89].

In the mesangial matrix, immunohistochemical studies show that the amount of type IV collagen changes according to the stage of DKD. In earlier lesions of diffuse glomerulosclerosis, mesangial staining for type IV collagen is increased while more advanced nodular glomerulosclerosis showed marked reduction in the mesangial staining for type IV collagen, suggesting that type IV collagen is progressively substituted for other collagen types such as type VI and type III during the transition from diffuse to nodular glomerulosclerosis[39,40,89]. However, an elevated mesangial staining for type IV collagen has been observed in specific nodular lesions, called non-mesangiolytic nodules, compared to normal kidney[33,71]. The amount of type IV collagen in nodular lesions may depend on the type of the lesion, mesangiolytic or non-mesangiolytic. In a study aimed to investigate collagen staining of mesangial nodules from 67 patients with DKD, type IV collagen staining was only robust in nodular lesions with strong PAS/periodic acid methenamine silver (PAMS) staining (non-mesangiolytic nodular lesions). In contrast, nodular lesions with faint PAS/PAMS staining (mesangiolytic nodular lesions) did not show type IV collagen. The amount of type IV collagen correlates with the PAS and PAMS staining pattern. Non-mesangiolytic nodules (with prominent PAS/PAMS staining) are strongly positive for type IV collagen whereas mesangiolytic nodules (with weak or negative PAS/PAMS staining) show weak or negative staining for type IV collagen[147]. Immunofluorescence studies performed in 918 kidney biopsy samples from patients with diabetes (T1D and T2D) show accumulation of α3 and α5 chains of type IV collagen in diffuse mesangial sclerosis while minimal amounts of these α3 and α5 chains were seen within the mesangium of control subjects[43].

In patients with diabetes, two large clinical investigations with different population groups (African American and European descent subjects) have shown that genetic variants in the gene that codes the α3 chain of type IV collagen (*COL4A3*) may modulate susceptibility to DKD and ESKD[149]. In 4885 African American patients with T2D, an association between the genetic variant R408H (rs34505188) in *COL4A3* and ESKD has been observed, suggesting that genetic changes in the *COL4A3* locus may contribute to ESKD susceptibility in patients with diabetes[149]. In 19406 T1D patients of European descent from 17 cohorts, a genome-wide association meta-analysis reveals that a single nucleotide polymorphism in the *COL4A3* gene is associated with protection from DKD (proteinuria and ESKD)[150].

Glomerular type V collagen in DKD: Immunohistochemical studies have documented an enrichment in mesangial type V collagen in diffuse glomerulosclerosis and nodular lesions in patients with DKD compared to control subjects. Increased staining for type V collagen is observed in advanced mesangial disease, compared to normal tissues and early mesangial disease.

Staining for type V collagen was strongly positive in all nodular lesions, mesangiolytic and non-mesangiolytic[39,40,137,147].

Glomerular type VI collagen in DKD: In patients with DKD, the amount of mesangial type VI collagen is elevated. Quantitation by radioimmunoassay reveals that the level of type VI collagen is 2.8-fold higher in the diabetic preparations compared to control subjects. Furthermore, the amount of mesangial type VI collagen increases with the progression of DKD. In earlier lesions of diffuse glomerulosclerosis, the contribution of type VI collagen deposition to the overall matrix expansion is minor. However, type VI collagen is a major component in the expanded mesangial matrix of nodular glomerulosclerosis. A marked increase in type VI collagen deposition is observed in nodular lesions where the strong positivity for type VI collagen is evenly distributed throughout the entire nodules[31,39,40,147].

Other factors that contribute to modulate the composition of kidney ECM in DKD

As kidney ECM remodeling is profoundly altered in patients with DKD, the expression of MMPs, TIMPs, integrins, integrin-associated proteins, and signaling pathways from growth factors have been reported abnormal among these patients. In addition, the nuclear factor-kappa-B (NF-κB) family of transcription factors and advanced glycation end-products (AGEs) have been proposed as potential contributors to the ECM disturbance present in patients with DKD.

MMPs and their inhibitors in patients with DKD: The expression of MMPs and their inhibitors is altered in patients with DKD. Clinical studies have suggested that these ECM components might be useful to evaluate the risk for cardiovascular disease, kidney disease, and all-cause mortality among patients with diabetes. In a cross-sectional study, T1D patients with cardiovascular disease showed higher levels of TIMP-1 compared to T1D patients without cardiovascular disease[151]. In a prospective study that followed 337 T1D patients for a median period of 12.3 years, elevated MMP-2 plasma levels were associated with higher incidence of cardiovascular events, but this relationship was attenuated after adjustment for estimated GFR, suggesting that kidney function may mediate the association[152]. In a cross-sectional pooled analysis of three groups of T1D patients, circulating MMP-1, MMP-2, and MMP-3 Levels were associated with arterial stiffening independent of confounding factors while no association with TIMPs was observed[153]. In a case-control study that evaluated 120 control women and 120 women with a history of gestational diabetes 3.7 years after delivery, both serum TIMP-1 Levels and arterial stiffness were higher in subjects with previous gestational diabetes compared to control individuals[154]. In T1D patients, MMPs and their inhibitors have been associated with albuminuria in a cross-sectional study[151], and with kidney function decline in a prospective study[152]. In a prospective observational cohort study, urinary excretion of MMP-7 was independently associated with higher mortality rate over a median follow-up period of 3.0 years, in T2D patients with DKD. In contrast, no association between serum MMP-7 Level and mortality was observed[155]. In T1D patients, the association of MMP-1, MMP-2 and MMP-3 with all-cause mortality was attenuated after adjustment for estimated GFR, suggesting that the known association between kidney function and mortality may mediate the relation between MMPs and death[152].

However, MMPs expression in glomeruli may be altered in other glomerular diseases, such as IgA nephropathy, which is associated with extensive changes of the glomerular ECM proteome, including higher abundance of MMP-9, MMP-2, α1 chain of type IV collagen, fibronectin, and β1-laminin[156].

Growth factors (TGF-β, PDGF, and VEGF) in patients with DKD: In T2D patients with albuminuria, serum TGF-β1 Level is higher compared to healthy controls and T2D patients with normal urinary albumin excretion rate, suggesting that serum TGF-β1 might be used to evaluate progression of DKD [157,158]. Several meta-analyses indicate a potential value of serum TGF-β1 Levels to evaluate the risk of DKD and the advance of the disease[159-161]. However, the administration of a neutralizing monoclonal antibody against TGF-β1 to T1D and T2D patients with DKD failed to slow progression of the disease compared to placebo in a randomized controlled clinical trial, suggesting that TGF-β1 is not a major determinant of kidney function decline in patients with diabetes[162].

The glomerular expression of PDGF-B and its receptor (PDGFR-β) is higher in T2D patients with DKD compared to normal kidneys, particularly in samples with mild mesangial expansion. In contrast, the expression of PDGF-A and its receptor (PDGFR-α) is comparable in normal kidneys and patients with DKD[163].

In patients with DKD, VEGF-A expression is lower compared to normal kidney tissue[164]. VEGF signaling has been reported to be differentially regulated in patients with DKD in a study that examined gene-expression changes in human DKD[165]. However, in a prospective study that recruited 155 T1D patients with proteinuria, plasma VEGF failed to predict kidney function decline over 3-year follow-up [166].

Integrins and integrin-associated proteins in patients with DKD: Glomerular integrin and integrin-linked kinase signaling pathways have been found differentially regulated in patients with DKD[165]. In addition, the expression of integrin-linked kinase in the mesangium is increased in kidney specimens from patients with diabetes and diffuse mesangial expansion, compared to control samples. In contrast, integrin-linked kinase level is reduced in glomeruli with advanced nodular sclerosis and global sclerosis, suggesting that integrin-linked kinase expression increases during early stages of DKD[114].

NF-κB in patients with DKD: NF-κB is a transcription factor that regulates the expression of several genes. NF-κB-inducing kinase activates the NF-κB signaling pathway. Dysregulation of NF-κB signaling has been implicated in DKD, but its role remains uncertain. In vitro studies using human proximal tubular epithelial cells (HK-2 cells) suggest a role for NF-κB pathway in modulating diabetes-induced disease in renal tubular epithelium[167,168].

Advanced glycation products in patients with DKD: AGEs are molecules that result from nonenzymatic glycation of proteins and lipids. They may bind to cell surface receptors (RAGEs). AGEs have been hypothesized to be involved in the development of human DKD, but their participation remain undefined. In kidney biopsies from patients with DKD, AGEs are detected in the expanded

mesangial matrix while they are not identified in control samples[169,170]. In addition, AGEs are identified in areas of glomerulosclerosis and arteriosclerosis in other diseases, such as FSGS, hypertensive nephrosclerosis, and lupus nephritis[169]. RAGE expression was detected in mesangial cells and glomerular epithelial cells, in both patients with DKD and control subjects[170]. In a cross-sectional study, the level of AGEs was positively associated with serum concentration of MMP-2, MMP3, and TIMP-1 while an inverse association with MMP-9 was observed in T1D patients[171].

CONCLUSION

Understanding mechanisms that regulate glomerular ECM injury and repair may contribute to develop therapeutic strategies for DKD and other kidney diseases. During adult life, mesangial cells produce mesangial matrix. The turnover of the GBM present at birth is unknown. Podocyte foot processes surround and attach entirely the GBM. Adult podocytes may sustain hypertrophy following the loss of adjacent cells to prevent bared GBM areas that compromise the filtration barrier. Glycosaminoglycans, such as heparan sulfate and hyaluronic acid, are major constituents of the glomerular ECM. The specific pattern of sulfation of glycosaminoglycans allows the identification of these molecules as "self" by complement components and avoid complement-mediated self-damage. Sialic acid is also present in glomerular ECM and may serve to a similar function. Fibronectin-1 is important for the normal deposition of other ECM components, such as collagen. Type IV, V, and VI collagens are predominant types of collagen normally present in the glomerular ECM while type III collagen appears in diseased states, such as diabetes and glomerulosclerosis. The composition and arrangement of the glomerular ECM is profoundly altered in patients with diabetes. The global quantity of glomerular ECM is increased while the amount of sulfated proteoglycans is reduced and hyaluronic acid is augmented, compared to control tissue. Fibronectin-1 is increased in early lesions of mesangial expansion. Likewise, the amount of fibronectin-1 in capillary walls and aorta is increased before the development of vascular disease in patients with diabetes. Mesangial type III, type V, and type VI collagen amount is elevated in patients with DKD and increases progressively with the advance of the disease. Genetic variants in the gene that codes the α3 chain of type IV collagen (*COL4A3*) may modulate susceptibility to DKD and ESKD.

ACKNOWLEDGEMENTS

We are very grateful to Ms. Gema Souto, MHS for her help and support during the writing of this manuscript.

FOOTNOTES

Author contributions: Adeva-Andany MM conceived and designed the review, performed the literature search and wrote the original draft of the manuscript; Carneiro-Freire N contributed to the writing and revision of intellectual content; both authors participated in the final version of the manuscript.

Conflict-of-interest statement: The authors declare that there is no conflict of interest.

Country/Territory of origin: Suriname

ORCID number: María M Adeva-Andany 0000-0002-9997-2568; Natalia Carneiro-Freire 0000-0003-4404-0541.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Saleem MA**, O'Hare MJ, Reiser J, Coward RJ, Inward CD, Farren T, Xing CY, Ni L, Mathieson PW, Mundel P. A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 2002; **13**: 630-638 [PMID: 11856766 DOI: 10.1681/ASN.V133630]

2 **Faul C**, Asanuma K, Yanagida-Asanuma E, Kim K, Mundel P. Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton. *Trends Cell Biol* 2007; **17**: 428-437 [PMID: 17804239 DOI: 10.1016/j.tcb.2007.06.006]

3 **Pollak MR**. Familial FSGS. *Adv Chronic Kidney Dis* 2014; **21**: 422-425 [PMID: 25168831 DOI: 10.1053/j.ackd.2014.06.001]

4 **Kliewe F**, Kaling S, Lötzsch H, Artelt N, Schindler M, Rogge H, Schröder S, Scharf C, Amann K, Daniel C, Lindenmeyer MT, Cohen CD, Endlich K, Endlich N. Fibronectin is up-regulated in podocytes by mechanical stress. *FASEB J* 2019; **33**: 14450-14460 [PMID: 31675484 DOI: 10.1096/fj.201900978RR]

5 **Baig S**, Paisey R, Dawson C, Barrett T, Maffei P, Hodson J, Rambhatla SB, Chauhan P, Bolton S, Dassie F, Francomano C, Marshall RP, Belal M, Skordilis K, Hayer M, Price AM, Cramb R, Edwards N, Steeds RP, Geberhiwot T. Defining renal phenotype in Alström syndrome. *Nephrol Dial Transplant* 2020; **35**: 994-1001 [PMID: 30307515 DOI: 10.1093/ndt/gfy293]

6 **Riemer T**, Nimptsch A, Nimptsch K, Schiller J. Determination of the glycosaminoglycan and collagen contents in tissue samples by high-resolution 1H NMR spectroscopy after DCl-induced hydrolysis. *Biomacromolecules* 2012; **13**: 2110-2117 [PMID: 22713080 DOI: 10.1021/bm300485j]

7 **Bergholt MS**, Serio A, Albro MB. Raman Spectroscopy: Guiding Light for the Extracellular Matrix. *Front Bioeng Biotechnol* 2019; **7**: 303 [PMID: 31737621 DOI: 10.3389/fbioe.2019.00303]

8 **Sherlock BE**, Chen J, Mansfield JC, Green E, Winlove CP. Biophotonic tools for probing extracellular matrix mechanics. *Matrix Biol Plus* 2021; **12**: 100093 [PMID: 34934939 DOI: 10.1016/j.mbplus.2021.100093]

9 **Reimann C**, Brangsch J, Colletini F, Walter T, Hamm B, Botnar RM, Makowski MR. Molecular imaging of the extracellular matrix in the context of atherosclerosis. *Adv Drug Deliv Rev* 2017; **113**: 49-60 [PMID: 27639968 DOI: 10.1016/j.addr.2016.09.005]

10 **Chow MJ**, Turcotte R, Lin CP, Zhang Y. Arterial extracellular matrix: a mechanobiological study of the contributions and interactions of elastin and collagen. *Biophys J* 2014; **106**: 2684-2692 [PMID: 24940786 DOI: 10.1016/j.bpj.2014.05.014]

11 **Pezzoli D**, Di Paolo J, Kumra H, Fois G, Candiani G, Reinhardt DP, Mantovani D. Fibronectin promotes elastin deposition, elasticity and mechanical strength in cellularised collagen-based scaffolds. *Biomaterials* 2018; **180**: 130-142 [PMID: 30036726 DOI: 10.1016/j.biomaterials.2018.07.013]

12 **Feng Q**, Li D, Li Q, Cao X, Dong H. Microgel assembly: Fabrication, characteristics and application in tissue engineering and regenerative medicine. *Bioact Mater* 2022; **9**: 105-119 [PMID: 34820559 DOI: 10.1016/j.bioactmat.2021.07.020]

13 **Suh EJ**, Remillard MY, Legesse-Miller A, Johnson EL, Lemons JM, Chapman TR, Forman JJ, Kojima M, Silberman ES, Coller HA. A microRNA network regulates proliferative timing and extracellular matrix synthesis during cellular quiescence in fibroblasts. *Genome Biol* 2012; **13**: R121 [PMID: 23259597 DOI: 10.1186/gb-2012-13-12-r121]

14 **Feng S**, Gao Y, Yin D, Lv L, Wen Y, Li Z, Wang B, Wu M, Liu B. Identification of Lumican and Fibromodulin as Hub Genes Associated with Accumulation of Extracellular Matrix in Diabetic Nephropathy. *Kidney Blood Press Res* 2021; **46**: 275-285 [PMID: 33887734 DOI: 10.1159/000514013]

15 **Wang Z**, Chen X, Li C, Tang W. Application of weighted gene co-expression network analysis to identify novel key genes in diabetic nephropathy. *J Diabetes Investig* 2022; **13**: 112-124 [PMID: 34245661 DOI: 10.1111/jdi.13628]

16 **Chen FM**, Liu X. Advancing biomaterials of human origin for tissue engineering. *Prog Polym Sci* 2016; **53**: 86-168 [PMID: 27022202 DOI: 10.1016/j.progpolymsci.2015.02.004]

17 **Daley WP**, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. *J Cell Sci* 2008; **121**: 255-264 [PMID: 18216330 DOI: 10.1242/jcs.006064]

18 **Karamanos NK**, Theocharis AD, Piperigkou Z, Manou D, Passi A, Skandalis SS, Vynios DH, Orian-Rousseau V, Ricard-Blum S, Schmelzer CEH, Duca L, Durbeej M, Afratis NA, Troeberg L, Franchi M, Masola V, Onisto M. A guide to the composition and functions of the extracellular matrix. *FEBS J* 2021; **288**: 6850-6912 [PMID: 33605520 DOI: 10.1111/febs.15776]

19 **Chiang CE**, Fang YQ, Ho CT, Assunção M, Lin SJ, Wang YC, Blocki A, Huang CC. Bioactive Decellularized Extracellular Matrix Derived from 3D Stem Cell Spheroids under Macromolecular Crowding Serves as a Scaffold for Tissue Engineering. *Adv Healthc Mater* 2021; **10**: e2100024 [PMID: 33890420 DOI: 10.1002/adhm.202100024]

20 **Wilks BT**, Evans EB, Howes A, Hopkins CM, Nakhla MN, Williams G, Morgan JR. Quantifying Cell-Derived Changes in Collagen Synthesis, Alignment, and Mechanics in a 3D Connective Tissue Model. *Adv Sci (Weinh)* 2022; **9**: e2103939 [PMID: 35102708 DOI: 10.1002/advs.202103939]

21 **Assunção M**, Yiu CHK, Wan HY, Wang D, Ker DFE, Tuan RS, Blocki A. Hyaluronic acid drives mesenchymal stromal cell-derived extracellular matrix assembly by promoting fibronectin fibrillogenesis. *J Mater Chem B* 2021; **9**: 7205-7215 [PMID: 33710248 DOI: 10.1039/d1tb00268f]

22 **Lin S**, Mequanint K. Bioreactor-induced mesenchymal progenitor cell differentiation and elastic fiber assembly in engineered vascular tissues. *Acta Biomater* 2017; **59**: 200-209 [PMID: 28690007 DOI: 10.1016/j.actbio.2017.07.012]

23 **Urbanczyk M**, Layland SL, Schenke-Layland K. The role of extracellular matrix in biomechanics and its impact on bioengineering of cells and 3D tissues. *Matrix Biol* 2020; **85-86**: 1-14 [PMID: 31805360 DOI: 10.1016/j.matbio.2019.11.005]

24 **Abraham VC**, Miller LN, Pratt SD, Putman B, Kim L, Gopalakrishnan SM, King A. Implementation of a human podocyte injury model of chronic kidney disease for profiling of renoprotective compounds. *Eur J Pharmacol* 2017; **815**: 219-232 [PMID: 28899697 DOI: 10.1016/j.ejphar.2017.09.006]

25 **Yoshida Y**, Miyazaki K, Kamiie J, Sato M, Okuizumi S, Kenmochi A, Kamijo K, Nabetani T, Tsugita A, Xu B, Zhang Y, Yaoita E, Osawa T, Yamamoto T. Two-dimensional electrophoretic profiling of normal human kidney glomerulus

proteome and construction of an extensible markup language (XML)-based database. *Proteomics* 2005; **5**: 1083-1096 [PMID: 15668994 DOI: 10.1002/pmic.200401075]

26 **Miyamoto M**, Yoshida Y, Taguchi I, Nagasaka Y, Tasaki M, Zhang Y, Xu B, Nameta M, Sezaki H, Cuellar LM, Osawa T, Morishita H, Sekiyama S, Yaoita E, Kimura K, Yamamoto T. In-depth proteomic profiling of the normal human kidney glomerulus using two-dimensional protein prefractionation in combination with liquid chromatography-tandem mass spectrometry. *J Proteome Res* 2007; **6**: 3680-3690 [PMID: 17711322 DOI: 10.1021/pr070203n]

27 **Lennon R**, Byron A, Humphries JD, Randles MJ, Carisey A, Murphy S, Knight D, Brenchley PE, Zent R, Humphries MJ. Global analysis reveals the complexity of the human glomerular extracellular matrix. *J Am Soc Nephrol* 2014; **25**: 939-951 [PMID: 24436468 DOI: 10.1681/ASN.2013030233]

28 **Hobeika L**, Barati MT, Caster DJ, McLeish KR, Merchant ML. Characterization of glomerular extracellular matrix by proteomic analysis of laser-captured microdissected glomeruli. *Kidney Int* 2017; **91**: 501-511 [PMID: 27988214 DOI: 10.1016/j.kint.2016.09.044]

29 **Suleiman H**, Zhang L, Roth R, Heuser JE, Miner JH, Shaw AS, Dani A. Nanoscale protein architecture of the kidney glomerular basement membrane. *Elife* 2013; **2**: e01149 [PMID: 24137544 DOI: 10.7554/eLife.01149]

30 **Beisswenger PJ**, Spiro RG. Studies on the human glomerular basement membrane. Composition, nature of the carbohydrate units and chemical changes in diabetes mellitus. *Diabetes* 1973; **22**: 180-193 [PMID: 4347583 DOI: 10.2337/diab.22.3.180]

31 **Mohan PS**, Carter WG, Spiro RG. Occurrence of type VI collagen in extracellular matrix of renal glomeruli and its increase in diabetes. *Diabetes* 1990; **39**: 31-37 [PMID: 2210058 DOI: 10.2337/diacare.39.1.31]

32 **Zhu D**, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM. Application of electron microscopic immunocytochemistry to the human kidney: distribution of type IV and type VI collagen in normal human kidney. *J Histochem Cytochem* 1994; **42**: 577-584 [PMID: 8157929 DOI: 10.1177/42.5.8157929]

33 **Tamsma JT**, van den Born J, Bruijn JA, Assmann KJ, Weening JJ, Berden JH, Wieslander J, Schrama E, Hermans J, Veerkamp JH. Expression of glomerular extracellular matrix components in human diabetic nephropathy: decrease of heparan sulphate in the glomerular basement membrane. *Diabetologia* 1994; **37**: 313-320 [PMID: 8174847 DOI: 10.1007/bf00398060]

34 **Groffen AJ**, Ruegg MA, Dijkman H, van de Velden TJ, Buskens CA, van den Born J, Assmann KJ, Monnens LA, Veerkamp JH, van den Heuvel LP. Agrin is a major heparan sulfate proteoglycan in the human glomerular basement membrane. *J Histochem Cytochem* 1998; **46**: 19-27 [PMID: 9405491 DOI: 10.1177/002215549804600104]

35 **Murata K**. Acidic glycosaminoglycans in human kidney tissue. *Clin Chim Acta* 1975; **63**: 157-169 [PMID: 126123 DOI: 10.1016/0009-8981(75)90158-8]

36 **Parthasarathy N**, Spiro RG. Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 1982; **31**: 738-741 [PMID: 6219021 DOI: 10.2337/diab.31.8.738]

37 **Shimomura H**, Spiro RG. Studies on macromolecular components of human glomerular basement membrane and alterations in diabetes. Decreased levels of heparan sulfate proteoglycan and laminin. *Diabetes* 1987; **36**: 374-381 [PMID: 2948855 DOI: 10.2337/diab.36.3.374]

38 **Oomura A**, Nakamura T, Arakawa M, Ooshima A, Isemura M. Alterations in the extracellular matrix components in human glomerular diseases. *Virchows Arch A Pathol Anat Histopathol* 1989; **415**: 151-159 [PMID: 2500770 DOI: 10.1007/bf00784353]

39 **Nerlich A**, Schleicher E. Immunohistochemical localization of extracellular matrix components in human diabetic glomerular lesions. *Am J Pathol* 1991; **139**: 889-899 [PMID: 1928305]

40 **Nerlich AG**, Schleicher ED, Wiest I, Specks U, Timpl R. Immunohistochemical localization of collagen VI in diabetic glomeruli. *Kidney Int* 1994; **45**: 1648-1656 [PMID: 7933812 DOI: 10.1038/ki.1994.216]

41 **Groffen AJ**, Buskens CA, van Kuppevelt TH, Veerkamp JH, Monnens LA, van den Heuvel LP. Primary structure and high expression of human agrin in basement membranes of adult lung and kidney. *Eur J Biochem* 1998; **254**: 123-128 [PMID: 9652404 DOI: 10.1046/j.1432-1327.1998.2540123.x]

42 **Groffen AJ**, Hop FW, Tryggvason K, Dijkman H, Assmann KJ, Veerkamp JH, Monnens LA, Van den Heuvel LP. Evidence for the existence of multiple heparan sulfate proteoglycans in the human glomerular basement membrane and mesangial matrix. *Eur J Biochem* 1997; **247**: 175-182 [PMID: 9249024 DOI: 10.1111/j.1432-1033.1997.00175.x]

43 **Kriz W**, Löwen J, Federico G, van den Born J, Gröne E, Gröne HJ. Accumulation of worn-out GBM material substantially contributes to mesangial matrix expansion in diabetic nephropathy. *Am J Physiol Renal Physiol* 2017; **312**: F1101-F1111 [PMID: 28228399 DOI: 10.1152/ajprenal.00020.2017]

44 **Babal P**, Slugen I, Danis D, Zaviacic M, Gardner WA Jr. Sialic acid expression in normal and diseased human kidney. *Acta Histochem* 1996; **98**: 71-77 [PMID: 9054191]

45 **Bowles WHD**, Gloster TM. Sialidase and Sialyltransferase Inhibitors: Targeting Pathogenicity and Disease. *Front Mol Biosci* 2021; **8**: 705133 [PMID: 34395532 DOI: 10.3389/fmolb.2021.705133]

46 **Fearon DT**. Regulation by membrane sialic acid of beta1H-dependent decay-dissociation of amplification C3 convertase of the alternative complement pathway. *Proc Natl Acad Sci U S A* 1978; **75**: 1971-1975 [PMID: 273923 DOI: 10.1073/pnas.75.4.1971]

47 **Blackmore TK**, Sadlon TA, Ward HM, Lublin DM, Gordon DL. Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H. *J Immunol* 1996; **157**: 5422-5427 [PMID: 8955190]

48 **Ram S**, Sharma AK, Simpson SD, Gulati S, McQuillen DP, Pangburn MK, Rice PA. A novel sialic acid binding site on factor H mediates serum resistance of sialylated Neisseria gonorrhoeae. *J Exp Med* 1998; **187**: 743-752 [PMID: 9480984 DOI: 10.1084/jem.187.5.743]

49 **Hellwage J**, Jokiranta TS, Friese MA, Wolk TU, Kampen E, Zipfel PF, Meri S. Complement C3b/C3d and cell surface polyanions are recognized by overlapping binding sites on the most carboxyl-terminal domain of complement factor H. *J Immunol* 2002; **169**: 6935-6944 [PMID: 12471127 DOI: 10.4049/jimmunol.169.12.6935]

50 **Feyzi E**, Saldeen T, Larsson E, Lindahl U, Salmivirta M. Age-dependent modulation of heparan sulfate structure and function. *J Biol Chem* 1998; **273**: 13395-13398 [PMID: 9593669 DOI: 10.1074/jbc.273.22.13395]

51 **Pangburn MK**, Müller-Eberhard HJ. Complement C3 convertase: cell surface restriction of beta1H control and generation of restriction on neuraminidase-treated cells. *Proc Natl Acad Sci U S A* 1978; **75**: 2416-2420 [PMID: 276881 DOI: 10.1073/pnas.75.5.2416]

52 **Meri S**, Pangburn MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation *via* a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci U S A* 1990; **87**: 3982-3986 [PMID: 1692629 DOI: 10.1073/pnas.87.10.3982]

53 **Blackmore TK**, Hellwage J, Sadlon TA, Higgs N, Zipfel PF, Ward HM, Gordon DL. Identification of the second heparin-binding domain in human complement factor H. *J Immunol* 1998; **160**: 3342-3348 [PMID: 9531293]

54 **Loeven MA**, Rops AL, Berden JH, Daha MR, Rabelink TJ, van der Vlag J. The role of heparan sulfate as determining pathogenic factor in complement factor H-associated diseases. *Mol Immunol* 2015; **63**: 203-208 [PMID: 25246018 DOI: 10.1016/j.molimm.2014.08.005]

55 **Colognato H**, Winkelmann DA, Yurchenco PD. Laminin polymerization induces a receptor-cytoskeleton network. *J Cell Biol* 1999; **145**: 619-631 [PMID: 10225961 DOI: 10.1083/jcb.145.3.619]

56 **Smith PS**, Fanning JC, Aarons I. The structure of the normal human glomerular basement membrane. Ultrastructural localization of type IV collagen and laminin. *Pathology* 1989; **21**: 254-258 [PMID: 2633114 DOI: 10.3109/00313028909061069]

57 **Zenker M**, Aigner T, Wendler O, Tralau T, Müntefering H, Fenski R, Pitz S, Schumacher V, Royer-Pokora B, Wühl E, Cochat P, Bouvier R, Kraus C, Mark K, Madlon H, Dötsch J, Rascher W, Maruniak-Chudek I, Lennert T, Neumann LM, Reis A. Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet* 2004; **13**: 2625-2632 [PMID: 15367484 DOI: 10.1093/hmg/ddh284]

58 **Hasselbacher K**, Wiggins RC, Matejas V, Hinkes BG, Mucha B, Hoskins BE, Ozaltin F, Nürnberg G, Becker C, Hangan D, Pohl M, Kuwertz-Bröking E, Griebel M, Schumacher V, Royer-Pokora B, Bakkaloglu A, Nürnberg P, Zenker M, Hildebrandt F. Recessive missense mutations in LAMB2 expand the clinical spectrum of LAMB2-associated disorders. *Kidney Int* 2006; **70**: 1008-1012 [PMID: 16912710 DOI: 10.1038/sj.ki.5001679]

59 **Weiss MA**, Ooi BS, Ooi YM, Engvall E, Ruoslahti E. Immunofluorescent localization of fibronectin in the human kidney. *Lab Invest* 1979; **41**: 340-347 [PMID: 385978]

60 **Dixon AJ**, Burns J, Dunnill MS, McGee JO. Distribution of fibronectin in normal and diseased human kidneys. *J Clin Pathol* 1980; **33**: 1021-1028 [PMID: 7002956 DOI: 10.1136/jcp.33.11.1021]

61 **Sottile J**, Hocking DC. Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. *Mol Biol Cell* 2002; **13**: 3546-3559 [PMID: 12388756 DOI: 10.1091/mbc.e02-01-0048]

62 **Assmann KJ**, Koene RA, Wetzels JF. Familial glomerulonephritis characterized by massive deposits of fibronectin. *Am J Kidney Dis* 1995; **25**: 781-791 [PMID: 7747733 DOI: 10.1016/0272-6386(95)90555-3]

63 **Castelletti F**, Donadelli R, Banterla F, Hildebrandt F, Zipfel PF, Bresin E, Otto E, Skerka C, Renieri A, Todeschini M, Caprioli J, Caruso RM, Artuso R, Remuzzi G, Noris M. Mutations in FN1 cause glomerulopathy with fibronectin deposits. *Proc Natl Acad Sci U S A* 2008; **105**: 2538-2543 [PMID: 18268355 DOI: 10.1073/pnas.0707730105]

64 **Ertoy Baydar D**, Kutlugun AA, Bresin E, Piras R. A case of familial glomerulopathy with fibronectin deposits caused by the Y973C mutation in fibronectin. *Am J Kidney Dis* 2013; **61**: 514-518 [PMID: 23219110 DOI: 10.1053/j.ajkd.2012.08.050]

65 **Ohtsubo H**, Okada T, Nozu K, Takaoka Y, Shono A, Asanuma K, Zhang L, Nakanishi K, Taniguchi-Ikeda M, Kaito H, Iijima K, Nakamura S. Identification of mutations in FN1 leading to glomerulopathy with fibronectin deposits. *Pediatr Nephrol* 2016; **31**: 1459-1467 [PMID: 27056061 DOI: 10.1007/s00467-016-3368-7]

66 **Bonting SL**, De Bruin H, Pollak VE. QUANTITATIVE HISTOCHEMISTRY OF THE NEPHRON. VI. HYDROXYPROLINE IN THE HUMAN GLOMERULUS. *J Clin Invest* 1961; **40**: 177-180 [PMID: 16695847 DOI: 10.1172/jci104242]

67 **Beisswenger PG**, Spiro RG. Human glomerular basement membrane: chemical alteration in diabetes mellitus. *Science* 1970; **168**: 596-598 [PMID: 5436594 DOI: 10.1126/science.168.3931.596]

68 **Kefalides NA**. Biochemical properties of human glomerular basement membrane in normal and diabetic kidneys. *J Clin Invest* 1974; **53**: 403-407 [PMID: 11344553 DOI: 10.1172/jci107573]

69 **Funabiki K**, Horikoshi S, Tomino Y, Nagai Y, Koide H. Immunohistochemical analysis of extracellular components in the glomerular sclerosis of patients with glomerulonephritis. *Clin Nephrol* 1990; **34**: 239-246 [PMID: 2073766]

70 **Yoshioka K**, Takemura T, Tohda M, Akano N, Miyamoto H, Ooshima A, Maki S. Glomerular localization of type III collagen in human kidney disease. *Kidney Int* 1989; **35**: 1203-1211 [PMID: 2671464 DOI: 10.1038/ki.1989.111]

71 **Razzaque MS**, Koji T, Taguchi T, Harada T, Nakane PK. In situ localization of type III and type IV collagen-expressing cells in human diabetic nephropathy. *J Pathol* 1994; **174**: 131-138 [PMID: 7965408 DOI: 10.1002/path.1711740209]

72 **Bongers EM**, Huysmans FT, Levtchenko E, de Rooy JW, Blickman JG, Admiraal RJ, Huygen PL, Cruysberg JR, Toolens PA, Prins JB, Krabbe PF, Borm GF, Schoots J, van Bokhoven H, van Remortele AM, Hoefsloot LH, van Kampen A, Knoers NV. Genotype-phenotype studies in nail-patella syndrome show that LMX1B mutation location is involved in the risk of developing nephropathy. *Eur J Hum Genet* 2005; **13**: 935-946 [PMID: 15928687 DOI: 10.1038/sj.ejhg.5201446]

73 **Oe Y**, Mishima E, Mori T, Okamoto K, Honkura Y, Nagasawa T, Yoshida M, Sato H, Suzuki J, Ikeda R, Sohara E, Uchida S, Katori Y, Miyazaki M. A Novel Mutation in LMX1B (p.Pro219Ala) Causes Focal Segmental Glomerulosclerosis with Alport Syndrome-like Phenotype. *Intern Med* 2021; **60**: 2991-2996 [PMID: 33814499 DOI: 10.2169/internalmedicine.6987-20]

74 **Hudson BG**, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med* 2003; **348**: 2543-2556 [PMID: 12815141 DOI: 10.1056/NEJMra022296]

75 **Edwards N**, Rice SJ, Raman S, Hynes AM, Srivastava S, Moore I, Al-Hamed M, Xu Y, Santibanez-Koref M, Thwaites DT, Gale DP, Sayer JA. A novel LMX1B mutation in a family with end-stage renal disease of 'unknown cause'. *Clin Kidney J* 2015; **8**: 113-119 [PMID: 25713721 DOI: 10.1093/ckj/sfu129]

76 **Isojima T**, Harita Y, Furuyama M, Sugawara N, Ishizuka K, Horita S, Kajiho Y, Miura K, Igarashi T, Hattori M, Kitanaka S. LMX1B mutation with residual transcriptional activity as a cause of isolated glomerulopathy. *Nephrol Dial Transplant*

2014; **29**: 81-88 [PMID: 24042019 DOI: 10.1093/ndt/gft359]
77 **Konomoto T**, Imamura H, Orita M, Tanaka E, Moritake H, Sato Y, Fujimoto S, Harita Y, Hisano S, Yoshiura K, Nunoi H. Clinical and histological findings of autosomal dominant renal-limited disease with LMX1B mutation. *Nephrology (Carlton)* 2016; **21**: 765-773 [PMID: 26560070 DOI: 10.1111/nep.12666]
78 **Andeen NK**, Schleit J, Blosser CD, Dorschner MO, Hisama FM, Smith KD. LMX1B-Associated Nephropathy With Type III Collagen Deposition in the Glomerular and Tubular Basement Membranes. *Am J Kidney Dis* 2018; **72**: 296-301 [PMID: 29246420 DOI: 10.1053/j.ajkd.2017.09.023]
79 **Silverman ME**, Goodman RM, Cuppage FE. The Nail-Patella syndrome. Clinical findings and ultrastructural observations in the kidney. *Arch Intern Med* 1967; **120**: 68-74 [PMID: 6028699 DOI: 10.1001/archinte.120.1.68]
80 **Boyer O**, Woerner S, Yang F, Oakeley EJ, Linghu B, Gribouval O, Tête MJ, Duca JS, Klickstein L, Damask AJ, Szustakowski JD, Heibel F, Matignon M, Baudouin V, Chantrel F, Champigneulle J, Martin L, Nitschké P, Gubler MC, Johnson KJ, Chibout SD, Antignac C. LMX1B mutations cause hereditary FSGS without extrarenal involvement. *J Am Soc Nephrol* 2013; **24**: 1216-1222 [PMID: 23687361 DOI: 10.1681/ASN.2013020171]
81 **Ghoumid J**, Petit F, Holder-Espinasse M, Jourdain AS, Guerra J, Dieux-Coeslier A, Figeac M, Porchet N, Manouvrier-Hanu S, Escande F. Nail-Patella Syndrome: clinical and molecular data in 55 families raising the hypothesis of a genetic heterogeneity. *Eur J Hum Genet* 2016; **24**: 44-50 [PMID: 25898926 DOI: 10.1038/ejhg.2015.77]
82 **Shimohata H**, Miyake Y, Yoshida Y, Usui J, Mori T, Sohara E, Uchida S, Hirayama K, Kobayashi M. LMX1B-associated nephropathy that showed myelin figures on electron microscopy. *CEN Case Rep* 2021; **10**: 588-591 [PMID: 34076843 DOI: 10.1007/s13730-021-00612-y]
83 **Lei L**, Oh G, Sutherland S, Abra G, Higgins J, Sibley R, Troxell M, Kambham N. Myelin bodies in LMX1B-associated nephropathy: potential for misdiagnosis. *Pediatr Nephrol* 2020; **35**: 1647-1657 [PMID: 32356190 DOI: 10.1007/s00467-020-04564-w]
84 **Duggal R**, Nada R, Rayat CS, Rane SU, Sakhuja V, Joshi K. Collagenofibrotic glomerulopathy-a review. *Clin Kidney J* 2012; **5**: 7-12 [PMID: 26069739 DOI: 10.1093/ndtplus/sfr144]
85 **Kurien AA**, Larsen CP, Cossey LN. Collagenofibrotic glomerulopathy. *Clin Kidney J* 2015; **8**: 543-547 [PMID: 26413279 DOI: 10.1093/ckj/sfv061]
86 **Aoki T**, Hayashi K, Morinaga T, Tomida H, Hishida M, Yamamoto S, Kajiwara N, Tamai H. Two brothers with collagenofibrotic glomerulopathy. *CEN Case Rep* 2015; **4**: 85-89 [PMID: 28509277 DOI: 10.1007/s13730-014-0145-y]
87 **Vogt BA**, Wyatt RJ, Burke BA, Simonton SC, Kashtan CE. Inherited factor H deficiency and collagen type III glomerulopathy. *Pediatr Nephrol* 1995; **9**: 11-15 [PMID: 7742208 DOI: 10.1007/bf00858956]
88 **Kleppel MM**, Santi PA, Cameron JD, Wieslander J, Michael AF. Human tissue distribution of novel basement membrane collagen. *Am J Pathol* 1989; **134**: 813-825 [PMID: 2650557]
89 **Zhu D**, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM. Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. *Kidney Int* 1994; **45**: 425-433 [PMID: 8164429 DOI: 10.1038/ki.1994.55]
90 **Barker DF**, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Tryggvason K. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990; **248**: 1224-1227 [PMID: 2349482 DOI: 10.1126/science.2349482]
91 **Voskarides K**, Damianou L, Neocleous V, Zouvani I, Christodoulidou S, Hadjiconstantinou V, Ioannou K, Athanasiou Y, Patsias C, Alexopoulos E, Pierides A, Kyriacou K, Deltas C. COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy. *J Am Soc Nephrol* 2007; **18**: 3004-3016 [PMID: 17942953 DOI: 10.1681/asn.2007040444]
92 **Parkin JD**, San Antonio JD, Pedchenko V, Hudson B, Jensen ST, Savige J. Mapping structural landmarks, ligand binding sites, and missense mutations to the collagen IV heterotrimers predicts major functional domains, novel interactions, and variation in phenotypes in inherited diseases affecting basement membranes. *Hum Mutat* 2011; **32**: 127-143 [PMID: 21280145 DOI: 10.1002/humu.21401]
93 **Kashtan CE**, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K, Nozu K, Renieri A, Rheault M, Wang F, Gross O. Alport syndrome: a unified classification of genetic disorders of collagen IV α345: a position paper of the Alport Syndrome Classification Working Group. *Kidney Int* 2018; **93**: 1045-1051 [PMID: 29551517 DOI: 10.1016/j.kint.2017.12.018]
94 **Savige J**, Storey H, Watson E, Hertz JM, Deltas C, Renieri A, Mari F, Hilbert P, Plevova P, Byers P, Cerkauskaite A, Gregory M, Cerkauskiene R, Ljubanovic DG, Becherucci F, Errichiello C, Massella L, Aiello V, Lennon R, Hopkinson L, Koziell A, Lungu A, Rothe HM, Hoefele J, Zacchia M, Martic TN, Gupta A, van Eerde A, Gear S, Landini S, Palazzo V, Al-Rabadi L, Claes K, Corveleyn A, Van Hoof E, van Geel M, Williams M, Ashton E, Belge H, Ars E, Bierzynska A, Gangemi C, Lipska-Ziętkiewicz BS. Consensus statement on standards and guidelines for the molecular diagnostics of Alport syndrome: refining the ACMG criteria. *Eur J Hum Genet* 2021; **29**: 1186-1197 [PMID: 33854215 DOI: 10.1038/s41431-021-00858-1]
95 **Barua M**, Paterson AD. Population-based studies reveal an additive role of type IV collagen variants in hematuria and albuminuria. *Pediatr Nephrol* 2022; **37**: 253-262 [PMID: 33635378 DOI: 10.1007/s00467-021-04934-y]
96 **Papazachariou L**, Papagregoriou G, Hadjipanagi D, Demosthenous P, Voskarides K, Koutsofti C, Stylianou K, Ioannou P, Xydakis D, Tzanakis I, Papadaki A, Kallivretakis N, Nikolakakis N, Perysinaki G, Gale DP, Diamantopoulos A, Goudas P, Goumenos D, Soloukides A, Boletis I, Melexopoulou C, Georgaki E, Frysira E, Komianou F, Grekas D, Paliouras C, Alivanis P, Vergoulas G, Pierides A, Daphnis E, Deltas C. Frequent COL4 mutations in familial microhematuria accompanied by later-onset Alport nephropathy due to focal segmental glomerulosclerosis. *Clin Genet* 2017; **92**: 517-527 [PMID: 28632965 DOI: 10.1111/cge.13077]
97 **Malone AF**, Phelan PJ, Hall G, Cetincelik U, Homstad A, Alonso AS, Jiang R, Lindsey TB, Wu G, Sparks MA, Smith SR, Webb NJ, Kalra PA, Adeyemo AA, Shaw AS, Conlon PJ, Jennette JC, Howell DN, Winn MP, Gbadegesin RA. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. *Kidney Int* 2014; **86**: 1253-1259 [PMID: 25229338 DOI: 10.1038/ki.2014.305]

98 **Xie J**, Wu X, Ren H, Wang W, Wang Z, Pan X, Hao X, Tong J, Ma J, Ye Z, Meng G, Zhu Y, Kiryluk K, Kong X, Hu L, Chen N. COL4A3 mutations cause focal segmental glomerulosclerosis. *J Mol Cell Biol* 2014; **6**: 498-505 [PMID: 25596306 DOI: 10.1093/jmcb/mju040]

99 **Yao T**, Udwan K, John R, Rana A, Haghighi A, Xu L, Hack S, Reich HN, Hladunewich MA, Cattran DC, Paterson AD, Pei Y, Barua M. Integration of Genetic Testing and Pathology for the Diagnosis of Adults with FSGS. *Clin J Am Soc Nephrol* 2019; **14**: 213-223 [PMID: 30647093 DOI: 10.2215/CJN.08750718]

100 **Dische FE**. Measurement of glomerular basement membrane thickness and its application to the diagnosis of thin-membrane nephropathy. *Arch Pathol Lab Med* 1992; **116**: 43-49 [PMID: 1734832]

101 **Kashtan CE**, Segal Y. Genetic disorders of glomerular basement membranes. *Nephron Clin Pract* 2011; **118**: c9-c18 [PMID: 21071975 DOI: 10.1159/000320876]

102 **Nogueira M**, Cartwright J Jr, Horn K, Doe N, Shappell S, Barrios R, Coroneos E, Truong LD. Thin basement membrane disease with heavy proteinuria or nephrotic syndrome at presentation. *Am J Kidney Dis* 2000; **35**: E15 [PMID: 10739808 DOI: 10.1016/s0272-6386(00)70033-3]

103 **Quinlan C**, Rheault MN. Genetic Basis of Type IV Collagen Disorders of the Kidney. *Clin J Am Soc Nephrol* 2021; **16**: 1101-1109 [PMID: 33849932 DOI: 10.2215/CJN.19171220]

104 **Hoorn EJ**, Taams NE, Hurskainen T, Salih M, Weening JJ, Jonkman MF, Pas HH, Schreurs MW. Bullous Pemphigoid With a Dual Pattern of Glomerular Immune Complex Disease. *Am J Kidney Dis* 2016; **67**: 302-306 [PMID: 26616334 DOI: 10.1053/j.ajkd.2015.10.015]

105 **Hurskainen T**, Moilanen J, Sormunen R, Franzke CW, Soininen R, Loeffek S, Huilaja L, Nuutinen M, Bruckner-Tuderman L, Autio-Harmainen H, Tasanen K. Transmembrane collagen XVII is a novel component of the glomerular filtration barrier. *Cell Tissue Res* 2012; **348**: 579-588 [PMID: 22457199 DOI: 10.1007/s00441-012-1368-x]

106 **Saarela J**, Rehn M, Oikarinen A, Autio-Harmainen H, Pihlajaniemi T. The short and long forms of type XVIII collagen show clear tissue specificities in their expression and location in basement membrane zones in humans. *Am J Pathol* 1998; **153**: 611-626 [PMID: 9708820 DOI: 10.1016/S0002-9440(10)65603-9]

107 **Enoksen ITT**, Svistounov D, Norvik JV, Stefansson VTN, Solbu MD, Eriksen BO, Melsom T. Serum Matrix Metalloproteinase 7 and accelerated GFR decline in a general non-diabetic population. *Nephrol Dial Transplant* 2021 [PMID: 34436577 DOI: 10.1093/ndt/gfab251]

108 **Głowińska-Olszewska B**, Urban M. Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. *Metabolism* 2007; **56**: 799-805 [PMID: 17512313 DOI: 10.1016/j.metabol.2007.01.011]

109 **Ishikawa J**, Hoshide S, Eguchi K, Ishikawa S, Pickering TG, Shimada K, Kario K. Plasma tissue inhibitor of matrix metalloproteinase-1 level is increased in normotensive non-dippers in association with impaired glucose metabolism. *Hypertens Res* 2008; **31**: 2045-2051 [PMID: 19098376 DOI: 10.1291/hypres.31.2045]

110 **Li Y**, Yang J, Dai C, Wu C, Liu Y. Role for integrin-linked kinase in mediating tubular epithelial to mesenchymal transition and renal interstitial fibrogenesis. *J Clin Invest* 2003; **112**: 503-516 [PMID: 12925691 DOI: 10.1172/jci17913]

111 **Aggeli AS**, Kitsiou PV, Tzinia AK, Boutaud A, Hudson BG, Tsilibary EC. Selective binding of integrins from different renal cell types to the NC1 domain of alpha3 and alpha1 chains of type IV collagen. *J Nephrol* 2009; **22**: 130-136 [PMID: 19229828]

112 **Karamessinis PM**, Tzinia AK, Kitsiou PV, Stetler-Stevenson WG, Michael AF, Fan WW, Zhou B, Margaritis LH, Tsilibary EC. Proximal tubular epithelial cell integrins respond to high glucose by altered cell-matrix interactions and differentially regulate matrixin expression. *Lab Invest* 2002; **82**: 1081-1093 [PMID: 12177247 DOI: 10.1097/01.lab.0000022224.86237.31]

113 **Kitsiou PV**, Tzinia AK, Stetler-Stevenson WG, Michael AF, Fan WW, Zhou B, Tsilibary EC. Glucose-induced changes in integrins and matrix-related functions in cultured human glomerular epithelial cells. *Am J Physiol Renal Physiol* 2003; **284**: F671-F679 [PMID: 12620921 DOI: 10.1152/ajprenal.00266.2002]

114 **Guo L**, Sanders PW, Woods A, Wu C. The distribution and regulation of integrin-linked kinase in normal and diabetic kidneys. *Am J Pathol* 2001; **159**: 1735-1742 [PMID: 11696434 DOI: 10.1016/s0002-9440(10)63020-9]

115 **van Roeyen CR**, Ostendorf T, Denecke B, Bokemeyer D, Behrmann I, Strutz F, Lichenstein HS, LaRochelle WJ, Pena CE, Chaudhuri A, Floege J. Biological responses to PDGF-BB *vs* PDGF-DD in human mesangial cells. *Kidney Int* 2006; **69**: 1393-1402 [PMID: 16557224 DOI: 10.1038/sj.ki.5000332]

116 **Dey N**, Ghosh-Choudhury N, Kasinath BS, Choudhury GG. TGFβ-stimulated microRNA-21 utilizes PTEN to orchestrate AKT/mTORC1 signaling for mesangial cell hypertrophy and matrix expansion. *PLoS One* 2012; **7**: e42316 [PMID: 22879939 DOI: 10.1371/journal.pone.0042316]

117 **Kim MK**, Maeng YI, Sung WJ, Oh HK, Park JB, Yoon GS, Cho CH, Park KK. The differential expression of TGF-β1, ILK and wnt signaling inducing epithelial to mesenchymal transition in human renal fibrogenesis: an immunohistochemical study. *Int J Clin Exp Pathol* 2013; **6**: 1747-1758 [PMID: 24040439]

118 **Yan Q**, Sui W, Xie S, Chen H, Zou G, Guo J, Zou H. Expression and role of integrin-linked kinase and collagen IV in human renal allografts with interstitial fibrosis and tubular atrophy. *Transpl Immunol* 2010; **23**: 1-5 [PMID: 20398767 DOI: 10.1016/j.trim.2010.04.001]

119 **Foster RR**, Hole R, Anderson K, Satchell SC, Coward RJ, Mathieson PW, Gillatt DA, Saleem MA, Bates DO, Harper SJ. Functional evidence that vascular endothelial growth factor may act as an autocrine factor on human podocytes. *Am J Physiol Renal Physiol* 2003; **284**: F1263-F1273 [PMID: 12620928 DOI: 10.1152/ajprenal.00276.2002]

120 **Simon M**, Gröne HJ, Jöhren O, Kullmer J, Plate KH, Risau W, Fuchs E. Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney. *Am J Physiol* 1995; **268**: F240-F250 [PMID: 7864162 DOI: 10.1152/ajprenal.1995.268.2.F240]

121 **Thomas S**, Vanuystel J, Gruden G, Rodríguez V, Burt D, Gnudi L, Hartley B, Viberti G. Vascular endothelial growth factor receptors in human mesangium in vitro and in glomerular disease. *J Am Soc Nephrol* 2000; **11**: 1236-1243 [PMID: 10864579 DOI: 10.1681/ASN.V1171236]

122 **Zhu X**, Wu S, Dahut WL, Parikh CR. Risks of proteinuria and hypertension with bevacizumab, an antibody against

vascular endothelial growth factor: systematic review and meta-analysis. *Am J Kidney Dis* 2007; **49**: 186-193 [PMID: 17261421 DOI: 10.1053/j.ajkd.2006.11.039]

123 **Sartelet H**, Toupance O, Lorenzato M, Fadel F, Noel LH, Lagonotte E, Birembaut P, Chanard J, Rieu P. Sirolimus-induced thrombotic microangiopathy is associated with decreased expression of vascular endothelial growth factor in kidneys. *Am J Transplant* 2005; **5**: 2441-2447 [PMID: 16162193 DOI: 10.1111/j.1600-6143.2005.01047.x]

124 **Kovalevich J**, Tracy B, Langford D. PINCH: More than just an adaptor protein in cellular response. *J Cell Physiol* 2011; **226**: 940-947 [PMID: 20945343 DOI: 10.1002/jcp.22437]

125 **Ghatak S**, Morgner J, Wickström SA. ILK: a pseudokinase with a unique function in the integrin-actin linkage. *Biochem Soc Trans* 2013; **41**: 995-1001 [PMID: 23863169 DOI: 10.1042/BST20130062]

126 **Kim YC**, Gonzalez-Nieves R, Cutler ML. Rsu1 contributes to cell adhesion and spreading in MCF10A cells *via* effects on P38 map kinase signaling. *Cell Adh Migr* 2015; **9**: 227-232 [PMID: 25482629 DOI: 10.4161/19336918.2014.972775]

127 **Yang H**, Lin L, Sun K, Zhang T, Chen W, Li L, Xie Y, Wu C, Wei Z, Yu C. Complex structures of Rsu1 and PINCH1 reveal a regulatory mechanism of the ILK/PINCH/Parvin complex for F-actin dynamics. *Elife* 2021; **10** [PMID: 33587032 DOI: 10.7554/eLife.64395]

128 **Górska A**, Mazur AJ. Integrin-linked kinase (ILK): the known vs. the unknown and perspectives. *Cell Mol Life Sci* 2022; **79**: 100 [PMID: 35089438 DOI: 10.1007/s00018-021-04104-1]

129 **Velyvis A**, Yang Y, Wu C, Qin J. Solution structure of the focal adhesion adaptor PINCH LIM1 domain and characterization of its interaction with the integrin-linked kinase ankyrin repeat domain. *J Biol Chem* 2001; **276**: 4932-4939 [PMID: 11078733 DOI: 10.1074/jbc.M007632200]

130 **Sepulveda JL**, Wu C. The parvins. *Cell Mol Life Sci* 2006; **63**: 25-35 [PMID: 16314921 DOI: 10.1007/s00018-005-5355-1]

131 **Tu Y**, Huang Y, Zhang Y, Hua Y, Wu C. A new focal adhesion protein that interacts with integrin-linked kinase and regulates cell adhesion and spreading. *J Cell Biol* 2001; **153**: 585-598 [PMID: 11331308 DOI: 10.1083/jcb.153.3.585]

132 **Fukuda T**, Guo L, Shi X, Wu C. CH-ILKBP regulates cell survival by facilitating the membrane translocation of protein kinase B/Akt. *J Cell Biol* 2003; **160**: 1001-1008 [PMID: 12654898 DOI: 10.1083/jcb.200212113]

133 **Krishnamurti U**, Chen Y, Michael A, Kim Y, Fan WW, Wieslander J, Brunmark C, Rondeau E, Sraer JD, Delarue F, Tsilibary EC. Integrin-mediated interactions between primary/T-sv40 immortalized human glomerular epithelial cells and type IV collagen. *Lab Invest* 1996; **74**: 650-657 [PMID: 8600316]

134 **Spiro RG**. Biochemistry of the renal glomerular basement membrane and its alterations in diabetes mellitus. *N Engl J Med* 1973; **288**: 1337-1342 [PMID: 4574722 DOI: 10.1056/nejm197306212882506]

135 **Goldstein JL**, Fialkow PJ. The Alström syndrome. Report of three cases with further delineation of the clinical, pathophysiological, and genetic aspects of the disorder. *Medicine (Baltimore)* 1973; **52**: 53-71 [PMID: 4689172 DOI: 10.1097/00005792-197301000-00003]

136 **Marshall JD**, Bronson RT, Collin GB, Nordstrom AD, Maffei P, Paisey RB, Carey C, Macdermott S, Russell-Eggitt I, Shea SE, Davis J, Beck S, Shatirishvili G, Mihai CM, Hoeltzenbein M, Pozzan GB, Hopkinson I, Sicolo N, Naggert JK, Nishina PM. New Alström syndrome phenotypes based on the evaluation of 182 cases. *Arch Intern Med* 2005; **165**: 675-683 [PMID: 15795345 DOI: 10.1001/archinte.165.6.675]

137 **Falk RJ**, Scheinman JI, Mauer SM, Michael AF. Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. *Diabetes* 1983; **32** Suppl 2: 34-39 [PMID: 6400667 DOI: 10.2337/diab.32.2.s34]

138 **Tervaert TW**, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E, Joh K, Noël LH, Radhakrishnan J, Seshan SV, Bajema IM, Bruijn JA; Renal Pathology Society. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* 2010; **21**: 556-563 [PMID: 20167701 DOI: 10.1681/ASN.2010010010]

139 **Schleicher E**, Wieland OH. Changes of human glomerular basement membrane in diabetes mellitus. *J Clin Chem Clin Biochem* 1984; **22**: 223-227 [PMID: 6726120 DOI: 10.1515/cclm.1984.22.3.223]

140 **Berenson GS**, Ruiz H, Dalferes ER Jr, Dugan FA, Radhakrishnamurthy B. Acid mucopolysaccharide changes in diabetic kidneys. *Diabetes* 1970; **19**: 161-170 [PMID: 4245198 DOI: 10.2337/diab.19.3.161]

141 **Zhao L**, Zhang Y, Liu F, Yang H, Zhong Y, Wang Y, Li S, Su Q, Tang L, Bai L, Ren H, Zou Y, Wang S, Zheng S, Xu H, Li L, Zhang J, Chai Z, Cooper ME, Tong N. Urinary complement proteins and risk of end-stage renal disease: quantitative urinary proteomics in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. *J Endocrinol Invest* 2021; **44**: 2709-2723 [PMID: 34043214 DOI: 10.1007/s40618-021-01596-3]

142 **Bonomo JA**, Palmer ND, Hicks PJ, Lea JP, Okusa MD, Langefeld CD, Bowden DW, Freedman BI. Complement factor H gene associations with end-stage kidney disease in African Americans. *Nephrol Dial Transplant* 2014; **29**: 1409-1414 [PMID: 24586071 DOI: 10.1093/ndt/gfu036]

143 **Valoti E**, Noris M, Perna A, Rurali E, Gherardi G, Breno M, Parvanova Ilieva A, Petrov Iliev I, Bossi A, Trevisan R, Dodesini AR, Ferrari S, Stucchi N, Benigni A, Remuzzi G, Ruggenenti P. Impact of a Complement Factor H Gene Variant on Renal Dysfunction, Cardiovascular Events, and Response to ACE Inhibitor Therapy in Type 2 Diabetes. *Front Genet* 2019; **10**: 681 [PMID: 31428128 DOI: 10.3389/fgene.2019.00681]

144 **Westberg NG**, Michael AF. Human glomerular basement membrane: chemical composition in diabetes mellitus. *Acta Med Scand* 1973; **1-2**: 39-47 [PMID: 4727247 DOI: 10.1111/j.0954-6820.1973.tb19411.x]

145 **Rasmussen LM**, Heickendorff L. Accumulation of fibronectin in aortas from diabetic patients. A quantitative immunohistochemical and biochemical study. *Lab Invest* 1989; **61**: 440-446 [PMID: 2796291]

146 **Kimmelstiel P**, Kim OJ, Beres J. Studies on renal biopsy specimens, with the aid of the electron microscope. I. Glomeruli in diabetes. *Am J Clin Pathol* 1962; **38**: 270-279 [PMID: 14456010 DOI: 10.1093/ajcp/38.3.270]

147 **Mise K**, Ueno T, Hoshino J, Hazue R, Sumida K, Yamanouchi M, Hayami N, Suwabe T, Hiramatsu R, Hasegawa E, Sawa N, Fujii T, Hara S, Wada J, Makino H, Takaichi K, Ohashi K, Ubara Y. Nodular lesions in diabetic nephropathy: Collagen staining and renal prognosis. *Diabetes Res Clin Pract* 2017; **127**: 187-197 [PMID: 28388509 DOI: 10.1016/j.diabres.2017.03.006]

148 **Alsaad KO**, Edrees B, Rahim KA, Alanazi A, Ahmad M, Aloudah N. Collagenofibrotic (Collagen Type III)

glomerulopathy in association with diabetic nephropathy. *Saudi J Kidney Dis Transpl* 2017; **28**: 898-905 [PMID: 28748894]

149 **Guan M**, Ma J, Keaton JM, Dimitrov L, Mudgal P, Stromberg M, Bonomo JA, Hicks PJ, Freedman BI, Bowden DW, Ng MC. Association of kidney structure-related gene variants with type 2 diabetes-attributed end-stage kidney disease in African Americans. *Hum Genet* 2016; **135**: 1251-1262 [PMID: 27461219 DOI: 10.1007/s00439-016-1714-2]

150 **Salem RM**, Todd JN, Sandholm N, Cole JB, Chen WM, Andrews D, Pezzolesi MG, McKeigue PM, Hiraki LT, Qiu C, Nair V, Di Liao C, Cao JJ, Valo E, Onengut-Gumuscu S, Smiles AM, McGurnaghan SJ, Haukka JK, Harjutsalo V, Brennan EP, van Zuydam N, Ahlqvist E, Doyle R, Ahluwalia TS, Lajer M, Hughes MF, Park J, Skupien J, Spiliopoulou A, Liu A, Menon R, Boustany-Kari CM, Kang HM, Nelson RG, Klein R, Klein BE, Lee KE, Gao X, Mauer M, Maestroni S, Caramori ML, de Boer IH, Miller RG, Guo J, Boright AP, Tregouet D, Gyorgy B, Snell-Bergeon JK, Maahs DM, Bull SB, Canty AJ, Palmer CNA, Stechemesser L, Paulweber B, Weitgasser R, Sokolovska J, Rovīte V, Pīrāgs V, Prakapiene E, Radzeviciene L, Verkauskiene R, Panduru NM, Groop LC, McCarthy MI, Gu HF, Möllsten A, Falhammar H, Brismar K, Martin F, Rossing P, Costacou T, Zerbini G, Marre M, Hadjadj S, McKnight AJ, Forsblom C, McKay G, Godson C, Maxwell AP, Kretzler M, Susztak K, Colhoun HM, Krolewski A, Paterson AD, Groop PH, Rich SS, Hirschhorn JN, Florez JC; SUMMIT Consortium, DCCT/EDIC Research Group, GENIE Consortium. Genome-Wide Association Study of Diabetic Kidney Disease Highlights Biology Involved in Glomerular Basement Membrane Collagen. *J Am Soc Nephrol* 2019; **30**: 2000-2016 [PMID: 31537649 DOI: 10.1681/ASN.2019030218]

151 **Peeters SA**, Engelen L, Buijs J, Chaturvedi N, Fuller JH, Schalkwijk CG, Stehouwer CD; EURODIAB Prospective Complications Study Group. Plasma levels of matrix metalloproteinase-2, -3, -10, and tissue inhibitor of metalloproteinase-1 are associated with vascular complications in patients with type 1 diabetes: the EURODIAB Prospective Complications Study. *Cardiovasc Diabetol* 2015; **14**: 31 [PMID: 25848912 DOI: 10.1186/s12933-015-0195-2]

152 **Peeters SA**, Engelen L, Buijs J, Jorsal A, Parving HH, Tarnow L, Rossing P, Schalkwijk CG, Stehouwer CDA. Plasma matrix metalloproteinases are associated with incident cardiovascular disease and all-cause mortality in patients with type 1 diabetes: a 12-year follow-up study. *Cardiovasc Diabetol* 2017; **16**: 55 [PMID: 28446168 DOI: 10.1186/s12933-017-0539-1]

153 **Peeters SA**, Engelen L, Buijs J, Chaturvedi N, Fuller JH, Jorsal A, Parving HH, Tarnow L, Theilade S, Rossing P, Schalkwijk CG, Stehouwer CDA. Circulating matrix metalloproteinases are associated with arterial stiffness in patients with type 1 diabetes: pooled analysis of three cohort studies. *Cardiovasc Diabetol* 2017; **16**: 139 [PMID: 29070037 DOI: 10.1186/s12933-017-0620-9]

154 **Vilmi-Kerälä T**, Lauhio A, Tervahartiala T, Palomäki O, Uotila J, Sorsa T, Palomäki A. Subclinical inflammation associated with prolonged TIMP-1 upregulation and arterial stiffness after gestational diabetes mellitus: a hospital-based cohort study. *Cardiovasc Diabetol* 2017; **16**: 49 [PMID: 28407807 DOI: 10.1186/s12933-017-0530-x]

155 **Afkarian M**, Zelnick LR, Ruzinski J, Kestenbaum B, Himmelfarb J, de Boer IH, Mehrotra R. Urine matrix metalloproteinase-7 and risk of kidney disease progression and mortality in type 2 diabetes. *J Diabetes Complications* 2015; **29**: 1024-1031 [PMID: 26412030 DOI: 10.1016/j.jdiacomp.2015.08.024]

156 **Paunas FTI**, Finne K, Leh S, Osman TA, Marti HP, Berven F, Vikse BE. Characterization of glomerular extracellular matrix in IgA nephropathy by proteomic analysis of laser-captured microdissected glomeruli. *BMC Nephrol* 2019; **20**: 410 [PMID: 31726998 DOI: 10.1186/s12882-019-1598-1]

157 **Ibrahim S**, Rashed L. Estimation of transforming growth factor-beta 1 as a marker of renal injury in type II diabetes mellitus. *Saudi Med J* 2007; **28**: 519-523 [PMID: 17457470]

158 **Shaker OG**, Sadik NA. Transforming growth factor beta 1 and monocyte chemoattractant protein-1 as prognostic markers of diabetic nephropathy. *Hum Exp Toxicol* 2013; **32**: 1089-1096 [PMID: 23515495 DOI: 10.1177/0960327112470274]

159 **Mou X**, Zhou DY, Ma JR, Liu YH, Chen HP, Hu YB, Shou CM, Chen JW, Liu WH, Ma GL. Serum TGF-β1 as a Biomarker for Type 2 Diabetic Nephropathy: A Meta-Analysis of Randomized Controlled Trials. *PLoS One* 2016; **11**: e0149513 [PMID: 26901047 DOI: 10.1371/journal.pone.0149513]

160 **Qiao YC**, Chen YL, Pan YH, Ling W, Tian F, Zhang XX, Zhao HL. Changes of transforming growth factor beta 1 in patients with type 2 diabetes and diabetic nephropathy: A PRISMA-compliant systematic review and meta-analysis. *Medicine (Baltimore)* 2017; **96**: e6583 [PMID: 28403088 DOI: 10.1097/MD.0000000000006583]

161 **Zhou T**, Li HY, Zhong H, Zhong Z. Relationship between transforming growth factor-β1 and type 2 diabetic nephropathy risk in Chinese population. *BMC Med Genet* 2018; **19**: 201 [PMID: 30458730 DOI: 10.1186/s12881-018-0717-3]

162 **Voelker J**, Berg PH, Sheetz M, Duffin K, Shen T, Moser B, Greene T, Blumenthal SS, Rychlik I, Yagil Y, Zaoui P, Lewis JB. Anti-TGF-*β*1 Antibody Therapy in Patients with Diabetic Nephropathy. *J Am Soc Nephrol* 2017; **28**: 953-962 [PMID: 27647855 DOI: 10.1681/ASN.2015111230]

163 **Uehara G**, Suzuki D, Toyoda M, Umezono T, Sakai H. Glomerular expression of platelet-derived growth factor (PDGF)-A, -B chain and PDGF receptor-alpha, -beta in human diabetic nephropathy. *Clin Exp Nephrol* 2004; **8**: 36-42 [PMID: 15067514 DOI: 10.1007/s10157-003-0265-8]

164 **Baelde HJ**, Eikmans M, Lappin DW, Doran PP, Hohenadel D, Brinkkoetter PT, van der Woude FJ, Waldherr R, Rabelink TJ, de Heer E, Bruijn JA. Reduction of VEGF-A and CTGF expression in diabetic nephropathy is associated with podocyte loss. *Kidney Int* 2007; **71**: 637-645 [PMID: 17264876 DOI: 10.1038/sj.ki.5002101]

165 **Woroniecka KI**, Park AS, Mohtat D, Thomas DB, Pullman JM, Susztak K. Transcriptome analysis of human diabetic kidney disease. *Diabetes* 2011; **60**: 2354-2369 [PMID: 21752957 DOI: 10.2337/db10-1181]

166 **Hovind P**, Tarnow L, Oestergaard PB, Parving HH. Elevated vascular endothelial growth factor in type 1 diabetic patients with diabetic nephropathy. *Kidney Int Suppl* 2000; **75**: S56-S61 [PMID: 10828763]

167 **Zhao Y**, Banerjee S, LeJeune WS, Choudhary S, Tilton RG. NF-κB-inducing kinase increases renal tubule epithelial inflammation associated with diabetes. *Exp Diabetes Res* 2011; **2011**: 192564 [PMID: 21869881 DOI: 10.1155/2011/192564]

168 **Bae EH**, Cho S, Joo SY, Ma SK, Kim SH, Lee J, Kim SW. 4-Hydroxy-2-hexenal-induced apoptosis in human renal proximal tubular epithelial cells. *Nephrol Dial Transplant* 2011; **26**: 3866-3873 [PMID: 21771752 DOI:

10.1093/ndt/gfr386]

169 **Tanji N**, Markowitz GS, Fu C, Kislinger T, Taguchi A, Pischetsrieder M, Stern D, Schmidt AM, D'Agati VD. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *J Am Soc Nephrol* 2000; **11**: 1656-1666 [PMID: 10966490 DOI: 10.1681/ASN.V1191656]

170 **Suzuki D**, Toyoda M, Yamamoto N, Miyauchi M, Katoh M, Kimura M, Maruyama M, Honma M, Umezono T, Yagame M. Relationship between the expression of advanced glycation end-products (AGE) and the receptor for AGE (RAGE) mRNA in diabetic nephropathy. *Intern Med* 2006; **45**: 435-441 [PMID: 16679697 DOI: 10.2169/internalmedicine.45.1557]

171 **Peeters SA**, Engelen L, Buijs J, Theilade S, Rossing P, Schalkwijk CG, Stehouwer CDA. Associations between advanced glycation endproducts and matrix metalloproteinases and its inhibitor in individuals with type 1 diabetes. *J Diabetes Complications* 2018; **32**: 325-329 [PMID: 29395841 DOI: 10.1016/j.jdiacomp.2017.12.011]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 521-531

DOI: 10.4239/wjd.v13.i7.521

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Association between urinary concentrations of bisphenol A substitutes and diabetes in adults

Rafael Moreno-Gómez-Toledano, Esperanza Vélez-Vélez, María I Arenas, Marta Saura, Ricardo J Bosch

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: He Z, China; Rojas A, Chile

Received: March 11, 2022
Peer-review started: March 11, 2022
First decision: April 17, 2022
Revised: April 26, 2022
Accepted: June 22, 2022
Article in press: June 22, 2022
Published online: July 15, 2022

Rafael Moreno-Gómez-Toledano, Marta Saura, Ricardo J Bosch, Universidad de Alcalá, Department of Biological Systems/Physiology Unit, Alcalá de Henares 28871, Spain

Esperanza Vélez-Vélez, Fundación Jiménez Díaz School of Nursing, Jiménez Díaz Foundation, Autonomous University of Madrid, Madrid 28040, Spain

María I Arenas, Universidad de Alcalá, Department of Biomedicine and Biotechnology,Alcalá de Henares 28871, Spain

Marta Saura, Centro de Investigación en Red de Enfermedades Cardiovasculares, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid 28034, Spain

Corresponding author: Rafael Moreno-Gómez-Toledano, PhD, Associate Research Scientist, Universidad de Alcalá, Department of Biological Systems/Physiology Unit, Campus Universitario - C/19, Av. de Madrid, Alcalá de Henares 28871, Spain. rafael.moreno@uah.es

Abstract

BACKGROUND

Due to new restrictions on the use of bisphenol A (BPA), industries are beginning to replace it with derived molecules such as bisphenol S and F (BPS and BPF). There is extensive evidence in the academic literature on the potential health effects of BPA, which is known to be a diabetogenic molecule. However, there are few publications related to new compounds derived from BPA.

AIM

To perform an epidemiological study of urinary BPS and BPF in the American National Health and Nutrition Examination Survey (NHANES) cohort, and analyze their possible relationship with diabetes mellitus.

METHODS

NHANES datasets from 2013 to 2016 were used due to the urinary BPF and BPS availability. Data from 3658 adults were analyzed to perform regression analysis exploring the possible relationship between BPA-derived compounds and diabetes.

RESULTS

Descriptive statistics, linear regression modeling, and logistic regression analysis revealed a significant relationship between urinary BPS, but not BPF, and diabetes

risk. Additionally, a relationship was observed between both compounds and hypertension and a slight relationship between BPF and dyslipidemia.

CONCLUSION

In the present study, a strong relationship between urinary BPS, not BPF, and diabetes risk has been determined. BPA substitute molecules do not exempt the population from potential health risks.

Key Words: Bisphenol S; Bisphenol F; Diabetes mellitus; National Health and Nutrition Examination Survey; Urine

Core Tip: Molecules derived from bisphenol A (increasing use in the plastic industry and the production of heat-sensitive tickets) could be related to pathologies such as diabetes (bisphenol S) and hypertension (bisphenol S and F).

Citation: Moreno-Gómez-Toledano R, Vélez-Vélez E, Arenas MI, Saura M, Bosch RJ. Association between urinary concentrations of bisphenol A substitutes and diabetes in adults. *World J Diabetes* 2022; 13(7): 521-531
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/521.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.521

INTRODUCTION

In the last decades, the demand and production of plastic polymers have increased substantially. Both its production and recycling involve the release of pollutants, xenobiotic compounds that should not be found in the air, rivers, or the human population[1,2]. One of the most important, due to its wide distribution and variety of biological effects, is bisphenol A (BPA)[3-6]. The European Chemicals Agency has recently included BPA within the Candidate List of substances of very high concern due to its properties as an endocrine disruptor and its potentially harmful effect on reproduction[7]. Furthermore, the European Union has restricted the use of this substance in thermal paper due to its potential danger to the health of exposed workers[8].

Therefore, the need to seek alternatives to BPA is a fact of vital importance for modern industry. Currently, two known compounds are bisphenol S (BPS) and bisphenol F (BPF), which can already be found in BPA-free packaging and thermal tickets regulated by European legislation[9,10]. The use of these derivatives does not imply a reduction in possible adverse effects *per se*; it only indicates the use of new materials whose safety has not yet been tested. Comparative studies between BPA substitutes have shown that both BPF and BPS are as hormonally active as BPA[9], so they could also be included in the category of endocrine disruptors.

Diabetes mellitus (DM) and its associated complications are a medical catastrophe of global dimensions[11]. The number of people affected has risen from 108 million in 1980[12] to almost 500 million today[13]. The latest estimates suggest that it could rise to 578 million in 2030 and 700 million in 2045[13]. Risk causes for the disease include a combination of genetic and metabolic factors. There are non-modifiable factors, such as ethnicity or age, and modifying factors, such as diet, obesity, or smoking [14]. The multiplicity of factors that influence the development of the disease implies that environmental pollutants could also affect it. There is evidence that BPA exposure correlates with the risk of developing DM[15].

However, new alternative compounds to BPA have only been used in modern industry for a short time. For this reason, few academic publications study its possible relationship with diabetes. The first pieces of evidence have been detected in a cellular experimental model[16], in males (but not females) of a murine experimental model[17], and in human cohorts from China[18,19] and France[20]. However, it has not yet been studied in one of the world's largest urinary bisphenol cohorts, the American National Health and Nutrition Examination Survey (NHANES). Studies in this cohort have demonstrated the presence of BPF and BPS in urine, observing positive and statistically significant relationships with disorders such as asthma[21], obesity[22-24], or depression[25]. Obesity is closely related to diabetes[26-28], so studying its relationship with new environmental pollutants is coherent and necessary. The present work aimed to correlate, for the first time in the NHANES cohort, diabetes with the urinary concentration of BPA substitutes using regression models.

MATERIALS AND METHODS

NHANES 2013-2016 population

The NHANES datasets from 2013 to 2016 were used in the present statistical model due to the urinary BPF and BPS availability. In the first phase, the data of all the study participants were extracted through the official website of the Centers for Disease Control and Prevention[29] (accessed December 01, 2021), obtaining 20146 individuals. Subsequently, the individuals with available BPS and BPF were selected, obtaining 5333 subjects, of which 3699 were adults (over 18 years of age). Data from 3658 patients could be used for regression models (complete data). Subsequently, two classifications were made: Group 1 was performed with individuals with and without diabetes. Groups 2 and 3 were performed by analyzing the individuals based on the concentration of urinary phenols (BPS for group 2 and BPF for group 3).

All individuals whose doctor had diagnosed them with diabetes, those taking blood glucose medication, and individuals with a fasting glucose value ≥ 126 mg/dL or hemoglobin A1c ≥ 6.5% were included in the diabetic group. The individuals classified according to the concentration of phenols were divided into four quartiles for BPS and BPF (Q1-Q4). BPS and BPF values were corrected for urinary creatinine to normalize variations due to hydration or glomerular filtration capacity[30].

Binary logistic regression models were corrected for factors such as age, sex, body mass index (BMI), smoking, hypertension, or dyslipidemia. All those patients diagnosed by their doctor, those with medication for hypertension, and individuals with systolic pressure ≥ 140 mmHg or systolic ≥ 90 mmHg were considered hypertensive. The patients with dyslipidemia were those with diagnosed cholesterol disorders, with prescribed medication or fasting total cholesterol ≥ 240 mg/dL[31]. For smoking, all individuals who answered affirmatively to the question "have you smoked more than 100 cigarettes in your life?" or individuals with a serum cotinine value greater than 10 mg/dL[32] were included.

Statistical analysis

The IBM SPSS Statistics 27 program was used for the statistical analyses to carry out linear regression and logistic regression analyses, and the GraphPad Prism 7.0 program was used for basic descriptive statistics and comparative analysis. In the comparative analysis of the diabetes subgroup, the Mann-Whitney test was used. In the case of classification based on the phenol quartile, the Kruskal-Wallis test was used. The linear regression analysis used the R-squared coefficient of determination to define the percentage of change in the dependent variable affected by the independent variable. The ANOVA test was used to validate the statistical significance of the coefficient. Finally, the β coefficients and their statistical significance were calculated.

Since the diabetes variable is dichotomous, a binary logistic regression model was used. BPF and BPS values were analyzed with the corresponding correction with urinary creatinine, using their logarithmic transformation to normalize the non-parametric distribution. Three different regression analyzes were performed for each parameter: (1) Individual; (2) Corrected for age, sex, and BMI; and (3) Corrected for the above parameters and smoking, hypertension, and dyslipidemia.

In the study of groups 2 and 3, a multinomial logistic regression model was used. As in the previous statistical model, age, sex, BMI, smoking, hypertension, and dyslipidemia were also included. In all cases, those results whose *P* value was less than 0.05 were interpreted as statistically significant.

RESULTS

General data

Descriptive statistical analyses showed, in addition to the expected differences related to blood glucose, interesting changes in BPS levels, significantly higher in diabetic patients. However, the BPF values did not show significant variations. In addition, diabetic patients had higher age, BMI, and systolic pressure and lower total cholesterol (Table 1).

Descriptive analyses of group 2 (distributed according to BPS quartile) showed that individuals with a higher concentration of BPS (Q4) had a significant increase in BMI, fasting glucose, and BPF than individuals with a lower level of BPS (Q1) (Table 2). In addition, the percentages showed a positive and dose-dependent relationship between the BPA quartile and the number of patients with diabetes, hypertension, and dyslipidemia. Interestingly, the percentage of men showed a negative trend with urinary BPS concentration, and a positive trend was observed between the percentage of individuals with diabetes, hypertension, or dyslipidemia, and urinary BPS concentration.

On the other hand, the descriptive analyses of group 3 (distributed according to the BPF quartile) showed significant age differences (in quartiles 2, 3, and 4), BMI (Q2 and Q3), and cotinine (the quartile 4 had a significantly higher concentration than the other three quartiles) (Table 3). In this group, no significant differences were observed in the parameters related to diabetes, but an interesting positive relationship was observed in the percentage of individuals with hypertension.

Table 1 Descriptive statistics of main variables analyzed in individuals of group 1 (diabetes)

	Non-diabetic	Diabetic
n	3017	641
Age	41.11 (40.48-41.75)	58.33 (57.15-59.53)[d]
Gender, % of men	46.6	51
BMI, kg/m^2	27.82 (27.6-28.04)	31.4 (30.86-31.94)[d]
Fasting glucose, mg/dL	98.08 (97.56-98.6)	149.6 (144.4-154.9)[d]
HbA1c, %	5.41 (5.40-5.43)	7.26 (7.14-7.39)[d]
Cotinine, serum, ng/mL	0.28 (0.24-0.31)	0.18 (0.13-0.24)[a]
Smoker, %	42.5	50.2
Systolic blood pressure, mmHg	121 (120.4-121.6)	130.2 (128.7-131.7)[d]
Diastolic blood pressure, mmHg	68.86 (68.42-69.31)	68.21 (67.17-69.26)
Hypertension, %	36.1	71.9
Dyslipidemia, %	35	66.6
Total cholesterol, mg/dL	187.4 (186-188.9)	180.7 (177.2-184.2)[c]
Bisphenol F, µg/g creatinine	0.41 (0.39-0.43)	0.43 (0.38-0.48)
Bisphenol S, µg/g creatinine	0.5 (0.48-0.52)	0.59 (0.53-0.64)[b]

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
[d]$P < 0.0001$.
The results are expressed as percentages (%) or as geometric mean (95%CI). BMI: Body mass index; HbA1c: Hemoglobin A1c.

Simple linear regression

Linear regression analyses were performed using fasting glucose and hemoglobin A1c (HbA1c) values to explore the relationship between diabetes and phenols. As shown in Table 4, the results were significant in the BPS group, while BPF did not show a statistical relationship with these parameters.

Binary logistic regression

The subsequent binomial logistic regression analysis performed on the dichotomous dependent variable diabetes confirmed the data observed in the linear regression (Table 5). Thus, it was observed that the urinary concentration of BPS, both individually and corrected for other factors, was an independent factor related to diabetes mellitus. However, this relationship could not be determined in the urinary concentration of BPF.

Multinomial logistic regression

This statistical analysis model showed a significant relationship between diabetes and BPS, but not BPF (Table 6). However, statistically significant data were only observed in the first two models (individual and corrected for sex, age, and BMI). Although it did not become significant when corrected for all the parameters, the resulting *P* value was 0.063.

Interestingly, in the BPS study, Q4 individuals showed a positive and significant relationship with gender, with an odds ratio (OR) (95%CI) of 1.94 (1.61-2.35) for women. An important relationship was also observed in the risk of suffering hypertension, with an OR of 1.26 (1.01-1.57). In the BPF study, the same significant relationship was observed in gender, with an OR of 2.13 (1.75-2.58) for women. Finally, a positive relationship was observed with smoking [OR of 1.78 (1.47-2.17)] and slightly negative with the BMI [0.98 (0.97-0.998)].

Complementary study of significant pathologies in regression models

Due to the results observed in the regression models and the trends observed in the descriptive statistics, a binomial logistic regression model was established, using hypertension or dyslipidemia as the dependent variable, in order to relate the risk of suffering from any of them depending on the concentration of urinary phenols. As shown in Table 7, urinary BPS is an independent factor related to hypertension. BPF, on the other hand, showed a statistically significant relationship when analyzed individually with both hypertension and dyslipidemia. This relationship held when correcting for age,

Table 2 Descriptive statistics of main variables analyzed in individuals of group 2 (bisphenol S)

BPS quartile	Q1	Q2	Q3	Q4
n	915	911	894	938
Age	42.88 (41.65-44.14)	42.42 (41.22-43.65)	44.5 (43.32-45.72)	45.8 (43.89-46.3)[d]
Gender, % of men	56.3	49.7	42.8	40.6
BMI, kg/m^2	27.76 (27.36-28.17)	28.41 (27.99-28.83)	28.94 (28.51-29.38)[c]	28.57 (28.15-29)[a]
Diabetes mellitus, %	15.3	16.6	18.1	20
Fasting glucose, mg/dL	103.7 (101.8-105.8)	104.5 (102.4-106.6)	108.2 (105.5-111)	108.8 (106.3-111.5)[a]
HbA1c, %	5.62 (5.57-5.68)	5.66 (5.61-5.72)	5.75 (5.69-5.81)	5.78 (5.71-5.85)
Smoker, %	43.6	44.8	42.4	44.5
Cotinine, serum, ng/mL	0.24 (0.19-0.32)	0.27 (0.21-0.35)	0.24 (0.18-0.31)	0.27 (0.21-0.35)
Hypertension, %	39	40.6	43.4	46.3
Systolic blood pressure, mmHg	120.6 (119.5-121.7)	123.1 (122-124.2)	122.5 (121.4-123.7)	123.9 (122.7-125.1)
Diastolic blood pressure, mmHg	68.31 (67.5-69.13)	69.36 (68.52-70.21)	69.18 (68.37-69.99)	68.18 (67.37-69)
Dyslipidemia, %	39.3	40.6	40.8	41.5
Total cholesterol, mg/dL	184.3 (131.6-187)	184.7 (182-187.3)	187.7 (184.9-190.5)	188.3 (185.7-190. 9)
Bisphenol F, μg/g creatinine	0.37 (0.34-0.41)	0.43 (0.39-0.47)[a]	0.41 (0.38-0.45)	0.45 (0.41-0.49)[b]

[a]$P < 0.05$, significant differences with respect to group Q1.
[b]$P < 0.01$, significant differences with respect to group Q1.
[c]$P < 0.001$, significant differences with respect to group Q1.
[d]$P < 0.05$, significant differences between Q2 and Q4.
The results are expressed as percentages (%) or as geometric mean (95%CI). BMI: Body mass index; HbA1c: Hemoglobin A1c; BPS: Bisphenol S; BPF: Bisphenol F.

sex, and BMI for hypertension, but not for dyslipidemia. Finally, no significant relationship was determined after correction for the rest of the parameters.

DISCUSSION

In the present work, it has been demonstrated, for the first time in the NHANES cohort, that BPS, but not BPF, is related to diabetes. The academic literature includes few publications that explore the BPS-diabetes or BPF-diabetes paradigm. There are only three relevant epidemiological studies; two studied type 2 diabetes[18,20], and the remaining investigated gestational diabetes[19].

Duan *et al*[18] considered that all individuals with fasting glucose ≥ 7.0 mmol/L or HbA1c ≥ 6.5% had type 2 diabetes mellitus. After performing the logistic regression analysis, they determined an OR (95%CI) of 1.73 (1.37-2.18) for the urinary BPS, analogous to the results observed in this study.

Rancière *et al*[20] conducted a longitudinal study analyzing the cases of type 2 diabetes developed over 9 years in the DESIR cohort. Due to the low rate of detection of urinary BPS (less than 15%), the statistical model was established comparing individuals with detectable levels of BPS with those in whom the compound had not been detected, obtaining a higher (significant) risk of developing type 2 diabetes in those individuals with detectable levels of BPS. The detection rate in the NHANES cohort was 57.1% and 88.4% for BPF and BPS, respectively[21]. The results also support and reaffirm those obtained in the present work, although the difference in the detection ratio is very striking. Völkel *et al*[33,34] exclusively quantified BPS-glucuronide, the main metabolized form, according to human pharmacokinetic models. In the case of the NHANES cohort, the total concentration of bisphenol was analyzed after previously deconjugating the metabolized forms (glucuronide and sulfate) with Helix pomatia enzymes.

Lastly, Zhang *et al*[19] analyzed BPS and BPF in a cohort of Chinese pregnant women to study their possible relationship with gestational diabetes mellitus. Interestingly, quantitative analyses detected BPS and BPF in most urine samples from pregnant women (greater than 90% in both cases). However, the regression models did not show significant relationships with either compound. They only determined a slight but significant increase in glucose related to urinary BPS concentration. Finally, when studying the relationship between blood glucose and urinary BPS according to fetal sex, they

Table 3 Descriptive statistics of main variables analyzed in individuals of group 3 (bisphenol F)

BPF quartile	Q1	Q2	Q3	Q4
n	912	912	922	912
Age	41.22 (40.04-42.42)	44.81 (43.59-46.06)[c]	45.22 (44.02-46.46)[d]	43.69 (42.5-44.92)[a]
Gender, % of men	58.2	44.5	43.7	43
BMI, kg/m^2	29.04 (28.61-29.48)	28.01 (27.6-28.43)[b]	28.04 (27.63-28.45)[b]	28.59 (28.16-29.02)
Diabetes mellitus, %	17.1	19.7	16.4	16.9
Fasting glucose, mg/dL	108.3 (105.9-110.7)	106.2 (103.9-108.6)	104.7 (102.6-106.9)	105.6 (5.63-5.75)
HbA1c, %	5.7 (5.64-5.75)	5.74 (5.68-5.81)	5.69 (5.63-5.75)	5.69 (5.63-5.75)
Smoker, %	38.6	40.9	46.4	49.3
Cotinine, serum, ng/mL	0.18 (0.14-0.23)	0.19 (0.15-0.25)	0.29 (0.22-0.38)	0.43 (0.33-0.57)[c,e,f]
Hypertension, %	39.7	42	43.3	44.4
Systolic blood pressure, mmHg	121.9 (120.9-123.1)	122.9 (121.8-124.1)	123 (121.8-124.1)	122.3 (121.1-123.5)
Diastolic blood pressure, mmHg	68.73 (67.93-69.53)	68.53 (67.76-69.31)	68.79 (67.94-69.64)	68.95 (68.1-69.82)
Dyslipidemia, %	37.6	41	43.4	40.2
Total cholesterol, mg/dL	185.3 (182.2-187.9)	186.9 (184.1-189.8)	186.7 (184.1-189.4)	185-9 (183.3-188.6)
BPS, μg/g creatinine	0.48 (0.44-0.51)	0.5 (0.46-0.54)	0.55 (0.51-0.59)	0.54 (0.50-0.58)

[a]$P < 0.05$, significant differences with respect to group Q1.
[b]$P < 0.01$, significant differences with respect to group Q1.
[c]$P < 0.001$, significant differences with respect to group Q1.
[d]$P < 0.0001$, significant differences with respect to group Q1.
[e]$P < 0.05$, significant differences between Q2 and Q4.
[f]$P < 0.05$, significant differences between Q3 and Q4.
The results are expressed as percentages (%) or as geometric mean (95%CI). BMI: Body mass index; HbA1c: Hemoglobin A1c; BPS: Bisphenol S; BPF: Bisphenol F.

Table 4 Simple linear regression with hemoglobin A1c and fasting glucose

	HbA1c			Fasting glucose		
Variable	Adjusted R^2	β_0	β	Adjusted R^2	β_0	β
[1]BPS	0.005[d]	5.835[d]	0.069[d]	0.006[c]	111.69[d]	2.48[d]
[1]BPF	0.000	5.795	0.006	0.000	109.65	-0.39

[c]$P < 0.001$.
[d]$P < 0.0001$.
[1]Log transformed.
HbA1c: Hemoglobin A1c; BPS: Bisphenol S; BPF: Bisphenol F.

observed that the relationship was more significant in the case of female fetuses.

The present study determined that there was a significant relationship between urinary BPS and diabetes. However, such a relationship was not observed with urinary BPF. From a molecular point of view, it is interesting to note that BPF, like BPA, has carbon and hydrogen atoms, while BPS also contains sulfur atoms[19]. There is conflicting evidence in the academic literature between BPA and diabetes. Thus, some studies observed a positive and significant relationship with diabetes mellitus[35, 36] or prediabetes[37], while others did not find a significant relationship[38]. In addition, there are even works, such as that by Wang *et al*[39], in which they determined that pregnant women with higher levels of urinary BPA had a lower risk of developing gestational diabetes.

Interestingly, both BPS and BPF (like BPA) have been shown to have pro-estrogenic and anti-androgenic activity[40]. In pancreatic cell cultures, it has been observed that both BPS and BPF can negatively affect insulin secretion and ion channels through a signaling mechanism that includes estrogen receptor beta[16]. A recent animal study conducted by Qiu *et al*[41] observed that BPF and BPS produced similar effects on the immune system in zebrafish. In an experimental non-obese diabetic

Table 5 Association between phenols and diabetes

	Diabetes	
Variable	OR (95%CI)	*P* value
[1]BPS (1)	1.115 (1.038-1.196)	0.003
[1]BPS (2)	1.109 (1.026-1.198)	0.009
[1]BPS (3)	1.099 (1.016-1.188)	0.018
[1]BPF (1)	1.020 (0.961-1.083)	0.513
[1]BPF (2)	1.005 (0.941-1.072)	0.890
[1]BPF (3)	0.991 (0.928-1.059)	0.795

[1]Log-transformed.
(1) Individual; (2) Corrected for age, sex, and body mass index; and (3) Corrected for the above parameters and smoking, hypertension, and dyslipidemia.
OR: Odds ratio; BPS: Bisphenol S; BPF: Bisphenol F.

Table 6 Association between diabetes and bisphenol S or F quartile

	Q1	Q2	Q3	Q4
Variable	Ref.	OR (95% CI)	OR (95%CI)	OR (95%CI)
[1]Diabetes (1)	Ref.	1.1 (0.86-1.41)	1.23 (0.96-1.57)	1.39 (1.09-1.77)[c]
[1]Diabetes (2)	Ref.	1.11 (0.85-1.46)	1.14 (0.87-1.48)	1.32 (1.01-1.71)[a]
[1]Diabetes (3)	Ref.	1.09 (0.83-1.43)	1.12 (0.86-1.47)	1.28 (0.99-1.67)
[2]Diabetes (1)	Ref.	1.19 (0.94-1.51)	0.95 (0.74-1.21)	0.98 (0.77-1.26)
[2]Diabetes (2)	Ref.	1.16 (0.90-1.51)	0.88 (0.68-1.15)	0.94 (0.72-1.23)
[2]Diabetes (3)	Ref.	1.17 (0.90-1.52)	0.86 (0.66-1.13)	0.92 (0.70-1.20)

[a]$P < 0.05$.
[c]$P < 0.001$.
[1]Bisphenol S (group 2).
[2]Bisphenol F (group 3).
(1) Individual; (2) Corrected for age, sex, and body mass index; and (3) Corrected for the above parameters and smoking, hypertension, and dyslipidemia.
OR: Odds ratio.

mouse model, it has been observed that BPS could negatively affect glucose homeostasis in males, while a protective effect was observed in females[17]. From a mechanistic point of view, bisphenols have the potential to affect the development of diabetes through different pathways. In addition to the classical estrogen receptors (ER-α, ER-β, and G protein-coupled receptor 30), BPA has been shown to have increased binding capacity to the estrogen-related receptor (ERR-γ)[43]. ERR-γ is important in diabetes since it plays an essential role in correctly maturing pancreatic β cells[44] and insulin secretion[45]. This receptor also plays a vital role in coordinating metabolic and endocrine signals, regulating hepatic glucose metabolism[46]. Previous work by our group demonstrated that this receptor participates in the loss of podocyte adhesion induced by BPA and is directly related to diabetic nephropathy[3]. On the other hand, it has been observed that both BPA and BPS can affect insulin cell signaling in skeletal muscle and adipose tissue (reducing the expression of insulin receptor substrate 1 and Akt phosphorylation)[43].

The linear regression model of the present work showed very significant values only with the BPS. However, the *R*-squared value was low (0.005) despite being significant. This data implies that the relationship between both variables is low; urinary BPS could only explain a tiny part of diabetes cases. Subsequent binomial and multinomial logistic regression models confirmed and reinforced the relationship between BPS and diabetes while ruling out the statistical relationship with urinary BPF. Nowadays, the vision of "one factor-one disease" could be considered obsolete. Numerous pathologies, such as diabetes, cannot be explained by the action of a single element since they are multifactorial. Therefore, the main idea extracted from the results is that BPS is an environmental factor related to diabetes.

Table 7 Association between hypertension or dyslipidemia and bisphenol S or F

	Hypertension		Dyslipidemia	
Variable	OR (95%CI)	*P* value	OR (95%CI)	*P* value
[1]BPS (1)	1.12 (1.06-1.18)	0.000	0.99 (0.99-1.11)	0.099
[1]BPS (2)	1.09 (1.02-1.17)	0.007	1.02 (0.95-1.08)	0.607
[1]BPS (3)	1.08 (1.01-1.16)	0.017	0.99 (0.937-1.065)	0.980
[1]BPF (1)	1.07 (1.02-1.12)	0.005	1.05 (1.005-1.1)	0.03
[1]BPF (2)	1.06 (1.001-1.12)	0.044	1.04 (0.98-1.1)	0.168
[1]BPF (3)	1.04 (0.99-1.11)	0.136	1.03 (0.98-1.09)	0.274

[1]Log-transformed.
(1) Individual; (2) Corrected for age, sex, and body mass index; and (3) Corrected for the above parameters and smoking, hypertension, and dyslipidemia.
OR: Odds ratio.

On the other hand, complementary studies on hypertension and dyslipidemia have shown interesting evidence. First, both derived compounds show interesting significant relationships with the risk of hypertension, especially BPS. As with diabetes, few works study the relationship between BPS or BPF and these diseases. Jiang *et al*[42] found a positive and significant relationship between individuals with higher levels of urinary BPS, but not BPF, with hypertension. On the other hand, the works of Liu *et al*[22] and Jacobson *et al*[24] found a significant relationship between urinary BPF[22] or both phenolic derivatives[24] and obesity in children and adolescents.

Due to the differences observed in the risks of predisposition to diseases, it could be stated that the compounds derived from BPA (despite having similar hormonal activity) could act on different cell signaling mechanisms, promoting the development or progression of different diseases.

CONCLUSION

The present study has determined a strong relationship between urinary BPS, not BPF, and diabetes risk. In the case of hypertension, both molecules could be involved in pathophysiological mechanisms, which, in the case of dyslipidemia, would be exclusive to BPF. Future studies will be necessary to delve into the paradigm and explore the relationship of the new BPA-derived molecules with other related diseases, such as kidney disease. BPA substitute molecules do not exempt the population from potential health risks.

ARTICLE HIGHLIGHTS

Research background

New restrictions on the use of bisphenol A (BPA) have conditioned the use of new derivative compounds by the plastics industry. The small amount of evidence for its possible effects on human health shows its need, especially in diseases such as diabetes, whose incidence has increased substantially in recent years.

Research motivation

The study of the urinary excretion of the new bisphenols and their possible relationship with human health is of particular importance. The present work aimed to provide new evidence that supports the need for restriction in using new molecules derived from BPA.

Research objectives

The work's objective was to analyze the relationship between urinary bisphenols and diabetes in one of the largest global cohorts, National Health and Nutrition Examination Survey (NHANES). The possible results could support the need to explore the signaling pathways involved in the pancreatic pathophysiology potentially induced by this class of molecules.

Research methods

By applying descriptive statistics, simple linear regressions, and logistic regression models, this study

aimed to analyze the data from the NHANES cohort in a novel way in a context that has been little studied in the academic literature.

Research results

After using all the tools and statistical models, the results have consistently pointed to bisphenol S as a risk factor for diabetes, excluding bisphenol F. On the other hand, the relationships observed with hypertension and dyslipidemia maintain the need to evaluate both molecules in the human health context.

Research conclusions

In a novel way in the NHANES cohort, the present study has shown that exposure to new bisphenols is directly related to diabetes.

Research perspectives

Future research should explore the causal relationship through longitudinal studies and evaluate the potential deleterious effects on other pathologies, such as kidney disease.

FOOTNOTES

Author contributions: Moreno-Gómez-Toledano R contributed to the conceptualization and writing of original draft; Moreno-Gómez-Toledano R, Vélez-Vélez E, Arenas MI, Saura M, and Bosch RJ contributed to the data curation, and manuscript writing, review, and editing; Moreno-Gómez-Toledano R and Vélez-Vélez E contributed to the formal analysis and methodology; Saura M and Bosch RJ contributed to the funding acquisition and project administration; Moreno-Gómez-Toledano R, Vélez-Vélez E, Arenas MI, Saura M, and Bosch RJ contributed to the investigation; all authors have read and agreed to the published version of the manuscript.

Supported by the Instituto de Salud Carlos III, No. PI15/02139; and Comunidad Autónoma de Madrid, No. B2017-BMD-3686.

Institutional review board statement: The study was reviewed and approved for publication by our Institutional Reviewer.

Conflict-of-interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data sharing statement: No additional data are available.

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: Spain

ORCID number: Rafael Moreno-Gómez-Toledano 0000-0002-4834-289X; Esperanza Vélez-Vélez 0000-0003-2786-416X; María I Arenas 0000-0002-7825-0654; Marta Saura 0000-0002-3977-6581; Ricardo J Bosch 0000-0002-9094-1204.

S-Editor: Zhang H
L-Editor: Wang TQ
P-Editor: Zhang H

REFERENCES

1 **Vandenberg LN**, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reprod Toxicol* 2007; **24**: 139-177 [PMID: 17825522 DOI: 10.1016/j.reprotox.2007.07.010]
2 **Vandenberg LN**, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect* 2010; **118**: 1055-1070 [PMID: 20338858 DOI: 10.1289/ehp.0901716]
3 **Moreno-Gómez-Toledano R**, Arenas MI, González-Martínez C, Olea-Herrero N, Reventún P, Di Nunzio M, Sánchez-Esteban S, Arilla-Ferreiro E, Saura M, Bosch RJ. Bisphenol A impaired cell adhesion by altering the expression of adhesion

and cytoskeleton proteins on human podocytes. *Sci Rep* 2020; **10**: 16638 [PMID: 33024228 DOI: 10.1038/s41598-020-73636-6]

4 **Moreno-Gómez-Toledano R**, Arenas MI, Muñoz-Moreno C, Olea-Herrero N, Reventun P, Izquierdo-Lahuerta A, Antón-Cornejo A, González-Santander M, Zaragoza C, Saura M, Bosch RJ. Comparison of the renal effects of bisphenol A in mice with and without experimental diabetes. Role of sexual dimorphism. *Biochim Biophys Acta Mol Basis Dis* 2022; **1868**: 166296 [PMID: 34718120 DOI: 10.1016/j.bbadis.2021.166296]

5 **Reventun P**, Sanchez-Esteban S, Cook A, Cuadrado I, Roza C, Moreno-Gomez-Toledano R, Muñoz C, Zaragoza C, Bosch RJ, Saura M. Bisphenol A induces coronary endothelial cell necroptosis by activating RIP3/CamKII dependent pathway. *Sci Rep* 2020; **10**: 4190 [PMID: 32144343 DOI: 10.1038/s41598-020-61014-1]

6 **Moreno-Gómez-Toledano R**, Sánchez-Esteban S, Cook A, Mínguez-Moratinos M, Ramírez-Carracedo R, Reventún P, Delgado-Marín M, Bosch RJ, Saura M. Bisphenol A Induces Accelerated Cell Aging in Murine Endothelium. *Biomolecules* 2021; **11** [PMID: 34680063 DOI: 10.3390/biom11101429]

7 **European Chemical Agency (ECHA)**. Candidate List of Substances of Very High Concern for Authorisation. 2014 [DOI: 10.1016/b978-0-12-386454-3.00547-9]

8 **Comisión Europea**. Reglamento (UE) 2016/2235 De La Comisión de 12 de diciembre de 2016. Diario Oficial de la Unión Europea (2016). [cited 2021 Nov 24]. Available from: https://www.boe.es/doue/2016/337/L00003-00005.pdf

9 **Rochester JR**, Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environ Health Perspect* 2015; **123**: 643-650 [PMID: 25775505 DOI: 10.1289/ehp.1408989]

10 **Skledar DG**, Schmidt J, Fic A, Klopčič I, Trontelj J, Dolenc MS, Finel M, Mašič LP. Influence of metabolism on endocrine activities of bisphenol S. *Chemosphere* 2016; **157**: 152-159 [PMID: 27213244 DOI: 10.1016/j.chemosphere.2016.05.027]

11 **Ritz E**, Rychlík I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. *Am J Kidney Dis* 1999; **34**: 795-808 [PMID: 10561134 DOI: 10.1016/S0272-6386(99)70035-1]

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

13 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

14 **Glovaci D**, Fan W, Wong ND. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. *Curr Cardiol Rep* 2019; **21**: 21 [PMID: 30828746 DOI: 10.1007/s11886-019-1107-y]

15 **Hwang S**, Lim JE, Choi Y, Jee SH. Bisphenol A exposure and type 2 diabetes mellitus risk: a meta-analysis. *BMC Endocr Disord* 2018; **18**: 81 [PMID: 30400886 DOI: 10.1186/s12902-018-0310-y]

16 **Marroqui L**, Martinez-Pinna J, Castellano-Muñoz M, Dos Santos RS, Medina-Gali RM, Soriano S, Quesada I, Gustafsson JA, Encinar JA, Nadal A. Bisphenol-S and Bisphenol-F alter mouse pancreatic β-cell ion channel expression and activity and insulin release through an estrogen receptor ERβ mediated pathway. *Chemosphere* 2021; **265**: 129051 [PMID: 33250229 DOI: 10.1016/j.chemosphere.2020.129051]

17 **Xu J**, Huang G, Guo TL. Bisphenol S Modulates Type 1 Diabetes Development in Non-Obese Diabetic (NOD) Mice with Diet- and Sex-Related Effects. *Toxics* 2019; **7** [PMID: 31234578 DOI: 10.3390/toxics7020035]

18 **Duan Y**, Yao Y, Wang B, Han L, Wang L, Sun H, Chen L. Association of urinary concentrations of bisphenols with type 2 diabetes mellitus: A case-control study. *Environ Pollut* 2018; **243**: 1719-1726 [PMID: 30408859 DOI: 10.1016/j.envpol.2018.09.093]

19 **Zhang W**, Xia W, Liu W, Li X, Hu J, Zhang B, Xu S, Zhou Y, Li J, Cai Z, Li Y. Exposure to Bisphenol a Substitutes and Gestational Diabetes Mellitus: A Prospective Cohort Study in China. *Front Endocrinol (Lausanne)* 2019; **10**: 262 [PMID: 31114544 DOI: 10.3389/fendo.2019.00262]

20 **Rancière F**, Botton J, Slama R, Lacroix MZ, Debrauwer L, Charles MA, Roussel R, Balkau B, Magliano DJ; D. E.S.I.R. Study Group. Exposure to Bisphenol A and Bisphenol S and Incident Type 2 Diabetes: A Case-Cohort Study in the French Cohort D.E.S.I.R. *Environ Health Perspect* 2019; **127**: 107013 [PMID: 31663775 DOI: 10.1289/EHP5159]

21 **Mendy A**, Salo PM, Wilkerson J, Feinstein L, Ferguson KK, Fessler MB, Thorne PS, Zeldin DC. Association of urinary levels of bisphenols F and S used as bisphenol A substitutes with asthma and hay fever outcomes. *Environ Res* 2020; **183**: 108944 [PMID: 31911000 DOI: 10.1016/j.envres.2019.108944]

22 **Liu B**, Lehmler HJ, Sun Y, Xu G, Sun Q, Snetselaar LG, Wallace RB, Bao W. Association of Bisphenol A and Its Substitutes, Bisphenol F and Bisphenol S, with Obesity in United States Children and Adolescents. *Diabetes Metab J* 2019; **43**: 59-75 [PMID: 30793552 DOI: 10.4093/dmj.2018.0045]

23 **Liu B**, Lehmler HJ, Sun Y, Xu G, Liu Y, Zong G, Sun Q, Hu FB, Wallace RB, Bao W. Bisphenol A substitutes and obesity in US adults: analysis of a population-based, cross-sectional study. *Lancet Planet Health* 2017; **1**: e114-e122 [PMID: 29308453 DOI: 10.1016/S2542-5196(17)30049-9]

24 **Jacobson MH**, Woodward M, Bao W, Liu B, Trasande L. Urinary Bisphenols and Obesity Prevalence Among U.S. Children and Adolescents. *J Endocr Soc* 2019; **3**: 1715-1726 [PMID: 31528831 DOI: 10.1210/js.2019-00201]

25 **Hao K**, Luo J, Sun J, Ge H, Wang Z. Associations of urinary bisphenol A and its alternatives bisphenol S and F concentrations with depressive symptoms among adults. *Chemosphere* 2021; **279**: 130573 [PMID: 33878692 DOI: 10.1016/j.chemosphere.2021.130573]

26 **Maggio CA**, Pi-Sunyer FX. Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am* 2003; **32**: 805-822, viii [PMID: 14711063 DOI: 10.1016/s0889-8529(03)00071-9]

27 **Polsky S**, Ellis SL. Obesity, insulin resistance, and type 1 diabetes mellitus. *Curr Opin Endocrinol Diabetes Obes* 2015; **22**: 277-282 [PMID: 26087341 DOI: 10.1097/MED.0000000000000170]

28 **Riobó Serván P**, Moreno Ruiz I. [Nutrition in chronic kidney disease]. *Nutr Hosp* 2019; **36**: 63-69 [PMID: 31368337 DOI: 10.20960/nh.02812]

29 **Centers for Disease Control and Prevention (CDC)**. National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey (NHANES). (2021). [cited 2021 Dec 1]. Available from: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

30 **Mage DT**, Allen RH, Kodali A. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. *J Expo Sci Environ Epidemiol* 2008; **18**: 360-368 [PMID: 17878925 DOI: 10.1038/sj.jes.7500614]

31 **Moon S**, Yu SH, Lee CB, Park YJ, Yoo HJ, Kim DS. Effects of bisphenol A on cardiovascular disease: An epidemiological study using National Health and Nutrition Examination Survey 2003-2016 and meta-analysis. *Sci Total Environ* 2021; **763**: 142941 [PMID: 33158523 DOI: 10.1016/j.scitotenv.2020.142941]

32 **Pirkle JL**, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. *JAMA* 1996; **275**: 1233-1240 [PMID: 8601954 DOI: 10.1001/jama.1996.03530400021033]

33 **Völkel W**, Colnot T, Csanády GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol* 2002; **15**: 1281-1287 [PMID: 12387626 DOI: 10.1021/tx025548t]

34 **Völkel W**, Bittner N, Dekant W. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos* 2005; **33**: 1748-1757 [PMID: 16103135 DOI: 10.1124/dmd.105.005454]

35 **Shankar A**, Teppala S. Relationship between urinary bisphenol A levels and diabetes mellitus. *J Clin Endocrinol Metab* 2011; **96**: 3822-3826 [PMID: 21956417 DOI: 10.1210/jc.2011-1682]

36 **Tosirisuk N**, Sakorn N, Jantarat C, Nosoongnoen W, Aroonpakmongkol S, Supornsilchai V. Increased bisphenol A levels in Thai children and adolescents with type 1 diabetes mellitus. *Pediatr Int* 2021; **64**: e14944 [PMID: 34342913 DOI: 10.1111/ped.14944]

37 **Sabanayagam C**, Teppala S, Shankar A. Relationship between urinary bisphenol A levels and prediabetes among subjects free of diabetes. *Acta Diabetol* 2013; **50**: 625-631 [PMID: 23636267 DOI: 10.1007/s00592-013-0472-z]

38 **İnce T**, Balcı A, Yalçın SS, Özkemahlı G, Erkekoglu P, Kocer-Gumusel B, Yurdakök K. Urinary bisphenol-A levels in children with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab* 2018; **31**: 829-836 [PMID: 29975667 DOI: 10.1515/jpem-2018-0141]

39 **Wang X**, Wang X, Chen Q, Luo ZC, Zhao S, Wang W, Zhang HJ, Zhang J, Ouyang F. Urinary Bisphenol A Concentration and Gestational Diabetes Mellitus in Chinese Women. *Epidemiology* 2017; **28** Suppl 1: S41-S47 [PMID: 29028674 DOI: 10.1097/EDE.0000000000000730]

40 **Park C**, Song H, Choi J, Sim S, Kojima H, Park J, Iida M, Lee Y. The mixture effects of bisphenol derivatives on estrogen receptor and androgen receptor. *Environ Pollut* 2020; **260**: 114036 [PMID: 31995776 DOI: 10.1016/j.envpol.2020.114036]

41 **Qiu W**, Shao H, Lei P, Zheng C, Qiu C, Yang M, Zheng Y. Immunotoxicity of bisphenol S and F are similar to that of bisphenol A during zebrafish early development. *Chemosphere* 2018; **194**: 1-8 [PMID: 29195089 DOI: 10.1016/j.chemosphere.2017.11.125]

42 **Jiang S**, Liu H, Zhou S, Zhang X, Peng C, Zhou H, Tong Y, Lu Q. Association of bisphenol A and its alternatives bisphenol S and F exposure with hypertension and blood pressure: A cross-sectional study in China. *Environ Pollut* 2020; **257**: 113639 [PMID: 31796315 DOI: 10.1016/j.envpol.2019.113639]

43 **Ahmed F**, Pereira MJ, Aguer C. Bisphenols and the Development of Type 2 Diabetes: The Role of the Skeletal Muscle and Adipose Tissue. *Environ* 2021; **8**: 35 [DOI: 10.3390/ENVIRONMENTS8040035]

44 **Yoshihara E**, Wei Z, Lin CS, Fang S, Ahmadian M, Kida Y, Tseng T, Dai Y, Yu RT, Liddle C, Atkins AR, Downes M, Evans RM. ERRγ Is Required for the Metabolic Maturation of Therapeutically Functional Glucose-Responsive β Cells. *Cell Metab* 2016; **23**: 622-634 [PMID: 27076077 DOI: 10.1016/j.cmet.2016.03.005]

45 **Lei Z**, JunHui L, PeiFeng L. Candidate genes mediated by estrogen-related receptor γ in pancreatic β cells. *J Biochem Mol Toxicol* 2019; **33**: e22390 [PMID: 31478280 DOI: 10.1002/jbt.22390]

46 **Kim DK**, Choi HS. Emerging role of the orphan nuclear receptor estrogen-related receptor gamma in liver metabolic diseases. *Liver Res* 2019; **3**: 99-105 [DOI: 10.1016/j.livres.2019.03.001]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 532-542

DOI: 10.4239/wjd.v13.i7.532

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Study

Efficacy and mechanism of anti-vascular endothelial growth factor drugs for diabetic macular edema patients

Yun-Fei Li, Qian Ren, Chao-Hui Sun, Li Li, Hai-Dong Lian, Rui-Xue Sun, Xian Su, Hua Yu

Specialty type: Ophthalmology

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): 0

P-Reviewer: Hilliard ME, United States; Kilanowska A, Poland; Ortega AL, Spain

Received: February 17, 2022
Peer-review started: February 17, 2022
First decision: April 17, 2022
Revised: April 26, 2022
Accepted: June 16, 2022
Article in press: June 16, 2022
Published online: July 15, 2022

Yun-Fei Li, Qian Ren, Chao-Hui Sun, Li Li, Rui-Xue Sun, Xian Su, Hua Yu, Department of Ophthalmology, Shijiazhuang City People's Hospital, Shijiazhuang 050031, Hebei Province, China

Hai-Dong Lian, Department of Ophthalmology, The First Affiliated Hospital of Shihezi University School of Medicine, Shihezi 832061, Xinjiang Uygur Autonomous Region, China

Corresponding author: Qian Ren, MD, Associate Chief Physician, Department of Ophthalmology, Shijiazhuang City People's Hospital, No. 365 Jianhua South Street, Yuhua District, Shijiazhuang 050031, Hebei Province, China. qr31535@163.com

Abstract

BACKGROUND

Diabetes is a serious public health concern in China, with 30% of patients developing retinopathy, and diabetic macular edema (DME) having the biggest impact on vision. High blood glucose level can cause retinal cell hypoxia, thus promoting vascular endothelial growth factor (VEGF) formation and increasing vascular permeability, which induces DME. Moreover, cell hypoxia can accelerate the rate of apoptosis, which leads to the aging of patients. In severe cases, optic cell apoptosis or retinal fibrosis and permanent blindness may occur.

AIM

To investigate and compare the efficacy, mechanism, and differences between two anti-VEGF drugs (Compaq and ranibizumab) in DME patients.

METHODS

Ninety-six patients with DME who attended our hospital from April 2018 to February 2020 were included and randomly divided into two groups (Compaq group and ranibizumab group). The groups received vitreal cavity injections of 0.5 mg Compaq and 0.5 mg ranibizumab, respectively, once a month. The best corrected visual acuity (BCVA), intraocular pressure (IOP), macular retinal thickness (CMT), macular choroidal thickness (SFCT), foveal no perfusion area (FAZ), superficial capillary density, deep capillary density, treatment effect, and adverse reactions were compared before and after treatment and between the two groups.

RESULTS

Before treatment and 1-mo post-treatment, there was no statistically significant

difference in the estimated BCVA in both groups ($P > 0.05$). BCVA decreased in the Compaq group 3 mo after treatment, and the difference was statistically significant ($P < 0.05$). Before treatment, and 1 mo and 3 mo post-treatment, there was no statistically significant difference in the estimated IOP in either group ($P > 0.05$). Before treatment and 1-mo post-treatment, there was no statistically significant difference in the estimated CMT, SFCT, or FAZ in either group ($P > 0.05$). CMT and SFCT values decreased in the Compaq group 3 mo post-treatment, and the difference was statistically significant ($P < 0.05$). Before treatment, and 1 mo and 3 mo post-treatment, there were no statistically significant differences in vascular density in the shallow or deep capillary plexi of the fovea, parafovea, or overall macular area between the two groups ($P > 0.05$). Marked efficient, effective, and invalid rates were 70.83% and 52.08%, 27.08% and 39.58%, and 2.08% and 8.33% in the Compaq and ranibizumab groups, respectively. The differences between the two groups were statistically significant ($P < 0.05$).

CONCLUSION

Anti-VEGF drugs can effectively improve CMT and SFCT, without affecting microcirculation, thus providing an effective and safe treatment for patients with DME.

Key Words: Diabetic macular edema; Vascular endothelial growth factor; Compaq; Ranibizumab; Optimally correct vision; Diabetes

Core Tip: The main pathological feature of diabetic macular edema (DME) is abnormal neovascularization throughout the retinal pigment epithelium. New vessels develop rapidly and are fragile, thus resulting to rupture and retinal detachment, macular edema, impaired, vision and blind spots. Without effective treatment, vision declines rapidly, causing irreversible impairment. Compaq has a strong affinity with vascular endothelial growth factor (VEGF) receptors, and as a novel VEGF biological agent, it has a relatively strong inhibition of vascular growth in ocular lesions. Our study investigated the effect and mechanism of anti-VEGF drugs in DME patients to improve clinical DME treatment.

Citation: Li YF, Ren Q, Sun CH, Li L, Lian HD, Sun RX, Su X, Yu H. Efficacy and mechanism of anti-vascular endothelial growth factor drugs for diabetic macular edema patients. *World J Diabetes* 2022; 13(7): 532-542
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/532.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.532

INTRODUCTION

Diabetic macular edema (DME), which manifests clinically as visual impairment, is a common complication of diabetes[1-3]. Long-term high blood glucose is the basis of DME, as it causes increased endothelial cell permeability in the tight junctions of retinal capillaries, allowing protein and fluid to accumulate in the area of the macula, resulting in macular edema. Previous studies have also found that along with leaking capillary endothelial cells, an increase in glycosylated hemoglobin due to high blood sugar is also a risk factor for cystoid macular edema. Strict blood glucose control within a reasonable range can slow down the development of DME.

Vascular endothelial growth factor (VEGF) play an important role in the pathophysiology of DME[4]. In a high blood glucose environment, glycosylation products increase, and active oxygen is at a relatively high level, leading to further diglyceride production. This activates protein kinase C, which mediates the generation of VEGF[5]. VEGF is the primary regulatory factor for neovascularization and vascular permeability, which characterizes DME. The interaction of vascular receptors on the endothelial cell surface can be inactivated through VEGF inhibition to prevent vascular endothelial hyperplasia, thereby reducing retinal neovascularization and blood vessel leakage in the macular area. Currently, according to evidence from multiple regions, the pharmaceutical drug Compaq (Chengdu Kanghong Biotechnology Co., LTD., Chengdu, China; National Drug approval S20130012)[6] acts directly on new vessels in retinal lesion tissue, thus reducing the incidence of blood vessel hyperplasia caused by photocoagulation. Moreover, inreverse rate of retinal tissue can be reduced by laser photocoagulation reduction. For these reasons, anti-VEGF drugs are recommended as the first-line treatment for DME to improve clinical manifestations. Examples of anti-VEGF drugs used for macular edema in China include bevacizumab, Compaq, and ranibizumab[7]. Vitreal cavity injection is the preferred route of administration for anti-VEGF drugs.

Compaq is a novel anti-VEGF drug developed independently in China, which can specifically bind to VEGF-A, VEGF-B, and placenta growth factors to inhibit the activation of VEGF families, thus preventing neovascularization and reducing blood retinal barrier (BRB) damage and minor microvasculature leakage. Studies have shown[8,9] Compaq as an efficacious DME treatment, but whether it can inhibit neovascularization, reduce the perfusion area in the macular area, and improve microcirculation remains unclear.

There are a few studies comparing the effects of Compaq with that of ranibizumab for DME administered *via* vitreal cavity injection. For this study, we used optical coherence tomography angiography (OCTA) to measure the treatment effect and mechanism of action in these two anti-VEGF drugs. Based on the segment frequency amplitude of B-scan correlation calculation and the real-time flow of red blood cells in retinal vascular correlation calculation, OCTA can remove the correlation of fixed organization, highlight regular blood flow images of the organization, and recombine all images to obtain structures, such as the retina and choroid blood vessels, to facilitate blood flow evaluation[10].

MATERIALS AND METHODS

Patients

A total of 96 patients with DME who visited our hospital from April 2018 to February 2020 were included in the study. Patients were randomly divided into two groups: Compaq (n = 48) and ranibizumab (n = 48) by assigning even and odd-numbered sequences. The inclusion criteria were as follows: (1) Age 54–79 years; (2) Diagnosed according to the criteria and treatment guidelines for diabetic retinopathy (DR)[11] (with DME within the proliferative phase of the DR lesion stage); (3) Hospital admission and receipt of fundoscopic angiography, with diagnosis by OCTA examination; (4) Retinal fovea thickness in the macular area > 250 μm; and (5) Ocular IOP range: 10-21 mmHg. The exclusion criteria were as follows: (1) Presence of eye tumors; (2) History of ocular trauma; (3) Retinal macular spots and tissue hyperplasia; (4) Reticular vein obstruction and senile macular lesions; (5) Hypertensive retinopathy; (6) Combined cataract and glaucoma; and (7) Other systematic major disease.

The study was approved by the Medical Ethics Committee of our hospital, and informed consent from the patients' families was obtained for the treatment plan of this study.

Therapeutic regimen

Patients were placed in the supine position, and the cornea and conjunctival sac were cleaned. Routine pre-anesthesia operations were performed, followed by surface anesthesia. Under the microscope (Nikon, Tokyo, Japan), the doctor opened the patient's eyelid. Compaq (0.10 mg/mL, 0.2 mL/injection) was injected into the vitreous chamber of patients in both groups. After injection, the needle was withdrawn slowly, and 0.5 mg/eye/time was infused in the vitreous cavity once per month for the first 3 mo (0.05 mL), followed by intravitreal administration once every 3 mo.

Basic treatment

All patients underwent lacrimal tract irrigation 3 d before surgery and were treated with levofloxacin eye drops four times a day for 5 d (Shentian Pharmaceutical Co., LTD. Noto Factor, Japan; National medicine approval number J20150106; 10 mL/branch/box) after surgery.

Observation indexes and evaluations

The best corrected visual acuity (BCVA), intraocular pressure (IOP), macular retinal thickness (CMT), macular choroidal thickness (SFCT), foveal no perfusion area (FAZ), superficial capillary density, deep capillary density, treatment effect, and adverse reactions were compared between the two groups before and after treatment.

BCVA was measured using a TDRS visual acuity chart at a distance of 4 m, and the maximum number of letters obtained was recorded at four timepoints: pre-treatment, and at 1, 3, and 6 mo post-treatment.

For IOP measurement, a non-contact ophthalmometer (model AD-1900; Neusoft Xikang Co., LTD., Shenyang, China) was used, and the average value of three inspections were taken.

Astigmatism was examined using the slit slice method, and an OCT instrument (American BD company production, model AU-300; GE Company, Chicago, Illinois) was used to scan the macular area within a range of 6 mm × 6 mm. Consequently, the retinal thickness of the macular fovea was measured.

Treatments were evaluated with reference to the diagnosis and treatment of diabetic retinopathy[12]. Treatments were considered markedly effective if edema had disappeared, retinal hemorrhage had been completely absorbed, and there was no obvious neovascularization, leakage, or perfusion in the macular area 3 mo after treatment. Treatments were considered effective if retinal hemorrhage had partially absorbed, vascular leakage was reduced, the perfusion density (PD) in the non-perfusion area was < 5, and no new vessels were assumed to be functioning. Lastly, treatments were considered invalid if patients did not meet the above criteria.

Ophthalmic testing included the following: (1) Routine examination: BCVA was measured using an international standard visual acuity chart; IOP was measured using a non-contact tonometer; and (2) OCT examination: A Zeiss Cirrus 5000 OCT instrument (Carl Zeiss Meditec, Jena, Germany) was used. CMT was measured with 2 PD on the optic disc temporal side and 1.5 PD below the macular fovea. Three measurements were obtained, and the average value was considered. SFCT was measured using the caliper function of the instrument. The retinal blood flow imaging mode was selected, and the scanning range of the macular area was 3 mm × 3 mm, scanning signal intensity index was > 45, and transverse and longitudinal scanning required 3 s. The patients were instructed not to move their eyes during the scan. Supporting analysis software was used to measure the vessel density of the shallow capillary plexus (SCP) and deep capillary plexus (DCP) in the FAZ within the scanning range of 3 mm × 3 mm in the macular area at the SCP level. All examinations were performed by the same physician and reviewed independently by two surgeons clinically experienced fundus imaging analysis.

Statistical analysis

A normal distribution test showed that the BCVA values of the patients in this study were consistent with an approximate normal distribution or normal distribution, and was expressed as mean ± SD. Test was used for comparisons between groups. The counting data were expressed as percentages, and comparisons were based on χ^2 test or the Mann–Whitney U test. Professional SPSS 21.0 software (IBM Corp., Armonk, NY) was used for data processing, and the significance level was set at $\alpha = 0.05$.

RESULTS

Baseline data between the two groups

The age, sex, body mass index, and BCVA before treatment and the distribution of the affected side were compared between the two groups, and no statistically significant difference was found ($P > 0.05$), as shown in Table 1.

Comparison of estimated BCVA and IOP values between the two groups

Before and 1-mo post-treatment, there was no statistically significant difference between the estimated value of BCVA in either group ($P > 0.05$). After 3 mo, a decrease was observed in the Compaq group, and the difference was statistically significant ($P < 0.05$). However, there was no statistically significant difference in the estimated value of IOP before, 1 mo, or 3 mo after the treatment in either group ($P > 0.05$), as shown in Table 2.

Comparison of estimated CMT, SFCT, and FAZ values between the two groups

There were no statistically significant differences in the estimated values of CMT, SFCT, or FAZ before or 1-mo post-treatment in either group ($P > 0.05$). Three months post-treatment, the estimated values of CMT and SFCT in the Compaq group were lower than those in the ranibizumab group, and the difference was statistically significant ($P < 0.05$) (Table 3).

Comparison of vascular density in the SCP and DCP between the two groups

Before, 1 mo, and 3 mo post-treatment, there were no statistically significant differences in the vascular density of the SCP and DCP of the fovea, parafovea, or overall macular area between the Compaq and ranibizumab groups ($P > 0.05$), as shown in Table 4 and Table 5.

Comparison of clinical efficiency between the two groups

Three months post-treatment, the rates of marked efficiency, effective, and invalid in the Compaq and ranibizumab groups were 70.83% and 52.08%, 27.08% and 39.58%, and 2.08% and 8.33%, respectively, and the difference between the two groups was statistically significant ($P < 0.05$), as shown in Table 6 and Figure 1A.

Comparison of adverse reaction rates between the two groups

The rates of adverse reactions in the Compaq and ranibizumab groups were 6.25% and 12.50%, respectively, and there was no statistically significant difference between the two groups ($P > 0.05$), as shown in Table 7 and Figure 1B.

Typical cases

A 71-year-old male patient, with a history of diabetes over 12 years, reported a significant decrease in visual acuity in the prior 6 mo. After admission, he was diagnosed with diabetic macular edema by fundus angiography and optical coherence tomography. Before treatment, the foveal thickness in the macular area was > 477.2 μm, and the BCVA value was 0.83 LogMAR. The patient was treated with intravitreal injection of Conbercept 0.5 mg once a month. The OCT examination results before and after treatment are shown in Figure 2. The patient's visual acuity BCVA recovered to 0.55 LogMAR 3 mo after

Table 1 Baseline data between the two groups

Baseline data	Compaq group (*n* = 48)	Ranibizumab group (*n* = 48)	t/χ^2 value	*P* value
Age (yr)	64.8 ± 7.2	66.3 ± 6.9	-1.042	0.300
BMI (kg/m^2)	23.5 ± 2.3	23.2 ± 2.8	0.574	0.568
Before treatment: BCVA (LogMAR)	0.78 ± 0.12	0.80 ± 0.11	-0.851	0.397
Gender, *n* (%)			2.043	0.153
Male	27 (56.25)	20 (41.67)		
Female	21 (43.75)	28 (58.33)		
Distribution of affected side, *n* (%)			0.667	0.414
Left	22 (45.83)	26 (54.17)		
Right	26 (54.17)	22 (45.83)		

BCVA: Best corrected visual acuity.

Table 2 Comparison of estimated values of best corrected visual acuity, intraocular pressure between the two groups (mean ± SD)

Groups	BCVA (LogMAR)			IOP (mmHg)		
	Before treatment	1 mo after treatment	3 mo after treatment	Before treatment	1 mo after treatment	3 mo after treatment
Compaq group (*n* = 48)	0.78 ± 0.12	0.72 ± 0.13	0.51 ± 0.10	16.84 ± 2.77	16.40 ± 2.81	16.39 ± 2.64
Ranibizumab group (*n* = 48)	0.80 ± 0.11	0.75 ± 0.14	0.57 ± 0.13	16.50 ± 2.80	16.72 ± 2.76	16.81 ± 2.82
t value	-0.851	-1.088	-2.535	0.598	-0.563	-0.753
P value	0.397	0.279	0.013	0.551	0.575	0.453

BCVA: Best corrected visual acuity; IOP: Intraocular pressure.

treatment.

DISCUSSION

In diabetes, the retina is prone to injury due to oxidative stress. A high blood glucose environment can increase active oxygen levels as well as oxygen production in the mitochondria, thus impeding balance between the deoxidation and neutralization of mitochondrial reactive oxygen species in the body. This increased oxidative stress level results in macular edema[13,14]. Research has verified that the excessive production of mitochondrial reactive oxygen species and the decrease of antioxidant enzymes promote the progression of diabetes.

As a novel anti-VEGF, developed independently in China, Compaq can be used to inhibit the increase of vascular wall permeability and neovascularization. The VEGF concentrations in the vitreous chamber and perivascular vessels on the retinal surface are abnormally elevated in DME patients, resulting in retinal neovascularization and increased vascular wall permeability, finally leading to edema. Our study showed that before treatment and 1-mo post-treatment, there was no statistical difference in BCVA between the two groups. However, 3 mo post-treatment, BCVA was significantly lower in the Compaq group than in the ranibizumab group.

Various kinds of anti-VEGF drugs are commonly used in DME treatment, including imported drugs, such as ranibizumab. These drugs bind to and inhibit VEGF receptors to prevent the formation of specific receptors of neovascularization, and therefore, blood glucose and its effects such as retinal capillary permeability can be reduced to improve vision[15]. Compaq eye injections have a significant effect on neovascularization inhibition to reduce VEGF concentration and vascular wall permeability in the eyes and reduce the infiltration of blood vessels; therefore, retinal edema can be absorbed and the degree of macular edema can be relieved to significantly improve visual performance. At present, Compaq is used globally in the field of ophthalmology to reduce macular central retina thickness and choroid thickness of the macular fovea to improve vision in DME patients. Compaq treatment has been

Table 3 Comparison of estimated values of macular retinal thickness, macular choroidal thickness, foveal no perfusion area between the two groups (mean ± SD)

Groups	Before treatment	1 mo after treatment	3 mo after treatment
CMT (μm)			
Compaq group (*n* = 48)	445.8 ± 89.6	372.1 ± 76.0	210.6 ± 66.4
Ranibizumab group (*n* = 48)	452.7 ± 93.2	384.0 ± 80.6	243.1 ± 73.5
t value	-0.370	-0.744	-2.273
P value	0.712	0.459	0.025
SFCT (μm)			
Compaq group (*n* = 48)	335.1 ± 55.9	323.4 ± 59.5	281.6 ± 54.0
Ranibizumab group (*n* = 48)	340.5 ± 58.3	330.5 ± 63.0	306.2 ± 57.3
t value	-0.463	-0.568	-2.165
P value	0.644	0.572	0.033
FAZ (mm^2)			
Compaq group (*n* = 48)	0.74 ± 0.10	0.72 ± 0.12	0.73 ± 0.11
Ranibizumab group (*n* = 48)	0.75 ± 0.12	0.74 ± 0.14	0.74 ± 0.11
t value	-0.444	-0.751	-0.445
P value	0.658	0.454	0.657

CMT: Macular retinal thickness; SFCT: Macular choroidal thickness; FAZ: Foveal no perfusion area.

Table 4 Comparison of vascular density in the shallow capillary plexus between the two groups (mean ± SD, %)

Groups	Before treatment	1 mo after treatment	3 mo after treatment
Fovea			
Compaq group (*n* = 48)	20.64 ± 4.40	20.30 ± 3.95	20.28 ± 3.77
Ranibizumab group (*n* = 48)	20.90 ± 4.83	20.48 ± 4.20	20.37 ± 4.14
t value	-0.276	-0.216	-0.111
P value	0.783	0.829	0.912
Parafovea			
Compaq group (*n* = 48)	38.56 ± 4.82	38.10 ± 4.50	37.73 ± 4.72
Ranibizumab group (*n* = 48)	39.10 ± 5.57	38.67 ± 5.53	38.38 ± 5.28
t value	-0.508	-0.554	-0.636
P value	0.613	0.581	0.526
Overall macular area			
Compaq group (*n* = 48)	35.74 ± 5.10	35.43 ± 4.85	34.92 ± 5.51
Ranibizumab group (*n* = 48)	36.30 ± 5.34	35.67 ± 5.11	34.58 ± 5.18
t value	-0.525	-0.236	0.311
P value	0.601	0.814	0.756

proven to be effective and safe[16].

DME treatment of the retina and choroid can sometimes lead to retinal capillary cell loss and degeneration and vascular endothelial cell hyperplasia in diabetes patients, thereby destroying the blood-retinal barrier[17]. Meanwhile, VEGF secretion can be increased, leading to retinal tight junction dysfunction and pericyte loss, as well as an impaired BRB; thus, vascular wall permeability can be increased, causing fluid to leak into retinal tissue and accumulate, resulting in macular edema. Severe cases may develop macular edema, thickening, vision loss, or even blindness. Some studies have found

Table 5 Comparison of vascular density in the deep capillary plexus between the two groups (mean ± SD, %)

Groups	Before treatment	1 mo after treatment	3 mo after treatment
Fovea			
Compaq group (n = 48)	18.58 ± 3.80	18.23 ± 3.75	17.86 ± 4.12
Ranibizumab group (n = 48)	19.14 ± 4.00	18.78 ± 4.24	18.47 ± 3.96
t value	-0.703	-0.673	-0.740
P value	0.484	0.502	0.461
Parafovea			
Compaq group (n = 48)	40.92 ± 5.73	40.51 ± 4.85	40.38 ± 5.22
Ranibizumab group (n = 48)	40.40 ± 5.51	40.10 ± 5.28	39.56 ± 4.87
t value	0.453	0.396	0.796
P value	0.651	0.693	0.428
Overall macular area			
Compaq group (n = 48)	39.64 ± 4.85	39.40 ± 4.77	38.78 ± 4.62
Ranibizumab group (n = 48)	40.43 ± 5.18	39.93 ± 5.03	39.52 ± 4.85
t value	-0.771	-0.530	-0.765
P value	0.442	0.598	0.446

Table 6 Comparison of clinical efficiency between the two groups, n (%)

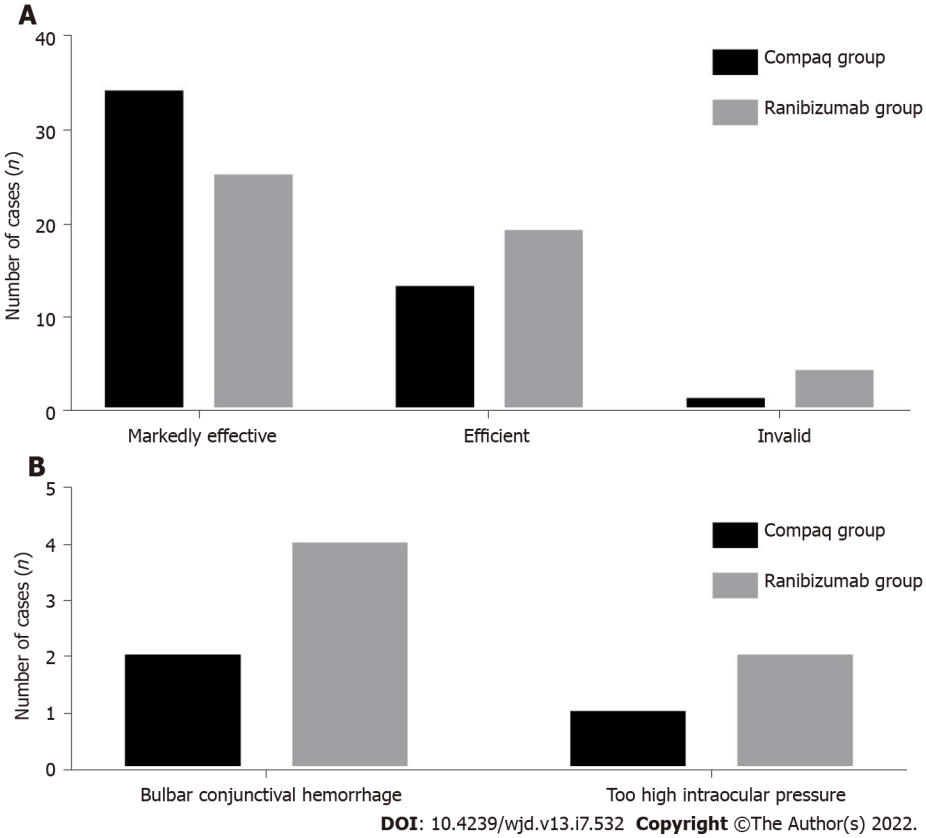
Groups	Markedly efficiency	Efficient	Invalid
Compaq group (n = 48)	34 (70.83)	13 (27.08)	1 (2.08)
Ranibizumab group (n = 48)	25 (52.08)	19 (39.58)	4 (8.33)
Z value	-1.993		
P value	0.046		

Table 7 Comparison of incidence of adverse reaction between the two groups, n (%)

Groups	Bulbar conjunctival hemorrhage	Too high intraocular pressure	Adverse reaction
Compaq group (n = 48)	2	1	3 (6.25)
Ranibizumab group (n = 48)	4	2	6 (12.50)
χ^2 value			1.333
P value			0.248

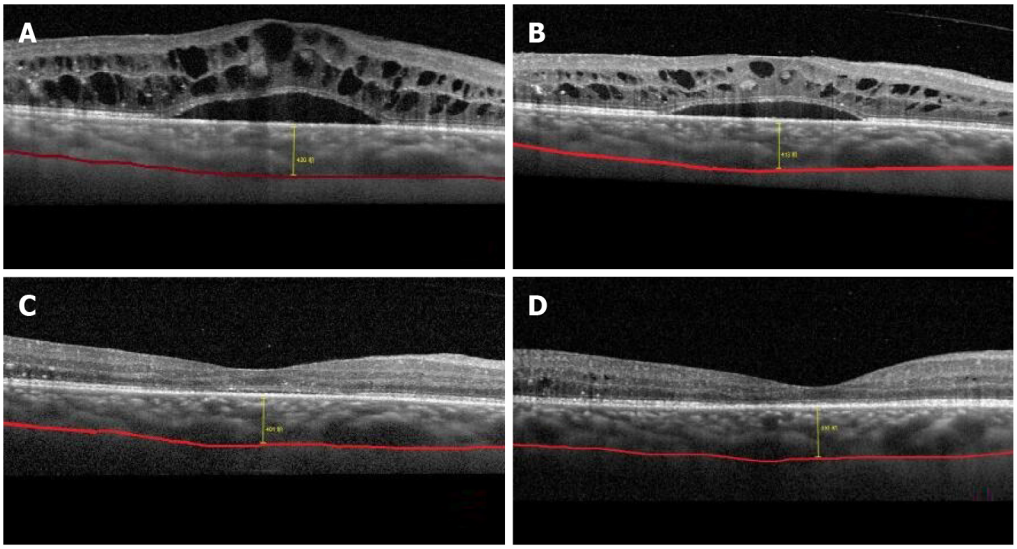
that DR patients may have a relatively thin choroid compared to DME patients. Upon OCTA examination, the thicknesses and changes in each retinal layer can be clearly observed, and the structural image and thickness of the choroid can be distinguished[18]. However, according to our study results, before treatment and 1-mo post-treatment, there was no statistical difference in CMT, SFCT, or FAZ level between the two groups. Three months post-treatment, the estimated values of CMT and SFCT in the Compaq group were significantly lower than those in the ranibizumab group.

Moreover, previous studies have shown that increased total cholesterol, triglycerides, and low-density lipoprotein is related to DME in diabetic patients. Anti-VEGF administered through vitreal cavity injection can improve glucose and lipid levels and decrease levels of oxidative stress throughout the body, which significantly reduces VEGF production, thus reducing retinal vein and artery diameter. This also reduces the permeability of retinal capillaries, inhibiting neovascularization and reducing the extent of damage in the BRB. The thicknesses of the macular central retina and choroid can be reduced once macular edema is relieved. After anti-VEGF treatment in DME, the detailed reasons for decreased macular central choroid thickness remain unclear, which may be because anti-VEGF drugs such as Compaq can inhibit signaling, and the activity of VEGF can be inhibited by binding to VEGF to reduce



DOI: 10.4239/wjd.v13.i7.532

Figure 1 Histogram of clinical efficiency and incidence of adverse reaction between the two groups. A: Histogram of clinical efficiency; B: Incidence of adverse reaction.



DOI: 10.4239/wjd.v13.i7.532

Figure 2 The optical coherence tomography test results of before and after patient treatment. A: The test result of optical coherence tomography before the treatment, where macular edema was obvious; B: Re-examination after 1 wk of treatment, where the macular edema is slightly relieved; C: Re-examination after 1 month of treatment, where the macular edema was significantly reduced; D: The condition of the patient 3 mo post-treatment, where the macular edema has nearly disappeared, and the choroid thickness has become significantly thinner.

respiration and edema caused by retinal vascular leakage, resulting in decreased macular central retina and choroid thickness for improved vision.

DME is a complex pathologic progress caused by multiple factors. Currently, it is believed that microvascular lesion in diabetes can cause blood flow changes in retinal microvasculature, and histanoxia can lead to aggravated inflammation, resulting in the release of various inflammatory factors,

such as prostaglandins, leukocyte trienes, intercellular adhesion molecule-1, integrin, and tumor necrosis factor α. These factors cause vascular endothelial injury and increased VEGF secretion, thus promoting macular edema[19]. Conversely, macular edema can aggravate histanoxia, and VEGF can be further increased to stimulate the growth of new blood vessels, as well as the formation of a microaneurysm, which becomes a negative feedback loop. Our study showed that, before and after treatment, there were no statistical differences in vascular density in the SCP and DCP in the overall macular area between the two groups. There was also no significant difference in treatment efficacy, indicating that the two drugs selected in our study had no influence on microcirculation, and were safe. Compaq can directly act on new blood vessels in retinal lesions and reduce the damage caused by laser photocoagulation on the retina, thereby resulting in a decreased inflammatory response rate in retinal tissue.

Some studies have shown that both ranibizumab and Compaq can reduce the macular FAZ area and increase vascular density in the SCP to improve microcirculation[20]. With the progression of diabetes, VEGF secretion can be abnormally increased and BRB irreversibility can be aggravated, resulting in the interrupted integrity of the macular arch, and forming of the microaneurysm. Normal vascular tissue is destroyed, and the overall vascular density of the macular area is also reduced. Reports on the quantitative observation of the FAZ area and vascular density using OCTA in the treatment of DME remain unclear.

Previous studies[21] used Conbercept for treatment and found that it can exert strong affinity and multi-target characteristics in the treatment, and can reach the target concentration in a short time. Anti-VEGF therapy decreased SFCT, which has become a relevant parameter for drug selection and follow-up evaluation. DME has a very large impact on the choroid and has a very large impact on the patient's visual acuity. Anti-VEGF drugs can inhibit the biological activity of abnormal VEGF in new blood vessels in the body. From the results of this study, it can be concluded that anti-VEGF drugs can effectively improve CMT and SFCT in patients with DME, restore good visual effects, and represent a safe and efficient treatment regimen for DME. At present, there is no accurate software for measuring choroidal thickness, and the measurement of SFCT is performed by highly qualified physicians. Inevitably, there will be some errors. Automatic analysis software may reduce these errors and reduce the number of times choroidal thickness is measured.

Previous studies on the effect of anti-VEGF drugs on DME have mostly analyzed the changes in visual acuity, central macular retinal thickness, and choroid thickness, while other studies have focused on the changes and correlation in eye axis before and after treatment[22]. This study is unique in that it observed and analyzed the effects of anti-VEGF drugs on SFCT, FAZ, and microcirculation. Concurrently, it also explored the improvements in conventional indicators, such as vision and intraocular pressure, which are of high clinical value.

This study was a clinical controlled study on the treatment of DME patients with vitreous injection of ranibizumab and Compaq. During the follow-up process, we discovered that both drugs could reduce CMT and SFCT to a certain extent and improve the visual acuity of patients. However, this study was a preliminary clinical application study with a small sample size and a short follow-up period; hence, further studies are warranted to confirm our findings.

CONCLUSION

In summary, anti-VEGF drugs can effectively improve CMT and SFCT, without affecting microcirculation, thus resulting in positive treatment outcomes.

ARTICLE HIGHLIGHTS

Research background

Diabetes is a serious public health concern in China, with 30% of patients developing retinopathy, and diabetic macular edema (DME) having the biggest impact on vision.

Research motivation

Compaq as an efficacious DME treatment, but whether it can inhibit neovascularization, reduce the perfusion area in the macular area, and improve microcirculation remains unclear.

Research objectives

This study aimed to investigate and compare the efficacy, mechanism, and differences between two anti-vascular endothelial growth factor (VEGF) drugs (Compaq and ranibizumab) in DME patients.

Research methods

Total 96 patients with DME were divided into two groups with different treatment modalities.

Research results

Marked efficient, effective, and invalid rates were 70.83% and 52.08%, 27.08% and 39.58%, and 2.08% and 8.33% in the Compaq and ranibizumab groups, respectively.

Research conclusions

Anti-VEGF drugs can effectively improve macular retinal thickness and macular choroidal thickness, without affecting microcirculation.

Research perspectives

This study was a preliminary clinical application study with a small sample size and a short follow-up period; hence, further studies are warranted to confirm our findings.

FOOTNOTES

Author contributions: Li YF and Yu H designed this study; Li YF wrote this manuscript; Li YF, Ren Q, Sun ZH, Li L, Lian HD, Sun RX, Sun X and Yu H were responsible for sorting the data; and all authors read and confirmed the revision of the manuscript.

Institutional review board statement: The study was reviewed and approved by the Shijiazhuang City People's Hospital Institutional Review Board (Approval No. 2017-22).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors have nothing to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number: Yun-Fei Li 0000-0003-3284-1667; Qian Ren 0000-0003-3792-1332; Chao-Hui Sun 0000-0002-0748-6117; Li Li 0000-0002-0721-375X; Hai-Dong Lian 0000-0001-6066-9951; Rui-Xue Sun 0000-0003-2828-5119; Xian Su 0000-0002-9853-5868; Hua Yu 0000-0001-7901-8235.

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

REFERENCES

1 **Alnagdy AA**, Abouelkheir HY, El-Khouly SE, Tarshouby SM. Impact of topical nonsteroidal anti-inflammatory drugs in prevention of macular edema following cataract surgery in diabetic patients. *Int J Ophthalmol* 2018; **11**: 616-622 [PMID: 29675380 DOI: 10.18240/ijo.2018.04.13]
2 **Rinaldi M**, dell'Omo R, Morescalchi F, Semeraro F, Gambicorti E, Cacciatore F, Chiosi F, Costagliola C. ILM peeling in nontractional diabetic macular edema: review and metanalysis. *Int Ophthalmol* 2018; **38**: 2709-2714 [PMID: 29090356 DOI: 10.1007/s10792-017-0761-6]
3 **Chaudhary S**, Zaveri J, Becker N. Proliferative diabetic retinopathy (PDR). *Dis Mon* 2021; **67**: 101140 [PMID: 33546872 DOI: 10.1016/j.disamonth.2021.101140]
4 **Kang YK**, Park HS, Park DH, Shin JP. Incidence and treatment outcomes of secondary epiretinal membrane following intravitreal injection for diabetic macular edema. *Sci Rep* 2020; **10**: 528 [PMID: 31953511 DOI: 10.1038/s41598-020-57509-6]
5 **Akkaya S**, Açıkalın B, Doğan YE, Çoban F. Subthreshold micropulse laser versus intravitreal anti-VEGF for diabetic macular edema patients with relatively better visual acuity. *Int J Ophthalmol* 2020; **13**: 1606-1611 [PMID: 33078112 DOI: 10.18240/ijo.2020.10.15]
6 **Mathis T**, Dot C, Mauget-Faÿsse M, Kodjikian L. Variation of choroidal thickness in diabetic macular edema: friend or foe? *Acta Ophthalmol* 2021; **99**: e282-e283 [PMID: 32588972 DOI: 10.1111/aos.14530]
7 **Busch C**, Fraser-Bell S, Zur D, Rodríguez-Valdés PJ, Cebeci Z, Lupidi M, Fung AT, Gabrielle PH, Giancipoli E, Chaikitmongkol V, Okada M, Laíns I, Santos AR, Kunavisarut P, Sala-Puigdollers A, Chhablani J, Ozimek M, Hilely A,

Unterlauft JD, Loewenstein A, Iglicki M, Rehak M; International Retina Group. Real-world outcomes of observation and treatment in diabetic macular edema with very good visual acuity: the OBTAIN study. *Acta Diabetol* 2019; **56**: 777-784 [PMID: 30903434 DOI: 10.1007/s00592-019-01310-z]

8 **Maloney MH**, Schilz SR, Herrin J, Sangaralingham LR, Shah ND, Barkmeier AJ. Risk of Systemic Adverse Events Associated with Intravitreal Anti-VEGF Therapy for Diabetic Macular Edema in Routine Clinical Practice. *Ophthalmology* 2019; **126**: 1007-1015 [PMID: 30292542 DOI: 10.1016/j.ophtha.2018.09.040]

9 **Mastropasqua L**, Di Staso S, D'Aloisio R, Mastropasqua A, Di Antonio L, Senatore A, Ciancaglini M, Di Nicola M, Di Martino G, Tognetto D, Toto L. Anatomical and functional changes after dexamethasone implant and ranibizumab in diabetic macular edema: a retrospective cohort study. *Int J Ophthalmol* 2019; **12**: 1589-1597 [PMID: 31637195 DOI: 10.18240/ijo.2019.10.11]

10 **Jenkins AJ**, Joglekar MV, Hardikar AA, Keech AC, O'Neal DN, Januszewski AS. Biomarkers in Diabetic Retinopathy. *Rev Diabet Stud* 2015; **12**: 159-195 [PMID: 26676667 DOI: 10.1900/RDS.2015.12.159]

11 **Cheung N**, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010; **376**: 124-136 [PMID: 20580421 DOI: 10.1016/S0140-6736(09)62124-3]

12 **Seery CW**, Betesh S, Guo S, Zarbin MA, Bhagat N, Wagner RS. Update on the Use of Anti-VEGF Drugs in the Treatment of Retinopathy of Prematurity. *J Pediatr Ophthalmol Strabismus* 2020; **57**: 351-362 [PMID: 33211892 DOI: 10.3928/01913913-20200824-02]

13 **Osathanugrah P**, Sanjiv N, Siegel NH, Ness S, Chen X, Subramanian ML. The Impact of Race on Short-term Treatment Response to Bevacizumab in Diabetic Macular Edema. *Am J Ophthalmol* 2021; **222**: 310-317 [PMID: 33045219 DOI: 10.1016/j.ajo.2020.09.042]

14 **Euswas N**, Phonnopparat N, Morasert K, Thakhampaeng P, Kaewsanit A, Mungthin M, Rangsin R, Sakboonyarat B. National trends in the prevalence of diabetic retinopathy among Thai patients with type 2 diabetes and its associated factors from 2014 to 2018. *PLoS One* 2021; **16**: e0245801 [PMID: 33481907 DOI: 10.1371/journal.pone.0245801]

15 **Ruiz-Moreno JM**, de Andrés-Nogales F, Oyagüez I. Cost-consequence analysis of extended loading dose of anti-VEGF treatment in diabetic macular edema patients. *BMC Ophthalmol* 2020; **20**: 371 [PMID: 32943041 DOI: 10.1186/s12886-020-01637-0]

16 **Starr MR**, Mahr MA, Smith WM, Iezzi R, Barkmeier AJ, Bakri SJ. Outcomes of Patients With Active Diabetic Macular Edema at the Time of Cataract Surgery Managed With Intravitreal Anti-Vascular Endothelial Growth Factor Injections. *Am J Ophthalmol* 2021; **229**: 194-199 [PMID: 33852907 DOI: 10.1016/j.ajo.2021.04.002]

17 **Yoshitake T**, Murakami T, Yoshitake S, Suzuma K, Dodo Y, Fujimoto M, Tsujikawa A. Anti-Fumarase Antibody as a Predictor of Functional Efficacy of Anti-VEGF Therapy for Diabetic Macular Edema. *Invest Ophthalmol Vis Sci* 2019; **60**: 787-794 [PMID: 30807637 DOI: 10.1167/iovs.18-26209]

18 **Imazeki M**, Noma H, Yasuda K, Motohashi R, Goto H, Shimura M. Anti-VEGF Therapy Reduces Inflammation in Diabetic Macular Edema. *Ophthalmic Res* 2021; **64**: 43-49 [PMID: 32454504 DOI: 10.1159/000508953]

19 **Jagadish P**, Dalziel D. Discharge outcomes of patients referred to specialist eye clinic from diabetic retinopathy screening in Northland (2014-15). *N Z Med J* 2017; **130**: 89-93 [PMID: 28207728]

20 **Rajeshuni N**, Zubair T, Ludwig CA, Moshfeghi DM, Mruthyunjaya P. Evaluation of Racial, Ethnic, and Socioeconomic Associations With Treatment and Survival in Uveal Melanoma, 2004-2014. *JAMA Ophthalmol* 2020; **138**: 876-884 [PMID: 32614376 DOI: 10.1001/jamaophthalmol.2020.2254]

21 **Ming S**, Xie K, Yang M, He H, Li Y, Lei B. Comparison of intravitreal dexamethasone implant and anti-VEGF drugs in the treatment of retinal vein occlusion-induced oedema: a meta-analysis and systematic review. *BMJ Open* 2020; **10**: e032128 [PMID: 32595145 DOI: 10.1136/bmjopen-2019-032128]

22 **Kelkar A**, Webers C, Shetty R, Kelkar J, Labhsetwar N, Pandit A, Malode M, Tidke S. Factors affecting compliance to intravitreal anti-vascular endothelial growth factor therapy in Indian patients with retinal vein occlusion, age-related macular degeneration, and diabetic macular edema. *Indian J Ophthalmol* 2020; **68**: 2143-2147 [PMID: 32971626 DOI: 10.4103/ijo.IJO_1866_19]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 543-552

DOI: 10.4239/wjd.v13.i7.543

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Elevated levels of fructosamine are independently associated with SARS-CoV-2 reinfection: A 12-mo follow-up study

Xiao-Yan Huang, Li-Juan Yang, Xiang Hu, Xing-Xing Zhang, Xiao Gu, Lin-Jia Du, Zhi-Ying He, Xue-Jiang Gu

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
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ford J, United States; Gluvic Z, Serbia; Guven M, Turkey
A-Editor: Liu X, China

Received: December 8, 2021
Peer-review started: December 8, 2021
First decision: April 18, 2022
Revised: April 29, 2022
Accepted: June 13, 2022
Article in press: June 13, 2022
Published online: July 15, 2022

Xiao-Yan Huang, Li-Juan Yang, Xiang Hu, Xing-Xing Zhang, Xiao Gu, Lin-Jia Du, Zhi-Ying He, Xue-Jiang Gu, Department of Endocrine and Metabolic Disease, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Xiao-Yan Huang, Department of Endocrine and Metabolic Disease, Yueqing People's Hospital, Affiliated Hospital of Wenzhou Medical University, Wenzhou 325600, Zhejiang Province, China

Corresponding author: Xue-Jiang Gu, MMed, Chief Doctor, Department of Endocrine and Metabolic Disease, The First Affiliated Hospital of Wenzhou Medical University, Shangcai Village, Nanbaixiang Street, Ouhai District, Wenzhou 325000, Zhejiang Province, China. guxuejiang@wmu.edu.cn

Abstract

BACKGROUND

The association between blood levels of fructosamine (FMN) and recurrent coronavirus disease 2019 (COVID-19) is currently unclear.

AIM

To investigate a prospective relationship between blood levels of FMN and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection.

METHODS

A total of 146 Chinese hospitalized patients infected with SARS-CoV-2 were consecutively collectively recruited and followed from January 2020 to May 2021. Diagnosis of COVID-19 and SARS-CoV-2 reinfection was based on the diagnostic criteria and treatment protocol in China. The levels of FMN were determined in blood and divided into tertiles based on their distribution in the cohort of COVID-19 patients. Multivariate-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated for SARS-CoV-2 reinfection across the tertiles of FMN levels. A Cox regression model was used to generate the HR for SARS-CoV-2 reinfection in the participants in the top tertile of FMN levels compared with those at the bottom. Disease-free survival was used as the time variable, and relapse was used as the state variable, adjusted for age, gender, influencing factors such as diabetes mellitus, hypertension, and corticosteroid therapy, and clinical indexes such as acute liver failure, acute kidney failure, white blood cell (WBC) count, C-reactive protein, prognostic nutritional index (PNI), and blood lipids. Kaplan-Meier analysis with log-rank tests was used to compare the survival rate

between patients with elevated FMN levels (FMN > 1.93 mmol/L, the top tertile) and those with nonelevated levels.

RESULTS

Clinical data for the 146 patients with confirmed COVID-19 [age 49 (39-55) years; 49% males] were analyzed. Eleven patients had SARS-CoV-2 reinfection. The SARS-CoV-2 reinfection rate in patients with elevated FMN levels was significantly higher than that in patients with nonelevated FMN (17% *vs* 3%; $P = 0.008$) at the end of the 12-mo follow-up. After adjustments for gender, age, diabetes mellitus, hypertension, corticosteroid therapy, WBC count, PNI, indexes of liver and renal function, and blood lipids, patients with nonelevated FMN levels had a lower risk of SARS-CoV-2 reinfection than those with elevated FMN levels (HR = 6.249, 95%CI: 1.377-28.351; $P = 0.018$). Kaplan-Meier analysis showed that the cumulative survival rate of patients infected with SARS-CoV-2 was higher in patients with nonelevated FMN levels than in those with elevated FMN levels (97% *vs* 83%; log rank $P = 0.002$).

CONCLUSION

Elevated levels of FMN are independently associated with SARS-CoV-2 reinfection, which highlights that patients with elevated FMN should be cautiously monitored after hospital discharge.

Key Words: Fructosamine; COVID-19; Reinfection; Blood

Core Tip: Diabetes is a risk factor for coronavirus disease 2019 (COVID-19), which results in increased severity and mortality but has no relationship with reinfection. The present study, for the first time, reported the relationship between severe acute respiratory syndrome coronavirus 2 reinfection and blood levels of fructosamine (FMN), an index reflecting recent glycemic control. Our results demonstrated that FMN levels may influence the prognosis of patients with COVID-19, and patients with high FMN levels should be followed closely to monitor reinfection.

Citation: Huang XY, Yang LJ, Hu X, Zhang XX, Gu X, Du LJ, He ZY, Gu XJ. Elevated levels of fructosamine are independently associated with SARS-CoV-2 reinfection: A 12-mo follow-up study. *World J Diabetes* 2022; 13(7): 543-552

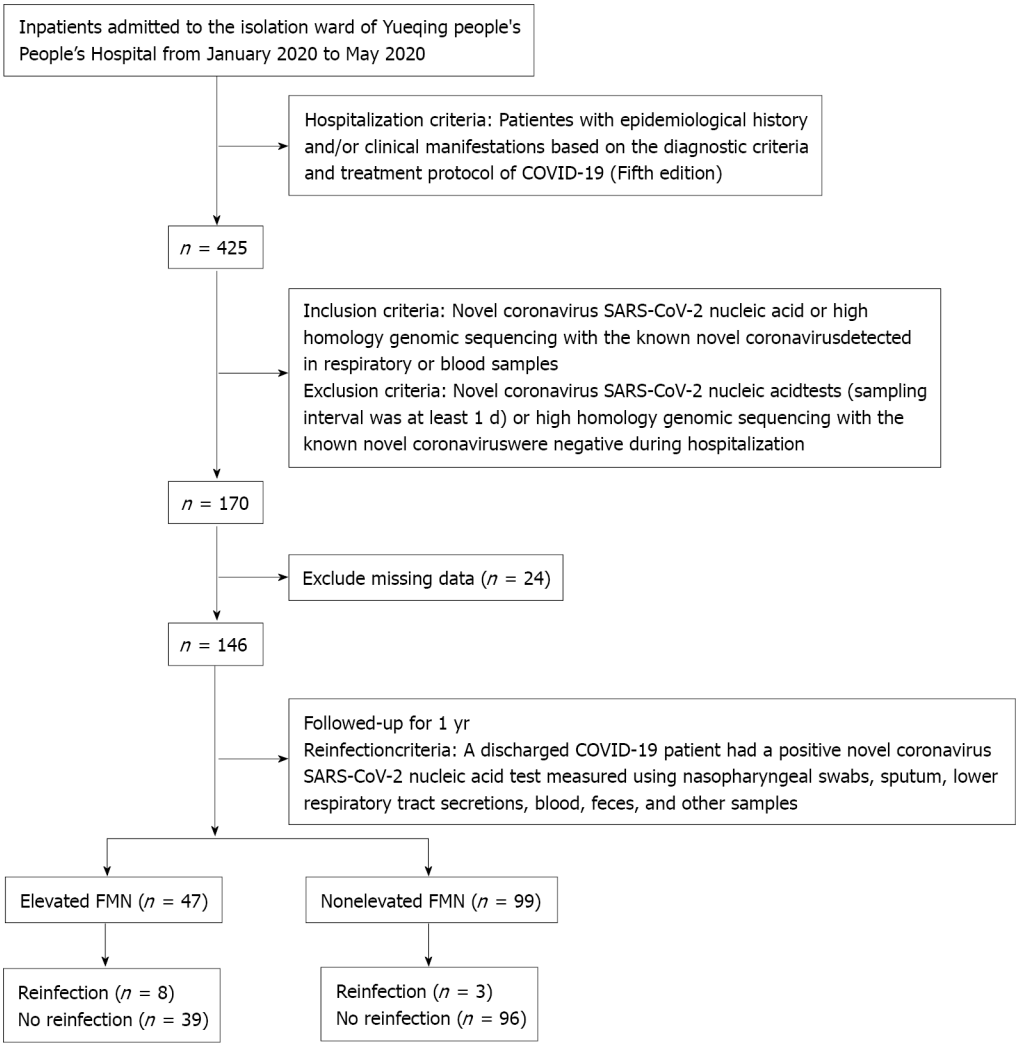
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/543.htm

DOI: https://dx.doi.org/10.4239/wjd.v13.i7.543

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was identified as an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019. It is mainly transmitted by droplets, contact, and aerosols in confined spaces[1,2]. It is highly infectious and widespread[3,4], with more than 505 million patients infected globally, with a cumulative mortality rate of 1.2%[5]. Diabetes is a risk factor for COVID-19, which results in increased severity and mortality[6-8]. A previous study found that of the 570 patients who died or were discharged from hospital, the mortality rate was 6.2% (of 386) for patients without diabetes or hyperglycemia, compared to 28.8% (of 184) for patients who had diabetes and/or uncontrolled hyperglycemia[9]. Hyperglycemia is considered a factor for severity of infection, including severe pneumonia, multiple organ failure, and death. In addition, hemoglobin (Hb)A1c level is an independent risk factor for death and a predictor of COVID-19 severity in patients with diabetes mellitus[10,11].

Fructosamine (FMN) reflects the overall glycemic control for the past 2-3 wk[12] and is strongly correlated with glucose and HbA1c levels[13,14]. HbA1c reflects overall glycemic control over the past 2-3 mo, and general blood glucose monitoring reflects glucose levels at the point. General blood glucose monitoring and HbA1c levels cannot accurately contribute to a prediction index for recent glycemic control. FMN level can be determined rapidly and better reflects recent glycemic control. It has also been associated with diabetic retinopathy, diabetic nephropathy, and long-term cardiovascular outcomes[15]. In addition, FMN levels are positively associated with the risk of periprosthetic joint infection and negatively associated with cancer risk. A previous study also demonstrated that FMN is a valuable marker for predicting adverse outcomes following total hip arthroplasty[16]. Hence, FMN correlates with diabetic complications, inflammation, and cancer. However, to date, no studies have



DOI: 10.4239/wjd.v13.i7.543

Figure 1 Flowchart of the study cohort. FMN: Fructosamine; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

demonstrated its association with COVID-19 risk or SARS-CoV-2 reinfection. The objective of the present study was to determine whether there is an association between FMN levels and COVID-19 risk and SARS-CoV-2 reinfection. This may provide a theoretical basis for the clinical treatment and prognosis of SARS-CoV-2 reinfection.

MATERIALS AND METHODS

Study cohort

Between January and May 2020, we enrolled 146 patients from the isolation ward of Yueqing People's Hospital, a designated isolation hospital for COVID-19. All the patients met the diagnostic criteria and treatment protocol for COVID-19 (5th edition). Elevated FMN was defined as levels higher than the upper tertile value of 1.93 mmol/L. The study cohort was divided into two groups based on FMN levels (Figure 1), *i.e.*, elevated FMN group (> 1.93 mmol/L; n = 47) and nonelevated FMN group (≤ 1.93 mmol/L; n = 99). All patients were followed from January 2020 to May 2021, with an average follow-up period of 1 year. The study protocol was approved by the Ethics Committee of Yueqing People's Hospital, Affiliated Hospital of Wenzhou Medical University (No. YQYY202100033).

Laboratory measurements

Venous blood samples were collected after an overnight fast of ≥ 8 h. All laboratory data were obtained from the first serum collection during hospitalization. The absolute value of peripheral white blood cells (WBCs), lymphocytes, serum creatinine, liver function indexes (alanine and aspartate aminotransferases), lipid profiles (total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, and low-

density lipoprotein cholesterol), and albumin were measured using standard methods. FMN levels were measured using the Roche automatic biochemical analyzer (Basel, Switzerland) and high performance liquid chromatography (Roche). The reference range for FMN was 1.15-2.25 mmol/L. Prognostic nutritional index (PNI) reflects the immune-nutritional status of patients and was determined by calculating serum albumin levels plus a fivefold total number of lymphocytes. PNI is associated with various cancers, such as lung, breast, and gynecological cancers[17].

Diagnostic criteria

The patients were diagnosed according to the Chinese Diagnostic Criteria and Treatment Protocol for COVID-19 (5th edition)[18].

Suspected cases: The patients were suspected to have COVID-19 based on a comprehensive analysis in combination with epidemiological history and clinical manifestations. The epidemiological history included: History of travel or residence in Wuhan and surrounding areas, or other communities where cases have been reported within 14 d before onset of illness; history of contact with a SARS-CoV-2-infected patient (positive for nucleic acid test) within 14 d before onset of illness; history of contact with patients with fever or respiratory symptoms from Wuhan and surrounding areas, or from communities where cases have been reported, within 14 d before onset of illness; and aggregation onset. Clinical manifestations included: Fever and/or respiratory symptoms; imaging features of SARS-CoV-2 pneumonia; normal or reduced total WBC count, or reduced lymphocyte count during the early stages of the disease. An individual with an epidemiological history and any two of the clinical manifestations were regarded as a suspected case. If there was no clear epidemiological history, three of the clinical manifestations should be satisfied.

Confirmed cases: Suspected cases with one of the following two tests being positive were regarded as confirmed cases: SARS-CoV-2 nucleic acid detected by real-time reverse transcription polymerase chain reaction in respiratory tract specimens or blood samples, and genomic sequencing of the respiratory or blood samples showing high homology with SARS-CoV-2.

Discharge criteria

A patient was discharged from isolation and transferred to other wards if his/her body temperature returned to normal and was stable for 3 d, respiratory symptoms improved significantly, lung imaging showed obvious improvement, and two nucleic acid tests were negative (sampling interval was at least 1 d).

Reinfection criteria

SARS-CoV-2 reinfection was defined when a discharged patient had a positive result on the SARS-CoV-2 nucleic acid test measured using nasopharyngeal swabs, sputum, lower respiratory tract secretions, blood, feces, and other samples.

Statistical analysis

IBM SPSS Statistics version 26 (IBM, Armonk, NY, United States) was used for statistical analyses. Normality of data distribution was determined by one-sample Kolmogorov-Smirnov test. Normally distributed data are expressed as the mean ± SD, and were determined using an independent group *t*-test. Non-normally distributed data are expressed as the median and interquartile range and were analyzed using the Mann-Whitney *U* test. The c^2 test was used for intergroup comparisons of categorical variables. Cox regression was used to determine the hazard ratio (HR) with 95% confidence interval (CI) for the positive reinfection across the tertiles of FMN levels, with the bottom tertile group as a reference. Kaplan-Meier analysis was used to determine the cumulative survival rate in patients with an FMN level higher than the top tertile compared with that in patients with nonelevated levels, tested using log-rank test. A two-sided P value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study cohort

Of the 146 patients with COVID-19, 72 were male (49%) and 74 were female (51%), with an average age of 49 years. Comparison between the nonelevated FMN and elevated FMN groups showed no significant difference in gender, respiratory failure, WBC count, C-reactive protein, PNI, alanine transferase, aspartate aminotransferase, serum creatinine, triglyceride, total cholesterol, high-density lipoprotein, or low-density lipoprotein ($P > 0.05$) (Table 1). The average age of patients with elevated FMN was higher than that of patients in the nonelevated FMN group [53 (43-58) years *vs* 47 (35-53) years, $P = 0.008$] (Table 1). There were significant differences in diabetes mellitus, hypertension, and corticosteroid therapy between the two groups ($P < 0.05$) (Table 1).

Table 1 Baseline characteristics of the study cohort

Variable	Total	Elevated FMN[1]	Nonelevated FMN[2]	*P* value
Patients, *n* (%)	146	47 (68)	99 (32)	
Gender, *n* (%)				0.319
Male	72 (49)	26 (36)	46 (64)	
Female	74 (51)	21 (28)	53 (72)	
Age (yr)	49 (39-55)	53 (43-58)	47 (35-53)	0.008
Diabetes mellitus, *n* (%)	17 (12)	14 (82)	3 (18)	0.000
Hypertension, *n* (%)	18 (12)	8 (44)	10 (56)	0.023
Respiratory failure, *n* (%)	12 (8)	6 (50)	6 (50)	0.291
Corticosteroid therapy, *n* (%)	30 (21)	5 (17)	25 (83)	0.041
WBC [(4.0 × 10^9/L)-(10.0 × 10^9/L)]	4.64 (3.63-5.82)	5.06 (3.85-6.45)	4.58 (3.45-5.40)	0.067
CRP (< 5 mg/L)	7.30 (5.0-25.60)	6.80 (5.0-34.90)	7.80 (5.0-23.60)	0.320
PNI	47.80 (44.26-50.58)	49.55 (46.05-50.95)	47.05 (44.05-49.55)	0.061
ALT (0-55 U/L)	20.50 (14.0-29.0)	22.00 (15.0-31.0)	19.00 (13.0-28.0)	0.138
AST (0-55 U/L)	23.00 (18.0-31.0)	25.00 (19.0-32.0)	22.00 (18.0-30.0)	0.016
SCR (45-84 μmol/L)	62 (50-74)	64 (55-73)	61 (50-75)	0.460
TC (3.60-5.70 mmol/L)	4.24 ± 0.77	4.11 ± 0.79	4.30 ± 0.76	0.176
TG (0.60-1.70 mmol/L)	1.16 (0.86-1.69)	1.22 (0.88-1.77)	1.14 (0.86-1.66)	0.239
HDL-C (1.09-2.27 mmol/L)	0.95 (0.80-1.16)	0.92 (0.76-1.16)	0.98 (0.83-1.16)	0.314
LDL-C (1.30-3.37 mmol/L)	2.30 (1.94-2.91)	2.22 (1.90-2.78)	2.32 (1.98-2.92)	0.242
Reinfection case, *n* (%)	11 (7.5)	8 (73)	3 (17)	0.008

[1]Upper third of fructosamine levels.
[2]Lower two-thirds of fructosamine levels.
Data are presented as the mean (SD) for normally distributed data and median (interquartile range) for non-normal distributed data. PNI = serum albumin (g/L) + 5 × lymphocyte count (× 10^9/L). *P* value was calculated using one-sample Kolmogorov-Smirnov test or *t*-test. WBC: White blood cell count; FMN: Fructosamine; CRP: C-reactive protein; PNI: Prognostic nutritional index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; SCR: Serum creatinine; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

Association of FMN levels with SARS-CoV-2 reinfection

The SARS-CoV-2 reinfection rate was significantly higher in patients in the elevated FMN group than in those in the nonelevated FMN group (17% *vs* 3%, $P = 0.008$) (Table 1). In the Cox regression model, disease-free survival (DFS) was used as the time variable, and reinfection was used as the state variable. After full adjustment, the elevated FMN group showed an increased risk of reinfection (HR = 6.249, 95%CI: 1.377-28.351, $P = 0.018$; P for trend < 0.05) (Table 2).

Association of FMN with cumulative DFS rate

Kaplan-Meier survival analysis showed that the cumulative DFS rate in the elevated FMN group was lower compared to that of the nonelevated FMN group (83% *vs* 97%, $P = 0.002$) (Figure 2). The survival rate was determined using the log-rank test, and the P for trend was < 0.05.

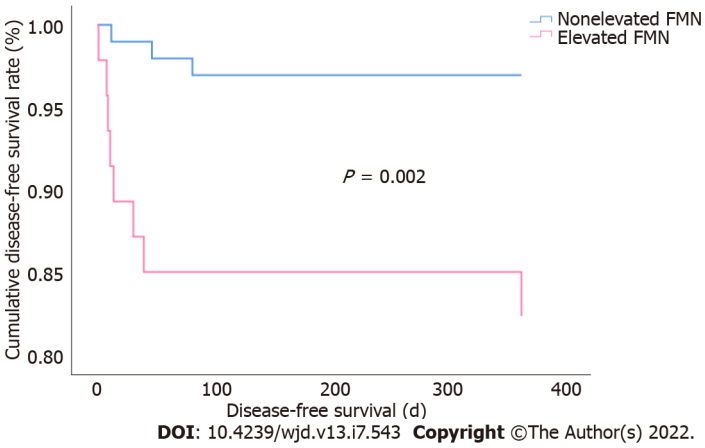
DISCUSSION

We found that patients with elevated FMN levels were older compared to patients in the nonelevated FMN group. Elevated FMN levels were positively associated with reinfection rate as well as HR for reinfection, while the cumulative DFS rate was lower in patients in the elevated FMN group. These results demonstrate that FMN levels may influence the prognosis of patients with COVID-19. COVID-19 is an acute inflammatory disease. Previous studies have demonstrated that patients with diabetes and severe disease were less likely to experience recurrence of SARS-CoV-2 infection[19]; however, patients with uncontrolled diabetes had an increased risk of reinfection[20]. Blood glucose monitoring reflects

Table 2 Association of fructosamine levels with SARS-CoV-2 reinfection

FMN dichotomy	B	SE	HR	95%CI	*P* value
Model 1	1.827	0.677	6.214	1.647-23.438	0.007
Model 2	1.898	0.759	6.674	1.507-29.544	0.012
Model 3	1.832	0.772	6.249	1.377-28.351	0.018

Model 1: Unadjusted. Model 2: Adjusted for age, gender, diabetes mellitus, corticosteroid therapy, and hypertension. Model 3: Adjusted for Model 2 and acute liver failure, acute kidney failure, white blood cell count, C-reactive protein, prognostic nutritional index, and blood lipids. *P* value for hazard ratio with 95% confidence interval was calculated using Cox regression models to indicate a significant association. FMN: Fructosamine; HR: Hazard ratio; CI: Confidence interval.



DOI: 10.4239/wjd.v13.i7.543

Figure 2 Association of fructosamine levels with cumulative disease-free survival rate. FMN: Fructosamine.

glucose levels at the point of testing and does not reflect overall blood glucose control. Compared to HbA1c, FMN can reflect blood glucose changes more recently. Previous studies have demonstrated that FMN is a good predictor of adverse events following total knee arthroplasty. Patients with high FMN levels were more likely to develop prosthetic joint infections compared to patients with low FMN levels. Unlike FMN, HbA1c does not show a significant association with complications[6]. FMN but not HbA1c is a significant predictor of infection in hemodialysis and diabetes patients with acute infections[21]. In our study, we found that FMN was associated with SARS-CoV-2 reinfection. Compared to patients with low FMN levels, patients with high FMN levels were found to have a higher reinfection rate. Patients with high FMN levels had a higher HR for reinfection, while patients with low FMN levels had higher cumulative DFS rates. It appears that FMN levels may predispose individuals to reinfection. Thus, the clinical focus should be on maintaining consistent euglycemia, using standard point-of-care glucose checks.

FMNs are advanced glycation end products (AGEs) generated when glucose reacts reversibly with amino groups in proteins. Reversible aldehyde imine intermediate is formed by the aldehyde group of carbohydrates and the N-terminal amino acids of proteins. However, irreversible AGEs are generated through a Maillard reaction[22]. Maillard reactions have been shown to impair cellular function[23]. FMN-3 kinase-related protein, designated as a potential longevity protein[24], can catalyze deglycation of Maillard intermediates directly downstream from FMN, thereby reducing AGE levels[25,26]. Several studies have demonstrated that AGE levels increase with age[27]. In our study, we found that older patients had higher FMN levels.

High FMN levels usually reflect hyperglycemia, which may lead to poor outcomes. Hyperglycemia enhances the expression of angiotensin-converting enzyme (ACE)2, which is the major cell entry receptor for SARS-CoV-2. ACE2 is widely expressed in the kidneys, lungs, and intestinal mucosal cells. SARS-CoV-2 can replicate abundantly in these sites and may contribute to reinfection[28] (Figure 3). Physiologically, hyperglycemia leads to a significant decrease in lymphocyte count, *i.e.*, $CD3^+$ and $CD4^+$ T cells, which in turn reduces humoral immunity mediated by macrophages and dendritic cells, and induces interleukin-6, tumor necrosis factor α, *etc.* to induce a cytokine storm[29]. This immunological disorder may increase the occurrence of antibody-dependent enhancement (ADE). In patients who are positive for coronavirus-specific antibodies or are infected by different virus strains, their antibodies may not neutralize the infection, but instead trigger FCγ receptor-mediated uptake of the virus, leading to an increase in virus numbers in the body[30,31] (Figure 4). Hence, ADE may be another pathological

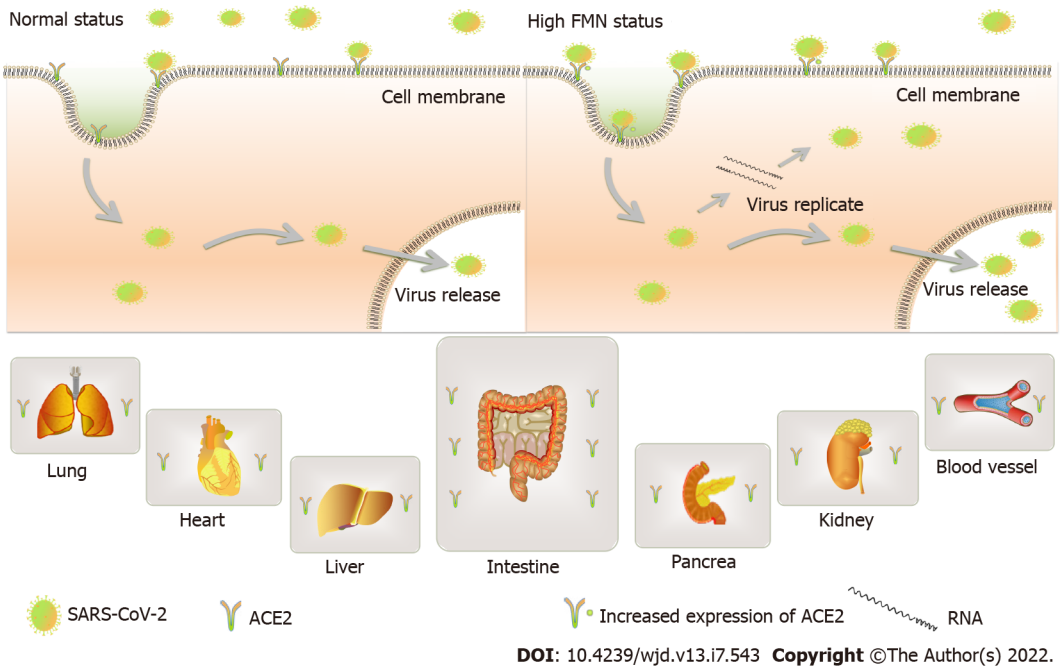


Figure 3 Potential pathways for reinfection in patients with high fructosamine levels and increased angiotensin-converting enzyme 2 expression. FMN: Fructosamine; ACE2: Angiotensin-converting enzyme 2; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

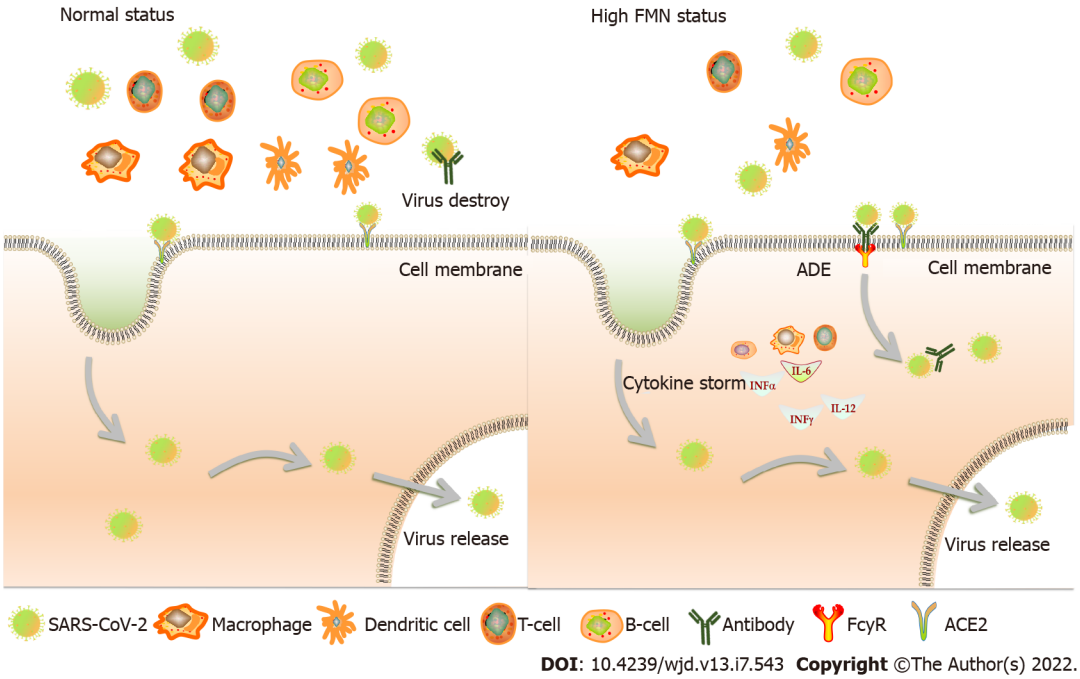


Figure 4 Potential pathways of reinfection in patients with high fructosamine levels with immunological disorders. FMN: Fructosamine; ACE2: Angiotensin-converting enzyme 2; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; INF: Interferon; IL: Interleukin; ADE: Antibody-dependent enhancement.

mechanism of positive securement of SARS-CoV-2.

There were some limitations to the present study, which may have introduced potential bias. First, the study was a prospective, single center, small cohort study. Additional multicenter studies using larger patient cohorts should be performed to validate our findings. Second, HbA1c data for some of the patients were not available, which affected our comparative analysis of HbA1c and FMN levels. Third, diabetes was not excluded in the inclusion criteria, but we adjusted for diabetes.

CONCLUSION

Elevated FMN levels were found to predispose COVID-19 patients to reinfection and hence should be followed closely to monitor reinfection.

ARTICLE HIGHLIGHTS

Research background

Diabetes is a risk factor for coronavirus disease 2019 (COVID-19) which results in increased severity and mortality but has no relationship with COVID-19 reinfection. No study has reported the relationship between COVID-19 reinfection and blood levels of fructosamine (FMN). The present study for the first time reported this relationship.

Research motivation

We mainly investigate the relationship between blood levels of FMN and COVID-19 reinfection.

Research objectives

We found that FMN levels may influence the prognosis of patients infected with COVID-19, which highlight that the hospitalization patients with elevated levels of FMN should be cautiously monitored at post discharge.

Research methods

A total of 146 inpatients from the designated isolation hospital for COVID-19 patients, who were satisfied based on the diagnostic criteria and treatment protocol of COVID-19 (Fifth edition). The study cohort was divided into two groups based on FMN levels, elevated FMN was defined as levels higher than its upper tertile value, with the average follow-up period being one year. Cox regression was used to determine the hazard ratios (HRs) with 95% confidence intervals for the positive reinfection across the tertiles of FMN levels. Kaplan-Meier analysis was used to determine the cumulative survival rate in the patients with higher than the top tertiles of FMN levels compared with those with non-elevated levels, tested using log-rank.

Research results

We found that patients with elevated FMN levels were older than the non-elevated FMN group. Elevated FMN levels were positively associated with reinfection rate as well as HR for reinfection, while the cumulative disease-free survival rate was lower for patients in the elevated FMN group. These results demonstrate that FMN levels may influence the prognosis of patients infected with COVID-19.

Research conclusions

Elevated levels of FMN are independently associated with COVID-19 reinfection, which highlight that the COVID-19 patients with elevated levels of FMN should be followed up closely to monitor reinfection.

Research perspectives

Additional multicenter, hemoglobin A1c data available studies using larger patient cohorts should be performed to validate our findings.

ACKNOWLEDGEMENTS

The authors would like to thank Yueqing People's Hospital, Affiliated Hospital of Wenzhou Medical University for the data support and Dr. Bo Yang for his careful statistical assistance.

FOOTNOTES

Author contributions: Gu XJ was the guarantor and designed the study; Huang XY and Hu X participated in the acquisition, analysis, interpretation of the data, and drafted the initial manuscript; Yang LY, Zhang XX, Gu X, Du LJ, and He ZY revised the article critically for important intellectual content.

Institutional review board statement: The study protocol was approved by the Ethics Committee of Yueqing People's Hospital, Affiliated Hospital of Wenzhou Medical University (No. YQYY202100033).

Informed consent statement: The informed consent statement was waived.

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

Data sharing statement: No additional data are available.

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: China

ORCID number: Xiao-Yan Huang 0000-0002-9737-5815; Li-Juan Yang 0000-0002-6000-6530; Xiang Hu 0000-0002-6619-187X; Xing-Xing Zhang 0000-0002-1460-1865; Xiao Gu 0000-0002-7607-1281; Lin-Jia Du 0000-0002-1950-5469; Zhi-Ying He 0000-0002-5609-518X; Xue-Jiang Gu 0000-0002-7629-7627.

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

REFERENCES

1 **Naqvi AAT**, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, Atif SM, Hariprasad G, Hasan GM, Hassan MI. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165878 [PMID: 32544429 DOI: 10.1016/j.bbadis.2020.165878]
2 **Huang YN**, Wang L, Cao MX, Huang ZC, Du R; Hu LG; Qu GB; Liang Y; Wang P. A review on the environmental transmission of novel coronavirus (SARS-CoV-2). *Environ Chem* 2021; **40**: 1945-1957 [DOI: 10.7524/j.issn.0254-6108.]
3 **Lu H**, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. *J Med Virol* 2020; **92**: 401-402 [PMID: 31950516 DOI: 10.1002/jmv.25678]
4 **Kirtipal N**, Bharadwaj S, Kang SG. From SARS to SARS-CoV-2, insights on structure, pathogenicity and immunity aspects of pandemic human coronaviruses. *Infect Genet Evol* 2020; **85**: 104502 [PMID: 32798769 DOI: 10.1016/j.meegid.2020.104502]
5 **World Health Organization**. WHO COVID-19 Dashboard, 2020. [cited 24 April 2022]. Available from: https://covid19.who.int/
6 **Singh AK**, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab Syndr* 2020; **14**: 303-310 [PMID: 32298981 DOI: 10.1016/j.dsx.2020.04.004]
7 **Kumar A**, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A. Is diabetes mellitus associated with mortality and severity of COVID-19? *Diabetes Metab Syndr* 2020; **14**: 535-545 [PMID: 32408118 DOI: 10.1016/j.dsx.2020.04.044]
8 **Ceriello A**, Standl E, Catrinoiu D, Itzhak B, Lalic NM, Rahelic D, Schnell O, Škrha J, Valensi P; Diabetes and Cardiovascular Disease (D&CVD) EASD Study Group. Issues of Cardiovascular Risk Management in People With Diabetes in the COVID-19 Era. *Diabetes Care* 2020; **43**: 1427-1432 [PMID: 32409501 DOI: 10.2337/dc20-0941]
9 **Bode B**, Garrett V, Messler J, McFarland R, Crowe J, Booth R, Klonoff DC. Glycemic Characteristics and Clinical Outcomes of COVID-19 Patients Hospitalized in the United States. *J Diabetes Sci Technol* 2020; **14**: 813-821 [PMID: 32389027 DOI: 10.1177/1932296820924469]
10 **Liu W**, Zhou Y, Song L, Zhou L, Hu Y. Analysis, clinical characteristics and death-related factors in patients with COVID-19 and diabetes. *J Med University of Chongqing***45**: 911-915 [DOI: 10.13406/j.cnki.cyxb.002628]
11 **Merzon E**, Green I, Shpigelman M, Vinker S, Raz I, Golan-Cohen A, Eldor R. Haemoglobin A1c is a predictor of COVID-19 severity in patients with diabetes. *Diabetes Metab Res Rev* 2021; **37**: e3398 [PMID: 32852883 DOI: 10.1002/dmrr.3398]
12 **Cefalu WT**, Bell-Farrow AD, Petty M, Izlar C, Smith JA. Clinical validation of a second-generation fructosamine assay. *Clin Chem* 1991; **37**: 1252-1256 [PMID: 1855298]
13 **Juraschek SP**, Steffes MW, Selvin E. Associations of alternative markers of glycemia with hemoglobin A(1c) and fasting glucose. *Clin Chem* 2012; **58**: 1648-1655 [PMID: 23019309 DOI: 10.1373/clinchem.2012.188367]
14 **Choi R**, Park MJ, Lee S, Lee SG, Lee EH. Association Among Glycemic Biomarkers in Korean Adults: Hemoglobin A1c, Fructosamine, and Glycated Albumin. *Clin Lab* 2021; **67** [PMID: 34258983 DOI: 10.7754/Clin.Lab.2020.201133]
15 **Rooney MR**, Tang O, Pankow JS, Selvin E. Glycaemic markers and all-cause mortality in older adults with and without diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia* 2021; **64**: 339-348 [PMID: 32990802 DOI: 10.1007/s00125-020-05285-3]
16 **Shohat N**, Goswami K, Breckenridge L, Held MB, Malkani AL, Shah RP, Schwarzkopf R, Parvizi J. Fructosamine is a valuable marker for glycemic control and predicting adverse outcomes following total hip arthroplasty: a prospective multi-

institutional investigation. *Sci Rep* 2021; **11**: 2227 [PMID: 33500515 DOI: 10.1038/s41598-021-81803-6]

17 **Hu X**, Deng H, Wang Y, Chen L, Gu X, Wang X. Predictive value of the prognostic nutritional index for the severity of coronavirus disease 2019. *Nutrition* 2021; **84**: 111123 [PMID: 33476998 DOI: 10.1016/j.nut.2020.111123]

18 **China Bureau of Disease Control And Prevention**. The diagnostic criteria and treatment protocol for COVID-19 (5th edition), 2020 Feb 21. [cited 2022 April 24]. Available from: http://www.nhc.gov.cn/jkj/s3577/202002/a5d6f7b8c48c451c87dba14889b30147.shtml

19 **Azam M**, Sulistiana R, Ratnawati M, Fibriana AI, Bahrudin U, Widyaningrum D, Aljunid SM. Recurrent SARS-CoV-2 RNA positivity after COVID-19: a systematic review and meta-analysis. *Sci Rep* 2020; **10**: 20692 [PMID: 33244060 DOI: 10.1038/s41598-020-77739-y]

20 **Pal R**, Banerjee M. Are people with uncontrolled diabetes mellitus at high risk of reinfections with COVID-19? *Prim Care Diabetes* 2021; **15**: 18-20 [PMID: 32800450 DOI: 10.1016/j.pcd.2020.08.002]

21 **Burekovic A**, Dizdarevic-Bostandzic A, Godinjak A. Poorly regulated blood glucose in diabetic patients--predictor of acute infections. *Med Arh* 2014; **68**: 163-166 [PMID: 25195343]

22 **Gounden V**, Ngu M, Anastasopoulou C, Jialal I. Fructosamine. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan– [PMID: 29262081]

23 **Voyer LE**, Alvarado C. [Maillard reaction. Pathogenic effects]. *Medicina (B Aires)* 2019; **79**: 137-143 [PMID: 31048279]

24 **Torres GG**, Nygaard M, Caliebe A, Blanché H, Chantalat S, Galan P, Lieb W, Christiansen L, Deleuze JF, Christensen K, Strauch K, Müller-Nurasyid M, Peters A, Nöthen MM, Hoffmann P, Flachsbart F, Schreiber S, Ellinghaus D, Franke A, Dose J, Nebel A. Exome-Wide Association Study Identifies FN3KRP and PGP as New Candidate Longevity Genes. *J Gerontol A Biol Sci Med Sci* 2021; **76**: 786-795 [PMID: 33491046 DOI: 10.1093/gerona/glab023]

25 **Szwergold B**. A Hypothesis: Fructosamine-3-Kinase-Related-Protein (FN3KRP) Catalyzes Deglycation of Maillard Intermediates Directly Downstream from Fructosamines. *Rejuvenation Res* 2021; **24**: 310-318 [PMID: 34314247 DOI: 10.1089/rej.2021.0009]

26 **Szwergold BS**, Bunker RD, Loomes KM. The physiological substrates of fructosamine-3-kinase-related-protein (FN3KRP) are intermediates of nonenzymatic reactions between biological amines and ketose sugars (fructation products). *Med Hypotheses* 2011; **77**: 739-744 [PMID: 21924559 DOI: 10.1016/j.mehy.2011.07.027]

27 **Schulze C**, Wetzel F, Kueper T, Malsen A, Muhr G, Jaspers S, Blatt T, Wittern KP, Wenck H, Käs JA. Stiffening of human skin fibroblasts with age. *Clin Plast Surg* 2012; **39**: 9-20 [PMID: 22099845 DOI: 10.1016/j.cps.2011.09.008]

28 **Kumar A**, Faiq MA, Pareek V, Raza K, Narayan RK, Prasoon P, Kumar P, Kulandhasamy M, Kumari C, Kant K, Singh HN, Qadri R, Pandey SN, Kumar S. Relevance of SARS-CoV-2 related factors ACE2 and TMPRSS2 expressions in gastrointestinal tissue with pathogenesis of digestive symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 patients. *Med Hypotheses* 2020; **144**: 110271 [PMID: 33254575 DOI: 10.1016/j.mehy.2020.110271]

29 **Chee YJ**, Tan SK, Yeoh E. Dissecting the interaction between COVID-19 and diabetes mellitus. *J Diabetes Investig* 2020; **11**: 1104-1114 [PMID: 32558211 DOI: 10.1111/jdi.13326]

30 **Ulrich H**, Pillat MM, Tárnok A. Dengue Fever, COVID-19 (SARS-CoV-2), and Antibody-Dependent Enhancement (ADE): A Perspective. *Cytometry A* 2020; **97**: 662-667 [PMID: 32506725 DOI: 10.1002/cyto.a.24047]

31 **Karthik K**, Senthilkumar TMA, Udhayavel S, Raj GD. Role of antibody-dependent enhancement (ADE) in the virulence of SARS-CoV-2 and its mitigation strategies for the development of vaccines and immunotherapies to counter COVID-19. *Hum Vaccin Immunother* 2020; **16**: 3055-3060 [PMID: 32845733 DOI: 10.1080/21645515.2020.1796425]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 553-565

DOI: 10.4239/wjd.v13.i7.553

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Factors associated with trabecular bone score in postmenopausal women with type 2 diabetes and normal bone mineral density

Olga N Fazullina, Anton I Korbut, Vadim V Klimontov

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

P-Reviewer: Bhattacharya S, India; Jones AR, Australia; Koo M, Taiwan

Received: February 15, 2022
Peer-review started: February 15, 2022
First decision: April 17, 2022
Revised: May 2, 2022
Accepted: June 24, 2022
Article in press: June 24, 2022
Published online: July 15, 2022

Olga N Fazullina, Anton I Korbut, Vadim V Klimontov, Laboratory of Endocrinology, Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk 630060, Russia

Corresponding author: Vadim V Klimontov, DSc, MD, PhD, Professor, Laboratory of Endocrinology, Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Timakov Street 2, Novosibirsk 630060, Russia. klimontov@mail.ru

Abstract

BACKGROUND

Osteoporosis and type 2 diabetes (T2D) have been recognized as a widespread comorbidity leading to excess mortality and an enormous healthcare burden. In T2D, bone mineral density (BMD) may underestimate the risk of low-energy fractures as bone quality is reduced. It was hypothesized that a decrease in the trabecular bone score (TBS), a parameter assessing bone microarchitecture, may be an early marker of impaired bone health in women with T2D.

AIM

To identify clinical and body composition parameters that affect TBS in postmenopausal women with T2D and normal BMD.

METHODS

A non-interventional cross-sectional comparative study was conducted. Potentially eligible subjects were screened at tertiary referral center. Postmenopausal women with T2D, aged 50-75 years, with no established risk factors for secondary osteoporosis, were included. BMD, TBS and body composition parameters were assessed by dual-energy X-ray absorptiometry. In women with normal BMD, a wide range of anthropometric, general and diabetes-related clinical and laboratory parameters were evaluated as risk factors for TBS decrease using univariate and multivariate regression analysis and analysis of receiver operating characteristic (ROC) curves.

RESULTS

Three hundred twelve women were initially screened, 176 of them met the inclusion criteria and underwent dual X-ray absorptiometry. Those with reduced BMD were subsequently excluded; 96 women with normal BMD were included in final analysis. Among them, 43 women (44.8%) showed decreased TBS values (≤

1.31). Women with TBS ≤ 1.31 were taller and had a lower body mass index (BMI) when compared to those with normal TBS (P = 0.008 and P = 0.007 respectively). No significant differences in HbA1c, renal function, calcium, phosphorus, alkaline phosphatase, PTH and 25(OH)D levels were found. In a model of multivariate linear regression analysis, TBS was positively associated with gynoid fat mass, whereas the height and androgen fat mass were associated negatively (all P < 0.001). In a multiple logistic regression, TBS ≤ 1.31 was associated with lower gynoid fat mass (adjusted odd ratio [OR], 0.9, 95% confidence interval [CI], 0.85-0.94, P < 0.001), higher android fat mass (adjusted OR, 1.13, 95%CI, 1.03-1.24, P = 0.008) and height (adjusted OR, 1.13, 95%CI, 1.05-1.20, P < 0.001). In ROC-curve analysis, height ≥ 162.5 cm (P = 0.04), body mass index ≤ 33.85 kg/m^2 (P = 0.002), gynoid fat mass ≤ 5.41 kg (P = 0.03) and android/gynoid fat mass ratio ≥ 1.145 (P < 0.001) were identified as the risk factors for TBS reduction.

CONCLUSION

In postmenopausal women with T2D and normal BMD, greater height and central adiposity are associated with impaired bone microarchitecture.

Key Words: Diabetes; Osteoporosis; Bone mineral density; Trabecular bone score; Obesity; Body composition

Core Tip: In this study, we assessed the impact of a wide range of general and diabetes-related parameters on trabecular bone score (TBS) in postmenopausal women with type 2 diabetes (T2D) and normal bone mineral density (BMD). A decrease in TBS was revealed in 44.8% of study participants. These data indicate that a substantial proportion of postmenopausal women with T2D and normal BMD may have impaired bone microarchitecture. Greater height and central adiposity turned out to be the risk factors for decreased TBS in these women.

Citation: Fazullina ON, Korbut AI, Klimontov VV. Factors associated with trabecular bone score in postmenopausal women with type 2 diabetes and normal bone mineral density. *World J Diabetes* 2022; 13(7): 553-565
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/553.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.553

INTRODUCTION

Type 2 diabetes (T2D) and bone fractures have been recognized as a widespread comorbidity leading to excess mortality and an enormous healthcare burden[1,2]. Recent data from the Continuous National Health and Nutrition Examination Survey (NHANES) indicate an increasing prevalence of osteoporosis and osteopenia in the US among T2D patients[3]. People with T2D have higher risk of vertebral and some non-vertebral fractures than non-diabetic individuals[4,5], regardless of normal or even increased bone mineral density (BMD)[6,7]. This "diabetic paradox" has been attributed to the modified effect of hyperglycemia, obesity and related factors on BMD[8]. As BMD assessment may lead to underestimation of a fracture risk in T2D, additional parameters of bone health should be taken into consideration.

In recent years, the Trabecular Bone Score (TBS) on lumbar spine dual X-ray absorptiometry (DXA) images is increasingly applied for the assessment of bone microarchitecture. It had been demonstrated that low TBS is associated with both prevalent and incident fractures; therefore, TBS was incorporated in the Fracture Risk Assessment tool (FRAX) algorithm[9]. The impaired bone microarchitecture is considered as a major contributor to fracture risk in T2D[10]. Accordingly, the utility of TBS for osteoporotic fracture risk assessment was shown in postmenopausal women with T2D[11,12]. Individuals with diabetes as compared to those without have significantly lower TBS[13,14]; the difference is greater in women[13]. It could be speculated that the reduction of TBS is an earlier event in the deterioration of bone health in T2D than BMD decrease. However, at present, data on TBS in postmenopausal women with T2D and normal BMD is limited, and predictors of the TBS decrease in these women need to be refined.

A growing body of evidence indicates the pivotal role of hyperglycemia-related biochemical abnormalities, as well as obesity and dysregulated adipokine production, in the pathogenesis of increased bone fragility in T2D[15,16]. Nevertheless, the role of diabetes-related factors and fat accumu-

lation at early stages of bone metabolic disease in T2D needs further research.

Therefore, in this study we aimed to identify clinical and body composition parameters that affect TBS in postmenopausal women with T2D and normal BMD.

MATERIALS AND METHODS

Design

A non-interventional cross-sectional comparative study was conducted.

To be included in the study, women had to meet the following criteria: (1) Caucasian origin; (2) Age 50-75 years; (3) Time since menopause ≥ 1 year; (4) Known T2D duration ≥ 1 year; and (5) Normal BMD assessed by DXA.

The following list of exclusion criteria was applied: Endocrine diseases other than T2D (hyperthyroidism, hypothyroidism, hyperparathyroidism, hypopituitarism, acromegaly, and Cushing syndrome); Rheumatic diseases (rheumatoid arthritis, psoriatic arthritis, spondyloarthritis, systemic lupus erythematosus, systemic sclerosis, vasculitis, and crystal-induced arthritis); Inflammatory bowel diseases, celiac disease, malabsorption or bariatric surgery in medical history; Chronic kidney disease with an estimated glomerular filtration rate (eGFR) less than 45 mL/min/1.73 m^2; Ever diagnosed with any kind of malignancy; Immobilization for more than one month in medical history; Treatment with thiazolidinediones, glucocorticoids, anticonvulsants or immunosuppressive drugs, postmenopausal hormonal replacement therapy, anti-osteoporotic therapy at the time of the study or in the past.

Potentially eligible subjects were screened at the clinic of Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (Novosibirsk, Russa), a tertiary referral center. All women underwent a detailed clinical examination, which included the assessment of glycemic control, in-depth screening/monitoring of diabetic complications and associated diseases. Women who met the inclusion criteria (1-4) and did not have the exclusion criteria underwent DXA to determine body composition, BMD and TBS. Those with abnormal BMD (T-score ≤ -1 SD) were excluded. The rest of the participants were divided into 2 groups: 1) women with normal TBS (>1.31); 2) women with TBS reduction (≤1.31). The cut-off TBS value was chosen according to the results of meta-analysis [17]. The risk factors for TBS reduction were estimated by univariate and multivariate regression analysis and analysis of receiver operating characteristic (ROC)-curves.

Ethical issues

The study protocol was approved by the Ethical Committee of the clinic of Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (protocol N. 104 from 20 December 2014). All study participants provided informed written consent prior to study enrollment.

Methods

DXA and fracture risk assessment: The BMD and T-score at the lumbar spine (L1–L4), femur, femoral neck and forearm were assessed by DXA (Lunar Prodigy Advance bone densitometer, GE healthcare, Madison, WI, United States; database NHANES III; the Least significant change is 0.028 g/cm^2 for L1-L4, 0.033 g/cm^2 for femur, and 0.055 g/cm^2 for radius 33%). The TBS was estimated with the use of TBS iNsight software (version 3.0.2.0, GE healthcare). The Body Composition software (GE healthcare) was applied for assessment of body composition parameters, including bone mineral component, fat mass and lean mass, and fat distribution. Fat distribution patterns were differentiated based on the ratio of fat mass in the abdominal and hip areas (android and gynoid fat mass respectively)[18].

The FRAX tool (web version 4.3, country-specific algorithm, https://www.sheffield.ac.uk/FRAX/tool.aspx?country=13) was used to determine the ten-year risk of low-energy fractures. Both TBS-unadjusted and TBS-adjusted FRAX scores were calculated.

Laboratory investigations: The measurements of the levels of glycated hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, uric acid, creatinine, calcium, phosphorus and alkaline phosphatase were performed with a biochemical analyzer AU480 (Beckman Coulter, Minneapolis, MN, United States). eGFR was calculated using the CKD-EPI formula (2009). Albumin concentrations were determined in the morning urine samples by immunoturbidimetric method with a fully automated chemistry analyzer BS-120 (Mindray, Shenzhen, China); the result was adjusted to excreted creatinine. Serum levels of PTH and 25(OH)D were measured by ELISA with the use of Access 2 Immunoassay System analyzer (Beckman Coulter) and Access Intact PHT, Access 25(OH) Vitamin D Total kits (Beckman Coulter).

Statistical analysis

Dell Statistica 13.0 (Dell Software, Aliso Viejo, CA, United States) was used for most of the applied statistical procedures. The sample size was calculated with a predetermined Type I error rate $\alpha = 0.05$,

power goal 1–β = 80% and standardized size effect 0.5 for clinical characteristics (age, duration of diabetes, age and duration of menopause, height, body weight, body mass index [BMI], waist-to-hip circumference), laboratory parameters (HbA1c, eGFR, calcium, phosphorus, 25(OH)D, PTH) and body composition (fat and lean mass, android and gynoid fat mass and percentage, android/gynoid fat mass ratio). The minimal number of participants in each group was defined as 34 persons. Assuming the prevalence of osteoporosis[19,20] and decreased TBS[21,22] in patients with T2D and using principles described previously[23,24], we estimated the minimal number of study participants as 150 individuals.

Quantitative data are presented as medians (lower quartiles; upper quartiles), frequencies are presented as percentages (%). The Kolmogorov–Smirnov (KS) test was applied to test the normality. As the majority of the quantitative parameters were not distributed normally, the non-parametric Mann–Whitney U-test was used for the group comparisons. The differences in discrete parameters were assessed using the χ^2 test. *P* values below 0.05 were considered as significant.

Spearman rank correlation analysis was applied to test associations between variables. Multiple linear regression analysis with backward elimination was used to reveal factors affecting TBS. The description of the model included beta coefficients with standard errors and *P* values, adjusted coefficient of determination (R^2), standard error of estimate and *P* value of the model.

Multiple logistic regression analysis with backward elimination was used to identify predictors of decreased TBS. The models with lower KS statistics p value and higher area under the curve (AUC), selectivity (Se), and specificity (Sp) were selected. Crude and adjusted odd ratio (OR), 95% confidence interval (CI) and *P* value were calculated for parameters included in the models.

To assess the parameters associated with decreased TBS, ROC-curve analysis was performed with IBM SPSS Statistics for Windows, Version 26.0 (International Business Machines Corporation, Armonk, NY, USA). The AUC with 95%CI and *P* value were calculated. The results were considered significant if the AUC with a lower border of 95%CI was above 0.5 and *P* value was below 0.05. The cut-off values were found with both Se and Sp above 0.55.

RESULTS

Study participants

Three hundred twelve women were initially screened, 176 of them met the inclusion criteria (1-4). These subjects underwent DXA with BMD and TBS assessment. According to DXA results, 17 women had osteoporosis and 63 had osteopenia; these individuals were excluded. Ultimately, 96 women with normal BMD were included in the final analysis.

The mean age of women was 64 years (range: 50-75 years) and mean time since menopause was 16 years (range: 1-37 years). Thirteen women were overweight, 79 were obese and four had a normal BMI. The BMI ranged from 19.1 to 50.2 kg/m^2 (median 33.6 kg/m^2). The duration of T2D varied from 1 to 48 years (median 15 years). All patients received antihyperglycemic therapy, including metformin (n = 80), sulfonylurea (n = 34), sodium glucose cotransporter 2 inhibitors (n = 26), dipeptidyl peptidase-4 inhibitors (n = 9), and insulin (n = 70), mostly in combinations. The mean level of HbA1c was 8.76% (72.2 mmol/mol), ranging from 5.61 to 13.64% (37.7 to 125.6 mmol/mol).

Characteristics of women with T2D depending on TBS values

The clinical characteristics of women with preserved and decreased TBS are presented in Table 1. Women with TBS ≤ 1.31 were taller and had a lower BMI when compared to those with normal TBS (*P* = 0.008 and *P* = 0.007 respectively). There was a trend towards greater age and longer diabetes duration in women with TBS ≤ 1.31 (*P* = 0.09 and *P* = 0.052 respectively). The levels of HbA1c were slightly higher in women with TBS ≤ 1.31, but the difference with women with TBS > 1.31 were not statistically significant (*P* = 0.13). No differences in HbA1c, eGFR, calcium, phosphorus, alkaline phosphatase, PTH and 25(OH)D levels were found between the groups. Most women, including 45 (84.9%) with TBS > 1.31 and 38 women (88.4%) with TBS ≤ 1.31, had 25(OH)D concentrations < 30 ng/mL. The prevalence of diabetic complications and diabetes-associated conditions, as well as antihyperglycemic therapy, did not differ between the groups.

Six women with TBS > 1.31 and 14 women with TBS ≤ 1.31 had at least one fracture in their medical history (χ^2 = 5.64, *P* = 0.02). Two women with TBS > 1.31 had a low-energy fracture (humerus, tibia) in anamnesis. In the group of patients with TBS ≤ 1.31, nine women reported low-energy fractures of spine (n = 2), radius (n = 4), femur neck (n = 1) and humerus (n = 2). A difference in the prevalence of low-energy fractures was statistically significant (χ^2=6.05, *P* = 0.01). At the same time, there were no differences in BMD and T-score between two groups (Table 2). The 10-year risk of low-grade hip fractures was higher in those with TBS ≤ 1.31 (all *P* < 0.0001). The inclusion of TBS data in the FRAX algorithm exacerbated the differences between the groups.

Women with reduced TBS had lower gynoid fat mass and higher android/gynoid fat mass ratio (*P* = 0.004 and *P* < 0.0001 respectively). No differences in trunk fat mass, lean mass and BMC were found (Table 3).

Table 1 Clinical characteristics of postmenopausal women with type 2 diabetes depending on trabecular bone score values

Parameter	Women with TBS > 1.31 (*n* = 53)	Women with TBS ≤ 1.31 (*n* = 43)	*P* value
Age (yr)	62 (59; 68)	65 (59; 72)	0.09
Age at menopause (yr)	50 (46; 53)	50 (45; 52.5)	0.6
Time since menopause (yr)	14 (10; 19)	17 (9; 21.5)	0.72
Diabetes duration (yr)	14 (10; 20)	19 (12; 23)	0.052
Height (cm)	160 (156; 165)	164 (160; 167)	0.008
Body weight (kg)	90 (81; 101)	84 (80; 93)	0.2
BMI (kg/m^2)	35.3 (32.5; 37.2)	32 (29.7; 34.9)	0.007
WHR	0.95 (0.93; 1.0)	1.02 (0.9; 1.05)	0.37
HbA1c (%)	8.5 (7.1; 9.3)	8.9 (7.7; 10.1)	0.13
Total cholesterol (mmol/L)	4.4 (4.1; 5.6)	5.1 (3.9; 5.7)	0.44
LDL-cholesterol (mmol/L)	2.9 (2.6; 3.7)	3.4 (2.7; 3.9)	0.21
HDL-cholesterol (mmol/L)	1.3 (1.1; 1.5)	1.2 (1.1; 1.5)	0.67
Triglycerides (mmol/L)	1.8 (1.1; 2.5)	2.1 (1.4; 2.7)	0.31
hsCRP (mmol/L)	3.1 (1.8; 8.3)	3.3 (1.5; 7.3)	0.71
Calcium (mmol/L)	2.4 (2.4; 2.5)	2.4 (2.4; 2.5)	0.96
Phosphorus (mmol/L)	1.2 (1.1; 1.4)	1.3 (1.2; 1.4)	0.11
Alkaline phosphatase (IU/L)	84.6 (67.3; 107.3)	81.1 (64.8; 98.5)	0.66
PTH (pg/mL)	32.4 (24; 45.4)	31.2 (15.3; 39.0)	0.36
25(OH)D (ng/mL)	21.3 (15.8; 26.5)	18.7 (12.4; 24.2)	0.07
eGFR (mL/min/1.73 m^2)	76 (55; 93)	72 (57; 92)	0.7
UACR (mg/mmoL)	0.6 (0.3; 1.1)	0.5 (0.3; 1.0)	0.18
Diabetic retinopathy, *n* (%)	24 (45.3%)	24 (55.8%)	0.38
CKD, *n* (%)	22 (41.5%)	20 (46.5%)	0.89
Diabetic neuropathy, *n* (%)	53 (100%)	43 (100%)	0.76
Peripheral artery disease, *n* (%)	19 (35.8%)	19 (48.3%)	0.42
Coronary artery disease, *n* (%)	17 (32.1%)	11 (27.6%)	0.59
Metformin, *n* (%)	43 (81.1%)	37 (86%)	0.68
Sulfonylurea, *n* (%)	20 (37.7%)	14 (32.6%)	0.67
DPP4 inhibitor, *n* (%)	3 (5.7%)	6 (14%)	0.49
SGLT2 inhibitor, *n* (%)	16 (30.2%)	10 (23.3%)	0.56
Insulin, *n* (%)	38 (71.7%)	32 (74.4%)	0.82
Fracture in medical history, *n* (%)	6 (11.3%)	14 (32.6%)	0.02
Low-energy fracture in medical history, *n* (%)	2 (3.8%)	9 (20.9%)	0.01

Data are presented as medians (25; 75 percentiles). TBS: Trabecular bone score; BMI: Body mass index; WHR: Waist-to-hip ratio; HbA1c: Hemoglobin A1c; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; hsCRP: High-sensitivity C-reactive protein; PTH: Parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D; eGFR: Estimated glomerular filtration rate; UACR: Urinary albumin-to-creatitine ratio; CKD: Chronic kidney disease; DPP4: Dipeptidyl peptidase-4; SGLT2: Sodium glucose cotransporter 2.

Associations of TBS with clinical and laboratory parameters

In observed women, TBS correlated positively with BMI (r = 0.33, P = 0.001), total fat mass (r = 0.26, P = 0.01) and gynoid fat mass (r = 0.39, P = 0.001). Height and android/gynoid fat mass ratio demonstrated inverse correlations with TBS (r = -0.26, P = 0.01 and r = -0.44, P = 0.00001 respectively), meanwhile, all assessed laboratory parameters, with the exception of 25(OH)D, did not show significant relationships.

Table 2 Dual X-ray absorptiometry parameters and Fracture Risk Assessment tool scores in postmenopausal women with type 2 diabetes depending on trabecular bone score values

Parameter	Women with TBS > 1.31 (*n* = 53)	Women with TBS ≤ 1.31 (*n* = 43)	*P* value
TBS	1.465 (1.39; 1.514)	1.206 (1.127; 1.271)	< 0.001
T-score, minimal	0.0 (-0.5; 0.5)	-0.2 (-0.6; 0.3)	0.42
T-score, L1-L4	0.9 (0.1; 1.7)	1.0 (0.1; 1.7)	0.84
T-score, femoral neck	0.1 (-0.2; 0.7)	-0.05 (-0.5; 0.5)	0.42
T-score, total femur	1.3 (0.85; 1.6)	1.3 (0.6; 1.8)	0.97
T-score, radius	0.3 (-0.3; 0.7)	-0.05 (-0.7; 0.7)	0.27
BMD, L1-L4 (g/cm^2)	1.278 (1.197; 1.387)	1.317 (1.205; 1.39)	0.86
BMD, neck (g/cm^2)	1.044 (1.011; 1.129)	1.045 (0.968; 1.105)	0.45
BMD, total femur (g/cm^2)	1.173 (1.099; 1.210)	1.178 (1.088; 1.237)	0.85
BMD, radius, (g/cm^2)	0.897 (0.849; 0.939)	0.873 (0.816; 0.938)	0.27
FRAX major (%)	6.1 (5.7; 6.8)	6.4 (5.7; 7.1)	0.38
FRAX hip (%)	0.1 (0.1; 0.3)	0.3 (0.1; 0.4)	0.08
FRAX major, TBS-adjusted (%)	5.1 (4.6; 6.0)	7.8 (6.9; 9.2)	< 0.001
FRAX hip, TBS-adjusted (%)	0.1 (0.0; 0.2)	0.4 (0.2; 0.6)	< 0.001

Data are presented as medians (25; 75 percentiles). TBS: Trabecular bone score; BMD: Bone mineral density; FRAX: The Fracture Risk Assessment Tool; FRAX hip: 10-year risk of hip low-energy fractures; FRAX major: 10-year risk of major low-energy fractures.

Table 3 Body composition parameters in postmenopausal women with type 2 diabetes depending on trabecular bone score values

Parameter	Women with TBS > 1.31 (*n* = 53)	Women with TBS ≤ 1.31 (*n* = 43)	*P* value
Total fat mass (%)	45.1 (41.7; 48.3)	43.7 (40.2; 46.2)	0.1
Total fat mass (kg)	40.4 (33.0; 40.4)	36.8 (32.4; 39.5)	0.12
Trunk fat mass (kg)	23.0 (18.8; 25.9)	21.9 (20.2; 25.1)	0.89
Android fat mass (kg)	4.0 (2.9; 4.6)	3.9 (3.5; 4.7)	0.47
Gynoid fat mass (kg)	5.8 (4.9; 6.9)	4.9 (4.3; 5.9)	0.004
Android/gynoid fat mass ratio	1.07 (0.99; 1.17)	1.18 (1.12; 1.29)	< 0.001
Lean mass (kg)	48.2 (44.4; 52.0)	47.7 (44.0; 52.1)	0.83
Bone mineral component (kg)	2.5 (2.4; 2.6)	2.5 (2.3; 2.7)	0.8

Data are presented as medians (25; 75 percentiles). TBS: trabecular bone score.

The levels of 25(OH)D demonstrated weak positive correlation with TBS (r = 0.21, P = 0.042). In addition, 25(OH)D correlated negatively with android fat mass (r = -0.20, P = 0.048), waist circumference (r = -0.24, P = 0.024), PTH (r = -0.34, P = 0.006), and alkaline phosphatase (r = -0.28, P = 0.007).

In a model of multivariate linear regression analysis, TBS was positively associated with gynoid fat mass (+0.007 per each 100 g), whereas the influence of height and androgen fat mass was negative (-0.008 per each cm and and -0.007 per each 100 g, respectively, Table 4). The same factors were identified in a multiple logistic regression model (Table 5). Thus, gynoid fat mass turned out to be a protective factor for TBS (-10% per each 100 g), while height and android fat mass were the risk factors for TBS reduction (+13% per each cm and each 100 g). However, the influence of android fat mass became significant only after being adjusted on height and gynoid fat mass. Moreover, the influence of all factors included in the logistic regression model increased after adjustment.

We have used ROC-analysis to estimate the cut-off values of the factors associated with TBS (Table 6). The height ≥ 162.5 cm, BMI ≤ 33.85 kg/m^2, gynoid fat mass ≤ 5.4 kg (≤ 43.2%), and android/gynoid fat mass ratio ≥ 1.15 were identified as the risk factors of decreased TBS.

Table 4 Factors associated with trabecular bone score in postmenopausal women with type 2 diabetes

Parameter	Coefficient β ± SE	*P* value
Height (cm)	-0.008 ± 0.002	< 0.001
Android fat (100 g)	-0.007 ± 0.002	< 0.001
Gynoid fat (100 g)	0.007 ± 0.002	< 0.001

The linear regression models with backward stepwise selection. Parameters of the model: Intercept 2.54 ± 0.39, adjusted R2 0.31, SE of estimate 0.14, *P* value < 0.001.

Table 5 Factors associated with decreased trabecular bone score in postmenopausal women with type 2 diabetes

Parameter	Crude OR, 95%CI, *P* value	Adjusted OR, 95%CI, *P* value
Height, cm	1.10 (1.02-1.19), *P* = 0.01	1.13 (1.03-1.24), *P* = 0.008
Android fat, 100 g	1.02 (0.98-1.05), *P* = 0.38	1.13 (1.05-1.20), *P* < 0.001
Gynoid fat, 100 g	0.96 (0.93-0.99), *P* = 0.01	0.90 (0.85-0.94), *P* < 0.001

The logistic regression models with forward stepwise selection. Parameters of the model: Intercept 19.0, Kolmogorov–Smirnov test *P* value < 0.001, area under the curve 0.82, Selectivity 0.74, Specificity 0.77, OR 7.69, 95%CI (3.08-19.2), *P* < 0.001 for cut-off value of logistic function = 0.47. 95%CI: 95% confidence interval; OR: Odd ratio.

Table 6 Risk factors of decreased trabecular bone score in postmenopausal women with type 2 diabetes estimated by receiver operating characteristic-analysis

Parameter	Cut-off points	Se	Sp	AUC ± SE (95%CI), *P* value	OR (95%CI), *P* value
Height (cm)	≥ 162.5	0.605	0.604	0.66 ± 0.06 (0.55-0.77), *P* = 0.009	2.33 (1.02-5.31), *P* = 0.04
BMI (kg/m^2)	≤ 33.85	0.70	0.62	0.66 ± 0.06 (0.55-0.77), *P* = 0.008	3.81 (1.62-8.96), *P* = 0.002
Gynoid fat (kg)	≤ 5.41	0.63	0.60	0.67 ± 0.06 (0.56-0.78), *P* = 0.004	2.49 (1.09-5.71), *P* = 0.03
Android fat mass (kg)	≥ 3.95	0.49	0.48	0.54 ± 0.06 (0.43-0.66), *P* = 0.46	0.88 (0.39-1.98), *P* = 0.76
Android/gynoid fat	≥ 1.145	0.70	0.71	0.75 ± 0.05 (0.66-0.85), *P* < 0.001	5.69 (2.35-13.79), *P* < 0.001

Sp: Specificity; Se: Sensitivity; AUC: Area under the curve; SE: Standard error; OR: Odd ratio; 95%CI: 95% Confidence interval; BMI: Body mass index.

DISCUSSION

In this study, we investigated the effects of a number of anthropometric parameters, general and diabetes-related clinical characteristics and body composition on bone microarchitecture, assessed by TBS, in postmenopausal women with T2D and normal BMD.

To date, several imaging modalities, including DXA, radiography, micro-computed tomography, high-resolution peripheral quantitative computed tomography (HR-pQCT), and high-resolution magnetic resonance imaging, have been proposed for bone quality assessment[25]. Among these methods, HR-pQCT and TBS are the most used tools to study the bone microarchitecture in diabetes [26]. HR-pQCT is a non-invasive three-dimensional imaging modality for assessment of bone microarchitecture and bone strength in the appendicular skeleton (*i.e.*, distal radius and tibia)[27]. In the Framingham-HR-pQCT study a modest deterioration in cortical bone and reductions in bone area in patients with T2D were revealed[28]. At the same time, in another population-based study by Nilsson *et al*[29] more favorable bone microarchitecture was observed in elderly women with T2D compared to non-diabetic subjects. TBS is a gray-level textural metric that can be extracted from the two-dimensional lumbar spine DXA image[30]. This analytical method for bone microarchitecture assessment is more available and less expensive than HRpQCT.

The normal range for TBS remains a matter of debate. In 2012, an international working group of TBS users proposed the following criteria: TBS ≥ 1.35 is considered to be normal; TBS between 1.20 and 1.35 indicates partially degraded microarchitecture; finally, TBS ≤ 1.20 defines degraded microarchitecture [31]. Later, based on the results of meta-analysis of 14 population cohort studies from North America, Asia, Australia, and Europe (n = 17809) estimated relationship between TBS and fracture risk, slightly

different criteria for assessing TBS have been proposed[17]. TBS > 1.31 was attributed to normal microarchitecture, TBS values between 1.23 and 1.31 were associated with partially degraded microarchitecture, and TBS < 1.23 was considered as an indicator of degraded microarchitecture. Taken into account that fractures are the most important clinical events related to the bone health, in this study we also used the cut-off value 1.31 to differentiate women with normal and degrade microarchitecture. This cut-off point has been also applied in recent osteoporosis studies[32,33]. Given the relatively small sample size, we did not distinguish a subgroup of patients with borderline TBS (1.23–1.31).

A significant proportion (44.8%) of women in our study showed TBS values less than 1.31. Earlier it was found that T2D women 50 years old and over had lower TBS but higher BMD when compared to non-diabetic women[11]. Postmenopausal women with newly diagnosed T2D showed a decrease in TBS and bone formation markers[34]. A recent study has demonstrated a negative association between TBS and pre-diabetes in subjects aged over 60 years and discordance between TBS and BMD in these subjects [35]. Therefore, the reduction of TBS may reflect an early stage of the impairment of bone health in diabetes. Previously an inverse association between age and TBS was observed in population studies in French and non-Hispanic white US women[36,37]. In this study we were unable to identify age as an independent risk factor for TBS reduction. This can be explained by the relatively small sample size, the upper age limit of 75 years, and the greater influence of other risk factors.

Our results indicate that greater height, lower BMI and gynoid fat mass, but higher android fat mass and android/gynoid fat mass ratio contribute to TBS decrease in women with T2D. A favorable effect of BMI and fat mass on BMD in postmenopausal women with T2D was documented in previous studies [38,39]. However, data on the effect of obesity on the bone metabolism, TBS and fracture risk are not so optimistic[40-42]. In disagreement with previously reported data[43], we observed a positive association between BMI and TBS. At the same time, we found negative association between android/gynoid fat mass ratio and TBS. Moreover, gynoid fat turned out to be a protective factor and android fat was a risk factor for TBS reduction. These findings provide further support to notion that not only fat mass, but also fat distribution, is important for bone health. Previously, inverse association between android fat and TBS was found in Chinese men[44]. Moon *et al*[40] have shown that TBS increase as visceral fat mass decrease in men and women with T2D. In the Newcastle Thousand Families Study an increase in total and, especially, visceral fat mass was associated with prevalent vertebral fracture irrespective of BMD in women aged about 62 years[41]. It was shown that abdominal fat is related to retarded bone formation and impaired bone quality in premenopausal women[42]. Therefore, central adiposity can be considered as a risk factor of bone fragility in T2D.

The association between abdominal obesity and impaired bone microarchitecture can be mediated *via* insulin resistance[43]. Increased bone marrow adiposity, the changes in adipokine production and low-grade inflammation are considered as the relevant mechanisms also[45]. In addition, vitamin D deficiency can worsen bone microarchitecture in women with T2D and abdominal obesity. In our cohort, 25(OH)D demonstrated negative correlation with waist circumference and abdominal fat mass. This data is in agreement with findings from recent meta-analysis of epidemiologic studies indicating an association between vitamin D deficiency and abdominal obesity[46]. Vitamin D deficiency in obese people is attributed to lower dietary intake of vitamin D, lesser skin exposure to sunlight, decreased vitamin absorption, impaired hydroxylation in adipose tissue and 25(OH)D accumulation in fat[47]. At the same time, it is believed that vitamin D deficiency can be associated with insulin resistance and related disorders[48,49].

The role of hyperglycemia as a factor contributing to the degradation of bone microarchitecture is widely discussed. The mechanisms of bone fragility in hyperglycemia include the accumulation of advanced glycation end products and collagen cross-linking, suppressed osteoid mineralization, reduced osteoblastogenesis, and retarded bone turnover[50]. Ho-Pham *et al*[13] reported that subjects with pre-diabetes have a decrease in TBS when compared with normal individuals. At the same time, Holloway *et al*[14] found no difference in TBS between subjects with normoglycaemia and impaired fasting glucose. A negative association between TBS and HbA1c has been reported in subjects with diabetes[51]. In the Maasticht study a negative association was found between HbA1c and parameters of bone health estimated by HR-pQCT in individuals with well-controlled T2D[52]. In our study, HbA1c was only slightly higher in patients with TBS ≤ 1.31. Even though we did not identify HbA1c as a risk factor for a decrease TBS, we cannot exclude the role of hyperglycemia in the deterioration of bone microarchitecture. Most of the patients had long-term diabetes and non-target glycemic control parameters on combined antidiabetic therapy. These factors could modify the effect of hyperglycemia on TBS. Besides, single HbA1c measurements were included in the analysis. Therefore, the effect of metabolic memory on bone structure cannot be ruled out.

The value of TBS as a predictor of low-energy fractures is a matter of increasing interest. It was demonstrated that in postmenopausal women with T2D TBS rather than BMD is associated with vertebral[53] and major osteoporotic fractures[11]. The FRAX score, being unadjusted to TBS, underestimates fracture risk in these women[54]. In our study, women with normal and reduced TBS demonstrated no differences in the unadjusted FRAX scores, although they were different in the prevalent fractures. As expected, incorporation of TBS values into the FRAX algorithm increased probability of the fractures in women with lower TBS. Therefore, TBS can help to improve the assessment of the risk of fractures in women with T2D and normal BMD. However, even after TBS

adjustment the risk of fractures may be underestimated. A recent population-based prospective study by Leslie *et al*[55] (the Manitoba BMD Registry) showed that a residual effect of diabetes on major osteoporotic fractures estimated with FRAX persists even after TBS adjustment, though the adjustment attenuated the effect of the disease. Adjustment for diabetes further improves the quality of fracture prediction.

The cross-sectional design and relatively small sample size are the limitations of our study. The recruitment of patients in one clinical center could lead to some sample bias. We could not differentiate visceral and subcutaneous adipose tissue with the applied DXA technique. As the used version of TBS iNsight software does not correct for extremes of BMI, we cannot exclude some underestimation of TBS in patients with obesity class 2 and 3[56].

At the same time, as far as we know, this is the first study estimating the risk factors for impaired bone microarchitecture assessed by TBS in postmenopausal women with T2D and normal BMD. Further studies of a larger size and prospective design are needed to establish the role of the identified factors as predictors of TBS reduction in these women. The value of TBS in the prediction of osteoporosis-related fractures in postmenopausal women with T2D and normal BMD is another challenge for future research.

CONCLUSION

In this study, we have revealed a decrease in the TBS values in 44.8% of postmenopausal women with T2D and normal BMD. These data indicate that a substantial proportion of postmenopausal women with T2D have impaired bone microarchitecture despite the normal BMD parameters. Greater height and central adiposity turned out to be the risk factors for impaired bone microarchitecture in these women. The results give further support to notion that estimation of TBS should be an essential element of DXA protocol in postmenopausal women with T2D.

ARTICLE HIGHLIGHTS

Research background

People with type 2 diabetes (T2D) have higher risk of vertebral and some non-vertebral fractures than non-diabetic individuals, regardless of normal or even increased bone mineral density (BMD). As BMD assessment may lead to underestimation of a fracture risk in T2D, additional parameters of bone health should be taken into consideration. The impaired bone microarchitecture is considered as a major contributor to fracture risk in T2D. Trabecular Bone Score (TBS) on lumbar spine dual X-ray absorptiometry (DXA) images is increasingly applied for the assessment of bone microarchitecture. Individuals with diabetes as compared to those without have significantly lower TBS.

Research motivation

At present, data on TBS in postmenopausal women with T2D and normal BMD is limited, and predictors of TBS decrease in these women need to be refined. In particular, the role of body composition and diabetes-related parameters as risk factors for deterioration of bone microarchitecture needs further research.

Research objectives

To identify clinical and body composition parameters that affect TBS in postmenopausal women with T2D and normal BMD.

Research methods

A non-interventional cross-sectional comparative study was conducted. Postmenopausal women with T2D, aged 50-75 years, with no established risk factors for secondary osteoporosis, were included. BMD, TBS and body composition parameters were assessed by DXA. In women with normal BMD, a wide range of anthropometric, general and diabetes-related clinical and laboratory parameters were evaluated as risk factors for TBS decrease using univariate and multivariate regression analysis and analysis of receiver operating characteristic (ROC) curves.

Research results

Among women with normal BMD, 44.8% showed decreased TBS values (≤ 1.31). Women with decreased TBS were taller and had a lower BMI when compared to those with normal TBS. No significant differences in HbA1c, renal function, calcium, phosphorus, alkaline phosphatase, PTH and 25(OH)D levels were found. In the models of multivariate regression analysis, TBS was positively associated with gynoid fat mass, whereas the height and androgen fat mass were associated negatively.

In the ROC-curve analysis, height ≥ 162.5 cm, body mass index < 33.85 kg/m^2, gynoid fat mass ≤ 5.41 kg and android/gynoid fat mass ratio ≥ 1.145 were identified as the risk factors for TBS reduction.

Research conclusions

The obtained results indicate that a substantial proportion of postmenopausal women with T2D and normal BMD may have impaired bone microarchitecture. Greater height and central adiposity turned out to be the risk factors for decreased TBS in these women. The results give further support to notion that estimation of TBS should be an essential element of DXA protocol in postmenopausal women with T2D.

Research perspectives

The prognostic value of TBS as a risk factor for fractures in patients with T2D and normal BMD needs further research. Prospective studies should determine the effect of changes in body weight and body composition on bone microarchitecture in these patients. The impact of hyperglycemia, glucose variability and metabolic memory, as well as various antihyperglycemic drugs, also needs to be clarified.

FOOTNOTES

Author contributions: Klimontov VV contributed to study conception and design, data analysis and interpretation; Fazullina ON and Korbut AI collected the data and performed data analysis and interpretation; Fazullina ON and Klimontov VV wrote the article; all authors approved the final version of the manuscript.

Institutional review board statement: The study protocol was approved by the Ethical Committee of the RICEL - Branch of IC&G SB RAS (protocol N. 104 from 20 December 2014).

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

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: Russia

ORCID number: Olga N Fazullina 0000-0002-5868-579X; Anton I Korbut 0000-0003-3502-5892; Vadim V Klimontov 0000-0002-5407-8722.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Tebé C**, Martínez-Laguna D, Carbonell-Abella C, Reyes C, Moreno V, Diez-Perez A, Collins GS, Prieto-Alhambra D. The association between type 2 diabetes mellitus, hip fracture, and post-hip fracture mortality: a multi-state cohort analysis. *Osteoporos Int* 2019; **30**: 2407-2415 [PMID: 31444526 DOI: 10.1007/s00198-019-05122-3]

2 **Sato M**, Ye W, Sugihara T, Isaka Y. Fracture risk and healthcare resource utilization and costs among osteoporosis patients with type 2 diabetes mellitus and without diabetes mellitus in Japan: retrospective analysis of a hospital claims database. *BMC Musculoskelet Disord* 2016; **17**: 489 [PMID: 27887655 DOI: 10.1186/s12891-016-1344-9]

3 **Xu Y**, Wu Q. Trends in osteoporosis and mean bone density among type 2 diabetes patients in the US from 2005 to 2014. *Sci Rep* 2021; **11**: 3693 [PMID: 33580184 DOI: 10.1038/s41598-021-83263-4]

4 **Moayeri A**, Mohamadpour M, Mousavi SF, Shirzadpour E, Mohamadpour S, Amraei M. Fracture risk in patients with type 2 diabetes mellitus and possible risk factors: a systematic review and meta-analysis. *Ther Clin Risk Manag* 2017; **13**: 455-468 [PMID: 28442913 DOI: 10.2147/TCRM.S131945]

5 **Koromani F**, Oei L, Shevroja E, Trajanoska K, Schoufour J, Muka T, Franco OH, Ikram MA, Zillikens MC, Uitterlinden

AG, Krestin GP, Anastassiades T, Josse R, Kaiser SM, Goltzman D, Lentle BC, Prior JC, Leslie WD, McCloskey E, Lamy O, Hans D, Oei EH, Rivadeneira F. Vertebral Fractures in Individuals With Type 2 Diabetes: More Than Skeletal Complications Alone. *Diabetes Care* 2020; **43**: 137-144 [PMID: 31658976 DOI: 10.2337/dc19-0925]

6 **Oei L**, Zillikens MC, Dehghan A, Buitendijk GH, Castaño-Betancourt MC, Estrada K, Stolk L, Oei EH, van Meurs JB, Janssen JA, Hofman A, van Leeuwen JP, Witteman JC, Pols HA, Uitterlinden AG, Klaver CC, Franco OH, Rivadeneira F. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. *Diabetes Care* 2013; **36**: 1619-1628 [PMID: 23315602 DOI: 10.2337/dc12-1188]

7 **Goldshtein I**, Nguyen AM, dePapp AE, Ish-Shalom S, Chandler JM, Chodick G, Shalev V. Epidemiology and correlates of osteoporotic fractures among type 2 diabetic patients. *Arch Osteoporos* 2018; **13**: 15 [PMID: 29502187 DOI: 10.1007/s11657-018-0432-x]

8 **Botella Martínez S**, Varo Cenarruzabeitia N, Escalada San Martin J, Calleja Canelas A. The diabetic paradox: Bone mineral density and fracture in type 2 diabetes. *Endocrinol Nutr* 2016; **63**: 495-501 [PMID: 27481443 DOI: 10.1016/j.endonu.2016.06.004]

9 **Harvey NC**, Glüer CC, Binkley N, McCloskey EV, Brandi ML, Cooper C, Kendler D, Lamy O, Laslop A, Camargos BM, Reginster JY, Rizzoli R, Kanis JA. Trabecular bone score (TBS) as a new complementary approach for osteoporosis evaluation in clinical practice. *Bone* 2015; **78**: 216-224 [PMID: 25988660 DOI: 10.1016/j.bone.2015.05.016]

10 **Palui R**, Pramanik S, Mondal S, Ray S. Critical review of bone health, fracture risk and management of bone fragility in diabetes mellitus. *World J Diabetes* 2021; **12**: 706-729 [PMID: 34168723 DOI: 10.4239/wjd.v12.i6.706]

11 **Leslie WD**, Aubry-Rozier B, Lamy O, Hans D; Manitoba Bone Density Program. TBS (trabecular bone score) and diabetes-related fracture risk. *J Clin Endocrinol Metab* 2013; **98**: 602-609 [PMID: 23341489 DOI: 10.1210/jc.2012-3118]

12 **Lin YC**, Wu J, Kuo SF, Cheung YC, Sung CM, Fan CM, Chen FP, Mhuircheartaigh JN. Vertebral Fractures in Type 2 Diabetes Patients: Utility of Trabecular Bone Score and Relationship With Serum Bone Turnover Biomarkers. *J Clin Densitom* 2020; **23**: 37-43 [PMID: 30773275 DOI: 10.1016/j.jocd.2019.01.003]

13 **Ho-Pham LT**, Nguyen TV. Association between trabecular bone score and type 2 diabetes: a quantitative update of evidence. *Osteoporos Int* 2019; **30**: 2079-2085 [PMID: 31214749 DOI: 10.1007/s00198-019-05053-z]

14 **Holloway KL**, De Abreu LLF, Hans D, Kotowicz MA, Sajjad MA, Hyde NK, Pasco JA. Trabecular Bone Score in Men and Women with Impaired Fasting Glucose and Diabetes. *Calcif Tissue Int* 2018; **102**: 32-40 [PMID: 28965154 DOI: 10.1007/s00223-017-0330-z]

15 **Napoli N**, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL; IOF Bone and Diabetes Working Group. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol* 2017; **13**: 208-219 [PMID: 27658727 DOI: 10.1038/nrendo.2016.153]

16 **Costantini S**, Conte C. Bone health in diabetes and prediabetes. *World J Diabetes* 2019; **10**: 421-445 [PMID: 31523379 DOI: 10.4239/wjd.v10.i8.421]

17 **McCloskey EV**, Odén A, Harvey NC, Leslie WD, Hans D, Johansson H, Barkmann R, Boutroy S, Brown J, Chapurlat R, Elders PJM, Fujita Y, Glüer CC, Goltzman D, Iki M, Karlsson M, Kindmark A, Kotowicz M, Kurumatani N, Kwok T, Lamy O, Leung J, Lippuner K, Ljunggren Ö, Lorentzon M, Mellström D, Merlijn T, Oei L, Ohlsson C, Pasco JA, Rivadeneira F, Rosengren B, Sornay-Rendu E, Szulc P, Tamaki J, Kanis JA. A Meta-Analysis of Trabecular Bone Score in Fracture Risk Prediction and Its Relationship to FRAX. *J Bone Miner Res* 2016; **31**: 940-948 [PMID: 26498132 DOI: 10.1002/jbmr.2734]

18 **Klimontov VV**, Bulumbaeva DM, Fazullina ON, Lykov AP, Bgatova NP, Orlov NB, Konenkov VI, Pfeiffer AFH, Pivovarova-Ramich O, Rudovich N. Circulating Wnt1-inducible signaling pathway protein-1 (WISP-1/CCN4) is a novel biomarker of adiposity in subjects with type 2 diabetes. *J Cell Commun Signal* 2020; **14**: 101-109 [PMID: 31782053 DOI: 10.1007/s12079-019-00536-4]

19 **DeShields SC**, Cunningham TD. Comparison of osteoporosis in US adults with type 1 and type 2 diabetes mellitus. *J Endocrinol Invest* 2018; **41**: 1051-1060 [PMID: 29353395 DOI: 10.1007/s40618-018-0828-x]

20 **Schacter GI**, Leslie WD. Diabetes and Osteoporosis: Part I, Epidemiology and Pathophysiology. *Endocrinol Metab Clin North Am* 2021; **50**: 275-285 [PMID: 34023043 DOI: 10.1016/j.ecl.2021.03.005]

21 **Dhaliwal R**, Cibula D, Ghosh C, Weinstock RS, Moses AM. Bone quality assessment in type 2 diabetes mellitus. *Osteoporos Int* 2014; **25**: 1969-1973 [PMID: 24718377 DOI: 10.1007/s00198-014-2704-7]

22 **Nikfarjam M**, Heshmat R, Gharibzadeh S, Ostovar A, Maleki V, Moludi J, Nabipour I, Shafiee G, Larijani B. The association between muscle indicators and bone mass density and related risk factors in the diabetic elderly population: Bushehr Elderly Health (BEH) Program. *J Diabetes Metab Disord* 2021; **20**: 1429-1438 [PMID: 34900794 DOI: 10.1007/s40200-021-00881-5]

23 **Fritz CO**, Morris PE, Richler JJ. Effect size estimates: current use, calculations, and interpretation. *J Exp Psychol Gen* 2012; **141**: 2-18 [PMID: 21823805 DOI: 10.1037/a0024338]

24 **Serdar CC**, Cihan M, Yücel D, Serdar MA. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem Med (Zagreb)* 2021; **31**: 010502 [PMID: 33380887 DOI: 10.11613/BM.2021.010502]

25 **Wang F**, Zheng L, Theopold J, Schleifenbaum S, Heyde CE, Osterhoff G. Methods for bone quality assessment in human bone tissue: a systematic review. *J Orthop Surg Res* 2022; **17**: 174 [PMID: 35313901 DOI: 10.1186/s13018-022-03041-4]

26 **Martínez-Montoro JI**, García-Fontana B, García-Fontana C, Muñoz-Torres M. Evaluation of Quality and Bone Microstructure Alterations in Patients with Type 2 Diabetes: A Narrative Review. *J Clin Med* 2022; **11** [PMID: 35456299 DOI: 10.3390/jcm11082206]

27 **Nishiyama KK**, Shane E. Clinical imaging of bone microarchitecture with HR-pQCT. *Curr Osteoporos Rep* 2013; **11**: 147-155 [PMID: 23504496 DOI: 10.1007/s11914-013-0142-7]

28 **Samelson EJ**, Demissie S, Cupples LA, Zhang X, Xu H, Liu CT, Boyd SK, McLean RR, Broe KE, Kiel DP, Bouxsein ML. Diabetes and Deficits in Cortical Bone Density, Microarchitecture, and Bone Size: Framingham HR-pQCT Study. *J Bone Miner Res* 2018; **33**: 54-62 [PMID: 28929525 DOI: 10.1002/jbmr.3240]

29 **Nilsson AG**, Sundh D, Johansson L, Nilsson M, Mellström D, Rudäng R, Zoulakis M, Wallander M, Darelid A, Lorentzon

M. Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study. *J Bone Miner Res* 2017; **32**: 1062-1071 [PMID: 27943408 DOI: 10.1002/jbmr.3057]

30 **Silva BC**, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, McCloskey EV, Kanis JA, Bilezikian JP. Trabecular bone score: a noninvasive analytical method based upon the DXA image. *J Bone Miner Res* 2014; **29**: 518-530 [PMID: 24443324 DOI: 10.1002/jbmr.2176]

31 **Cormier C**, Lamy O, Poriau S. TBS in routine clinial practice: proposals of use. Switzerland: Medimaps Group, 2012. [cited 1 February 2022]. Available from: https://sanova.at/wp-content/uploads/2020/08/Atlas_TBS.pdf

32 **Binkley N**, Morin SN, Martineau P, Lix LM, Hans D, Leslie WD. Frequency of normal bone measurement in postmenopausal women with fracture: a registry-based cohort study. *Osteoporos Int* 2020; **31**: 2337-2344 [PMID: 32778934 DOI: 10.1007/s00198-020-05576-w]

33 **Panahi N**, Ostovar A, Fahimfar N, Aghaei Meybodi HR, Gharibzadeh S, Arjmand B, Sanjari M, Khalagi K, Heshmat R, Nabipour I, Soltani A, Larijani B. Factors associated with TBS worse than BMD in non-osteoporotic elderly population: Bushehr elderly health program. *BMC Geriatr* 2021; **21**: 444 [PMID: 34315430 DOI: 10.1186/s12877-021-02375-8]

34 **Li H**, Wen Y, Liu P, Zhang L, Zhang X, Liu Y, Ma B, Kuang H, Wang J, Song L. Characteristics of bone metabolism in postmenopausal women with newly diagnosed type 2 diabetes mellitus. *Clin Endocrinol (Oxf)* 2021; **95**: 430-438 [PMID: 34008210 DOI: 10.1111/cen.14501]

35 **Ebrahimpur M**, Sharifi F, Nezhad FA, Bagherzadeh M, Ostovar A, Shafiee G, Heshmat R, Mehrdad N, Razi F, Khashayar P, Nabipour I, Larijani B. Effect of diabetes on BMD and TBS values as determinants of bone health in the elderly: Bushehr Elderly Health program. *J Diabetes Metab Disord* 2019; **18**: 99-106 [PMID: 31275880 DOI: 10.1007/s40200-019-00395-1]

36 **Dufour R**, Winzenrieth R, Heraud A, Hans D, Mehsen N. Generation and validation of a normative, age-specific reference curve for lumbar spine trabecular bone score (TBS) in French women. *Osteoporos Int* 2013; **24**: 2837-2846 [PMID: 23681084 DOI: 10.1007/s00198-013-2384-8]

37 **Simonelli C**, Leib E, Mossman N, Winzenrieth R, Hans D, McClung M. Creation of an age-adjusted, dual-energy x-ray absorptiometry-derived trabecular bone score curve for the lumbar spine in non-Hispanic US White women. *J Clin Densitom* 2014; **17**: 314-319 [PMID: 24582086 DOI: 10.1016/j.jocd.2013.09.002]

38 **Raška I Jr**, Rašková M, Zikán V, Škrha J. Body composition is associated with bone and glucose metabolism in postmenopausal women with type 2 diabetes mellitus. *Physiol Res* 2017; **66**: 99-111 [PMID: 27782739 DOI: 10.33549/physiolres.933310]

39 **Chain A**, Crivelli M, Faerstein E, Bezerra FF. Association between fat mass and bone mineral density among Brazilian women differs by menopausal status: The Pró-Saúde Study. *Nutrition* 2017; **33**: 14-19 [PMID: 27908545 DOI: 10.1016/j.nut.2016.08.001]

40 **Moon HU**, Lee N, Chung YS, Choi YJ. Reduction of visceral fat could be related to the improvement of TBS in diabetes mellitus. *J Bone Miner Metab* 2020; **38**: 702-709 [PMID: 32399674 DOI: 10.1007/s00774-020-01107-z]

41 **Hind K**, Pearce M, Birrell F. Total and Visceral Adiposity Are Associated With Prevalent Vertebral Fracture in Women but Not Men at Age 62 Years: The Newcastle Thousand Families Study. *J Bone Miner Res* 2017; **32**: 1109-1115 [PMID: 28261864 DOI: 10.1002/jbmr.3085]

42 **Cohen A**, Dempster DW, Recker RR, Lappe JM, Zhou H, Zwahlen A, Müller R, Zhao B, Guo X, Lang T, Saeed I, Liu XS, Guo XE, Cremers S, Rosen CJ, Stein EM, Nickolas TL, McMahon DJ, Young P, Shane E. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study. *J Clin Endocrinol Metab* 2013; **98**: 2562-2572 [PMID: 23515452 DOI: 10.1210/jc.2013-1047]

43 **Hayón-Ponce M**, García-Fontana B, Avilés-Pérez MD, González-Salvatierra S, Andújar-Vera F, Moratalla-Aranda E, Muñoz-Torres M. Lower trabecular bone score in type 2 diabetes mellitus: A role for fat mass and insulin resistance beyond hyperglycaemia. *Diabetes Metab* 2021; **47**: 101276 [PMID: 34517124 DOI: 10.1016/j.diabet.2021.101276]

44 **Lv S**, Zhang A, Di W, Sheng Y, Cheng P, Qi H, Liu J, Yu J, Ding G, Cai J, Lai B. Assessment of Fat distribution and Bone quality with Trabecular Bone Score (TBS) in Healthy Chinese Men. *Sci Rep* 2016; **6**: 24935 [PMID: 27112305 DOI: 10.1038/srep24935]

45 **Gkastaris K**, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. *J Musculoskelet Neuronal Interact* 2020; **20**: 372-381 [PMID: 32877973]

46 **Hajhashemy Z**, Foshati S, Saneei P. Relationship between abdominal obesity (based on waist circumference) and serum vitamin D levels: a systematic review and meta-analysis of epidemiologic studies. *Nutr Rev* 2022; **80**: 1105-1117 [PMID: 34537844 DOI: 10.1093/nutrit/nuab070]

47 **Migliaccio S**, Di Nisio A, Mele C, Scappaticcio L, Savastano S, Colao A; Obesity Programs of nutrition, Education, Research and Assessment (OPERA) Group. Obesity and hypovitaminosis D: causality or casualty? *Int J Obes Suppl* 2019; **9**: 20-31 [PMID: 31391922 DOI: 10.1038/s41367-019-0010-8]

48 **Szymczak-Pajor I**, Drzewoski J, Śliwińska A. The Molecular Mechanisms by Which Vitamin D Prevents Insulin Resistance and Associated Disorders. *Int J Mol Sci* 2020; **21** [PMID: 32932777 DOI: 10.3390/ijms21186644]

49 **Theik NWY**, Raji OE, Shenwai P, Shah R, Kalluri SR, Bhutta TH, Hannoodee H, Al Khalili M, Khan S. Relationship and Effects of Vitamin D on Metabolic Syndrome: A Systematic Review. *Cureus* 2021; **13**: e17419 [PMID: 34589329 DOI: 10.7759/cureus.17419]

50 **Romero-Díaz C**, Duarte-Montero D, Gutiérrez-Romero SA, Mendivil CO. Diabetes and Bone Fragility. *Diabetes Ther* 2021; **12**: 71-86 [PMID: 33185853 DOI: 10.1007/s13300-020-00964-1]

51 **Kim JH**, Choi HJ, Ku EJ, Kim KM, Kim SW, Cho NH, Shin CS. Trabecular bone score as an indicator for skeletal deterioration in diabetes. *J Clin Endocrinol Metab* 2015; **100**: 475-482 [PMID: 25368976 DOI: 10.1210/jc.2014-2047]

52 **de Waard EAC**, de Jong JJA, Koster A, Savelberg HHCM, van Geel TA, Houben AJHM, Schram MT, Dagnelie PC, van der Kallen CJ, Sep SJS, Stehouwer CDA, Schaper NC, Berendschot TTJM, Schouten JSAG, Geusens PPMM, van den Bergh JPW. The association between diabetes status, HbA1c, diabetes duration, microvascular disease, and bone quality of the distal radius and tibia as measured with high-resolution peripheral quantitative computed tomography-The Maastricht

Study. *Osteoporos Int* 2018; **29**: 2725-2738 [PMID: 30209523 DOI: 10.1007/s00198-018-4678-3]

53 **Yamamoto M**, Yamauchi M, Sugimoto T. Prevalent vertebral fracture is dominantly associated with spinal microstructural deterioration rather than bone mineral density in patients with type 2 diabetes mellitus. *PLoS One* 2019; **14**: e0222571 [PMID: 31525243 DOI: 10.1371/journal.pone.0222571]

54 **Majumdar SR**, Leslie WD, Lix LM, Morin SN, Johansson H, Oden A, McCloskey EV, Kanis JA. Longer Duration of Diabetes Strongly Impacts Fracture Risk Assessment: The Manitoba BMD Cohort. *J Clin Endocrinol Metab* 2016; **101**: 4489-4496 [PMID: 27603908 DOI: 10.1210/jc.2016-2569]

55 **Leslie WD**, Johansson H, McCloskey EV, Harvey NC, Kanis JA, Hans D. Comparison of Methods for Improving Fracture Risk Assessment in Diabetes: The Manitoba BMD Registry. *J Bone Miner Res* 2018; **33**: 1923-1930 [PMID: 29953670 DOI: 10.1002/jbmr.3538]

56 **Amnuaywattakorn S**, Sritara C, Utamakul C, Chamroonrat W, Kositwattanarerk A, Thamnirat K, Ongphiphadhanakul B. Simulated increased soft tissue thickness artefactually decreases trabecular bone score: a phantom study. *BMC Musculoskelet Disord* 2016; **17**: 17 [PMID: 26757709 DOI: 10.1186/s12891-016-0886-1]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 566-580

DOI: 10.4239/wjd.v13.i7.566

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Prospective Study

Relationship between quality of life and adolescent glycolipid metabolism disorder: A cohort study

Xiao-Hua Liang, Yang-Ling Ren, Xiao-Yue Liang, Jing-Yu Chen, Ping Qu, Xian Tang

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): D
Grade E (Poor): 0

P-Reviewer: Kuru Çolak T, Turkey; Sibiya N, South Africa; Teixeira KN, Brazil; Xiao JB, Spain

Received: December 20, 2021
Peer-review started: December 20, 2021
First decision: April 18, 2022
Revised: April 29, 2022
Accepted: June 20, 2022
Article in press: June 20, 2022
Published online: July 15, 2022

Xiao-Hua Liang, Yang-Ling Ren, Xiao-Yue Liang, Ping Qu, Xian Tang, Department of Clinical Epidemiology and Biostatistics, Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Child Health and Nutrition, Chongqing 400016, China

Jing-Yu Chen, Ultrasound Department of Children's Hospital of Chongqing Medical University, Children's Hospital of Chongqing Medical University, Chongqing 400014, China

Corresponding author: Xiao-Hua Liang, MD, PhD, Associate Research Scientist, Department of Clinical Epidemiology and Biostatistics, Children's Hospital of Chongqing Medical University, National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Child Health and Nutrition, No. 136 2nd Street, Yuzhong District, Chongqing 400016, China.
xiaohualiang@hospital.cqmu.edu.cn

Abstract

BACKGROUND

The prevalence of glucolipid metabolic disorders (GLMDs) in children and adolescents has a recognized association with cardiovascular diseases and type 2 diabetes mellitus in adulthood. Therefore, it is important to enhance our understanding of the risk factors for GLMD in childhood and adolescence.

AIM

To explore the relationship between quality of life (QoL) and adolescent GLMD.

METHODS

This study included 1956 samples in 2019 from a cohort study established in 2014. The QoL scale and glycolipid indexes were collected during follow-up; other covariates of perinatal factors, physical measures, and socioeconomic indicators were collected and adjusted. A generalized linear regression model and logistic regression model were used to analyse the correlation between QoL and GLMD.

RESULTS

Higher scores of QoL activity opportunity, learning ability and attitude, attitude towards doing homework, and living convenience domains correlated negatively with insulin and homeostasis model assessment insulin resistance (IR) levels. Psychosocial factors, QoL satisfaction factors, and total QoL scores had significant

protective effects on insulin and IR levels. Activity opportunity, learning ability and attitude, attitude towards doing homework domains of QoL, psychosocial factor, and total score of QoL correlated positively with high density lipoprotein. In addition, the attitude towards doing homework domain was a protective factor for dyslipidaemia, IR > 3, and increased fasting blood glucose; four factors, QoL and total QoL score correlated significantly negatively with IR > 3. In subgroup analyses of sex, more domains of QoL correlated with insulin and triglyceride levels, dyslipidaemia, and IR > 3 in females. Poor QoL was associated with an increased prevalence of GLMD, and the effect was more pronounced in males than in females. Measures to improve the QoL of adolescents are essential to reduce rates of GLMD.

CONCLUSION

Our study revealed that QoL scores mainly correlate negatively with the prevalence of GLMD in adolescents of the healthy population. The independent relationship between QoL and GLMD can be illustrated by adjusting for multiple covariates that may be associated with glycaemic index. In addition, among females, more QoL domains are associated with glycaemic index.

Key Words: Quality of life; Insulin resistance; Lipids; Metabolic abnormality

Core Tip: Persistent abnormalities of glucose and lipid metabolism in childhood have a well-established association with adulthood cardiovascular diseases. Previous conclusions about the association between quality of life (QoL) and glycolipid metabolism disorder (GLMD) were almost all based on adults with type 2 diabetes or dyslipidaemia, whereas there is limited evidence for the association between QoL and GLMD in healthy children and adolescents. This study found that a poor QoL score was associated with increased insulin, triglyceride, and IR levels, and the association was more significant in males than in females. In addition, seven domains, four factors, and total QoL score were negatively associated with abnormalities in glucose and lipid metabolism. Measures to improve the QoL of adolescents are essential to reduce the prevalence of GLMD.

Citation: Liang XH, Ren YL, Liang XY, Chen JY, Qu P, Tang X. Relationship between quality of life and adolescent glycolipid metabolism disorder: A cohort study. *World J Diabetes* 2022; 13(7): 566-580
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/566.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.566

INTRODUCTION

The increased prevalence of glucolipid metabolic disorders (GLMDs) in children and adolescents has a well-established association with cardiovascular diseases (CVDs) and type 2 diabetes (T2D) in adulthood[1]. Therefore, it is important to increase our understanding of the risk factors for GLMD during childhood and adolescence. Previous studies have illustrated that risk factors for GLMD in children include unhealthy dietary habits[2], genetic factors[3,4], poor prenatal exposure to high maternal fasting blood glucose (FBG) levels[5], and gestational diabetes. Overall, conclusions about insulin resistance (IR) and quality of life (QoL) are controversial. The results of Schlotz *et al*[6] showed that IR is associated with lower health-related QoL only in physical health domains[6]. However, a cohort study reported that participants with IR had deteriorated health-related QoL involving physical functioning, emotional role limitations, social functioning, pain, and general health perception, and a more significant correlation was found in males[7]. Several previous studies[8,9] have found that limited trials have reported health-related QoL (HRQoL) in diabetes mellitus, and diabetes affects several dimensions of QoL, such as physical, social well-being, and emotional, compared with a control group [10,11]. Additionally, the primary intervention of pravastatin plus intensive dietary advice might improve the QoL of patients with hyperlipidaemia[12,13]. Several intervention trials[10,14,15] of patients with T2D found disease special-QoL and HRQoL to be improved after treatment, accompanied by decreased FPG, triglyceride (TG), and insulin levels. A systematic review also illustrated that diabetes self-management education may improve the QoL of diabetes by decreasing glycosylated haemoglobin (HbA1c) levels[11]. Therefore, previous conclusions suggest that hyperlipidaemia and impaired fasting glycaemia may impact QoL. Moreover, a study showed that lower QoL impacts the ability to achieve a good HbA1c level[16]. Diverse QoL survey tools have been used in previous studies, with most of these assessment tools being focused on disease-specific QoL[11,17], whereas there are few

scales for measuring the global health or general health of healthy subjects[7]. QoL includes multidimensional terms, which represent satisfaction with life status and describe a subject's functioning in physical, emotional, and social domains. Little evidence about the relationship between QoL and GLMD has been reported, especially in children and adolescents, which is an important stage of growth.

To our knowledge, there are no studies exploring the correlation between QoL and GLMD in healthy children aged 10-14 years from a rural-urban cohort study. The hypothesis of this study is that QoL affect GLMD in children and adolescents. The aim of this cohort study was to explore the correlation of QoL scores with GLMD in adolescents, providing an excellent opportunity to identify independent risk factors for GLMD after adjusting for multiple variables, such as perinatal variables, socioeconomic status (SES), anthropometric measures, and other biochemical indexes.

MATERIALS AND METHODS

Subjects

Two-stage stratified cluster sampling was used to select children from urban and rural areas of Chongqing; then, two regions *per* county were randomly chosen; and finally, all children living in the selected region were informed and included if they were satisfied the inclusion criteria. In addition, a bidirectional cohort in which retrospective and prospective variables were adjusted was used to evaluate the relationship between QoL and GLMD. At baseline, children who met all the following criteria were recruited: (1) Aged 6-9 years in 2014; (2) Resided in the selected areas for more than 6 mo; (3) Did not have serious diseases (*e.g.*, nephropathy, CVD, or cancer); and (4) Consent both from the parents and children for participation. At baseline, all participants in grades 1 and 2 were recruited mainly from two elementary schools. The class head teacher delivered questionnaires to children who signed informed consent forms, and the children took the questionnaires home and completed them with their parents, after which the teacher collected the questionnaires. Two thousand one hundred and thirty-six children with venous blood samples were analysed in this study. After excluding 117 children without FBG or insulin data and 60 children without QoL information, 1959 children with complete data were analysed (Figure 1). This study was approved by the Institutional Review Board of the Children's Hospital of Chongqing Medical University, and all subjects and their parents/guardians signed informed consent forms.

Demographic variables

Demographic information and SES (parents' occupation and education level, and family income) were collected. Bachelor's and master's degrees were combined, as there were few parents with the latter. Therefore, parental education level was measured on a four-point scale [≤ 9 years (elementary and middle school), 9-12, 12-15, and > 15 years]. Perinatal variables included maternal obesity and maternal increased weight during pregnancy. Family history of obesity or CVD was investigated using a self-filled questionnaire. In addition, sleeping quality and dietary intake of vegetables, red meat, and salt were surveyed; the detailed protocol was published in a previous paper[18,19].

The questionnaire is valid and reliable, has been used in more than 20000 children, has been modified several times after each survey, and has been described in detail in our previous publications[19-21]. The questionnaire included information on demographics, perinatal status, SES, dietary intake, physical activity, and sleep quality; it was completed both by the children and their parents or guardians according to the protocol.

Physical examination

Anthropometric indexes of height, weight, and waist circumference were measured by well-trained paediatric nurses, and the detailed protocol for these measurements has been introduced in our previous papers[19,22,23].

Biochemical indexes

Venous blood (3 mL) was drawn in the morning after at least 12 h of fasting from subjects who provided informed consent[24]. FBG, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), TGs, fasting insulin (FI), and glycosylated haemoglobin were measured within 2 h after blood draw; details are provided in other publications[19]. In 2019, FI was measured using a Siemens Centaur XP.

QoL questionnaire

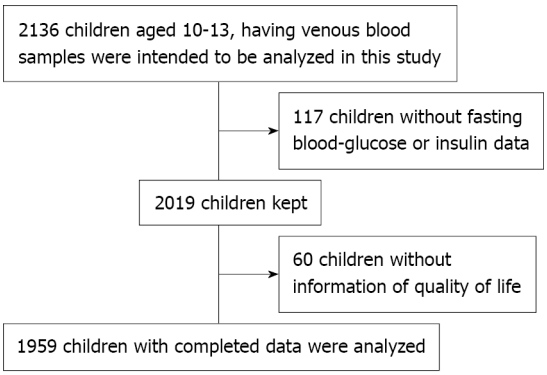
The QoL Scale for Children and Adolescents (QLSCA) with 49 items, which is suitable for children aged 7-18 years old, was used to investigate the QoL of children[25]. QLSCA includes four factors and 13 domains, which have been introduced in our previous publication[25]. A four-point scale was used with the QLSCA, with a randomized bidirectional response (positive or negative) item by item to limit the bias of the response. Individual item values were recorded in the same direction prior to analysis.

Table 1 General characteristics of glycolipid metabolism study in adolescents (mean ± SD)

Variable	Male	Female	F	*P* value
Anthropometric measures				
Age, yr	11.21 ± 0.64	11.13 ± 0.68	7.55	0.006
Height, cm	151.52 ± 8.53	152.08 ± 7.30	2.41	0.121
Weight, kg	45.31 ± 11.87	43.46 ± 10.00	13.81	< 0.001
Waist circumference, cm	68.17 ± 11.07	63.76 ± 8.52	96.30	< 0.001
FBG, mmol/L	4.49 ± 0.45	4.42 ± 0.41	14.81	< 0.001
HbA1c, %	5.37 ± 0.20	5.37 ± 0.19	0.04	0.843
Insulin, pmol/L	82.91 ± 81.09	85.30 ± 70.01	0.48	0.486
Insulin resistance index	2.42 ± 2.65	2.41 ± 2.13	0.01	0.952
Creatinine, mmol/L	52.86 ± 10.60	53.96 ± 28.20	1.26	0.261
Uric acid, μmol/L	333.51 ± 83.13	305.13 ± 66.72	65.16	< 0.001
TG, mean, mmol/L	1.04 ± 0.52	1.09 ± 0.49	4.41	0.036
HDL-C, mmol/L	1.43 ± 0.32	1.43 ± 0.30	0.00	0.994
LDL-C, mmol/L	1.84 ± 0.43	1.84 ± 0.44	0.02	0.893
Physical activity, min/day	3.52 ± 0.62	3.52 ± 0.57	0.01	0.939
Sleep score	45.08 ± 5.85	45.97± 6.38	10.36	0.001
Dietary intake, g/day				
Cereals and potatoes	183.48 ± 173.6	160.72 ± 164.5	8.86	0.003
Vegetables	207.71 ± 197.7	216.41 ± 213.8	0.88	0.349
Red meat	159.26 ± 199.9	152.11 ± 204.2	0.61	0.434
Nutritional supplements	20.05 ± 32.04	19.29 ± 32.57	0.27	0.603
Increased BMI during pregnancy, kg/m2	1.82 ± 0.75	1.82 ± 0.75	0.05	0.829
13 domains of QoL				
Self-satisfaction	50.83 ± 11.09	49.33 ± 11.48	8.60	0.003
Relationship of teacher and pupil	53.62 ± 10.20	53.81 ± 9.64	0.19	0.665
Physical feeling	50.32 ± 10.53	49.60 ± 11.00	2.16	0.142
Companionship	54.34 ± 9.88	53.14 ± 10.96	6.49	0.011
Parenthood	52.13 ± 10.83	50.73 ± 11.76	7.43	0.007
Physical activity ability	50.11 ± 10.96	50.13 ± 10.23	0.01	0.966
Learning ability and attitude	52.34 ± 9.92	51.41 ± 10.33	4.14	0.042
Self-esteem	51.18 ± 11.20	49.75 ± 10.84	8.21	0.004
Negative emotion	48.23 ± 10.79	47.14 ± 11.57	4.61	0.032
Attitude towards doing homework	51.44 ± 9.23	51.59 ± 9.00	0.13	0.716
Activity opportunity	54.99 ± 9.40	54.39 ± 9.64	1.88	0.170
Living convenience	54.41 ± 7.88	54.54 ± 7.47	0.14	0.704
Other	50.99 ± 10.01	50.60 ± 10.13	0.71	0.401
Four factors of QoL				
Psychosocial factor	64.71 ± 10.26	65.25 ± 10.17	1.36	0.244
Physical and mental health factor	36.00 ± 6.02	35.77 ± 5.90	0.73	0.393
Living environment factor	24.36 ± 4.20	23.73 ± 4.28	10.58	0.001
Quality of life satisfaction factor	25.13 ± 4.31	24.72 ± 4.42	4.38	0.037

Mother's education, yr, *n* (%)				
Approximately 9	314 (31.15)	325 (34.17)	2.35	0.308
Approximately 12	360 (35.71)	315 (33.12)		
≥ 15	334 (33.13)	311 (32.70)		
Father's education, yr, *n* (%)				
Approximately 9	277 (27.48)	250 (26.29)	6.22	0.045
Approximately 12	338 (33.53)	369 (38.80)		
≥ 15	393 (38.99)	332 (34.91)		
Income, Yuan/year, *n* (%)				
Approximately 25000	155 (15.38)	141 (14.83)	2.74	0.741
Approximately 50000	166 (16.47)	168 (17.67)		
Approximately 100000	236 (23.41)	245 (25.76)		
Approximately 150000	190 (18.85)	163 (17.14)		
Approximately 200000	117 (11.61)	106 (11.15)		
> 200000	144 (14.29)	128 (13.46)		
Region				
Urban, *n* (%)	764 (75.79)	728 (76.55)	0.16	0.694
Rural, *n* (%)	244 (24.21)	223 (23.45)		

IR: Insulin resistance; FBG: Fasting blood glucose; HbA1c: Glycosylated haemoglobin; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; QoL: Quality of life; BMI: Body mass index.



DOI: 10.4239/wjd.v13.i7.566

Figure 1 Flow chart of participants analyzed in this study.

Response values were summed and normalized to the age-, sex-, and urban-rural-specific norms of Chinese individuals into a score ranging from 0-100 (normalized with a mean of 50 and SD of 10), whereby higher scores represent better QoL[26]. The domain scores and factor scores between males and females are displayed in Table 1.

Diagnostic criteria

Children were diagnosed with increased blood glucose if their FBG was ≥ 5.6 mmol/L[27]. Dyslipidaemia was indicated if any one of the following criteria were met[28]: (1) TC ≥ 200 mg/dL; (2) TG ≥ 130 mg/dL; (3) LDL-C ≥ 130 mg/dL; and (4) HDL-C ≤ 40 mg/dL. Moreover, IR was defined by homeostasis model assessment (HOMA)-IR > 3.0[29], which was calculated by the formula (FI mU/L) × (FBG mmol/L)/22.5. Maternal overweight/obesity was defined as a body mass index greater than 24 kg/m^2[30]. Maternal pregnancy weight gain was defined by the guidelines of the institute of medicine [31], as gaining 12.5-18.0 kg, 11.5-16.0 kg, 7.0-11.5 kg, and 5.0-9.0 kg for underweight, normal weight, overweight, and obesity, respectively.

Table 2 Effect of dimensions of quality of life on glycolipid metabolism disorder

Variable		FI		IR		TG		HDL	
		β (95%CI)	*P* value	β (95%CI)	*P* value	β (95%CI)	*P* value	β (95%CI)	*P* value
Model 1 (domains of QoL)	Self-satisfaction	-0.399 (-0.696, -0.102)	0.009	-0.011 (-0.021, -0.001)	0.028	-0.003 (-0.005, -0.001)	0.004	0.000 (-0.001, 0.002)	0.509
	Relationship of teacher and pupil	-0.272 (-0.607, 0.063)	0.112	-0.006 (-0.017, 0.005)	0.252	-0.004 (-0.006, -0.001)	0.002	0.001 (-0.000, 0.002)	0.177
	Activity opportunity	-0.452 (-0.761, -0.143)	0.004	-0.013 (-0.023, -0.003)	0.014	-0.003 (-0.005, -0.000)	0.017	0.001 (-0.000, 0.002)	0.098
	Physical activity ability	-0.608 (-0.920, -0.295)	< 0.001	-0.014 (-0.024, -0.004)	0.008	-0.004 (-0.007, -0.002)	< 0.001	0.002 (0.001, 0.003)	0.098
	Learning ability and attitude	-0.391 (-0.721, -0.061)	0.020	-0.010 (-0.021, 0.001)	0.075	-0.002 (-0.004, 0.000)	0.109	0.002 (0.001, 0.003)	0.002
	Attitude towards doing homework	-0.684 (-1.05, -0.319)	<0.001	-0.018 (-0.030, -0.006)	0.003	-0.005 (-0.008, -0.003)	< 0.001	0.002 (0.000, 0.003)	0.033
	Living convenience	-0.469 (-0.905, -0.034)	0.035	-0.016 (-0.030, -0.001)	0.030	-0.003 (-0.005, 0.000)	0.093	0.001 (-0.001, 0.003)	0.311
Model 2 (domains of QoL)	Self-satisfaction	-0.352 (-0.641, -0.063)	0.017	-0.009 (-0.019, 0.000)	0.054	-0.003 (-0.005, -0.001)	0.006	0.001 (-0.001, 0.002)	0.330
	Relationship of teacher and pupil	-0.327 (-0.646, -0.007)	0.045	-0.008 (-0.019, 0.002)	0.127	-0.003 (-0.006, -0.001)	0.003	0.001 (-0.000, 0.002)	0.203
	Activity opportunity	-0.421 (-0.719, -0.123)	0.006	-0.012 (-0.022, -0.002)	0.018	-0.003 (-0.005, -0.001)	0.011	0.001 (0.000, 0.003)	0.027
	Physical activity ability	-0.394 (-0.696, -0.091)	0.011	-0.008 (-0.018, 0.002)	0.113	-0.003 (-0.005, -0.001)	0.005	0.001 (-0.000, 0.002)	0.192
	Learning ability and attitude	-0.442 (-0.758, -0.126)	0.006	-0.011 (-0.021, -0.000)	0.046	-0.001 (-0.004, 0.001)	0.190	0.002 (0.001, 0.004)	0.001
	Attitude towards doing homework	-0.720 (-1.07, -0.373)	< 0.001	-0.019 (-0.031, -0.008)	0.001	-0.005 (-0.008, -0.003)	< 0.001	0.002 (0.001, 0.004)	0.005
	Living convenience	-0.413 (-0.825, -0.001)	0.049	-0.014 (-0.028, -0.000)	0.043	-0.002 (-0.005, 0.001)	0.127	0.001 (-0.001, 0.003)	0.186

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, height, weight, vegetable intaking, red meat intaking, salt intake, sleeping quality, father's education, mother's education, household income, urban-rural areas, maternal increased weight during pregnancy, and maternal obesity. FI: Fasting insulin; IR: Insulin resistance; TG: Triglyceride; HDL: High density lipoprotein cholesterol; QoL: Quality of life.

Statistical analysis

Continuous variables, such as insulin, HOMA-IR, and TG, which did not conform to a normal distribution, were subjected to natural logarithmic transformation before analyses. The relationship between QoL and GLMD was analysed with a generalized linear model. Two models were used to adjust for covariates: Model 1 adjusted for age and sex, and Model 2 adjusted for covariates of height, weight, vegetable intake, red meat intake, salt intake, sleeping quality, father's education, mother's education, household income, urban-rural areas, maternal increased weight during pregnancy, and maternal obesity, which may reflect the independent effect of QoL on blood glucose and lipid indexes. In addition, a logistic regression model was used to analyse the relationship between QoL and GLMD with two models to adjust for covariates.

The results were analysed with SAS 9.4 software (Copyright© 2020 SAS Institute Inc. Cary, NC, United States). An α level of 0.05 was defined as a significant difference.

RESULTS

General characteristics

The general characteristics of the subjects are presented in Table 1. A total of 1956 samples were included. The mean age was 11.21 ± 0.64 years, and 51.53% (1008/1956) were males. The 13 domains, four factors, and total score of QoL, biochemical indexes, and anthropometric, perinatal, and SES variables between males and females are shown in Table 1.

Table 3 Effect of four factors of quality of life on glycolipid metabolism disorder

Variable		FI		IR		TG		HDL	
		β (95%CI)	*P* value	β (95%CI)	*P* value	β (95%CI)	*P* value	β (95%CI)	*P* value
Model 1 (four factors of QoL)	Physical and mental health factor	-0.222 (-0.547, 0.104)	0.181	-0.005 (-0.016, 0.006)	0.364	-0.003 (-0.005, -0.000)	0.017	0.001 (-0.000, 0.003)	0.064
	Psychosocial factor	-0.835 (-1.390, -0.278)	0.003	-0.021 (-0.039, -0.002)	0.026	-0.006 (-0.010, -0.002)	0.002	0.002 (-0.000, 0.005)	0.052
	Living environment factor	-0.821 (-1.610, -0.036)	0.040	-0.014 (-0.039, 0.012)	0.298	-0.007 (-0.012, -0.002)	0.012	0.004 (0.001, 0.008)	0.008
	Quality of life satisfaction factor	-0.848 (-1.610, -0.082)	0.030	-0.023 (-0.048, 0.002)	0.074	-0.006 (-0.011, -0.001)	0.031	0.001 (-0.002, 0.004)	0.507
	Total score of QoL	-0.440 (-0.710, -0.169)	0.002	-0.012 (-0.020, -0.003)	0.011	-0.004 (-0.006, -0.002)	< 0.001	0.001 (0.000, 0.002)	0.040
Model 2 (four factors of QoL)	Physical and mental health factor	-0.272 (-0.588, 0.044)	0.092	-0.006 (-0.017, 0.004)	0.229	-0.002 (-0.005, -0.000)	0.039	0.001 (-0.000, 0.002)	0.122
	Psychosocial factor	-0.906 (-1.450, -0.367)	0.001	-0.023 (-0.041, -0.005)	0.011	-0.007 (-0.010, -0.003)	0.001	0.003 (0.001, 0.005)	0.008
	Living environment factor	-0.916 (-1.690, -0.143)	0.020	-0.018 (-0.044, 0.008)	0.172	-0.005 (-0.011, 0.000)	0.067	0.003 (-0.001, 0.006)	0.126
	Quality of life satisfaction factor	-0.965 (-1.710, -0.217)	0.012	-0.027 (-0.052, -0.002)	0.035	-0.006 (-0.011, -0.001)	0.027	0.002 (-0.001, 0.005)	0.239
	Total score of QoL	-0.441 (-0.705, -0.177)	0.001	-0.011 (-0.020, -0.003)	0.010	-0.004 (-0.006, -0.002)	0.001	0.001 (0.000, 0.002)	0.021

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, height, weight, vegetable intaking, red meat intaking, salt intake, sleeping quality, father's education, mother's education, household income, urban-rural areas, maternal increased weight during pregnancy, and maternal obesity. FI: Fasting insulin; IR: Insulin resistance; TG: Triglyceride; HDL: High density lipoprotein cholesterol; QoL: Quality of life.

Association between QoL and glycolipid indexes

Tables 2 and 3 display the effect of QoL on glycolipid metabolism in adolescents. Adolescents with higher domain scores of living convenience had lower FBG than their counterparts ($P < 0.05$). TG and HDL-C were higher in adolescents who had a negative attitude towards doing homework ($P < 0.05$), and the impact of living convenience and attitude towards doing homework on glycolipid indexes (TG and HDL-C) was also significant after adjusting for multiple factors. However, the impact of QoL factors on LDL-C and TC was not significant ($P > 0.05$) (Supplementary Tables 1 and 2). Levels of insulin and IR were lower in adolescents with a higher factor score of psychosocial, living environment, and QoL satisfaction than their counterparts ($P < 0.05$). Moreover, adolescents with higher psychosocial factor scores and total QoL scores had decreased TGs and increased HDL-C compared with their counterparts after adjusting for covariates ($P < 0.05$).

The effect of QoL on glycolipid metabolism indexes by sex is shown in Supplementary Tables. The results in Supplementary Tables 3 and 4 illustrate the relationship between QoL and indexes (FI, IR, TG, and HDL). Scores of attitude towards doing homework and living convenience were negative for FI, IR, and TG ($P < 0.05$ or $P < 0.001$), and the association of attitude towards doing homework with IR/TG was also significant after adjusting for covariates in Model 2 ($P < 0.05$). Moreover, the relationship between total QoL score and FI/TG was negative ($P < 0.05$). Higher scores of activity opportunity, physical activity ability, learning ability, and attitude and lower levels of FI, TG, and HDL were found for females ($P < 0.05$); in Model 2, the score of attitude towards doing homework correlated negatively with IR level ($P = 0.018$).

The results showed that the total score of QoL was a negative factor for FI [β (95%CI): -0.441 (-0.705, -0.177)], IR [β (95%CI): -0.011 (-0.020, -0.003)], and TG [β (95%CI): -0.004 (-0.006, -0.002)] but a positive factor for HDL [β (95%CI): 0.001 (0.000, 0.002)].

In addition, the association between the four factors of QoL and the prevalence of glycolipid metabolism indexes by sex is shown in Supplementary Tables 5 and 6. The relationship between the living convenience score and FBG was negative ($P < 0.05$). However, significant relationships for females were only found in Model 1.

Association between QoL and GLMD prevalence

The results in Tables 4 and 5 indicated the relationship between QoL and GLMD in adolescents. The attitude towards doing homework domain score was a protective factor for dyslipidaemia [OR (95%CI): 0.984 (0.972, 0.995); $P = 0.004$], and the relationship was significant even after adjusting for covariates

Table 4 Logistic regression analysis of dimensions of quality of life and glycolipid metabolism disorder

Variable		Dyslipidemia		IR > 3		Increased FBG	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Total							
Model 1 (domains of QoL)	Self-satisfaction	0.994 (0.984, 1.003)	0.177	0.987 (0.978, 0.997)	0.009	1.009 (0.991, 1.027)	0.344
	Relationship of teacher and pupil	0.991 (0.981, 1.002)	0.095	0.987 (0.977, 0.998)	0.025	1.012 (0.991, 1.032)	0.264
	Activity opportunity	0.989 (0.980, 0.999)	0.031	0.988 (0.978, 0.998)	0.023	1.006 (0.988, 1.025)	0.527
	Physical activity ability	0.988 (0.978, 0.998)	0.020	0.981 (0.971, 0.992)	0.001	1.013 (0.994, 1.031)	0.184
	Learning ability and attitude	0.990 (0.979, 1.000)	0.057	0.989 (0.978, 1.000)	0.047	1.003 (0.984, 1.023)	0.736
	Attitude towards doing homework	0.984 (0.972, 0.995)	0.004	0.986 (0.974, 0.998)	0.026	0.978 (0.959, 0.997)	0.022
	Living convenience	0.997(0.984,1.011)	0.712	0.990(0.976,1.004)	0.158	0.993(0.970,1.018)	0.587
Model 2 (domains of QoL)	Self-satisfaction	0.993 (0.984, 1.003)	0.183	0.986 (0.975, 0.996)	0.007	1.008 (0.989, 1.026)	0.408
	Relationship of teacher and pupil	0.992 (0.981, 1.003)	0.154	0.984 (0.972, 0.996)	0.008	1.007 (0.987, 1.029)	0.480
	Activity opportunity	0.988 (0.978, 0.998)	0.017	0.986 (0.975, 0.997)	0.016	1.007 (0.988, 1.026)	0.497
	Physical activity ability	0.994 (0.984, 1.005)	0.268	0.985 (0.973, 0.997)	0.012	1.013 (0.994, 1.033)	0.184
	Learning ability and attitude	0.990 (0.979, 1.001)	0.072	0.986 (0.974, 0.998)	0.018	1.003 (0.983, 1.023)	0.770
	Attitude towards doing homework	0.982 (0.970, 0.994)	0.003	0.983 (0.971, 0.996)	0.012	0.978 (0.958, 0.997)	0.024
	Living convenience	0.997 (0.983, 1.011)	0.685	0.990 (0.975, 1.005)	0.195	0.996 (0.972, 1.021)	0.765
Male							
Model 1 (domains of QoL)	Self-satisfaction	0.996 (0.983, 1.009)	0.538	0.990 (0.977, 1.004)	0.173	1.001 (0.980, 1.023)	0.927
	Relationship of teacher and pupil	0.998 (0.983, 1.012)	0.759	0.994 (0.978, 1.009)	0.414	1.003 (0.979, 1.027)	0.836
	Activity opportunity	0.995 (0.981, 1.009)	0.478	0.993 (0.978, 1.008)	0.370	0.999 (0.977, 1.022)	0.942
	Physical activity ability	0.993 (0.980, 1.007)	0.341	0.984 (0.970, 0.999)	0.037	1.006 (0.984, 1.028)	0.623
	Learning ability and attitude	0.996 (0.981, 1.011)	0.597	0.999 (0.983, 1.016)	0.915	0.992 (0.969, 1.016)	0.503
	Attitude towards doing homework	0.983 (0.968, 0.998)	0.031	0.996 (0.979, 1.013)	0.636	0.980 (0.957, 1.004)	0.102
	Living convenience	1.008 (0.988, 1.027)	0.444	0.988 (0.968, 1.008)	0.229	0.982 (0.955, 1.010)	0.211
Model 2 (domains of QoL)	Self-satisfaction	0.996 (0.982, 1.010)	0.570	0.991 (0.976, 1.006)	0.241	1.003 (0.981, 1.026)	0.766
	Relationship of teacher and pupil	0.999 (0.984, 1.014)	0.888	0.991 (0.975, 1.008)	0.313	1.000 (0.976, 1.025)	0.982
	Activity opportunity	0.993 (0.978, 1.008)	0.365	0.992 (0.976, 1.008)	0.332	1.004 (0.981, 1.027)	0.762
	Physical activity ability	1.002 (0.988, 1.017)	0.745	0.991 (0.975, 1.007)	0.282	1.009 (0.986, 1.033)	0.438
	Learning ability and attitude	0.996 (0.981, 1.012)	0.645	0.998 (0.980, 1.015)	0.793	0.994 (0.970, 1.018)	0.604
	Attitude towards doing homework	0.980 (0.964, 0.996)	0.017	0.996 (0.977, 1.014)	0.650	0.983 (0.959, 1.008)	0.180
	Living convenience	1.013 (0.992, 1.034)	0.222	0.994 (0.973, 1.016)	0.603	0.987 (0.958, 1.016)	0.363
Female							

Model 1 (domains of QoL)	Self-satisfaction	0.991 (0.978, 1.004)	0.195	0.985 (0.972, 0.998)	0.022	1.024 (0.992, 1.056)	0.146
	Relationship of teacher and pupil	0.983 (0.968, 0.999)	0.035	0.980 (0.965, 0.996)	0.012	1.029 (0.991, 1.068)	0.139
	Activity opportunity	0.984 (0.971, 0.998)	0.021	0.984 (0.970, 0.998)	0.021	1.017 (0.986, 1.050)	0.292
	Physical activity ability	0.982 (0.967, 0.997)	0.016	0.977 (0.962, 0.993)	0.004	1.025 (0.993, 1.058)	0.133
	Learning ability and attitude	0.984 (0.969, 0.999)	0.032	0.980 (0.965, 0.995)	0.009	1.023 (0.991, 1.057)	0.164
	Attitude towards doing homework	0.984 (0.968, 1.001)	0.062	0.977 (0.960, 0.994)	0.007	0.972 (0.942, 1.004)	0.084
	Living convenience	0.986 (0.967, 1.006)	0.174	0.991 (0.971, 1.012)	0.387	1.018 (0.972, 1.067)	0.448
Model 2 (domains of QoL)	Self-satisfaction	0.991 (0.978, 1.005)	0.230	0.981 (0.966, 0.996)	0.011	1.014 (0.981, 1.049)	0.404
	Relationship of teacher and pupil	0.985 (0.969, 1.001)	0.060	0.975 (0.958, 0.992)	0.005	1.017 (0.977, 1.058)	0.420
	Activity opportunity	0.984 (0.970, 0.998)	0.026	0.982 (0.967, 0.997)	0.022	1.009 (0.977, 1.043)	0.573
	Physical activity ability	0.986 (0.971, 1.002)	0.097	0.976 (0.959, 0.994)	0.007	1.019 (0.983, 1.056)	0.307
	Learning ability and attitude	0.984 (0.969, 1.000)	0.046	0.974 (0.958, 0.991)	0.003	1.016 (0.982, 1.051)	0.362
	Attitude towards doing homework	0.983 (0.966, 1.000)	0.052	0.972 (0.954, 0.991)	0.003	0.965 (0.934, 0.997)	0.031
	Living convenience	0.983 (0.963, 1.004)	0.106	0.984 (0.963, 1.007)	0.167	1.013 (0.964, 1.064)	0.613

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, height, weight, vegetable intaking, red meat intaking, salt intake, sleeping quality, father's education, mother's education, household income, urban-rural areas, maternal increased weight during pregnancy, and maternal obesity. IR: Insulin resistance; FBG: Fasting blood glucose; QoL: Quality of life.

[OR (95%CI): 0.982 (0.970, 0.994); P = 0.003]. This relationship was also statistically significant in males [OR (95%CI): 0.983 (0.968, 0.998); P = 0.031]. However, among females, there were other factors of significance, such as relationship of teacher and pupil [OR (95%CI): 0.985 (0.969, 1.001); P = 0.060], activity opportunity [OR (95%CI): 0.984 (0.970, 0.998); P = 0.026], and learning ability and attitude [OR (95%CI): 0.984 (0.969, 1.000); P = 0.046]. After adjusting for covariates in Model 1 and Model 2, self-satisfaction, the relationship of teacher and pupil, activity opportunity, physical activity ability, learning ability and attitude, and attitude towards doing homework were protective factors for IR > 3 in all participants and in females, but only the physical activity ability domain score was significant in males [OR (95%CI): 0.984 (0.970, 0.999); P = 0.037] in Model 1. Attitude towards the homework domain was a protective factor for FBG in Model 2 for all subjects and females (P < 0.05) (Table 4).

The relationship between the four factors of QoL and the prevalence of GLMD was not significant in males (P > 0.05). Psychosocial factor [OR (95%CI): 0.976 (0.959, 0.994); P = 0.008] [OR (95%CI): 0.980 (0.962, 0.998); P = 0.034] and total score of QoL [OR (95%CI): 0.990 (0.982, 0.999); P = 0.025] [OR (95%CI): 0.988 (0.979, 0.997); P = 0.007] were protective factors for dyslipidaemia and IR > 3, respectively, with statistical significance in the total cohort in Model 1. In addition, higher score of psychosocial factor [OR (95%CI): 0.971 (0.946, 0.996); P = 0.024], living environment factor [OR (95%CI): 0.952 (0.919, 0.987); P = 0.008], and total score of QoL [OR (95%CI): 0.985 (0.973, 0.997); P = 0.014] in females was related to a lower prevalence of dyslipidaemia in Model 2. In Model 2, adjusted for covariates, all factors of QoL were protective factors for IR > 3 (P < 0.05) (Table 5).

DISCUSSION

The association between QoL and the prevalence of GLMD was illustrated using a large-sample-size childhood health cohort study. By adjusting for multiple covariates that may correlate with glycolipid indexes, the independent relationship between QoL and GLMD was demonstrated. In addition, more domains of QoL correlated with glycolipid indexes in females.

Our study revealed that QoL scores mainly correlate negatively with the prevalence of GLMD in adolescents. Research on the relationship between QoL and GLMD in the healthy population is limited. According to a previous cross-sectional study that included 74 diabetic adolescents[32], no significant relationship between QoL and HbA1c levels was observed. However, a cross-sectional study found that QoL scores correlated with an increase in the components of MS, with the physical health domain of

Table 5 Logistic regression analysis of four factors of quality of life and glycolipid metabolism disorder

Variables		Dyslipidemia		IR		Increased FBG	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Total							
Model 1 (four factors of QoL)	Physical and mental health factor	0.992 (0.982, 1.002)	0.120	0.991 (0.980, 1.002)	0.113	1.020 (1.000, 1.041)	0.049
	Psychosocial factor	0.976 (0.959, 0.994)	0.008	0.980 (0.962, 0.998)	0.034	0.995 (0.964, 1.027)	0.767
	Living environment factor	0.977 (0.952, 1.001)	0.065	0.979 (0.954, 1.006)	0.121	1.077 (1.026, 1.130)	0.003
	Quality of life satisfaction factor	0.992 (0.968, 1.017)	0.531	0.972 (0.948, 0.997)	0.028	1.033 (0.986, 1.082)	0.168
	Total score of QoL	0.990 (0.982, 0.999)	0.025	0.988 (0.979, 0.997)	0.007	1.009 (0.993, 1.025)	0.267
Model 2 (four factors of QoL)	Physical and mental health factor	0.994 (0.983, 1.005)	0.296	0.988 (0.976, 1.000)	0.047	1.015 (0.994, 1.036)	0.154
	Psychosocial factor	0.973 (0.953, 0.993)	0.010	0.973 (0.953, 0.993)	0.010	0.992 (0.960, 1.025)	0.620
	Living environment factor	0.988 (0.962, 1.015)	0.387	0.971 (0.942, 1.000)	0.053	1.060 (1.008, 1.115)	0.024
	Quality of life satisfaction factor	0.992 (0.966, 1.017)	0.520	0.961 (0.934, 0.988)	0.005	1.024 (0.977, 1.074)	0.320
	Total score of QoL	0.991 (0.982, 1.000)	0.043	0.985 (0.976, 0.995)	0.004	1.009 (0.992, 1.026)	0.317
Male							
Model 1 (four factors of QoL)	Physical and mental health factor	0.999 (0.985, 1.014)	0.899	0.999 (0.983, 1.015)	0.913	1.016 (0.992, 1.042)	0.196
	Psychosocial factor	0.981 (0.957, 1.005)	0.124	0.998 (0.972, 1.025)	0.889	0.982 (0.945, 1.021)	0.355
	Living environment factor	1.001 (0.966, 1.038)	0.935	0.987 (0.950, 1.026)	0.514	1.060 (0.998, 1.125)	0.057
	Quality of life satisfaction factor	1.002 (0.968, 1.037)	0.916	0.986 (0.950, 1.023)	0.459	1.018 (0.962, 1.077)	0.537
	Total score of QoL	0.995 (0.983, 1.008)	0.470	0.995 (0.982, 1.009)	0.482	1.003 (0.983, 1.023)	0.799
Model 2 (four factors of QoL)	Physical and mental health factor	1.002 (0.986, 1.017)	0.824	0.999 (0.982, 1.016)	0.895	1.013 (0.988, 1.039)	0.301
	Psychosocial factor	0.994 (0.965, 1.024)	0.686	0.994 (0.965, 1.024)	0.686	0.983 (0.945, 1.024)	0.414
	Living environment factor	1.021 (0.982, 1.062)	0.299	0.989 (0.947, 1.033)	0.633	1.047 (0.983, 1.114)	0.154
	Quality of life satisfaction factor	1.001 (0.964, 1.039)	0.969	0.982 (0.943, 1.022)	0.374	1.016 (0.959, 1.077)	0.580
	Total score of QoL	0.997 (0.984, 1.010)	0.645	0.996 (0.982, 1.011)	0.601	1.005 (0.984, 1.026)	0.631
Female							
Model 1 (four factors of QoL)	Physical and mental health factor	0.984 (0.970, 0.999)	0.037	0.983 (0.968, 0.998)	0.026	1.025 (0.990, 1.061)	0.160
	Psychosocial factor	0.971 (0.946, 0.996)	0.024	0.962 (0.936, 0.988)	0.004	1.020 (0.965, 1.079)	0.477
	Living environment factor	0.952 (0.919, 0.987)	0.008	0.970 (0.935, 1.006)	0.105	1.102 (1.016, 1.195)	0.019
	Quality of life satisfaction factor	0.983 (0.950, 1.017)	0.329	0.958 (0.925, 0.992)	0.016	1.056 (0.976, 1.143)	0.173

	Total score of QoL	0.985 (0.973, 0.997)	0.014	0.980 (0.968, 0.993)	0.002	1.020 (0.992, 1.049)	0.156
Model 2 (four factors of QoL)	Physical and mental health factor	0.987 (0.971, 1.003)	0.103	0.976 (0.960, 0.993)	0.007	1.011 (0.974, 1.049)	0.565
	Psychosocial factor	0.956 (0.929, 0.984)	0.003	0.956 (0.929, 0.984)	0.003	1.006 (0.950, 1.065)	0.846
	Living environment factor	0.962 (0.925, 1.000)	0.048	0.951 (0.912, 0.992)	0.019	1.072 (0.982, 1.171)	0.120
	Quality of life satisfaction factor	0.984 (0.948, 1.021)	0.393	0.941 (0.905, 0.978)	0.002	1.025 (0.942, 1.115)	0.568
	Total score of QoL	0.985 (0.972, 0.998)	0.024	0.975 (0.961, 0.989)	0.001	1.012 (0.982, 1.043)	0.445

Model 1: Adjusted for age and sex. >Model 2: Adjusted for age, sex, height, weight, vegetable intaking, red meat intaking, salt intake, sleeping quality, father's education, mother's education, household income, urban-rural areas, maternal increased weight during pregnancy, and maternal obesity. IR: Insulin resistance; FBG: Fasting blood glucose; QoL: Quality of life.

QoL having the most significant association[33]. In our study, six domains, four factors, and the total QoL score were correlated significantly negatively with glycolipid indexes, and the effect was independent of obesity. To our knowledge, this is the first cohort study with a large sample size to explore the relationship of QoL with GLMD in adolescents.

The association between QoL and GLMD may be impacted by sex. Scores on several domains of QoL are reportedly lower in females than in males[34-37]. Longitudinal studies have shown a significant relationship between weight and QoL only in females[38]. However, one study found that females and males have similar psychological characteristics[39]. Overall, numerous studies have detected significant sex differences in awareness and mental health[40,41]. For instance, in terms of personality, males score higher than females in self-acceptance and autonomy, whereas females score higher than males in personal growth and positive relationships with others[42]. Our study adds more evidence about the sex difference in the association between QoL and GLMD; overall, more domains of QoL correlated with GLMD in females.

Several mechanisms may explain why QoL may impact GLMD. Physical and psychological health and social well-being are encompassed in HRQoL[43]. Previous study results show that an increase in total HbA1c is related to a decrease in QoL[44]. In addition, research has found that better QoL is associated with better healthy dietary patterns and behaviours in children and adolescents[45]. Irrational diets may induce FBG increases. For example, a high-fat diet induces IR, triggering accumulation of diacylglycerol and ceramide levels in the liver and inhibiting the insulin signalling pathway [46]. Some studies have suggested that physical activity and mental health are positively associated with QoL[44]; it is well known that exercise enhances insulin signalling independent of PI3K and that glucose transport and GLUT4 translocation are enhanced as skeletal muscle contraction is stimulated by insulin [47]. Similarly, better education may lead to greater confidence, a sense of security, and building better relationships with others, contributing to mental health[48]. The mechanisms through which IR influences emotional regulation are being revealed by animal and human studies, and the brain requires glucose as an essential energy source[49,50].

In conclusion, GLMD prevalence and high glycolipid levels are elevated in adolescents with low QoL scores. To our knowledge, this is the first study to explore the relationship of QoL with glycolipid indexes from a large-sample-size cohort study of adolescents, and the correlation was significant after adjusting for multiple covariates. Our study emphasizes the importance of improving QoL in children and adolescents and provides scientific evidence for educational institutions to improve the educational model to enhance the QoL of school-age children. However, our study illustrates the relationship between QoL and glycolipid indexes from a nearly cross-sectional perspective, and a further well-designed large-sample-size cohort study or randomized controlled trial study should be conducted to examine the causal relationships.

CONCLUSION

Our study reveals that QoL scores mainly correlate negatively with the prevalence of GLMD in adolescents of the healthy population. The independent relationship between QoL and GLMD can be illustrated by adjusting for multiple covariates that may be associated with glycaemic index. In addition, more QoL domains are associated with glycaemic index in females.

ARTICLE HIGHLIGHTS

Research background

The prevalence of glucolipid metabolic disorders (GLMDs) in children and adolescents has a recognized association with cardiovascular diseases and type 2 diabetes mellitus in adulthood. Therefore, it is important to increase our understanding of the risk factors for GLMD in childhood and adolescence.

Research motivation

Quality of life (QoL) includes multidimensional terms, which represent satisfaction with life status and describe a subject's functioning in physical, emotional, and social domains. Little evidence about the relationship between QoL and GLMD has been reported, especially in children and adolescents, which is an important stage of growth.

Research objectives

The aim of this cohort study was to explore the correlation of QoL scores and personality traits with GLMD in adolescents, providing an excellent opportunity to identify independent risk factors for GLMD after adjusting for multiple variables, such as perinatal variables, socioeconomic status, anthropometric measures, and other biochemical indexes.

Research methods

Two-stage stratified cluster sampling was used to select children from urban and rural areas of Chongqing; two regions *per* county were randomly chosen; and finally, all children living in the selected region were informed and included if they met the inclusion criteria.

Research results

Our study revealed that QoL scores mainly correlate negatively with the prevalence of GLMD in adolescents.

Research conclusions

The prevalence of GLMD and high glycolipid levels are increased in adolescents with features of low QoL scores. Our study adds more evidence about sex difference in the association between QoL and GLMD, and more domains of QoL correlate with GLMD in females.

Research perspectives

Our study illustrates the relationship between QoL and glycolipid indexes from a nearly cross-sectional perspective, and a further well-designed cohort study with a large sample size or randomized controlled trial should be conducted to explore the causal relationships.

FOOTNOTES

Author contributions: Liang XH conceived of and designed the study; Qu P and Chen JY participated in the acquisition of the data; Liang XH analysed the data; Liang XH, Ren YL, and Liang XY drafted and revised the manuscript; all authors critically reviewed and approved the final paper.

Supported by Intelligent Medicine Research Project of Chongqing Medical University, No. ZHYX202109; The Major Health Project of Chongqing Science and Technology Bureau, No. CSTC2021jscx-gksb-N0001; Research and Innovation Team of Chongqing Medical University, No. W0088; Joint Medical Research Project of Chongqing Municipal Health Commission and Chongqing Science and Technology Bureau, No. 2020MSXM062; National Key Research and Development Project of the Ministry of Science and Technology of the People's Republic of China, No. 2017YFC0211705; and Young Scientists Fund Program of the National Natural Science Foundation of China, No. 81502826.

Institutional review board statement: The study was reviewed and approved by the Chongqing Jiulongpo District Center for Disease Control and Prevention Institutional Review Board, No. 2013-006.

Clinical trial registration statement: This study is registered at ChiCTR. The registration identification number is ChiCTR2100048861.

Informed consent statement: Informed consent was obtained from all patients for inclusion in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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

Data sharing statement: Anonymised participant data will be shared after approval by the corresponding author, following a reasonable submitted request. Data are available from Xiao-Hua Liang (Clinical Epidemiology and Biostatistics Department, Children's Hospital of Chongqing Medical University, No.136 2nd Zhongshan Road, Yuzhong District, Chongqing, China, 400016; Email: xiaohualiang@hospital.cqmu.edu.cn or liangxiaohua666@sina.com).

CONSORT 2010 statement: The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

Country/Territory of origin: China

ORCID number: Xiao-Hua Liang 0000-0003-3867-9779; Yang-Ling Ren 0000-0002-2684-5066; Xiao-Yue Liang 0000-0001-8774-5010; Jing-Yu Chen 0000-0002-0712-7974; Ping Qu 0000-0002-6728-746X; Xian Tang 0000-0002-1141-0844.

S-Editor: Fan JR
L-Editor: Wang TQ
P-Editor: Fan JR

REFERENCES

1 **Koskinen JS**, Kytö V, Juonala M, Viikari JSA, Nevalainen J, Kähönen M, Lehtimäki T, Hutri-Kähönen N, Laitinen T, Tossavainen P, Jokinen E, Magnussen CG, Raitakari OT. Childhood risk factors and carotid atherosclerotic plaque in adulthood: The Cardiovascular Risk in Young Finns Study. *Atherosclerosis* 2020; **293**: 18-25 [PMID: 31830725 DOI: 10.1016/j.atherosclerosis.2019.11.029]

2 **Lorzadeh E**, Sangsefidi ZS, Mirzaei M, Hosseinzadeh M. Dietary Habits and their Association with Metabolic Syndrome in a sample of Iranian adults: A population-based study. *Food Sci Nutr* 2020; **8**: 6217-6225 [PMID: 33282272 DOI: 10.1002/fsn3.1918]

3 **Brown AE**, Walker M. Genetics of Insulin Resistance and the Metabolic Syndrome. *Curr Cardiol Rep* 2016; **18**: 75 [PMID: 27312935 DOI: 10.1007/s11886-016-0755-4]

4 **Zhu X**, Chen D, Xiu M, Li S, Zhang XY. Serum BDNF levels, glycolipid metabolism in deficit schizophrenia: A case-control study. *Asian J Psychiatr* 2022; **69**: 103003 [PMID: 34999534 DOI: 10.1016/j.ajp.2022.103003]

5 **Scholtens DM**, Metzger BE. Response to Comment on Scholtens *et al*. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal Glycemia and Childhood Glucose Metabolism. Diabetes Care 2019;42:381-392. *Diabetes Care* 2019; **42**: e128-e129 [PMID: 31221713 DOI: 10.2337/dci19-0024]

6 **Schlotz W**, Ambery P, Syddall HE, Crozier SR, Sayer AA, Cooper C, Phillips DI; Hertfordshire Cohort Study Group. Specific associations of insulin resistance with impaired health-related quality of life in the Hertfordshire Cohort Study. *Qual Life Res* 2007; **16**: 429-436 [PMID: 17091361 DOI: 10.1007/s11136-006-9129-5]

7 **Kazukauskiene N**, Podlipskyte A, Varoneckas G, Mickuviene N. Health-related quality of life and insulin resistance over a 10-year follow-up. *Sci Rep* 2021; **11**: 24294 [PMID: 34934126 DOI: 10.1038/s41598-021-03791-x]

8 **Leach MJ**, Kumar S. Cinnamon for diabetes mellitus. *Cochrane Database Syst Rev* 2012; CD007170 [PMID: 22972104 DOI: 10.1002/14651858.CD007170.pub2]

9 **Hemmingsen B**, Gimenez-Perez G, Mauricio D, Roqué I Figuls M, Metzendorf MI, Richter B. Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2017; **12**: CD003054 [PMID: 29205264 DOI: 10.1002/14651858.CD003054.pub4]

10 **Tajaddini A**, Roshanravan N, Mobasseri M, Aeinehchi A, Sefid-Mooye Azar P, Hadi A, Ostadrahimi A. Saffron improves life and sleep quality, glycaemic status, lipid profile and liver function in diabetic patients: A double-blind, placebo-controlled, randomised clinical trial. *Int J Clin Pract* 2021; **75**: e14334 [PMID: 33960081 DOI: 10.1111/ijcp.14334]

11 **Cunningham AT**, Crittendon DR, White N, Mills GD, Diaz V, LaNoue MD. The effect of diabetes self-management education on HbA1c and quality of life in African-Americans: a systematic review and meta-analysis. *BMC Health Serv Res* 2018; **18**: 367 [PMID: 29769078 DOI: 10.1186/s12913-018-3186-7]

12 **Taylor F**, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, Ward K, Ebrahim S. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013; CD004816 [PMID: 23440795 DOI: 10.1002/14651858.CD004816.pub5]

13 **Lindholm LH**, Ekbom T, Dash C, Isacsson A, Scherstén B. Changes in cardiovascular risk factors by combined pharmacological and nonpharmacological strategies: the main results of the CELL Study. *J Intern Med* 1996; **240**: 13-22 [PMID: 8708586 DOI: 10.1046/j.1365-2796.1996.492831000.x]

14 **Florez H**, Pan Q, Ackermann RT, Marrero DG, Barrett-Connor E, Delahanty L, Kriska A, Saudek CD, Goldberg RB, Rubin RR; Diabetes Prevention Program Research Group. Impact of lifestyle intervention and metformin on health-related

quality of life: the diabetes prevention program randomized trial. *J Gen Intern Med* 2012; **27**: 1594-1601 [PMID: 22692637 DOI: 10.1007/s11606-012-2122-5]

15 **Nasiri Lari Z**, Hajimonfarednejad M, Riasatian M, Abolhassanzadeh Z, Iraji A, Vojoud M, Heydari M, Shams M. Efficacy of inhaled Lavandula angustifolia Mill. Essential oil on sleep quality, quality of life and metabolic control in patients with diabetes mellitus type II and insomnia. *J Ethnopharmacol* 2020; **251**: 112560 [PMID: 31931160 DOI: 10.1016/j.jep.2020.112560]

16 **Cochran J**, Conn VS. Meta-analysis of quality of life outcomes following diabetes self-management training. *Diabetes Educ* 2008; **34**: 815-823 [PMID: 18832286 DOI: 10.1177/0145721708323640]

17 **Peña-Purcell NC**, Jiang L, Ory MG, Hollingsworth R. Translating an evidence-based diabetes education approach into rural african-american communities: the "wisdom, power, control" program. *Diabetes Spectr* 2015; **28**: 106-115 [PMID: 25987809 DOI: 10.2337/diaspect.28.2.106]

18 **Tong J**, Ren Y, Liu F, Liang F, Tang X, Huang D, An X, Liang X. The Impact of PM2.5 on the Growth Curves of Children's Obesity Indexes: A Prospective Cohort Study. *Front Public Health* 2022; **10**: 843622 [PMID: 35392463 DOI: 10.3389/fpubh.2022.843622]

19 **Wang Q**, Qu P, Chen J, Tang X, Hao G, Liang X. Associations Between Physical Activity and Hypertension in Chinese Children: A Cross-Sectional Study From Chongqing. *Front Med (Lausanne)* 2021; **8**: 771902 [PMID: 34977073 DOI: 10.3389/fmed.2021.771902]

20 **Liang X**, Chen J, An X, Liu F, Liang F, Tang X, Qu P. The impact of PM2.5 on children's blood pressure growth curves: A prospective cohort study. *Environ Int* 2022; **158**: 107012 [PMID: 34991268 DOI: 10.1016/j.envint.2021.107012]

21 **Liang X**, Xiao L, Luo Y, Xu J. Prevalence and risk factors of childhood hypertension from birth through childhood: a retrospective cohort study. *J Hum Hypertens* 2020; **34**: 151-164 [PMID: 31666662 DOI: 10.1038/s41371-019-0282-z]

22 **Liang X**, Tang X, Xi B, Qu P, Ren Y, Hao G. Abdominal obesity-related lipid metabolites may mediate the association between obesity and glucose dysregulation. *Pediatr Res* 2022 [PMID: 35437306 DOI: 10.1038/s41390-022-02074-z]

23 **Liang X**, Hao G, Xiao L, Luo S, Zhang G, Tang X, Qu P, Li R. Association Between Extraversion Personality With the Blood Pressure Level in Adolescents. *Front Cardiovasc Med* 2022; **9**: 711474 [PMID: 35310998 DOI: 10.3389/fcvm.2022.711474]

24 **Liang X**, Chen M, Wang D, Wen J, Chen J. Vitamin A deficiency indicating as low expression of LRAT may be a novel biomarker of primary hypertension. *Clin Exp Hypertens* 2021; **43**: 151-163 [PMID: 33052059 DOI: 10.1080/10641963.2020.1833023]

25 **Liang X**, Zhang P, Luo S, Zhang G, Tang X, Liu L. The association of quality of life and personality characteristics with adolescent metabolic syndrome: a cohort study. *Health Qual Life Outcomes* 2021; **19**: 160 [PMID: 34103067 DOI: 10.1186/s12955-021-01797-7]

26 **Wu HR**, Liu PL, Meng H. Norm, Reliability and Validity of Children and Adolescents' QOL scale. *Zhongguo Xuexiao Weisheng* 2006; **27**: 4 [DOI: 10.1002/nur.20045]

27 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; **36** Suppl 1: S67-S74 [PMID: 23264425 DOI: 10.2337/dc13-S067]

28 **Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents**; National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* 2011; **128** Suppl 5: S213-S256 [PMID: 22084329 DOI: 10.1542/peds.2009-2107C]

29 **Yin J**, Li M, Xu L, Wang Y, Cheng H, Zhao X, Mi J. Insulin resistance determined by Homeostasis Model Assessment (HOMA) and associations with metabolic syndrome among Chinese children and teenagers. *Diabetol Metab Syndr* 2013; **5**: 71 [PMID: 24228769 DOI: 10.1186/1758-5996-5-71]

30 **Hu L**, Huang X, You C, Li J, Hong K, Li P, Wu Y, Wu Q, Wang Z, Gao R, Bao H, Cheng X. Prevalence of overweight, obesity, abdominal obesity and obesity-related risk factors in southern China. *PLoS One* 2017; **12**: e0183934 [PMID: 28910301 DOI: 10.1371/journal.pone.0183934]

31 **Johnson J**, Clifton RG, Roberts JM, Myatt L, Hauth JC, Spong CY, Varner MW, Wapner RJ, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Samuels P, Sciscione A, Harper M, Tolosa JE, Saade G, Sorokin Y; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network*. Pregnancy outcomes with weight gain above or below the 2009 Institute of Medicine guidelines. *Obstet Gynecol* 2013; **121**: 969-975 [PMID: 23635732 DOI: 10.1097/AOG.0b013e31828aea03]

32 **Afshar M**, Mohammadi MR. Investigating the relationship between quality of life, self-care capability and HbA1c level in diabetic adolescents. *J Kashan Univer Med Sci* 2014; **18**: 8 [DOI: 10.5812/ircmj.21110]

33 **Amiri P**, Deihim T, Taherian R, Karimi M, Gharibzadeh S, Asghari-Jafarabadi M, Shiva N, Azizi F. Factors Affecting Gender Differences in the Association between Health-Related Quality of Life and Metabolic Syndrome Components: Tehran Lipid and Glucose Study. *PLoS One* 2015; **10**: e0143167 [PMID: 26625120 DOI: 10.1371/journal.pone.0143167]

34 **Swanson SA**, Crow SJ, Le Grange D, Swendsen J, Merikangas KR. Prevalence and correlates of eating disorders in adolescents. Results from the national comorbidity survey replication adolescent supplement. *Arch Gen Psychiatry* 2011; **68**: 714-723 [PMID: 21383252 DOI: 10.1001/archgenpsychiatry.2011.22]

35 **Mond JM**, Hay PJ. Functional impairment associated with bulimic behaviors in a community sample of men and women. *Int J Eat Disord* 2007; **40**: 391-398 [PMID: 17497705 DOI: 10.1002/eat.20380]

36 **Striegel RH**, Bedrosian R, Wang C, Schwartz S. Why men should be included in research on binge eating: results from a comparison of psychosocial impairment in men and women. *Int J Eat Disord* 2012; **45**: 233-240 [PMID: 22031213 DOI: 10.1002/eat.20962]

37 **Bentley C**, Mond J, Rodgers B. Sex differences in psychosocial impairment associated with eating-disordered behavior: what if there aren't any? *Eat Behav* 2014; **15**: 609-614 [PMID: 25218359 DOI: 10.1016/j.eatbeh.2014.08.015]

38 **Garner RE**, Feeny DH, Thompson A, Bernier J, McFarland BH, Huguet N, Kaplan MS, Orpana H, Ross NA, Blanchard C. Bodyweight, gender, and quality of life: a population-based longitudinal study. *Qual Life Res* 2012; **21**: 813-825 [PMID: 21842378 DOI: 10.1007/s11136-011-9989-1]

39 **Hyde JS**. Gender similarities and differences. *Annu Rev Psychol* 2014; **65**: 373-398 [PMID: 23808917 DOI: 10.1146/annurev-psych-010213-115057]

40 **Tennant R**, Hiller L, Fishwick R, Platt S, Joseph S, Weich S, Parkinson J, Secker J, Stewart-Brown S. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): development and UK validation. *Health Qual Life Outcomes* 2007; **5**: 63 [PMID: 18042300 DOI: 10.1186/1477-7525-5-63]

41 **Matud MP**, Bethencourth JM, Ibáñez I, Fortes D. Gender and psychological well-being in older adults. *Int Psychogeriatr* 2020; **32**: 1293-1302 [PMID: 32456745 DOI: 10.1017/S1041610220000824]

42 **Matud MP**, López-Curbelo M, Fortes D. Gender and Psychological Well-Being. *Int J Environ Res Public Health* 2019; **16** [PMID: 31547223 DOI: 10.3390/ijerph16193531]

43 **Solans M**, Pane S, Estrada MD, Serra-Sutton V, Berra S, Herdman M, Alonso J, Rajmil L. Health-related quality of life measurement in children and adolescents: a systematic review of generic and disease-specific instruments. *Value Health* 2008; **11**: 742-764 [PMID: 18179668 DOI: 10.1111/j.1524-4733.2007.00293.x]

44 **Parsa P**, Ahmadinia-Tabesh R, Mohammadi Y, Khorami N. Investigating the relationship between quality of life with lipid and glucose levels in Iranian diabetic patients. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S879-S883 [PMID: 28755844 DOI: 10.1016/j.dsx.2017.07.009]

45 **Wu XY**, Zhuang LH, Li W, Guo HW, Zhang JH, Zhao YK, Hu JW, Gao QQ, Luo S, Ohinmaa A, Veugelers PJ. The influence of diet quality and dietary behavior on health-related quality of life in the general population of children and adolescents: a systematic review and meta-analysis. *Qual Life Res* 2019; **28**: 1989-2015 [PMID: 30875010 DOI: 10.1007/s11136-019-02162-4]

46 **Zabielski P**, Hady HR, Chacinska M, Roszczyc K, Gorski J, Blachnio-Zabielska AU. The effect of high fat diet and metformin treatment on liver lipids accumulation and their impact on insulin action. *Sci Rep* 2018; **8**: 7249 [PMID: 29739997 DOI: 10.1038/s41598-018-25397-6]

47 **Orchard TJ**, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, Fowler S; Diabetes Prevention Program Research Group. The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann Intern Med* 2005; **142**: 611-619 [PMID: 15838067]

48 **Iwanowicz-Palus G**, Zarajczyk M, Bień A. The relationship between health-related quality of life, acceptance of illness and characteristics of pregnant women with hyperglycemia. *Health Qual Life Outcomes* 2020; **18**: 325 [PMID: 33008418 DOI: 10.1186/s12955-020-01582-y]

49 **Mergenthaler P**, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. *Trends Neurosci* 2013; **36**: 587-597 [PMID: 23968694 DOI: 10.1016/j.tins.2013.07.001]

50 **Hopkins DF**, Williams G. Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity. *Diabet Med* 1997; **14**: 1044-1050 [PMID: 9455932 DOI: 10.1002/(SICI)1096-9136(199712)14:12<1044::AID-DIA508>3.0.CO;2-F]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 581-583

DOI: 10.4239/wjd.v13.i7.581

ISSN 1948-9358 (online)

LETTER TO THE EDITOR

More studies are necessary to establish the effectiveness of Jinhuang powder in the treatment of diabetic foot

Ya-Wen Ye, Zi-Yun Yan, Lian-Ping He, Cui-Ping Li

Specialty type: Peripheral vascular disease

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
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Corvino A, Italy; Primadhi RA, Indonesia; Złoch M
A-Editor: Lin FY, China

Received: February 28, 2022
Peer-review started: February 28, 2022
First decision: April 17, 2022
Revised: April 18, 2022
Accepted: June 14, 2022
Article in press: June 14, 2022
Published online: July 15, 2022

Ya-Wen Ye, Zi-Yun Yan, Lian-Ping He, Cui-Ping Li, School of Medicine, Taizhou University, Taizhou 318000, Zhejiang Province, China

Corresponding author: Cui-Ping Li, MD, Teacher, School of Medicine, Taizhou University, No. 1139 Shifu Avenue, Taizhou 318000, Zhejiang Province, China. lcpyxy@tzc.edu.cn

Abstract

Diabetes mellitus is a common global public health problem that can cause serious illness and premature death. Diabetic foot ulcer, one of the complications of diabetes, is a major cause of morbidity and mortality and is associated with many other devastating complications. Previous study found that a group of traditional Chinese medicine (TCM) can be used for treating diabetic foot ulcers. More and more attention is being paid to the use of Chinese medicine to heal diabetic feet. Under the guidance of relevant theories of traditional Chinese medicine, more studies are needed to reveal the key active components and related signal pathways of TCM in the treatment of diabetic foot ulcer. One clinical study explored the treatment of diabetic foot with infection combined moist exposed burn ointment with Jinhuang powder. However, large-scale multi-center, double blind, randomized, placebo-controlled clinical trials and animal studies are necessary to establish the effectiveness of Jinhuang powder in the treatment of diabetic foot.

Key Words: Diabetic foot; Jinhuang powder; Traditional Chinese medicine

Core Tip: In recent years, most diabetic foot patients in China are adopting traditional Chinese medicine and western medicine. The short duration of clinical follow-up was not sufficient to explain the efficacy of the treatment, and the safety of the treatment was not mentioned. Large multicenter, double-blind, randomized, placebo controlled clinical trials and animal studies are necessary to determine Jinhuang powder as supplement combined with other Chinese medicine or western medicine as an effective and safe therapy for diabetic foot.

Citation: Ye YW, Yan ZY, He LP, Li CP. More studies are necessary to establish the effectiveness of Jinhuang powder in the treatment of diabetic foot. *World J Diabetes* 2022; 13(7): 581-583
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/581.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.581

TO THE EDITOR

This is a comment on "This is a comment on "Clinical Study of moist exposed burn ointment (MEBO) Combined with Jinhuang powder for Diabetic Foot with Infection"[1]. We were pleased that read the research article by Hong-Bo Zhan, *et al*[1]. Their work highlights that the use of Jinhuang powder as supplement combined with MEBO as an effective and safe therapy for diabetic foot. This study provides important clues to the treatment of foot infection, ulcer.

Diabetic foot ulcers are one of the most challenging complications of diabetes[2-4]. Previous study found that a group of traditional Chinese medicine (TCM) (*e.g.*, herbal medicine foot bath decoction[5], TCM injections[6,7], Chinese herbal medicine ulcer oil[8], moxibustion[9], Astragali Radix and Rehmanniae Radix Mixture[10], the peptide compounds of Wuguchong[11], Astragali Radix and Rehmanniae Radix[12,13]) played an important role in the treatment of the disease. Some study also found that interventional radiology plays a crucial role in the treatment of diabetic foot disease[14]. However, the study only focuses on Jinhuang powder has improved the efficacy and safety of MEBO in the treatment of diabetic foot. Thus, some questions still need further be discussed.

In recent years, most diabetic foot patients in China are adopting TCM and western medicine[5,15]. A series of systematic review articles showed that TCM can increase the clinical effective rate of conventional therapies by 27%[6], regulate the signaling pathways to promote diabetic wound healing[16]. An experiment on albino Wistar rats found that Astragali Radix and Rehmanniae Radix in the ratio of 2:1 significantly enhance the circulating CD34+/VEGFR2+/CD45-EPCs levels in diabetic foot ulcer[17]. Another study confirmed the effect of the peptide compounds of Wuguchong in treating diabetic ulcers to a certain extent[11]. However, therapeutic effect criterion is observing wound surface and assessing degree of pain. The research evaluation index was single and lacked objective evaluation.

Another problem of this study was research design. It was only a single-center trial, no double blindness, no placebo group. The short duration of clinical follow-up was not sufficient to explain the efficacy of the treatment, and the safety of the treatment was not mentioned. Large multicenter, double-blind, randomized, placebo controlled clinical trials and animal studies are necessary to determine Jinhuang powder as supplement combined with other Chinese medicine or western medicine as an effective and safe therapy for diabetic foot.

Increasing attention is being given to the use of Chinese medicine for healing diabetic foot. Under the guidance of relevant theories of TCM, more studies are needed to reveal the key active components and related signal pathways of TCM in the treatment of diabetic foot ulcer, so as to promote the further research and clinical application of TCM[16].

Overall, MEBO combined with Jinhuang powder is more effective than MEBO alone in treating diabetic foot. However, large-scale multi-center, double blind, randomized, placebo-controlled clinical trials and animal studies are also necessary to establish the effectiveness of Jinhuang powder in the treatment of diabetic foot.

FOOTNOTES

Author contributions: Li CP and He LP conceived of the presented idea and provided critical feedback to the final manuscript; Li CP, Ye YW and Yan ZY wrote and revised the manuscript; Li CP and He LP approved the main conceptual ideas and proof outline; all authors provided final edits and approved the manuscript.

Supported by the research work was supported by the Taizhou philosophy and social science planning project, No. 19GHZ05; and the Taizhou Science and technology planning project, No. 1902ky85.

Conflict-of-interest statement: The authors have no conflict of interest to disclose.

Country/Territory of origin: China

ORCID number: Ya-Wen Ye 0000-0002-5099-4678; Zi-Yun Yan 0000-0002-4695-7989; Lian-Ping He 0000-0002-9627-5599; Cui-Ping Li 0000-0002-9410-1835.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Zhan HB**, Sun QQ, Yan L, Cai J. Clinical Study of MEBO Combined with Jinhuang Powder for Diabetic Foot with Infection. *Evid Based Complement Alternat Med* 2021; **2021**: 5531988 [PMID: 34335813 DOI: 10.1155/2021/5531988]
2 **Doğruel H**, Aydemir M, Balci MK. Management of diabetic foot ulcers and the challenging points: An endocrine view. *World J Diabetes* 2022; **13**: 27-36 [PMID: 35070057 DOI: 10.4239/wjd.v13.i1.27]
3 **Soyoye DO**, Abiodun OO, Ikem RT, Kolawole BA, Akintomide AO. Diabetes and peripheral artery disease: A review. *World J Diabetes* 2021; **12**: 827-838 [PMID: 34168731 DOI: 10.4239/wjd.v12.i6.827]
4 **Nagoba B**, Gavkare A, Rayate A, Mumbre S, Rao A, Warad B, Nanaware N, Jamadar N. Role of an acidic environment in the treatment of diabetic foot infections: A review. *World J Diabetes* 2021; **12**: 1539-1549 [PMID: 34630906 DOI: 10.4239/wjd.v12.i9.1539]
5 **Zhang Y**, Yuan H, Kang J, Xie H, Long X, Qi L, Xie C, Gong G. Clinical study for external washing by traditional Chinese medicine in the treatment of multiple infectious wounds of diabetic foot: Study protocol clinical trial (SPIRIT compliant). *Medicine (Baltimore)* 2020; **99**: e19841 [PMID: 32332634 DOI: 10.1097/MD.0000000000019841]
6 **Tan L**, Shi Q, Liu C, Zhang J, Wang H, Zhai J. Traditional Chinese Medicine Injections in the Treatment of Diabetic Foot: A Systematic Review and Meta-Analysis. *Evid Based Complement Alternat Med* 2018; **2018**: 4730896 [PMID: 30402123 DOI: 10.1155/2018/4730896]
7 **Huang YY**, Jiang M, Zhang C, Wang Z, He D, Guo YM, Tian JP, Yu XC, Lu AP. Benefits of Chinese Medicine Among Patients with Diabetic Foot: An Expert Review from Clinical Studies. *Curr Vasc Pharmacol* 2015; **13**: 520-525 [PMID: 25360838 DOI: 10.2174/1570161112666141014152811]
8 **Jia H**, Yang B, Li Y, Liang C, Lu H, Lin D, Ju S. Chinese medicine ulcer oil promotes the healing of diabetic foot ulcers. *J Int Med Res* 2018; **46**: 2679-2686 [PMID: 29916286 DOI: 10.1177/0300060518769529]
9 **Tay JS**, Kim YJ. Efficacy of moxibustion in diabetes peripheral neuropathy. *Medicine (Baltimore)* 2021; **100**: e28173 [PMID: 34889293 DOI: 10.1097/MD.0000000000028173]
10 **Fan S**, Shi X, Wang A, Hou T, Li K, Diao Y. Evaluation of the key active ingredients of 'Radix Astragali and Rehmanniae Radix Mixture' and related signaling pathways involved in ameliorating diabetic foot ulcers from the perspective of TCM-related theories. *J Biomed Inform* 2021; **123**: 103904 [PMID: 34474187 DOI: 10.1016/j.jbi.2021.103904]
11 **Taowen P**, Shuyuan F, Xiaoli S, Annan W, Feng Q, Yizhong Z, Jing L, Bin L, Kun L, Yunpeng D. Study on the action mechanism of the peptide compounds of Wuguchong on diabetic ulcers, based on UHPLC-Q-TOF-MS, network pharmacology and experimental validation. *J Ethnopharmacol* 2022; **288**: 114974 [PMID: 35033625 DOI: 10.1016/j.jep.2022.114974]
12 **Zhang Q**, Fong CC, Yu WK, Chen Y, Wei F, Koon CM, Lau KM, Leung PC, Lau CB, Fung KP, Yang M. Herbal formula Astragali Radix and Rehmanniae Radix exerted wound healing effect on human skin fibroblast cell line Hs27 *via* the activation of transformation growth factor (TGF-β) pathway and promoting extracellular matrix (ECM) deposition. *Phytomedicine* 2012; **20**: 9-16 [PMID: 23083814 DOI: 10.1016/j.phymed.2012.09.006]
13 **Ko CH**, Yi S, Ozaki R, Cochrane H, Chung H, Lau W, Koon CM, Hoi SW, Lo W, Cheng KF, Lau CB, Chan WY, Leung PC, Chan JC. Healing effect of a two-herb recipe (NF3) on foot ulcers in Chinese patients with diabetes: a randomized double-blind placebo-controlled study. *J Diabetes* 2014; **6**: 323-334 [PMID: 24330156 DOI: 10.1111/1753-0407.12117]
14 **Reekers JA**. The Role of Interventional Radiology in the Treatment of Arterial Diabetic Foot Disease. *Cardiovasc Intervent Radiol* 2016; **39**: 1369-1371 [PMID: 27435578 DOI: 10.1007/s00270-016-1337-y]
15 **Lee AL**, Chen BC, Mou CH, Sun MF, Yen HR. Association of Traditional Chinese Medicine Therapy and the Risk of Vascular Complications in Patients With Type II Diabetes Mellitus: A Nationwide, Retrospective, Taiwanese-Registry, Cohort Study. *Medicine (Baltimore)* 2016; **95**: e2536 [PMID: 26817897 DOI: 10.1097/MD.0000000000002536]
16 **Zhou X**, Guo Y, Yang K, Liu P, Wang J. The signaling pathways of traditional Chinese medicine in promoting diabetic wound healing. *J Ethnopharmacol* 2022; **282**: 114662 [PMID: 34555452 DOI: 10.1016/j.jep.2021.114662]
17 **Tam JC**, Ko CH, Lau KM, To MH, Kwok HF, Siu WS, Lau CP, Chan WY, Leung PC, Fung KP, Lau CB. Enumeration and functional investigation of endothelial progenitor cells in neovascularization of diabetic foot ulcer rats with a Chinese 2-herb formula. *J Diabetes* 2015; **7**: 718-728 [PMID: 25350858 DOI: 10.1111/1753-0407.12230]

World Journal of Diabetes

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

World J Diabetes 2022 July 15; 13(7): 584-586

DOI: 10.4239/wjd.v13.i7.584

ISSN 1948-9358 (online)

LETTER TO THE EDITOR

Epidemiology for public health practice: The application of spatial epidemiology

Longjian Liu, Garvita Nagar, Ousmane Diarra, Stephanie Shosanya, Geeta Sharma, David Afesumeh, Akshatha Krishna

Specialty type: Health care sciences and services

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

P-Reviewer: Aslam MS, Malaysia; Fakhradiyev I, Kazakhstan; Harsini PA, Iran; Kadriyan H, Indonesia; Mohammadi A; Welter J, Switzerland

Received: February 12, 2022
Peer-review started: February 12, 2022
First decision: April 17, 2022
Revised: May 5, 2022
Accepted: June 22, 2022
Article in press: June 22, 2022
Published online: July 15, 2022

Longjian Liu, Garvita Nagar, Ousmane Diarra, Stephanie Shosanya, Geeta Sharma, David Afesumeh, Akshatha Krishna, Epidemiology and Biostatistics, Drexel University Dornsife School of Public Health, Philadelphia, PA 19104, United States

Corresponding author: Longjian Liu, MD, MSc, PhD, Doctor, Epidemiology and Biostatistics, Drexel University Dornsife School of Public Health, 3215 Market ST, Philadelphia, PA 19104, United States. ll85@drexel.edu

Abstract

Spatial epidemiology is the description and analysis of geographic patterns and variations in disease risk factors, morbidity and mortality with respect to their distributions associated with demographic, socioeconomic, environmental, health behavior, and genetic risk factors, and time-varying changes. In the Letter to Editor, we had a brief description of the practice for the mortality and the space-time patterns of John Snow's map of cholera epidemic in London, United Kingdom in 1854. This map is one of the earliest public heath practices of developing and applying spatial epidemiology. In the early history, spatial epidemiology was predominantly applied in infectious disease and risk factor studies. However, since the recent decades, noncommunicable diseases have become the leading cause of death in both developing and developed countries, spatial epidemiology has been used in the study of noncommunicable disease. In the Letter, we addressed two examples that applied spatial epidemiology to cluster and identify stroke belt and diabetes belt across the states and counties in the United States. Similar to any other epidemiological study design and analysis approaches, spatial epidemiology has its limitations. We should keep in mind when applying spatial epidemiology in research and in public health practice.

Key Words: Diabetes mellitus; Spatial epidemiology; Diabetes belt; Public health practice

Core Tip: This is a Letter to the Editor on the article published in *World Journal of Diabetes* 2021; 12: 1042, entitled: Spatial epidemiology of diabetes: Methods and Insights. Spatial epidemiology is a new sub-field of epidemiology. We read with great interest this paper, and would like to further address the application of spatial epidemiology for public health practice.

Citation: Liu L, Nagar G, Diarra O, Shosanya S, Sharma G, Afesumeh D, Krishna A. Epidemiology for public health practice: The application of spatial epidemiology. *World J Diabetes* 2022; 13(7): 584-586
URL: https://www.wjgnet.com/1948-9358/full/v13/i7/584.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i7.584

TO THE EDITOR

Dear Editor, we read with great interest the recently published review paper, entitled "Spatial epidemiology of diabetes: Methods and Insights" by Cuadros *et al*[1], in *World Journal of Diabetes* 2021. The investigators reviewed spatial methods used to understand the spatial structure of the disease and identify the potential geographical drivers of the spatial distribution of diabetes mellitus. Their report serves as a good review on the concepts and methods of spatial epidemiology. In this letter we aimed to briefly address few examples of the historical public health practice in the United Kingdom and the application of spatial epidemiology in the recent decades in the United States, and to address the potential limitations when applying this technique in research and in public health practice.

The method used in "*analysis of geographic variation in disease*" could be tracked back to more than 150 years ago, for example, the renowned study of cholera epidemic in London, United Kingdom In 1854. John Snow, a physician, used mapping approach to trace the source of the Broad Street cholera outbreak (or Golden Square outbreak) in central London[1]. In the United States, an example is that a "stroke belt" or "stroke alley" was identified in early 1980s using spatial analysis approach and to define a 11-state region, where the states had age-adjusted stroke mortality rates more than 10% above the national average[2,3]. In 2011, a study by Barker *et al*[4] identified a geographically coherent region of the United States, where the prevalence of diagnosed diabetes mellitus is especially high. This area is also known as the "diabetes belt". The "diabetes belt" consisted of 644 counties in 15 mostly southern states. A further analysis indicated that the prevalence of obesity and sedentary lifestyle (two modifiable risk factors for diabetes) was significantly higher in the diabetes belt than in the rest of the United States[5].

However, it should be noted that similar to the other analytical techniques, spatial epidemiology also has potential limitations[5,6]. First, the basic analysis approach of spatial epidemiology is based on ecological analysis design. Exposures and responses are measured only for aggregates rath than individuals. Therefore, findings from the analysis are subject to have ecological fallacy[5-8]. For example, results from an ecological analysis suggested that there was a significant correlation between increased state-level stroke prevalence and state-level stroke mortality. However, of the study states, several states that had higher state-level stroke prevalence rates did not have high stroke mortality rates, which led to a relatively weaker association than results from analyses using individual-level data[2]. Second, most spatial epidemiological studies apply age-adjusted rates to examine and map the variations in disease rates across geographic areas, such as neighborhoods, communities, districts, counties, states and countries at a global level. However, the calculation of age-adjusted rate is based on the proportion of age distributions across the geographics defined areas. If the proportions of age distributions vary widely between the comparison areas or regions, a simple weighted age-adjusted rate may be meaningless and may lead to an inappropriate comparison[5]. Third, data from disease registries, such as a small regional cancer registry, disease surveillance, or data from hospital electronic health records in a specific township is susceptible to information bias as a result of limited sources. Fourth, data protection and confidentiality should be kept in mind, specifically if mapping disease across small areas, such as small neighborhoods. It is likely that the number of persons at risk (*i.e.*, denominators) and the number of cases (*i.e.*, numerators) are too small to be used[8,9]. In the situation, a combined sample should be considered[10]. Lastly, confounding effects on the study association between exposures and outcomes should be considered and controlled appropriately in spatial epidemiological study.

In conclusion, the application of spatial epidemiology plays a pivotal role in advancing our understanding of the geographic distributions of specific disease and disease risk factors, which significantly contributes to disease control and prevention at population and community levels. However, the limitations of the study design and analysis approaches should be kept in mind when applying it in research and in public health practice.

FOOTNOTES

Author contributions: Liu L drafted the Letter; Nagar G, Diarra O, Shosanya S, Sharma G, Afesumeh D, and Krishna A critically reviewed the Letter.

Conflict-of-interest statement: The authors have no conflict of interest to disclose.

Country/Territory of origin: United States

ORCID number: Longjian Liu 0000-0001-7956-7111; Garvita Nagar 0000-0002-3572-9024; Ousmane Diarra 0000-0002-0643-2598; Stephanie Shosanya 0000-0002-9089-8592; Geeta Sharma 0000-0003-2856-5663; David Afesumeh 0000-0002-5519-5939; Akshatha Krishna 0000-0002-6063-0747.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Cuadros DF**, Li J, Musuka G, Awad SF. Spatial epidemiology of diabetes: Methods and insights. *World J Diabetes* 2021; **12**: 1042-1056 [PMID: 34326953 DOI: 10.4239/wjd.v12.i7.1042]
2 **Bingham P**, Verlander NQ, Cheal MJ. John Snow, William Farr and the 1849 outbreak of cholera that affected London: a reworking of the data highlights the importance of the water supply. *Public Health* 2004; **118**: 387-394 [PMID: 15313591 DOI: 10.1016/j.puhe.2004.05.007]
3 **Liao Y**, Greenlund KJ, Croft JB, Keenan NL, Giles WH. Factors explaining excess stroke prevalence in the US Stroke Belt. *Stroke* 2009; **40**: 3336-3341 [PMID: 19679841 DOI: 10.1161/STROKEAHA.109.561688]
4 **Barker LE**, Kirtland KA, Gregg EW, Geiss LS, Thompson TJ. Geographic distribution of diagnosed diabetes in the U.S.: a diabetes belt. *Am J Prev Med* 2011; **40**: 434-439 [PMID: 21406277 DOI: 10.1016/j.amepre.2010.12.019]
5 **Lanska DJ**, Kuller LH. The geography of stroke mortality in the United States and the concept of a stroke belt. *Stroke* 1995; **26**: 1145-1149 [PMID: 7604404 DOI: 10.1161/01.str.26.7.1145]
6 **Elliott P**, Wartenberg D. Spatial epidemiology: current approaches and future challenges. *Environ Health Perspect* 2004; **112**: 998-1006 [PMID: 15198920 DOI: 10.1289/ehp.6735]
7 **Beale L**, Abellan JJ, Hodgson S, Jarup L. Methodologic issues and approaches to spatial epidemiology. *Environ Health Perspect* 2008; **116**: 1105-1110 [PMID: 18709139 DOI: 10.1289/ehp.10816]
8 **Szklo M**. Epidemiology: Beyond the Basics. *Am J Epidemiol* 2001; **153**: 821-822 [DOI: 10.1093/aje/153.8.821]
9 **Liu L**, Wang F, Gracely EJ, Moore K, Melly S, Zhang F, Sato PY, Eisen HJ. Burden of Uncontrolled Hyperglycemia and Its Association with Patients Characteristics and Socioeconomic Status in Philadelphia, USA. *Health Equity* 2020; **4**: 525-532 [PMID: 34095699 DOI: 10.1089/heq.2020.0076]
10 **Liu L**, Núñez AE. Multilevel and urban health modeling of risk factors for diabetes mellitus: a new insight into public health and preventive medicine. *Adv Prev Med* 2014; **2014**: 246049 [PMID: 25431678 DOI: 10.1155/2014/246049]

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