ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2022 November 15; 13(11): 900-1000

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

Contents

Monthly Volume 13 Number 11 November 15, 2022

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

Observational Study

World Journal of Diabetes

Monthly Volume 13 Number 11 November 15, 2022

Contents

NAME OF JOURNAL

World Journal of Diabetes

ISSN

ISSN 1948-9358 (online)

LAUNCH DATE

June 15, 2010

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Lu Cai, Md. Shahidul Islam, Jian-Bo Xiao, Michael Horowitz

EDITORIAL BOARD MEMBERS

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

PUBLICATION DATE

November 15, 2022

INSTRUCTIONS TO AUTHORS

https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS

https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS

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

PUBLICATION MISCONDUCT

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

ARTICLE PROCESSING CHARGE

https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS

https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION

https://www.f6publishing.com

E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 900-911

DOI: 10.4239/wjd.v13.i11.900

ISSN 1948-9358 (online)

REVIEW

Type 2 diabetes and bone fragility in children and adults

Maria Felicia Faienza, Paola Pontrelli, Giacomina Brunetti

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

P-Reviewer: Lee KS, South Korea; Munteanu C, Romania; Zhao CF, China

Received: May 30, 2022
Peer-review started: May 30, 2022
First decision: August 7, 2022
Revised: August 17, 2022
Accepted: October 11, 2022
Article in press: October 11, 2022
Published online: November 15, 2022

Maria Felicia Faienza, Department of Biomedical Sciences and Human Oncology, Pediatric Unit, University of Bari Aldo Moro, Bari 70124, Italy

Paola Pontrelli, Division of Nephrology, Department of Emergency and Organ Transplantation, University of Bari Aldo Moro, Bari 70124, Italy

Giacomina Brunetti, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, Bari 70125, Italy

Corresponding author: Giacomina Brunetti, PhD, Associate Professor, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, *Via* Orabona, 4, Bari 70125, Italy. giacomina.brunetti@uniba.it

Abstract

Type 2 diabetes (T2D) is a global epidemic disease. The prevalence of T2D in adolescents and young adults is increasing alarmingly. The mechanisms leading to T2D in young people are similar to those in older patients. However, the severity of onset, reduced insulin sensitivity and defective insulin secretion can be different in subjects who develop the disease at a younger age. T2D is associated with different complications, including bone fragility with consequent susceptibility to fractures. The purpose of this systematic review was to describe T2D bone fragility together with all the possible involved pathways. Numerous studies have reported that patients with T2D show preserved, or even increased, bone mineral density compared with controls. This apparent paradox can be explained by the altered bone quality with increased cortical bone porosity and compromised mechanical properties. Furthermore, reduced bone turnover has been described in T2D with reduced markers of bone formation and resorption. These findings prompted different researchers to highlight the mechanisms leading to bone fragility, and numerous critical altered pathways have been identified and studied. In detail, we focused our attention on the role of microvascular disease, advanced glycation end products, the senescence pathway, the Wnt/β-catenin pathway, the osteoprotegerin/receptor-activator of nuclear factor kappa B ligand, osteonectin and fibroblast growth factor 23. The understanding of type 2 myeloid bone fragility is an important issue as it could suggest possible interventions for the prevention of poor bone quality in T2D and/or how to target these pathways when bone disease is clearly evident.

Key Words: Type 2 diabetes; Bone remodeling; Cytokines; Bone fragility; Bone mineral density; Chronic kidney disease

Core Tip: Type 2 diabetes (T2D) patients show increased susceptibility to bone fractures, despite their bone mineral density being normal or increased, leading to difficult identification for clinicians. The prevalence of T2D in adolescents and young adults is increasing alarmingly. Different researchers highlighted the mechanisms leading to bone fragility, and different critical altered pathways have been identified and studied. In this review, we described the different metabolic pathways responsible for bone fragility in patients with T2D. They can be useful for its management, although further studies are needed to deepen our understanding of the mechanisms underlying bone fragility in T2D.

Citation: Faienza MF, Pontrelli P, Brunetti G. Type 2 diabetes and bone fragility in children and adults. *World J Diabetes* 2022; 13(11): 900-911
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/900.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.900

INTRODUCTION

The prevalence of type 2 diabetes (T2D) mellitus in adolescents and young adults is increasing alarmingly. Data from the SEARCH study showed an annual increase of approximately 7% in the incidence of T2D among people aged 10-years-old to 19-years-old in the United States, with increases in all ethnic groups[1]. Increases in children, adolescents and young adults with T2D have been described across most regions of the world[2]. The highest T2D incidence rates in youth have been registered in Canadian First Nations, American Indian and Navajo nation, Australian Aboriginal and Torres Strait Islander and African American populations (31-94/100000 each year), while youths from non-Hispanic Caucasian populations (*i.e.,* United States and Europe) display the lowest incidence rates (0.1-0.8/100000 each year). Studies show the highest prevalence in youth from Mexico and Brazil, indigenous populations in Canada and the United States, together with Black populations in the Americas (160-3300/100000). Conversely, the lowest prevalence was registered in European populations (0.6-2.7/100000)[2].

A recent literature review examined country-specific prevalence and incidence data of youth-onset T2D published between 2008 and 2019[3]. The highest prevalence rates of youth-onset T2D were observed in China (520 cases/100000 people) and the United States (212 cases/100000) and the lowest in Denmark (0.6 cases/100000) and Ireland (1.2 cases/100000). However, the highest incidence rates were reported in Taiwan (63 cases/100000) and the United Kingdom (33.2 cases/100000), with the lowest in Fiji (0.43 cases/100000) and Austria (0.6 cases/100000). These differences in epidemiology data may be partially explained by variations in the diagnostic criteria used within studies, screening recommendations within national guidelines and race/ethnicity within countries.

The main predisposing risk factors for the development of T2D in pediatric age are represented by obesity, family history and sedentary lifestyle[4]. The mechanisms leading to T2D in young people are similar to those in older patients. However, the severity of onset, reduced insulin sensitivity and defective insulin secretion can be different in subjects who develop the disease at a younger age[5]. In particular, the phase of nutrient-induced insulin secretion might be impaired earlier in children and adolescents than in older subjects[6].

The comorbidities associated with T2D in young people include hypertension, cardiovascular disease, kidney impairment and retinopathy. Furthermore, psychosocial problems are often observed[7]. Altered bone quality has been reported in patients with T2D, possible mechanisms for the effect of T2D on bone mineral density (BMD) include the toxic effects of hyperglycemia, which may impair differentiation and proliferation of osteoblasts[8]. In addition, hyperglycemia can increase urine calcium excretion, which inhibits bone formation[8]. Thus, the objective of this review was to describe T2D bone fragility together with all the possible involved pathways.

T2D AND BONE IMPAIRMENT

Most studies have found that patients with T2D have preserved, or even increased, BMD compared with controls but display bone fragility with consequent increased susceptibility to fractures[9-11]. This apparent paradox is due to the altered bone quality in these patients. In detail, the spine trabecular bone score is decreased in patients with T2D, and it is a predictor of fracture risk independently of the BMD [12].

Furthermore, different studies have evaluated T2D effects on bone microarchitecture of the peripheral skeleton (radius and tibia) through high-resolution peripheral quantitative computed tomography. These studies have generally shown preserved, or even improved, trabecular bone microarchitecture in patients with T2D compared with controls[12-17]. Furthermore, some[14,15,17-19], but not all[16,20,21], studies report augmented cortical porosity in patients with T2D, and interestingly this parameter independently predicts fracture risk.

Another aspect of bone quality that might be impaired in patients with T2D is the mechanical characteristics that can be assessed through the measurement of the bone material strength index. However, discordant results have been reported on this issue. In detail, some authors found reduced bone material strength index in T2D in comparison with controls[20,22,23], whereas others reported no significant variation[16]. These different results could be associated to the different comorbidities characterizing T2D.

Several studies have noted reduced bone turnover in patients with T2D[20,24,25]. It has been reported that patients with T2D have reduced markers of bone formation [serum levels of procollagen type 1 amino-terminal propeptide and osteocalcin (OC)] as well as resorption (carboxy-terminal telopeptide of type 1 collagen)[20,24,26,27]. Moreover, Starup-Linde *et al*[28] demonstrated an inverse relationship between glycemic control (hemoglobin A1c) and OC levels and a similar trend for carboxy-terminal telopeptide of type 1 collagen and procollagen type 1 amino-terminal propeptide. N-terminal telopeptide of type 1 collagen and bone-specific alkaline phosphatase levels are not significantly different between patients with T2D and controls[29]. Very recently, it has been reported that thyroid homeostasis could affect bone turnover markers[30] and that follicle-stimulating hormone levels may contribute to the suppression of the same markers[31].

MECHANISMS AND MOLECULAR PATHWAYS INVOLVED IN T2D BONE FRAGILITY

An indication of mutual regulatory control of both bone and glycemic homeostasis recognizes the close interplay between these two systems. The common regulatory mechanisms involve microvascular disease, advanced glycation end products (AGEs), osteoprotegerin (OPG)/receptor-activator of nuclear factor kappa B ligand (RANKL), the Wnt/β-catenin pathway, osteonectin and fibroblast growth factor 23 (FGF23) (Table 1).

Microvascular disease

Microvascular disease is a common complication (retinopathy, nephropathy or neuropathy) of diabetes [32]. Angiopathy has been demonstrated in the iliac crest of diabetic patients[33]. Recently, decreased microvascular blood flow has been demonstrated to be linked with cortical porosity in patients with T2D, suggesting that microvascular disease negatively affects bone microarchitecture in T2D[16]. Consistently, cortical porosity of the distal radius and tibia is most pronounced in patients with T2D with microvascular disease[19]. In contrast, in 2022 it was found that the poorest femoral trabecular microarchitecture was associated with vascular complications in patients with T2D[34]. Patients with T2D with microvascular disease display a significantly lower trabecular bone score, after adjusting for confounders. Moreover, multivariable analysis demonstrated a significant correlation between low 25(OH) vitamin D levels and microvascular disease[35].

Several mechanisms have been proposed to explain how microvascular disease is associated with bone fragility in T2D. It is important to remember that skeletal blood flow provides growth factors, hormones, oxygen and nutrients affecting bone remodeling, suggesting that alteration in microvasculature leads to bone impairment. In the same manner as perivascular cells show stem-cell like properties and may differentiate in osteoblastic cells, blood vessels also release factors affecting the differentiation and activity of osteoblasts and osteoclasts[36]. Blood flow promotes angiogenesis and thus osteogenesis. Bone blood flow is reduced in T2D rats[37], and hypoxia increases the canal network in rat cortical bone [38], suggesting that insufficient oxygen and blood flow associated with microvascular disease alters bone microarchitecture. The recruitment of osteoprogenitors from blood vessels is fundamental for bone formation following osteoclast resorption[39]. Thus, microvascular disease could uncouple resorption and formation in cortical bone by impairing osteoprogenitor recruitment. However, further studies are needed to deepen our understanding of the mechanisms and in particular whether bone fragility is a comorbidity of T2D or a complication (this item is a matter of debate)[40].

AGEs

Hyperglycemia disturbs both bone cells and the extracellular matrix. The presence of glucose determines the production of intermediate products, which eventually generate the irreversible accumulation of AGE[41]. AGE accumulation leads to the synthesis of defective collagens as well as of reactive oxygen species, with consequent structural changes in the bone[42]. In detail, considering the organic bone matrix, these products lead to diminished bone strength[43,44]. Elevated AGE levels are associated with increased fracture risk[45].

Table 1 Mechanisms of bone fragility in type 2 diabetes

Cytokines/factors	Mechanisms	Bone effect
Microvascular disease	Reduced bone vasculature, blood flow and oxygen supply	Increased fracture risk
AGEs	Osteoclast and osteoblast alterations	Poor bone quality, impaired biomechanical properties, and occurrence of fracture
Senescence pathways	Osteocyte impairment	Reduced biomechanical strength, defective bone microarchitecture and increased risk of fracture
Wnt/β-catenin pathway	High levels of sclerostin and DKK1 in T2D. Involvement in CKD-MBD	Impairment of bone cell activity in murine and human models
OPG/RANKL	Decreased OPG/RANKL ratio	Suppressed bone turnover
Osteonectin	High levels of osteonectin	Albuminuria is linked to higher levels of osteonectin
Osteocalcin	Reduced levels in T2D	Decreased bone formation. Bone fracture, involved in T2D and kidney complication
FGF23/klotho	High FGF23 and low klotho levels in T2D	Dysregulation of mineral metabolism, bone fractures. FGF23 is linked to bone fragility; reduced klotho levels are predictors for CKD-MBD

AGEs: Advanced glycation end products; CKD-MBD: Chronic kidney disease-mineral and bone disorder; DKK1: Dickkopf-related protein 1; FGF23: Fibroblast growth factor 23; OPG/RANKL: Osteoprotegerin/receptor-activator of nuclear factor kappa B ligand; T2D: Type 2 diabetes.

The AGE-receptor for AGE (RAGE) binding generates reactive oxygen species production, macrophage and platelet activation, vascular inflammation and inflammatory cell migration[46]. All these events are involved in the onset and progression of typical macro- and microangiopathy associated with diabetes, thus leading to brittle bones with diminished strength and less capability to deform before fracturing[47].

RAGE is also expressed by immune cells and incites activation of the nuclear factor kappa-light-chain-enhancer of activated B cells, a central transcription factor of the immune and inflammatory response[46]. The AGE-RAGE interaction in immune cells leads to the increased expression of chemokines and adhesion molecules, secreting further RAGE ligands, supporting the inflammatory tissue response, regulating the activated macrophage reaction to enhance the destructive signals in the tissues and inhibiting the repair and remodeling responses[46]. AGEs may determine osteoclastogenesis and osteoblast alterations in the bone microenvironment due to the increase in inflammatory cytokines, leading to osteoporosis[48].

In detail, pentosidine, the most studied AGE, accumulates in the trabecular and cortical bone in patients with T2D and negatively affects their bone strength as well as probably leading to functional changes in osteoblasts and the bone mineralization process[49,50]. Consequently, trabecular and cortical bones show impaired biomechanical properties and decreased strength, together with altered osteoblast activity as well as adhesion to the collagen matrix and thus negatively affect bone homeostasis[45,50-52].

AGE bone content correlates with worse bone microarchitecture, including lower volumetric BMD, bone volume/total volume and increased trabecular separation/spacing[53]. High concentrations of AGEs blunt insulin-like growth factor I- mediated osteoblast stimulation and determines the resistance of osteoblasts to insulin-like growth factor I effects[54]. Consistently, insulin-like growth factor I serum levels have been found to be inversely correlated with the occurrence of vertebral fractures in T2D postmenopausal women[55].

The role of cellular senescence in mediating skeletal fragility in T2D

Different forms of stress can lead a cell to enter an irreversible permanent growth arrest known as senescence[56]. This is triggered by cyclin-dependent kinase inhibitors, remarkably p16Ink4a and p21Cip1, that antagonize the activity of cyclin-dependent kinases to stop cell proliferation[57,58]. Senescent cells display a transformed gene expression profile with an increase in senescent cell anti-apoptotic pathways as well as a senescence-associated secretory phenotype[59], typically consisting of proinflammatory cytokines, chemokines and matrix remodeling proteins[60,61]. A premature increase in senescent cells is evident in T2D, especially pancreatic β cells and bone[62,63]. In particular, osteocyte senescence has been demonstrated using an inducible obese mouse model of T2D. These mice display bone quality alterations quite similar to bones from humans with T2D, such as reduced biomechanical strength, defective cortical bone microarchitecture and low bone formation rates[63]. Furthermore, in this model, senescent osteocytes were identified for the high levels of p16Ink4a and p21Cip1, senescence-associated distension of satellites, increased telomere-associated foci (another cell marker of senescence) as well as typical increased expression of proinflammatory senescence-associated secretory phenotype and nuclear factor kappa-light-chain-enhancer of activated B cells[63].

Additionally, cellular senescence in T2D has been linked to the incidence of fracture in murine models and patients[64,65]. In detail, using a murine model of T2D reflecting both hyperinsulinemia caused by insulin resistance induced by a high-fat diet and insulinopenia induced by low dose streptozotocin, increased density of senescent cells has been demonstrated in the callus area in fracture healing [64]. Additionally, the same authors reported that cells of the osteoblastic lineage cultured with sera from patients with T2D displayed increased expression of the p53 responsive genes that are typical of a senescent microenvironment[64]. The decreased levels of serum senescent miR-31-5p in older diabetic women is linked to incidents of fragility fracture and can significantly predict fracture risk if combined with femoral neck and BMD measurements[65].

The Wnt/β-catenin pathway

The Wnt/β-catenin pathway activation promotes osteoblastogenesis and bone formation but inhibits osteoclastogenesis. Dickkopf-related protein 1 and sclerostin (encoded by *Sost*) antagonize the Wnt/β-catenin pathway by binding to low-density lipoprotein receptor-related protein 5 or 6, thus inhibiting osteoblastogenesis and promoting osteoclastogenesis[66].

Bone expression of sclerostin and Dickkopf-related protein 1 has been demonstrated to be high in T2D rat models[67,68]. Circulating sclerostin levels have also been found to be increased in patients with T2D[69] and correlated to the decrease in bone formation markers[70]. In contrast, in T2D postmenopausal women the high circulating levels of sclerostin are related to vertebral fractures[71]. Interestingly, T2D postmenopausal women with previous fractures display thinner cortical bone, together with a tendency towards larger volumetric bone density and elevated circulating levels of sclerostin compared with diabetic women without fractures and nondiabetic controls with fractures [72]. More recently, Piccoli *et al*[53] reported that *Sost* expression in RNA extracts from the femoral head of patients with T2D is significantly increased compared with the controls, although circulating sclerostin levels were found to be higher in T2D subjects but not statistically significant.

OPG/RANKL

OPG is a soluble tumor necrosis factor receptor superfamily member originally discovered in bone[73,74]. It is an anti-resorptive cytokine that works by binding and neutralizing the receptor activator for RANKL. RANKL is a molecule that induces osteoclast differentiation and activity[73,74]. The OPG/RANKL axis is also linked to the regulation of glucose homeostasis[75,76]. In detail, hyperglycemia downregulates RANKL expression, which inhibits the differentiation and activity of osteoclasts[73,76].

The duration of diabetes seems to negatively affect bone metabolism, but poor glycemic control (hemoglobin A1c ≥ 7.5%) has also been shown to be associated with an increased risk of fracture[77]. Decreased levels of RANKL have been reported in diabetic patients compared to healthy subjects[78]. This seems to be due to the increased number of immature osteoblasts and osteoclasts[79]. Other authors have reported that serum RANKL levels are reduced and OPG increased in diabetic patients with respect to nondiabetics and prediabetic subjects[80,81].

Furthermore, it has also been reported that high RANKL levels are related to a significantly increased risk of T2D development[82]. However, other authors did not measure significant differences in RANKL levels between patients with T2D and controls[29]. Human osteoblast cultures from cancellous bone biopsies of diabetic patients displayed a decreased RANKL/OPG ratio compared to the controls, suggesting that the bone turnover process is suppressed[83].

Osteonectin

Osteonectin is produced by osteoblasts and high osteonectin serum levels represent a marker of bone formation[84]. Osteonectin induces osteoblast differentiation, commitment and survival. *In vivo*, osteonectin-knock out and haploinsufficient mice show osteopenia with low bone turnover, a decreased number of osteoblasts as well as a reduced bone formation rate[85,86]. Additionally, Dole *et al*[87] reported that a single nucleotide polymorphism in the 3′ untranslated region of osteonectin determined variability in bone mass by modulating its expression. Patients with albuminuria had significantly higher levels of osteonectin compared with normoalbuminuric patients[88].

T2D AND BONE-KIDNEY CROSS-TALK: THE ROLE OF BONE-DERIVED HORMONES

Chronic kidney disease (CKD) represents a serious complication of T2D and impacts 25%-40% of the diabetic population[89], thus leading to end stage renal disease with the need for dialysis or kidney transplantation[90]. Although kidney replacement therapy improves long-term survival and quality of life in CKD patients, this survival highlights bone fragility as an emerging complication[91]. In a large cohort of patients with CKD followed between 1990 and 1999, Bal *et al*[92] demonstrated that the

fracture risk was higher with a prolonged period of dialysis before transplantation, and both epidermal growth factor receptor decrease and albuminuria increase were considered important risk factors for fracture[93]. Bone fragility in CKD patients is dependent on several risk factors, and literature data demonstrate the impact of age, race (Caucasian) and sex, low body mass index < 23 kg/m^2, glucocorticoid duration and immunosuppressive agents[94]. However, in addition to the described factors and dialysis vintage, diabetes and pancreas replacement therapy are also important risk factors for bone fragility[95,96].

In 2009, the Clinical Practice Guidelines for the Diagnosis, Evaluation, Prevention, and Treatment of CKD-Mineral and Bone Disorder (CKD-MBD) were originally published by Kidney Disease: Improving Global Outcomes[97]. This clinical syndrome defines a systemic disorder in CKD patients responsible for abnormalities in mineral metabolism, bone remodeling and vascular calcification. Despite the completion of several key clinical trials since the 2009 publication of the CKD-MBD guidelines, large gaps in the knowledge still remain[98]. Prospective studies are needed to determine the value of BMD and bone biomarkers as predictors of fractures[99] as well as the impact of different therapeutic approaches on bone fragility, especially in patients with both diabetes and kidney disease. Recent studies have demonstrated that CKD patients with T2D are at increased risk of bone diseases[100], which could involve FGF23.

FGF23

Ribeiro *et al*[101] described how the FGF23/klotho axis is a predictive factor for fractures in patients with T2D with early CKD and demonstrated that α-klotho and FGF23 independently influenced the occurrence of bone fractures. FGF23 is a bone-derived hormone secreted by osteocytes that regulates phosphate and vitamin D metabolism. It acts in the kidney through FGF receptors and klotho, thus preventing renal tubular reabsorption of phosphorus. FGF23 plays an important role in the development of bone and mineral disorders, and many studies over recent years, including patients with CKD and diabetes, have demonstrated that FGF23 levels increase in CKD patients and have an impact on bone disease, cardiovascular disease and all causes of mortality[102]. FGF23 can also induce secondary hyperparathyroidism by increasing the 24-hydroxylation of vitamin D, and these changes are associated with an increased risk of fracture in dialysis[103]. FGF23 levels are also further raised in CKD patients with diabetes who had had a previous fracture[101], thus underlying the association of a history of prior fracture with increased risk of hip fracture, as observed in all dialysis patients[104]. Moreover, FGF23 may also promote insulin secretion and insulin resistance[105], thus influencing the risk of adverse outcomes, especially under CKD conditions[106]. Thus FGF23 could represent a potential biomarker for CKD progression in diabetes[107] and be associated with multiple risk factors [108], including bone fragility.

FGF23 signaling on target tissues is mediated by FGF receptors and klotho, which functions as a coreceptor to increase the binding affinity of FGF23 for FGF receptors. Klotho can also circulate as a secreted protein and a physiologically active hormone. It has been demonstrated that insulin can stimulate the release of klotho by inducing the cleavage of the extracellular domain of klotho by ADAM10 and ADAM17 in the kidney[109]. Cleaved klotho can thus regulate both the phosphorus and calcium metabolism in the kidney and mineral homeostasis in the body through 1-alpha hydroxylase activity as well as parathyroid hormone and FGF23 secretion[110]. Klotho expression is significantly reduced by several kidney injuries such as glomerulonephritis, acute kidney injury, ischemia/reperfusion injury and delayed graft function[111,112], chronic allograft dysfunction[113,114] and renal cell carcinoma[115]. Low klotho levels are also associated with accelerated aging that can promote dysregulated mineral metabolism and osteoporosis. Thus, reduced klotho levels are considered early factors in the development of CKD-MBD[116,117]. Klotho levels are also compromised in patients with early CKD and diabetes[101], while lower levels of klotho seem to be an independent predictive factor for bone fracture[101].

Sclerostin and OC

The presence of diabetes may also increase sclerostin, an osteocyte-specific protein that inhibits bone formation, and higher serum sclerostin levels are associated with increased fracture rates[118]. Thus, sclerostin has been described as an important factor contributing to CKD-MBD[119]. In diabetic patients with CKD, sclerostin levels start to increase in the CKD-G3 stage, while patients in the CKD-G4/5 stages have dramatically increased levels of circulating sclerostin[120].

OC is another bone-derived hormone whose levels reflect the ability of osteoblasts to form bones [121]. OC is directly associated with glucose metabolism and experimental models show that OC can increase insulin production by pancreatic β cells and insulin sensitivity in peripheral tissues[122]. Moreover, insulin receptor signaling increases the production of OC in osteoblasts[123]. OC levels have been recently associated with the risk of incident diabetes and kidney complications, while increased levels have been described in CKD patients[124,125]. In early CKD patients with diabetes, OC levels independently influence the occurrence of bone fracture[101]. However, further studies are needed to confirm the specific role of OC in the context of diabetes and CKD.

Further research is also needed to assess the diagnostic and prognostic value of these bone turnover biomarkers in the field of CKD-MBD in the context of diabetes. However, the described hormones represent important factors for the development of bone diseases in the context of CKD and may be considered as targets for future clinical trials.

CONCLUSION

The studies reported in the present review describe altered bone quality and the possible mechanisms underlying its pathophysiology. Patients with T2D frequently display bone fragility, which is often an underdiagnosed condition in these subjects. The understanding of its pathophysiology is an important issue as it could suggest possible interventions for the prevention of poor bone quality in T2D. Additionally, the discovery of its pathophysiology could help to target these pathways when bone disease is clearly evident. Thus, the simultaneous use of anti-diabetic drugs and bone treating agents could help to ameliorate the quality of life of patients with T2D. This issue is of particular interest considering the life extension observed. Nevertheless, the possible interventions to improve bone quality in T2D require further investigation, which could determine different treatment approaches through personalized medicine.

FOOTNOTES

Author contributions: Faienza MF wrote the clinical implications; Pontrelli P explored the bone-kidney axis; Brunetti G performed the majority of the writing and coordinated the writing of the paper.

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

Country/Territory of origin: Italy

ORCID number: Maria Felicia Faienza 0000-0002-1899-8337; Paola Pontrelli 0000-0002-7654-8318; Giacomina Brunetti 0000-0002-0681-1432.

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

REFERENCES

1 **Mayer-Davis EJ**, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, Imperatore G, Linder B, Marcovina S, Pettitt DJ, Pihoker C, Saydah S, Wagenknecht L; SEARCH for Diabetes in Youth Study. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. *N Engl J Med* 2017; **376**: 1419-1429 [PMID: 28402773 DOI: 10.1056/NEJMoa1610187]
2 **Sayyed A**. International Diabetes Federation Diabetes Atlas – 10th Edition. [cited 40 April 2022]. Available from: https://www.metabolichealthdigest.com/international-diabetes-federation-diabetes-atlas-10th-edition/
3 **Lynch JL**, Barrientos-Pérez M, Hafez M, Jalaludin MY, Kovarenko M, Rao PV, Weghuber D. Country-Specific Prevalence and Incidence of Youth-Onset Type 2 Diabetes: A Narrative Literature Review. *Ann Nutr Metab* 2020; **76**: 289-296 [PMID: 32980841 DOI: 10.1159/000510499]
4 **Lascar N**, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S. Type 2 diabetes in adolescents and young adults. *Lancet Diabetes Endocrinol* 2018; **6**: 69-80 [PMID: 28847479 DOI: 10.1016/S2213-8587(17)30186-9]
5 **Gungor N**, Bacha F, Saad R, Janosky J, Arslanian S. Youth type 2 diabetes: insulin resistance, beta-cell failure, or both? *Diabetes Care* 2005; **28**: 638-644 [PMID: 15735201 DOI: 10.2337/diacare.28.3.638]
6 **Druet C**, Tubiana-Rufi N, Chevenne D, Rigal O, Polak M, Levy-Marchal C. Characterization of insulin secretion and resistance in type 2 diabetes of adolescents. *J Clin Endocrinol Metab* 2006; **91**: 401-404 [PMID: 16291705 DOI: 10.1210/jc.2005-1672]
7 **Zuckerman Levin N**, Cohen M, Phillip M, Tenenbaum A, Koren I, Tenenbaum-Rakover Y, Admoni O, Hershkovitz E, Haim A, Mazor Aronovitch K, Zangen D, Strich D, Brener A, Yeshayahu Y, Schon Y, Rachmiel M, Ben-Ari T, Levy-Khademi F, Tibi R, Weiss R, Lebenthal Y, Pinhas-Hamiel O, Shehadeh N. Youth-onset type 2 diabetes in Israel: A national cohort. *Pediatr Diabetes* 2022; **23**: 649-659 [PMID: 35521999 DOI: 10.1111/pedi.13351]

8 **Thrailkill KM**, Lumpkin CK Jr, Bunn RC, Kemp SF, Fowlkes JL. Is insulin an anabolic agent in bone? *Am J Physiol Endocrinol Metab* 2005; **289**: E735-E745 [PMID: 16215165 DOI: 10.1152/ajpendo.00159.2005]

9 **Bonds DE**, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, Johnson KC, Margolis KL. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab* 2006; **91**: 3404-3410 [PMID: 16804043 DOI: 10.1210/jc.2006-0614]

10 **Ma L**, Oei L, Jiang L, Estrada K, Chen H, Wang Z, Yu Q, Zillikens MC, Gao X, Rivadeneira F. Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. *Eur J Epidemiol* 2012; **27**: 319-332 [PMID: 22451239 DOI: 10.1007/s10654-012-9674-x]

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 **Patsch JM**, Burghardt AJ, Yap SP, Baum T, Schwartz AV, Joseph GB, Link TM. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res* 2013; **28**: 313-324 [PMID: 22991256 DOI: 10.1002/jbmr.1763]

13 **Heilmeier U**, Cheng K, Pasco C, Parrish R, Nirody J, Patsch JM, Zhang CA, Joseph GB, Burghardt AJ, Schwartz AV, Link TM, Kazakia G. Cortical bone laminar analysis reveals increased midcortical and periosteal porosity in type 2 diabetic postmenopausal women with history of fragility fractures compared to fracture-free diabetics. *Osteoporos Int* 2016; **27**: 2791-2802 [PMID: 27154435 DOI: 10.1007/s00198-016-3614-7]

14 **Burghardt AJ**, Issever AS, Schwartz AV, Davis KA, Masharani U, Majumdar S, Link TM. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2010; **95**: 5045-5055 [PMID: 20719835 DOI: 10.1210/jc.2010-0226]

15 **Yu EW**, Putman MS, Derrico N, Abrishamanian-Garcia G, Finkelstein JS, Bouxsein ML. Defects in cortical microarchitecture among African-American women with type 2 diabetes. *Osteoporos Int* 2015; **26**: 673-679 [PMID: 25398431 DOI: 10.1007/s00198-014-2927-7]

16 **Samakkarnthai P**, Sfeir JG, Atkinson EJ, Achenbach SJ, Wennberg PW, Dyck PJ, Tweed AJ, Volkman TL, Amin S, Farr JN, Vella A, Drake MT, Khosla S. Determinants of Bone Material Strength and Cortical Porosity in Patients with Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32556277 DOI: 10.1210/clinem/dgaa388]

17 **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]

18 **Paccou J**, Ward KA, Jameson KA, Dennison EM, Cooper C, Edwards MH. Bone Microarchitecture in Men and Women with Diabetes: The Importance of Cortical Porosity. *Calcif Tissue Int* 2016; **98**: 465-473 [PMID: 26686695 DOI: 10.1007/s00223-015-0100-8]

19 **Shanbhogue VV**, Hansen S, Frost M, Jørgensen NR, Hermann AP, Henriksen JE, Brixen K. Compromised cortical bone compartment in type 2 diabetes mellitus patients with microvascular disease. *Eur J Endocrinol* 2016; **174**: 115-124 [PMID: 26537860 DOI: 10.1530/EJE-15-0860]

20 **Farr JN**, Drake MT, Amin S, Melton LJ 3rd, McCready LK, Khosla S. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res* 2014; **29**: 787-795 [PMID: 24123088 DOI: 10.1002/jbmr.2106]

21 **Shu A**, Yin MT, Stein E, Cremers S, Dworakowski E, Ives R, Rubin MR. Bone structure and turnover in type 2 diabetes mellitus. *Osteoporos Int* 2012; **23**: 635-641 [PMID: 21424265 DOI: 10.1007/s00198-011-1595-0]

22 **Furst JR**, Bandeira LC, Fan WW, Agarwal S, Nishiyama KK, McMahon DJ, Dworakowski E, Jiang H, Silverberg SJ, Rubin MR. Advanced Glycation Endproducts and Bone Material Strength in Type 2 Diabetes. *J Clin Endocrinol Metab* 2016; **101**: 2502-2510 [PMID: 27115060 DOI: 10.1210/jc.2016-1437]

23 **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]

24 **Starup-Linde J**, Vestergaard P. Biochemical bone turnover markers in diabetes mellitus - A systematic review. *Bone* 2016; **82**: 69-78 [PMID: 25722065 DOI: 10.1016/j.bone.2015.02.019]

25 **Tonks KT**, White CP, Center JR, Samocha-Bonet D, Greenfield JR. Bone Turnover Is Suppressed in Insulin Resistance, Independent of Adiposity. *J Clin Endocrinol Metab* 2017; **102**: 1112-1121 [PMID: 28324004 DOI: 10.1210/jc.2016-3282]

26 **Starup-Linde J**. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. *Front Endocrinol (Lausanne)* 2013; **4**: 21 [PMID: 23482417 DOI: 10.3389/fendo.2013.00021]

27 **Starup-Linde J**, Lykkeboe S, Handberg A, Vestergaard P, Høyem P, Fleischer J, Hansen TK, Poulsen PL, Laugesen E. Glucose variability and low bone turnover in people with type 2 diabetes. *Bone* 2021; **153**: 116159 [PMID: 34461287 DOI: 10.1016/j.bone.2021.116159]

28 **Starup-Linde J**, Lykkeboe S, Gregersen S, Hauge EM, Langdahl BL, Handberg A, Vestergaard P. Differences in biochemical bone markers by diabetes type and the impact of glucose. *Bone* 2016; **83**: 149-155 [PMID: 26555635 DOI: 10.1016/j.bone.2015.11.004]

29 **Hygum K**, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL. MECHANISMS IN ENDOCRINOLOGY: Diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *Eur J Endocrinol* 2017; **176**: R137-R157 [PMID: 28049653 DOI: 10.1530/EJE-16-0652]

30 **Chen Y**, Zhang W, Chen C, Wang Y, Wang N, Lu Y. Thyroid and bone turnover markers in type 2 diabetes: results from the METAL study. *Endocr Connect* 2022; **11** [PMID: 35196256 DOI: 10.1530/EC-21-0484]

31 **Zha KX**, An ZM, Ge SH, Cai J, Zhou Y, Ying R, Zhou J, Gu T, Guo H, Zhao Y, Wang NJ, Lu YL. FSH may mediate the association between HbA1c and bone turnover markers in postmenopausal women with type 2 diabetes. *J Bone Miner Metab* 2022; **40**: 468-477 [PMID: 35059887 DOI: 10.1007/s00774-021-01301-7]

32 **Barrett EJ**, Liu Z, Khamaisi M, King GL, Klein R, Klein BEK, Hughes TM, Craft S, Freedman BI, Bowden DW, Vinik AI, Casellini CM. Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *J Clin Endocrinol Metab*

2017; **102**: 4343-4410 [PMID: 29126250 DOI: 10.1210/jc.2017-01922]
33 **Bartl R**, Moser W, Burkhardt R, Sandel P, Kamke W, Mähr G, Adelmann BC. [Diabetic osteomyelopathy: histobioptic data of bone and bone marrow in diabetes mellitus (author's transl)]. *Klin Wochenschr* 1978; **56**: 743-754 [PMID: 682529 DOI: 10.1007/BF01476763]
34 **Cirovic A**, Jadzic J, Djukic D, Djonic D, Zivkovic V, Nikolic S, Djuric M, Milovanovic P. Increased Cortical Porosity, Reduced Cortical Thickness, and Reduced Trabecular and Cortical Microhardness of the Superolateral Femoral Neck Confer the Increased Hip Fracture Risk in Individuals with Type 2 Diabetes. *Calcif Tissue Int* 2022 [PMID: 35871240 DOI: 10.1007/s00223-022-01007-6]
35 **Maamar El Asri M**, Pariente Rodrigo E, Díaz-Salazar de la Flor S, Pini Valdivieso S, Ramos Barrón MC, Olmos Martínez JM, Hernández Hernández JL. Trabecular bone score and 25-hydroxyvitamin D levels in microvascular complications of type 2 diabetes mellitus. *Med Clin (Barc)* 2022; **158**: 308-314 [PMID: 34238580 DOI: 10.1016/j.medcli.2021.04.027]
36 **Chen J**, Hendriks M, Chatzis A, Ramasamy SK, Kusumbe AP. Bone Vasculature and Bone Marrow Vascular Niches in Health and Disease. *J Bone Miner Res* 2020; **35**: 2103-2120 [PMID: 32845550 DOI: 10.1002/jbmr.4171]
37 **Stabley JN**, Prisby RD, Behnke BJ, Delp MD. Type 2 diabetes alters bone and marrow blood flow and vascular control mechanisms in the ZDF rat. *J Endocrinol* 2015; **225**: 47-58 [PMID: 25817711 DOI: 10.1530/JOE-14-0514]
38 **Matsumoto T**, Ando N, Tomii T, Uesugi K. Three-dimensional cortical bone microstructure in a rat model of hypoxia-induced growth retardation. *Calcif Tissue Int* 2011; **88**: 54-62 [PMID: 20848090 DOI: 10.1007/s00223-010-9415-7]
39 **Delaisse JM**, Andersen TL, Kristensen HB, Jensen PR, Andreasen CM, Søe K. Re-thinking the bone remodeling cycle mechanism and the origin of bone loss. *Bone* 2020; **141**: 115628 [PMID: 32919109 DOI: 10.1016/j.bone.2020.115628]
40 **Shanbhogue VV**, Hansen S, Frost M, Brixen K, Hermann AP. Bone disease in diabetes: another manifestation of microvascular disease? *Lancet Diabetes Endocrinol* 2017; **5**: 827-838 [PMID: 28546096 DOI: 10.1016/S2213-8587(17)30134-1]
41 **Singh R**, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia* 2001; **44**: 129-146 [PMID: 11270668 DOI: 10.1007/s001250051591]
42 **Strollo R**, Rizzo P, Spoletini M, Landy R, Hughes C, Ponchel F, Napoli N, Palermo A, Buzzetti R, Pozzilli P, Nissim A. HLA-dependent autoantibodies against post-translationally modified collagen type II in type 1 diabetes mellitus. *Diabetologia* 2013; **56**: 563-572 [PMID: 23160643 DOI: 10.1007/s00125-012-2780-1]
43 **Hernandez CJ**, Tang SY, Baumbach BM, Hwu PB, Sakkee AN, van der Ham F, DeGroot J, Bank RA, Keaveny TM. Trabecular microfracture and the influence of pyridinium and non-enzymatic glycation-mediated collagen cross-links. *Bone* 2005; **37**: 825-832 [PMID: 16140600 DOI: 10.1016/j.bone.2005.07.019]
44 **Ahmed N**. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract* 2005; **67**: 3-21 [PMID: 15620429 DOI: 10.1016/j.diabres.2004.09.004]
45 **Epstein S**, Defeudis G, Manfrini S, Napoli N, Pozzilli P; Scientific Committee of the First International Symposium on Diabetes and Bone. Diabetes and disordered bone metabolism (diabetic osteodystrophy): time for recognition. *Osteoporos Int* 2016; **27**: 1931-1951 [PMID: 26980458 DOI: 10.1007/s00198-015-3454-x]
46 **Manigrasso MB**, Juranek J, Ramasamy R, Schmidt AM. Unlocking the biology of RAGE in diabetic microvascular complications. *Trends Endocrinol Metab* 2014; **25**: 15-22 [PMID: 24011512 DOI: 10.1016/j.tem.2013.08.002]
47 **Huebschmann AG**, Regensteiner JG, Vlassara H, Reusch JE. Diabetes and advanced glycoxidation end products. *Diabetes Care* 2006; **29**: 1420-1432 [PMID: 16732039 DOI: 10.2337/dc05-2096]
48 **Sanguineti R**, Puddu A, Mach F, Montecucco F, Viviani GL. Advanced glycation end products play adverse proinflammatory activities in osteoporosis. *Mediators Inflamm* 2014; **2014**: 975872 [PMID: 24771986 DOI: 10.1155/2014/975872]
49 **Saito M**, Fujii K, Soshi S, Tanaka T. Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation-induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. *Osteoporos Int* 2006; **17**: 986-995 [PMID: 16552468 DOI: 10.1007/s00198-006-0087-0]
50 **Sanguineti R**, Storace D, Monacelli F, Federici A, Odetti P. Pentosidine effects on human osteoblasts in vitro. *Ann N Y Acad Sci* 2008; **1126**: 166-172 [PMID: 18448811 DOI: 10.1196/annals.1433.044]
51 **McCarthy AD**, Uemura T, Etcheverry SB, Cortizo AM. Advanced glycation endproducts interefere with integrin-mediated osteoblastic attachment to a type-I collagen matrix. *Int J Biochem Cell Biol* 2004; **36**: 840-848 [PMID: 15006636 DOI: 10.1016/j.biocel.2003.09.006]
52 **Kume S**, Kato S, Yamagishi S, Inagaki Y, Ueda S, Arima N, Okawa T, Kojiro M, Nagata K. Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. *J Bone Miner Res* 2005; **20**: 1647-1658 [PMID: 16059636 DOI: 10.1359/JBMR.050514]
53 **Piccoli A**, Cannata F, Strollo R, Pedone C, Leanza G, Russo F, Greto V, Isgrò C, Quattrocchi CC, Massaroni C, Silvestri S, Vadalà G, Bisogno T, Denaro V, Pozzilli P, Tang SY, Silva MJ, Conte C, Papalia R, Maccarrone M, Napoli N. Sclerostin Regulation, Microarchitecture, and Advanced Glycation End-Products in the Bone of Elderly Women With Type 2 Diabetes. *J Bone Miner Res* 2020; **35**: 2415-2422 [PMID: 32777114 DOI: 10.1002/jbmr.4153]
54 **McCarthy AD**, Etcheverry SB, Cortizo AM. Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-I and its binding proteins: role in osteoblast development. *Acta Diabetol* 2001; **38**: 113-122 [PMID: 11827431 DOI: 10.1007/s005920170007]
55 **Kanazawa I**, Yamaguchi T, Sugimoto T. Serum insulin-like growth factor-I is a marker for assessing the severity of vertebral fractures in postmenopausal women with type 2 diabetes mellitus. *Osteoporos Int* 2011; **22**: 1191-1198 [PMID: 20532480 DOI: 10.1007/s00198-010-1310-6]
56 **Khosla S**, Farr JN, Tchkonia T, Kirkland JL. The role of cellular senescence in ageing and endocrine disease. *Nat Rev Endocrinol* 2020; **16**: 263-275 [PMID: 32161396 DOI: 10.1038/s41574-020-0335-y]
57 **Alcorta DA**, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci U S A* 1996; **93**: 13742-13747

[PMID: 8943005 DOI: 10.1073/pnas.93.24.13742]
58 **Beauséjour CM**, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J* 2003; **22**: 4212-4222 [PMID: 12912919 DOI: 10.1093/emboj/cdg417]
59 **Wissler Gerdes EO**, Zhu Y, Tchkonia T, Kirkland JL. Discovery, development, and future application of senolytics: theories and predictions. *FEBS J* 2020; **287**: 2418-2427 [PMID: 32112672 DOI: 10.1111/febs.15264]
60 **Coppé JP**, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010; **5**: 99-118 [PMID: 20078217 DOI: 10.1146/annurev-pathol-121808-102144]
61 **Acosta JC**, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, Athineos D, Kang TW, Lasitschka F, Andrulis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AP, Carroll T, Capper D, Pritchard C, Inman GJ, Longerich T, Sansom OJ, Benitah SA, Zender L, Gil J. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* 2013; **15**: 978-990 [PMID: 23770676 DOI: 10.1038/ncb2784]
62 **Aguayo-Mazzucato C**, Andle J, Lee TB Jr, Midha A, Talemal L, Chipashvili V, Hollister-Lock J, van Deursen J, Weir G, Bonner-Weir S. Acceleration of β Cell Aging Determines Diabetes and Senolysis Improves Disease Outcomes. *Cell Metab* 2019; **30**: 129-142.e4 [PMID: 31155496 DOI: 10.1016/j.cmet.2019.05.006]
63 **Eckhardt BA**, Rowsey JL, Thicke BS, Fraser DG, O'Grady KL, Bondar OP, Hines JM, Singh RJ, Thoreson AR, Rakshit K, Lagnado AB, Passos JF, Vella A, Matveyenko AV, Khosla S, Monroe DG, Farr JN. Accelerated osteocyte senescence and skeletal fragility in mice with type 2 diabetes. *JCI Insight* 2020; **5** [PMID: 32267250 DOI: 10.1172/jci.insight.135236]
64 **Figeac F**, Tencerova M, Ali D, Andersen TL, Appadoo DRC, Kerckhofs G, Ditzel N, Kowal JM, Rauch A, Kassem M. Impaired Bone Fracture Healing in Type 2 Diabetes Is Caused by Defective Functions of Skeletal Progenitor Cells. *Stem Cells* 2022; **40**: 149-164 [PMID: 35257177 DOI: 10.1093/stmcls/sxab011]
65 **Heilmeier U**, Hackl M, Schroeder F, Torabi S, Kapoor P, Vierlinger K, Eiriksdottir G, Gudmundsson EF, Harris TB, Gudnason V, Link TM, Grillari J, Schwartz AV. Circulating serum microRNAs including senescent miR-31-5p are associated with incident fragility fractures in older postmenopausal women with type 2 diabetes mellitus. *Bone* 2022; **158**: 116308 [PMID: 35066213 DOI: 10.1016/j.bone.2021.116308]
66 **Brunetti G**, D'Amato G, Chiarito M, Tullo A, Colaianni G, Colucci S, Grano M, Faienza MF. An update on the role of RANKL-RANK/osteoprotegerin and WNT-ß-catenin signaling pathways in pediatric diseases. *World J Pediatr* 2019; **15**: 4-11 [PMID: 30343446 DOI: 10.1007/s12519-018-0198-7]
67 **Kim JY**, Lee SK, Jo KJ, Song DY, Lim DM, Park KY, Bonewald LF, Kim BJ. Exendin-4 increases bone mineral density in type 2 diabetic OLETF rats potentially through the down-regulation of SOST/sclerostin in osteocytes. *Life Sci* 2013; **92**: 533-540 [PMID: 23357248 DOI: 10.1016/j.lfs.2013.01.001]
68 **Nuche-Berenguer B**, Moreno P, Portal-Nuñez S, Dapía S, Esbrit P, Villanueva-Peñacarrillo ML. Exendin-4 exerts osteogenic actions in insulin-resistant and type 2 diabetic states. *Regul Pept* 2010; **159**: 61-66 [PMID: 19586609 DOI: 10.1016/j.regpep.2009.06.010]
69 **Gennari L**, Merlotti D, Valenti R, Ceccarelli E, Ruvio M, Pietrini MG, Capodarca C, Franci MB, Campagna MS, Calabrò A, Cataldo D, Stolakis K, Dotta F, Nuti R. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. *J Clin Endocrinol Metab* 2012; **97**: 1737-1744 [PMID: 22399511 DOI: 10.1210/jc.2011-2958]
70 **Gaudio A**, Privitera F, Battaglia K, Torrisi V, Sidoti MH, Pulvirenti I, Canzonieri E, Tringali G, Fiore CE. Sclerostin levels associated with inhibition of the Wnt/β-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012; **97**: 3744-3750 [PMID: 22855334 DOI: 10.1210/jc.2012-1901]
71 **Yamamoto M**, Yamauchi M, Sugimoto T. Elevated sclerostin levels are associated with vertebral fractures in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2013; **98**: 4030-4037 [PMID: 23894157 DOI: 10.1210/jc.2013-2143]
72 **Heilmeier U**, Carpenter DR, Patsch JM, Harnish R, Joseph GB, Burghardt AJ, Baum T, Schwartz AV, Lang TF, Link TM. Volumetric femoral BMD, bone geometry, and serum sclerostin levels differ between type 2 diabetic postmenopausal women with and without fragility fractures. *Osteoporos Int* 2015; **26**: 1283-1293 [PMID: 25582311 DOI: 10.1007/s00198-014-2988-7]
73 **Wang Y**, Liu Y, Huang Z, Chen X, Zhang B. The roles of osteoprotegerin in cancer, far beyond a bone player. *Cell Death Discov* 2022; **8**: 252 [PMID: 35523775 DOI: 10.1038/s41420-022-01042-0]
74 **Takegahara N**, Kim H, Choi Y. RANKL biology. *Bone* 2022; **159**: 116353 [PMID: 35181574 DOI: 10.1016/j.bone.2022.116353]
75 **Wittrant Y**, Gorin Y, Woodruff K, Horn D, Abboud HE, Mohan S, Abboud-Werner SL. High d(+)glucose concentration inhibits RANKL-induced osteoclastogenesis. *Bone* 2008; **42**: 1122-1130 [PMID: 18378205 DOI: 10.1016/j.bone.2008.02.006]
76 **Duan P**, Yang M, Wei M, Liu J, Tu P. Serum Osteoprotegerin Is a Potential Biomarker of Insulin Resistance in Chinese Postmenopausal Women with Prediabetes and Type 2 Diabetes. *Int J Endocrinol* 2017; **2017**: 8724869 [PMID: 28255300 DOI: 10.1155/2017/8724869]
77 **Dede AD**, Tournis S, Dontas I, Trovas G. Type 2 diabetes mellitus and fracture risk. *Metabolism* 2014; **63**: 1480-1490 [PMID: 25284729 DOI: 10.1016/j.metabol.2014.09.002]
78 **Secchiero P**, Corallini F, Pandolfi A, Consoli A, Candido R, Fabris B, Celeghini C, Capitani S, Zauli G. An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. *Am J Pathol* 2006; **169**: 2236-2244 [PMID: 17148684 DOI: 10.2353/ajpath.2006.060398]
79 **Sassi F**, Buondonno I, Luppi C, Spertino E, Stratta E, Di Stefano M, Ravazzoli M, Isaia G, Trento M, Passera P, Porta M, Isaia GC, D'Amelio P. Type 2 diabetes affects bone cells precursors and bone turnover. *BMC Endocr Disord* 2018; **18**: 55 [PMID: 30089481 DOI: 10.1186/s12902-018-0283-x]
80 **Panezai J**, Altamash M, Engström PE, Larsson A. Association of Glycated Proteins with Inflammatory Proteins and Periodontal Disease Parameters. *J Diabetes Res* 2020; **2020**: 6450742 [PMID: 31998807 DOI: 10.1155/2020/6450742]
81 **Cherian P**, Al-Khairi I, Jamal M, Al-Sabah S, Ali H, Dsouza C, Alshawaf E, Al-Ali W, Al-Khaledi G, Al-Mulla F, Abu-Farha M, Abubaker J. Association Between Factors Involved in Bone Remodeling (Osteoactivin and OPG) With Plasma

Levels of Irisin and Meteorin-Like Protein in People With T2D and Obesity. *Front Endocrinol (Lausanne)* 2021; **12**: 752892 [PMID: 34777249 DOI: 10.3389/fendo.2021.752892]

82 **Kiechl S**, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, Moschen AR, Muscogiuri G, Sorice GP, Kireva T, Summerer M, Wirtz S, Luther J, Mielenz D, Billmeier U, Egger G, Mayr A, Oberhollenzer F, Kronenberg F, Orthofer M, Penninger JM, Meigs JB, Bonora E, Tilg H, Willeit J, Schett G. Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med* 2013; **19**: 358-363 [PMID: 23396210 DOI: 10.1038/nm.3084]

83 **Miranda C**, Giner M, Montoya MJ, Vázquez MA, Miranda MJ, Pérez-Cano R. Influence of high glucose and advanced glycation end-products (ages) levels in human osteoblast-like cells gene expression. *BMC Musculoskelet Disord* 2016; **17**: 377 [PMID: 27582133 DOI: 10.1186/s12891-016-1228-z]

84 **Rosset EM**, Bradshaw AD. SPARC/osteonectin in mineralized tissue. *Matrix Biol* 2016; **52-54**: 78-87 [PMID: 26851678 DOI: 10.1016/j.matbio.2016.02.001]

85 **Boskey AL**, Moore DJ, Amling M, Canalis E, Delany AM. Infrared analysis of the mineral and matrix in bones of osteonectin-null mice and their wildtype controls. *J Bone Miner Res* 2003; **18**: 1005-1011 [PMID: 12817752 DOI: 10.1359/jbmr.2003.18.6.1005]

86 **Delany AM**, Amling M, Priemel M, Howe C, Baron R, Canalis E. Osteopenia and decreased bone formation in osteonectin-deficient mice. *J Clin Invest* 2000; **105**: 915-923 [PMID: 10749571 DOI: 10.1172/JCI7039]

87 **Dole NS**, Kapinas K, Kessler CB, Yee SP, Adams DJ, Pereira RC, Delany AM. A single nucleotide polymorphism in osteonectin 3' untranslated region regulates bone volume and is targeted by miR-433. *J Bone Miner Res* 2015; **30**: 723-732 [PMID: 25262637 DOI: 10.1002/jbmr.2378]

88 **Xu L**, Niu M, Yu W, Xia W, Gong F, Wang O. Associations between FGF21, osteonectin and bone turnover markers in type 2 diabetic patients with albuminuria. *J Diabetes Complications* 2017; **31**: 583-588 [PMID: 27916484 DOI: 10.1016/j.jdiacomp.2016.11.012]

89 **Afkarian M**, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, de Boer IH. Clinical Manifestations of Kidney Disease Among US Adults With Diabetes, 1988-2014. *JAMA* 2016; **316**: 602-610 [PMID: 27532915 DOI: 10.1001/jama.2016.10924]

90 **Zhou Z**, Chaudhari P, Yang H, Fang AP, Zhao J, Law EH, Wu EQ, Jiang R, Seifeldin R. Healthcare Resource Use, Costs, and Disease Progression Associated with Diabetic Nephropathy in Adults with Type 2 Diabetes: A Retrospective Observational Study. *Diabetes Ther* 2017; **8**: 555-571 [PMID: 28361464 DOI: 10.1007/s13300-017-0256-5]

91 **Jepson C**, Hsu JY, Fischer MJ, Kusek JW, Lash JP, Ricardo AC, Schelling JR, Feldman HI; Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. Incident Type 2 Diabetes Among Individuals With CKD: Findings From the Chronic Renal Insufficiency Cohort (CRIC) Study. *Am J Kidney Dis* 2019; **73**: 72-81 [PMID: 30177484 DOI: 10.1053/j.ajkd.2018.06.017]

92 **Ball AM**, Gillen DL, Sherrard D, Weiss NS, Emerson SS, Seliger SL, Kestenbaum BR, Stehman-Breen C. Risk of hip fracture among dialysis and renal transplant recipients. *JAMA* 2002; **288**: 3014-3018 [PMID: 12479766 DOI: 10.1001/jama.288.23.3014]

93 **Daya N**, Voskertchian A, Schneider ALC, Ballew S, McAdams DeMarco M, Coresh J, Appel LJ, Selvin E, Grams ME. Kidney Function and Fracture Risk: The Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis* 2016; **67**: 218-226 [PMID: 26250781 DOI: 10.1053/j.ajkd.2015.06.020]

94 **Alshayeb HM**, Josephson MA, Sprague SM. CKD-mineral and bone disorder management in kidney transplant recipients. *Am J Kidney Dis* 2013; **61**: 310-325 [PMID: 23102732 DOI: 10.1053/j.ajkd.2012.07.022]

95 **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]

96 **Hofbauer LC**, Busse B, Eastell R, Ferrari S, Frost M, Müller R, Burden AM, Rivadeneira F, Napoli N, Rauner M. Bone fragility in diabetes: novel concepts and clinical implications. *Lancet Diabetes Endocrinol* 2022; **10**: 207-220 [PMID: 35101185 DOI: 10.1016/S2213-8587(21)00347-8]

97 **Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group**. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl* 2009; S1-130 [PMID: 19644521 DOI: 10.1038/ki.2009.188]

98 **Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group**. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl (2011)* 2017; **7**: 1-59 [PMID: 30675420 DOI: 10.1016/j.kisu.2017.04.001]

99 **Ketteler M**, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, Moe SM, Shroff R, Tonelli MA, Toussaint ND, Vervloet MG, Leonard MB. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. *Kidney Int* 2017; **92**: 26-36 [PMID: 28646995 DOI: 10.1016/j.kint.2017.04.006]

100 **Ray S**, Beatrice AM, Ghosh A, Pramanik S, Bhattacharjee R, Ghosh S, Raychaudhury A, Mukhopadhyay S, Chowdhury S. Profile of chronic kidney disease related-mineral bone disorders in newly diagnosed advanced predialysis diabetic kidney disease patients: A hospital based cross-sectional study. *Diabetes Metab Syndr* 2017; **11** Suppl 2: S931-S937 [PMID: 28728874 DOI: 10.1016/j.dsx.2017.07.019]

101 **Ribeiro AL**, Mendes F, Carias E, Rato F, Santos N, Neves PL, Silva AP. FGF23-klotho axis as predictive factors of fractures in type 2 diabetics with early chronic kidney disease. *J Diabetes Complications* 2020; **34**: 107476 [PMID: 31708378 DOI: 10.1016/j.jdiacomp.2019.107476]

102 **Yeung SMH**, Bakker SJL, Laverman GD, De Borst MH. Fibroblast Growth Factor 23 and Adverse Clinical Outcomes in Type 2 Diabetes: a Bitter-Sweet Symphony. *Curr Diab Rep* 2020; **20**: 50 [PMID: 32857288 DOI: 10.1007/s11892-020-01335-7]

103 **Desbiens LC**, Sidibé A, Ung RV, Fortier C, Munger M, Wang YP, Bisson SK, Marquis K, Agharazii M, Mac-Way F. FGF23-klotho axis, bone fractures, and arterial stiffness in dialysis: a case-control study. *Osteoporos Int* 2018; **29**: 2345-

2353 [PMID: 29959497 DOI: 10.1007/s00198-018-4598-2]
104 **Jadoul M**, Albert JM, Akiba T, Akizawa T, Arab L, Bragg-Gresham JL, Mason N, Prutz KG, Young EW, Pisoni RL. Incidence and risk factors for hip or other bone fractures among hemodialysis patients in the Dialysis Outcomes and Practice Patterns Study. *Kidney Int* 2006; **70**: 1358-1366 [PMID: 16929251 DOI: 10.1038/sj.ki.5001754]
105 **Nakashima A**, Yokoyama K, Kawanami D, Ohkido I, Urashima M, Utsunomiya K, Yokoo T. Association between resistin and fibroblast growth factor 23 in patients with type 2 diabetes mellitus. *Sci Rep* 2018; **8**: 13999 [PMID: 30228288 DOI: 10.1038/s41598-018-32432-z]
106 **Takashi Y**, Kawanami D. The Role of Bone-Derived Hormones in Glucose Metabolism, Diabetic Kidney Disease, and Cardiovascular Disorders. *Int J Mol Sci* 2022; **23** [PMID: 35216490 DOI: 10.3390/ijms23042376]
107 **Titan SM**, Zatz R, Graciolli FG, dos Reis LM, Barros RT, Jorgetti V, Moysés RM. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin J Am Soc Nephrol* 2011; **6**: 241-247 [PMID: 20966122 DOI: 10.2215/CJN.04250510]
108 **Silva AP**, Mendes F, Fragoso A, Jeronimo T, Pimentel A, Gundlach K, Büchel J, Santos N, Neves PL. Altered serum levels of FGF-23 and magnesium are independent risk factors for an increased albumin-to-creatinine ratio in type 2 diabetics with chronic kidney disease. *J Diabetes Complications* 2016; **30**: 275-280 [PMID: 26750742 DOI: 10.1016/j.jdiacomp.2015.11.006]
109 **Chen CD**, Podvin S, Gillespie E, Leeman SE, Abraham CR. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. *Proc Natl Acad Sci U S A* 2007; **104**: 19796-19801 [PMID: 18056631 DOI: 10.1073/pnas.0709805104]
110 **Sakan H**, Nakatani K, Asai O, Imura A, Tanaka T, Yoshimoto S, Iwamoto N, Kurumatani N, Iwano M, Nabeshima Y, Konishi N, Saito Y. Reduced renal α-Klotho expression in CKD patients and its effect on renal phosphate handling and vitamin D metabolism. *PLoS One* 2014; **9**: e86301 [PMID: 24466013 DOI: 10.1371/journal.pone.0086301]
111 **Castellano G**, Intini A, Stasi A, Divella C, Gigante M, Pontrelli P, Franzin R, Accetturo M, Zito A, Fiorentino M, Montinaro V, Lucarelli G, Ditonno P, Battaglia M, Crovace A, Staffieri F, Oortwijn B, van Amersfoort E, Pertosa G, Grandaliano G, Gesualdo L. Complement Modulation of Anti-Aging Factor Klotho in Ischemia/Reperfusion Injury and Delayed Graft Function. *Am J Transplant* 2016; **16**: 325-333 [PMID: 26280899 DOI: 10.1111/ajt.13415]
112 **Mezzolla V**, Pontrelli P, Fiorentino M, Stasi A, Pesce F, Franzin R, Rascio F, Grandaliano G, Stallone G, Infante B, Gesualdo L, Castellano G. Emerging biomarkers of delayed graft function in kidney transplantation. *Transplant Rev (Orlando)* 2021; **35**: 100629 [PMID: 34118742 DOI: 10.1016/j.trre.2021.100629]
113 **Infante B**, Bellanti F, Correale M, Pontrelli P, Franzin R, Leo S, Calvaruso M, Mercuri S, Netti GS, Ranieri E, Brunetti ND, Grandaliano G, Gesualdo L, Serviddio G, Castellano G, Stallone G. mTOR inhibition improves mitochondria function/biogenesis and delays cardiovascular aging in kidney transplant recipients with chronic graft dysfunction. *Aging (Albany NY)* 2021; **13**: 8026-8039 [PMID: 33758105 DOI: 10.18632/aging.202863]
114 **Tataranni T**, Biondi G, Cariello M, Mangino M, Colucci G, Rutigliano M, Ditonno P, Schena FP, Gesualdo L, Grandaliano G. Rapamycin-induced hypophosphatemia and insulin resistance are associated with mTORC2 activation and Klotho expression. *Am J Transplant* 2011; **11**: 1656-1664 [PMID: 21672148 DOI: 10.1111/j.1600-6143.2011.03590.x]
115 **Gigante M**, Lucarelli G, Divella C, Netti GS, Pontrelli P, Cafiero C, Grandaliano G, Castellano G, Rutigliano M, Stallone G, Bettocchi C, Ditonno P, Gesualdo L, Battaglia M, Ranieri E. Soluble Serum αKlotho Is a Potential Predictive Marker of Disease Progression in Clear Cell Renal Cell Carcinoma. *Medicine (Baltimore)* 2015; **94**: e1917 [PMID: 26559258 DOI: 10.1097/MD.0000000000001917]
116 **Kuro-O M**. The Klotho proteins in health and disease. *Nat Rev Nephrol* 2019; **15**: 27-44 [PMID: 30455427 DOI: 10.1038/s41581-018-0078-3]
117 **Hu MC**, Kuro-o M, Moe OW. Klotho and chronic kidney disease. *Contrib Nephrol* 2013; **180**: 47-63 [PMID: 23652549 DOI: 10.1159/000346778]
118 **Poole KE**, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Löwik CW, Reeve J. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J* 2005; **19**: 1842-1844 [PMID: 16123173 DOI: 10.1096/fj.05-4221fje]
119 **Figurek A**, Rroji M, Spasovski G. Sclerostin: a new biomarker of CKD-MBD. *Int Urol Nephrol* 2020; **52**: 107-113 [PMID: 31612420 DOI: 10.1007/s11255-019-02290-3]
120 **Kim SH**, Yoon SY, Lim SK, Rhee Y. The effect of renal dysfunction on circulating sclerostin level in patients with type 2 diabetes. *Int J Endocrinol* 2014; **2014**: 715908 [PMID: 25053944 DOI: 10.1155/2014/715908]
121 **Yoshimura N**, Hashimoto T, Sakata K, Morioka S, Kasamatsu T, Cooper C. Biochemical markers of bone turnover and bone loss at the lumbar spine and femoral neck: the Taiji study. *Calcif Tissue Int* 1999; **65**: 198-202 [PMID: 10441650 DOI: 10.1007/s002239900682]
122 **Lee NK**, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; **130**: 456-469 [PMID: 17693256 DOI: 10.1016/j.cell.2007.05.047]
123 **Wei J**, Ferron M, Clarke CJ, Hannun YA, Jiang H, Blaner WS, Karsenty G. Bone-specific insulin resistance disrupts whole-body glucose homeostasis *via* decreased osteocalcin activation. *J Clin Invest* 2014; **124**: 1-13 [PMID: 24642469 DOI: 10.1172/JCI72323]
124 **Ye X**, Yu R, Jiang F, Hou X, Wei L, Bao Y, Jia W. Osteocalcin and Risks of Incident Diabetes and Diabetic Kidney Disease: A 4.6-Year Prospective Cohort Study. *Diabetes Care* 2022; **45**: 830-836 [PMID: 35090006 DOI: 10.2337/dc21-2113]
125 **Bacchetta J**, Boutroy S, Guebre-Egziabher F, Juillard L, Drai J, Pelletier S, Richard M, Charrié A, Carlier MC, Chapurlat R, Laville M, Fouque D. The relationship between adipokines, osteocalcin and bone quality in chronic kidney disease. *Nephrol Dial Transplant* 2009; **24**: 3120-3125 [PMID: 19515806 DOI: 10.1093/ndt/gfp262]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 912-920

DOI: 10.4239/wjd.v13.i11.912

ISSN 1948-9358 (online)

MINIREVIEWS

Orthotic approach to prevention and management of diabetic foot: A narrative review

Min Cheol Chang, Yoo Jin Choo, In Sik Park, Myung Woo Park, Du Hwan Kim

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

P-Reviewer: Miranda C, Italy; Ran X, China

Received: July 15, 2022
Peer-review started: July 15, 2022
First decision: August 7, 2022
Revised: August 24, 2022
Accepted: October 11, 2022
Article in press: October 11, 2022
Published online: November 15, 2022

Min Cheol Chang, Yoo Jin Choo, Rehabilitation Medicine, Yeungnam University, Daegu 42415, South Korea

In Sik Park, Korean Podiatry and Pedorthics Institute, Goyang 10442, South Korea

Myung Woo Park, Du Hwan Kim, Physical Medicine and Rehabilitation, Chung-Ang University, Seoul 06973, South Korea

Corresponding author: Du Hwan Kim, MD, PhD, Associate Professor, Physical Medicine and Rehabilitation, Chung-Ang University, 102, Heukseok-ro, Dongjak-gu, Seoul 06973, South Korea. ri-pheonix@hanmail.net

Abstract

Diabetic foot is a common complication affecting more than one-fifth of patients with diabetes. If not treated in time, it may lead to diabetic foot ulcers or Charcot arthropathy. For the management of diabetic foot, shoe modifications and orthoses can be used to reduce pressure on the affected foot or provide the foot with increased stability. In addition, the shoe modifications and orthotic devices can relieve patient discomfort during walking. Appropriate shoe modifications include changing the insole material, modifying the heel height, adding a steel shank or rocker sole, and using in-depth shoes. Alternatively, a walking brace or ankle-foot orthosis can be used to reduce the pressure on the affected foot. The purpose of this narrative review was to provide a reference guide to support clinicians in prescribing shoe modifications and foot orthoses to treat diabetic foot ulcers and Charcot arthropathy.

Key Words: Diabetic foot; Foot ulcers; Charcot arthropathy; Shoes; Foot orthosis

Core Tip: Footwear modifications and orthosis in diabetic foot management are aimed to prevent ulcers, protect the foot from external stimuli, and regulate the pressure on the foot. Types of shoe modifications include using an in-depth shoe, combination of insole materials, lifting the heel, applying a rocker sole, and applying an extended steel shank or flare or stabilizer. Orthosis includes prefabricated removable walking brace (such as control ankle motion walker, pneumatic walker, and conformer walking boot), Arizona ankle-foot orthosis, patellar-tendon-bearing orthosis, and Charcot restraint orthotic walker.

Citation: Chang MC, Choo YJ, Park IS, Park MW, Kim DH. Orthotic approach to prevention and management of diabetic foot: A narrative review. *World J Diabetes* 2022; 13(11): 912-920
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/912.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.912

INTRODUCTION

Diabetic foot is an infection, ulceration, or destruction of the tissues of the foot. It is associated with neuropathy and/or peripheral artery disease in the lower extremity of a person with diabetes mellitus [1]. The global annual incidence of diabetic foot ulcers in patients with diabetes was reported to be approximately 2%-5%, with a lifetime risk ranging from 15%-20%[2]. According to a report by the American Diabetes Association, 20% of patients with diabetes are hospitalized because of foot problems [3]. The main underlying causes of diabetic foot ulcer are peripheral neuropathy and ischemia due to peripheral vascular disease[4,5]. Once a foot ulcer develops, it may progress to foot infection, which if incurable, could eventually require limb amputation[4]. Most diabetic foot ulcers are caused by repetitive trauma due to weight-bearing or poorly fitting footwear, and ulcers often recur if the weight-bearing is not suppressed and the biomechanical abnormality of the foot is not controlled[5,6]. Prevention is important for effective management of the diabetic foot, with methods including continuous education and proper footwear for the patients with diabetes[6]. Such methods have been reported to prevent approximately 80% of all limb amputations due to diabetes[7]. Unfortunately, however, many patients are unaware of the diabetic foot, have never received proper education on its prevention, for example by wearing adequate shoes, or often receive treatment only after the problems, such as foot ulcers and neuropathic arthropathy, occur[8]. Therefore, it is essential for clinicians dealing with diabetic foot to be aware of the possible feet abnormalities due to diabetic complications and the importance of educating the patients with diabetes, including wearing the right footwear.

Another disease that can occur frequently as a complication of diabetes is Charcot arthropathy. The pathogenesis of Charcot arthropathy is not clearly known yet. It is believed to be a phenomenon in which the bone is weakened due to severe bone deficiency caused by autonomic nervous system abnormality or a local increase in blood flow to the bone[9]. Weakened bones are prone to fractures even under a very small weight, and the ligaments may also weaken, resulting in dislocation or subluxation [10]. Patients with Charcot arthropathy fail to sense pain in their limbs and continue to apply their weight, leading to bone deformity that further changes the normal force transmission path; this increases the chance of a fracture or dislocation and, consequently, deforms the weight-bearing part [11]. Over time, the process stops, and the deformity becomes permanent. Owing to the dislocation, the load cannot be distributed effectively and stably during walking, resulting in movement limitations, and the persisting bone protrusion eventually causes foot ulcers[12]. Furthermore, Charcot foot is characterized by a collapsed arch[9,10]. The non-surgical treatment for Charcot arthropathy is based on off-loading and edema control principles. The goal is to prevent the foot ulcers by minimizing mechanical stress, reducing edema, and creating structural stability, all of which help in restricting the weight-bearing and stabilizing the joint until the Charcot arthropathy is sufficiently controlled[5].

Orthoses play a very important role in treating diabetic feet that have already developed foot ulcers and Charcot arthropathy. Orthotic devices not only provide stability, limit the joint movement, and control foot deformity, but also relieve the load and evenly distribute the pressure on the foot. As a result, orthoses can effectively heal foot ulcers and control the symptoms of Charcot arthropathy. This review describes the management of diabetic feet using properly fitting shoes and orthoses.

FOOT ULCER CLASSIFICATION

Wagner classified foot ulcers from Category 0-3 based on the loss of protective sensation, foot deformity, and history of ulcer or ischemia[13]. Category 0 applies to cases where none of the following applies: Loss of protective sensation, deformity, callus, weakness, or history of ulceration or ischemia. Such cases are dealt with educating the patients on basic foot care and recommending conventional footwear. Category 1 solely involves the loss of protective sensation, and the use of in-depth shoes or

sneakers, non-molded soft inlays, and total contact orthoses is recommended. Category 2 involves foot deformity along with the loss of protective sensation and requires the use of in-depth shoes or sneakers, custom-molded foot orthoses, and external shoe modifications, if necessary. Category 3 involves all three factors, namely loss of protective sensation, foot deformity, and history of ulcer or ischemia, and requires custom-fabricated, pressure-dissipating accommodative foot orthoses, with additional recommendations for inlay-depth, soft-leather, adjustable-lacing shoes, and external shoe modifications, if necessary[13]. As illustrated above, the number of requirements for orthoses or properly fitting footwear and the complexity of prescriptions increase with the rising risk of foot ulcers.

ADAPTED FOOTWEAR

Ill-fitting footwear is a common cause of foot ulcers, whereas therapeutic footwear plays an important role in reducing the likelihood of foot ulcers[14]. Foot ulcers recur in about half the patients who wear shoes without modifications, compared to a recurrence rate of approximately 20% when an appropriate protective footwear is worn[5]. While prescribing adapted footwear for patients with diabetes, the goals are to protect the feet from the external environment, relieve excessive pressure, reduce impact and shear force, control foot deformity, and stabilize the movement[14]. The primary role of adapted footwear is to protect the feet from further harm by reducing the pressure on the affected area rather than treating the foot ulcers themselves and preventing their occurrence or recurrence[14,15]. The goal should be to reduce the pressure by at least 30% or to less than 200 kPa, where the pressure on the sole is the highest[16].

GENERAL PRINCIPLES FOR PRESCRIBING SHOES FOR PATIENTS WITH DIABETES

The shoe should conform to the shape of the foot, and there should be enough space inside, similar to an in-depth shoe. To determine the appropriate shoe size, the overall foot length, arch length, and foot width should be measured[17]. The shoes must be manufactured to accommodate the first and fifth metatarsophalangeal joints, which represent the widest part of the foot, while the shoe length should be such that there is a space of 1.3-1.6 cm between the longest toe and the tip of the shoe[17]. Additionally, the ball of the shoe should match in width with the ball of the foot, and the counter should not press the starting point of the Achilles tendon[8,17]. The insole should be removable and of triple-depth; leather insole is not recommended, as the primary aim is to minimize shear and friction[8]. The heel of the shoe is manufactured to be typically 2.5-5 cm high. If the height of the heel is over 5 cm, the pressure on the forefoot increases excessively[8,18]. Regarding the plantar surface, especially when the forefoot has an ulcer, a rigid rocker or a rigid rocker sole can reduce the pressure and help in healing of the ulcer[8]. It is recommended to purchase the shoes in the afternoon when the feet are swollen. In addition to checking the shoe size, the shoes must be tried on to check if it can correctly support the shape and the size of the feet when weight load is applied[8,17].

IN-DEPTH SHOE

In-depth shoes are usually blucher-type Oxford shoes that are 0.6-1.3 cm deeper than the conventional shoes. This extra space helps when using insoles and foot orthoses, which are necessary in cases of foot deformity due to Charcot arthropathy[8,17]. The in-depth shoes should have a light weight, good shock absorption capacity, and strong heel counter[8]. In the past, the upper material was mostly made of soft leather, but nowadays, breathable synthetic material is commonly used[8]. In addition, the relatively softer insoles are layered, and the lower the density of the insole, the more is the cushioning at the interface between the foot and the insole[19]. The insole is generally thermoformed to contour the patient's foot, and the outer sole is also modified to further reduce the pressure[19]. In addition to the Oxford shoes, sneakers may also be used. Sneakers have several advantages in terms of the depth, removable insoles, variety of ball widths offered by manufacturers, and diversity of design compared to that of the traditional Oxford shoes, allowing the patient to choose a model according to their personal preference[20]. When purchasing a ready-made footwear, the shoes should be modified if the foot deformity is severe. If the shoes cannot be modified, they must be custom-made[8].

SHOE INSOLE

Well-made custom insoles are necessary to properly distribute the pressure around the foot deformity. One of the ways is to increase the ambient pressure in order to relieve the pressure on a certain part of

the foot; however, this approach tends to be inaccurate and can result in damage to the foot because of an increase in the partial pressure[8]. The insole serves as the backbone of the shoe and secures the upper part to the sole[17]. The insole is manufactured in an accommodative form and divided into a soft, semi-rigid, or rigid material. Soft materials include cross-linked polyethylene foam, open-cell polyurethane foam, sponge rubber, and closed-cell expanded rubber. Although these soft materials are excellent for pressure distribution, they wear out quickly and have poor durability[21]. Semi-rigid materials include firm cross-linked polyethylene foam, ethylene vinyl acetate, and cork composite, which have a longer lifespan than the soft materials. They also function as a support in addition to providing shock absorption and cushioning. Semi-rigid materials are fabricated as custom insoles with three or more layers and typically used together with soft materials to provide a combination of support and compliance[22]. Soft and moldable polyethylene foam is used in the area beneath the plantar surface. Urethane polymer is used in the middle layer to prevent wear and absorb shock. Rigid ethylene vinyl acetate or cork is used as the bottommost layer for support and control of movement[8]. Insoles with rigid materials are made of thermoplastics, acrylics, and carbon fiber composites. Although they are highly durable and offer ample support and control, the rigid materials are difficult to modify and have much lesser shock absorption capacity and offer reduced cushioning and protection. In general, the use of rigid materials is contraindicated for patients with diabetes and neuropathy or a history of foot ulcers[23]. Compression paper or leather is commonly used to make insoles for patients with diabetes. The high-strength compression paper insoles are light in weight and inexpensive. Leather insoles are highly durable and adapt well to the plantar surface, absorb moisture, and provide excellent ventilation. However, the leather insoles are not always used owing to their relatively higher price and heavier weight compared to those of compression paper[17].

EXTERNAL SHOE MODIFICATIONS

Rocker sole

Rocker soles are an effective modification method for changing the plantar pressure and improving gait [17,24]. Rocker soles help to transition smoothly from heel-strike to toe-off without bending the shoe or foot. From the biomechanical perspective, this improves the overall gait by restoring the movement of the foot or ankle joint that was lost due to foot pain or deformation. This also relieves the pressure in a specific area of the plantar surface[8]. The apex of the rocker sole should be located proximal to the area where the pressure should be relieved, and the front end of the rocker sole should be arched from the proximal part of the metatarsal head to the distal end of the outsole. If there is an angle in the rocker sole instead of an arch, the gait will not be smooth[17]. Several types of rocker soles are available, all of which require appropriate modifications to meet the needs of the user. For example, the double rocker sole is a soft rocker sole without an outsole in the midfoot region. The forefoot rocker sole has a rocker angle only in the toe area. The heel-to-toe rocker sole has a rocker angle on both the heels and toes[16, 17].

Solid ankle cushion heel

The shoe heel provides stability to the foot heel and distributes the force applied on the foot to the entire sole[17]. The typical height of the shoe heel is 1.5-2 cm for men and 2.5-3 cm for women, but this could be modified according to the user's needs[25]. Most shoe heels are made up of rigid materials, but, if needed, a flexible heel may also be used to allow some plantar flexion[18]. A typical example is the solid ankle cushion heel (SACH), which has a wedge-shaped shock-absorbing material inserted into the heel. The SACH acts as a buffer during heel contact and mechanically increases the heel traction during the gait cycle, creating a smooth transition from heel-strike to toe-off[26]. Typically, the angle of the SACH is within 30°[17]. The SACH is indicated for patients with ankle or hindfoot stiffness due to metatarsal ulcers or Charcot deformity. It is also used in cases of degenerative arthritis or ankle fixation[17,26].

Extended steel shank

The extended steel shank, made of spring steel or carbon graphite, is located between the layers of the sole from the heel to the toes and serves to reinforce the midfoot region of the shoe[27]. The extended steel shank is typically used in combination with rocker soles to help improve their performance. In addition, the shank prevents the bending of the shoe, restricts the toe and midfoot movement, and further reinforces the driving force after a toe-off during gait[8,17]. However, since the extended steel shank can be easily bent owing to the properties of the material, thermoforming may also be combined for increased rigidity[17].

Flare and stabilizer

The flare and stabilizer modify the inside or outside of the shoe to stabilize the foot and serve as a support for the shoe[28,29]. A flare, typically made of ethylene vinyl acetate, is a structure added to the heel and sole of a shoe to widen the support surface of the shoe[17]. The flare, when used in combination with the rocker sole, also serves to increase the stability while walking[8]. A stabilizer is an

extension made of hardened resin or crepe that is added to the side of the sole to provide greater stability than flares do. A stabilizer is used for patients with severe instability on the medial or lateral side of the hindfoot or midfoot[30].

ORTHOSES

The International Working Group on Diabetic Foot (IWGDF) has published the off-loading guidelines for the appropriate treatment of diabetic foot ulcers[31]. According to this guideline, for people with diabetic ulcers, a removable knee-high off-loading device with an adequate foot-device interface should be selected as the first-choice treatment. Furthermore, a total contact cast (TCC) or a nonremovable knee-high walker is recommended depending on the patient's preference or the level of foot deformity. If nonremovable knee-high off-loading devices are contraindicated, a removable knee-high or ankle-high off-loading device is recommended[31].

Previous studies have reported that using a knee-high off-loading device is a faster treatment approach for foot ulcers than using other off-loading devices[32,33]. However, in actual clinical practice, knee-high off-loading devices are not commonly used, because they are contraindicated in at least half of the patients with diabetic foot ulcers with ischemia or infection[34]. Furthermore, many clinicians consider knee-high off-loading devices to be less effective than other types of devices[34]. However, according to the IWGDF guideline, since knee-high off-loading devices are contraindicated only in severe ischemia or infection, they can be used for mild or moderate ischemia or infection[31]. Additionally, TCC has traditionally been considered a gold standard off-loading treatment option, and knee-high off-loading devices are the first suggested option for patients with diabetic foot. Nevertheless, TCC and knee-high off-loading devices are under-used due to the perception that they are time-consuming to make and not cost-effective. However, since instant TCC and removable cast walkers are commonly used nowadays, it is necessary to improve the clinicians' expertise and awareness of orthoses that can be used in diabetic foot treatment[34-36]. Lastly, some barriers may be related to the patients themselves. The patients often perform weight-bearing activities at home that can strain their feet, as they misunderstand that off-loading treatment should be followed outside the home only[34,37]. Moreover, patients who fail to perceive the seriousness of diabetic foot ulcers are less motivated to use the off-loading devices[37,38]. These challenges can be addressed by educating the patients on their condition and the importance of the off-loading devices[35]. The poor mobility and stability that occur when patients use knee-high off-loading devices can also be problematic. The use of the device may result in a difference in length between the legs making it difficult to walk. For such cases, the shoe height, instead of the device, can be modified or mobility aids, such as a frame, can be used simultaneously[34]. The following paragraphs describe the off-loading devices available for diabetic foot ulcers and Charcot arthropathy.

NONREMOVABLE OFF-LOADING DEVICE

A typical example of a nonremovable walker is the TCC. The TCC is considered the standard treatment for managing neuropathic plantar ulcers and is also used to protect the foot in the early stages of vulnerability to Charcot fracture-dislocation[31,39]. The TCC relieves the pressure and load on the foot by distributing the weight across the entire sole and can prevent the risk of injury to bony prominences, such as the malleolus and tibia[40]. However, its limitations include the requirement of a skilled cast technician to apply the TCC appropriately, high manufacturing costs, and time-consuming process[40]. Moreover, the TCC is contraindicated in the very elderly and patients with infection or severe ischemia, visual or balance problems, varicose veins, or contralateral foot ulcers[40].

PREFABRICATED REMOVABLE WALKING BRACE

A prefabricated removable walking brace is used to treat diabetic feet with ulcers and Charcot arthropathy. The types of walking braces include the boot-type control ankle motion walker that controls ankle movement and uses an arch filler[41,42] (Figure 1A), the pneumatic walker that applies pneumatic pressure to reduce edema and prevent callus formation[43] (Figure 1B), and the conformer walking boot that consists of a molded inner liner that wraps around the foot and leg[44]. All walking braces consist of rigid rocker soles and a protective insole made up of materials such as Plastazote® foams, propylene terephthalate, and Spenco®. The walking braces are designed to immobilize all joints of the ankle and foot as would a TCC[8]. The general advantage of walking braces is that they are easy to wear, making it possible to manage the affected area at a relatively lower cost. However, if the foot deformity is severe, applying them can be difficult, and their large volume may reduce patient compliance[8].

DOI: 10.4239/wjd.v13.i11.912 **Copyright** ©The Author(s) 2022.

Figure 1 Types of refabricated removable walking brace and general appearance of orthosis. A: Control ankle motion walker; B: Pneumatic walker; C: Arizona ankle-foot orthosis; D: Patella-tendon-bearing orthosis.

ARIZONA ANKLE-FOOT ORTHOSIS

The Arizona ankle-foot orthosis (AFO) is mainly used for non-surgical management of posterior tibial tendon dysfunction or spastic deformity[45] (Figure 1C). It is also used in cases of ankle instability, arthritis, Charcot arthropathy, and diabetic peripheral neuropathy[8]. The Arizona AFO, typically custom-made from leather and polypropylene, extends proximal to the mid-axis of the tibia and distal to the metatarsal heads. The Arizona AFO plays a role in minimizing valgus alignment of the hindfoot, lateral calcaneal displacement, and dislocation of the medial ankle or restoring inadequate kinematics by supporting the calf and midfoot[46,47].

PATELLAR-TENDON-BEARING ORTHOSIS

The patellar-tendon-bearing (PTB) orthosis is used for partial weight-bearing of the lower extremities while restricting ankle movement[48] (Figure 1D). It supports the patellar tendon so that 60%-70% of the weight is supported by the knee joint and approximately 30% by the ankle joint[18]. A PTB orthosis can be applied to diabetic feet with fractures of the tibia or fibula, foot ulcers, and Charcot arthropathy[17]. The PTB orthosis is custom-made using a plastic-type brim based on the same principle as that used for making a PTB socket for below-knee prosthesis. The load is distributed to the ankle joint of the orthosis through the medial and lateral uprights. In general, the PTB orthosis is designed to restrict the movement of the ankle joint and provide internal and external stability[18]. Although this orthosis has the advantage of easily responding to changes in foot circumference caused by severe fluctuations in lower-extremity edemas, its heavy weight may limit patient compliance[8].

CHARCOT RESTRAINT ORTHOTIC WALKER

The Charcot restraint orthotic walker (CROW) is an orthosis manufactured to completely cover the feet and legs and can be applied to diabetic feet with Charcot arthropathy[8]. In Charcot arthropathy, it is used to prevent the development of ulcers by evenly distributing pressure to the entire leg and foot[49]. For a severe rocker sole deformity in the midfoot, it may be necessary to limit or eliminate the movement of the midfoot and hindfoot to restrict the entire foot and ankle joints. In this case, the CROW eliminates the movement of the foot and ankle joints, reduces the load and shear force applied to the foot, and protects the deformed foot from further damage[50]. The CROW orthosis is similar to a bivalved TCC and consists of a rigid polypropylene anterior and posterior shell with a dorsiflexion stop, heel lift, and rocker sole to counteract the nutcracker effect[8]. Since the CROW orthosis is fastened with a Velcro® strap, it is easy to detach, making it convenient for wound management, including wound cleaning[49]. However, as the CROW orthosis is bulky, it potentially limits patient compliance and sometimes leads to knee and lumbar pain due to immobility of the lower extremities[51].

CONCLUSION

In patients with diabetes, a foot ulcer or Charcot arthropathy is often the first step to amputation of the lower extremity, which becomes a major obstacle in the patient's life. To prevent foot ulcers, thorough and repeated patient education on diabetic feet is necessary in addition to preventive skin care and, above all, prescription of appropriate footwear. Once foot ulcers and Charcot arthropathy occur, it is extremely important to prescribe appropriate footwear and orthoses for the diabetic feet to effectively treat the foot lesion and prevent further deterioration. We believe that this review can serve as a reference guide for medical staff when prescribing appropriate footwear and orthoses for patients with diabetes.

FOOTNOTES

Author contributions: Chang MC and Choo YJ consulted the literature and wrote the first draft, revised, and improved the manuscript; Chang MC, Choo YJ, Park IS, Park MW and Kim DH designed, checked, modified, and finalized the manuscript; and all authors have proofread the revised manuscript.

Supported by the National Research Foundation of Korea Grant that was funded by the Korean Government, No. NRF-2019M3E5D1A02069399.

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

Country/Territory of origin: South Korea

ORCID number: Min Cheol Chang 0000-0002-7629-7213; Yoo Jin Choo 0000-0002-3820-2279; In Sik Park 0000-0002-9800-9577; Myung Woo Park 0000-0001-7531-8433; Du Hwan Kim 0000-0002-9980-8549.

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

REFERENCES

1 **van Netten JJ**, Bus SA, Apelqvist J, Lipsky BA, Hinchliffe RJ, Game F, Rayman G, Lazzarini PA, Forsythe RO, Peters EJG, Senneville É, Vas P, Monteiro-Soares M, Schaper NC; International Working Group on the Diabetic Foot. Definitions and criteria for diabetic foot disease. *Diabetes Metab Res Rev* 2020; **36** Suppl 1: e3268 [PMID: 31943705 DOI: 10.1002/dmrr.3268]
2 **Chun DI**, Kim S, Kim J, Yang HJ, Kim JH, Cho JH, Yi Y, Kim WJ, Won SH. Epidemiology and Burden of Diabetic Foot Ulcer and Peripheral Arterial Disease in Korea. *J Clin Med* 2019; **8** [PMID: 31130633 DOI: 10.3390/jcm8050748]
3 **Skrepnek GH**, Mills JL Sr, Lavery LA, Armstrong DG. Health Care Service and Outcomes Among an Estimated 6.7 Million Ambulatory Care Diabetic Foot Cases in the U.S. *Diabetes Care* 2017; **40**: 936-942 [PMID: 28495903 DOI: 10.2337/dc16-2189]
4 **Amin N**, Doupis J. Diabetic foot disease: From the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. *World J Diabetes* 2016; **7**: 153-164 [PMID: 27076876 DOI: 10.4239/wjd.v7.i7.153]
5 **Boulton AJM**, Armstrong DG, Kirsner RS, Attinger CE, Lavery LA, Lipsky BA, Mills JL Sr, Steinberg JS. Diagnosis and Management of Diabetic Foot Complications. Arlington (VA): American Diabetes Association; 2018 [PMID: 30958663 DOI: 10.2337/db20182-1]
6 **Uccioli L**, Faglia E, Monticone G, Favales F, Durola L, Aldeghi A, Quarantiello A, Calia P, Menzinger G. Manufactured shoes in the prevention of diabetic foot ulcers. *Diabetes Care* 1995; **18**: 1376-1378 [PMID: 8721941 DOI: 10.2337/diacare.18.10.1376]
7 **Boulton AJ**, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005; **366**: 1719-1724 [PMID: 16291066 DOI: 10.1016/S0140-6736(05)67698-2]
8 **Park GY**. Orthotic management for diabetic foot. *J Korean Soc Prosthet Orthot* 2009; **3**: 21-26
9 **Ferreira RC**. Diabetic Foot. Part 2: Charcot Neuroarthropathy. *Rev Bras Ortop (Sao Paulo)* 2020; **55**: 397-403 [PMID: 32904836 DOI: 10.1055/s-0039-3402460]
10 **Mumoli N**, Camaiti A. Charcot foot. *CMAJ* 2012; **184**: 1392 [PMID: 22431900 DOI: 10.1503/cmaj.111972]
11 **Marmolejo VS**, Arnold JF, Ponticello M, Anderson CA. Charcot Foot: Clinical Clues, Diagnostic Strategies, and Treatment Principles. *Am Fam Physician* 2018; **97**: 594-599 [PMID: 29763252]

12 **Shah MK**, Hugghins SY. Charcot's joint: an overlooked diagnosis. *J La State Med Soc* 2002; **154**: 246-50; discussion 250 [PMID: 12440752]

13 **Wagner FW Jr**. The diabetic foot. *Orthopedics* 1987; **10**: 163-172 [PMID: 3809012 DOI: 10.3928/0147-7447-19870101-28]

14 **Gao Y**, Wang C, Chen D, Huang H, Chen L, Liu G, Lin S, Liu M, Wen X, Cho JH, Chen Y, Li Y, Ran X. Effects of novel diabetic therapeutic footwear on preventing ulcer recurrence in patients with a history of diabetic foot ulceration: study protocol for an open-label, randomized, controlled trial. *Trials* 2021; **22**: 151 [PMID: 33597005 DOI: 10.1186/s13063-021-05098-8]

15 **Bus SA**, Waaijman R, Arts M, de Haart M, Busch-Westbroek T, van Baal J, Nollet F. Effect of custom-made footwear on foot ulcer recurrence in diabetes: a multicenter randomized controlled trial. *Diabetes Care* 2013; **36**: 4109-4116 [PMID: 24130357 DOI: 10.2337/dc13-0996]

16 **Ahmed S**, Barwick A, Butterworth P, Nancarrow S. Footwear and insole design features that reduce neuropathic plantar forefoot ulcer risk in people with diabetes: a systematic literature review. *J Foot Ankle Res* 2020; **13**: 30 [PMID: 32498719 DOI: 10.1186/s13047-020-00400-4]

17 **Jeong DY**, Kim JH, Kang P, Jang IS. Pedorthics. Seoul: Koonja, 2010

18 **Kim JH**, Park YS, Song JC, Shin HS, Chang YJ. Prosthetics and orthotics. 3rd ed. Seoul: Topmed, 2006

19 **Tadinada R**, DuBourdieu C, Khan T, Armstrong DG, Ramya Tadinada C, DuBourdieu TK. Diabetic foot risk classification and shoe recommendations: Lessons from SALSA at Rancho Los Amigos. *Diabet Foot J* 2020; **23**: 64-69

20 **Oh-Park M**. Use of athletic footwear, therapeutic shoes, and foot orthoses in psychiatric practice. *PM&R STARS* 2001; **15**: 569-585

21 **Brodsky JW**, Kourosh S, Stills M, Mooney V. Objective evaluation of insert material for diabetic and athletic footwear. *Foot Ankle* 1988; **9**: 111-116 [PMID: 3229697 DOI: 10.1177/107110078800900303]

22 **Bowker JH**, Pfeifer MA. Levin and O'Neal's The diabetic foot. 6th ed. St. Mosby: Louis, 2001

23 **Michael JW**. Lower limb orthosis. In: Goldberg B, Hsu J. Atlas of Orthoses and Assistive Devices. 3rd ed. St. Mosby: Louis, 1997: 209-216

24 **López-Moral M**, Lázaro-Martínez JL, García-Morales E, García-Álvarez Y, Álvaro-Afonso FJ, Molines-Barroso RJ. Clinical efficacy of therapeutic footwear with a rigid rocker sole in the prevention of recurrence in patients with diabetes mellitus and diabetic polineuropathy: A randomized clinical trial. *PLoS One* 2019; **14**: e0219537 [PMID: 31295292 DOI: 10.1371/journal.pone.0219537]

25 **Bus SA**, Zwaferink JB, Dahmen R, Busch-Westbroek T. State of the art design protocol for custom made footwear for people with diabetes and peripheral neuropathy. *Diabetes Metab Res Rev* 2020; **36** Suppl 1: e3237 [PMID: 31845547 DOI: 10.1002/dmrr.3237]

26 **Elattar O**, Smith T, Ferguson A, Farber D, Wapner K. Uses of braces and orthotics for conservative management of foot and ankle disorders. *Foot Ankle Orthop* 2018; 1-12 [DOI: 10.1177/2473011418780700]

27 **Murphy DP**, Webster JB, Lovegreen W, Simoncini A. Braddom's physical medicine and rehabilitation. 6th ed. Amsterdam: Elsevier, 2021: 229-247.e2

28 **Baxi O**, Yeranosian M, Lin A, Munoz M, Lin S. Orthotic management of neuropathic and dysvascular feet. 5th ed. Amsterdam: Elsevier, 2019: 268-276.e2

29 **Masiero S**, Mastrocostas M, Musumeci A. Orthoses in older patients. In: Masiero S, Carraro U. Rehabilitation medicine for elderly patients. Practical issues in geriatrics. Cham: Springer, 2018

30 **Paton JS**, Thomason K, Trimble K, Metcalfe JE, Marsden J. Effect of a forefoot off-loading postoperative shoe on muscle activity, posture, and static balance. *J Am Podiatr Med Assoc* 2013; **103**: 36-42 [PMID: 23328851 DOI: 10.7547/1030036]

31 **Bus SA**, Armstrong DG, van Deursen RW, Lewis JE, Caravaggi CF, Cavanagh PR; International Working Group on the Diabetic Foot. IWGDF guidance on footwear and offloading interventions to prevent and heal foot ulcers in patients with diabetes. *Diabetes Metab Res Rev* 2016; **32** Suppl 1: 25-36 [PMID: 26813614 DOI: 10.1002/dmrr.2697]

32 **Fife CE**, Carter MJ, Walker D, Thomson B, Eckert KA. Diabetic foot ulcer off-loading: The gap between evidence and practice. Data from the US Wound Registry. *Adv Skin Wound Care* 2014; **27**: 310-316 [PMID: 24932951 DOI: 10.1097/01.ASW.0000450831.65667.89]

33 **Zhang Y**, Cramb S, McPhail SM, Pacella R, van Netten JJ, Cheng Q, Derhy PH, Kinnear EM, Lazzarini PA; Diabetic Foot Working Group, Queensland Statewide Diabetes Clinical Network, Australia. Factors Associated With Healing of Diabetes-Related Foot Ulcers: Observations From a Large Prospective Real-World Cohort. *Diabetes Care* 2021; **44**: e143-e145 [PMID: 34074651 DOI: 10.2337/dc20-3120]

34 **Lazzarini PA**, Jarl G. Knee-High Devices Are Gold in Closing the Foot Ulcer Gap: A Review of Offloading Treatments to Heal Diabetic Foot Ulcers. *Medicina (Kaunas)* 2021; **57** [PMID: 34577864 DOI: 10.3390/medicina57090941]

35 **Lazzarini PA**, Jarl G, Gooday C, Viswanathan V, Caravaggi CF, Armstrong DG, Bus SA. Effectiveness of offloading interventions to heal foot ulcers in persons with diabetes: a systematic review. *Diabetes Metab Res Rev* 2020; **36** Suppl 1: e3275 [PMID: 32176438 DOI: 10.1002/dmrr.3275]

36 **Bus SA**, Armstrong DG, Gooday C, Jarl G, Caravaggi C, Viswanathan V, Lazzarini PA; International Working Group on the Diabetic Foot (IWGDF). Guidelines on offloading foot ulcers in persons with diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020; **36** Suppl 1: e3274 [PMID: 32176441 DOI: 10.1002/dmrr.3274]

37 **van Netten JJ**, Seng L, Lazzarini PA, Warnock J, Ploderer B. Reasons for (non-)adherence to self-care in people with a diabetic foot ulcer. *Wound Repair Regen* 2019; **27**: 530-539 [PMID: 31107578 DOI: 10.1111/wrr.12728]

38 **Ploderer B**, Brown R, Seng LSD, Lazzarini PA, van Netten JJ. Promoting Self-Care of Diabetic Foot Ulcers Through a Mobile Phone App: User-Centered Design and Evaluation. *JMIR Diabetes* 2018; **3**: e10105 [PMID: 30305266 DOI: 10.2196/10105]

39 **Griffiths DA**, Kaminski MR. Duration of total contact casting for resolution of acute Charcot foot: a retrospective cohort study. *J Foot Ankle Res* 2021; **14**: 44 [PMID: 34130722 DOI: 10.1186/s13047-021-00477-5]

40 **Faglia E**, Caravaggi C, Clerici G, Sganzaroli A, Curci V, Vailati W, Simonetti D, Sommalvico F. Effectiveness of removable walker cast versus nonremovable fiberglass off-bearing cast in the healing of diabetic plantar foot ulcer: a

randomized controlled trial. *Diabetes Care* 2010; **33**: 1419-1423 [PMID: 20357377 DOI: 10.2337/dc09-1708]

41 **Ritz G**, Rowland WD, Rowland JW. Use of the Cam Walker in treating diabetic ulcers. A case report. *J Am Podiatr Med Assoc* 1996; **86**: 253-256 [PMID: 8699346 DOI: 10.7547/87507315-86-6-253]

42 **Sprinchorn AE**, O'Sullivan R, Beischer AD. Transient bone marrow edema of the foot and ankle and its association with reduced systemic bone mineral density. *Foot Ankle Int* 2011; **32**: S508-S512 [PMID: 21733459 DOI: 10.3113/FAI.2011.0508]

43 **Caravaggi C**, Sganzaroli A, Fabbi M, Cavaiani P, Pogliaghi I, Ferraresi R, Capello F, Morabito A. Nonwindowed nonremovable fiberglass off-loading cast versus removable pneumatic cast (AircastXP Diabetic Walker) in the treatment of neuropathic noninfected plantar ulcers: a randomized prospective trial. *Diabetes Care* 2007; **30**: 2577-2578 [PMID: 17563333 DOI: 10.2337/dc07-0990]

44 **Pollo FE**, Brodsky JW, Crenshaw SJ, Kirksey C. Plantar pressures in fiberglass total contact casts vs. a new diabetic walking boot. *Foot Ankle Int* 2003; **24**: 45-49 [PMID: 12540081 DOI: 10.1177/107110070302400107]

45 **Augustin JF**, Lin SS, Berberian WS, Johnson JE. Nonoperative treatment of adult acquired flat foot with the Arizona brace. *Foot Ankle Clin* 2003; **8**: 491-502 [PMID: 14560901 DOI: 10.1016/s1083-7515(03)00036-6]

46 **Ringleb SI**, Armstrong T, Berglund LJ, Kitaoka HB, Kaufman KR. Stiffness of the Arizona ankle-foot orthosis before and after modification for gait analysis. *J Prost Orthotics* 2009; **21**: 204-207 [DOI: 10.1097/JPO.0b013e3181bfb28e]

47 **Sung KS**, Yu IS. Acquired adult flatfoot: pathophysiology, diagnosis, and nonoperative treatment. *J Korean Foot Ankle Soc* 2014; **18**: 87-92 [DOI: 10.14193/jkfas.2014.18.3.87]

48 **Berwin JT**, Burton TM, Taylor J, McGregor AH, Roche A. Plantar loading forces while walking in a below-knee cast with an attached loadbearing frame. *Foot Ankle Int* 2015; **36**: 722-729 [PMID: 25712122 DOI: 10.1177/1071100715572258]

49 **Keast DH**, Vair AH. Use of the charcot restraint orthotic walker in treatment of neuropathic foot ulcers: a case series. *Adv Skin Wound Care* 2013; **26**: 549-552 [PMID: 24253211 DOI: 10.1097/01.ASW.0000437640.22278.2e]

50 **Pinzur MS**, Dart HC. Pedorthic management of the diabetic foot. *Foot Ankle Clin* 2001; **6**: 205-214 [PMID: 11488049 DOI: 10.1016/s1083-7515(03)00089-5]

51 **Oh-Park M**, Kim DD, Lee JM. Footwear and insole. In: Ko YJ, Kang SY. Physical Medicine & Rehabilitation. 1st ed. Seoul: Jungmunkag, 2009: 286-289

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 921-925

DOI: 10.4239/wjd.v13.i11.921

ISSN 1948-9358 (online)

MINIREVIEWS

Effects of Chios mastic gum on cardiometabolic risk factors

Athanasia K Papazafiropoulou

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

P-Reviewer: Kosmas C, United States; Yang L, China

Received: July 24, 2022
Peer-review started: July 24, 2022
First decision: September 12, 2022
Revised: September 16, 2022
Accepted: October 11, 2022
Article in press: October 11, 2022
Published online: November 15, 2022

Athanasia K Papazafiropoulou, The First Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, Piraeus 18536, Greece

Corresponding author: Athanasia K Papazafiropoulou, MD, MSc, PhD, Attending Doctor, Research Scientist, The First Department of Internal Medicine and Diabetes Center, Tzaneio General Hospital of Piraeus, 1 Zanni and Afentouli Street, Piraeus 18536, Greece.
pathan@ath.forthnet.gr

Abstract

Chios mastic gum (CMG), the resin produced by the trunk of *Pistachia lentiscus* var Chia, has been used for culinary and medicinal purposes since antiquity. Despite the fact that *Pistacia* species are widely distributed throughout the Mediterranean basin and in the circum-Mediterranean regions, CMG is a distinctive resin of the mastic trees grown exclusively in the southern part of the island of Chios. CMG has been used for centuries as a spice, a cosmetic, but its most important usage has been as a strong phytotherapeutic therapy, primarily for the management of gastrointestinal diseases. Recently, there are studies demonstrating that CMG has hypolipidemic, cardioprotective and antidiabetic properties. Therefore, the aim of the present review is to summarize the existing literature data regarding the potential beneficial effects of CMG on cardiometabolic risk factors.

Key Words: Chios mastic gum; Glucose; Cardioprotection; Low-density lipoprotein-cholesterol; Triglycerides

Core tip: Chios mastic gum (CMG), the resin produced by the trunk of *Pistachia lentiscus* var Chia, has been used for centuries as a strong phytotherapeutic therapy, primarily for the management of gastrointestinal diseases. Recently, there are studies demonstrating that it has hypolipidemic, cardioprotective and antidiabetic properties. Therefore, the aim of the present review is to summarize the existing literature data regarding the potential beneficial effects of CMG on cardiometabolic risk factors.

Citation: Papazafiropoulou AK. Effects of Chios mastic gum on cardiometabolic risk factors. *World J Diabetes* 2022; 13(11): 921-925
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/921.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.921

INTRODUCTION

The aromatic resin known as Chios mastic gum (CMG) is made by the evergreen plant *Pistacia lentiscus* var Chia (Anacardiaceae). Mastic is traditionally produced by making shallow slits in the bark and trunk of the shrub using specific implements called ceditria[1]. Despite the fact that *Pistacia* species are widely distributed throughout the Mediterranean basin and in the circum-Mediterranean regions, CMG is a distinctive resin of the mastic trees grown exclusively in the southern part of the island of Chios, which is situated in the central Aegean Sea close to the coastline of Minor Asia. The fact that mastic is only produced in one location of the island and nowhere else in the greater Mediterranean region may be explained by thousands of years of selective cultivation and the particular microenvironment. The mastic tree's cultivation and resin harvesting are part of the area's cultural heritage, and the total production comes from 24 settlements (Mastichochoria in Greek)[1].

CMG has been used for centuries as a spice, a cosmetic, but its most important usage has been as a strong phytotherapeutic therapy, primarily for the management of gastrointestinal diseases. Galenos and Dioscourides, two ancient Greek physicians, highlighted its benefits and suggested using it. Furthermore, the need for CMG has always held a special place in folk medicine throughout Europe and Asia during the Byzantine and Medieval eras, and afterwards in formal Pharmacopeias[2]. The first research revealing the resin's positive characteristics on gastrointestinal inflammations, and particularly those caused by *Helicobacter pylori*, were published in the 1980s, reigniting the scientific community's interest in CMG[3].

The most prevalent and traditional therapeutic application of mastic in the treatment of gastrointestinal diseases has been extensively studied in recent decades by several scientific investigations that have focused on CMG. Its antibacterial, anti-inflammatory, antioxidant, hypolipidemic, antidiabetic, and anticancer activities have since been the subject of several investigations[2]. Therefore, the aim of the present review is to summarize the existing literature data regarding the potential beneficial effects of CMG on cardiometabolic risk factors.

CHEMICAL COMPOSITION OF CMG

Numerous chemicals have been isolated and identified after a detailed analysis of the chemical makeup of CMG[8-12]. However, ongoing study continues to uncover novel substances, as seen in the case of mastichinoic acid A, a new tetracyclic triterpenoid that was recently discovered from CMG[13]. About 25% of the total CMG is made up of poly-b-myrcene, a sticky and insoluble polymer. From CMG, a number of triterpenoids have been identified. More specifically, acidic and neutral fractions can be obtained from complete mastic gum extract (without the polymer). All significant triterpenic acids, including masticadienonic, isomasticadienonic, oleanonic acid, moronic acid, masticadienolic acid, and oleanolic acid, are included in the acidic fraction. Triterpenic neutral substances such as oleanolic aldehyde, 28-norolean-17-en-3-one, tirucallol, b-amyrone, isomasticadienolic aldehyde, and dammaradienone are included in the neutral fraction.

Other substances with smaller amounts include verbenone, a-terpinolene, and linalool, which support the antibacterial properties of mastic oil, and camphene, which has hypolipidemic properties [14]. Gallic acid traces have also been found. It is amazing that research describing the antibacterial, hypolipidemic, and anti-inflammatory properties of mastic gum or mastic oil have shown the presence of synergy phenomenon, where the combination of many substances is more potent than any one ingredient alone. With herbal products that include numerous different active ingredients, this synergy phenomenon occurs frequently.

EFFECTS OF CMG ON LIPIDS METABOLISM

Human low-density lipoprotein cholesterol (LDL-C) has been shown to be resistant to copper-induced oxidation *in vitro* through the powerful antioxidant effects of CMG[15]. Peripheral blood mononuclear cells are cytotoxic when exposed to oxidized LDL-C without the presence of CMG, an d whole polar extract of CMG prevents this from happening. While mastic complete polar extract increases glutathione (GSH) levels and lowers CD36 expression, oxidized LDL-C decreases GSH levels and increases CD36 expression[16]. Rats susceptible to detergent-induced hyperlipidemia and naïve rats have both been

used to study the hypolipidemic effects of mastic gum essential oil (MGO). The levels of blood total cholesterol, LDL-C, and triglycerides were decreased in a dose-dependent manner after MGO treatment to untrained rats. MGO injection resulted in a significant decrease in the levels of total cholesterol, LDL-C, and triglycerides in hyperlipidemic rats[16].

In a different study, complete CMG was given as a powder and blended with food for 8 wk in low and high doses to examine the hypolipidemic effects of CMG on diabetic mice. The serum levels of triglycerides, total cholesterol, and LDL-C were all significantly lower in the low-dose group whereas high-density lipoprotein cholesterol (HDL-C) levels were significantly higher. Triglyceride levels were considerably lower in the high-dose group[17].

When administered to hypercholesterolemic rabbits, complete mastic extract without polymer and neutral mastic fraction (NMF) decreased total cholesterol levels by 47% and 88%, respectively, exhibiting strong hypolipidemic actions[18]. Healthy adults over the age of 50 years have received total mastic extract. Subjects were divided into two groups at random and given either a mastic solution (low dose) for 12 mo or a daily dose of 5 g of mastic powder (high dose) for 18 mo. The high-dose group showed a decrease in blood total cholesterol, LDL-C, total cholesterol/HDL-C ratio, apolipoprotein A-1, and apolipoprotein B, but no change in the apoB/apoA-1 ratio[19].

In a prospective, randomized, placebo-controlled, pilot study, healthy volunteers' total cholesterol and blood sugar levels were considerably reduced over the course of 8 wk by taking three capsules per day containing 330 mg CMG. It is important to note that, despite the absence of side effects, overweight and obese people in particular shown excellent tolerance. CMG activity decreases when polymer is absent. Measurements of cholesterol levels in healthy individuals did not reveal any appreciable reduction after taking mastic gum capsules free of polymers[20].

A major limitation of the above human studies was that the rest of the diet of the participants (apart from the addition of CMG) was not controlled and, therefore, any effect of possible diet changes on the results of the study could not be excluded. It should be mentioned that the effects of CMG on lipids in humans are rather minor and should not be overstated as unique, as there are other natural substances, such as sterols and stanols, that have been shown to cause significant reductions in LDL-C.

EFFECTS OF CMG ON CARDIOPROTECTIVE ACTIVITY

Cardiovascular disease risk appears to be decreased by CMG. Perhaps one of the underlying causes of this function is the potent antioxidant activity of CMG and its ability to inhibit the buildup of the oxidized LDL in cells, which can cause atherosclerosis[16]. Two essential adhesion molecules can be decreased by the neutral fraction of CMG (25–200 g/mL) and, more specifically, the chemical tirucallol (0.1–100 mmol/L), according to research in human aortic endothelial cells [vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1]. Due to the buildup of monocytes in the artery innermost layer, VCAM-1 and ICAM-1 are linked to the early development of atherosclerosis [21]. In another study, male 12-wk-old diabetic mice were divided into groups receiving low and high doses of CMG. The high-dose CMG group (n = 12) received 500 mg/kg body weight for the same duration as the low dose CMG group (n = 12) for a total of 8 wk. CMG lowered serum lipid and glucose levels in both groups[22]. In 2018, the authors showed that administering CMG to renovascular hypertensive rats at a dose of 40 mg/kg/d for 2 wk after the onset of hypertension lowered their blood pressure. The results showed a relationship between reduced levels of renin, C-reactive protein, and interleukin-6, as well as increased vascular and cardiac remodeling[23].

In a different *in vivo* experiment, for 6 wk, rabbits were fed a specific diet supplemented with the NMF and the total mastic extract without polymer (TMEWP) at the same dose. In rabbits that were given a normal diet while under anesthesia, both extracts appeared to diminish the size of the infarct, and in hypercholesterolemic rabbits, they both had antiatheromatic and hypolipidemic effects. For TMEWP and NMF, the reduction in total cholesterol levels was 47% and 88%, respectively[24].

The beneficial benefits of CMG on peripheral and aortic blood pressure hemodynamics in hypertensive patients were established in a randomized double-blind case-controlled crossover design, hinting potential downregulation of the proteasome system and the NADPH oxidase 2 pro-oxidant pathway. The subjects consumed 2800 mg of CMG orally (four tablets of 700 mg or a placebo), and they had evaluations during two subsequent visits spaced by 1 wk[25]. Another pilot investigation also suggested that CMG powder may play a role in human *in vivo* hepato- or cardioprotection. In the group consuming daily 5 g of mastic powder for 18 mo, a reduction in blood total cholesterol, LDL-C, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B, serum glutamyl oxaloacetic transaminase, serum glutamic pyruvic transaminase, and -glutamyl transferase levels was seen[19]. Since apolipoprotein A-1 is a major component of the HDL-C complex (protective fat removal particles), and thus acts as a cardioprotective molecule, the above observed reduction in the study by Triantafyllou *et al*[19] has to be translated carefully to daily clinical practice especially in patients with increased cardiovascular risk.

EFFECTS OF CMG ON GLUCOSE METABOLISM

The antidiabetic benefit of CMG is a recent discovery, and there is not a lot of evidence to back it up yet. Triantafyllou *et al*[19] presented the first concrete proof of glucose-lowering activity, showing that in the low-dose group, male patients' glucose levels were markedly reduced. According to Georgiadis *et al* [17], CGM had an unexpectedly strong antidiabetic effect, significantly decreasing blood sugar levels in both the low- and high-dose groups of mice. It is noteworthy to note that, in line with Triantafyllou *et al* [19], they found that the low-dose group performed better than the high-dose group. According to a recent study, CMG consumption had positive benefits on blood lipid indicators and insulin resistance in healthy Japanese men. More particularly, 30 min of additional activity three times per week enhanced the effect of the mastic powder intake on insulin, which was lowered by 5 g/d for 6 mo[26].

CONCLUSION

CMG has a wide spectrum of antimicrobial, antioxidant, hypolipidemic, anti-inflammatory, and antidiabetic activities. Several studies have shown that CMG exerts beneficial effects on lipid and glucose metabolism. However, further studies are required to clarify the formula and the active compounds of CMG that have potential cardioprotective effects as well as their use in clinical practice.

FOOTNOTES

Author contributions: Papazafiropoulou AK contributed to writing the manuscript.

Conflict-of-interest statement: The author reports no relevant conflicts of interest for this article.

Country/Territory of origin: Greece

ORCID number: Athanasia K Papazafiropoulou 0000-0002-7596-4942.

S-Editor: Wang JJ
L-Editor: Kerr C
P-Editor: Wang JJ

REFERENCES

1 **Pachi VK**, Mikropoulou EV, Gkiouvetidis P, Siafakas K, Argyropoulou A, Angelis A, Mitakou S, Halabalaki M. Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review. *J Ethnopharmacol* 2020; **254**: 112485 [PMID: 32092498 DOI: 10.1016/j.jep.2019.112485]
2 **Paraschos S**, Mitakou S, Skaltsounis AL. Chios gum mastic: A review of its biological activities. *Curr Med Chem* 2012; **19**: 2292-2302 [PMID: 22414110 DOI: 10.2174/092986712800229014]
3 **Al-Habbal MJ**, Al-Habbal Z, Huwez FU. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. *Clin Exp Pharmacol Physiol* 1984; **11**: 541-544 [PMID: 6395994 DOI: 10.1111/j.1440-1681.1984.tb00864.x]
4 **Papageorgiou VP**, Bakola-Christianopoulou MN, Apazidou KK, Psarros EE. Gas chromatographic analysis of the acidic triterpenic fraction of mastic gum. *J Chromatogr* 1997; **769**: 262-273 [DOI: 10.1016/S0021-9673(96)01032-1]
5 **Magiatis P**, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia. *Planta Med* 1999; **65**: 749-752 [PMID: 10630120 DOI: 10.1055/s-2006-960856]
6 **Kalliora AC**, Milona A, Chiou A, Petsios DG, Andrikopoulos NK. Detection and identification of simple phenolics in Pistacia lentiscus resin. *J Liq Chromatogr Relat Technol* 2004; **27**: 289-300 [DOI: 10.1081/JLC-120027100]
7 **Koutsoudaki C**, Krsek M, Rodger A. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia. *J Agric Food Chem* 2005; **53**: 7681-7685 [PMID: 16190616 DOI: 10.1021/jf050639s]
8 **Assimopoulou AN**, Papageorgiou VP. GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of Pistacia species. Part I. Pistacia lentiscus var. Chia. *Biomed Chromatogr* 2005; **19**: 285-311 [PMID: 15651084 DOI: 10.1002/bmc.454]
9 **Paraschos S**, Magiatis P, Mitakou S, Petraki K, Kalliaropoulos A, Maragkoudakis P, Mentis A, Sgouras D, Skaltsounis

AL. In vitro and *in vivo* activities of Chios mastic gum extracts and constituents against Helicobacter pylori. *Antimicrob Agents Chemother* 2007; **51**: 551-559 [PMID: 17116667 DOI: 10.1128/AAC.00642-06]

10 **Paraschos S**, Magiatis P, Skaltsounis AL. Application of chiral GC-MS analysis in the detection of natural products adulteration–The example of Chios mastic gum essential oil. *Planta Med* 2008; **74**: 297 [DOI: 10.1055/s-0028-1084905]

11 **Paraschos S**, Magiatis P, Skaltsounis AL, Smyrnioudis I. Quality profile of Chios mastic (mastiha) essential oil. *Planta Med* 2011; **77**: PE42 [DOI: 10.1055/s-0031-1282373]

12 **Arabi Monfared A**, Yazdanpanah M, Zareshahrabadi Z, Pakshir K, Ghahartars M, Mehrabani D, Yazdanpanah S, Iraji A, Zomorodian K. Chemical composition and antifungal activities of aromatic water of *Zataria multiflora* Boiss. *Curr Med Mycol* 2021; **7**: 29-35 [PMID: 35528627 DOI: 10.18502/CMM.7.3.7255]

13 **Gao JB**, Li GY, Huang J, Wang HY, Zhang K, Wang JH. A new tetracyclic triterpenoid compound from Mastich. *J Asian Nat Prod Res* 2013; **15**: 400-403 [PMID: 23470034 DOI: 10.1080/10286020.2013.769524]

14 **Vallianou I**, Peroulis N, Pantazis P, Hadzopoulou-Cladaras M. Camphene, a plant-derived monoterpene, reduces plasma cholesterol and triglycerides in hyperlipidemic rats independently of HMG-CoA reductase activity. *PLoS One* 2011; **6**: e20516 [PMID: 22073134 DOI: 10.1371/journal.pone.0020516]

15 **Andrikopoulos NK**, Kaliora AC, Assimopoulou AN, Papapeorgiou VP. Biological activity of some naturally occurring resins, gums and pigments against *in vitro* LDL oxidation. *Phytother Res* 2003; **17**: 501-507 [PMID: 12748987 DOI: 10.1002/ptr.1185]

16 **Dedoussis GV**, Kaliora AC, Psarras S, Chiou A, Mylona A, Papadopoulos NG, Andrikopoulos NK. Antiatherogenic effect of Pistacia lentiscus *via* GSH restoration and downregulation of CD36 mRNA expression. *Atherosclerosis* 2004; **174**: 293-303 [PMID: 15136059 DOI: 10.1016/j.atherosclerosis.2004.02.011]

17 **Georgiadis I**, Karatzas T, Korou LM, Agrogiannis G, Vlachos IS, Pantopoulou A, Tzanetakou IP, Katsilambros N, Perrea DN. Evaluation of Chios mastic gum on lipid and glucose metabolism in diabetic mice. *J Med Food* 2014; **17**: 393-399 [PMID: 24404977 DOI: 10.1089/jmf.2013.0069]

18 **Paraschos S**, Andreadou I, Iliodromitis EK, Zoga A, Magiatis P, Mitaku S, Kaklamanis L, Skaltsounis AL, Kremastinos DT. Antiatheromatic and hypolipidemic activity of Chios Mastic Gum in anesthetized rabbits. *Planta Med* 2008; **74**: PA343 [DOI: 10.1055/s-0028-1084340]

19 **Triantafyllou A**, Chaviaras N, Sergentanis TN, Protopapa E, Tsaknis J. Chios mastic gum modulates serum biochemical parameters in a human population. *J Ethnopharmacol* 2007; **111**: 43-49 [PMID: 17150319 DOI: 10.1016/j.jep.2006.10.031]

20 **Kartalis A**, Didagelos M, Georgiadis I, Benetos G, Smyrnioudis N, Marmaras H, Voutas P, Zotika C, Garoufalis S, Andrikopoulos G. Effects of Chios mastic gum on cholesterol and glucose levels of healthy volunteers: A prospective, randomized, placebo-controlled, pilot study (CHIOS-MASTIHA). *Eur J Prev Cardiol* 2016; **23**: 722-729 [PMID: 26311707 DOI: 10.1177/2047487315603186]

21 **Loizou S**, Paraschos S, Mitakou S, Chrousos GP, Lekakis I, Moutsatsou P. Chios mastic gum extract and isolated phytosterol tirucallol exhibit anti-inflammatory activity in human aortic endothelial cells. *Exp Biol Med (Maywood)* 2009; **234**: 553-561 [PMID: 19234052 DOI: 10.3181/0811-RM-338]

22 **Tzani A**, Bletsa E, Doulamis IP, Korou LM, Konstantopoulos P, Vlachos IS, Georgiadis I, Perrea DN. Hypolipidemic, hepatoprotective and anti-inflammatory role of Chios Mastic gum in Streptozotocin-induced diabetic mice with fatty liver disease. *Hell Atheroscler Soc* 2017 [DOI: 10.23803/HJA.V7I4.87]

23 **Tzani AI**, Doulamis IP, Konstantopoulos PS, Pasiou ED, Daskalopoulou A, Iliopoulos DC, Georgiadis IV, Kavantzas N, Kourkoulis SK, Perrea DN. Chios mastic gum decreases renin levels and ameliorates vascular remodeling in renovascular hypertensive rats. *Biomed Pharmacother* 2018; **105**: 899-906 [PMID: 30021383 DOI: 10.1016/j.biopha.2018.06.067]

24 **Andreadou I**, Mitakou S, Paraschos S, Efentakis P, Magiatis P, Kaklamanis L, Halabalaki M, Skaltsounis L, Iliodromitis EK. "Pistacia lentiscus L." reduces the infarct size in normal fed anesthetized rabbits and possess antiatheromatic and hypolipidemic activity in cholesterol fed rabbits. *Phytomedicine* 2016; **23**: 1220-1226 [PMID: 27316396 DOI: 10.1016/j.phymed.2016.06.002]

25 **Kontogiannis C**, Georgiopoulos G, Loukas K, Papanagnou ED, Pachi VK, Bakogianni I, Laina A, Kouzoupis A, Karatzi K, Trougakos IP, Stamatelopoulos K. Chios mastic improves blood pressure haemodynamics in patients with arterial hypertension: Implications for regulation of proteostatic pathways. *Eur J Prev Cardiol* 2019; **26**: 328-331 [PMID: 30160513 DOI: 10.1177/2047487318796985]

26 **Fukazawa T**, Smyrnioudis I, Konishi M, Takahashi M, Kim HK, Nishimaki M, Xiang M, Sakamoto S. Effects of Chios mastic gum and exercise on physical characteristics, blood lipid markers, insulin resistance, and hepatic function in healthy Japanese men. *Food Sci Biotechnol* 2018; **27**: 773-780 [PMID: 30263803 DOI: 10.1007/s10068-018-0307-3]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 926-939

DOI: 10.4239/wjd.v13.i11.926

ISSN 1948-9358 (online)

MINIREVIEWS

Advances in neovascularization after diabetic ischemia

Yue Cai, Guang-Yao Zang, Yan Huang, Zhen Sun, Li-Li Zhang, Yong-Jiang Qian, Wei Yuan, Zhong-Qun Wang

Specialty type: Cardiac and cardiovascular systems

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

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

P-Reviewer: Amin A, United Arab Emirates; Hettiarachchi P, Sri Lanka; Jovandaric MZ, Serbia

Received: August 5, 2022
Peer-review started: August 5, 2022
First decision: August 20, 2022
Revised: September 9, 2022
Accepted: November 2, 2022
Article in press: November 2, 2022
Published online: November 15, 2022

Yue Cai, Guang-Yao Zang, Zhen Sun, Li-Li Zhang, Yong-Jiang Qian, Wei Yuan, Zhong-Qun Wang, Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang 212000, Jiangsu Province, China

Yan Huang, Department of Ophthalmology, Affiliated Hospital of Jiangsu University, Zhenjiang 212000, Jiangsu Province, China

Corresponding author: Zhong-Qun Wang, PhD, Professor, Researcher, Department of Cardiology, Affiliated Hospital of Jiangsu University, No. 438 Jiefang Road, Jingkou District, Zhenjiang 212000, Jiangsu Province, China. wangtsmc@126.com

Abstract

With the high incidence of diabetes around the world, ischemic complications cause a serious influence on people's production and living. Neovascularization plays a significant role in its development. Therefore, neovascularization after diabetic ischemia has aroused attention and has become a hot spot in recent years. Neovascularization is divided into angiogenesis represented by atherosclerosis and arteriogenesis characterized by coronary collateral circulation. When mononuclear macrophages successively migrate to the ischemia anoxic zone after ischemia or hypoxia, they induce the secretion of cytokines, such as vascular endothelial growth factor and hypoxia-inducible factor, activate signaling pathways such as classic Wnt and phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) pathways, trigger oxidative stress response, activate endothelial progenitor cells or enter the glycolysis or lactic acid process and promote the formation of new blood vessels, remodeling them into mature blood vessels and restoring blood supply. However, the hypoglycemic condition has different impacts on neovascularization. Consequently, this review aimed to introduce the mechanisms of neovascularization after diabetic ischemia, increase our understanding of diabetic ischemic complications and their therapies and provide more treatment options for clinical practice and effectively relieve patients' pain. It is believed that in the near future, neovascularization will bring more benefits and hope to patients with diabetes.

Key Words: Diabetes mellitus; Angiogenesis; Arteriogenesis; Ischemia; Hypoxia

Core Tip: This review aimed to give an overview of neovascularization in patients with diabetes. First, we introduced the basic concepts and influencing factors of neovascularization, including angiogenesis and arteriogenesis. Second, the mechanisms regarding cytokines, classical and novel signaling pathways, glycolysis and lactic acid process and so on described in detail. Then, the neovascularization after diabetic ischemia was further described in combination with the complications of diabetes, such as diabetic atherosclerosis, diabetic retinopathy, diabetic nephropathy and diabetic foot ulcer. Last but not least, the treatment plans listed, with advantages and disadvantages, that may offer more treatment options.

Citation: Cai Y, Zang GY, Huang Y, Sun Z, Zhang LL, Qian YJ, Yuan W, Wang ZQ. Advances in neovascularization after diabetic ischemia. *World J Diabetes* 2022; 13(11): 926-939
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/926.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.926

INTRODUCTION

Diabetes mellitus is a complex, heterogeneous, whole-body chronic metabolic disease; it is predicted that by 2045, the number of patients with diabetes, aged 20-79 years, will increase to 783 million[1]. Diabetes-related complications include microvascular plaque formation, ischemia and hypoxia caused by atherosclerosis. They are characterized by severe arterial ischemia, increased risk of amputation of peripheral artery disease and so on. Promoting neovascularization to restore the blood flow of the ischemic area is conducive to disease outcomes while avoiding risk. Hence, it is an urgent issue for scientific researchers.

NEOVASCULARIZATION AFTER ISCHEMIA

Neovascularization is caused by angiogenesis and arteriogenesis. Angiogenesis refers to the budding of new blood vessels in the vascular bed in an original way to form new vasculature, mainly in the capillaries[2]. Arteriogenesis is the formation of new arteries by expanding lumen diameter and remodeling tube walls to restore blood flow in the ischemic area. The new route gradually disappears when the previously blocked artery is recanalized[3].

Angiogenesis

Physiological angiogenesis mainly occurs in embryo development, endometrial thickening and wound healing; the process depends on the ratio of proangiogenic factors to antiangiogenic factors. Pathological angiogenesis is often triggered in disease states, such as atherosclerosis, tumors, systemic lupus erythematosus, *etc*[4], to form abnormal blood vessels with thinner walls and higher permeability. Angiogenesis includes the following steps: (1) Endothelial cells sprout under the action of angiogenic factors[5]; (2) Pericytes aggregate if their absence leads to increased lumen permeability[6] and vascular instability; and (3) The basement membrane is reconstructed to develop mature and stable blood vessels.

Atherosclerosis can lead to severe complications such as myocardial infarction (MI), resulting in heart failure. Endothelial cells, which sprout under the action of angiogenic factors, significantly improve the patient's recovery, which is the focus of current research.

After MI, blood congestion, thromboembolism and compression of the surrounding tissue, proinflammatory factors are released, leading to impaired endothelial integrity, loss of myocardial cells, endothelial cell damage, increased capillary permeability and secretion of proinflammatory cytokines to activate white blood cells and endothelial cells. This results in the release of a large number of inflammatory factors into the infarcted myocardium, thus promoting myocardial inflammation[7]. The formation of neovascularization in the ischemic infarct area to provide nutrients and oxygen is the key to post-MI repair. Neovascularization in the surrounding area of infarction increases vascular density and extends to the core area of infarction[8], and hypoxia plays an essential role in this process.

The most studied factor is hypoxia-inducible factor-1α (HIF-1α), whose reduced protein expression under high glucose conditions leads to increased MI[9]. In anoxic environments, the HIF-1α/vascular endothelial growth factor (VEGF) pathway acts by releasing angiogenic factors. After MI, reactive oxygen species (ROS) in cardiac fibroblasts increase by about 50%, resulting in mutations in the HIF phenotype marked by scar contraction and dysfunction[10]. HIF activation induces VEGF release, which activates endothelial cells (ECs) through the paracrine mechanism, and can be expressed in ECs to participate in angiogenesis. In addition, angiopoietin-like protein 4 stabilizes VEGF receptor 2/Ca^{2+}-dependent cell adhesion molecule 5 complex to maintain endothelial structural integrity and promote

macrophage transformation into a repair phenotype to enhance boundary region angiogenesis.

Arteriogenesis

Arteriogenesis refers to the growth of new arteries or the derived collateral vessels, mainly involved in the active proliferation of ECs and smooth muscle cells, resulting in lumen enlargement and wall remodeling. It comprises primarily two stages[3]. In the early stage, the diameter of lateral branches and tube walls increases under the influence of fluid shear stress. Subsequently, monocytes promote lumen remodeling by secreting metalloproteinases and cytokines. In addition, M1-type macrophages promote the progression of myocardial inflammation[11]. The release of inflammatory factors under ischemia and hypoxia promotes the progression of inflammation and the occurrence of glycolysis. Under this action, fibroblasts are transformed into ECs, triggering epigenetic modification. ECs and fibroblasts contribute to the formation of arteries[12-14].

Human coronary circulation has an extensive anastomotic network. Even one-third of ordinary people have collateral circulation to cope with MI caused by transient vascular occlusion[15]. Further, 20%-25% of patients with coronary artery disease can prevent MI, improve survival and reduce mortality by promoting normal blood flow through collateral circulation during coronary artery occlusion. Approximately 1 in 5 patients cannot tolerate percutaneous coronary intervention or coronary artery bypass grafting. Therefore, collateral growth promotion is a promising therapeutic strategy targeting arteriogenesis. In the absence of coronary artery disease, these arteries are only 100-200 μm in diameter, and the lumen is impassable. When coronary artery disease causes a major artery occlusion, the collateral arteries are remodeled and the lumen is expanded to 100-800 μm in diameter to serve as part of the major artery[16], which is in line with normal routes and has one to two layers of smooth muscle cells. At the same time, the expansion of the diameter of the tube is accompanied by a decrease in the number of collateral arteries. The myocardial protection of large blood flow is more significant in the collateral arteries than in the new capillaries surrounding the infarct area[17].

Although collateral maturation is essential for preserving cardiac function, the related markers are still lacking[18]. Although the influence of coronary artery collateral formation and prognosis has been controversial at present[19], some studies showed[20] that patients with MI having coronary collateral circulation have more severe stenosis and worse cardiac function. Therefore, from a macro point of view, it is believed that collateral circulation benefits at least one-fifth of patients who cannot undergo percutaneous coronary intervention and coronary artery bypass grafting. Physiological or pathological vascular reconstruction and blood flow redistribution can prevent excessive MI and reduce injury to the body. This therapeutic strategy has broad research prospects and is worth further exploration to benefit patients.

Impact factors

As mentioned earlier, neovascularization is affected by various proangiogenic/antiangiogenic factors. The mechanisms and roles of proangiogenic factors, such as HIF, macrophages, VEGF family, noncoding RNA and hepatocyte growth factor, and antiangiogenic factors, such as thrombospondin-1 and interleukin 12 (IL-12), summarized in this study (Tables 1 and 2)[7,21-44].

MECHANISMS OF NEOVASCULARIZATION AFTER DIABETIC ISCHEMIA

Glycolysis

Studies have shown that 80% of ATP in ECs is produced by glycolysis[45], mainly due to the limited number of mitochondria. The energy generated needs to be supplied to the distal tissues. In addition, the ATP generation by glycolysis is faster than that by oxidative phosphorylation. Therefore, glycolysis is the primary energy supply for ECs in normal and hypoxic states. Hypoxia or lack of nutrition can promote the production of VEGF, fibroblast growth factor and other angiogenic factors[46], the concentration and the activity of hexokinase and membrane expression of glucose transporter protein 1. The ECs differentiate into specific cells under high VEGF levels, forming platelet pseudopodia and filiform pseudopodia, promoting migration and stem cell proliferation. Stable glycolysis produces lactic acid [47], promoting VEGF expression and inducing angiogenesis under multiple effects.

6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) transcription through HIF-1α is induced by hypoxia, which is an essential regulator of glycolysis and can be phosphorylated by activating kinases such as mitogen-activated protein kinase[48]. PFKFB3 can promote the synthesis of fructose-2 6-diphosphate, activate phosphofructokinase 1 and promote glycolysis. The Notch-Delta-like ligand 4 signaling pathway can promote stem cell extension, tip cell growth and blood vessel germination[49].

Some intermediate products of glycolysis enter the pentose phosphate pathway. The NADPH produced during this process is necessary for nitric oxide (NO) biosynthesis, promoting angiogenesis. When macrophage metabolism shifts to glycolysis, M1 proinflammatory macrophages facilitate glycolysis, interrupting the tricarboxylic acid cycle and producing lactic acid rather than metabolizing pyruvate to acetyl-CoA. Anti-inflammatory M2 macrophages inhibit the pentose phosphate pathway.

Table 1 Mechanisms and functions of proangiogenic factors

Trigger factors	Mechanism	Function	Ref.
HIF	When hypoxia occurs, HIF-α dimerizes with HIF-β, binding to hypoxia response elements in the nucleus, transcribing thousands of genes and promoting angiogenesis	Promote angiogenesis and increase vascular density; stimulate collateral vessel compensatory formation; regulate EPO and other downstream factors; mobilize endothelial progenitor cells	[21-24]
Macrophages	Macrophages are divided into M1 type, which is proinflammatory and phagocytic, and M2 type, which is anti-inflammatory and promotes angiogenesis	Transform into perivascular cells to control vascular permeability; remodel extracellular matrix to provide conduit for apical cells and promote blood vessel germination; endothelial cells and trim abnormal blood vessels	[25-30]
Monocytes	Angiogenesis is directly dependent on the number of circulating monocytes	Induce HIF-mediated release of chemokines and growth factors to stimulate angiogenesis; express angiogenin receptor Tie-2 and exacerbate inflammation	[7, 31]
VEGF family	VEGF-A regulates angiogenesis, vascular permeability and inflammation. VEGF-B regulates angiogenesis and apoptosis. VEGF-C and VEGF-D regulate lymphangiogenesis, apoptosis and fiber formation	VEGF activates a variety of downstream signaling pathways and promotes the proliferation, migration and vascular remodeling of ECs; activate ERK1/2 and promote angiogenesis	[32-34]
Noncoding RNAs	Many noncoding RNAs regulate complex processes of angiogenesis	MiR-25-3p enhances endothelial permeability and angiogenesis. MiR-590-5p subtype NF90 has angiogenic effects	[35, 36]
HGF	Stimulates angiogenesis by inducing endothelial cell proliferation, migration and tubular blood vessel formation	HGF significantly increases the expression of VEGF, decreases the activation of NF-κB and vascular leakage, promotes angiogenesis, is anti-inflammatory, is anti-oxidative and reduces vascular permeability	[37]
Angiotensin II	Angiogenesis is induced by activation of angiotensin 1 receptor and nicotinamide adenine dinucleotide phosphate oxidase	Induction of angiotensin II synthesis can lead to a proangiogenic state	[38]
asTF	asTF is widely expressed in macrophages and neovascularization in AS plaques	asTF affects all key stages of angiogenesis, including proliferation, migration and differentiation and induces increased levels of HIF and VEGF to promote angiogenesis	[39]
Classical Wnt pathway	Under the influence of Wnt factor, β-catenin isolates and enters the nucleus, binding to TCF/LEF and initiating transcription of downstream genes	The Wnt pathway promotes angiogenesis by regulating endothelial cell proliferation	[40, 41]

AS: Atherosclerosis; asTF: Alternatively spliced tissue factor; EC: Endothelial cell; EPO: Erythropoietin; HGF: Hepatocyte growth factor; HIF: Hypoxia inducible factor; MiR: MicroRNA; NF-κB: Nuclear factor kappa-B; VEGF: Vascular endothelial growth factor.

Table 2 Mechanisms and functions of the antiangiogenic factors

Inhibitory factor	Mechanism	Function	Ref.
Platelet reactive protein-1 (TSP-1)	TSP-1 levels increase under hypoxia	TSP-1 inhibits angiogenesis by stimulating endothelial cell apoptosis and inhibiting endothelial cell migration and proliferation as well as inhibiting VEGF and eNOS	[42, 43]
IL-12	IL-12 stimulates the expression of proinflammatory and antiangiogenic genes in monocytes	Neutralization of IL-12 can enhance angiogenesis in ischemic areas and reduce body dysfunction	[7, 44]
Noncoding RNAs	Noncoding RNA has the dual role of promoting and inhibiting angiogenesis	Upregulation of MiR-15a and MiR-16 reduce Tie2 protein levels and inhibit angiogenesis	[35, 36]

eNOS: Endothelial nitric oxide synthase; IL-12: Interleukin 12; MiR: MicroRNA; TSP-1: Thrombospondin-1; VEGF: Vascular endothelial growth factor.

Lactylation

Lactic acid has long been considered as a metabolic waste. Recent studies have shown that lactic acid can be expressed as a signaling molecule in wound healing and angiogenesis[50]. Lactic acid can modify histones to regulate macrophage polarity and expression of tissue repair genes such as arginase-1[51]. In inflammatory diseases such as atherosclerosis, macrophages secrete proinflammatory cytokines such as tumor necrosis factor γ and interleukin 12 to cause extensive damage to surrounding tissue, and anaerobic glycolysis causes lactic acid accumulation[51]. The macrophage polarity transition is a hallmark of the disease, and lactic acid converts macrophages from a proinflammatory phenotype into a repairing phenotype, removes cell debris and promotes wound healing. Inflammatory macrophages undergo modifications, which promote the repair characteristics of macrophages in response to inflammatory damage[52]. In addition, lactic acid can covalently couple with various histone lysine residues during histone acetylation to promote the transcription of homeostasis-related genes. In the late stage of

lactic acid and histone lactate modification and accumulation, the cells switch to a steady-state phenotype, in which inflammatory genes are difficult to induce.

Studies have shown that lactic acid can promote the secretion of VEGF, activate the nuclear factor kappa-B/C-X-C motif chemokine 8 pathway and stabilize HIF-1α, playing a role in promoting angiogenesis signaling molecules. The overall function of lactic acid is to transform the inflammatory phenotype of macrophages into a repair phenotype. We hypothesized that lactic acid was associated with angiogenesis. However, the integration mechanism of lactic acid and hypoxia, such as HIF, chromatin remodeling and other processes extending to angiogenesis, is still unclear and needs further research (Figure 1).

Oxidative stress

When the body is subjected to various diabetic stimuli, the mitochondria is stimulated to produce superoxide, leading to the formation of the powerful oxidant nitrite, which damages DNA and depletes intracellular NAD (+)[53], resulting in a pathological state. Two common mechanisms[54] contribute to increased oxidative stress[55] in diabetes: One is an increase in free radical production and the other is a decrease in the levels of protective endogenous antioxidants. Also, natural antioxidants include dandelion[56], saffron[57,58], hawthorn[59], vitamin C and vitamin E[60]. However, rhizoma polygonate in traditional Chinese medicine can dephosphorylate DNA to damage DNA[61]. In addition, hyperglycemia activates nuclear factor kappa-B, which can lead to changes in the inflammatory response, upregulation of COX-2, inducible NO synthase (NOS)[62], tumor necrosis factor α and interleukin 1, promotion of cell proliferation and inhibition of cell death. The increased expression of inducible NOS catalyzes the production of large amounts of NO[63]. The inhibition of TLR2/4 signaling can avoid nuclear factor kappa-B translocation, ultimately reducing cell apoptosis[64]. The hyperglycemic environment can stimulate the mitochondrial respiratory chain to produce a large number of oxygen free radicals, activate protein kinases C[65] and promote the NADPH-related processes of oxidative stress, leading to endothelial cell apoptosis. A small number of ROS can maintain normal physiological function[66]; however, an excess of ROS causes oxidative stress[67], which can activate multiple stress kinases and related proteases and affect their activities[68], aggravate cytotoxicity and attack cells, leading to endothelial progenitor cell senescence, apoptosis and inhibition of migration and proliferation. Superoxide anions and H_2O_2 in the ROS family play a major role in this process. In addition, the activity of endodermal NOS is reduced, the metabolism of tetrahydrobiopurine (BH4) is abnormal, and dihydrobiopterin (BH2) cannot be recovered in diabetes, resulting in a lower level of BH4[69]. NOS induces the formation of many superoxide anions instead of NO, aggravating oxidative stress. Advanced glycation end products lead to an imbalance in ROS production and clearance and increased endothelial permeability[70]. Oxidative stress impairs angiogenesis through multiple mechanisms.

Endothelial progenitor cells

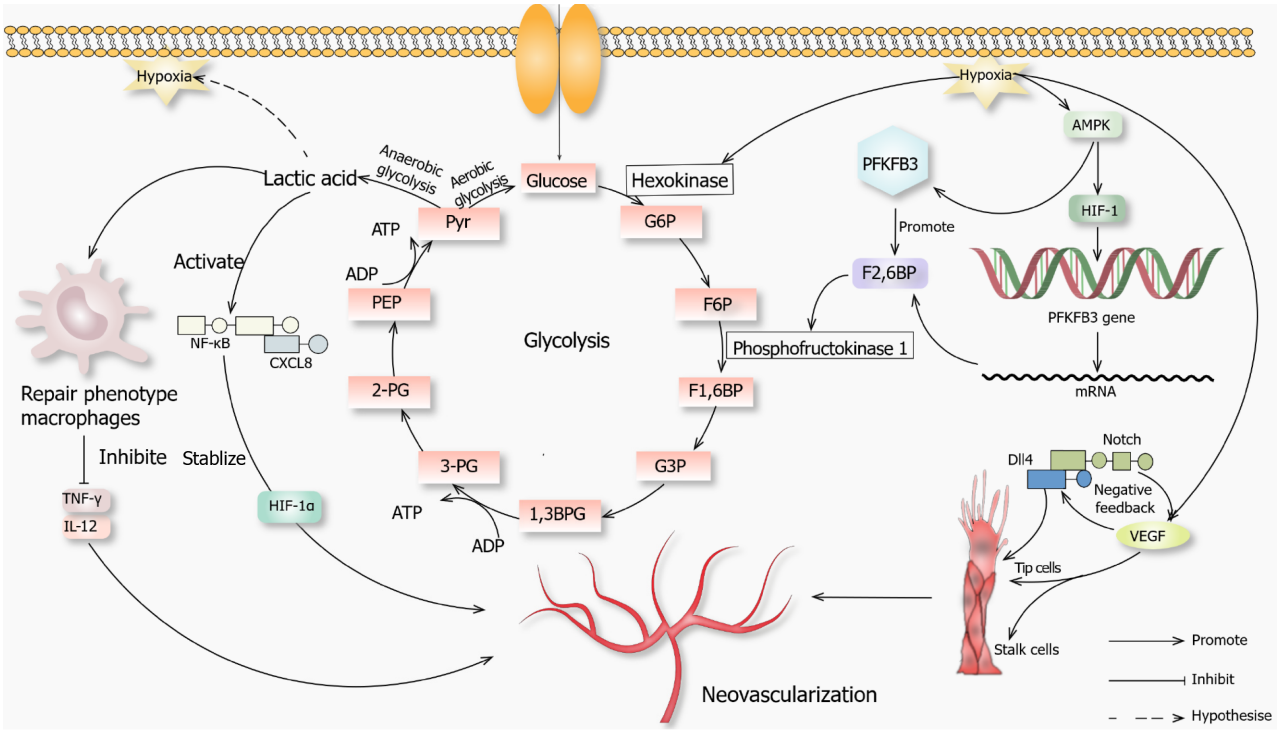
Endothelial progenitor cells (EPCs) are fusiform cells with limited proliferative capacity in the early stage and cells with high proliferative capacity in the late stage[71]. During tissue ischemia, EPCs can be mobilized from bone marrow to damaged blood vessels for vascular repair or angiogenesis, or the number of circulating EPCs increases *via* various factors, including VEGF, stromal cell-derived factor-1 or stem cell factor. EPCs can proliferate, migrate, adhere and differentiate into ECs, repair damaged ECs and secrete angiogenic factors such as VEGF to promote angiogenesis in ischemic tissues[72].

In diabetes, endothelial dysfunction and delayed angiogenesis promote the occurrence and development of diabetic vascular complications. In the high glucose environment, the number of EPCs is reduced and their functions are impaired[73]. In addition, EPCs are less responsive to ischemia, VEGF, stromal cell-derived factor-1 and other stimuli, and the mobilization mechanism is damaged. EPCs may also secrete antiangiogenic factors. The high glucose environment leads to the excessive production of ROS, excessive activation of NADPH oxidase and a significant decrease in the levels of manganese-containing superoxide dismutase and other antioxidant enzymes, resulting in EPC dysfunction[74]. The excessive production of ROS significantly increases the levels of oxLDL, inhibits Akt phosphorylation and endothelial NOS (eNOS) expression, decreases NO activity, inhibits the PI3K/Akt/eNOS signaling pathway and induces the apoptosis of EPCs, migration of EPCs and formation of functional defects in the lumen[75]. In addition, the severe inflammatory environment of diabetes causes impaired adhesion and proliferation of EPCs as well as neovascularization.

However, when patients with diabetes suffer from vascular complications, the number and function of EPCs in different parts (microvessels and large vessels) are different. For example, when such patients suffer from peripheral artery disease, the number of EPCs decreases. However, the proliferative capacity of EPCs is increased in patients with proliferative retinopathy.

Slit2/Roundabout 1/PI3K/Akt/VEGF signaling pathways

Endothelial cell-derived Slit2 plays a proangiogenic role in EC migration and lumen formation through its Roundabout 1 (Robo1) receptor. High glucose levels directly induce Slit2 production or Slit2/Robo1 binding. Robo1 inhibits the activation of the PI3K/Akt pathways and HIF-1α/VEGF signaling pathways and inhibits angiogenesis. PI3K inhibitors also inhibit the HIF-1α/VEGF signaling



DOI: 10.4239/wjd.v13.i11.926

Figure 1 Novel mechanisms of neovascularization. When glucose enters the cell through channels that initiate glycolysis in an aerobic environment, when the anaerobic glycolysis, lactic acid can cause macrophages to convert to a repair phenotype. These macrophages will not produce inflammatory factors such as tumor necrosis factor γ and interleukin 12, which promotes angiogenesis. Lactic acid can activate the nuclear factor kappa-B-C-X-C motif chemokine 8 pathway, promote vascular endothelial growth factor (VEGF) secretion, stabilize hypoxia-inducible factor-1α (HIF-1α), and promote angiogenesis. When hypoxia occurs in the environment, it promotes hexokinase, which promotes glycolysis, and AMPK, which promotes hypoxia-inducible factor-1. It then acts on 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3. AMPK also acts directly on 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3, and they jointly promote fructose-2,6-biphosphatase, which acts on phosphofructokinase 1 and promotes glycolysis. Hypoxia also promotes VEGF secretion, and a high concentration of VEGF activates the Notch-DLL4 pathway, directly or indirectly promoting the proliferation of tip cells. VEGF also promotes the growth of stalk cells, and both promote angiogenesis. 1,3BPG: 1,3 diphosphoglycerate; 2-PG: 2-phosphoglycerate; 3-PG: 3-phosphoglycerate; F1,6BP: Fructose 1,6-diphosphate; F2,6BP: Fructose 2,6-diphosphate; F6P: Fructose 6 phosphate; G3P: Glyceraldehyde-3-phosphate; G6P: Glucose 6-phosphate; PEP: Phosphoenolpyruvate; Pyr: Pyruvic acid.

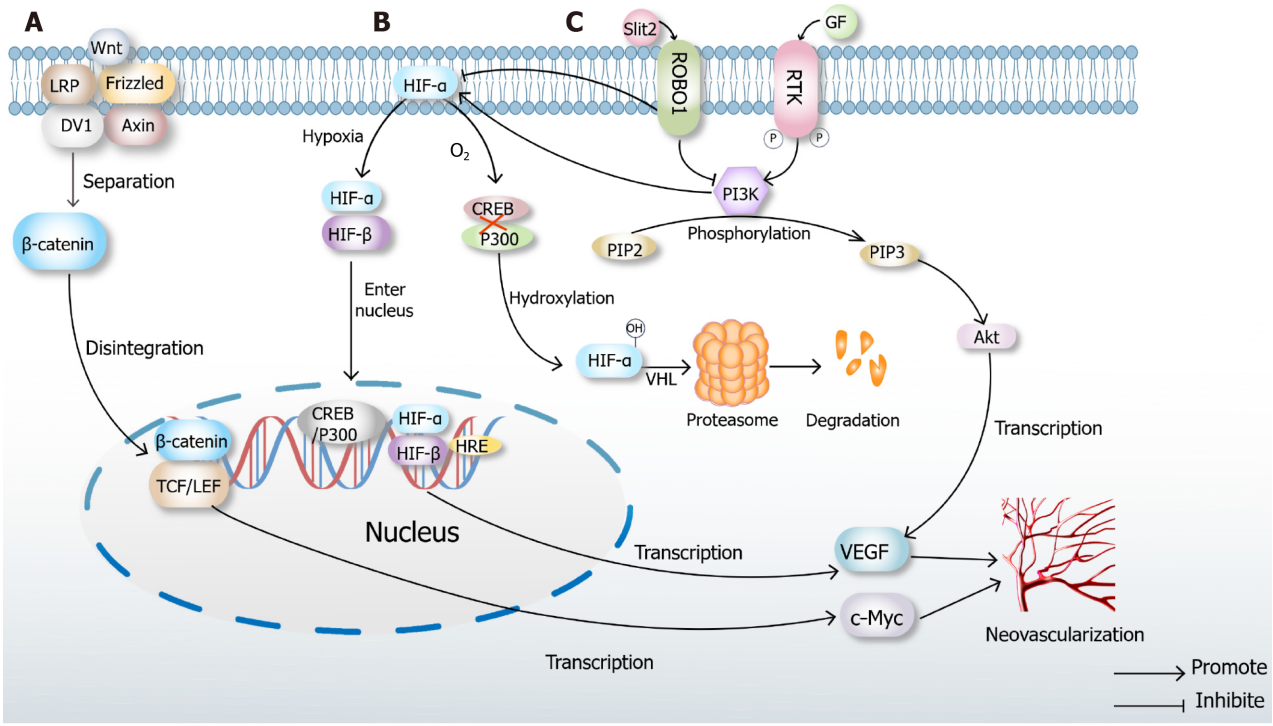
pathway. Hence, Robo1 may be a potential therapeutic target in diabetic ischemic complications with abnormal angiogenesis, such as diabetic nephropathy and diabetic retinal disease[76]. The interference of this signaling pathway can inhibit angiogenesis (Figure 2).

NEOVASCULARIZATION AFTER DIABETIC ISCHEMIA

Diabetes atherosclerosis

Atherosclerotic plaques lead to local hypoxia and trigger angiogenesis of the outer membrane of the vessel. The newly formed vessels are immature and leaky due to the lack of a tight connection between the pericellular cells and ECs. Lipids, inflammatory factors and red blood cells invade the plaques, leading to vessel rupture within the plaques. Proteolytic enzymes released by neovascularization promote inflammatory cell infiltration, and inflammatory factors trigger angiogenesis[77]. At the same time, intraplaque bleeding leads to a rapid increase in plaque volume, the fibrous cap changes to a contraction phenotype, and the fibrous cap becomes thinner[78], increasing the risk of plaque rupture [79]. However, Brezinski *et al*[80,81] put forward an opposite view that plaque rupture is caused by insufficient angiogenesis. The axial extension of neovascularization allows the healing of most plaques, while insufficient angiogenesis cannot sustain the growth of ECs in long necrotic plaques, resulting in plaque rupture and acute coronary syndrome.

Under high glucose conditions, the senescence and death of EPCs are accelerated, the PI3K/Akt/eNOS signaling pathway is inhibited, advanced glycation end products are increased, and fibrin formation is accelerated[82,83]. This triggers endoplasmic reticulum stress and oxidative stress, resulting in insulin resistance and accelerated atheromatous plaque formation. The endothelial cell differentiation is blocked, and angiogenesis is damaged (Figure 3).



DOI: 10.4239/wjd.v13.i11.926

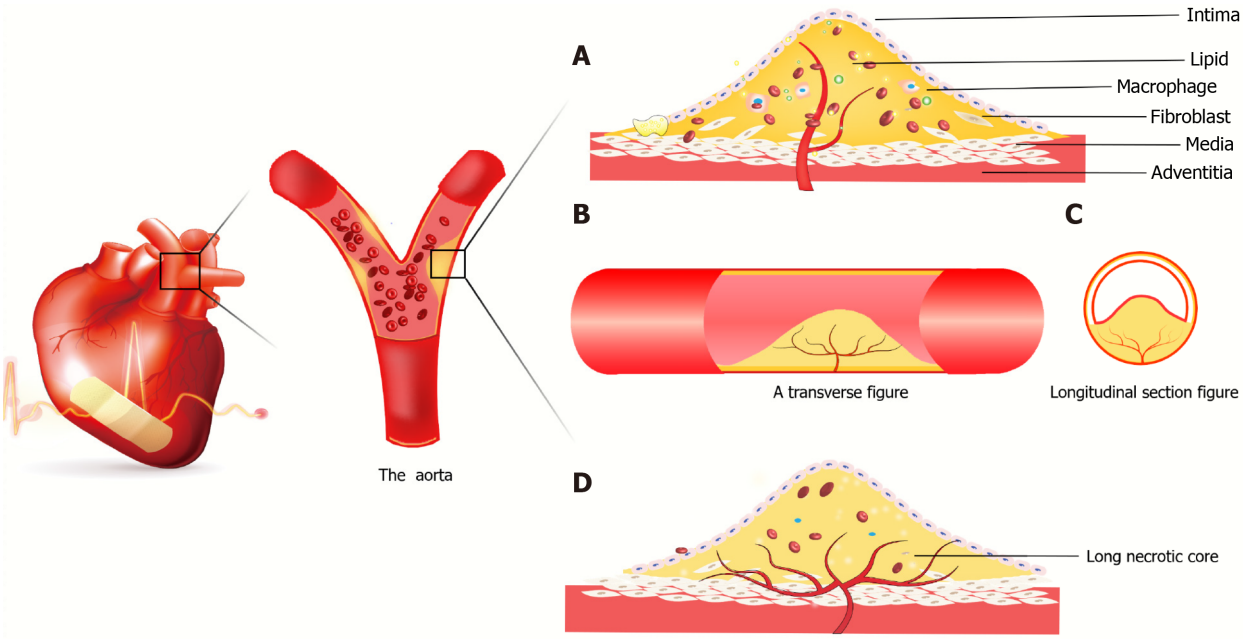
Figure 2 Classical mechanisms of neovascularization. A: Classical Wnt pathway. When there is no Wnt signal, LRP, Frizzled, DV1 and Axin are closely combined with β-catenin. In the presence of Wnt signaling, the complex disintegrates, and β-catenin enters the nucleus, binds to TCF/LEF, transcribes multiple downstream signals such as c-Myc and ultimately promotes neovascularization; B: Under normoxic conditions, hypoxia-inducible factor (HIF)-α inhibits the binding of cAMP-response element binding protein (CREB) and P300, which causes hydroxylation of HIF, then leads to proteasome degradation and inactivation after VHL ubiquitination. When hypoxia occurs, prolyl hydroxylase domains become inactive, allowing HIF-α to migrate to the nucleus, where it dimerizes with HIF-β. The dimer binds to the hypoxia response elements of specific genes in DNA, transcribes thousands of genes such as vascular endothelial growth factor (VEGF) and promotes neovascularization; C: High glucose induces Slit2/Roundabout 1 (Robo1) binding, and Robo1 inhibits the activation of phosphatidylinositol 3kinase (PI3K) /protein kinase B (Akt) and HIF-1α/VEGF signaling pathway and inhibits angiogenesis. PI3K inhibitors also inhibit the HIF-1α/VEGF signaling pathway. After activation of the PI3K/Akt signaling pathway, a variety of cytokines including VEGF will be transcribed to promote angiogenesis.

Diabetic retinopathy

Diabetic retinopathy (DR) can be divided into early nonproliferative DR and late proliferative DR in terms of progression, leading to pathological retinal angiogenesis[84]. Neovascularization extends along the surface of the retina into the vitreous cavity. Still, such vessels are fragile and easily broken, easily leading to vitreous hemorrhage, retinal detachment or macular nonperfusion and related photoreceptor dysfunction[85]. The earliest change is the thickening of the vascular basement membrane. In a high glucose environment, the basement membrane hardens and changes the elasticity of blood vessels, affecting the retinal blood flow and the dynamic balance between the inside and outside of blood vessels[86].

After the blood glucose level increases, the blood-retinal barrier degrades briefly in a few days or weeks, and then Muller cells are activated. Pericytes are lost in about 2 mo, followed by ECs, leading to vascular degeneration 6 mo after diabetes[85]. Vascular degeneration is caused by the loss of pericytes that control vascular patency, resulting in decreased vascular patency, vascular blockage, endothelial cell fusion/degeneration and finally basal membrane dissolution, vascular degeneration and formation of capillaries without ECs. However, under physiological conditions, ECs of retinal capillaries express a high level of tight connections, limiting the circulation of nutrients, soluble factors and cells into tissues [87], and vascular degeneration destroys this structural function.

Consistent with numerous pathological processes, in a hypoxic and ischemic environment, angiogenesis-related factors are promoted, retinal neovascularization occurs, and newly formed blood capillaries migrate to other capillaries and merge to form new blood capillaries[87]. However, the high glucose environment can promote the development of diabetic retinal neovascularization. Proliferative retinopathy is accompanied by tractional retinal detachment and vision loss. Therefore, the treatment aims to inhibit neovascularization. The standard treatment methods include laser therapy and anti-VEGF therapy. The intravitreal injection of VEGF inhibitors has excellent short-term safety. However, whether long-term anti-VEGF may have long-term adverse effects on the function of retinal neurons is unclear.



DOI: 10.4239/wjd.v13.i11.926 **Copyright** ©The Author(s) 2022.

Figure 3 Atherosclerotic plaque forms in coronary vessels. A: New blood vessels from the outer membrane to the media and intima, macrophages and smooth muscle cells gobble up from the film in lipid foam cell formation. The red blood cells, cytokines, *etc* extend the blood vessels into the plaque. It is because the spin-off of new blood vessels leads to unstable plaques, plaque hemorrhage and rupture resulting in acute coronary syndrome; B: Axial slice of intravascular plaque and neovascularization grows axially in the plaque; C: Transverse view of intravascular plaque; D: It is believed that neovascularization grows in the axial direction in plaques. Due to insufficient growth of neovascularization, long necrotic plaques are ruptured due to ischemia and hypoxia in the plaque, resulting in acute coronary syndrome.

Diabetic nephropathy

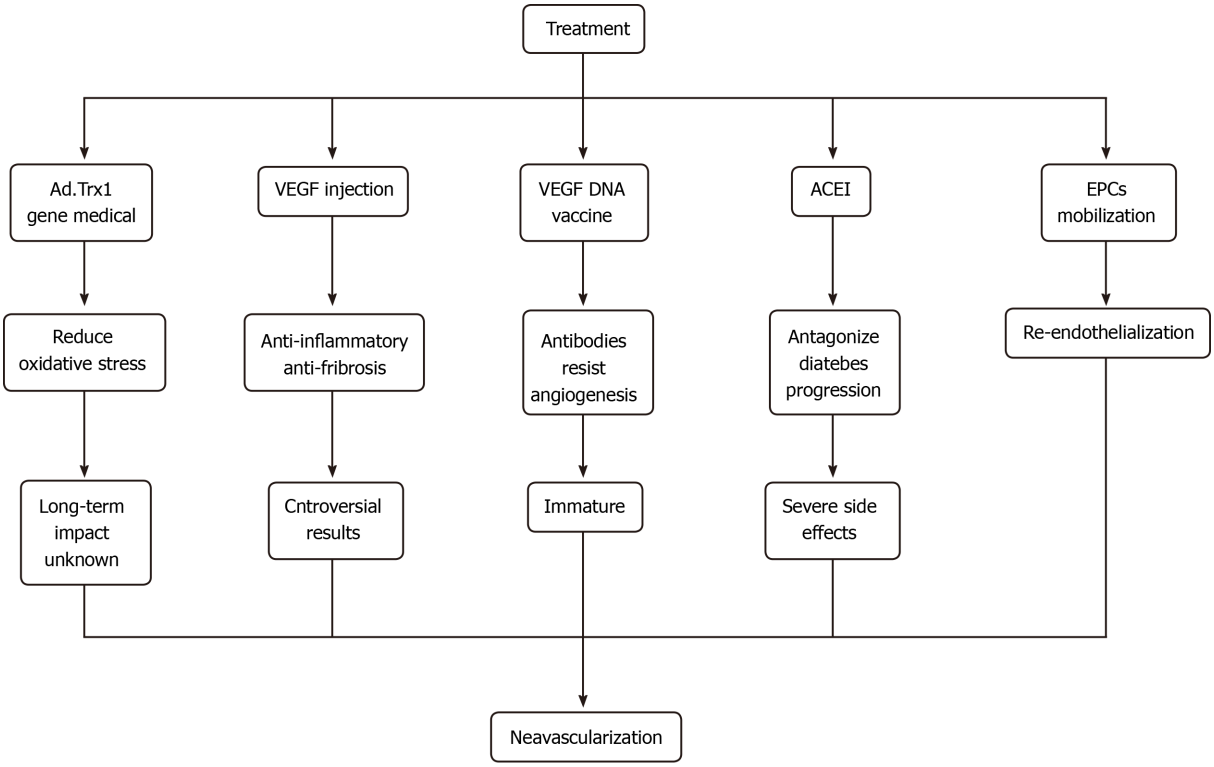
In the early stage of diabetic nephropathy, hypoxia induces HIF generation, and endothelial growth factor or angiopoietin maintains renal vascular density, resulting in increased abnormal angiogenesis, vascular immaturity, plasma protein leakage, increased proteinuria and significantly increased glomerular filtration rate. In the late stage, glomerular capillaries are sparse, and the production of nephrogenic erythropoietin is increased, which aggravates renal hypoxia, and glomerular cells lose vitality[88]. In this process, eNOS activity declines, the utilization rate of NO decreases, oxidative stress abates HIF activation, and VEGF expression is significantly lowered. Also, the levels of antiangiogenetic factors such as platelet response protein 1 and endothelial inhibition significantly increase, the levels of inflammatory cytokines increase, and VEGF expression is inhibited. Further, EPC function is impaired, the inflammatory response is enhanced, and the expression of adherence factors is upregulated, which is accompanied by impaired capillary ECs, endothelial barrier dysfunction and reduced angiogenesis, resulting in abnormal angiogenesis and vascular leakage. Podocytes are an important source of growth factors that regulate endothelial cell proliferation and angiogenesis. Their number increases in the early stage of diabetes and decreases in the late stage. The number of mesangial cells increases, the capillary basement membrane thickens, and the capillary number and area increase, directly or indirectly resulting in glomerular hyperplasia and mesangial expansion.

At present, no precise treatment is available for diabetic nephropathy to inhibit angiogenesis. VEGFA inhibitors can be used, but their levels need to be maintained at an appropriate level *in vivo*[89]. A deviation from moderate levels can cause damage.

Diabetic foot ulcer

Diabetic foot ulcer (DFU) is characterized by neuropathy caused by hyperglycemic levels, arterial stenosis caused by lipid deposition, and ischemic lesions of lower extremities. The DFU healing process can be divided into three overlapping phases: early steady-state and inflammation, arteriogenesis and matrix deposition; mid-late reshaping; and epithelial cell remodeling[90]. Neutrophil granulocyte and macrophages produce cytokines and promote cell proliferation; fibroblasts are rich in collagen fibers and induce angiogenesis and vascularization[91]. Neurological and ischemic lesions lead to impaired healing.

DFU arteriogenesis is reduced in diabetes. Hypoxia and ROS decrease transcriptional activities of HIF, VEGF, angiopoietin 2 and fibroblast growth factor and inhibit collateral development and arteriogenesis after limb ischemia[92]. Hyperglycemic levels result in impaired growth factor production and macrophage function, collagen accumulation inhibition and poor migration and proliferation of



DOI: 10.4239/wjd.v13.i11.926

Figure 4 Cutting-edge approaches to therapeutic angiogenesis. ACEI: Angiotensin converting enzyme inhibitors; EPCs: Endothelial progenitor cells; VEGF: Vascular endothelial growth factor.

keratinocytes and fibroblasts, leading to impaired angiogenesis[91]. At the same time, wound infection reduces the active matrix metalloproteinase 8 (MMP-8) level, increases the active MMP-9 level, inhibits laminin, enhances keratinocyte migration, reduces arteriogenesis and slows wound healing[93].

For the treatment of DFU, the current strategy is to increase arteriogenesis, eliminate oxidative stress and ulcer infection[94]. The best strategy for DFU treatment is to inhibit MMP-9 without affecting MMP-8, which can reduce inflammation and increase arteriogenesis. Applying prolyl hydroxylase domain inhibitors with clinical potential can stabilize HIF and increase its activity to promote arteriogenesis. Mesenchymal stem cells produce growth hormones that drive arteriogenesis and re-epithelialization.

TREATMENT

For treating diabetic ischemic neovascularization, the most crucial way is to control blood glucose levels. On this basis, various therapeutic methods, including gene therapy and vaccine research, have been proposed, which have broad prospects. For example, Ad.trx1 gene therapy can stabilize the microenvironment in the myocardium, reduce oxidative stress and cell death and induce neovascularization and maturation. This is a novel treatment that may improve disease progression and patient recovery[95]. Injection of VEGF and hepatocyte growth factor can promote neovascularization and exert anti-inflammatory and anti-fibrotic effects, but gene therapy is still controversial due to its insignificant effect and needs further study.

Antiangiogenesis antibodies can be generated by the intramuscular injection of the VEGF DNA vaccine; however, the technology is still immature, and the efficacy and long-term impact are not apparent. Hence, further clinical research is needed. Also, angiotensin-converting enzyme inhibitors can antagonize inflammation, increase the number of EPCs and improve the mobilization ability in patients with diabetes. However, this treatment causes an irritating cough, bilateral renal artery stenosis and other adverse side effects[96]. In addition, EPC mobilization promotes re-endothelialization, repairs damaged ECs, promotes angiogenesis and restores blood flow[97,98]. It is believed that with further research, more treatments can be developed to benefit mankind (Figure 4).

CONCLUSION

The advantages and disadvantages of neovascularization to the body are based on different environments. For , MI, peripheral arterial disease, coronary collateral circulation and DFU, neovascularization needs to be promoted to restore the perfusion of the ischemic area and reduce body damage. Neovascularization should be inhibited for DR and tumors to reduce the risk of retinal stripping or tumor metastasis. Although the involvement of neovascularization in disease pathogenesis is still not specific, it has huge prospects for treating ischemia in diabetes.

FOOTNOTES

Author contributions: Cai Y, Zang GY, Huang Y and Sun Z designed, collected and interpreted data and drafted, revised and approved the manuscript; Zhang LL, Qian YJ, Yuan W and Wang ZQ interpreted data and drafted, revised and approved the manuscript.

Supported by the National Natural Science Foundation of China, No. 82070455; the Related Foundation of Jiangsu Province, No. BK20201225; the Medical Innovation Team Project of Jiangsu Province, No. CXTDA2017010; and the Postgraduate Research and Practice Innovation Program of Jiangsu Province, No. KYCX20_3051.

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

Country/Territory of origin: China

ORCID number: Zhong-Qun Wang 0000-0003-3590-9313.

S-Editor: Gong ZM
L-Editor: Filipodia
P-Editor: Gong ZM

REFERENCES

1 **International Diabetes Federation**. IDF diabetes atlas[EB/OL]. [DOI: 10.1136/bmjdrc-2021-002122]
2 **Zhu L**, Lama S, Tu L, Dusting GJ, Wang JH, Liu GS. TAK1 signaling is a potential therapeutic target for pathological angiogenesis. *Angiogenesis* 2021; **24**: 453-470 [PMID: 33973075 DOI: 10.1007/s10456-021-09787-5]
3 **Annex BH**, Cooke JP. New Directions in Therapeutic Angiogenesis and Arteriogenesis in Peripheral Arterial Disease. *Circ Res* 2021; **128**: 1944-1957 [PMID: 34110899 DOI: 10.1161/CIRCRESAHA.121.318266]
4 **Akwii RG**, Sajib MS, Zahra FT, Mikelis CM. Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology. *Cells* 2019; **8** [PMID: 31108880 DOI: 10.3390/cells8050471]
5 **Dalton AC**, Shlamkovitch T, Papo N, Barton WA. Constitutive Association of Tie1 and Tie2 with Endothelial Integrins is Functionally Modulated by Angiopoietin-1 and Fibronectin. *PLoS One* 2016; **11**: e0163732 [PMID: 27695111 DOI: 10.1371/journal.pone.0163732]
6 **Xiong YY**, Gong ZT, Tang RJ, Yang YJ. The pivotal roles of exosomes derived from endogenous immune cells and exogenous stem cells in myocardial repair after acute myocardial infarction. *Theranostics* 2021; **11**: 1046-1058 [PMID: 33391520 DOI: 10.7150/thno.53326]
7 **Wu X**, Reboll MR, Korf-Klingebiel M, Wollert KC. Angiogenesis after acute myocardial infarction. *Cardiovasc Res* 2021; **117**: 1257-1273 [PMID: 33063086 DOI: 10.1093/cvr/cvaa287]
8 **Howangyin KY**, Silvestre JS. Diabetes mellitus and ischemic diseases: molecular mechanisms of vascular repair dysfunction. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1126-1135 [PMID: 24675660 DOI: 10.1161/ATVBAHA.114.303090]
9 **Janbandhu V**, Tallapragada V, Patrick R, Li Y, Abeygunawardena D, Humphreys DT, Martin EMMA, Ward AO, Contreras O, Farbehi N, Yao E, Du J, Dunwoodie SL, Bursac N, Harvey RP. Hif-1a suppresses ROS-induced proliferation of cardiac fibroblasts following myocardial infarction. *Cell Stem Cell* 2022; **29**: 281-297.e12 [PMID: 34762860 DOI: 10.1016/j.stem.2021.10.009]
10 **Hutchings G**, Kruszyna Ł, Nawrocki MJ, Strauss E, Bryl R, Spaczyńska J, Perek B, Jemielity M, Mozdziak P, Kempisty B, Nowicki M, Krasiński Z. Molecular Mechanisms Associated with ROS-Dependent Angiogenesis in Lower Extremity Artery Disease. *Antioxidants (Basel)* 2021; **10** [PMID: 34066926 DOI: 10.3390/antiox10050735]
11 **Meng S**, Zhou G, Gu Q, Chanda PK, Ospino F, Cooke JP. Transdifferentiation Requires iNOS Activation: Role of RING1A S-Nitrosylation. *Circ Res* 2016; **119**: e129-e138 [PMID: 27623813 DOI: 10.1161/CIRCRESAHA.116.308263]

12 **Chanda PK**, Meng S, Lee J, Leung HE, Chen K, Cooke JP. Nuclear *S*-Nitrosylation Defines an Optimal Zone for Inducing Pluripotency. *Circulation* 2019; **140**: 1081-1099 [PMID: 31412725 DOI: 10.1161/CIRCULATIONAHA.119.042371]

13 **Lai L**, Reineke E, Hamilton DJ, Cooke JP. Glycolytic Switch Is Required for Transdifferentiation to Endothelial Lineage. *Circulation* 2019; **139**: 119-133 [PMID: 30586707 DOI: 10.1161/CIRCULATIONAHA.118.035741]

14 **Möbius-Winkler S**, Uhlemann M, Adams V, Sandri M, Erbs S, Lenk K, Mangner N, Mueller U, Adam J, Grunze M, Brunner S, Hilberg T, Mende M, Linke AP, Schuler G. Coronary Collateral Growth Induced by Physical Exercise: Results of the Impact of Intensive Exercise Training on Coronary Collateral Circulation in Patients With Stable Coronary Artery Disease (EXCITE) Trial. *Circulation* 2016; **133**: 1438-48; discussion 1448 [PMID: 26979085 DOI: 10.1161/CIRCULATIONAHA.115.016442]

15 **Liu R**, Zhao H, Wu S, Li H. Incomplete protective effect of coronary collateral circulation for acute myocardial infarction patients. *Medicine (Baltimore)* 2020; **99**: e22750 [PMID: 33120776 DOI: 10.1097/MD.0000000000022750]

16 **Seiler C**, Stoller M, Pitt B, Meier P. The human coronary collateral circulation: development and clinical importance. *Eur Heart J* 2013; **34**: 2674-2682 [PMID: 23739241 DOI: 10.1093/eurheartj/eht195]

17 **Chu AA**, Li W, Zhu YQ, Meng XX, Liu GY. Effect of coronary collateral circulation on the prognosis of elderly patients with acute ST-segment elevation myocardial infarction treated with underwent primary percutaneous coronary intervention. *Medicine (Baltimore)* 2019; **98**: e16502 [PMID: 31374011 DOI: 10.1097/MD.0000000000016502]

18 **Nathoe HM**, Koerselman J, Buskens E, van Dijk D, Stella PR, Plokker TH, Doevendans PA, Grobbee DE, de Jaegere PP; Octopus Study Group. Determinants and prognostic significance of collaterals in patients undergoing coronary revascularization. *Am J Cardiol* 2006; **98**: 31-35 [PMID: 16784916 DOI: 10.1016/j.amjcard.2006.01.050]

19 **Fei Y**, Hou J, Xuan W, Zhang C, Meng X. The relationship of plasma miR-503 and coronary collateral circulation in patients with coronary artery disease. *Life Sci* 2018; **207**: 145-151 [PMID: 29870767 DOI: 10.1016/j.lfs.2018.06.001]

20 **Elias J**, Hoebers LPC, van Dongen IM, Claessen BEPM, Henriques JPS. Impact of Collateral Circulation on Survival in ST-Segment Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention With a Concomitant Chronic Total Occlusion. *JACC Cardiovasc Interv* 2017; **10**: 906-914 [PMID: 28473112 DOI: 10.1016/j.jcin.2017.01.026]

21 **Lucero García Rojas EY**, Villanueva C, Bond RA. Hypoxia Inducible Factors as Central Players in the Pathogenesis and Pathophysiology of Cardiovascular Diseases. *Front Cardiovasc Med* 2021; **8**: 709509 [PMID: 34447792 DOI: 10.3389/fcvm.2021.709509]

22 **Ullah K**, Wu R. Hypoxia-Inducible Factor Regulates Endothelial Metabolism in Cardiovascular Disease. *Front Physiol* 2021; **12**: 670653 [PMID: 34290616 DOI: 10.3389/fphys.2021.670653]

23 **Pan Z**, Ma G, Kong L, Du G. Hypoxia-inducible factor-1: Regulatory mechanisms and drug development in stroke. *Pharmacol Res* 2021; **170**: 105742 [PMID: 34182129 DOI: 10.1016/j.phrs.2021.105742]

24 **Matuszewska K**, Pereira M, Petrik D, Lawler J, Petrik J. Normalizing Tumor Vasculature to Reduce Hypoxia, Enhance Perfusion, and Optimize Therapy Uptake. *Cancers (Basel)* 2021; **13** [PMID: 34503254 DOI: 10.3390/cancers13174444]

25 **Karger A**, Nandigama R, Stenzinger A, Grimminger F, Pullamsetti SS, Seeger W, Savai R. Hidden Treasures: Macrophage Long Non-Coding RNAs in Lung Cancer Progression. *Cancers (Basel)* 2021; **13** [PMID: 34439281 DOI: 10.3390/cancers13164127]

26 **Zheng K**, Niu W, Lei B, Boccaccini AR. Immunomodulatory bioactive glasses for tissue regeneration. *Acta Biomater* 2021; **133**: 168-186 [PMID: 34418539 DOI: 10.1016/j.actbio.2021.08.023]

27 **Alvarez-Argote S**, O'Meara CC. The Evolving Roles of Cardiac Macrophages in Homeostasis, Regeneration, and Repair. *Int J Mol Sci* 2021; **22** [PMID: 34360689 DOI: 10.3390/ijms22157923]

28 **Martin P**, Gurevich DB. Macrophage regulation of angiogenesis in health and disease. *Semin Cell Dev Biol* 2021; **119**: 101-110 [PMID: 34330619 DOI: 10.1016/j.semcdb.2021.06.010]

29 **Liu J**, Geng X, Hou J, Wu G. New insights into M1/M2 macrophages: key modulators in cancer progression. *Cancer Cell Int* 2021; **21**: 389 [PMID: 34289846 DOI: 10.1186/s12935-021-02089-2]

30 **Hong H**, Tian XY. The Role of Macrophages in Vascular Repair and Regeneration after Ischemic Injury. *Int J Mol Sci* 2020; **21** [PMID: 32878297 DOI: 10.3390/ijms21176328]

31 **Olingy CE**, Dinh HQ, Hedrick CC. Monocyte heterogeneity and functions in cancer. *J Leukoc Biol* 2019; **106**: 309-322 [PMID: 30776148 DOI: 10.1002/JLB.4RI0818-311R]

32 **Zhou Y**, Zhu X, Cui H, Shi J, Yuan G, Shi S, Hu Y. The Role of the VEGF Family in Coronary Heart Disease. *Front Cardiovasc Med* 2021; **8**: 738325 [PMID: 34504884 DOI: 10.3389/fcvm.2021.738325]

33 **Apte RS**, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell* 2019; **176**: 1248-1264 [PMID: 30849371 DOI: 10.1016/j.cell.2019.01.021]

34 **Melincovici CS**, Boşca AB, Şuşman S, Mărginean M, Mihu C, Istrate M, Moldovan IM, Roman AL, Mihu CM. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol* 2018; **59**: 455-467 [PMID: 30173249]

35 **Razavi ZS**, Asgarpour K, Mahjoubin-Tehran M, Rasouli S, Khan H, Shahrzad MK, Hamblin MR, Mirzaei H. Angiogenesis-related non-coding RNAs and gastrointestinal cancer. *Mol Ther Oncolytics* 2021; **21**: 220-241 [PMID: 34095461 DOI: 10.1016/j.omto.2021.04.002]

36 **Ghafouri-Fard S**, Shoorei H, Mohaqiq M, Taheri M. Non-coding RNAs regulate angiogenic processes. *Vascul Pharmacol* 2020; **133-134**: 106778 [PMID: 32784009 DOI: 10.1016/j.vph.2020.106778]

37 **Shimamura M**, Nakagami H, Sanada F, Morishita R. Progress of Gene Therapy in Cardiovascular Disease. *Hypertension* 2020; **76**: 1038-1044 [PMID: 32772646 DOI: 10.1161/HYPERTENSIONAHA.120.14478]

38 **Xie S**, Wang Y, Huang Y, Yang B. Mechanisms of the antiangiogenic effects of aspirin in cancer. *Eur J Pharmacol* 2021; **898**: 173989 [PMID: 33657423 DOI: 10.1016/j.ejphar.2021.173989]

39 **Giannarelli C**, Alique M, Rodriguez DT, Yang DK, Jeong D, Calcagno C, Hutter R, Millon A, Kovacic JC, Weber T, Faries PL, Soff GA, Fayad ZA, Hajjar RJ, Fuster V, Badimon JJ. Alternatively spliced tissue factor promotes plaque angiogenesis through the activation of hypoxia-inducible factor-1α and vascular endothelial growth factor signaling.

Circulation 2014; **130**: 1274-1286 [PMID: 25116956 DOI: 10.1161/CIRCULATIONAHA.114.006614]
40 **Nusse R**, Clevers H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* 2017; **169**: 985-999 [PMID: 28575679 DOI: 10.1016/j.cell.2017.05.016]
41 **Foulquier S**, Daskalopoulos EP, Lluri G, Hermans KCM, Deb A, Blankesteijn WM. WNT Signaling in Cardiac and Vascular Disease. *Pharmacol Rev* 2018; **70**: 68-141 [PMID: 29247129 DOI: 10.1124/pr.117.013896]
42 **Dou M**, Chen Y, Hu J, Ma D, Xing Y. Recent Advancements in CD47 Signal Transduction Pathways Involved in Vascular Diseases. *Biomed Res Int* 2020; **2020**: 4749135 [PMID: 32733941 DOI: 10.1155/2020/4749135]
43 **Bazzazi H**, Zhang Y, Jafarnejad M, Isenberg JS, Annex BH, Popel AS. Computer Simulation of TSP1 Inhibition of VEGF-Akt-eNOS: An Angiogenesis Triple Threat. *Front Physiol* 2018; **9**: 644 [PMID: 29899706 DOI: 10.3389/fphys.2018.00644]
44 **Xu Y**, Yan Y, Geng T, Wang C, Xu Y, Yang P, Yan J. CD137-CD137L Signaling Affects Angiogenesis by Mediating Phenotypic Conversion of Macrophages. *J Cardiovasc Pharmacol* 2020; **75**: 148-154 [PMID: 31651672 DOI: 10.1097/FJC.0000000000000772]
45 **Zhang W**, Zhang X, Huang S, Chen J, Ding P, Wang Q, Li L, Lv X, Zhang P, Zhou D, Wen W, Wang Y, Lei QY, Wu J, Hu W. FOXM1D potentiates PKM2-mediated tumor glycolysis and angiogenesis. *Mol Oncol* 2021; **15**: 1466-1485 [PMID: 33314660 DOI: 10.1002/1878-0261.12879]
46 **De Bock K**, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Eelen G, Phng LK, Betz I, Tembuyser B, Brepoels K, Welti J, Geudens I, Segura I, Cruys B, Bifari F, Decimo I, Blanco R, Wyns S, Vangindertael J, Rocha S, Collins RT, Munck S, Daelemans D, Imamura H, Devlieger R, Rider M, Van Veldhoven PP, Schuit F, Bartrons R, Hofkens J, Fraisl P, Telang S, Deberardinis RJ, Schoonjans L, Vinckier S, Chesney J, Gerhardt H, Dewerchin M, Carmeliet P. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 2013; **154**: 651-663 [PMID: 23911327 DOI: 10.1016/j.cell.2013.06.037]
47 **Russo S**, Kwiatkowski M, Govorukhina N, Bischoff R, Melgert BN. Meta-Inflammation and Metabolic Reprogramming of Macrophages in Diabetes and Obesity: The Importance of Metabolites. *Front Immunol* 2021; **12**: 746151 [PMID: 34804028 DOI: 10.3389/fimmu.2021.746151]
48 **Irizarry-Caro RA**, McDaniel MM, Overcast GR, Jain VG, Troutman TD, Pasare C. TLR signaling adapter BCAP regulates inflammatory to reparatory macrophage transition by promoting histone lactylation. *Proc Natl Acad Sci U S A* 2020; **117**: 30628-30638 [PMID: 33199625 DOI: 10.1073/pnas.2009778117]
49 **Yuan C**, Wu C, Xue R, Jin C, Zheng C. Suppression of human colon tumor by EERAC through regulating Notch/DLL4/Hes pathway inhibiting angiogenesis *in vivo*. *J Cancer* 2021; **12**: 5914-5922 [PMID: 34476005 DOI: 10.7150/jca.61581]
50 **Dichtl S**, Lindenthal L, Zeitler L, Behnke K, Schlösser D, Strobl B, Scheller J, El Kasmi KC, Murray PJ. Lactate and IL6 define separable paths of inflammatory metabolic adaptation. *Sci Adv* 2021; **7** [PMID: 34162546 DOI: 10.1126/sciadv.abg3505]
51 **Yang L**, Gao L, Nickel T, Yang J, Zhou J, Gilbertsen A, Geng Z, Johnson C, Young B, Henke C, Gourley GR, Zhang J. Lactate Promotes Synthetic Phenotype in Vascular Smooth Muscle Cells. *Circ Res* 2017; **121**: 1251-1262 [PMID: 29021296 DOI: 10.1161/CIRCRESAHA.117.311819]
52 **Alvarez R**, Mandal D, Chittiboina P. Canonical and Non-Canonical Roles of PFKFB3 in Brain Tumors. *Cells* 2021; **10** [PMID: 34831136 DOI: 10.3390/cells10112913]
53 **Al-Shamsi M**, Amin A, Adeghate E. Effect of vitamin C on liver and kidney functions in normal and diabetic rats. *Ann N Y Acad Sci* 2006; **1084**: 371-390 [PMID: 17151316 DOI: 10.1196/annals.1372.031]
54 **Al Shamsi MS**, Amin A, Adeghate E. Beneficial effect of vitamin E on the metabolic parameters of diabetic rats. *Mol Cell Biochem* 2004; **261**: 35-42 [PMID: 15362483 DOI: 10.1023/b:mcbi.0000028735.79172.9b]
55 **Amin A**, Lotfy M, Mahmoud-Ghoneim D, Adeghate E, Mohamed A. Al-Akhras, Al-Saadi M, Al-Rahmoun S, Hameed R. Pancreas-protective effects of chlorella in STZ-induced diabetic animal model: insights into the mechanism. *J Diabetes Mellitus* 2011; **1**: 36-45 [DOI: 10.4236/jdm.2011.13006]
56 **Hamza AA**, Mohamed MG, Lashin FM, Amin A. Dandelion prevents liver fibrosis, inflammatory response, and oxidative stress in rats. *JoBAZ* 2020; **81**: 43 [DOI: 10.1186/s41936-020-00177-9]
57 **Nelson DR**, Hrout AA, Alzahmi AS, Chaiboonchoe A, Amin A, Salehi-Ashtiani K. Molecular Mechanisms behind Safranal's Toxicity to HepG2 Cells from Dual Omics. *Antioxidants (Basel)* 2022; **11** [PMID: 35740022 DOI: 10.3390/antiox11061125]
58 **Abdalla Y**, Abdalla A, Hamza AA, Amin A. Safranal Prevents Liver Cancer Through Inhibiting Oxidative Stress and Alleviating Inflammation. *Front Pharmacol* 2021; **12**: 777500 [PMID: 35177980 DOI: 10.3389/fphar.2021.777500]
59 **Hamza AA**, Lashin FM, Gamel M, Hassanin SO, Abdalla Y, Amin A. Hawthorn Herbal Preparation from *Crataegus oxyacantha* Attenuates In Vivo Carbon Tetrachloride -Induced Hepatic Fibrosis *via* Modulating Oxidative Stress and Inflammation. *Antioxidants (Basel)* 2020; **9** [PMID: 33255507 DOI: 10.3390/antiox9121173]
60 **Al-Shamsi M**, Amin A, Adeghate E. Vitamin E ameliorates some biochemical parameters in normal and diabetic rats. *Ann N Y Acad Sci* 2006; **1084**: 411-431 [PMID: 17151319 DOI: 10.1196/annals.1372.033]
61 **Benassi E**, Fan HY, Sun QL, Dukenbayev K, Wang Q, Shaimoldina A, Tassanbiyeva A, Nurtay L, Nurkesh A, Kutzhanova A, Mu CL, Dautov A, Razbekova M, Kabylda A, Yang Q, Li ZY, Amin A, Li XG, Xie YQ. Generation of particle assemblies mimicking enzymatic activity by processing of herbal food: the case of rhizoma polygonati and other natural ingredients in traditional Chinese medicine. *Nanoscale Adv* 2021; **3**: 2222-2235 [DOI: 10.1039/D0NA00958J]
62 **Hamza AA**, Fikry EM, Abdallah W, Amin A. Mechanistic insights into the augmented effect of bone marrow mesenchymal stem cells and thiazolidinediones in streptozotocin-nicotinamide induced diabetic rats. *Sci Rep* 2018; **8**: 9827 [PMID: 29959408 DOI: 10.1038/s41598-018-28029-1]
63 **Hamza AA**, Hassanin SO, Hamza S, Abdalla A, Amin A. Polyphenolic-enriched olive leaf extract attenuated doxorubicin-induced cardiotoxicity in rats *via* suppression of oxidative stress and inflammation. *JoBAZ* 2021; **82**: 54 [DOI: 10.1186/s41936-021-00251-w]
64 **Abdel-Latif R**, Heeba GH, Hassanin SO, Waz S, Amin A. TLRs-JNK/ NF-κB Pathway Underlies the Protective Effect of

the Sulfide Salt Against Liver Toxicity. *Front Pharmacol* 2022; **13**: 850066 [PMID: 35517830 DOI: 10.3389/fphar.2022.850066]

65 **El-Dakhly SM**, Salama AAA, Hassanin SOM, Yassen NN, Hamza AA, Amin A. Aescin and diosmin each alone or in low dose- combination ameliorate liver damage induced by carbon tetrachloride in rats. *BMC Res Notes* 2020; **13**: 259 [PMID: 32460808 DOI: 10.1186/s13104-020-05094-2]

66 **Kim YW**, Byzova TV. Oxidative stress in angiogenesis and vascular disease. *Blood* 2014; **123**: 625-631 [PMID: 24300855 DOI: 10.1182/blood-2013-09-512749]

67 **Amin A**, Hamza AA, Daoud S, Hamza W. Spirulina protects against cadmium-induced hepatotoxicity in rats. *Am J Pharmacol Toxicol* 2006; **1**: 21-25 [DOI: 10.3844/ajptsp.2006.21.25]

68 **Nurtay L**, Sun QL, Mu CL, Cao ZS, Wang Q, Liang ZS, Ma CP, Li XG, Amin A, Xie YQ. Rhizoma polygonati from Mount Tai: nutritional value and usefulness as a traditional Chinese medicine, source of herbzyme, and potential remediating agent for COVID-19 and chronic and hidden hunger. *Acupuncture Herb Med* 2021; **1**: 31-38 [DOI: 10.1097/hm9.0000000000000008]

69 **Soula M**, Weber RA, Zilka O, Alwaseem H, La K, Yen F, Molina H, Garcia-Bermudez J, Pratt DA, Birsoy K. Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. *Nat Chem Biol* 2020; **16**: 1351-1360 [PMID: 32778843 DOI: 10.1038/s41589-020-0613-y]

70 **Kang Q**, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biol* 2020; **37**: 101799 [PMID: 33248932 DOI: 10.1016/j.redox.2020.101799]

71 **Chopra H**, Hung MK, Kwong DL, Zhang CF, Pow EHN. Insights into Endothelial Progenitor Cells: Origin, Classification, Potentials, and Prospects. *Stem Cells Int* 2018; **2018**: 9847015 [PMID: 30581475 DOI: 10.1155/2018/9847015]

72 **Lopes-Coelho F**, Silva F, Gouveia-Fernandes S, Martins C, Lopes N, Domingues G, Brito C, Almeida AM, Pereira SA, Serpa J. Monocytes as Endothelial Progenitor Cells (EPCs), Another Brick in the Wall to Disentangle Tumor Angiogenesis. *Cells* 2020; **9** [PMID: 31906296 DOI: 10.3390/cells9010107]

73 **Jarajapu YP**, Grant MB. The promise of cell-based therapies for diabetic complications: challenges and solutions. *Circ Res* 2010; **106**: 854-869 [PMID: 20299675 DOI: 10.1161/CIRCRESAHA.109.213140]

74 **Kaushik K**, Das A. Endothelial progenitor cell therapy for chronic wound tissue regeneration. *Cytotherapy* 2019; **21**: 1137-1150 [PMID: 31668487 DOI: 10.1016/j.jcyt.2019.09.002]

75 **Hu Y**, Tao R, Chen L, Xiong Y, Xue H, Hu L, Yan C, Xie X, Lin Z, Panayi AC, Mi B, Liu G. Exosomes derived from pioglitazone-pretreated MSCs accelerate diabetic wound healing through enhancing angiogenesis. *J Nanobiotechnology* 2021; **19**: 150 [PMID: 34020670 DOI: 10.1186/s12951-021-00894-5]

76 **Liu J**, Hou W, Guan T, Tang L, Zhu X, Li Y, Hou S, Zhang J, Chen H, Huang Y. Slit2/Robo1 signaling is involved in angiogenesis of glomerular endothelial cells exposed to a diabetic-like environment. *Angiogenesis* 2018; **21**: 237-249 [PMID: 29299781 DOI: 10.1007/s10456-017-9592-3]

77 **Sedding DG**, Boyle EC, Demandt JAF, Sluimer JC, Dutzmann J, Haverich A, Bauersachs J. Vasa Vasorum Angiogenesis: Key Player in the Initiation and Progression of Atherosclerosis and Potential Target for the Treatment of Cardiovascular Disease. *Front Immunol* 2018; **9**: 706 [PMID: 29719532 DOI: 10.3389/fimmu.2018.00706]

78 **Ma Q**, Reiter RJ, Chen Y. Role of melatonin in controlling angiogenesis under physiological and pathological conditions. *Angiogenesis* 2020; **23**: 91-104 [PMID: 31650428 DOI: 10.1007/s10456-019-09689-7]

79 **Parma L**, Baganha F, Quax PHA, de Vries MR. Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis. *Eur J Pharmacol* 2017; **816**: 107-115 [PMID: 28435093 DOI: 10.1016/j.ejphar.2017.04.028]

80 **Brezinski ME**. Comparing the Risk Factors of Plaque Rupture and Failed Plaque Healing in Acute Coronary Syndrome. *JAMA Cardiol* 2019; **4**: 329-331 [PMID: 30865209 DOI: 10.1001/jamacardio.2019.0312]

81 **Brezinski M**, Willard F, Rupnick M. Inadequate Intimal Angiogenesis as a Source of Coronary Plaque Instability: Implications for Healing. *Circulation* 2019; **140**: 1857-1859 [PMID: 31790293 DOI: 10.1161/CIRCULATIONAHA.119.042192]

82 **Wang Y**, Jiang C, Shang Z, Qiu G, Yuan G, Xu K, Hou Q, He Y, Liu Y. AGEs/RAGE Promote Osteogenic Differentiation in Rat Bone Marrow-Derived Endothelial Progenitor Cells *via* MAPK Signaling. *J Diabetes Res* 2022; **2022**: 4067812 [PMID: 35155684 DOI: 10.1155/2022/4067812]

83 **Gryszczyńska B**, Budzyń M, Begier-Krasińska B, Osińska A, Boruczkowski M, Kaczmarek M, Bukowska A, Iskra M, Kasprzak MP. Association between Advanced Glycation End Products, Soluble RAGE Receptor, and Endothelium Dysfunction, Evaluated by Circulating Endothelial Cells and Endothelial Progenitor Cells in Patients with Mild and Resistant Hypertension. *Int J Mol Sci* 2019; **20** [PMID: 31412635 DOI: 10.3390/ijms20163942]

84 **Ji L**, Tian H, Webster KA, Li W. Neurovascular regulation in diabetic retinopathy and emerging therapies. *Cell Mol Life Sci* 2021; **78**: 5977-5985 [PMID: 34230991 DOI: 10.1007/s00018-021-03893-9]

85 **Hammes HP**. Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond. *Diabetologia* 2018; **61**: 29-38 [PMID: 28942458 DOI: 10.1007/s00125-017-4435-8]

86 **Roy S**, Kim D. Retinal capillary basement membrane thickening: Role in the pathogenesis of diabetic retinopathy. *Prog Retin Eye Res* 2021; **82**: 100903 [PMID: 32950677 DOI: 10.1016/j.preteyeres.2020.100903]

87 **Marziano C**, Genet G, Hirschi KK. Vascular endothelial cell specification in health and disease. *Angiogenesis* 2021; **24**: 213-236 [PMID: 33844116 DOI: 10.1007/s10456-021-09785-7]

88 **Li T**, Shen K, Li J, Leung SWS, Zhu T, Shi Y. Glomerular Endothelial Cells Are the Coordinator in the Development of Diabetic Nephropathy. *Front Med (Lausanne)* 2021; **8**: 655639 [PMID: 34222276 DOI: 10.3389/fmed.2021.655639]

89 **Zhang A**, Fang H, Chen J, He L, Chen Y. Role of VEGF-A and LRG1 in Abnormal Angiogenesis Associated With Diabetic Nephropathy. *Front Physiol* 2020; **11**: 1064 [PMID: 32982792 DOI: 10.3389/fphys.2020.01064]

90 **Vouillarmet J**, Bourron O, Gaudric J, Lermusiaux P, Millon A, Hartemann A. Lower-extremity arterial revascularization: Is there any evidence for diabetic foot ulcer-healing? *Diabetes Metab* 2016; **42**: 4-15 [PMID: 26072053 DOI: 10.1016/j.diabet.2015.05.004]

91 **Gorecka J**, Kostiuk V, Fereydooni A, Gonzalez L, Luo J, Dash B, Isaji T, Ono S, Liu S, Lee SR, Xu J, Liu J, Taniguchi R,

Yastula B, Hsia HC, Qyang Y, Dardik A. The potential and limitations of induced pluripotent stem cells to achieve wound healing. *Stem Cell Res Ther* 2019; **10**: 87 [PMID: 30867069 DOI: 10.1186/s13287-019-1185-1]

92 **Catrina SB**, Zheng X. Disturbed hypoxic responses as a pathogenic mechanism of diabetic foot ulcers. *Diabetes Metab Res Rev* 2016; **32** Suppl 1: 179-185 [PMID: 26453314 DOI: 10.1002/dmrr.2742]

93 **Chang M**, Nguyen TT. Strategy for Treatment of Infected Diabetic Foot Ulcers. *Acc Chem Res* 2021; **54**: 1080-1093 [PMID: 33596041 DOI: 10.1021/acs.accounts.0c00864]

94 **An T**, Chen Y, Tu Y, Lin P. Mesenchymal Stromal Cell-Derived Extracellular Vesicles in the Treatment of Diabetic Foot Ulcers: Application and Challenges. *Stem Cell Rev Rep* 2021; **17**: 369-378 [PMID: 32772239 DOI: 10.1007/s12015-020-10014-9]

95 **Samuel SM**, Thirunavukkarasu M, Penumathsa SV, Koneru S, Zhan L, Maulik G, Sudhakaran PR, Maulik N. Thioredoxin-1 gene therapy enhances angiogenic signaling and reduces ventricular remodeling in infarcted myocardium of diabetic rats. *Circulation* 2010; **121**: 1244-1255 [PMID: 20194885 DOI: 10.1161/CIRCULATIONAHA.109.872481]

96 **Yang JX**, Pan YY, Wang XX, Qiu YG, Mao W. Endothelial progenitor cells in age-related vascular remodeling. *Cell Transplant* 2018; **27**: 786-795 [PMID: 29882417 DOI: 10.1177/0963689718779345]

97 **Kshitiz**, Ellison DD, Suhail Y, Afzal J, Woo L, Kilic O, Spees J, Levchenko A. Dynamic secretome of bone marrow-derived stromal cells reveals a cardioprotective biochemical cocktail. *Proc Natl Acad Sci US A* 2019; **116**: 14374-14383 [PMID: 31239339 DOI: 10.1073/pnas.1902598116]

98 **George J**, Goldstein E, Abashidze S, Deutsch V, Shmilovich H, Finkelstein A, Herz I, Miller H, Keren G. Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. *Eur Heart J* 2004; **25**: 1003-1008 [PMID: 15191769 DOI: 10.1016/j.ehj.2004.03.026]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 940-948

DOI: 10.4239/wjd.v13.i11.940

ISSN 1948-9358 (online)

MINIREVIEWS

Nutritional supplementation on wound healing in diabetic foot: What is known and what is new?

Andrea Da Porto, Cesare Miranda, Gabriele Brosolo, Giorgio Zanette, Andrea Michelli, Roberto Da Ros

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): D
Grade E (Poor): 0

P-Reviewer: Basiri R, United States; Rai V, United States; Shalaby MN, Egypt

Received: August 11, 2022
Peer-review started: August 11, 2022
First decision: September 4, 2022
Revised: September 13, 2022
Accepted: October 11, 2022
Article in press: October 11, 2022
Published online: November 15, 2022

Andrea Da Porto, Gabriele Brosolo, Department on Internal Medicine, University of Udine, Udine 33100, Italy

Cesare Miranda, Giorgio Zanette, Department of Internal Medicine, Clinic of Endocrinology and Metabolism Diseases Azienda Sanitaria Friuli Occidentale, Pordenone 33170, Italy

Andrea Michelli, Roberto Da Ros, Department of Internal Medicine , SC Diabete e Centro Trattamento Piede Diabetico, Monfalcone 34074, Gorizia, Italy

Corresponding author: Andrea Da Porto, MD, MSc, Doctor, Department on Internal Medicine, University of Udine, Via Colugna, Udine 33100, Italy. daporto.andrea@gmail.com

Abstract

Non-healing diabetic foot ulcers (DFU) are the most notable and striking complications of diabetes mellitus. More than 25% of nonhealing DFU can ultimately lead to amputation of the lower extremity within 6-18 mo after the first manifestation of the wound. Although wound healing is complex, nutritional status is crucial in soft tissue repair. Malnutrition is highly prevalent and overlooked in patients with diabetes and chronic wounds. Moreover, to date, we do not have clear recommendations or evidence about the use of nutritional supplements for improving wound healing in patients with DFU. In this article the authors briefly analyzed the current evidence on the use of nutritional supplements of proteins or amino acids, fatty acids, probiotics, vitamins, and trace elements in the wound healing process in patients with DFU.

Key Words: Malnutrition; Supplements; Diabetic foot; Diabetes; Wound healing; Nutritional therapy

Core Tip: Malnutrition is common in patients with diabetes and chronic wounds. To date we do not have clear recommendations or evidence about the use of nutritional supplements for improving wound healing in patients with diabetic foot ulcers (DFU). This paper aimed to evaluate current evidence regarding the use of Nutritional supplementation on wound healing in patients with DFU.

Citation: Da Porto A, Miranda C, Brosolo G, Zanette G, Michelli A, Ros RD. Nutritional supplementation on wound healing in diabetic foot: What is known and what is new? *World J Diabetes* 2022; 13(11): 940-948
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/940.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.940

INTRODUCTION

The Global Burden of Disease study[1], estimated that 131.0 million (1.77%) people worldwide had diabetes-related lower-extremity complications (DRLECs) in 2016, equal to 34% of the diabetes population. Among these patients almost 105.6 million had neuropathy; 18.6 million had foot ulcers, and 6.8 million undergone to amputation. Moreover, it is known than 84% of lower extremity amputations are preceded by a foot ulcer[2], however ulcer prevention is still an overlooked opportunity[3].

In patients with diabetes known risk factors for developing ulcers are peripheral arterial disease, peripheral neuropathy, repeated trauma, history of previous ulcers, and/or amputation. Male subjects, patients with a longer duration of the disease and a low socioeconomic level have the highest risk[4-7].

Non-healing diabetic foot ulcers (DFU) are the one of the most relevant and dangerous complications of diabetes mellitus[2]. A diabetic foot ulcer is defined as nonhealing in the case of a wound area reduction of less than 50% after 4 weeks of standard care[8]. More than a quarter of non-healing DFU may in the end leads to lower extremity amputation within 6-18 mo after the wound's outset[9].

Although wound healing is regulated by complex mechanisms, the outcome of the process is the complete repair of the damaged tissue. It is known that infection, wound depth, size, and duration negatively impact healing process and are therefore associated with poor outcomes and possible amputation[10].

Nutritional status is crucial in wound healing. The presence of a wound has a negative impact on nutritional status due to the metabolic cost of repairing damaged tissue, in addition to nutrient loss through wound inflammatory exudate. Malnutrition is highly prevalent in patients with chronic diabetic foot wounds[11], and specific micronutrient deficiencies are common and associated with impaired wound healing and increase the risk of amputation in people with DFU[12,13]. Malnutrition is known to impair the inflammatory phase of the healing process, reducing fibroblast proliferation and collagen synthesis, increasing the risk of developing infections, decreasing T lymphocyte function, phagocytic activity, complement, and antibody levels[14]**.** Moreover, nutritional defect of polyunsaturated fatty acids (PUFAs) and consequent reduced synthesis of the essential fatty acid-derived resolvins, (crucial regulators of inflammatory phase of wound healing) are particularly deleterious in people with DFU where diabetes itself is associated with an overall decreased production of pro-resolving lipid mediators[15].

Therefore, nutritional interventions can improve DFU healing. Current Australian guidelines recommend the evaluation of nutritional status when progress towards DFU closure is not made[16] and the use of oral nutritional supplements (ONS) when an oral diet is not sufficient to meet nutritional requirements. However, to date, we do not have clear recommendations or evidence on the use of nutritional supplements to improve wound healing in patients with DFU.

This article aimed to evaluate current evidence regarding the use of Nutritional (Proteins, Aminoacids, Omega 3, probiotics, vitamins, and Trace Elements) supplementation on wound healing in patients with DFU (Table 1).

PROTEIN AND AMINO ACIDS

Proteins and amino acids are the most frequent nutritional supplementation used to improve the healing of diabetic foot wounds, starting from the evidence of the positive effects of these compounds in wound healing. We identified four randomised controlled trial (RCT) studies that supplemented with protein or amino acid mixture in patients with diabetic foot. Eneroth 2004[17] supplemented 27 malnourished patients with diabetic foot ulcers with 20 g protein per 200 mL bottle and compared them with a placebo (26 patients). It is unknown whether there is a difference in the proportion of ulcers healed at the end of the study period, or an absolute change in individual parameters of ulcer dimensions, or ulcer area, over time, for those treated, or not treated with an ONS. Secondary outcomes too, amputation and adverse events (death), didn't demonstrate a difference. Armstrong *et al*[18], an RCT with 270 patients, experimented with supplementation with arginine, glutamine, and b-hydroxy-b-methyl butyrate on foot ulcer healing in people with diabetes. No differences were found between groups in wound closure or time to wound healing after 16 wk. Subgroup analysis shows that the addition of arginine, glutamine, and b-hydroxy-b-methyl butyrate in addition to the standard of care can improve the healing of diabetic foot ulcers in patients with the risk of limb hypoperfusion and/or

Table 1 Strengths and Limitations of the main randomised controlled trial that evaluated the effects of specific nutritional supplement on wound healing in patients with diabetic foot ulcers

Ref.	Study design and Number ofparticipants	Nutrient(s) or Supplement Studied	Aim of study	Main results	Limitations
Eneroth *et al*[17], 2004	Double-blind RCT, 53 patients	20 g protein per 200 mL bottle, 1 kcal/mL	To determine the effects of supplementation on wound healing at 6 mo in subjects with DFU	10/27 (37%) participants in the oral nutritional supplement group healed at 6 mo compared with 12/26 (46%) in placebo group	The sample size was small; certainty of the evidence is very low
Armstrong [18], 2014	Double-blind RCT, 270 patients	Arginine,glutamine and β-hydroxy-β-methylbutyrate	To determine the effects of supplementation on proportion of ulcers healed at 16 wk	65/129 (50%) participants in the arginine, glutamine and β-hydroxy-β-methylbutyrate supplement group healed, compared with 65/141 (46%) in the placebo group	Certainty of the evidence is very low; no difference in quality of life, new ulcers, amputation
Wong *et al* [19], 2014	RCT, 27 patients	Amino acid mixture containing (beta)-hydroxy (beta)-methylbutyrate (HMB), arginine and glutamine	Compare pressure ulcer healing rates	Wound area did not decrease significantly in the short term for both groups. The proportion of viable tissues increased within 2 wk on HMB, arginine and glutamine supplementation; pressure Ulcer Scale for Healing scores showed significant improvement within 1 wk of supplementation for the experimental group	The sample size was small. Observation time was short. Amino acid does not appear to reduce wound size but to improve healing process
Basiri *et al* [20], 2022	RCT, 29 patients	Nutritional supplementation (total energy of 500 calories, 28 g of protein, and essential vitamins and minerals) and education	Effects of nutrition supplementation and education on inflammatory biomarkers	The mean plasma concentration of IL-6 significantly decreased in the treatment group	The sample size was small
Basiri *et al* [20], 2022	RCT, 29 patients	Nutritional supplementation (total energy of 500 calories, 28 g of protein, and essential vitamins and minerals) and education	Nutrient-dense formula combined with nutrition education on wound healing	Treatment group experienced a faster wound healing rate (6.43 mm^2 /wk more reduction in the wound area) than the control group. The mean reduction in the wound area during the first four weeks of the study was almost 13-fold greater in the treatment group compared to the control group	The sample size was small
Sipahi *et al* [22], 2013	Retrospective analysis, 11 patients	Beta-hydroxy-beta-methylbutyrate, arginine and glutamine	Wound healing in diabetic dialysis patients	Healing was observed on the wound depth score of 7 (63.6%) patients and on wound appearance score of 8 (72.7%) patients out of 11	Not RCT; the sample size was small
Soleimani *et al*[31], 2017	Double-blind RCT, 60 patients	Omega-3 PUFA	To determine the effects of flaxseed oil omega-3 fattyacids supplementation on wound healing and metabolic profiles insubjects with DFU	Significant decrease in ulcer length, width, and depth	The sample size was small. The authors did not report data on ulcer healing (percentage of ulcers healed), cost of surgery, quality of life, adverse events, development of any new foot ulcers, amputation rate, incidence osteomyelitis from surgery
Mohseni *et al*[37], 2017	Double-blind RCT, 60 patients	Probiotics (Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, and Bifidobacterium bifidum)	To determine the effects of oral probiotic therapy on wound healing andmetabolic status in adult patients with diabetes	Significant improvement in length, width, and depth of ulcers in treatment *vs* placebo groups	The sample size was small. The authors did not report data on ulcer healing (percentage of ulcers healed), quality of life, adverse events, development of any new foot ulcers, amputation rate
Gunton *et al* [39], 2021	Double-blind RCT, 19 patients	Vitamin C 500 mg daily in a slow-release capsule	To determine the effects of oral vitamin C supplement on wound healing	Healing at 8 wk was significantly better in the vitamin C group (median 100 v. -14 %, P = 0.041); healing without amputation occurred in all patients in the vitamin C group	The sample size was extremely small
Yarahmadi *et al*[40], 2021	Double-blind RCT, 25 patients	Oral vitamin E and C or placebo	To determine the effects of oral vitamin C and E supplement on wound	Significantly higher wound size reduction in intervention (P = 0.019); significant decrease in	The sample size was small. All patients were treated even with platelet-rich

			healing	prooxidant-antioxidant balance and hs-CRP in the intervention group ($P < 0.05$)	plasma-fibrin glue dressing and vitamin E
Razzaghi *et al*[46], 2018	Double-blind RCT, 60 patients	50000 IU vitamin D supplements every 2 wk	To determine the effects of oral vitamin D supplement on wound healing an on markers of Inflammation and Insulin resistance	After 12 wk vitamin D treatment resulted in treatment Greater Reduction in ulcer length, width, depth, and erythema rate *vs* placebo	The sample size was small
Afzali *et al* [47], 2019	Double-blind RCT, 70 patients	250 mg magnesium oxide	To determine the effects of oral magnesium oxide + vitamin E supplement on wound healing	Beneficial effects on ulcer size after 12 wk of treatment	The sample size was small
Razzaghi *et al*[46], 2018	Double-blind RCT, 57 patients	250 mg magnesium oxide plus 400 IU vitamin E	To determine the effects of oral magnesium oxide supplement on wound healing	Magnesium plus vitamin E supplements for 12 wk reduced ulcer length and depth *vs* placebo	The sample size was small
Momen-Heravi *et al* [48], 2017	Double-blind RCT, 60 patients	220 mg zinc sulfate supplements	To determine the effects of oral zinc supplement on wound healing	Zinc supplementation for 12 wk was associated with significant reductions in ulcer length and width	The sample size was small

RCT: Randomised controlled trial; DFU: Diabetic foot ulcers; PUFA: Polyunsaturated fatty acids.

hypoalbuminemia. Wong *et al*[19], use a mixture of amino acid containing (beta)-hydroxy (beta)-methyl butyrate (HMB), arginine, and glutamine in diabetic patients with pressure ulcers without obtaining wound area reduction , but demonstrates a better proportion of viable tissues on HMB, arginine, and glutamine supplementation group. Recently Basiri *et al*[20], evaluated the effects on wound healing of a treatment regime based on nutritional education in combination with a nutritional supplement (total of 500 kilocalories, 28 g of protein, and essential vitamins and minerals) in a group of patients with DFU getting a faster wound healing rate in treated group. The mean reduction in wound area during the first four weeks was almost 13 times greater in patients treated with nutritional supplement compared to the control group. Moreover, the same authors demonstrated in another publication that nutritional intervention could have favorable effects on controlling inflammation in patients with DFU[21]. Finally, we included in this analysis a retrospective study, from Sipahi *et al*[22] for interesting results in diabetic dialysis patients supplemented with aminoacid (beta-hydroxy-beta-methyl butyrate, arginine, and glutamine) obtaining healing in most of the patients (63.6%). Dialysis always represents a challenge in the healing of the diabetic foot.

In summary, the results of the current studies did not give a clear effect of proteins and amino acids in the healing of diabetic foot wounds, but there is some evidence of efficacy with improvement in the healing process. The nutritional status of the participants was not an inclusion/exclusion criterion in the studies included in the review. As such, the supplement was generally given randomly irrespective of the presence or absence of malnutrition at inclusion), thus, the baseline nutritional status of the participants is independently correlated with the severity of infection, increased risk of poor outcome, poor prognosis after vascular interventions and it even predicts amputation. Furthermore, it is unclear what the minimum duration is required to achieve a measurable impact on the wound once the serum level of a particular nutrient has improved beyond the deficient range.

POLYUNSATURATED OMEGA-3 FATTY ACIDS

Long-chain Omega-3 PUFA and their bioactive metabolites (resolvins, marine, protectin) have long been considered as powerful negative modulators of acute inflammation and/or inducers of its resolution [23], while the role they play in the wound healing process is still largely unknown. Interest is growing in the understanding of the specific effects of PUFA derived metabolites on the cellular and molecular mechanisms involved in the modulation of inflammatory process[24]. The most bioactive PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that can be obtained directly from many dietary sources as fish and algae, or indirectly through the endogenous metabolic conversion of their precursor, as linolenic acid contained in high concentrations in vegetables and nuts.

Data on the effects of omega-3 fatty acids from flaxseed oil on wound healing in human studies are scarce. Additionally, the studies[25-27] that have evaluated the effects of fish oil supplements (rich in omega-3) on wound healing in patients without diabetes have produced mixed results.

Regarding the consequence of omega-3 fatty acids on inflammatory factors, a meta-analysis showed that supplementation with marine-based omega-3 induce a significative reduction of C-reactive protein,

IL-6, and tumor necrosis factor α[28].

McDaniel *et al*[26,27,29] showed that supplementing omega-3 for 4 wk in a healthy subjects resulted in increased production of pro-inflammatory cytokines, including IL-1ß at wound sites representing a potential therapeutic option for people with chronic wounds[26-29].

As recommended in the Expert consensus and guidance of the American limb preservation society on nutrition interventions in adults with diabetic foot ulcers, mono and PUFAs, including EPA, DHA, and arachidonic acid should be considered because they contribute to membrane fluidity, membrane and intracellular signals, and modulation of apoptotic pathways[30] (ALPS Nutrition Interventions in Adults with Diabetic Foot Ulcers Expert Consensus and Guidance).

In a double-blind RCT, Soleimani *et al*[31] evaluated the effects of daily omega-3 fatty acids from flax seeds for 12 wk in 60 subjects with grade 3 DFU according to "Wagner-Meggitt's criteria". Participants were assigned to receive 1 g twice daily of omega-3 PUFA or a placebo for 12 wk. A significant reduction in ulcer width, length, and depth and an increase in insulin sensitivity and total antioxidant capacity was observed in the intervention compared to the control group. As reported by Moore *et al*[32] the study of Soleimani *et al*[31] had several limitations. The sample size was small. The authors did not report percentage of ulcers healed, cost of surgery, quality of life, amputation rate, and incidence of osteomyelitis from surgery.

PROBIOTICS

An emerging treatment line for wound healing is the use of probiotics, defined by the International Scientific Association of Probiotics and Prebiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[33].

The most common probiotics used as supplements include members of the Lactobacillus spp, Bifidobacterium genera[34], strains from other bacterial species (Propionibacterium acidilactici, Lactococcus lactis, Leuconostoc mesenteroides, Bacillus subtilis, Streptococcus thermophilus) and yeasts (Saccharomycesboulardii)[35].

Perioperative supplementation of probiotics demonstrated accelerated skin healing in diabetic rats, probably due to a reduction of the inflammatory response, increased neovascularization, and increased type I collagen deposition. Supplementation with probiotics also prevents weight loss and improves glycemic control in animal models[36].

In a double-blind RCT, the effects of daily probiotics supplementation for 12 wk were examined in 60 patients with DFU with cellulitis on wound healing[37]. Participants were randomly assigned to receive a probiotic capsule or placebo daily for 12 wk.

The study founds improvement in length, width, and depth of the ulcers in patients treated with probiotic supplement compared with placebo. Additionally, compared to placebo, a significant decrease in HbA1c (-0.6% ± 0.5% *vs* -0.2% ± 0.4%), total cholesterol and CRP levels was observed in treated patients. This study had several limitations: the sample size was small; the authors did not report data on ulcer healing, quality of life, adverse events, and amputation rate.

Studies on probiotic use are limited, for this reason more robust evidence are needed to examine the effects of probiotics on wound healing in patients with DFU.

VITAMINS AND TRACE ELEMENTS

Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that plays a pivotal role in many biological processes involved in wound healing, including collagen formation, regulation of the immune system, and maintenance of cartilage and bones.

The recommended dietary allowance for adults and older is 90 mg daily for men and 75 mg for women**.** Deficiency of Ascorbic Acid has been associated with increased severity of DFU in a prospective study of 131 patients with diabetic foot[12]. Intriguing data derive from case series of people with diabetes and poorly healing lower limb ulcers in whom there was prompt ulcer healing with vitamin C replacement[38].

Recent evidence from some small randomized controlled trials has raised the idea of a possible benefit of vitamin C supplements on wound healing in DFU. An Australian placebo-controlled randomized trial founds that active treatment with Vitamin C 500 mg daily in a slow-release capsule for 8 wk results in a better percentage reduction in ulcer volume in 7 patients with DFU[39].

With similar findings Yarahmadi *et al*[40] demonstrate that supplements of vitamin E (200IU/2 d) and C (250IU/2 d) for eight weeks significantly increase wound healing in patients with non-healing DFU by enhancing the wound healing process and reducing oxidative stress in 13 patients treated within platelet-rich plasma-fibrin glue dressing plus[40].

Therefore, although seminal evidence is promising and of interest for future research, more convincing and robust evidence on the role of ascorbic acid in wound healing is required.

Vitamin D

Vitamin D is a fat-soluble vitamin that has long been known to help the body absorb certain calcium and phosphorus; both are essential for the building of bone. Circulating levels of 25 (OHD) are low in patients with DFU[12] or patients with severe peripheral artery disease[41]. Furthermore, vitamin D insufficiency has been associated with impaired inflammatory responses, oxidative stress, and wound healing. A meta-analysis of observational studies reports that severe vitamin D deficiency is associated with foot ulceration[42] in people with diabetes. However, evidence of the benefits of vitamin D supplementation in DFU is still lacking. To date, we have only a few RCTs (most of them for the same study group) that evaluated the effects of Vitamin D supplementation on wound healing in DFU. A double-blind, placebo-controlled, randomized clinical trial, conducted among 60 patients with grade 3 DFU according to "Wagner-Meggitt" criteria, showed that after 12 wk of intervention, compared to placebo, vitamin D supplementation resulted in a significant reduction in ulcer length, width, depth, and erythema rate[43]. A very similar study with a comparable number of participants confirmed that a supplement of 60000 IU/weekly of cholecalciferol had a beneficial effect on wound healing when compared with a placebo[44].

More convincing and robust evidence is required on the role of vitamin D supplementation in wound healing.

Trace elements

The nutritional relevance of trace elements has grown rapidly due to a better understanding of their biological functions. They are involved with many enzymatic processes such as catalysis, oxidation-reduction, and cellular transport. Therefore, trace elements have a pivotal role in the synthesis and structural stabilization of both proteins and nucleic acid needed for wound healing. In wound healing, the most important trace elements are zinc, copper, chromium, selenium, and magnesium, which are believed to be essential for the regeneration of physiological tissue in humans[45]. However, only a few randomized controlled trials have tested the effect of trace element supplements on wound healing in DFU.

Effects of magnesium supplements on wound healing in people with diabetes were evaluated in 2 small randomized, double-blind, placebo-controlled trial. The first[46] was carried out among 70 subjects with grade 3 DFU according to the "Wagner-Meggitt" criteria and subjects treated with 250 mg of magnesium oxide had beneficial effects on ulcer size after 12 wk of treatment. In a similar study 57 patients with grade 3, DFU were randomized to take either 250 mg magnesium oxide plus 400 IU vitamin E (n = 29) or a placebo per day (n = 28) for 12 wk. Compared to placebo, taking magnesium plus vitamin E supplements reduced ulcer length and depth[47].

Zinc was tough to improve wound healing due to its effects on insulin resistance, inflammation, and oxidative stress. The effect of zinc supplements on wound healing in DFU was evaluated in a randomized, double-blind, placebo-controlled trial conducted among 60 patients with grade 3 diabetic foot ulcers[48]. Participants were randomly assigned to take either 220 mg zinc sulfate supplements containing 50 mg elemental zinc or a placebo daily for 12 wk. Compared with the placebo, zinc supplementation was associated with significant reductions in ulcer length and width.

CONCLUSION

Current evidence supports that poor nutritional status is common and associated with the impaired healing process in patients with DFU. However, the number of good quality RCTs evaluating nutritional supplementation in DFU patients is limited with varying effects[30,49,50]. Patients with diabetic foot ulcers require good multidisciplinary care to optimize wound healing, but often an important and overlooked aspect of this is their nutritional status. However, evidence for the impact of nutritional interventions on foot ulcer healing in people with diabetes remains uncertain, with studies showing no clear benefit or harm. For this reason, the IWGDF Guidelines on the use of interventions to improve healing of diabetic foot ulcers (2019) recommend not using interventions aimed at correcting the nutritional status of people with diabetic foot ulcers, to improve healing, preferring it as the best standard of care[10]. Instead, according to the new Australian guidelines on wound healing interventions to improve wound healing, the nutritional status of the foot ulcer should be reviewed, and adequate daily nutritional needs should be met as part of the best standard of care "to ensure that this recommendation has not been misinterpreted[15]. In conclusion more and well-designed studies are needed to better clarify the potential impact of nutritional supplementation on the healing of foot ulcers in people with diabetes.

PERSPECTIVES

Author's suggestion for future research and design of proper RCT study: (1) Complete evaluation of nutritional status at the beginning, evidence, and agreement on malnutrition, and evidence if the population examined have a specific nutritional defect; (2) guarantee to all patient's real optimal standard therapy; (3) choose uniformity of wound conditions, don't mix heterogeneous kinds of wounds. Consider that some conditions are time-limited, for example, choosing "infected wounds" we don't define a homogeneous group, with this definition we could include neuropathic, ischemic, traumatic, or mixed lesions with very different characteristics; (4) adequate duration of supplementation and study too, since mean wound healing of diabetic foot is approximately 3 mo, it is not possible to evaluate healing in a shorter period; (5) evaluate evidence of supplementation with blood samples that demonstrate direct change in parameters; and (6) choose multiple parameters, other than healing, to evaluate the effects of supplementation: Rate of wound reduction, percentage of granulation tissue.

FOOTNOTES

Author contributions: Da Porto A and Miranda C came up with ideas and constructs; Da Porto A, Miranda C, and Da Ros R wrote the manuscript; Michelli A, Brosolo G, and Zanette G approved the main conceptual ideas and made corrections; all authors provided final edits and approved the manuscript.

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

Country/Territory of origin: Italy

ORCID number: Andrea Da Porto 0000-0001-9995-0169.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Zhang Y**, Lazzarini PA, McPhail SM, van Netten JJ, Armstrong DG, Pacella RE. Global Disability Burdens of Diabetes-Related Lower-Extremity Complications in 1990 and 2016. *Diabetes Care* 2020; **43**: 964-974 [PMID: 32139380 DOI: 10.2337/dc19-1614]
2 **Pecoraro RE**, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care* 1990; **13**: 513-521 [PMID: 2351029 DOI: 10.2337/diacare.13.5.513]
3 **Miranda C**, Da Ros R. Prevention of Diabetic Foot Ulcer: A Neglected Opportunity. *Transl Med UniSa* 2020; **22**: 50-51 [PMID: 32523910]
4 **Boyko EJ**, Ahroni JH, Stensel V, Forsberg RC, Davignon DR, Smith DG. A prospective study of risk factors for diabetic foot ulcer. The Seattle Diabetic Foot Study. *Diabetes Care* 1999; **22**: 1036-1042 [PMID: 10388963 DOI: 10.2337/diacare.22.7.1036]
5 **Boulton AJ**, Armstrong DG, Albert SF, Frykberg RG, Hellman R, Kirkman MS, Lavery LA, Lemaster JW, Mills JL Sr, Mueller MJ, Sheehan P, Wukich DK; American Diabetes Association; American Association of Clinical Endocrinologists. Comprehensive foot examination and risk assessment: a report of the task force of the foot care interest group of the American Diabetes Association, with endorsement by the American Association of Clinical Endocrinologists. *Diabetes Care* 2008; **31**: 1679-1685 [PMID: 18663232 DOI: 10.2337/dc08-9021]
6 **van Netten JJ**, Price PE, Lavery LA, Monteiro-Soares M, Rasmussen A, Jubiz Y, Bus SA; International Working Group on the Diabetic Foot. Prevention of foot ulcers in the at-risk patient with diabetes: a systematic review. *Diabetes Metab Res Rev* 2016; **32** Suppl 1: 84-98 [PMID: 26340966 DOI: 10.1002/dmrr.2701]
7 **Miranda C**, Da Ros R, Marfella R. Update on prevention of diabetic foot ulcer. *Arch Med Sci Atheroscler Dis* 2021; **6**: e123-e131 [PMID: 34381913 DOI: 10.5114/amsad.2021.107817]
8 **Sheehan P**, Jones P, Giurini JM, Caselli A, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Plast Reconstr Surg* 2006; **117**: 239S-244S [PMID: 16799391 DOI: 10.1097/01.prs.0000222891.74489.33]
9 **Singh N**, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005; **293**: 217-228 [PMID: 15644549 DOI: 10.1001/jama.293.2.217]
10 **Rayman G**, Vas P, Dhatariya K, Driver V, Hartemann A, Londahl M, Piaggesi A, Apelqvist J, Attinger C, Game F;

International Working Group on the Diabetic Foot (IWGDF). Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020; **36** Suppl 1: e3283 [PMID: 32176450 DOI: 10.1002/dmrr.3283]

11 **Lauwers P**, Dirinck E, Van Bouwel S, Verrijken A, Van Dessel K, Van Gils C, Sels M, Peiffer F, Van Schil P, De Block C, Hendriks J. Malnutrition and its relation with diabetic foot ulcer severity and outcome: a review. *Acta Clin Belg* 2022; **77**: 79-85 [PMID: 32727304 DOI: 10.1080/17843286.2020.1800315]

12 **Pena G**, Kuang B, Cowled P, Howell S, Dawson J, Philpot R, Fitridge R. Micronutrient Status in Diabetic Patients with Foot Ulcers. *Adv Wound Care (New Rochelle)* 2020; **9**: 9-15 [PMID: 31871826 DOI: 10.1089/wound.2019.0973]

13 **Brookes JDL**, Jaya JS, Tran H, Vaska A, Werner-Gibbings K, D'Mello AC, Wong J, Lemoh CN, Saunder AC, Yii MK. Broad-Ranging Nutritional Deficiencies Predict Amputation in Diabetic Foot Ulcers. *Int J Low Extrem Wounds* 2020; **19**: 27-33 [PMID: 31549527 DOI: 10.1177/1534734619876779]

14 **Katona P**, Katona-Apte J. The interaction between nutrition and infection. *Clin Infect Dis* 2008; **46**: 1582-1588 [PMID: 18419494 DOI: 10.1086/587658]

15 **Shofler D**, Rai V, Mansager S, Cramer K, Agrawal DK. Impact of resolvin mediators in the immunopathology of diabetes and wound healing. *Expert Rev Clin Immunol* 2021; **17**: 681-690 [PMID: 33793355 DOI: 10.1080/1744666X.2021.1912598]

16 **Chen P**, Carville K, Swanson T, Lazzarini PA, Charles J, Cheney J, Prentice J; Australian Diabetes-related Foot Disease Guidelines & Pathways Project. Australian guideline on wound healing interventions to enhance healing of foot ulcers: part of the 2021 Australian evidence-based guidelines for diabetes-related foot disease. *J Foot Ankle Res* 2022; **15**: 40 [PMID: 35610723 DOI: 10.1186/s13047-022-00544-5]

17 **Eneroth M**, Larsson J, Oscarsson C, Apelqvist J. Nutritional supplementation for diabetic foot ulcers: the first RCT. *J Wound Care* 2004; **13**: 230-234 [PMID: 15214141 DOI: 10.12968/jowc.2004.13.6.26627]

18 **Armstrong DG**, Hanft JR, Driver VR, Smith AP, Lazaro-Martinez JL, Reyzelman AM, Furst GJ, Vayser DJ, Cervantes HL, Snyder RJ, Moore MF, May PE, Nelson JL, Baggs GE, Voss AC; Diabetic Foot Nutrition Study Group. Effect of oral nutritional supplementation on wound healing in diabetic foot ulcers: a prospective randomized controlled trial. *Diabet Med* 2014; **31**: 1069-1077 [PMID: 24867069 DOI: 10.1111/dme.12509]

19 **Wong A**, Chew A, Wang CM, Ong L, Zhang SH, Young S. The use of a specialised amino acid mixture for pressure ulcers: a placebo-controlled trial. *J Wound Care* 2014; **23**: 259-260, 262 [PMID: 24810310 DOI: 10.12968/jowc.2014.23.5.259]

20 **Basiri R**, Spicer MT, Levenson CW, Ormsbee MJ, Ledermann T, Arjmandi BH. Nutritional Supplementation Concurrent with Nutrition Education Accelerates the Wound Healing Process in Patients with Diabetic Foot Ulcers. *Biomedicines* 2020; **8** [PMID: 32756299 DOI: 10.3390/biomedicines8080263]

21 **Basiri R**, Spicer M, Levenson C, Ledermann T, Akhavan N, Arjmandi B. Improving Dietary Intake of Essential Nutrients Can Ameliorate Inflammation in Patients with Diabetic Foot Ulcers. *Nutrients* 2022; **14** [PMID: 35745123 DOI: 10.3390/nu14122393]

22 **Sipahi S**, Gungor O, Gunduz M, Cilci M, Demirci MC, Tamer A. The effect of oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine and glutamine on wound healing: a retrospective analysis of diabetic haemodialysis patients. *BMC Nephrol* 2013; **14**: 8 [PMID: 23311725 DOI: 10.1186/1471-2369-14-8]

23 **Ohue-Kitano R**, Yasuoka Y, Goto T, Kitamura N, Park SB, Kishino S, Kimura I, Kasubuchi M, Takahashi H, Li Y, Yeh YS, Jheng HF, Iwase M, Tanaka M, Masuda S, Inoue T, Yamakage H, Kusakabe T, Tani F, Shimatsu A, Takahashi N, Ogawa J, Satoh-Asahara N, Kawada T. α-Linolenic acid-derived metabolites from gut lactic acid bacteria induce differentiation of anti-inflammatory M2 macrophages through G protein-coupled receptor 40. *FASEB J* 2018; **32**: 304-318 [PMID: 28904023 DOI: 10.1096/fj.201700273R]

24 **Serini S**, Calviello G. New Insights on the Effects of Dietary Omega-3 Fatty Acids on Impaired Skin Healing in Diabetes and Chronic Venous Leg Ulcers. *Foods* 2021; **10** [PMID: 34681353 DOI: 10.3390/foods10102306]

25 **Jung JY**, Kwon HH, Hong JS, Yoon JY, Park MS, Jang MY, Suh DH. Effect of dietary supplementation with omega-3 fatty acid and gamma-linolenic acid on acne vulgaris: a randomised, double-blind, controlled trial. *Acta Derm Venereol* 2014; **94**: 521-525 [PMID: 24553997 DOI: 10.2340/00015555-1802]

26 **McDaniel JC**, Belury M, Ahijevych K, Blakely W. Omega-3 fatty acids effect on wound healing. *Wound Repair Regen* 2008; **16**: 337-345 [PMID: 18471252 DOI: 10.1111/j.1524-475X.2008.00388.x]

27 **McDaniel JC**, Szalacha L, Sales M, Roy S, Chafee S, Parinandi N. EPA + DHA supplementation reduces PMN activation in microenvironment of chronic venous leg ulcers: A randomized, double-blind, controlled study. *Wound Repair Regen* 2017; **25**: 680-690 [PMID: 28758717 DOI: 10.1111/wrr.12558]

28 **Li K**, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor α: a meta-analysis. *PLoS One* 2014; **9**: e88103 [PMID: 24505395 DOI: 10.1371/journal.pone.0088103]

29 **McDaniel JC**, Massey K, Nicolaou A. Fish oil supplementation alters levels of lipid mediators of inflammation in microenvironment of acute human wounds. *Wound Repair Regen* 2011; **19**: 189-200 [PMID: 21362086 DOI: 10.1111/j.1524-475X.2010.00659.x]

30 **Estimated website**. Guidelinecentral. [cited July 7 2022]. Available from: https://www.GuidelineCental.com

31 **Soleimani Z**, Hashemdokht F, Bahmani F, Taghizadeh M, Memarzadeh MR, Asemi Z. Clinical and metabolic response to flaxseed oil omega-3 fatty acids supplementation in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. *J Diabetes Complications* 2017; **31**: 1394-1400 [PMID: 28716357 DOI: 10.1016/j.jdiacomp.2017.06.010]

32 **Moore ZE**, Corcoran MA, Patton D. Nutritional interventions for treating foot ulcers in people with diabetes. *Cochrane Database Syst Rev* 2020; **7**: CD011378 [PMID: 32677037 DOI: 10.1002/14651858.CD011378.pub2]

33 **Hill C**, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 506-514 [PMID: 24912386 DOI: 10.1038/nrgastro.2014.66]

34 **Fijan S**, Frauwallner A, Langerholc T, Krebs B, Ter Haar Née Younes JA, Heschl A, Mičetić Turk D, Rogelj I. Efficacy of

Using Probiotics with Antagonistic Activity against Pathogens of Wound Infections: An Integrative Review of Literature. *Biomed Res Int* 2019; **2019**: 7585486 [PMID: 31915703 DOI: 10.1155/2019/7585486]

35 **Fijan S**. Microorganisms with claimed probiotic properties: an overview of recent literature. *Int J Environ Res Public Health* 2014; **11**: 4745-4767 [PMID: 24859749 DOI: 10.3390/ijerph110504745]

36 **Campos LF**, Tagliari E, Casagrande TAC, Noronha L, Campos ACL, Matias JEF. EFFECTS OF PROBIOTICS SUPPLEMENTATION ON SKIN WOUND HEALING IN DIABETIC RATS. *Arq Bras Cir Dig* 2020; **33**: e1498 [PMID: 32667528 DOI: 10.1590/0102-672020190001e1498]

37 **Mohseni S**, Bayani M, Bahmani F, Tajabadi-Ebrahimi M, Bayani MA, Jafari P, Asemi Z. The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. *Diabetes Metab Res Rev* 2018; **34** [PMID: 29193662 DOI: 10.1002/dmrr.2970]

38 **Christie-David DJ**, Gunton JE. Vitamin C deficiency and diabetes mellitus - easily missed? *Diabet Med* 2017; **34**: 294-296 [PMID: 27859562 DOI: 10.1111/dme.13287]

39 **Gunton JE**, Girgis CM, Lau T, Vicaretti M, Begg L, Flood V. Vitamin C improves healing of foot ulcers: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* 2021; **126**: 1451-1458 [PMID: 32981536 DOI: 10.1017/S0007114520003815]

40 **Yarahmadi A**, Saeed Modaghegh MH, Mostafavi-Pour Z, Azarpira N, Mousavian A, Bonakdaran S, Jarahi L, Samadi A, Peimani M, Hamidi Alamdari D. The effect of platelet-rich plasma-fibrin glue dressing in combination with oral vitamin E and C for treatment of non-healing diabetic foot ulcers: a randomized, double-blind, parallel-group, clinical trial. *Expert Opin Biol Ther* 2021; **21**: 687-696 [PMID: 33646060 DOI: 10.1080/14712598.2021.1897100]

41 **Da Porto A**, Cavarape A, Catena C, Colussi G, Casarsa V, Sechi LA. Interactions between vitamin D levels, cardiovascular risk factors, and atherothrombosis markers in patients with symptomatic peripheral artery disease. *Vasc Med* 2021; **26**: 315-316 [PMID: 33448908 DOI: 10.1177/1358863X20979360]

42 **Dai J**, Jiang C, Chen H. Vitamin D and diabetic foot ulcer: a systematic review and metanalysis. *Nutr Diabetes* 2019; **8**: 5

43 **Razzaghi R**, Pourbagheri H, Momen-Heravi M, Bahmani F, Shadi J, Soleimani Z, Asemi Z. The effects of vitamin D supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. *J Diabetes Complications* 2017; **31**: 766-772 [PMID: 27363929 DOI: 10.1016/j.jdiacomp.2016.06.017]

44 **Kamble A**, Ambad R, Padamwar M, Kakade A, Yeola M. To study the effect of oral vitamin D supplements on wound healing in patient with diabetic foot ulcer and its effect on lipid metabolism. *Int J Res Pharm Sci* 2020; **11**: 2701-2706

45 **Söderberg TA**. Trace Elements in Normal and Impaired Wound Healing. In: Altmeyer P, Hoffmann K, el Gammal S, Hutchinson J. Wound Healing and Skin Physiology. Berlin, Heidelberg: Springer, 1995 [DOI: 10.1007/978-3-642-77882-7_1]

46 **Razzaghi R**, Pidar F, Momen-Heravi M, Bahmani F, Akbari H, Asemi Z. Magnesium Supplementation and the Effects on Wound Healing and Metabolic Status in Patients with Diabetic Foot Ulcer: a Randomized, Double-Blind, Placebo-Controlled Trial. *Biol Trace Elem Res* 2018; **181**: 207-215 [PMID: 28540570 DOI: 10.1007/s12011-017-1056-5]

47 **Afzali H**, Jafari Kashi AH, Momen-Heravi M, Razzaghi R, Amirani E, Bahmani F, Gilasi HR, Asemi Z. The effects of magnesium and vitamin E co-supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. *Wound Repair Regen* 2019; **27**: 277-284 [PMID: 30693609 DOI: 10.1111/wrr.12701]

48 **Momen-Heravi M**, Barahimi E, Razzaghi R, Bahmani F, Gilasi HR, Asemi Z. The effects of zinc supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial. *Wound Repair Regen* 2017; **25**: 512-520 [PMID: 28395131 DOI: 10.1111/wrr.12537]

49 **Ye J**, Mani R. A Systematic Review and Meta-Analysis of Nutritional Supplementation in Chronic Lower Extremity Wounds. *Int J Low Extrem Wounds* 2016; **15**: 296-302 [PMID: 27810941 DOI: 10.1177/1534734616674624]

50 **Pomrinse SD**. Hospital beds in New York City--"the numbers game". *Bull N Y Acad Med* 1979; **55**: 874-885 [PMID: 291457 DOI: 10.1177/1534734617737659]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 949-961

DOI: 10.4239/wjd.v13.i11.949

ISSN 1948-9358 (online)

MINIREVIEWS

Combination therapy of hydrogel and stem cells for diabetic wound healing

Jia-Na Huang, Hao Cao, Kai-Ying Liang, Li-Ping Cui, Yan Li

Specialty type: Engineering, biomedical

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: Jeyaraman M, India; Prasetyo EP, Indonesia

Received: August 28, 2022
Peer-review started: August 28, 2022
First decision: September 12, 2022
Revised: September 25, 2022
Accepted: November 2, 2022
Article in press: November 2, 2022
Published online: November 15, 2022

Jia-Na Huang, Hao Cao, Kai-Ying Liang, Yan Li, School of Biomedical Engineering, Shenzhen Campus of Sun Yat-sen University, Shenzhen 518107, Guangdong Province, China

Jia-Na Huang, Hao Cao, Kai-Ying Liang, Yan Li, Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, Sun Yat-sen University, Guangzhou 510006, Guangdong Province, China

Li-Ping Cui, Endocrinology Department, Panyu Central Hospital, Guangzhou 511400, Guangdong Province, China

Corresponding author: Yan Li, PhD, Associate Professor, School of Biomedical Engineering, Shenzhen Campus of Sun Yat-sen University, No. 66 Gongchang Road, Shenzhen 518107, Guangdong Province, China. liyan99@mail.sysu.edu.cn

Abstract

Diabetic wounds (DWs) are a common complication of diabetes mellitus; DWs have a low cure rate and likely recurrence, thus affecting the quality of patients' lives. As traditional therapy cannot effectively improve DW closure, DW has become a severe clinical medical problem worldwide. Unlike routine wound healing, DW is difficult to heal because of its chronically arrested inflammatory phase. Although mesenchymal stem cells and their secreted cytokines can alleviate oxidative stress and stimulate angiogenesis in wounds, thereby promoting wound healing, the biological activity of mesenchymal stem cells is compromised by direct injection, which hinders their therapeutic effect. Hydrogels form a three-dimensional network that mimics the extracellular matrix, which can provide shelter for stem cells in the inflammatory microenvironment with reactive oxygen species in DW, and maintains the survival and viability of stem cells. This review summarizes the mechanisms and applications of stem cells and hydrogels in treating DW; additionally, it focuses on the different applications of therapy combining hydrogel and stem cells for DW treatment.

Key Words: Combination therapy; Mesenchymal stem cells; Hydrogel; Diabetic wound; Cells delivery; Wound healing

Core Tip: Diabetic wounds are a common diabetes mellitus complication with a low cure rate and likely recurrence. Although stem cell therapy is suitable for diabetic wound healing, simple transplantation methods, such as intravenous, subcutaneous, intramuscular, and local injection, are not conducive to cell survival, thus resulting in compromised efficacy. To improve the outcome of stem cell therapy, researchers have designed different types of hydrogels for stem cell delivery to ensure cell viability and paracrine functions. Herein, we discuss the current roles and applications of hydrogel and stem cell combination therapy for diabetic wound treatment.

Citation: Huang JN, Cao H, Liang KY, Cui LP, Li Y. Combination therapy of hydrogel and stem cells for diabetic wound healing. *World J Diabetes* 2022; 13(11): 949-961
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/949.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.949

INTRODUCTION

Diabetes mellitus (DM) is a significant global public health burden because of its high incidence and mortality rates[1]. In 2019, 1.5 million people died of DM[2]. Diabetic wounds (DWs) are one of the most concerning complications of DM and affect up to 25% of diabetic patients[3]. In addition to causing patient suffering, DW has a low cure rate and high amputation rate, and thus it places a long-term burden on society[4].

DW is difficult to heal because its healing process is unlike that of normal wounds. Normal wound healing typically includes three phases: Inflammation, proliferation, and remodeling. Various cells, growth factors, and cytokines play important roles in each phase to ensure a smooth wound healing progress[5]. Owing to the elevated levels of reactive oxygen species (ROS), impaired immune function, and cellular dysfunction in the DW microenvironment, the healing stage stagnates in the inflammatory phase[6]. In addition, the peripheral arterial disease leads to a lack of blood perfusion and hypoxia within wounds, thereby increasing ROS release[5]. ROS also induces the expression of extracellular matrix (ECM) degradation enzymes that degrade ECM, thus precluding the normal matrix-cell interaction required for wound healing and prolonging the inflammation phase of DW healing[5].

DW healing remains a clinical challenge because of several complications in the DW microenvironment, including oxidative stress, chronic inflammation, and angiogenic dysfunction[7]. Current clinical treatments (standard care) involve glycemic control, offloading, debridement, and infection management, which are painful and insufficient for curing DWs[8]. Therefore, new approaches for improving DW healing must be developed. The application of functional hydrogel dressings or scaffolds is a promising advanced therapy[9].

Hydrogels are three-dimensional (3D) networks with a high water content and have been intensively studied because they can be functionalized and have good biocompatibility. Several studies have shown that hydrogels provide a moist environment, contribute to cell migration and tissue regeneration, and promote wound healing[10]. Therefore, hydrogels are considered ideal dressings for DWs[11]. Furthermore, hydrogels provide antioxidant, antibacterial, proangiogenic, and proliferative functions owing to the sustained release of bioactive agents encapsulated in hydrogels. Stem cells are bioactive agents that promote wound healing and are effective in skin regeneration[12].

Stem cells possess self-renewal and differentiation abilities and are essential for post-injury skin repair[13]. Thus, stem cell therapy has become a promising new approach for treating DWs. Local injection of the cell suspension or stent implantation stimulates neovascularization, accelerates wound closure, prevents wound contracture and scar formation, and ultimately improves wound healing[14]. However, the outcome of stem cell therapy is hindered by the poor bioactivity of stem cells and thus the low amounts of secreted cytokines in the hyperglycemic inflammatory microenvironment of DWs. Effective stem cell delivery remains a challenge[15].

To achieve better healing outcomes combining hydrogel and stem cell treatment is one of the most promising therapies for DWs[16]. Although various reviews on stem cell therapy or hydrogel therapy for DWs have been reported, reviews on combined therapy are limited. Herein, we review the mechanisms of DW therapy combining hydrogel and stem cells and focus on preclinical studies of therapy combining hydrogel and stem cells for DWs.

FUNCTIONAL HYDROGELS FOR DW TREATMENT

Wound dressings play an essential role in DWs[2]. Hydrogels have become appealing and promising among various wound dressings owing to their high moisture retention, biocompatibility, and similarities to living tissues[17]. Hydrogels accelerate wound healing by maintaining gas exchange in

the wound, reducing pain by absorbing exudates, preventing infection, and maintaining a moist environment for cell migration. In addition, hydrogels have been used as delivery systems to minimize drug toxicity and improve drug delivery efficiency[2]. Functional hydrogels, such as antioxidant, immune regulation, and vascularization hydrogels, have been designed according to the wound microenvironment of DWs.

DWs are often accompanied by oxidative and antioxidant imbalance *in vivo*. Hydrogels are designed to alleviate excessive oxidative reactions. Self-antioxidant materials, such as 2-hydroxyethyl methacrylate[18] and polyvinyl alcohol, can directly act on wounds; additionally, gel-loaded antioxidant drugs, such as curcumin[19], or bioactive substances can be used to achieve antioxidant effects. These materials act as reducing agents.

Because the inflammatory phase has an active defense response to external stimuli, the inflammatory response aids in cleaning the wound during the healing process[20]. However, in chronic wounds, such as DWs, owing to repeated tissue damage, cytokines continue to recruit immune cells to the wound, thereby resulting in an excessive inflammatory response and blocked healing[21]. Therefore, the inhibition of excessive immune responses is also considered. Hydrogels, such as sodium alginate and zwitterionic hydrogels, can provide a protective microenvironment for wounds and regulate the transformation of macrophages between proinflammatory and anti-inflammatory[20]. Meanwhile, anti-inflammatory drug-loaded hydrogel dressings have a local sustained-release effect[22]. Responsive hydrogels that can change their properties according to environmental clues to achieve sustained release of entrapped drugs are also desirable.

Angiogenesis is essential for tissue regeneration, whereas the formation of healthy blood vessels is hindered by various microenvironment conditions in DWs[23]. Therefore, promoting blood vessel formation is conducive to DW healing. Studies have shown that some hydrogel materials, such as chitosan and hyaluronic acid, regulate the activity and distribution of cytokines or growth factors[24]. These materials simulate the microenvironment of the ECM, thereby promoting tissue formation. Bioactive components, including epidermal growth factor and vascular endothelial growth factor, can also be encapsulated by hydrogels, which can promote the regeneration of blood vessels[25].

In general, the mechanism of hydrogels in DWs is relatively clear and positively affects DW healing.

CURRENT STUDIES OF MESENCHYMAL STEM CELLS FOR DW HEALING

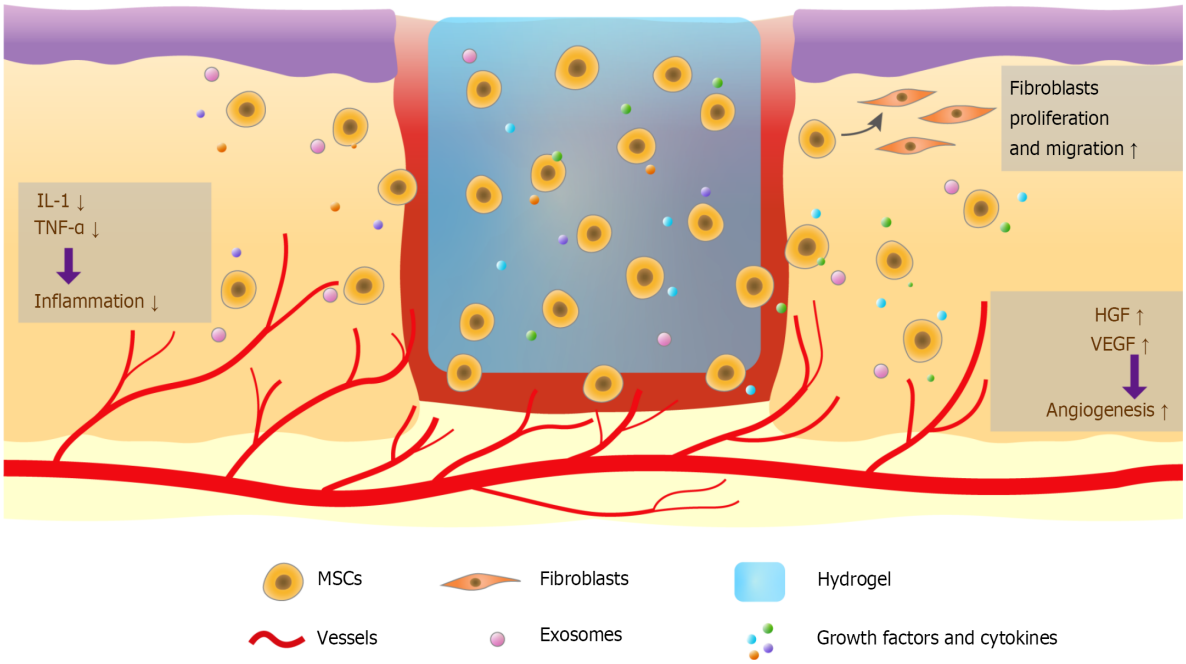
In addition to selecting different hydrogel materials, drugs, and biological factors, using stem cells to treat DWs is desirable. Stem cells can asymmetrically replicate and differentiate into different cell types [26]. With the unlimited replication capacity, they can provide numerous "sister" stem cells[15]. Furthermore, because stem cells secrete pro-regenerative cytokines, stem cell therapy, which treats diseases or injuries by administering stem cells into damaged tissues, has been used as an intervention for DWs[27]. Stem cells used for wound healing and tissue regeneration include embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells (MSCs)[15].

Allogeneic, xenogeneic, and autologous MSCs have been widely used in skin regeneration and wound healing owing to their significant proliferation, migration ability, and long-term self-renewal potential[28]. Considering the impaired function of MSCs derived from patients with diabetes and the risk of tissue rejection, allogeneic MSCs are more widely used[29].

MSCs that are locally injected into wounds are involved in various stages of wound healing. They reduce inflammatory responses through immunomodulation and growth factor production[15], accelerate neovascularization and epithelialization, and stimulate collagen synthesis[30], thereby accelerating wound healing[30]. Additionally, clinical studies have demonstrated the efficacy of MSCs in treating diabetic ulcers[30]. For example, injecting allogeneic MSCs into the dermis-epidermal junction[31] or subcutaneous and intramuscular tissue around wounds[32] facilitated DW healing in patients.

The potential benefits of MSC therapy have been demonstrated in several studies. Although simple transplantation methods, such as intravenous, subcutaneous, intramuscular, and local injection of MSCs, have achieved some preclinical and clinical success[5], MSC performance still has numerous limitations. Premature senescence and apoptosis of MSCs transplanted in DWs are some of the biggest limitations[33,34]. Owing to hyperglycemia caused by DM, DWs generate a chronic inflammatory microenvironment and accumulate advanced glycation end products, which is not conducive to the survival of stem cells[35] and increases the degradation of growth factors secreted by the effector cells, thus compromising efficacy[36]. Hence, the delivery strategy must be optimized to ensure cell viability, paracrine function, and differentiation function, which in turn ensures MSC therapy outcomes.

Abundant evidence has shown that using hydrogels to deliver MSCs improves DW healing. Hydrogels are ideal carrier systems for stem cells because they produce a relatively uniform distribution of transplanted cells and retain high water content, close to that of the native tissue, thus improving the retention and survival of stem cells at transplantation sites. Transplanted stem cells can exert their functions through paracrine signals and differentiate into the various cell types required in healthy tissues (Figure 1).



DOI: 10.4239/wjd.v13.i11.949 **Copyright** ©The Author(s) 2022.

Figure 1 Therapy combining hydrogels and mesenchymal stem cells promotes diabetic wound healing. Mesenchymal stem cells (MSCs) in hydrogels are long-lasting in the wound and regulate wound healing. These cells release exosomes, growth factors, and cytokines, reduce the levels of interleukin-1, tumor necrosis factor-α, and other pro-inflammatory cytokines to modulate the inflammatory response, enhance angiogenesis *via* increasing vascular endothelial growth factor and hepatocyte growth factor, and promote fibroblast and keratinocyte migration. MSCs can also be transdifferentiated into other cell types to increase wound closure. MSCs: Mesenchymal stem cells; IL-1: Interleukin-1; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor.

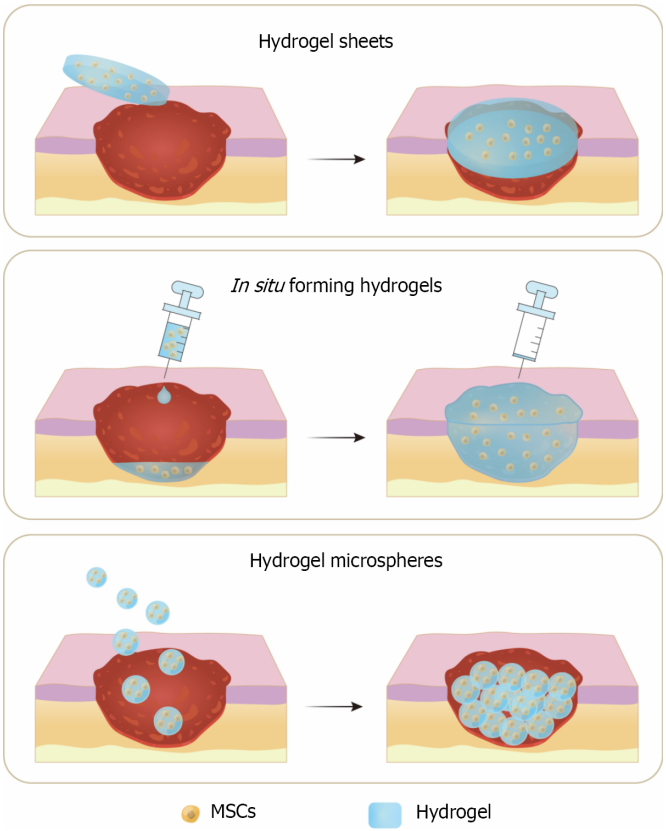
APPLICATIONS OF COMBINATION THERAPY OF HYDROGEL AND STEM CELLS FOR DW HEALING

As previously discussed, although stem cell therapy has promising potentials for DW healing, the lack of an optimal delivery strategy is one of the biggest obstacles to its therapeutic efficacy. Traditional injection of MSCs always results in low cell viability and transient engraftment, whereas using advanced biomaterial scaffolds (such as films, nanofibers, and hydrogels)[13] to maintain cellular viability, proliferation, and differentiation has received considerable attention[37]. Hydrogels have physical and biological characteristics similar to those of natural tissues[36]; this renders them as ideal candidates for stem cell delivery. Inspired by the encouraging outcomes of hydrogels on DW healing and their function as a carrier system for drugs, the efficacy of MSCs has been improved with hydrogels [37]. For a successful clinical application of the therapy, the optimal hydrogel composition for cell delivery must be considered, and appropriate application methods to ensure stem cell viability and promote DW healing must be designed. Currently, the most common application methods of hydrogels and stem cell combination therapy for DW healing are divided into hydrogel sheets, *in situ* forming hydrogels, and hydrogel microspheres (MS) (Figure 2).

HYDROGEL SHEETS

Applying hydrogel sheets on wounds is a convenient stem cell delivery method, wherein hydrogels are typically preformed in molds, with stem cells seeded onto or inside hydrogels. Rustad *et al*[38] seeded MSCs onto collagen-pullulan hydrogels and significantly accelerated wound healing and skin appendage recovery in mice within 11 d. The amount of microangiogenesis was approximately doubled in wounds treated with MSC-seeded hydrogel sheets compared with those treated with MSC injection. Given that the biomimetic hydrogel provides a functional niche to augment the regenerative potential of MSCs, the implanted MSCs differentiated into dermal fibroblasts, pericytes, and endothelial cells, which contribute to wound healing[38]. In another study, Guo *et al*[39] demonstrated the improved retention and survival rate of MSCs in hydrogel sheets when transplanted into mouse hearts compared to cell suspension alone. Cells were observed inside the hydrogel sheets for over 9 d in ICR mice.

In vitro culturing of stem cells within hydrogels was found to promote cell adhesion and enhance stem cell functions by supporting normal phenotype maintenance and empowering the transdifferen-



DOI: 10.4239/wjd.v13.i11.949 **Copyright** ©The Author(s) 2022.

Figure 2 Three application methods of hydrogels and mesenchymal stem cells combination therapy for diabetic wound healing. A: Hydrogel sheets preformed before application; B: *In situ* forming hydrogels injected at the wound for sol-gel transition; C: Hydrogel microspheres applied onto the diabetic wound. MSCs: Mesenchymal stem cells.

tiation capacity into specific skin lineages compared with the immediate transplantation of stem cell-seeded hydrogels[40]. Da Silva *et al*[41] pre-cultured adipose-derived stem cells (ADSCs) in hyaluronic acid-based sponge hydrogel in neurogenic/standard media for 14 d before transplantation onto the DWs of mice. Wounds treated with pre-cultured ADSCs-loaded spongy hydrogels improved wound closure rates compared to the untreated control and acellular spongy hydrogel groups after healing for 4 wk. The hydrogel sheet promoted the polarization of M1-type macrophages to the M2 type (anti-inflammatory) and improved successful neoinnervation.

Because of the high concentration of inflammatory cytokines in the DW microenvironment, which impairs the activity of MSCs and degrades growth factors secreted by stem cells, single functional hydrogel sheets may not be sufficient for DW healing[42]. To be more suitable for DW treatment, hydrogel sheets that inhibit inflammatory responses or protease activity are more effective[43]. Ahmed *et al*[44] studied the wound healing efficacy of bone marrow-derived mesenchymal stem cells (BMSCs) delivered by nitric oxide (NO)-releasing hydrogels on diabetic rabbits. As an endogenous molecule, NO increased angiogenesis and improved immune responses during acute infections. NO-releasing hydrogels increased the viability and proliferation of BMSCs under oxidative stress. In addition to improving collagen deposition and promoting re-epithelialization and angiogenic activity, the NO-releasing hydrogel with BMSC treatment upregulated the expression of growth and cytoactive factors for DW healing within 16 d[44].

In addition to traditional manufacturing technology, 3D bioprinting builds special structures layer-by-layer according to a predetermined computer model that better fits the skin's architecture and geometry, providing hydrogel sheets with more complex structures[45]. Xia *et al*[46] developed curcumin-incorporated 3D bioprinting gelatin methacryloyl (GelMA) to seed ADSCs and promote DW healing within 21 d. Curcumin encapsulation in 10% GelMA hydrogel exhibited inhibitory effects on ROS generation and ADSC apoptosis, and living cells were detected after scaffolds embedded with ADSCs were implanted into the backs of nude mice for 21 d. Further, the scaffold increased the amount of collagen deposition and induced angiogenesis in DWs[46]. In addition to 3D bioprinting, multifunctional hydrogel sheets with complex 3D structures can be produced by folding or weaving microfiber-shaped hydrogels[47]. Hydrogel sheets can also be easily functionalized, such as the thermally responsive release of stem cells or drugs[48] for oxidative stress resistance, antibacterial activity, and other functions.

Because stem cells can be cultured separately and the hydrogel sheet is easy to handle, combination therapy with hydrogel sheets and MSCs is easily translated into a clinical setting[49]. According to a clinical report, Ravari *et al*[50] applied BMSCs along with platelets, fibrin glue, and bone marrow-impregnated collagen matrix onto wounds, which resulted in the complete wound closure in 3 of 8 patients with aggressive, refractory DWs within 4 wk of treatment. Additionally, topical administration of placenta-derived mesenchymal stem cells in a sodium alginate hydrogel completely healed diabetic foot ulcers[51]. However, this clinical case report must be evaluated further because of the limited sample size of the report. Although functionalizing or changing shapes is very convenient, hydrogel sheets must be pre-formed before application. Because hydrogel sheets are not conducive to long-term storage and the bonding between the sheets and wound surface is limited, *in situ* forming hydrogels have attracted attention.

IN SITU FORMING HYDROGELS

In situ forming hydrogels are another mainstream application of combination therapy, with stem cells suspended in the precursor solution before application[52]. After the mixed precursor solution is injected into the wound site, the hydrogel containing stem cells is formed *in situ* on wound beds *via* chemical bonds[53]. Compared with hydrogel sheets, injectable hydrogels are more flexible in their application; this flexibility allows them to adapt to complex-shaped wounds and fit closely[54]. Eke *et al*[55] designed a precursor solution composed of GelMA and methacrylated hyaluronic acid containing ADSCs, which can be crosslinked within 40 s of ultraviolet irradiation to form hydrogels *in situ*. Reportedly, the hydrogel promoted cell proliferation, and *in vivo* studies revealed a three-fold increase in vascularization for the ADSC-loaded hydrogel group compared to the hydrogels without cells.

However, because ultraviolet irradiation may induce chromosomal and genetic instability[56], ultraviolet-crosslinked hydrogels on exposed wounds negatively affect cell viability and differentiation [57], which is detrimental to wound healing. Owing to its high biocompatibility and specificity[58], enzymatic crosslinking has received considerable attention[59]. Yao *et al*[52] developed a gelatin-hydroxyphenyl hydrogel with the dual enzyme crosslinking of horseradish peroxidase and galactose oxidase, and the hydrogel encapsulated with BMSCs achieved gelation within 5 min at the wound site. The gelatin-hydroxyphenyl hydrogel provides a friendly 3D microenvironment for BMSCs, thereby improving the transplanted cells' survival and accelerating wound closure[52].

Given that frequently studied natural hydrogels, such as gelatin, collagen, or hyaluronic acid, contain a single component of ECM, their potential to provide the optimum microenvironment for stem cell proliferation and differentiation is limited[60]. ECM maintains the original components of the native tissue and is considered an ideal scaffold for tissue regeneration[61]. Chen *et al*[62] developed an ECM-derived hydrogel from human decellularized adipose tissue matrix to deliver ADSCs to DWs. The hydrogel was prepared *via* pepsin digestion and pH neutralization. The paracrine activity of ADSCs encapsulated in the hydrogel was enhanced, whereas the secretion of hepatocyte growth factor increased, thus promoting neovascularization during wound healing[62]. Compared with the untreated control, local ADSC injection, and acellular hydrogel groups, treatment with ADSC-hydrogel composites accelerated wound closure in diabetic mice and restored cutaneous appendages within 14 d [62].

For better DW healing outcomes, specific materials are co-entrapped inside the hydrogel for hemostasis and anti-inflammatory properties, and the stem cell viability in the hydrogel can reach an ideal state by optimizing its mechanical strength. Xu *et al*[63] encapsulated MSCs in an injectable hydrogel system of GelMA and chitosan-catechol cross-linked with dithiothreitol to repair full-thickness DWs. Chitosan-catechol has a good hemostatic effect, and zinc ions were introduced into the hydrogel to enhance angiogenesis. The cell adhesion, proliferation, and differentiation potency of umbilical cord-derived mesenchymal stem cells *in vitro* were well maintained in GelMA with optimal stiffness. At the same time, the hydrogel-umbilical cord-derived mesenchymal stem cells combined treatment promoted DW healing by inhibiting the inflammatory factors TNF-α and IL-1β *in vivo*, with a wound closure rate of 92.2% within 14 d. Compared with the untreated control, local umbilical cord-derived mesenchymal stem cell injection, and acellular hydrogel groups, collagen deposition was significantly abundant on day 7, whereas the most vascular regeneration with the earliest hair follicle formation was found on day 14[63].

Dispersive MSCs are usually loaded inside hydrogels. Recently, 3D MSC spheroids were found to possess better differentiation potential than dispersive MSCs[64], which exhibited enhanced vascularization and anti-inflammatory effects[65], thereby promoting wound closure[66]. Yang *et al*[67] combined injectable thermosensitive chitosan/collagen/β-glycerophosphate hydrogels with 3D MSC spheroids, rapidly converted to a gel by physical cross-linking at body temperature, and then completely covered the wound surface and fitted to any shape of the wound bed. Compared with the local 2D monolayer MSC injection and 2D monolayer MSC-encapsulated hydrogel groups, angiogenic factors were much higher for wounds treated with 3D MSC spheroid-encapsulated hydrogel (almost 3-fold), and neovascularization was enhanced, thereby achieving complete re-epithelialization within 3

wk of implantation[67].

Although *in situ* forming hydrogels adapt to complex-shaped wounds and fit tightly, thus enabling flexible use at the wound bed, the bulk hydrogel formed at the wound site produces poor tissue infiltration and thus low stem cell survival. Compared with *in situ* forming hydrogels, hydrogel MSs have a larger specific surface area and more specific functions, thus playing an essential role in the medical field.

HYDROGEL MS

Hydrogel MSs exhibit good dispersion and stability in physiological environments with a high drug-loading capacity[68]. Their drug-carrying[69] and bioactive factors[70] are highly effective in wound healing. We previously demonstrated that antibiotic and growth factor separately loaded alginate/$CaCO_3$ MSs prepared using microfluidic technology sustainably released drugs and exhibited pH sensitivity. These MSs were embedded in the regenerated tissue and functioned as scaffold materials. They improved wound healing with thicker granulation tissue and stimulated angiogenesis, ideally meeting the requirements of different stages of wound healing[71]. Lei *et al*[70] developed biohybrid agarose MSs conjugated with basic fibroblast growth factor, which achieved local growth factor delivery, stimulated angiogenesis, and enhanced wound healing in diabetic mice.

The special geometry of hydrogel MSs is conducive to the diffusion of nutrients and wastes[72]. MSs that deliver stem cells can release stem cells, thereby promoting proliferation and differentiation of surrounding cells and enhancing the formation of integrated functional tissues[73]. Stem cell-loaded MSs have been applied in various tissue systems, including cartilage[74], bone[75], bone marrow[72], and brain[76]. Intracerebral implantation of stem cells using MSs in the rat brain improved stroke treatment[76]. Mao *et al*[72] demonstrated that microgel encapsulation sustained MSC survival after intravenous injection in mice and enhanced the immunoregulatory capacity of MSCs in a bone marrow transplantation model.

Considering that our previous study demonstrated that hydrogel MSs act as scaffolds and gradually integrate into regenerated skin tissue, we designed gelatin MSs encapsulated with ADSCs from rats (rADSC/MS) with an ideal mechanical strength and degradation rate that matched tissue regeneration to improve DW healing[77]. Gelatin MSs promoted the adhesion and proliferation of fibroblast cells and maintained the viability of encapsulated rADSCs. Slowly released exosomes from rADSCs were eventually internalized by HUVECs, which suggested a potential exosome mechanism for improving wound healing. The implanted rADSC/MS gradually integrated into the regenerated skin tissue, thus facilitating the arrangement of neat collagen fibers. Compared with the untreated group and the MS group, rADSCs embedded in rADSC/MS promoted M2 macrophage polarization and recovery of peripheral nerves, formed larger blood vessels, and eventually generated a dermis close to normal tissue within 14 d[77].

Previous studies have demonstrated that hydrogels provide a functional niche for MSCs, which enhances MSC regeneration potential and promotes wound healing. Preclinical studies on the combined treatment of DWs with hydrogels and stem cells are summarized in Table 1.

CONCLUSION

This review discussed the benefits associated with therapy combining hydrogels and MSCs for DW healing. Researchers have explored different application methods for stem cell delivery with hydrogels, including hydrogel sheets, *in situ* forming hydrogels, and hydrogel MSs. In addition to providing a friendly microenvironment for stem cells, this strategy enhances the adhesion between the dressing and wound and facilitates the function of stem cells, ultimately benefiting vascular and neural regeneration in DWs. Among these application methods, hydrogel MSs have the advantages of a larger specific surface area, more uniform dispersibility, and more specific functions; additionally, they can effectively deliver various types and functions of cells into the wound. Therefore, hydrogel MSs loaded with stem cells are expected to play an important role in clinical practice.

Therapy combining hydrogels and MSCs has shown great potential for DW healing. However, the plasticity of MSCs has led to their double-sidedness for clinical applications. Although the multi-differentiation ability provides them with good application prospects, it increases the risk of tumorigenicity [78]. As a solution, cell-free treatments, such as exosomes and artificial cell products derived from the MSCs secretome have attracted recent interest. Exosomes and secretomes retain the paracrine factors of stem cells[7]. Although extensive studies have explored the combination therapies of hydrogels and MSCs for DW healing, additional work is required to optimize parameters, such as the storage and transport stability of cells, and avoid their tumorigenic and immunogenic risks. Further improvement and testing of this technology *in vivo* will also contribute to the clinical transformation of combination therapy.

Table 1 Summary of studies regarding therapy combining hydrogels and stem cells for diabetic wound healing

Stem cell information, types, dosage in cells/wound	Hydrogel composition	Hydrogel types	Application methods	Animal	Wound size diameter, location	Full re-epithelialization efficiency	Outcome	Ref.
UMSCs from human, xenogeneic, 1 × 10^6	Self-assembled nanopeptide hydrogels based on RADA16-I, RGD, and KLT peptide solutions	Self-assembled nanopeptide hydrogels with easy biomimetic functionalization	Cells were encapsulated into the *in situ* forming hydrogels	NOD/SCID mice	8 mm, dorsal	10 d	Accelerated skin wound healing by inhibiting inflammation and promoting angiogenesis	[14]
BMSCs from rats, allogenic, 2 × 10^5	N-chitosan/ HA-ALD hydrogel	Hemostasis and antimicrobial hydrogels	Cells were encapsulated into the *in situ* forming hydrogels	STZ-induced diabetic rats	5 mm, foot	12 d	Promoted wound healing; stimulated the secretion of growth factors from rBMSCs, and modulated the inflammatory environment by inhibiting the expression of M1 macrophages and promoting the expression of M2 macrophages, resulting in granulation tissue formation, collagen deposition, nucleated cell proliferation, neovascularization	[37]
ADSCs from human, xenogeneic, 3 × 10^5	GG-HA spongy hydrogel	Vascularization hydrogels	Cells were seeded onto the top of spongy-like hydrogel sheets	STZ-induced diabetic mice	9 mm, dorsal	4 wk	Accelerated excisional skin wound healing; induced the healing phase switch from the inflammatory to the proliferative phase; presented a thicker epidermis with a high number of proliferative keratinocytes in the basal layer; increased the number of intraepidermal nerve fibers in the regenerated epidermis	[41]
BMSCs from rabbits, allogenic, 1 × 10^6	SNAP-loaded chitosan-PVA hydrogel	Vascularization hydrogels	Cells were intradermally injected and topically covered with hydrogel sheets	Alloxan monohydrate induced diabetic rabbits	20 mm, dorsal	14 d	Augmented the wound closure, decreased inflammation, and upregulated expression of CD31, VEGF and TGFβ-1; promoted angiogenesis by forming new capillaries and improving the microvascular and vessel maturation; showed an abundant expression of collagen type I on day 14	[44]
ADSCs from human, xenogeneic, 5 × 10^5	Curcumin-incorporated 3D bioprinting GelMA hydrogel	Antioxidant hydrogels	Cells were encapsulated into hydrogel sheets	STZ-induced diabetic nude mice	15 mm, dorsal	21 d	Promoted wound healing; improved hADSCs apoptosis and increased the amount of collagen	[46]
ADSCs from human, xenogeneic, 2.5 × 10^5	hDAM hydrogel	Intact ECM-derived hydrogels from living tissues	Cells were suspended in the *in situ* forming	KK/Upj-Ay/J mice (diabetic mice)	8 mm, dorsal	14 d	Accelerated wound closure and improved skin architecture regeneration,	[62]

			hydrogels				including better restoration of cutaneous appendages, increase of dermis thickness, and augmenting neovascularization	
UMSCs from human, xenogeneic, 5 × 10^6	GelMA/Chi-C hydrogel	Vascularization hydrogels	Cells were mixed with the *in situ* forming hydrogels	Diabetic mice (db/db)	8 mm, dorsal	14 d	Promoted the wound healing process by inhibiting protein expression of TNF-α and IL-1β to decrease inflammation. Accelerated angiogenesis and re-epithelialization, promoted collagen deposition, and induced regeneration of skin appendages such as hair follicles	[63]
PDSCs from human, xenogeneic, 1 × 10^6	Chitosan/collagen/β-GP hydrogel	Thermosensitive and pH-responsive hydrogels	3D spheroids were encapsulated in the *in situ* forming hydrogels	Diabetic mice (db/db)	7 mm, dorsal	3 wk	Accelerated wound closure by enhancing angiogenesis and paracrine effects. The hydrogel provided an environment favorable for the attachment and proliferation of encapsulated hPDSCs, accelerating cell proliferation and paracrine factor secretion	[67]
ADSCs from rats, allogenic, 5 × 10^5	Gelatin hydrogel	Adaptive hydrogel microspheres with degradation rates well-matched to tissue regeneration	Hydrogel microspheres	STZ-induced diabetic rats	8 mm, dorsal	14 d	Significantly accelerated wound healing by promoting M2 macrophage polarization, collagen deposition, angiogenesis associated with peripheral nerve recovery, and hair follicle formation. The microspheres well embedded in the tissue, exhibited good biocompatibility and adaptive biodegradation rates	[77]
BMSCs from human, xenogeneic, 5 × 10^5	PEGDA hydrogel	Bioinert synthetic hydrogels	Cells were encapsulated into hydrogel sheets	Genetically diabetic mice (BKS.Cg-m +/+Leprdb/J)	1 cm × 1 cm[1], dorsal	14 d	Accelerated wound healing; the co-encapsulation of hBMSCs and insulin secreting cells resulted in healing wounds without scab or scar	[79]
ADSCs from human, xenogeneic, 3 × 10^5	PEG-gelatin hydrogel	Vascularization hydrogels	Cells were mixed with the *in situ* forming hydrogels	Diabetic mice (db/db)	6 mm, dorsal	15 d	Significantly accelerated wound closure; the encapsulated cells attached and diffused well inside the hydrogel, improving cell retention *in vivo*; reduced inflammatory cell infiltration and enhanced neovascularization	[80]

[1]Wound size (side length × side length).

3D: Three dimensional; ADSCs: Adipose-derived stem cells; β-GP: β-glycerophosphate; BMSCs: Bone marrow-derived mesenchymal stem cells; Chi-C:

Chitosan-catechol; ECM: Extracellular matrix; GelMA: Gelatin methacryloyl; GG-HA: Gellan gum-hyaluronic acid; HA-ALD: Hyaluronic acid-aldehyde; hADSCs: Human adipose-derived stem cells; hBMSCs: Human bone marrow-derived mesenchymal stem cells; hDAM: Human decellularized adipose tissue matrix; hPDSCs: Human placenta-derived mesenchymal stem cells; N-chitosan: N-carboxyethyl chitosan; PDSCs: Placenta-derived mesenchymal stem cells; PEG: Poly(ethylene glycol); PEGDA: Polyethylene glycol diacrylate; PVA: Polyvinyl alcohol; rBMSCs: Rat bone marrow-derived mesenchymal stem cells; SNAP: S-nitroso-N-acetyl-penicillamine; STZ: Streptozotocin; UMSCs: Umbilical cord-derived mesenchymal stem cells; VEGF: Vascular endothelial growth factor.

FOOTNOTES

Author contributions: Li Y designed the research study; Huang JN and Cao H searched the literature and drafted the initial manuscript; Liang KY and Cui LP provided further revision; all authors have read and approved the final version of the manuscript.

Supported by the Shenzhen Basic Research Project, No. JCYJ20190807155805818; and the Foundation of Guangdong Provincial Key Laboratory of Sensor Technology and Biomedical Instrument, No. 2020B1212060077.

Conflict-of-interest statement: There are no conflicts of interest to report.

Country/Territory of origin: China

ORCID number: Yan Li 0000-0003-3115-8380.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Chen YL

REFERENCES

1 **Yu FX**, Lee PSY, Yang L, Gao N, Zhang Y, Ljubimov AV, Yang E, Zhou Q, Xie L. The impact of sensory neuropathy and inflammation on epithelial wound healing in diabetic corneas. *Prog Retin Eye Res* 2022; **89**: 101039 [PMID: 34991965 DOI: 10.1016/j.preteyeres.2021.101039]
2 **Alven S**, Peter S, Mbese Z, Aderibigbe BA. Polymer-Based Wound Dressing Materials Loaded with Bioactive Agents: Potential Materials for the Treatment of Diabetic Wounds. *Polymers (Basel)* 2022; **14** [PMID: 35215637 DOI: 10.3390/polym14040724]
3 **Dasari N**, Jiang A, Skochdopole A, Chung J, Reece EM, Vorstenbosch J, Winocour S. Updates in Diabetic Wound Healing, Inflammation, and Scarring. *Semin Plast Surg* 2021; **35**: 153-158 [PMID: 34526862 DOI: 10.1055/s-0041-1731460]
4 **Han Y**, Sun T, Tao R, Han Y, Liu J. Clinical application prospect of umbilical cord-derived mesenchymal stem cells on clearance of advanced glycation end products through autophagy on diabetic wound. *Eur J Med Res* 2017; **22**: 11 [PMID: 28340602 DOI: 10.1186/s40001-017-0253-1]
5 **Cho H**, Blatchley MR, Duh EJ, Gerecht S. Acellular and cellular approaches to improve diabetic wound healing. *Adv Drug Deliv Rev* 2019; **146**: 267-288 [PMID: 30075168 DOI: 10.1016/j.addr.2018.07.019]
6 **Li D**, Wu N. Mechanism and application of exosomes in the wound healing process in diabetes mellitus. *Diabetes Res Clin Pract* 2022; **187**: 109882 [PMID: 35487341 DOI: 10.1016/j.diabres.2022.109882]
7 **Holl J**, Kowalewski C, Zimek Z, Fiedor P, Kaminski A, Oldak T, Moniuszko M, Eljaszewicz A. Chronic Diabetic Wounds and Their Treatment with Skin Substitutes. *Cells* 2021; **10** [PMID: 33804192 DOI: 10.3390/cells10030655]
8 **Glover K**, Stratakos AC, Varadi A, Lamprou DA. 3D scaffolds in the treatment of diabetic foot ulcers: New trends vs conventional approaches. *Int J Pharm* 2021; **599**: 120423 [PMID: 33647412 DOI: 10.1016/j.ijpharm.2021.120423]
9 **Wu H**, Ni R, Shi Y, Hu Y, Shen Z, Pang Q, Zhu Y. The Promising Hydrogel Candidates for Preclinically Treating Diabetic Foot Ulcer: A Systematic Review and Meta-Analysis. *Adv Wound Care (New Rochelle)* 2022 [PMID: 35229628 DOI: 10.1089/wound.2021.0162]
10 **Liang Y**, He J, Guo B. Functional Hydrogels as Wound Dressing to Enhance Wound Healing. *ACS Nano* 2021 [PMID: 34374515 DOI: 10.1021/acsnano.1c04206]
11 **Gao D**, Zhang Y, Bowers DT, Liu W, Ma M. Functional hydrogels for diabetic wound management. *APL Bioeng* 2021; **5**: 031503 [PMID: 34286170 DOI: 10.1063/5.0046682]
12 **Verdi J**, Shirian S, Saleh M, Khadem Haghighian H, Kavianpour M. Mesenchymal Stem Cells Regenerate Diabetic Foot Ulcers: A Review Article. *World J Plast Surg* 2022; **11**: 12-22 [PMID: 35592239 DOI: 10.52547/wjps.11.1.12]
13 **Yu Q**, Qiao GH, Wang M, Yu L, Sun Y, Shi H, Ma TL. Stem Cell-Based Therapy for Diabetic Foot Ulcers. *Front Cell Dev*

Biol 2022; **10**: 812262 [PMID: 35178389 DOI: 10.3389/fcell.2022.812262]
14 **Xue J**, Sun N, Liu Y. Self-Assembled Nano-Peptide Hydrogels with Human Umbilical Cord Mesenchymal Stem Cell Spheroids Accelerate Diabetic Skin Wound Healing by Inhibiting Inflammation and Promoting Angiogenesis. *Int J Nanomedicine* 2022; **17**: 2459-2474 [PMID: 35669002 DOI: 10.2147/IJN.S363777]
15 **Nourian Dehkordi A**, Mirahmadi Babaheydari F, Chehelgerdi M, Raeisi Dehkordi S. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Res Ther* 2019; **10**: 111 [PMID: 30922387 DOI: 10.1186/s13287-019-1212-2]
16 **Rezaie F**, Momeni-Moghaddam M, Naderi-Meshkin H. Regeneration and Repair of Skin Wounds: Various Strategies for Treatment. *Int J Low Extrem Wounds* 2019; **18**: 247-261 [PMID: 31257948 DOI: 10.1177/1534734619859214]
17 **Francesko A**, Petkova P, Tzanov T. Hydrogel Dressings for Advanced Wound Management. *Curr Med Chem* 2018; **25**: 5782-5797 [PMID: 28933299 DOI: 10.2174/0929867324666170920161246]
18 **Singh B**, Dhiman A. Designing bio-mimetic moxifloxacin loaded hydrogel wound dressing to improve antioxidant and pharmacology properties. *RSC Adv* 2015; **5**: 44666-44678 [DOI: 10.1039/c5ra06857f]
19 **Gong C**, Wu Q, Wang Y, Zhang D, Luo F, Zhao X, Wei Y, Qian Z. A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing. *Biomaterials* 2013; **34**: 6377-6387 [PMID: 23726229 DOI: 10.1016/j.biomaterials.2013.05.005]
20 **Wang H**, Xu Z, Zhao M, Liu G, Wu J. Advances of hydrogel dressings in diabetic wounds. *Biomater Sci* 2021; **9**: 1530-1546 [PMID: 33433534 DOI: 10.1039/d0bm01747g]
21 **Frykberg RG**, Banks J. Challenges in the Treatment of Chronic Wounds. *Adv Wound Care (New Rochelle)* 2015; **4**: 560-582 [PMID: 26339534 DOI: 10.1089/wound.2015.0635]
22 **Tao SC**, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan Wound Dressings Incorporating Exosomes Derived from MicroRNA-126-Overexpressing Synovium Mesenchymal Stem Cells Provide Sustained Release of Exosomes and Heal Full-Thickness Skin Defects in a Diabetic Rat Model. *Stem Cells Transl Med* 2017; **6**: 736-747 [PMID: 28297576 DOI: 10.5966/sctm.2016-0275]
23 **Nazarnezhad S**, Baino F, Kim HW, Webster TJ, Kargozar S. Electrospun Nanofibers for Improved Angiogenesis: Promises for Tissue Engineering Applications. *Nanomaterials (Basel)* 2020; **10** [PMID: 32824491 DOI: 10.3390/nano10081609]
24 **Jiang Y**, Zhang X, Zhang W, Wang M, Yan L, Wang K, Han L, Lu X. Infant Skin Friendly Adhesive Hydrogel Patch Activated at Body Temperature for Bioelectronics Securing and Diabetic Wound Healing. *ACS Nano* 2022 [PMID: 35549213 DOI: 10.1021/acsnano.2c00662]
25 **Augustine R**, Hasan A, Dalvi YB, Rehman SRU, Varghese R, Unni RN, Yalcin HC, Alfkey R, Thomas S, Al Moustafa AE. Growth factor loaded in situ photocrosslinkable poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/gelatin methacryloyl hybrid patch for diabetic wound healing. *Mater Sci Eng C Mater Biol Appl* 2021; **118**: 111519 [PMID: 33255074 DOI: 10.1016/j.msec.2020.111519]
26 **Assinck P**, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury. *Nat Neurosci* 2017; **20**: 637-647 [PMID: 28440805 DOI: 10.1038/nn.4541]
27 **Zarei F**, Negahdari B, Eatemadi A. Diabetic ulcer regeneration: stem cells, biomaterials, growth factors. *Artif Cells Nanomed Biotechnol* 2018; **46**: 26-32 [PMID: 28355923 DOI: 10.1080/21691401.2017.1304407]
28 **Liu R**, Dong R, Chang M, Liang X, Wang HC. Adipose-Derived Stem Cells for the Treatment of Diabetic Wound: From Basic Study to Clinical Application. *Front Endocrinol (Lausanne)* 2022; **13**: 882469 [PMID: 35898452 DOI: 10.3389/fendo.2022.882469]
29 **Kita A**, Saito Y, Miura N, Miyajima M, Yamamoto S, Sato T, Yotsuyanagi T, Fujimiya M, Chikenji TS. Altered regulation of mesenchymal cell senescence in adipose tissue promotes pathological changes associated with diabetic wound healing. *Commun Biol* 2022; **5**: 310 [PMID: 35383267 DOI: 10.1038/s42003-022-03266-3]
30 **Krasilnikova OA**, Baranovskii DS, Lyundup AV, Shegay PV, Kaprin AD, Klabukov ID. Stem and Somatic Cell Monotherapy for the Treatment of Diabetic Foot Ulcers: Review of Clinical Studies and Mechanisms of Action. *Stem Cell Rev Rep* 2022; **18**: 1974-1985 [PMID: 35476187 DOI: 10.1007/s12015-022-10379-z]
31 **Uzun E**, Güney A, Gönen ZB, Özkul Y, Kafadar İH, Günay M, Mutlu M. Intralesional allogeneic adipose-derived stem cells application in chronic diabetic foot ulcer: Phase I/2 safety study. *Foot Ankle Surg* 2021; **27**: 636-642 [PMID: 32826167 DOI: 10.1016/j.fas.2020.08.002]
32 **Suzdaltseva Y**, Zhidkih S, Kiselev SL, Stupin V. Locally Delivered Umbilical Cord Mesenchymal Stromal Cells Reduce Chronic Inflammation in Long-Term Nonhealing Wounds: A Randomized Study. *Stem Cells Int* 2020; **2020**: 5308609 [PMID: 32148521 DOI: 10.1155/2020/5308609]
33 **Gong JH**, Dong JY, Xie T, Lu SL. The Influence of AGEs Environment on Proliferation, Apoptosis, Homeostasis, and Endothelial Cell Differentiation of Human Adipose Stem Cells. *Int J Low Extrem Wounds* 2017; **16**: 94-103 [PMID: 28682730 DOI: 10.1177/1534734617701575]
34 **Munir H**, Ward LSC, McGettrick HM. Mesenchymal stem cells as endogenous regulators of inflammation. In: Owens B, Lakins M. Advances in experimental medicine and biology. 2018: 73-98
35 **Wagers AJ**. The stem cell niche in regenerative medicine. *Cell Stem Cell* 2012; **10**: 362-369 [PMID: 22482502 DOI: 10.1016/j.stem.2012.02.018]
36 **Youngblood RL**, Truong NF, Segura T, Shea LD. It's All in the Delivery: Designing Hydrogels for Cell and Non-viral Gene Therapies. *Mol Ther* 2018; **26**: 2087-2106 [PMID: 30107997 DOI: 10.1016/j.ymthe.2018.07.022]
37 **Bai H**, Kyu-Cheol N, Wang Z, Cui Y, Liu H, Feng Y, Zhao Y, Lin Q, Li Z. Regulation of inflammatory microenvironment using a self-healing hydrogel loaded with BM-MSCs for advanced wound healing in rat diabetic foot ulcers. *J Tissue Eng* 2020; **11**: 2041731420947242 [PMID: 32913623 DOI: 10.1177/2041731420947242]
38 **Rustad KC**, Wong VW, Sorkin M, Glotzbach JP, Major MR, Rajadas J, Longaker MT, Gurtner GC. Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold. *Biomaterials* 2012; **33**: 80-90 [PMID: 21963148 DOI: 10.1016/j.biomaterials.2011.09.041]
39 **Guo R**, Wan F, Morimatsu M, Xu Q, Feng T, Yang H, Gong Y, Ma S, Chang Y, Zhang S, Jiang Y, Wang H, Chang D,

Zhang H, Ling Y, Lan F. Cell sheet formation enhances the therapeutic effects of human umbilical cord mesenchymal stem cells on myocardial infarction as a bioactive material. *Bioact Mater* 2021; **6**: 2999-3012 [PMID: 33732969 DOI: 10.1016/j.bioactmat.2021.01.036]

40 **Da Silva LP**, Reis RL, Correlo VM, Marques AP. Hydrogel-based strategies to advance therapies for chronic skin wounds. In: Yamush ML. Annual review of biomedical engineering. 201: 145-169

41 **Da Silva LP**, Santos TC, Rodrigues DB, Pirraco RP, Cerqueira MT, Reis RL, Correlo VM, Marques AP. Stem Cell-Containing Hyaluronic Acid-Based Spongy Hydrogels for Integrated Diabetic Wound Healing. *J Invest Dermatol* 2017; **137**: 1541-1551 [PMID: 28259681 DOI: 10.1016/j.jid.2017.02.976]

42 **Bjarnsholt T**, Kirketerp-Møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K, Høiby N, Givskov M. Why chronic wounds will not heal: a novel hypothesis. *Wound Repair Regen* 2008; **16**: 2-10 [PMID: 18211573 DOI: 10.1111/j.1524-475X.2007.00283.x]

43 **Chen S**, Shi J, Zhang M, Chen Y, Wang X, Zhang L, Tian Z, Yan Y, Li Q, Zhong W, Xing M. Mesenchymal stem cell-laden anti-inflammatory hydrogel enhances diabetic wound healing. *Sci Rep* 2015; **5**: 18104 [PMID: 26643550 DOI: 10.1038/srep18104]

44 **Ahmed R**, Afreen A, Tariq M, Zahid AA, Masoud MS, Ahmed M, Ali I, Akram Z, Hasan A. Bone marrow mesenchymal stem cells preconditioned with nitric-oxide-releasing chitosan/PVA hydrogel accelerate diabetic wound healing in rabbits. *Biomed Mater* 2021; **16** [PMID: 33075764 DOI: 10.1088/1748-605X/abc28b]

45 **Radmanesh S**, Shabangiz S, Koupaei N, Hassanzadeh-Tabrizi SA. 3D printed bio polymeric materials as a new perspective for wound dressing and skin tissue engineering applications: a review. *J Polym Res* 2022; **29** [DOI: 10.1007/s10965-022-02899-6]

46 **Xia S**, Weng T, Jin R, Yang M, Yu M, Zhang W, Wang X, Han C. Curcumin-incorporated 3D bioprinting gelatin methacryloyl hydrogel reduces reactive oxygen species-induced adipose-derived stem cell apoptosis and improves implanting survival in diabetic wounds. *Burns Trauma* 2022; **10**: tkac001 [PMID: 35291229 DOI: 10.1093/burnst/tkac001]

47 **Tian C**, Zhang X, Zhao G. Vitrification of stem cell-laden core-shell microfibers with unusually low concentrations of cryoprotective agents. *Biomater Sci* 2019; **7**: 889-900 [PMID: 30608077 DOI: 10.1039/c8bm01231h]

48 **Zhang X**, Tian C, Chen Z, Zhao G. Hydrogel-based multifunctional dressing combining magnetothermally responsive drug delivery and stem cell therapy for enhanced wound healing. *Adv Therap* 2020; **3**: 2000001 [DOI: 10.1002/adtp.202000001]

49 **Sivaraj D**, Chen K, Chattopadhyay A, Henn D, Wu W, Noishiki C, Magbual NJ, Mittal S, Mermin-Bunnell AM, Bonham CA, Trotsyuk AA, Barrera JA, Padmanabhan J, Januszyk M, Gurtner GC. Hydrogel Scaffolds to Deliver Cell Therapies for Wound Healing. *Front Bioeng Biotechnol* 2021; **9**: 660145 [PMID: 34012956 DOI: 10.3389/fbioe.2021.660145]

50 **Ravari H**, Hamidi-Almadari D, Salimifar M, Bonakdaran S, Parizadeh MR, Koliakos G. Treatment of non-healing wounds with autologous bone marrow cells, platelets, fibrin glue and collagen matrix. *Cytotherapy* 2011; **13**: 705-711 [PMID: 21284564 DOI: 10.3109/14653249.2011.553594]

51 **Zeng X**, Tang Y, Hu K, Jiao W, Ying L, Zhu L, Liu J, Xu J. Three-week topical treatment with placenta-derived mesenchymal stem cells hydrogel in a patient with diabetic foot ulcer: A case report. *Medicine (Baltimore)* 2017; **96**: e9212 [PMID: 29390468 DOI: 10.1097/MD.0000000000009212]

52 **Yao M**, Zhang J, Gao F, Chen Y, Ma S, Zhang K, Liu H, Guan F. New BMSC-Laden Gelatin Hydrogel Formed in Situ by Dual-Enzymatic Cross-Linking Accelerates Dermal Wound Healing. *ACS Omega* 2019; **4**: 8334-8340 [PMID: 31459921 DOI: 10.1021/acsomega.9b00878]

53 **Dimatteo R**, Darling NJ, Segura T. In situ forming injectable hydrogels for drug delivery and wound repair. *Adv Drug Deliv Rev* 2018; **127**: 167-184 [PMID: 29567395 DOI: 10.1016/j.addr.2018.03.007]

54 **Miguel SP**, Ribeiro MP, Brancal H, Coutinho P, Correia IJ. Thermoresponsive chitosan-agarose hydrogel for skin regeneration. *Carbohydr Polym* 2014; **111**: 366-373 [PMID: 25037363 DOI: 10.1016/j.carbpol.2014.04.093]

55 **Eke G**, Mangir N, Hasirci N, MacNeil S, Hasirci V. Development of a UV crosslinked biodegradable hydrogel containing adipose derived stem cells to promote vascularization for skin wounds and tissue engineering. *Biomaterials* 2017; **129**: 188-198 [PMID: 28343005 DOI: 10.1016/j.biomaterials.2017.03.021]

56 **Dahle J**, Kvam E, Stokke T. Bystander effects in UV-induced genomic instability: antioxidants inhibit delayed mutagenesis induced by ultraviolet A and B radiation. *J Carcinog* 2005; **4**: 11 [PMID: 16091149 DOI: 10.1186/1477-3163-4-11]

57 **Ovsianikov A**, Malinauskas M, Schlie S, Chichkov B, Gittard S, Narayan R, Löbler M, Sternberg K, Schmitz KP, Haverich A. Three-dimensional laser micro- and nano-structuring of acrylated poly(ethylene glycol) materials and evaluation of their cytoxicity for tissue engineering applications. *Acta Biomater* 2011; **7**: 967-974 [PMID: 20977947 DOI: 10.1016/j.actbio.2010.10.023]

58 **Mude L**, Sanapalli BKR, V AN, Singh SK, Karri VVSR. Overview of in situ gelling injectable hydrogels for diabetic wounds. *Drug Dev Res* 2021; **82**: 503-522 [PMID: 33432634 DOI: 10.1002/ddr.21788]

59 **Teixeira LS**, Feijen J, van Blitterswijk CA, Dijkstra PJ, Karperien M. Enzyme-catalyzed crosslinkable hydrogels: emerging strategies for tissue engineering. *Biomaterials* 2012; **33**: 1281-1290 [PMID: 22118821 DOI: 10.1016/j.biomaterials.2011.10.067]

60 **Spang MT**, Christman KL. Extracellular matrix hydrogel therapies: In vivo applications and development. *Acta Biomater* 2018; **68**: 1-14 [PMID: 29274480 DOI: 10.1016/j.actbio.2017.12.019]

61 **Bo Q**, Yan L, Li H, Jia Z, Zhan A, Chen J, Yuan Z, Zhang W, Gao B, Chen R. Decellularized dermal matrix-based photo-crosslinking hydrogels as a platform for delivery of adipose derived stem cells to accelerate cutaneous wound healing. *Mater De* 2020; **196**: 109152 [DOI: 10.1016/j.matdes.2020.109152]

62 **Chen Z**, Zhang B, Shu J, Wang H, Han Y, Zeng Q, Chen Y, Xi J, Tao R, Pei X, Yue W. Human decellularized adipose matrix derived hydrogel assists mesenchymal stem cells delivery and accelerates chronic wound healing. *J Biomed Mater Res A* 2021; **109**: 1418-1428 [PMID: 33253453 DOI: 10.1002/jbm.a.37133]

63 **Xu H**, Wang J, Wu D, Qin D. A hybrid hydrogel encapsulating human umbilical cord mesenchymal stem cells enhances diabetic wound healing. *J Mater Sci Mater Med* 2022; **33**: 60 [PMID: 35849219 DOI: 10.1007/s10856-022-06681-4]

64 **Tsai CC**, Hong YJ, Lee RJ, Cheng NC, Yu J. Enhancement of human adipose-derived stem cell spheroid differentiation in an in situ enzyme-crosslinked gelatin hydrogel. *J Mater Chem B* 2019; **7**: 1064-1075 [PMID: 32254774 DOI:

10.1039/c8tb02835d]
65 **Bartosh TJ**, Ylöstalo JH, Mohammadipoor A, Bazhanov N, Coble K, Claypool K, Lee RH, Choi H, Prockop DJ. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. *Proc Natl Acad Sci U S A* 2010; **107**: 13724-13729 [PMID: 20643923 DOI: 10.1073/pnas.1008117107]
66 **Wang X**, Jiang B, Sun H, Zheng D, Zhang Z, Yan L, Li E, Wu Y, Xu RH. Noninvasive application of mesenchymal stem cell spheres derived from hESC accelerates wound healing in a CXCL12-CXCR4 axis-dependent manner. *Theranostics* 2019; **9**: 6112-6128 [PMID: 31534540 DOI: 10.7150/thno.32982]
67 **Yang M**, He S, Su Z, Yang Z, Liang X, Wu Y. Thermosensitive Injectable Chitosan/Collagen/β-Glycerophosphate Composite Hydrogels for Enhancing Wound Healing by Encapsulating Mesenchymal Stem Cell Spheroids. *ACS Omega* 2020; **5**: 21015-21023 [PMID: 32875238 DOI: 10.1021/acsomega.0c02580]
68 **Liu J**, Chen Z, Wang J, Li R, Li T, Chang M, Yan F, Wang Y. Encapsulation of Curcumin Nanoparticles with MMP9-Responsive and Thermos-Sensitive Hydrogel Improves Diabetic Wound Healing. *ACS Appl Mater Interfaces* 2018; **10**: 16315-16326 [PMID: 29687718 DOI: 10.1021/acsami.8b03868]
69 **Shao Z**, Yin T, Jiang J, He Y, Xiang T, Zhou S. Wound microenvironment self-adaptive hydrogel with efficient angiogenesis for promoting diabetic wound healing. *Bioact Mater* 2023; **20**: 561-573 [PMID: 35846841 DOI: 10.1016/j.bioactmat.2022.06.018]
70 **Lei L**, Zhu Y, Qin X, Chai S, Liu G, Su W, Lv Q, Li D. Magnetic biohybrid microspheres for protein purification and chronic wound healing in diabetic mice. Chem Eng J 2021; 425: 130671 [DOI: 10.1016/j.cej.2021.130671]
71 **Shi M**, Zhang H, Song T, Liu X, Gao Y, Zhou J, Li Y. Sustainable Dual Release of Antibiotic and Growth Factor from pH-Responsive Uniform Alginate Composite Microparticles to Enhance Wound Healing. *ACS Appl Mater Interfaces* 2019; **11**: 22730-22744 [PMID: 31141337 DOI: 10.1021/acsami.9b04750]
72 **Mao AS**, Özkale B, Shah NJ, Vining KH, Descombes T, Zhang L, Tringides CM, Wong SW, Shin JW, Scadden DT, Weitz DA, Mooney DJ. Programmable microencapsulation for enhanced mesenchymal stem cell persistence and immunomodulation. *Proc Natl Acad Sci U S A* 2019; **116**: 15392-15397 [PMID: 31311862 DOI: 10.1073/pnas.1819415116]
73 **Cui X**, Alcala-Orozco CR, Baer K, Li J, Murphy CA, Durham M, Lindberg G, Hooper GJ, Lim KS, Woodfield TBF. 3D bioassembly of cell-instructive chondrogenic and osteogenic hydrogel microspheres containing allogeneic stem cells for hybrid biofabrication of osteochondral constructs. *Biofabrication* 2022; **14** [PMID: 35344942 DOI: 10.1088/1758-5090/ac61a3]
74 **Sulaiman S**, Chowdhury SR, Fauzi MB, Rani RA, Yahaya NHM, Tabata Y, Hiraoka Y, Binti Haji Idrus R, Min Hwei N. 3D Culture of MSCs on a Gelatin Microsphere in a Dynamic Culture System Enhances Chondrogenesis. *Int J Mol Sci* 2020; **21** [PMID: 32294921 DOI: 10.3390/ijms21082688]
75 **Yang H**, Wang S, Bian H, Xing X, Yu J, Wu X, Zhang L, Liang X, Lu A, Huang C. Extracellular matrix-mimicking nanofibrous chitosan microspheres as cell micro-ark for tissue engineering. *Carbohydr Polym* 2022; **292**: 119693 [PMID: 35725181 DOI: 10.1016/j.carbpol.2022.119693]
76 **Ghuman H**, Matta R, Tompkins A, Nitzsche F, Badylak SF, Gonzalez AL, Modo M. ECM hydrogel improves the delivery of PEG microsphere-encapsulated neural stem cells and endothelial cells into tissue cavities caused by stroke. *Brain Res Bull* 2021; **168**: 120-137 [PMID: 33373665 DOI: 10.1016/j.brainresbull.2020.12.004]
77 **Shi M**, Gao Y, Lee L, Song T, Zhou J, Yan L, Li Y. Adaptive Gelatin Microspheres Enhanced Stem Cell Delivery and Integration With Diabetic Wounds to Activate Skin Tissue Regeneration. *Front Bioeng Biotechnol* 2022; **10**: 813805 [PMID: 35433645 DOI: 10.3389/fbioe.2022.813805]
78 **Zhao Z**, Wang Z, Li G, Cai Z, Wu J, Wang L, Deng L, Cai M, Cui W. Injectable microfluidic hydrogel microspheres for cell and drug delivery. Adv Funct Mater 2021; 31: 2103339 [DOI: 10.1002/adfm.202103339]
79 **Aijaz A**, Teryek M, Goedken M, Polunas M, Olabisi RM. Coencapsulation of ISCs and MSCs Enhances Viability and Function of both Cell Types for Improved Wound Healing. *Cell Mol Bioeng* 2019; **12**: 481-493 [PMID: 31719928 DOI: 10.1007/s12195-019-00582-3]
80 **Dong Y**, Rodrigues M, Kwon SH, Li X, A S, Brett EA, Elvassore N, Wang W, Gurtner GC. Acceleration of Diabetic Wound Regeneration using an In Situ-Formed Stem-Cell-Based Skin Substitute. *Adv Healthc Mater* 2018; **7**: e1800432 [PMID: 30004192 DOI: 10.1002/adhm.201800432]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 962-971

DOI: 10.4239/wjd.v13.i11.962

ISSN 1948-9358 (online)

MINIREVIEWS

Role of defensins in diabetic wound healing

Zhi-Xiang Tan, Rui Tao, Si-Cheng Li, Bing-Zheng Shen, Lan-Xia Meng, Zhan-Yong Zhu

Specialty type: Engineering, biomedical

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

P-Reviewer: Dziegielewska-Gesiak S, Poland; Salim A, Pakistan

Received: September 14, 2022
Peer-review started: September 14, 2022
First decision: October 5, 2022
Revised: October 22, 2022
Accepted: October 31, 2022
Article in press: October 31, 2022
Published online: November 15, 2022

Zhi-Xiang Tan, Rui Tao, Si-Cheng Li, Zhan-Yong Zhu, Department of Plastic Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Bing-Zheng Shen, Department of Pharmacy, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Lan-Xia Meng, Department of Neurology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Corresponding author: Zhan-Yong Zhu, MD, PhD, Associate Chief Physician, Doctor, Surgeon, Department of Plastic Surgery, Renmin Hospital of Wuhan University, No. 238 Jiefang Road, Wuhan 430060, Hubei Province, China. zyzhu@whu.edu.cn

Abstract

The adverse consequences resulting from diabetes are often presented as severe complications. Diabetic wounds are one of the most commonly occurring complications in diabetes, and the control and treatment of this is costly. Due to a series of pathophysiological mechanisms, diabetic wounds remain in the inflammatory phase for a prolonged period of time, and face difficulty in entering the proliferative phase, thus leading to chronic non-healing wounds. The current consensus on the treatment of diabetic wounds is through multidisciplinary comprehensive management, however, standard wound treatment methods are still limited and therefore, more effective methods are required. In recent years, defensins have been found to play diverse roles in a variety of diseases; however, the molecular mechanisms underlying these activities are still largely unknown. Defensins can be constitutively or inductively produced in the skin, therefore, their local distribution is affected by the microenvironment of these diabetic wounds. Current evidence suggests that defensins are involved in the diabetic wound pathogenesis, and can potentially promote the early completion of each stage, thus making research on defensins a promising area for developing novel treatments for diabetic wounds. In this review, we describe the complex function of human defensins in the development of diabetic wounds, and suggest potential therapeutic benefits.

Key Words: Defensin; Diabetic wound; Wound healing; Inflammation; Re-epithelialization; Tissue regeneration

Core Tip: Although previous studies have suggested that defensins have a function in the promotion of wound healing, their mechanism is still unclear. In this review, we discuss the potential role of various defensins in refractory diabetic wounds and their properties, including immunoregulation, promotion of re-epithelialization, collagen deposition, vascular regeneration, and neurological recovery, as well as antimicrobial activity.

Citation: Tan ZX, Tao R, Li SC, Shen BZ, Meng LX, Zhu ZY. Role of defensins in diabetic wound healing. *World J Diabetes* 2022; 13(11): 962-971
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/962.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.962

INTRODUCTION

Diabetes was estimated to affect over 536.6 million people worldwide in 2021, and an increase in prevalence occurs at a faster rate among middle-income countries[1]. Diabetes mellitus gradually causes a series of complications, such as neuropathy, retinopathy, nephropathy, cardiovascular diseases, and diabetic wounds, as it develops. Due to lesions in the small blood vessels and peripheral nerves that are particularly prominent in the feet, diabetic wounds are usually presented as diabetic foot ulcers (DFUs), and are characterized by a delayed tissue growth and increased susceptibility to infection. Patients with DFUs have an increased risk of lower limb amputation, which has poor short-term prognosis associated with high mortality[2]. Statistically, the five-year mortality and direct costs of care for patients with DFUs have been comparable to that of cancer[3]. The large patient population and medical expenditure urgently requires an effective treatment method.

The mechanisms for diabetic wound development involve multifactorial etiologies, including a hyperglycemic microenvironment, abnormal host immune resistance, and neuropathy (Figure 1)[4]. These mechanisms influence each other, instead of occurring independently, causing irreversible diabetic complications. The local damage to vessels and nerves, reduced growth factors expression, and lower collagen accumulation contribute to repeated outbreaks and the protracted course of diabetic wounds, leading to further infections. Although current treatments, including glycemic control, anti-infective treatment, and advanced dressing application, promote wound healing by regulating the local microenvironment, they also have disadvantages, such as protracted treatment periods, high costs, and occasionally inefficient results[5]. Hence, studying methods that promote diabetic wound healing is ongoing.

With concern about antibiotic resistance growing more prominent, antimicrobial peptides (AMPs) have garnered attention as a new method of antibacterial therapy, including development of different formulation strategies for effective delivery to wounds, including AMPs loaded in nanoparticles, hydrogels, creams, gels, *etc*. As a representative AMP, the defensins properties are gradually being researched (Table 1)[6-23]. Human defensins are divided into α-defensins and β-defensins[23]. Human α-defensins mainly occur in neutrophils (human neutrophil peptide1-4, HNP1-4) or small intestinal Paneth cells (human defensin5-6, HD5-6). More extensively, 31 human β-defensins (HBDs) have been described, and HBD1-4 is most widely-studied[24]. Reportedly, the direct primary role of defensins is controlling microbial infections by killing bacteria and modulating the immune system. Moreover, defensins play different roles in different environments within the body, such as infected wounds, malignancy, atherosclerosis, pulmonary fibrosis, *etc*. In this review, we focused on investigating their mechanism of action in wound healing, especially chronic diabetic wounds.

Although little is known about defensins involvement in diabetic wounds, existing studies indicate that they play potential roles in complex pathophysiological changes of diabetic wounds[25,26]. This review summarizes and analyzes known experimental data about the role of defensins in diabetic wound healing, particularly for inflammation, cell proliferation and migration, regeneration of blood vessels and nerves, and antibacterial activities. Research articles on the role of defensins in diabetic wounds, published between inception and September 10, 2022, were collected from various search engines, such as PubMed, Google Scholar, Web of Science, and Science Direct using the following keywords: AMPs, defensins, host defense peptides, diabetic, refractory, and chronic wounds, wound healing, *etc*. Identified studies and relevant citations within these studies were reviewed.

MULTIFACTORIAL MECHANISM OF DEFENSINS DURING WOUND HEALING

The response to tissue injury involves multiple cellular and extracellular events, including inflammation, re-epithelialization, and angiogenesis, followed by fibroplasia with collagen synthesis, and tissue remodeling. Defensins may be a multifactorial modulator in the management of this process,

Table 1 Defensins play multiple roles in different diseases

Defensin		Main cellular source	Action	
α-defensin	HNP1	Neutrophils; monocytes; macrophages; natural killer cells	Increase the healing rate of MRSA-infected wounds[6]; promote hemostasis[7]; r/affect the cardiovascular system[8]; inhibit thrombus formation[9]	Anti-infection and immunoregulation [23]
	HNP2-3		Anti-tumor activity[10]	
	HNP4	Neutrophils	Characterize benign and malignant salivary gland tumors[11]	
	HD5-6	Intestinal Paneth cells	Reverse dyslipidemia and improve glucoregulatory capacity [12]; anti-tumor ability[13]; amyloid inhibitor[14]	
β-defensin	HBD1	Epithelial cells; monocytes; macrophages	Anti-tumor activity[15]; potentiate osteoclastogenesis[16]	Induce the secretion of angiogenin[54]; anti-infection and immunoregulation [23]
	HBD2		Accelerate wound healing[17]; Oncolytic activity[18]; reduce alcoholic liver injury[19]	
	HBD3		Accelerate wound healing[20]; induce IL-8 release and apoptosis in airway smooth muscle cells[21]	
	HBD4	Epithelial cells	Stimulate/suppress cancer cell proliferation and viability[22]	

HNP: Human neutrophil peptide; HBD: Human β-defensins.

which interferes in diabetic wounds (Table 2, Figure 2).

Defensins triggered by inflammation

The first phase of wound healing is the inflammatory phase, characterized by platelet aggregation and leukocytes migration, including neutrophils and macrophages that secrete defensins and consequently clear the wound area[27]. Studies suggest defensins promote recruitment and accumulation of leukocytes at inflammatory sites, and simultaneously release a series of chemokines[28,29]. In diabetic wounds, the number of neutrophils increases abnormally and macrophage polarization is suppressed, leading to an excessive inflammatory expression[30,31]. As defensins are released in response to inflammation from neutrophils and macrophages, which act as a signal to instigate recruitment of immune cells, a positive-feedback loop is created. HBDs can reportedly participate in degranulation of mast cells and induce secretion of proinflammatory factors by keratinocytes *via* the p38 and ERK1/2 MAPK pathways activation[32,33]. Through the same action sites, HNPs produce vasoactive by-products in endothelial cells *via* ROS-dependent mechanisms, and stimulate the increased expression of IL-6 and IL-8 by activating p42/44 MAPK pathways[34,35].

In contrast, studies investigating associations between defensins and inflammatory mediators exhibited controversial results. HBDs have demonstrated an immunosuppressive effect by down-regulating the TIR, TRAF-6, and NF-κB of TLR signaling pathways[36]. Moreover, HBDs contribute to their anti-inflammatory ability by inducing M2 macrophage differentiation[37]. Previous experiments established that HBDs can be beneficial in inflammatory diseases, such as periodontitis, considering its anti-inflammatory properties[38]. A study on HNPs from dying neutrophils exhibited an immunosuppressive effect of the α-defensins that inhibited macrophage stimulation[39]. The HNP1 "bipolar effect" represents the reduction of inflammatory responses with a physiological dose, enhanced expression of inflammatory factors with a high dose, and significant reductions in cell viability and interleukin-10 expression with increased concentration levels[40]. Overall, defensins perform different functions under different conditions, including concentration levels[40]. Defensins are often used as disease-related markers as dysregulation of their levels is caused by immune system disorders and effectors produced themselves or through associated cells[41]. However, the causal relationship and sequence of cascades remain unclear. Several studies emphasized the relationship between delayed wound healing and uncontrolled inflammatory responses, and defensins as efficient adjustors playing a regulatory role in the process.

Defensins promote skin reconstruction

Failure to re-epithelialize is one of the most significant indicators for chronic wounds. Re-epithelialization is achieved through keratinocyte migration, proliferation, and differentiation. The HNP1, HBD2, HBD3, and HBD4 can reportedly induce proliferation and migration of keratinocytes, which can consequently secrete HBDs, thereby promoting reconstruction of the cellular barrier to accelerate wound healing[42,43]. Subsequent studies reported that HBD3 enhances phosphorylation of the FGFR1/JAK2/STAT3 pathways to promote keratinocyte proliferation and migration[20]. HBD1 potentially acts as a relevant transcription factor by protecting keratinocytes from apoptosis during epithelial reorganization[44]. In other words, defensins have properties that promote wound epithelial-

Table 2 Defensins play a potential role in wound healing

Stage	Defensin	Activation
Inflammation	HNP1-2, HBD1-3[28]	Recruitment of leukocytes
	HNP1-4[29]	Secretion of inflammatory cytokines like IL-8
	HBD2-4[32]	Activation of the p38 and ERK1/2 MAPK pathways
	HNP1, HBD2[35]	Activation of the p42/44 MAPK pathways
	HBD2, HBD3[36]	Down-regulate the TIR, TRAF-6, NF-κB of TLR signaling pathways
	HBD3[37]	Induce M2 macrophage differentiation
Re-epithelialization	HNP1[43], HBD2-3[42]	Induce keratinocyte migration and proliferation
	HBD1[44]	Protect keratinocytes from apoptosis
Collagen synthesis	HNP1[45]	Enhance extracellular matrix deposition
	HBD3[20,37]	Increase the expression of MMP-2, and down-regulate the expression of MMP-9
Fibroplasia	HNP1[45], HBD2[46]	Promote the proliferation and activation of fibroblasts
Angiogenesis	HNP1[51], HBD2[52], HBD3[53]	Induce VEGF
	HBD1-4[54]	Induce angiogenin
	HNPs[55]	Inhibit adhesion and migration of endothelial cell
Nerve reconstruction	HNP1[40]	Promote the recovery of neurological function
	HBD3[57]	Modulate the expression of nerve elongation factors
Antimicrobial activity	HNP1-4, HBD1-4[61]	Exhibit a broad range of antimicrobial properties
	HBD2[60]	Reduce biofilm formation
	HNP1-3[62]	Neutralize bacterial toxins
	HNP1, HBD1, HBD3[63]	Show synergy of action with antibiotics

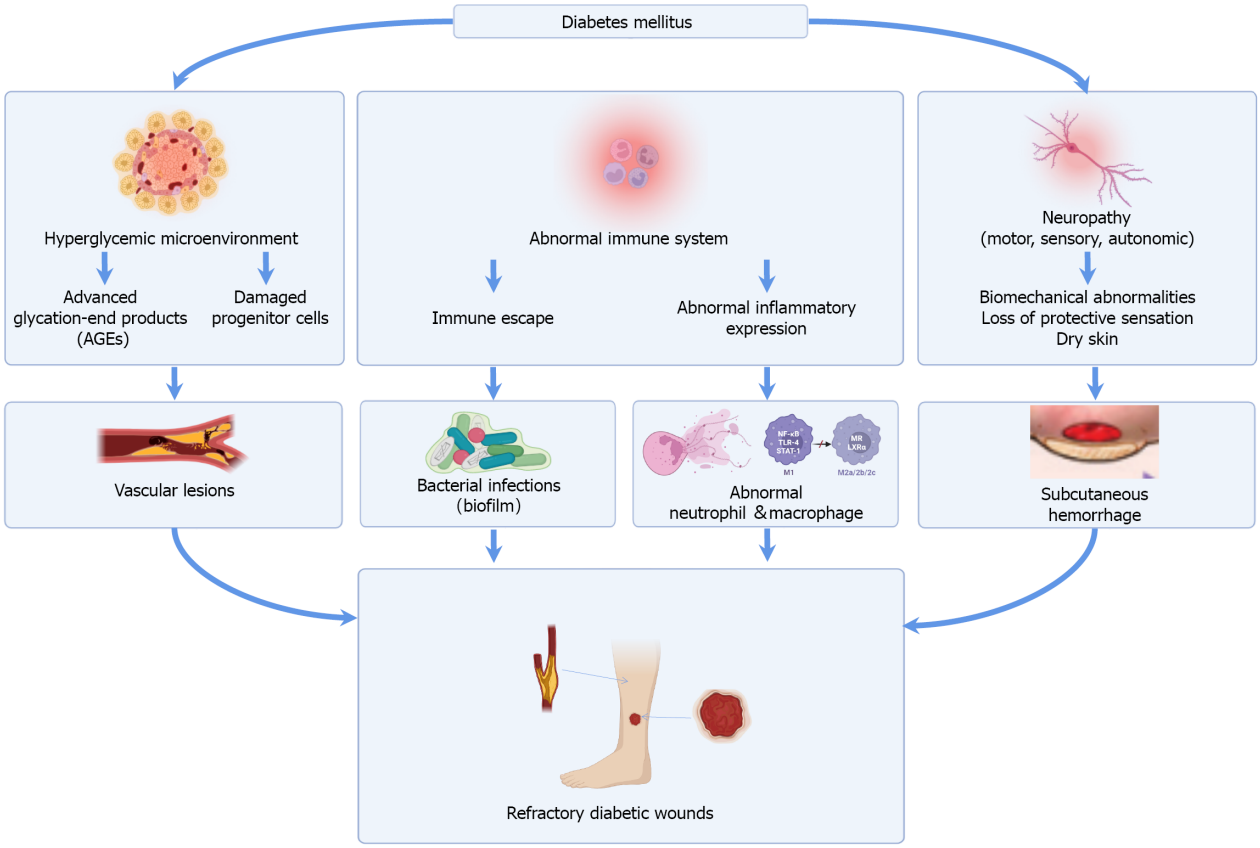
HNP: Human neutrophil peptide; HBD: Human β-defensins; MMP: Matrix metalloproteinase.

ization by affecting keratinocyte activity, and thus facilitating early wound closure.

Furthermore, defensins seemingly play an important role in fibroblasts and collagen matrix accumulation, which is essential for dermal reconstitution. HNP1 can reportedly promote proliferation and activation of fibroblasts more effectively than HBD2 at the same concentration, and the increased collagen gene expression can only be observed by its stimulation[45]. A study also proved that HBDs indirectly stimulate fibroblast migration by activating protein kinase C[46]. High levels of pro-inflammatory cytokines and inflammatory chemokines in diabetic wounds lead to an increased production of matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9, thereby inhibiting extracellular matrix formation and dermis reconstruction[47,48]. Studies have suggested that the use of an inhibitor for MMP-2 and MMP-9 accelerates wound healing in diabetic mice by maintaining the balance between systematic inflammation and cytokine biosynthesis[49]. HBD3 may potentially reverse the pathological condition as they have shown an inhibitory effect on MMP-9, which may result from cytotoxicity for dendritic cells in high concentrations[50]. Instead, HBD3 reportedly increases the expression of MMP-2, which is essential for angiogenesis and prolonged matrix remodeling[20]. To explain these contradictory findings, further clarification and a comprehensive analysis on the mechanism of wound healing is necessary, as well as verification through specific experiments.

Defensins involved in regeneration of blood vessels and nerves

Angiogenesis is a vital physiological process in wound healing and largely regulated by growth factors, specifically vascular endothelial growth factor (VEGF) and angiogenin. HNP1, HBD2, and HBD3 was proven to bind to cell surface receptor proteins, thus promoting VEGF expression and improvement of vascularization[51-53]. The novel role of HBDs in angiogenesis was also identified, revealing that HBD1-4 increases secretion of angiogenin dose-dependently[54]. However, the opposing actions can be described as a consequence of on-site recruitment of distinct subpopulations from circulation. HNPs can reportedly inhibit adhesion and migration of endothelial cells, and block VEGF-induced endothelial cell proliferation and capillary formation upon inflammatory stimulation[55]. These studies have shed a light on the mechanistic complexity of HNPs angiogenesis.



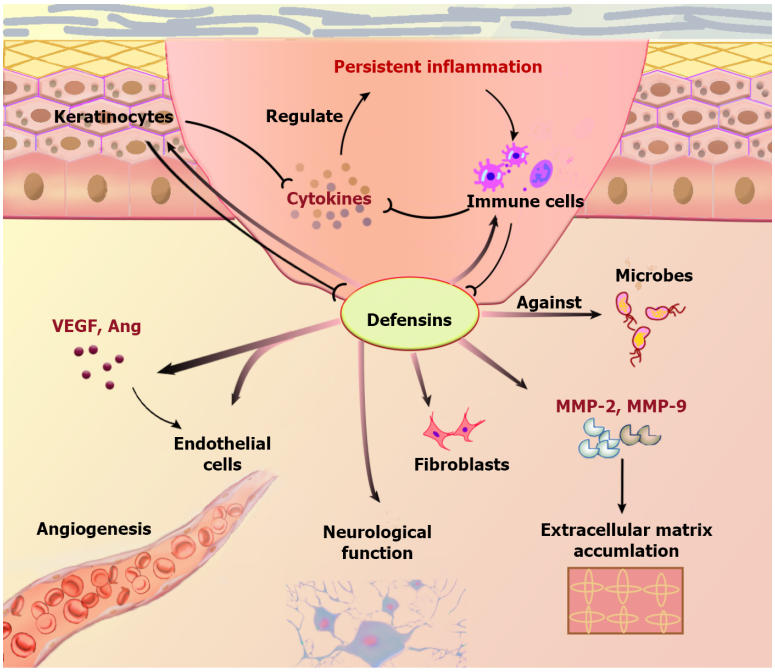
DOI: 10.4239/wjd.v13.i11.962

Figure 1 Mechanism of refractory diabetic wounds. The mainstream views include: Hyperglycemic microenvironment, abnormal immune system and neuropathy. Hyperglycemic microenvironment results in the complex formation of advanced glycation end products (AGEs) and cytokines, as well as circulating progenitor cell dysfunction. AGEs can significantly inhibit the proliferation of endothelial cells and alter the structure of collagen and elastin in the vascular wall, causing microvascular injury in the wound. The hallmarks of abnormal immune system are polymorphonuclear cell dysfunction, late neutrophil infiltration and suppressed macrophage polarization. As a result, diabetic wound healing is delayed and susceptible to bacterial infections and even biofilm formation. Neuropathy is occasion of subcutaneous hemorrhage, ultimately leads to skin breakdown.

Neuropathy caused by diabetes is the influencing factor for subcutaneous hemorrhage underneath the callus formation, ultimately leading to skin breakdown[56]. Studies have proven that HNP1 administration can promote recovery of neurological function following sciatic nerve injury[40]. Additionally, HBD3 modulates the expression of nerve elongation factors that are involved in epidermal hyperinnervation and hypersensitivity to warm sensations[57]. As a result, application of defensins can help prevent delayed treatment due to peripheral neuropathy and difficulty in mastering wound conditions in patients with diabetic wounds.

Defensins exhibit antimicrobial activity

Healing of refractory diabetic wounds is often associated with susceptibility to bacterial infections and formation of biofilms[58]. As a class of small cationic molecule peptides with broad-spectrum antimicrobial activity, defensins are produced to eliminate invading pathogens during the initial stages of wound formation[59]. While the important role of the pore-formation mechanism has been recognized in many studies, other mechanisms, such as disruption of cell wall synthesis, metabolic activity, ATP and nucleic acid synthesis, and amino acid uptake, have also been proposed in recent years[60]. HNPs and HBDs both exhibit a strong tendency to eliminate various pathogens, including *Staphylococcus aureus* and *Escherichia coli*, which often invades chronic wounds[61]. Specifically, HBD2 exhibits biofilm inhibitory activity by inducing structural changes that interfere with the biofilm precursor's transport into the extracellular space[60]. Additionally, HNPs were proven to protect leukocytes from neutralization by gram-positive pathogenic bacterial toxins[62]. Furthermore, they can potentially avoid the emergence of resistance when implemented with other antimicrobial therapies[63]. Defensins are not only more effective against drug-resistant bacteria, as compared to antibiotics, but can also preserve the resident bacteria, despite the lack of target specificity as an intractable problem preventing their use as a therapeutic drug[64].



DOI: 10.4239/wjd.v13.i11.962

Figure 2 Role of defensins in diabetic wound healing. VEGF: Vascular endothelial growth factor; Ang: Angiogenin.

CONCLUSION

Although there is ambiguity regarding its exact role, refractory healing of diabetic wounds is speculated result from interactions between multiple pathophysiological changes in the microenvironment of hyperglycemic and persistent inflammation. This affects immune cell function and composition of defensins at the wound site. In human skin, HBD1 is constitutively expressed in epithelial cells, while inducible HNP1-4 by neutrophils and HBD2-3 by keratinocytes mainly[65]. It was obtained through a biopsy that HBD2-4 were overexpressed in the border area of DFUs[25]. Studies generally agree that inadequate HBD expression is associated with poor wound healing, and many methods that promote diabetic wound healing are seemingly carried out by promoting defensins expression[66,67]. In diabetic wounds, higher HNP1, HNP3, and HNP4 expressions are more common in the central part than in the marginal areas, thus causing a significant increase in IL-8 expression under the influence of advanced glycation end products (AGEs)[29].

Defensins affect the expression and secretion of cytokines, cell proliferation, migration, and apoptosis, and are also involved in all stages of wound healing. Contrary to its proven activity in fighting pathogens and promoting tissue reconstruction, the role of defensins in inflammation and vascularization remains unclear. This discrepancy could be due to pro-inflammatory and anti-inflammatory properties being attributed to HBDs at lower concentrations[28,29], compared to antibacterial effects at higher concentrations exhibited in different experiments[48]. Thus, effects of defensins may vary depending on concentration. Furthermore, HNP1 and HBD3 exhibit increased cytotoxic effects with the increased concentration, which can also be related to a greater hydrophobicity[43,68]. Therefore, changing the local distribution or structure of defensins can have beneficial effects and prevent toxic side effects.

Studying every type of defensins within a single experiment is difficult. Additionally, certain defensins can exhibit different or contradictory effects within the same environment due to differences in experimental complexity and aims of the experiment. These factors create a huge obstacle in horizontal comparison among similar experiments. Cytotoxicity caused by defensins is difficult to assess, which indicates that topical application may be more appropriate than the systemic administration. Considering the unstable biochemical properties of defensins, topical application alone may be insufficient. To overcome this limitation, researchers are studying biological dressings as an alternative; however, formulation of an ideal material has not yet been achieved. However, animal studies on defensins exhibit improved healing outcomes, and display stable effects through application of new materials or genetic engineering methods[17,20,69]. These findings present defensins as a promising therapeutic approach owing to modern techniques, such as development of new materials to efficiently load active factors and novel protein sequences to highlight their beneficial effects.

Defensins regulates chronic inflammation, tissue regeneration, angiogenesis, and nerve recovery, as well as antimicrobial properties; therefore, they are a promising treatment for diabetic wounds. There is an urgent need to find the appropriate dosing regimens and develop new biological dressing altern-

atives to incorporate active factors. Hence, further preclinical investigations are necessary to understand extensive molecular mechanisms of defensins in the treatment of diabetic wounds, and consequently determine suitable therapeutic strategies.

FOOTNOTES

Author contributions: Tan ZX and Tao R wrote the manuscript and proposed research subtopics; Shen BZ was responsible for navigating the literature, sharing the relevant studies, and drawing the tables included in this review; Meng LX and Li SC drew the figures in the manuscript, formatted citations and compiled references, verified spelling, punctuation, and grammatical errors; Zhu ZY revised and formatted the body of the manuscript, and coordinated the whole work.

Supported by the Scientific Research Project of Hubei Health Committee, No. WJ2021F106.

Conflict-of-interest statement: Authors declare no conflicts of interests for this article.

Country/Territory of origin: China

ORCID number: Zhi-Xiang Tan 0000-0002-1614-133X; Rui Tao 0000-0001-8782-4421; Si-Cheng Li 0000-0003-4354-0288; Bing-Zheng Shen 0000-0003-1205-0139; Lan-Xia Meng 0000-0001-5307-671X; Zhan-Yong Zhu 0000-0003-4323-4862.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

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

2 **Rathnayake A**, Saboo A, Malabu UH, Falhammar H. Lower extremity amputations and long-term outcomes in diabetic foot ulcers: A systematic review. *World J Diabetes* 2020; **11**: 391-399 [PMID: 32994867 DOI: 10.4239/wjd.v11.i9.391]

3 **Armstrong DG**, Swerdlow MA, Armstrong AA, Conte MS, Padula WV, Bus SA. Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer. *J Foot Ankle Res* 2020; **13**: 16 [PMID: 32209136 DOI: 10.1186/s13047-020-00383-2]

4 **Pouget C**, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance. *Microorganisms* 2020; **8** [PMID: 33066595 DOI: 10.3390/microorganisms8101580]

5 **Holl J**, Kowalewski C, Zimek Z, Fiedor P, Kaminski A, Oldak T, Moniuszko M, Eljaszewicz A. Chronic Diabetic Wounds and Their Treatment with Skin Substitutes. *Cells* 2021; **10** [PMID: 33804192 DOI: 10.3390/cells10030655]

6 **Bolatchiev A**, Baturin V, Bazikov I, Maltsev A, Kunitsina E. Effect of antimicrobial peptides HNP-1 and hBD-1 on Staphylococcus aureus strains in vitro and in vivo. *Fundam Clin Pharmacol* 2020; **34**: 102-108 [PMID: 31313350 DOI: 10.1111/fcp.12499]

7 **Kim KC**, Lee W, Lee J, Cha HJ, Hwang BH. Newly Identified HNP-F from Human Neutrophil Peptide-1 Promotes Hemostasis. *Biotechnol J* 2019; **14**: e1800606 [PMID: 30927490 DOI: 10.1002/biot.201800606]

8 **Higazi M**, Abdeen S, Abu-Fanne R, Heyman SN, Masarwy A, Bdeir K, Maraga E, Cines DB, Higazi AA. Opposing effects of HNP1 (α-defensin-1) on plasma cholesterol and atherogenesis. *PLoS One* 2020; **15**: e0231582 [PMID: 32302327 DOI: 10.1371/journal.pone.0231582]

9 **McDaniel JK**, Abdelgawwad MS, Hargett A, Renfrow MB, Bdeir K, Cao W, Cines DB, Zheng XL. Human neutrophil peptide-1 inhibits thrombus formation under arterial flow *via* its terminal free cysteine thiols. *J Thromb Haemost* 2019; **17**: 596-606 [PMID: 30741476 DOI: 10.1111/jth.14407]

10 **Ferdowsi S**, Pourfathollah AA, Amiri F, Rafiee MH, Aghaei A. Evaluation of anticancer activity of α-defensins purified from neutrophils trapped in leukoreduction filters. *Life Sci* 2019; **224**: 249-254 [PMID: 30935951 DOI: 10.1016/j.lfs.2019.03.072]

11 **Winter J**, Pantelis A, Kraus D, Reckenbeil J, Reich R, Jepsen S, Fischer HP, Allam JP, Novak N, Wenghoefer M. Human α-defensin (DEFA) gene expression helps to characterise benign and malignant salivary gland tumours. *BMC Cancer* 2012; **12**: 465 [PMID: 23050799 DOI: 10.1186/1471-2407-12-465]

12 **Larsen IS**, Fritzen AM, Carl CS, Agerholm M, Damgaard MTF, Holm JB, Marette A, Nordkild P, Kiens B, Kristiansen K, Wehkamp J, Jensen BAH. Human Paneth cell α-defensin-5 treatment reverses dyslipidemia and improves glucoregulatory

capacity in diet-induced obese mice. *Am J Physiol Endocrinol Metab* 2019; **317**: E42-E52 [PMID: 30860877 DOI: 10.1152/ajpendo.00019.2019]

13 **Panjeta A**, Preet S. Anticancer potential of human intestinal defensin 5 against 1, 2-dimethylhydrazine dihydrochloride induced colon cancer: A therapeutic approach. *Peptides* 2020; **126**: 170263 [PMID: 31981594 DOI: 10.1016/j.peptides.2020.170263]

14 **Tang Y**, Zhang D, Gong X, Zheng J. Repurposing of intestinal defensins as multi-target, dual-function amyloid inhibitors *via* cross-seeding. *Chem Sci* 2022; **13**: 7143-7156 [PMID: 35799805 DOI: 10.1039/d2sc01447e]

15 **Sun CQ**, Arnold RS, Hsieh CL, Dorin JR, Lian F, Li Z, Petros JA. Discovery and mechanisms of host defense to oncogenesis: targeting the β-defensin-1 peptide as a natural tumor inhibitor. *Cancer Biol Ther* 2019; **20**: 774-786 [PMID: 30900935 DOI: 10.1080/15384047.2018.1564564]

16 **Makeudom A**, Supanchart C, Montreekachon P, Khongkhunthian S, Sastraruji T, Krisanaprakornkit J, Krisanaprakornkit S. The antimicrobial peptide, human β-defensin-1, potentiates in vitro osteoclastogenesis *via* activation of the p44/42 mitogen-activated protein kinases. *Peptides* 2017; **95**: 33-39 [PMID: 28709835 DOI: 10.1016/j.peptides.2017.07.004]

17 **Mi B**, Liu J, Liu Y, Hu L, Panayi AC, Zhou W, Liu G. The Designer Antimicrobial Peptide A-hBD-2 Facilitates Skin Wound Healing by Stimulating Keratinocyte Migration and Proliferation. *Cell Physiol Biochem* 2018; **51**: 647-663 [PMID: 30463067 DOI: 10.1159/000495320]

18 **Bindra GK**, Williams SA, Lay FT, Baxter AA, Poon IKH, Hulett MD, Phan TK. Human β-Defensin 2 (HBD-2) Displays Oncolytic Activity but Does Not Affect Tumour Cell Migration. *Biomolecules* 2022; **12** [PMID: 35204765 DOI: 10.3390/biom12020264]

19 **Warner JB**, Larsen IS, Hardesty JE, Song YL, Warner DR, McClain CJ, Sun R, Deng Z, Jensen BAH, Kirpich IA. Human Beta Defensin 2 Ameliorated Alcohol-Associated Liver Disease in Mice. *Front Physiol* 2021; **12**: 812882 [PMID: 35153819 DOI: 10.3389/fphys.2021.812882]

20 **Takahashi M**, Umehara Y, Yue H, Trujillo-Paez JV, Peng G, Nguyen HLT, Ikutama R, Okumura K, Ogawa H, Ikeda S, Niyonsaba F. The Antimicrobial Peptide Human β-Defensin-3 Accelerates Wound Healing by Promoting Angiogenesis, Cell Migration, and Proliferation Through the FGFR/JAK2/STAT3 Signaling Pathway. *Front Immunol* 2021; **12**: 712781 [PMID: 34594328 DOI: 10.3389/fimmu.2021.712781]

21 **Wang W**, Qu X, Dang X, Shang D, Yang L, Li Y, Xu D, Martin JG, Hamid Q, Liu J, Chang Y. Human β-defensin-3 induces IL-8 release and apoptosis in airway smooth muscle cells. *Clin Exp Allergy* 2017; **47**: 1138-1149 [PMID: 28437599 DOI: 10.1111/cea.12943]

22 **Gerashchenko OL**, Zhuravel EV, Skachkova OV, Khranovska NN, Filonenko VV, Pogrebnoy PV, Soldatkina MA. Biologic activities of recombinant human-beta-defensin-4 toward cultured human cancer cells. *Exp Oncol* 2013; **35**: 76-82 [PMID: 23828379]

23 **Fruitwala S**, El-Naccache DW, Chang TL. Multifaceted immune functions of human defensins and underlying mechanisms. *Semin Cell Dev Biol* 2019; **88**: 163-172 [PMID: 29501617 DOI: 10.1016/j.semcdb.2018.02.023]

24 **Park MS**, Kim JI, Lee I, Park S, Bae JY, Park MS. Towards the Application of Human Defensins as Antivirals. *Biomol Ther (Seoul)* 2018; **26**: 242-254 [PMID: 29310427 DOI: 10.4062/biomolther.2017.172]

25 **Rivas-Santiago B**, Trujillo V, Montoya A, Gonzalez-Curiel I, Castañeda-Delgado J, Cardenas A, Rincon K, Hernandez ML, Hernández-Pando R. Expression of antimicrobial peptides in diabetic foot ulcer. *J Dermatol Sci* 2012; **65**: 19-26 [PMID: 22047630 DOI: 10.1016/j.jdermsci.2011.09.013]

26 **Sanapalli BKR**, Yele V, Kalidhindi RSR, Singh SK, Gulati M, Karri VVSR. Human beta defensins may be a multifactorial modulator in the management of diabetic wound. *Wound Repair Regen* 2020; **28**: 416-421 [PMID: 31777130 DOI: 10.1111/wrr.12785]

27 **Petkovic M**, Mouritzen MV, Mojsoska B, Jenssen H. Immunomodulatory Properties of Host Defence Peptides in Skin Wound Healing. *Biomolecules* 2021; **11** [PMID: 34203393 DOI: 10.3390/biom11070952]

28 **Prasad SV**, Fiedoruk K, Daniluk T, Piktel E, Bucki R. Expression and Function of Host Defense Peptides at Inflammation Sites. *Int J Mol Sci* 2019; **21** [PMID: 31877866 DOI: 10.3390/ijms21010104]

29 **Meng L**, Guo X, Yang X, Liu H, Yu M, Wu Y, Zhu Z. Human α defensins promote the expression of the inflammatory cytokine interleukin-8 under high-glucose conditions: Novel insights into the poor healing of diabetic foot ulcers. *J Biochem Mol Toxicol* 2019; **33**: e22351 [PMID: 31158307 DOI: 10.1002/jbt.22351]

30 **Wong SL**, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, Kahn CR, Wagner DD. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med* 2015; **21**: 815-819 [PMID: 26076037 DOI: 10.1038/nm.3887]

31 **Louiselle AE**, Niemiec SM, Zgheib C, Liechty KW. Macrophage polarization and diabetic wound healing. *Transl Res* 2021; **236**: 109-116 [PMID: 34089902 DOI: 10.1016/j.trsl.2021.05.006]

32 **Niyonsaba F**, Ushio H, Nagaoka I, Okumura K, Ogawa H. The human beta-defensins (-1, -2, -3, -4) and cathelicidin LL-37 induce IL-18 secretion through p38 and ERK MAPK activation in primary human keratinocytes. *J Immunol* 2005; **175**: 1776-1784 [PMID: 16034119 DOI: 10.4049/jimmunol.175.3.1776]

33 **Chen X**, Niyonsaba F, Ushio H, Hara M, Yokoi H, Matsumoto K, Saito H, Nagaoka I, Ikeda S, Okumura K, Ogawa H. Antimicrobial peptides human beta-defensin (hBD)-3 and hBD-4 activate mast cells and increase skin vascular permeability. *Eur J Immunol* 2007; **37**: 434-444 [PMID: 17230440 DOI: 10.1002/eji.200636379]

34 **Syeda F**, Tullis E, Slutsky AS, Zhang H. Human neutrophil peptides upregulate expression of COX-2 and endothelin-1 by inducing oxidative stress. *Am J Physiol Heart Circ Physiol* 2008; **294**: H2769-H2774 [PMID: 18441204 DOI: 10.1152/ajpheart.00211.2008]

35 **Li J**, Zhu HY, Beuerman RW. Stimulation of specific cytokines in human conjunctival epithelial cells by defensins HNP1, HBD2, and HBD3. *Invest Ophthalmol Vis Sci* 2009; **50**: 644-653 [PMID: 18806299 DOI: 10.1167/iovs.08-1838]

36 **Semple F**, Dorin JR. β-Defensins: multifunctional modulators of infection, inflammation and more? *J Innate Immun* 2012; **4**: 337-348 [PMID: 22441423 DOI: 10.1159/000336619]

37 **Cui D**, Lyu J, Li H, Lei L, Bian T, Li L, Yan F. Human β-defensin 3 inhibits periodontitis development by suppressing inflammatory responses in macrophages. *Mol Immunol* 2017; **91**: 65-74 [PMID: 28886588 DOI: 10.1016/j.molimm.2017.08.012]

38 **Li L**, Jiang H, Chen R, Zhou J, Xiao Y, Zhang Y, Yan F. Human β-defensin 3 gene modification promotes the osteogenic differentiation of human periodontal ligament cells and bone repair in periodontitis. *Int J Oral Sci* 2020; **12**: 13 [PMID: 32350241 DOI: 10.1038/s41368-020-0078-6]

39 **Miles K**, Clarke DJ, Lu W, Sibinska Z, Beaumont PE, Davidson DJ, Barr TA, Campopiano DJ, Gray M. Dying and necrotic neutrophils are anti-inflammatory secondary to the release of alpha-defensins. *J Immunol* 2009; **183**: 2122-2132 [PMID: 19596979 DOI: 10.4049/jimmunol.0804187]

40 **Yuan YS**, Niu SP, Yu F, Zhang YJ, Han N, Lu H, Yin XF, Xu HL, Kou YH. Intraoperative single administration of neutrophil peptide 1 accelerates the early functional recovery of peripheral nerves after crush injury. *Neural Regen Res* 2020; **15**: 2108-2115 [PMID: 32394969 DOI: 10.4103/1673-5374.282270]

41 **Donnarumma G**, Paoletti I, Fusco A, Perfetto B, Buommino E, de Gregorio V, Baroni A. β-Defensins: Work in Progress. In: Advances in Microbiology, Infectious Diseases and Public Health: Volume 2. Donelli G (editor). Cham: Springer International Publishing; 2016. pp. 59-76.

42 **Niyonsaba F**, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, Nagaoka I, Okumura K, Ogawa H. Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol* 2007; **127**: 594-604 [PMID: 17068477 DOI: 10.1038/sj.jid.5700599]

43 **Gursoy UK**, Könönen E, Luukkonen N, Uitto VJ. Human neutrophil defensins and their effect on epithelial cells. *J Periodontol* 2013; **84**: 126-133 [PMID: 22443519 DOI: 10.1902/jop.2012.120017]

44 **Prado-Montes de Oca E**. Human beta-defensin 1: a restless warrior against allergies, infections and cancer. *Int J Biochem Cell Biol* 2010; **42**: 800-804 [PMID: 20100591 DOI: 10.1016/j.biocel.2010.01.021]

45 **Oono T**, Shirafuji Y, Huh WK, Akiyama H, Iwatsuki K. Effects of human neutrophil peptide-1 on the expression of interstitial collagenase and type I collagen in human dermal fibroblasts. *Arch Dermatol Res* 2002; **294**: 185-189 [PMID: 12111349 DOI: 10.1007/s00403-002-0310-6]

46 **van Kilsdonk JWJ**, Jansen PAM, van den Bogaard EH, Bos C, Bergers M, Zeeuwen PLJM, Schalkwijk J. The Effects of Human Beta-Defensins on Skin Cells in vitro. *Dermatology* 2017; **233**: 155-163 [PMID: 28689201 DOI: 10.1159/000477346]

47 **Rousselle P**, Braye F, Dayan G. Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Adv Drug Deliv Rev* 2019; **146**: 344-365 [PMID: 29981800 DOI: 10.1016/j.addr.2018.06.019]

48 **Dai J**, Shen J, Chai Y, Chen H. IL-1*β* Impaired Diabetic Wound Healing by Regulating MMP-2 and MMP-9 through the p38 Pathway. *Mediators Inflamm* 2021; **2021**: 6645766 [PMID: 34054346 DOI: 10.1155/2021/6645766]

49 **Gao M**, Nguyen TT, Suckow MA, Wolter WR, Gooyit M, Mobashery S, Chang M. Acceleration of diabetic wound healing using a novel protease-anti-protease combination therapy. *Proc Natl Acad Sci U S A* 2015; **112**: 15226-15231 [PMID: 26598687 DOI: 10.1073/pnas.1517847112]

50 **Raina M**, Bates AM, Fischer CL, Progulske-Fox A, Abbasi T, Vali S, Brogden KA. Human beta defensin 3 alters matrix metalloproteinase production in human dendritic cells exposed to Porphyromonas gingivalis hemagglutinin B. *J Periodontol* 2018; **89**: 361-369 [PMID: 29543996 DOI: 10.1002/JPER.17-0366]

51 **Baroni A**, Donnarumma G, Paoletti I, Longanesi-Cattani I, Bifulco K, Tufano MA, Carriero MV. Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. *Peptides* 2009; **30**: 267-272 [PMID: 19041917 DOI: 10.1016/j.peptides.2008.11.001]

52 **Petrov V**, Funderburg N, Weinberg A, Sieg S. Human β defensin-3 induces chemokines from monocytes and macrophages: diminished activity in cells from HIV-infected persons. *Immunology* 2013; **140**: 413-420 [PMID: 23829433 DOI: 10.1111/imm.12148]

53 **Amenomori M**, Mukae H, Ishimatsu Y, Sakamoto N, Kakugawa T, Hara A, Hara S, Fujita H, Ishimoto H, Hayashi T, Kohno S. Differential effects of human neutrophil peptide-1 on growth factor and interleukin-8 production by human lung fibroblasts and epithelial cells. *Exp Lung Res* 2010; **36**: 411-419 [PMID: 20715983 DOI: 10.3109/01902141003714049]

54 **Umehara Y**, Takahashi M, Yue H, Trujillo-Paez JV, Peng G, Nguyen HLT, Okumura K, Ogawa H, Niyonsaba F. The Antimicrobial Peptides Human β-Defensins Induce the Secretion of Angiogenin in Human Dermal Fibroblasts. *Int J Mol Sci* 2022; **23** [PMID: 35955934 DOI: 10.3390/ijms23158800]

55 **Seignez C**, Phillipson M. The multitasking neutrophils and their involvement in angiogenesis. *Curr Opin Hematol* 2017; **24**: 3-8 [PMID: 27755126 DOI: 10.1097/MOH.0000000000000300]

56 **Armstrong DG**, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. *N Engl J Med* 2017; **376**: 2367-2375 [PMID: 28614678 DOI: 10.1056/NEJMra1615439]

57 **Umehara Y**, Kamata Y, Tominaga M, Niyonsaba F, Ogawa H, Takamori K. Antimicrobial peptides human LL-37 and β-defensin-3 modulate the expression of nerve elongation factors in human epidermal keratinocytes. *J Dermatol Sci* 2017; **88**: 365-367 [PMID: 28843623 DOI: 10.1016/j.jdermsci.2017.07.010]

58 **Afonso AC**, Oliveira D, Saavedra MJ, Borges A, Simões M. Biofilms in Diabetic Foot Ulcers: Impact, Risk Factors and Control Strategies. *Int J Mol Sci* 2021; **22** [PMID: 34361044 DOI: 10.3390/ijms22158278]

59 **Gao X**, Ding J, Liao C, Xu J, Liu X, Lu W. Defensins: The natural peptide antibiotic. *Adv Drug Deliv Rev* 2021; **179**: 114008 [PMID: 34673132 DOI: 10.1016/j.addr.2021.114008]

60 **Parducho KR**, Beadell B, Ybarra TK, Bush M, Escalera E, Trejos AT, Chieng A, Mendez M, Anderson C, Park H, Wang Y, Lu W, Porter E. The Antimicrobial Peptide Human Beta-Defensin 2 Inhibits Biofilm Production of *Pseudomonas aeruginosa* Without Compromising Metabolic Activity. *Front Immunol* 2020; **11**: 805 [PMID: 32457749 DOI: 10.3389/fimmu.2020.00805]

61 **Pachón-Ibáñez ME**, Smani Y, Pachón J, Sánchez-Céspedes J. Perspectives for clinical use of engineered human host defense antimicrobial peptides. *FEMS Microbiol Rev* 2017; **41**: 323-342 [PMID: 28521337 DOI: 10.1093/femsre/fux012]

62 **Cardot-Martin E**, Casalegno JS, Badiou C, Dauwalder O, Keller D, Prévost G, Rieg S, Kern WV, Cuerq C, Etienne J, Vandenesch F, Lina G, Dumitrescu O. α-Defensins partially protect human neutrophils against Panton-Valentine leukocidin produced by Staphylococcus aureus. *Lett Appl Microbiol* 2015; **61**: 158-164 [PMID: 25963798 DOI: 10.1111/lam.12438]

63 **Bolatchiev A**. Antibacterial activity of human defensins against *Staphylococcus aureus* and *Escherichia coli*. *PeerJ* 2020;

8: e10455 [PMID: 33304659 DOI: 10.7717/peerj.10455]

64 **Pasupuleti M**, Schmidtchen A, Malmsten M. Antimicrobial peptides: key components of the innate immune system. *Crit Rev Biotechnol* 2012; **32**: 143-171 [PMID: 22074402 DOI: 10.3109/07388551.2011.594423]

65 **Steinstraesser L**, Koehler T, Jacobsen F, Daigeler A, Goertz O, Langer S, Kesting M, Steinau H, Eriksson E, Hirsch T. Host defense peptides in wound healing. *Mol Med* 2008; **14**: 528-537 [PMID: 18385817 DOI: 10.2119/2008-00002.Steinstraesser]

66 **Hozzein WN**, Badr G, Badr BM, Allam A, Ghamdi AA, Al-Wadaan MA, Al-Waili NS. Bee venom improves diabetic wound healing by protecting functional macrophages from apoptosis and enhancing Nrf2, Ang-1 and Tie-2 signaling. *Mol Immunol* 2018; **103**: 322-335 [PMID: 30366166 DOI: 10.1016/j.molimm.2018.10.016]

67 **Badr G**. Camel whey protein enhances diabetic wound healing in a streptozotocin-induced diabetic mouse model: the critical role of β-Defensin-1, -2 and -3. *Lipids Health Dis* 2013; **12**: 46 [PMID: 23547923 DOI: 10.1186/1476-511X-12-46]

68 **Liu S**, Zhou L, Li J, Suresh A, Verma C, Foo YH, Yap EP, Tan DT, Beuerman RW. Linear analogues of human beta-defensin 3: concepts for design of antimicrobial peptides with reduced cytotoxicity to mammalian cells. *Chembiochem* 2008; **9**: 964-973 [PMID: 18350527 DOI: 10.1002/cbic.200700560]

69 **Luo G**, Sun Y, Zhang J, Xu Z, Lu W, Wang H, Zhang Y, Li H, Mao Z, Ye S, Cheng B, Fang X. Nanodefensin-encased hydrogel with dual bactericidal and pro-regenerative functions for advanced wound therapy. *Theranostics* 2021; **11**: 3642-3660 [PMID: 33664853 DOI: 10.7150/thno.53089]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 972-985

DOI: 10.4239/wjd.v13.i11.972

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Basic Study

Dietary N$^{\varepsilon}$-(carboxymethyl) lysine affects cardiac glucose metabolism and myocardial remodeling in mice

Zhong-Qun Wang, Zhen Sun

Specialty type: Cell biology

Provenance and peer review: Unsolicited 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): 0
Grade E (Poor): 0

P-Reviewer: Jovandaric MZ, Serbia; Mai S, Italy

Received: August 16, 2022
Peer-review started: August 16, 2022
First decision: September 4, 2022
Revised: September 15, 2022
Accepted: October 11, 2022
Article in press: October 11, 2022
Published online: November 15, 2022

Zhong-Qun Wang, Zhen Sun, Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, Jiangsu Province, China

Corresponding author: Zhen Sun, PhD, Doctor, Department of Cardiology, Affiliated Hospital of Jiangsu University, No. 438 Jiefang Road, Zhenjiang 212001, Jiangsu Province, China. 1398041019@qq.com

Abstract

BACKGROUND

Myocardial remodeling is a key factor in the progression of cardiovascular disease to the end stage. In addition to myocardial infarction or stress overload, dietary factors have recently been considered associated with myocardial remodeling. N$^{\varepsilon}$-(carboxymethyl)lysine (CML) is a representative foodborne toxic product, which can be ingested *via* daily diet. Therefore, there is a marked need to explore the effects of dietary CML on the myocardium.

AIM

To explore the effects of dietary CML (dCML) on the heart.

METHODS

C57 BL/6 mice were divided into a control group and a dCML group. The control group and the dCML group were respectively fed a normal diet or diet supplemented with CML for 20 wk. Body weight and blood glucose were recorded every 4 wk. ^{18}F-fluorodeoxyglucose (FDG) was used to trace the glucose uptake in mouse myocardium, followed by visualizing with micro-positron emission tomography (PET). Myocardial remodeling and glucose metabolism were also detected. *In vitro,* H9C2 cardiomyocytes were added to exogenous CML and cultured for 24 h. The effects of exogenous CML on glucose metabolism, collagen I expression, hypertrophy, and apoptosis of cardiomyocytes were analyzed.

RESULTS

Our results suggest that the levels of fasting blood glucose, fasting insulin, and serum CML were significantly increased after 20 wk of dCML. Micro-PET showed that ^{18}F-FDG accumulated more in the myocardium of the dCML group than in the control group. Histological staining revealed that dCML could lead to myocardial fibrosis and hypertrophy. The indexes of myocardial fibrosis, apoptosis, and hypertrophy were also increased in the dCML group, whereas the activities of glucose metabolism-related pathways and citrate synthase (CS) were

significantly inhibited. In cardiomyocytes, collagen I expression and cellular size were significantly increased after the addition of exogenous CML. CML significantly promoted cellular hypertrophy and apoptosis, while pathways involved in glucose metabolism and level of *Cs* mRNA were significantly inhibited.

CONCLUSION

This study reveals that dCML alters myocardial glucose metabolism and promotes myocardial remodeling.

Key Words: Diet; Myocardial remodeling; Glucose metabolism; Nε-(carboxymethyl)lysine; C57 BL/6 mice

Core Tip: N^{ε}-(carboxymethyl)lysine (CML) exists in daily diet and is harmful to health. We established *in vitro* and *in vivo* models to investigate the effects of dietary CML (dCML) on the heart. We found that long-term dCML induced insulin resistance and elevated serum CML level. ^{18}F-fluorodeoxyglucose imaging indicated that dCML promoted myocardial glucose uptake, but the glucose metabolism was disrupted. Myocardial fibrosis, apoptosis, and hypertrophy were significantly enhanced by dCML. In the cell model, CML supplementation promoted cardiomyocyte apoptosis, cellular hypertrophy, and collagen I expression, and also inhibited pathways involved in glucose metabolism.

Citation: Wang ZQ, Sun Z. Dietary N^{ε}-(carboxymethyl) lysine affects cardiac glucose metabolism and myocardial remodeling in mice. *World J Diabetes* 2022; 13(11): 972-985
URL: https://www.wjgnet.com/1948-9358/full/v13/i11/972.htm
DOI: https://dx.doi.org/10.4239/wjd.v13.i11.972

INTRODUCTION

Cardiovascular disease is the leading cause of mortality worldwide[1]. Myocardial remodeling can lead to decreased cardiac function, which is an important factor in increasing mortality due to cardiovascular disease[2]. Cardiac remodeling is characterized by cardiomyocyte hypertrophy, apoptosis, fibrosis, and increased fibrocollagen deposition[3]. Short-term compensatory remodeling increases cardiac contractility, whereas long-term sustained pathological remodeling leads to a decline in cardiac function or even heart failure[4]. Cardiac remodeling can be caused by myocardial infarction, stress overload, inflammatory cardiomyopathy, idiopathic dilated cardiomyopathy, or diabetes[5]. Although these causes differ, they share similar mechanisms such as oxidative stress, endoplasmic reticulum stress, and inflammatory response[6]. Attempts have been made to improve myocardial remodeling with drugs or other interventions, albeit with unsatisfactory results[7].

Recent studies have shown that in addition to diseases such as diabetes and coronary heart disease, foodborne factors are also associated with myocardial remodeling[8]. Nakamura *et al*[9] found that carbohydrate content in the diet could affect myocardial remodeling. Zeng *et al*[10] reported that a high-fat diet promoted myocardial remodeling. Therefore, it is important to identify the key pathogenic components in the diet for the prevention and treatment of myocardial remodeling.

Advanced glycation end products (AGEs) are a class of heterogeneous irreversible products formed by non-enzymatic reactions[11]. AGEs can accumulate in various tissues resulting in adverse health effects by increasing disease pathogenesis[12,13]. AGEs can accumulate *via* endogenous and exogenous mechanisms. Food is the main source of exogenous AGEs[14]. N^{ε}-(carboxymethyl)lysine (CML) is considered a representative of food-derived AGEs[15]. CML has been found in a variety of foods such as milk, bakery products, and coffee. Ahmed *et al*[16] reported that the concentration of CML is 877 ± 47 nM in pasteurized milk. Assar *et al*[17] reported that bread crust contains 46.1 mg/kg of CML. Ingestion of CML *via* routine diet is substantially higher than the level of CML in plasma and tissues[18]. Daily exposure to high levels of CML is a health risk for humans[19]. The dietary intake of CML is positively correlated with prevalent vertebral fractures[20]. Studies have also shown that foodborne CML can accelerate the progression of atherosclerosis, Alzheimer's disease, and other diseases[21]. Our previous studies reported that long-term exposure to CML leads to osteogenic differentiation of vascular smooth muscle cells and calcification in diabetic plaques[12]. Exogenous CML can lead to the continuous evolution of atherosclerotic plaques[22]. However, it is currently unknown whether CML intake affects myocardial remodeling.

Glucose metabolism is an important energy source for heart activities. Physiologically, cardiomyocytes uptake and transport glucose *via* the glucose transporter (Glut) family and obtain energy *via* aerobic glucose oxidation[23]. Under pathological conditions such as myocardial infarction, glucose metabolism is altered and glucose uptake is increased to counteract the decline in cardiac function[24]. Impaired glucose metabolism is an independent risk factor for the progression of heart failure[25]. However, whether foodborne factors have an impact on myocardial glucose metabolism needs further study.

In the present study, we hypothesized that dietary CML (dCML) can lead to myocardial glucose metabolism dysfunction and myocardial remodeling. We fed mice a CML-supplemented diet and used ^{18}F-fluorodeoxyglucose (FDG) to track the glucose uptake by the mouse myocardium *in vitro* and *in vivo*. Our study provides new insights into the relationship between dCML and myocardial remodeling.

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Experimental Animal Use Ethics Committee of Jiangsu University and followed the ARRIVE guidelines. Ten-wk-old male C57 BL/6 mice (Cavens, ChangZhou, China) were stored in a light:dark (12:12) cycle environment at a temperature of 26 °C and humidity of 70%. Mice were randomly divided into two groups: control (Ctrl) group and dCML group (n = 25). Mice in the Ctrl group were freely administered a standard pelleted diet (XieTong, Nanjing, China). The dCML group received a daily standard pelleted diet mixed with CML (1 g/kg)[26]. The body weight and blood glucose of mice were measured every 4 wk. Blood glucose was detected with a glucose meter (Roche, Basel, Switzerland). After 20 wk of feeding, the fasting insulin levels of the mice were detected *via* the enzyme-linked immunoassay (ELISA) (Mercodia, Sweden), and the homeostatic model assessment insulin resistance (HOMA-IR) was calculated [HOMA-IR = glucose (mmol/L) × insulin (mIU/L)]/22.5. Then the mice were subjected to the oral glucose tolerance test (OGTT)[27]. Each mouse was fasted for 8 h prior to the tests. During fasting, mice had access to adequate water. The serum CML level in mice was detected *via* ELISA (Cloud-Clone, Wuhan, China). The operation steps followed the instructions. The citrate synthase (CS) in mouse tissues was detected with the Citrate Synthase Activity Detection Kit (Solarbio, Beijing, China). The mouse tissues were homogenized and centrifuged at 11000 g for 10 min. The protein concentration of supernatants was measured using the bicinchoninic acid assay. The samples were reacted with the detection reagent for 10 s or 2 min, and the absorbance at 412 nm was measured. Then the activity was calculated and expressed as nmol/min/mg protein.

Histology

Mice were euthanized with carbon dioxide (CO_2). Mouse hearts were isolated and the left ventricles were used for subsequent analysis. Masson's trichrome staining was performed to assess the degree of myocardial fibrosis in mice using an appropriate kit (Solarbio). Myocardial glycogen accumulation was measured using the Glycogen Periodic Acid Schiff (PAS) Stain Kit (Solarbio)[28]. The cytoplasm and nuclei were stained with hematoxylin and eosin (H&E) dye according to the manufacturer's instructions (Solarbio), followed by imaging using a microscope (Olympus, Tokyo, Japan). Areas testing positive with Masson's trichrome and PAS stains were measured using ImageJ software.

Positron emission tomography imaging

To assess glucose uptake in the mouse myocardium *in vivo*, ^{18}F-FDG was used to trace the glucose metabolism and visualized with micro-positron emission tomography (PET) (Inveon; Siemens, Munich, Germany)[29]. Mice were fasted for 12 h before scanning. The mice were weighed and anesthetized with isoflurane/oxygen mixture (15-20 mL/L). ^{18}F-FDG (7.4 MBq, 150 μL) was injected through the tail vein. Micro-PET scanning was performed 2 h after injection for 10 min. The scanned images were iteratively reconstructed with ordered set expectation maximization three-dimensional software. The average level of radioactive material uptake in the cardiac region was analyzed using ASIProVM software. The mean of standard uptake value (SUV_{mean}) was calculated according to the formula: SUV_{mean} = cardiac radioactive material uptake (μCi/g)/[total injected dose (μCi)/body weight (g)]. Similarly, the *in vitro* micro-PET scanning of mouse hearts was also performed[30]. Two hours after the injection of ^{18}F-FDG, the mice were euthanized with CO_2 and the hearts were isolated. The isolated heart was placed in a tube filled with ultrasound gel and scanned *via* micro-PET. The calculation of SUV_{mean} of isolated mouse hearts was the same as described above.

Cell culture

H9C2 cells (Procell, Wuhan, China) were cultured in Dulbecco's Modified Eagle Medium supplemented with 100 mL/L fetal bovine serum[31]. Cells were divided into a Ctrl group and a CML group, followed by seeding of 2 × 10^5 cells and addition of 10 mmol/L CML to the CML group, and incubated for 24 h.

Cell viability was detected with Cell Counting Kit-8 (C0037; Beyotime, Shanghai, China). All cells were cultured at 37 °C with 50 mL/L CO_2. Collagen I content in H9C2 cells was assessed *via* immunocytochemical staining. The SP Rabbit & Mouse HRP Kit (CoWin Century, Beijing, China) was used for immunocytochemical staining. Briefly, after fixing in 40 g/L paraformaldehyde for 30 min, the cell samples were washed with phosphate-buffered saline. The samples were incubated with 100 mL/L goat serum and 2.5 mL/L Triton X-100 at room temperature, followed by incubation with primary and secondary antibodies. The primary antibody used for the staining was: anti-collagen I (1:500, 14695-1-AP; Proteintech, Rosemont, IL, United States). To assess the cardiomyocyte area, cells were labeled with Phalloidin (P5282; Sigma-Aldrich, St. Louis, MO, United States). After fixing in 40 g/L paraformaldehyde, the cells were incubated with 2.5 mL/L Triton X-100 for 15 min, followed by labeling with Phalloidin (5 μmol/L) to analyze cell morphology. The nuclei were subsequently stained with DAPI. All stained images were acquired under a microscope (Olympus) and quantified with a computer-assisted image analysis system. Six high-resolution fields in each independent experiment were randomly selected and the area of at least 100 cells was calculated.

Western blotting

The experimental steps were performed as previously described[32]. Protein samples were prepared using RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Beyotime). Proteins were transferred to PVDF membranes after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After blocking in 50 g/L milk powder for 1 h, the membranes were incubated with suitable diluted primary antibodies overnight. The membranes were incubated with horseradish peroxidase-labeled secondary antibodies and imaged using a chemiluminescence system (Amersham Imager 600; GE Healthcare, Chicago, IL, United States). The acquired images were analyzed using ImageJ software. Primary antibodies used for western blotting were anti-CML (1:1000, ab125145; Abcam, Cambridge, United Kingdom), anti-collagen I (1:500, 14695-1-AP; Proteintech), anti-Glut-1 (1:2000, 21829-1-AP; Proteintech), anti-Glut-4 (1:2000, 66846-1-Ig; Proteintech), anti-Akt (1:5000, 10176-2-AP; Proteintech), anti-B-cell leukemia/lymphoma 2 (Bcl-2) (1:2000, 26593-1-AP; Proteintech), anti-Bcl-2-associated X, apoptosis regulator (BAX) (1:2000, 50599-2-Ig; Proteintech), anti-Akt (phospho-Ser473; 1:5000, 66444-1-Ig; Proteintech), anti-AMP-activated protein kinase (AMPK) (1:5000, 66536-1-Ig; Proteintech), anti-phospho-AMPK (phospho-Thr183 and Thr172; 1:5000, ab133448; Abcam) and anti-β-actin (1:2000, ET1702-67; HUABIO, Hangzhou, China).

Quantitative PCR

Total RNA from tissues/cells was obtained with the RNA-Quick Purification Kit (ES-RN001; YISHAN BIOTECH, Shanghai, China). The mRNA was reverse-transcribed into cDNA using a reverse transcriptase kit (R222; Vazyme Biotech, Nanjing, China)[33]. The SYBR qPCR Master Mix Kit (Q311, Vazyme Biotech) was used to detect the level of each gene. All experimental operations were carried out according to the manufacturer's instructions. The primer sequences are shown in Table 1.

Statistical analysis

All data were presented as the mean ± SD. The differences between the two groups were analyzed with the Student's *t*-test. Statistical significance was set at $P < 0.05$. All data were analyzed using SPSS 22.0 software.

RESULTS

Dietary CML affects glucose metabolism and insulin resistance in mice

After feeding the mice a diet supplemented with CML, we assessed their body weight and blood glucose every 4 wk until week 20. Body weight of the Ctrl group increased with feeding time, whereas the weight gain of the dCML group slowed down from the 8th wk of feeding (Figure 1A). There was no significant difference in body weight between the Ctrl group and the dCML group. From the 12th wk of feeding, the fasting blood glucose of mice in the dCML group was significantly higher than in the Ctrl group (Figure 1B). Further, we detected the glucose tolerance of mice. The OGTT test showed that the blood glucose AUC of the dCML group was significantly increased compared with the Ctrl group, suggesting impaired glucose tolerance of the dCML group (Figure 1C and D). There were significant differences in fasting insulin and HOMA-IR values between the two groups of mice. The fasting insulin and HOMA-IR levels of the dCML group were higher than those of the Ctrl group, suggesting insulin resistance in the dCML group (Figure 1E and F). Similarly, the serum CML level of mice in the dCML group was also significantly increased due to the consumption of CML, which was 2.6-fold higher than that in the Ctrl group (Figure 1G).

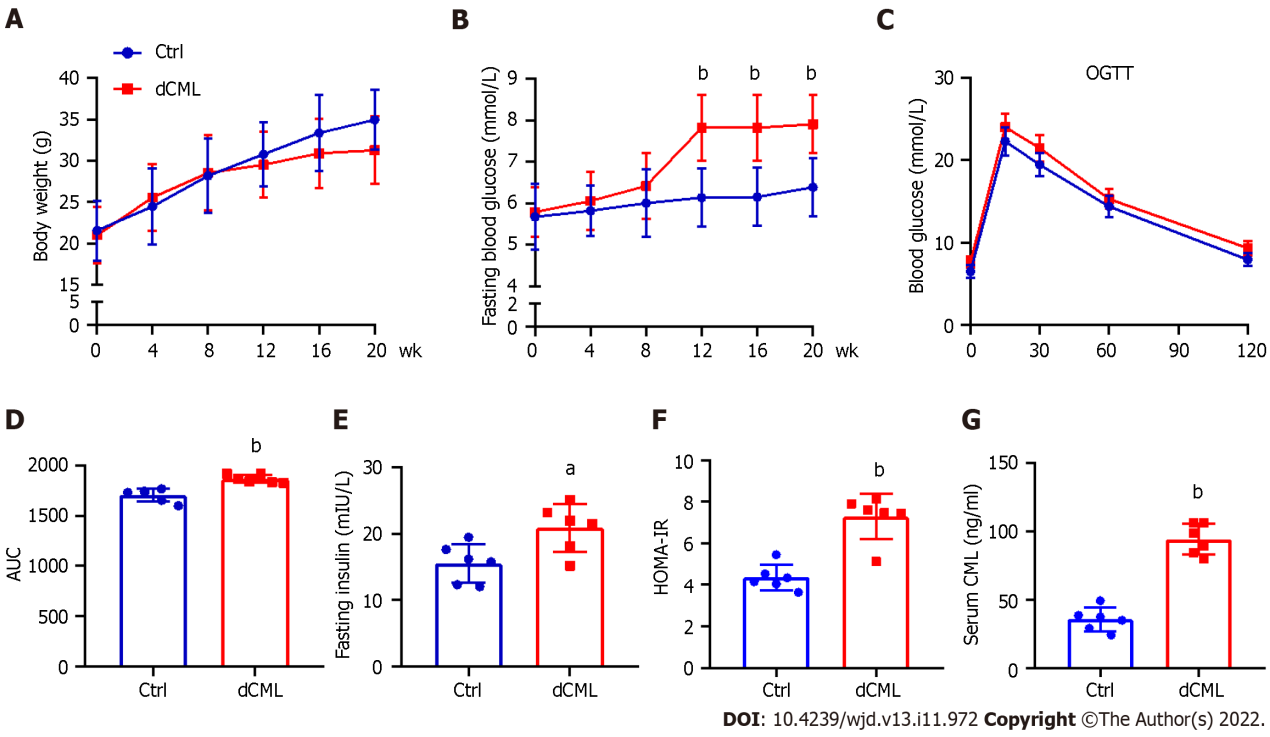
Dietary CML promotes glucose uptake in mouse myocardium

^{18}F-FDG is a glucose analog that can be used to track the glucose uptake in tissues and organs. Micro-

Table 1 Sequences of the primers used for quantitative PCR

Gene	Forward, 5'-3'	Reverse, 5'-3'
Bax (mouse)	GAACAGATCATGAAGACAGGG	CAGTTCATCTCCAATTCGCC
Bcl-2 (mouse)	AGGGGGAAACACCAGAATC	GGTAGCGACGAGAGAAGT
ANP (mouse)	CCTGTGTACAGTGCGGTGTC	CCTAGAAGCACTGCCGTCTC
Glut-1 (mouse)	ACGCCCCCCAGAAGGTTAT	GCGTGGTGAGTGTGGTGGAT
Glut-4 (mouse)	TTGCACACGGCTTCCGAACG	GATCTGCTGGAAACCCGACGG
β-actin (mouse)	TCTTGGGTATGGAATCCTGTG	ATCTCCTTCTGCATCCTGTCA
Bax (rat)	TGCAGAGGATGATTGCTGAC	GATCAGCTCGGGCACTTTAG
Bcl-2 (rat)	AGTGGGATGCGGGAGATGTG	GGGGCCGTACAGTTCCACAA
ANP (rat)	CCGTATACAGTGCGGTGTCCAAC	TCATCGGTCTGCTCGCTCAGG
Glut-1 (rat)	GCCTGAGACCAGTTGAAAGCAC	CTGCTTAGGTAAAGTTACAGGAG
Glut-4 (rat)	AGGCACCCTCACTACCCTTT	AGCATAGCCCTTTTCCTTCC
Cs (rat)	GGAACACACTCAACTCGGGA	ACCCCACTGTGAGCATCTACG
β-actin (rat)	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

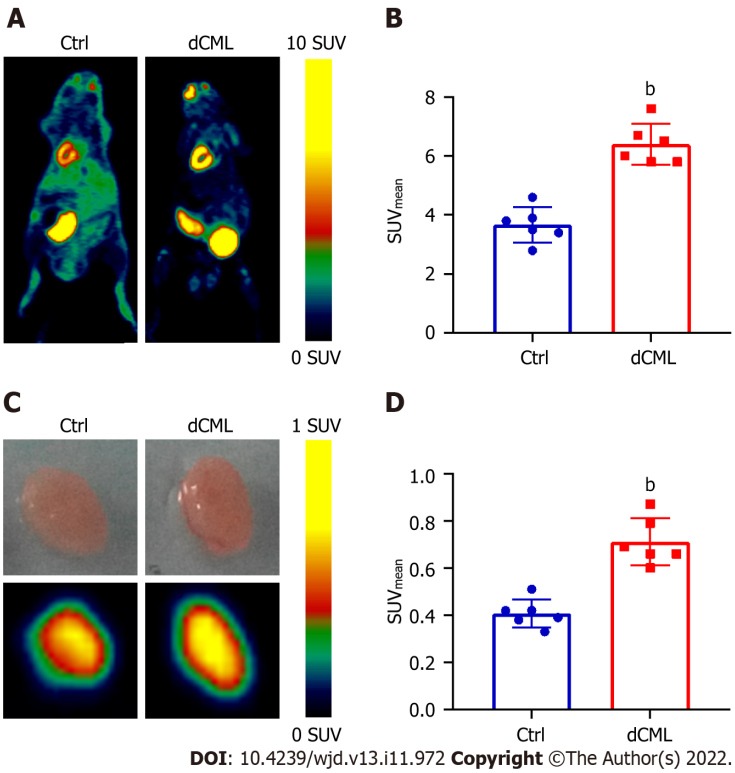
ANP: Atrial natriuretic peptide; BAX: Bcl-2-associated X; Bcl-2: B-cell leukemia/lymphoma 2; Cs: Citrate synthase; Glut: Glucose transporter.



DOI: 10.4239/wjd.v13.i11.972

Figure 1 Dietary Nᵋ-(carboxymethyl)lysine increases blood glucose and induces insulin resistance in mice. A: Body weight of mice; B: Fasting blood glucose of mice; C: Oral glucose tolerance test (OGTT) test of mice; D: Area under the curve (AUC) of OGTT test; E: Fasting insulin of mice; F: Mouse homeostatic model assessment insulin resistance; G: Mouse serum Nᵋ-(carboxymethyl)lysine (CML) level. Ctrl: Control; dCML: Dietary CML; $n = 6$. $^{a}P < 0.05$, $^{b}P < 0.01$, compared with the Ctrl group.

PET scanning was performed to detect the uptake of ^{18}F-FDG in the mouse myocardium. Compared with the Ctrl group, the myocardial SUV_{mean} of the dCML group was significantly increased (6.40 ± 0.70 *vs* 3.67 ± 0.60; $P < 0.01$) (Figure 2A and B). Then the mouse hearts were isolated and detected *via* micro-PET scanning *in vitro*. The SUV_{mean} of hearts in the dCML group was still significantly higher than that in the Ctrl group (0.71 ± 0.10 *vs* 0.41 ± 0.06; $P < 0.01$) (Figure 2C and D), suggesting that the glucose uptake of the myocardium was increased after supplementation with the CML diet.



DOI: 10.4239/wjd.v13.i11.972 **Copyright** ©The Author(s) 2022.

Figure 2 Myocardial glucose uptake is increased after dietary N$^{\varepsilon}$-(carboxymethyl)lysine. A and B: Micro-positron emission tomography scanning of ^{18}F-fluorodeoxyglucose (FDG) accumulation in mouse myocardium; C and D: Uptake of ^{18}F-FDG by isolated mouse hearts of the control (Ctrl) group and dietary N$^{\varepsilon}$-(carboxymethyl)lysine (dCML) group. SUV: Standard uptake value. $n = 6$. $^{b}P < 0.01$, compared with the Ctrl group.

Dietary CML promotes myocardial remodeling in mice

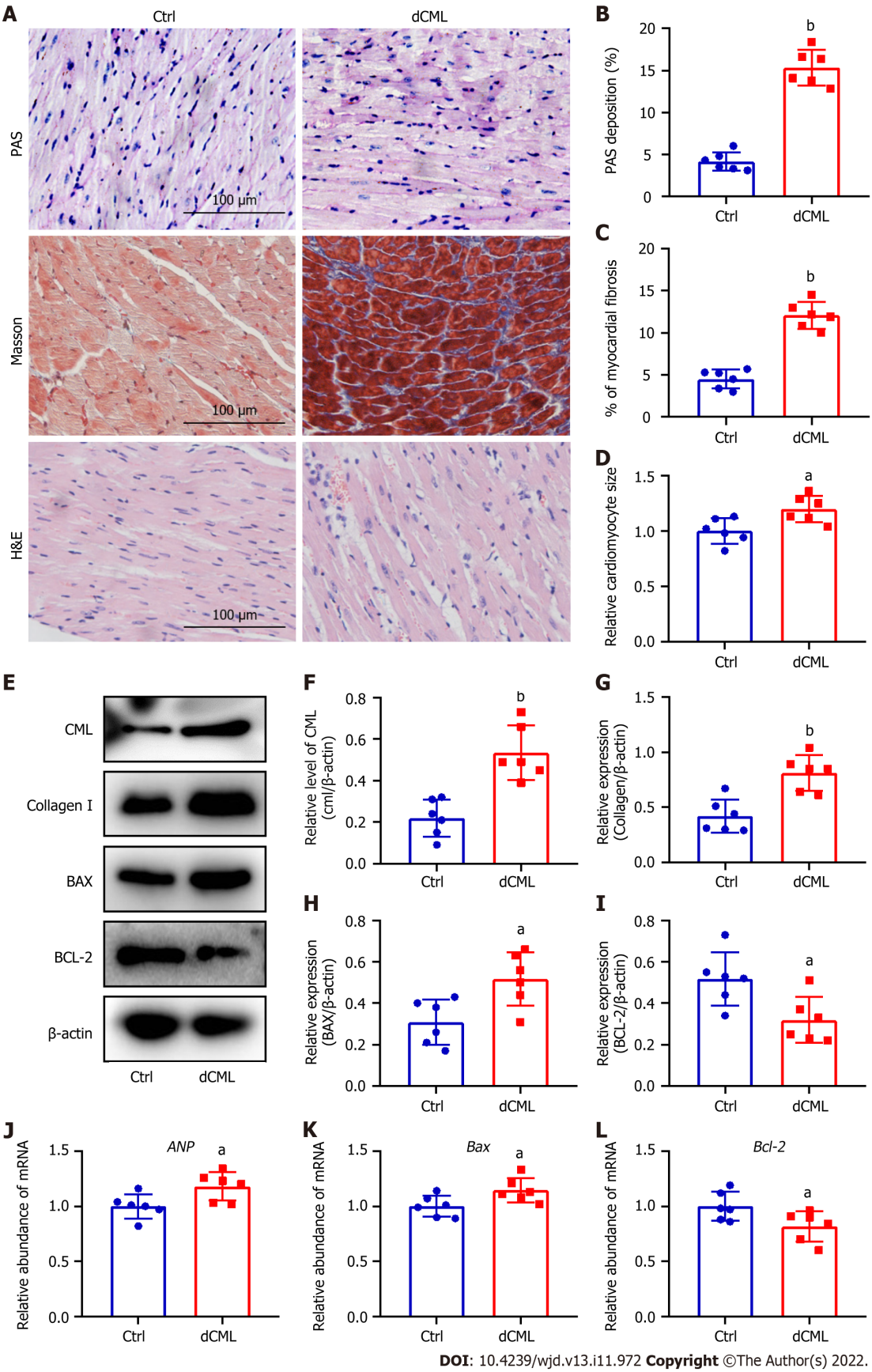
Histopathological changes in the myocardium of mice were detected after 20 wk of dCML. Glycogen PAS staining showed a significant increase in the accumulation of glycogen in the myocardium of the dCML group, which was 3.7-fold higher than that of the Ctrl group, suggesting impaired glucose metabolism (Figure 3A and B). Masson staining indicated that myocardial fibrosis was aggravated in the dCML group (Figure 3A and C). H&E staining showed that the cell area in the myocardium of the dCML group was 1.32-fold higher than that of the Ctrl group (Figure 3A and D). The level of CML in the myocardium of the dCML group also significantly increased (Figure 3E and F). Protein levels of collagen I and the apoptosis regulator BAX were significantly upregulated, whereas the anti-apoptotic factor Bcl-2 was significantly inhibited (Figure 3E, G, H and I). The mRNA level of the cardiac hypertrophy indicator atrial natriuretic peptide (ANP) was significantly increased in the dCML group (Figure 3J). Compared with the Ctrl group, *Bax* mRNA was significantly upregulated, whereas *Bcl-2* was significantly downregulated in the dCML group, suggesting increased myocardial apoptosis in the dCML group (Figure 3K and L).

Dietary CML inhibits glucose metabolic signaling pathways in mouse myocardium

Glut-1 and Glut-4 play a key roles in myocardial glucose transport[34]. Glut-1 mRNA and protein levels were significantly increased in the mouse myocardium of the dCML group, whereas Glut-4 was significantly decreased (Figure 4A-E). Akt and AMPK signaling are key regulatory pathways in glucose metabolism[34,35]. Compared with the Ctrl group, the dCML group showed significant inhibition of Akt and AMPK activities of the myocardium (Figure 4C, F and G). These results suggest that the myocardial glucose metabolism of mice is impaired after dCML. CS is the rate-limiting enzyme in the aerobic oxidation of glucose. The activity of CS was significantly inhibited in the dCML group (Figure 4H).

Exogenous CML inhibits glucose metabolism and promotes fibrosis, hypertrophy, and apoptosis in cardiomyocytes

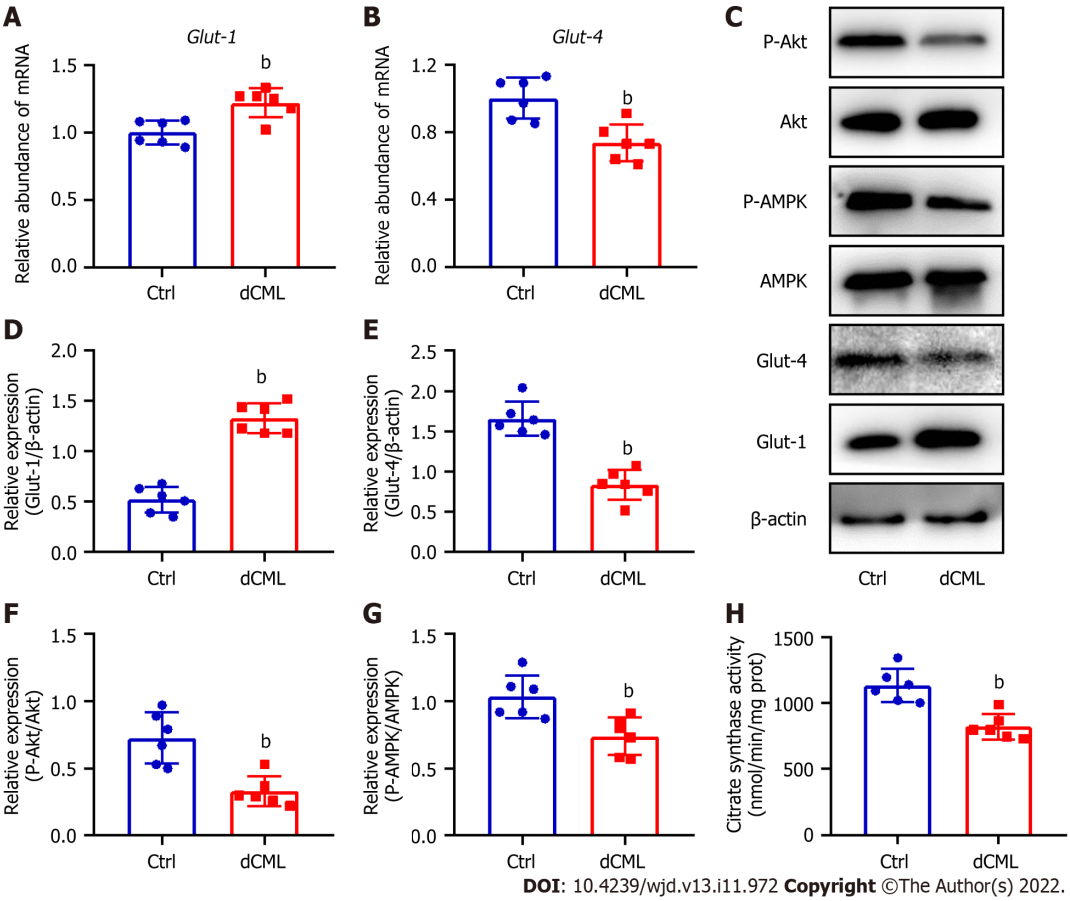
Given that dCML inhibits myocardial glucose metabolism and promotes myocardial remodeling in mice, we further investigated the direct effects of CML *in vitro*. Exogenous stimulation with 10 mmol/L CML did not affect the viability of H9C2 cells (Figure 5A). Exogenous CML stimulation significantly decreased Glut-4 expression and significantly reduced the levels of Akt and AMPK phosphorylation in H9C2 cardiomyocytes, whereas the mRNA and protein levels of Glut-1 were significantly increased (Figure 5B-H). Immunocytochemical staining indicated that CML increased the content of collagen I in



DOI: 10.4239/wjd.v13.i11.972

Figure 3 Dietary Nᵋ-(carboxymethyl)lysine increases myocardial fibrosis, hypertrophy and apoptosis in mice. A: Mouse myocardial glycogen Periodic Acid Schiff (PAS) staining, Masson's trichrome staining, and hematoxylin and eosin staining; Scale 100 μm; B and C: Percentage of PAS-positive and fibrotic areas in the mouse myocardium; D: Relative area of myocardial cells in the myocardium; E-I: Western blotting and its relative level of Nᵋ-(carboxymethyl)lysine (CML), collagen I, B-cell leukemia/lymphoma 2 (Bcl-2) and Bcl-2-associated X (BAX) in the mouse myocardium; J-L: Atrial natriuretic peptide (*ANP*), *Bax*, and *Bcl-2* mRNA

levels in the mouse myocardium. dCML: Dietary CML. $n = 6$. $^{a}P < 0.05$, $^{b}P < 0.01$, compared with the control (Ctrl) group.



DOI: 10.4239/wjd.v13.i11.972

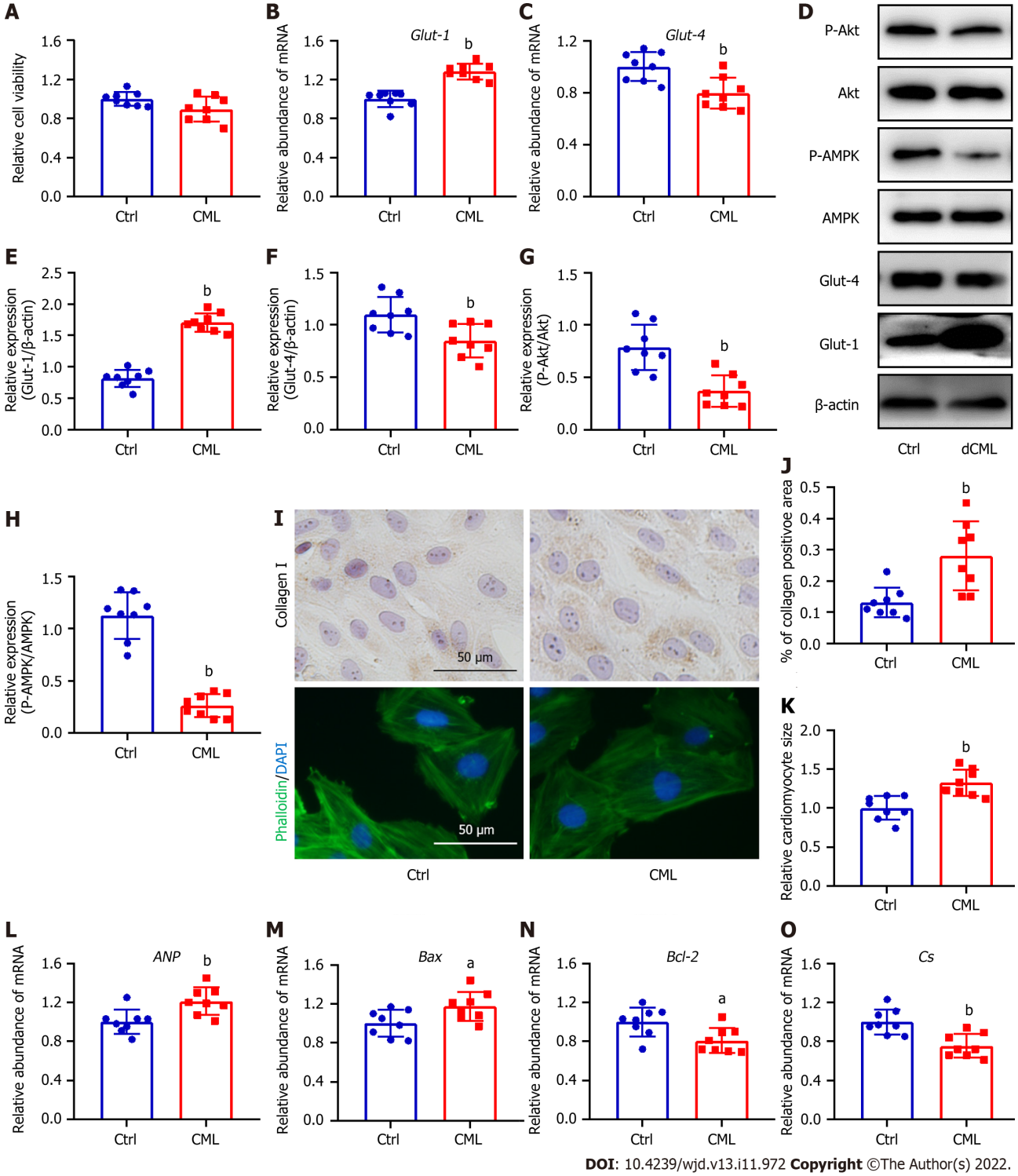
Figure 4 Dietary N$^{\varepsilon}$-(carboxymethyl)lysine impairs glucose metabolism in mouse myocardium. A and B: mRNA levels of glucose transporter (*Glut*) *-1* and *Glut-4* in the mouse myocardium; C-G: Western blotting and its relative quantification of Glut-1, Glut-4, phospho-Akt, and phospho-AMP-activated protein kinase (AMPK) in the mouse myocardium; H: Citrate synthase (CS) activity in the mouse myocardium. dCML: Dietary N$^{\varepsilon}$-(carboxymethyl)lysine; Bcl-2: B-cell leukemia/lymphoma 2; BAX: Bcl-2-associated X; ANP: Atrial natriuretic peptide. $n = 6$. $^{b}P < 0.01$, compared with the control (Ctrl) group.

cardiomyocytes (Figure 5I and J). Phalloidin staining showed that the cardiomyocyte area in the CML group was significantly increased, and the expression of cardiac hypertrophy marker ANP was also significantly upregulated (Figure 5I, K and L). Meanwhile, the mRNA level of the apoptosis indicator *Bax* was increased in the CML group, and the level of the anti-apoptotic marker *Bcl-2* was significantly downregulated, suggesting that CML induced cardiomyocyte apoptosis (Figure 5M and N). The *Cs* mRNA level was also decreased in the CML group (Figure 5O).

DISCUSSION

This study investigated the effects of dCML on myocardial glucose metabolism and myocardial remodeling using experimental mouse models and cells. In the *in vivo* model, myocardial glucose uptake was tracked using ^{18}F-FDG micro-PET scans. The glucose uptake in the dCML group was significantly increased. Histological staining and detection of related molecular indicators suggested that dCML inhibited glucose metabolism in the myocardium and promoted myocardial fibrosis, cardiac hypertrophy, and apoptosis (Figure 6). *In vitro*, H9C2 cardiomyocytes were treated with exogenous CML to analyze changes in cardiac remodeling and glucose metabolism. Consistent with the *in vivo* evidence, CML inhibited glucose metabolism and promoted hypertrophy, collagen I expression, and apoptosis of cardiomyocytes. Our study may reveal new clues for underlying foodborne factors associated with myocardial injury and provide new ideas for the prevention and treatment of myocardial remodeling.

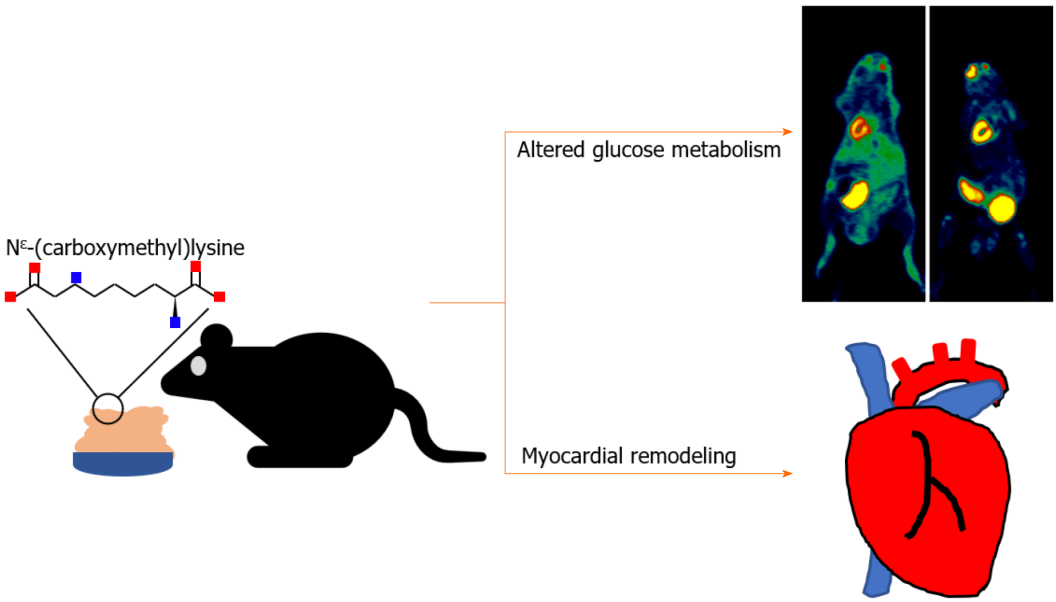
AGEs are a class of non-enzymatic reaction products composed of complex components, and the pathogenic role of AGEs has been previously reported[11,12]. The effects of AGEs may be receptor-



DOI: 10.4239/wjd.v13.i11.972

Figure 5 Exogenous Nᵋ-(carboxymethyl)lysine inhibits the glucose metabolism and promotes collagen I expression, hypertrophy and apoptosis in H9C2 cells. A: Cell viability after the simulation of Nᵋ-(carboxymethyl)lysine (CML); B and C: Quantitative PCR detection of glucose transporter (*Glut*) *-1* and *Glut-4* mRNA; D-H: Relative expression of Glut-1, Glut-4, phospho-Akt, and phospho-AMP-activated protein kinase (AMPK) in H9C2 cells; I: Upper: Detection of collagen I content with immunocytochemical staining; bottom: Phalloidin-labeled H9C2 cardiomyocytes; J: Quantification of collagen I-positive areas; K: Quantitative analysis of cardiomyocyte area; L-O: Atrial natriuretic peptide *(ANP)*, Bcl-2-associated X *(Bax)*, B-cell leukemia/lymphoma 2 *(Bcl-2)*, and citrate synthase *(CS)* mRNA levels in H9C2 cardiomyocytes. dCML: Dietary CML. $n = 8$ independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$, compared with the control (Ctrl) group.

dependent or receptor-independent. In the receptor-independent pathway, AGEs cross-link with the extracellular matrix and change the physicochemical properties, which affects cell physiology and tissue function[36]. In the receptor-dependent pathway, AGEs bind to cell surface receptors, change the original signal transmission pathway, and lead to pathological outcomes[37]. In addition to AGEs, the precursors of AGEs, such as methylglyoxal, also accumulate in the body. These precursors can play a direct pathogenic role or continue to form AGEs[38]. Adverse effects of dietary AGEs have been



DOI: 10.4239/wjd.v13.i11.972 **Copyright** ©The Author(s) 2022.

Figure 6 Dietary Nᵋ-(carboxymethyl)lysine alters myocardial glucose metabolism and promotes myocardial remodeling.

previously reported. Wang *et al*[39] found that dietary AGEs disrupted gut microbiota and induced insulin resistance. Thornton *et al*[40] suggested that dietary AGEs affected ovarian function. A western diet rich in AGEs can also induce changes in the cardiovascular system[41]. Given that AGEs are multi-component, but whether each component has a similar effect is unclear. It is still unknown which component plays the most critical role. CML is one of the most active components of AGEs. Due to its ease of formation, CML is also found in high concentrations in food[42]. Therefore, our study explored the role of dCML, and showed that dCML can lead to disorders of myocardial glucose metabolism and myocardial remodeling. This finding may provide more evidence underlying the negative effects of foodborne AGEs.

Some studies have also reported the limited pathogenic role of dietary AGEs. Koyama *et al*[43] found no significant association between dietary AGEs and all-cause mortality in adults with diabetes. The double blind parallel study by Linkens *et al*[44] suggested that a short-term AGE diet did not affect the sensitivity, secretion and clearance of insulin, vascular function, and overall inflammation in individuals with abdominal obesity. The Maastricht Study also revealed that dietary AGEs are not associated with stiffness of the aorta or carotid arteries[45]. These studies are population-based, suggesting additional confounding factors than in animal models. Moreover, surveys of dietary structure or dietary interventions in these subjects were conducted for relatively short periods of time, which may not be sufficient to represent the long-term dietary habits of individuals. In our study, we focused on myocardial glucose uptake and its remodeling and found adverse effects of dCML. We also evaluated the systemic effects of dCML on mice. Exposure to 20 wk of dCML resulted in glucose intolerance and insulin resistance, but the fasting glucose did not reach the level of diabetes, suggesting a progressive pathogenic effect of dCML. It may take more than 20 wk of a CML diet to further increase blood glucose and worsen insulin resistance. Also, the weight gain of dCML mice became more obvious starting from the 12th wk. Prolonged dCML time may induce significant changes in mouse body weight.

Myocardium is one of the most energy-consuming organs, with 70% of the energy supply of adult myocardium derived from ATP produced by fatty acid oxidation, and glucose metabolism plays a secondary but important role[46]. Under physiological conditions, glucose is converted to pyruvate. ATP is produced *via* tricarboxylic acid cycle and respiratory chain. Under specific pathological conditions, glucose is the main energy substrate as a result of the reorganization of enzymes involved in energy metabolism. However, glycolysis is the main energy source rather than aerobic oxidation. The energy provided by glycolysis does not meet the long-term needs of myocardial activity. The overall cardiac metabolic activity is subsequently reduced, eventually leading to cardiomyocyte apoptosis and malignant remodeling of the myocardium[47]. However, an increase or decrease in myocardial glucose metabolism is also associated with specific pathological changes. For example, in diabetic cardiomyopathy, the lipotoxicity caused by diabetes increases the fatty acid metabolism in the heart, thus inhibiting glucose metabolism[48]. The interaction between fatty acids and glucose metabolism is also known as Randle Cycle[49]. However, the relationship between myocardial metabolic reprogramming and myocardial pathological remodeling is unclear. Whether myocardial metabolic disturbance is a cause or a consequence of myocardial remodeling is still inconclusive. In this study, we observed impaired myocardial glucose metabolism but increased glucose uptake after long-term dCML in mice.

The glucose metabolic pathways Akt and AMPK were significantly inhibited. The long-term dCML may alter the metabolic substrates for myocardial energy supply. CS, an enzyme initiating the tricarboxylic acid cycle, was also inhibited after exposure to dCML. Therefore, the myocardium has to absorb more glucose to provide adequate substrates for energy metabolism. The specific mechanism will be further explored in future studies.

To analyze the glucose uptake in the myocardium, we used micro-PET imaging based on the ^{18}F-FDG probe. Since ^{18}F-FDG was synthesized in 1969, it has been widely used in the diagnosis, staging and prognostic assessment of clinical diseases[50]. FDG is a glucose analog and is therefore involved in glucose processing *in vivo*. Under pathological conditions, inflammation or hypoxia can lead to impaired glucose metabolism but increased glucose uptake to provide adequate energy. Therefore, ^{18}F-FDG usually accumulates in the lesions. In the study of cardiovascular disease, ^{18}F-FDG imaging also plays an important role. ^{18}F-FDG is the reference standard for molecular imaging of myocardial inflammation[51]. Our study found an increased ^{18}F-FDG uptake but impaired glucose transport and metabolism in the myocardium of dCML mice, which may be related to the elevated levels of inflammation. Consistent with our study, in the spontaneously hypertensive rat model, myocardial ^{18}F-FDG imaging SUV was elevated, whereas glucose aerobic oxidation-related transporters and metabolic pathways were significantly inhibited[29].

We investigated the detrimental effects of dietary CML on myocardial remodeling to draw attention to the CML content in the diet. However, this study has some limitations. The exploration of specific mechanisms needs to be further continued in the future, and clinical evidence is also needed. We speculate that dietary CML may also promote myocardial remodeling through non-receptor and receptor approaches. CML could increase collagen cross-linking in the extracellular matrix and bind to its receptors to activate related signals. In the future, we will further explore the mechanism of cardiac remodeling induced by dietary CML to identify effective targets for intervention. There is an endogenous CML generation system in human body. Compared with reducing endogenous CML, reducing exogenous CML from dietary sources appears to be more controllable. Previous studies have also reported some CML inhibitors, such as antioxidants and aminoguanidine[15]. However, these additives may change the original methods of food production, and their high cost and unclear safety also limit the application. Therefore, in the future, we will also focus on strategies to inhibit CML in diet preparation.

CONCLUSION

Our study focused on the adverse effects of food-derived CML on myocardial glucose metabolism and remodeling. Long-term dCML leads to impaired myocardial glucose metabolism and induces myocardial hypertrophy, fibrosis, and apoptosis. This study offers new clues associated with myocardial remodeling and also provides an experimental basis for dietary planning to prevent cardiovascular disease prevention.

ARTICLE HIGHLIGHTS

Research background

N$^{\varepsilon}$-(carboxymethyl)lysine (CML), a major component of advanced glycation end products, exists in the daily diet and poses a threat to health after ingestion. It is necessary to evaluate the effect of dietary CML on the heart.

Research motivation

Previous studies have confirmed that the toxic metabolite CML can cause pathological changes in a variety of tissues such as blood vessels and bones. Foodborne CML, as the main source of CML, may lead to cardiac injuries.

Research objectives

To investigate the effects of dietary CML on cardiac remodeling and glucose metabolism.

Research methods

C57 BL/6 mice received a 20-wk CML diet (1 g/kg). The body weight, fasting blood glucose, fasting insulin and serum CML levels of mice were recorded. Exogenous CML was given to establish an *in vitro* H9C2 cell model. Micro-positron emission tomography was used to evaluate the glucose uptake of the mouse heart. Myocardial remodeling and glucose metabolism were detected by histological/cytological staining, Western blotting, and polymerase chain reaction.

Research results

The 20 wk of CML diet could cause insulin resistance in mice and increase CML levels in serum and heart. Myocardial fibrosis, hypertrophy and apoptosis in mice were significantly aggravated after dietary CML. Moreover, dietary CML increased myocardial glucose uptake but disrupted glucose metabolism. *In vitro,* exogenous CML inhibited glucose metabolism-related signaling pathways and promoted H9C2 cell hypertrophy, apoptosis and collagen I expression.

Research conclusions

Dietary CML promoted cardiac remodeling and abnormal glucose metabolism.

Research perspectives

This study emphasizes the cardiac hazards of dietary CML and provides new suggestions for the diet preparation in the prevention and treatment cardiovascular diseases.

ACKNOWLEDGEMENTS

We thank the Experimental Animal Center of Jiangsu University for guiding animal experiments.

FOOTNOTES

Author contributions: Sun Z designed the study and wrote the manuscript; Wang ZQ performed the experiments, analyzed the data, and prepared the images; all authors edited and approved the final version of the article.

Supported by the National Natural Science Foundation of China, No. 82070455; Natural Science Foundation of Jiangsu Province, No. BK20201225; Medical Innovation Team Project of Jiangsu Province, No. CXTDA2017010; and Research and Innovation Funding Project for College Students in Experimental Animal Center of Jiangsu University.

Institutional animal care and use committee statement: All animal experiments were approved by the Experimental Animal Use Ethics Committee of Jiangsu University.

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

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

ORCID number: Zhong-Qun Wang 0000-0003-3590-9313; Zhen Sun 0000-0003-3368-1976.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Chen YX

REFERENCES

1 **Virani SS**, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Cheng S, Delling FN, Elkind MSV, Evenson KR, Ferguson JF, Gupta DK, Khan SS, Kissela BM, Knutson KL, Lee CD, Lewis TT, Liu J, Loop MS, Lutsey PL, Ma J, Mackey J, Martin SS, Matchar DB, Mussolino ME, Navaneethan SD, Perak AM, Roth GA, Samad Z, Satou GM, Schroeder EB, Shah SH, Shay CM, Stokes A, VanWagner LB, Wang NY, Tsao CW; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. *Circulation* 2021; **143**: e254-e743 [PMID: 33501848 DOI: 10.1161/CIR.0000000000000950]

2 **Alam P**, Maliken BD, Jones SM, Ivey MJ, Wu Z, Wang Y, Kanisicak O. Cardiac Remodeling and Repair: Recent

Approaches, Advancements, and Future Perspective. *Int J Mol Sci* 2021; **22** [PMID: 34884909 DOI: 10.3390/ijms222313104]

3 **Cohn JN**, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* 2000; **35**: 569-582 [PMID: 10716457 DOI: 10.1016/s0735-1097(99)00630-0]

4 **Li G**, Shao Y, Guo HC, Zhi Y, Qiao B, Ma K, Du J, Lai YQ, Li Y. MicroRNA-27b-3p down-regulates FGF1 and aggravates pathological cardiac remodelling. *Cardiovasc Res* 2022; **118**: 2139-2151 [PMID: 34358309 DOI: 10.1093/cvr/cvab248]

5 **Xiong R**, Li N, Chen L, Wang W, Wang B, Jiang W, Geng Q. STING protects against cardiac dysfunction and remodelling by blocking autophagy. *Cell Commun Signal* 2021; **19**: 109 [PMID: 34749750 DOI: 10.1186/s12964-021-00793-0]

6 **Feng Y**, Zhang Y, Xiao H. AMPK and cardiac remodelling. *Sci China Life Sci* 2018; **61**: 14-23 [PMID: 29170891 DOI: 10.1007/s11427-017-9197-5]

7 **Brown RD**, Ambler SK, Mitchell MD, Long CS. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. *Annu Rev Pharmacol Toxicol* 2005; **45**: 657-687 [PMID: 15822192 DOI: 10.1146/annurev.pharmtox.45.120403.095802]

8 **Mellor KM**, Ritchie RH, Davidoff AJ, Delbridge LM. Elevated dietary sugar and the heart: experimental models and myocardial remodeling. *Can J Physiol Pharmacol* 2010; **88**: 525-540 [PMID: 20555422 DOI: 10.1139/y10-005]

9 **Nakamura M**, Odanovic N, Nakada Y, Dohi S, Zhai P, Ivessa A, Yang Z, Abdellatif M, Sadoshima J. Dietary carbohydrates restriction inhibits the development of cardiac hypertrophy and heart failure. *Cardiovasc Res* 2021; **117**: 2365-2376 [PMID: 33070172 DOI: 10.1093/cvr/cvaa298]

10 **Zeng H**, Vaka VR, He X, Booz GW, Chen JX. High-fat diet induces cardiac remodelling and dysfunction: assessment of the role played by SIRT3 loss. *J Cell Mol Med* 2015; **19**: 1847-1856 [PMID: 25782072 DOI: 10.1111/jcmm.12556]

11 **Plemmenos G**, Piperi C. Pathogenic Molecular Mechanisms in Periodontitis and Peri-Implantitis: Role of Advanced Glycation End Products. *Life (Basel)* 2022; **12** [PMID: 35207505 DOI: 10.3390/Life12020218]

12 **Wang Z**, Jiang Y, Liu N, Ren L, Zhu Y, An Y, Chen D. Advanced glycation end-product Nε-carboxymethyl-Lysine accelerates progression of atherosclerotic calcification in diabetes. *Atherosclerosis* 2012; **221**: 387-396 [PMID: 22305260 DOI: 10.1016/j.atherosclerosis.2012.01.019]

13 **Wang ZQ**, Jing LL, Yan JC, Sun Z, Bao ZY, Shao C, Pang QW, Geng Y, Zhang LL, Li LH. Role of AGEs in the progression and regression of atherosclerotic plaques. *Glycoconj J* 2018; **35**: 443-450 [PMID: 29987432 DOI: 10.1007/s10719-018-9831-x]

14 **Chen Y**, Guo TL. Dietary advanced glycation end-products elicit toxicological effects by disrupting gut microbiome and immune homeostasis. *J Immunotoxicol* 2021; **18**: 93-104 [PMID: 34436982 DOI: 10.1080/1547691X.2021.1959677]

15 **Han L**, Li L, Li B, Zhao D, Li Y, Xu Z, Liu G. Review of the characteristics of food-derived and endogenous ne-carboxymethyllysine. *J Food Prot* 2013; **76**: 912-918 [PMID: 23643138 DOI: 10.4315/0362-028X.JFP-12-472]

16 **Ahmed N**, Mirshekar-Syahkal B, Kennish L, Karachalias N, Babaei-Jadidi R, Thornalley PJ. Assay of advanced glycation endproducts in selected beverages and food by liquid chromatography with tandem mass spectrometric detection. *Mol Nutr Food Res* 2005; **49**: 691-699 [PMID: 15945118 DOI: 10.1002/mnfr.200500008]

17 **Assar SH**, Moloney C, Lima M, Magee R, Ames JM. Determination of Nepsilon-(carboxymethyl)lysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids* 2009; **36**: 317-326 [PMID: 18389168 DOI: 10.1007/s00726-008-0071-4]

18 **Henle T**. AGEs in foods: do they play a role in uremia? *Kidney Int Suppl* 2003; S145-S147 [PMID: 12694332 DOI: 10.1046/j.1523-1755.63.s84.16.x]

19 **Liu X**, Zheng L, Zhang R, Liu G, Xiao S, Qiao X, Wu Y, Gong Z. Toxicological evaluation of advanced glycation end product Nε-(carboxymethyl)lysine: Acute and subacute oral toxicity studies. *Regul Toxicol Pharmacol* 2016; **77**: 65-74 [PMID: 26921796 DOI: 10.1016/j.yrtph.2016.02.013]

20 **Waqas K**, Chen J, van der Eerden BCJ, Ikram MA, Uitterlinden AG, Voortman T, Zillikens MC. Dietary Advanced Glycation End-Products (dAGEs) Intake and Bone Health: A Cross-Sectional Analysis in the Rotterdam Study. *Nutrients* 2020; **12** [PMID: 32784487 DOI: 10.3390/nu12082377]

21 **Delgado-Andrade C**, Fogliano V. Dietary Advanced Glycosylation End-Products (dAGEs) and Melanoidins Formed through the Maillard Reaction: Physiological Consequences of their Intake. *Annu Rev Food Sci Technol* 2018; **9**: 271-291 [PMID: 29350563 DOI: 10.1146/annurev-food-030117-012441]

22 **Wang Z**, Bao Z, Ding Y, Xu S, Du R, Yan J, Li L, Sun Z, Shao C, Gu W. Nε-carboxymethyl-lysine-induced PI3K/Akt signaling inhibition promotes foam cell apoptosis and atherosclerosis progression. *Biomed Pharmacother* 2019; **115**: 108880 [PMID: 31035012 DOI: 10.1016/j.biopha.2019.108880]

23 **Tardy-Cantalupi I**, Montessuit C, Papageorgiou I, Remondino-Müller A, Assimacopoulos-Jeannet F, Morel DR, Lerch R. Effect of transient ischemia on the expression of glucose transporters GLUT-1 and GLUT-4 in rat myocardium. *J Mol Cell Cardiol* 1999; **31**: 1143-1155 [PMID: 10336852 DOI: 10.1006/jmcc.1999.0952]

24 **Tran DH**, Wang ZV. Glucose Metabolism in Cardiac Hypertrophy and Heart Failure. *J Am Heart Assoc* 2019; **8**: e012673 [PMID: 31185774 DOI: 10.1161/JAHA.119.012673]

25 **Marsico F**, Gargiulo P, Marra AM, Parente A, Paolillo S. Glucose Metabolism Abnormalities in Heart Failure Patients: Insights and Prognostic Relevance. *Heart Fail Clin* 2019; **15**: 333-340 [PMID: 31079691 DOI: 10.1016/j.hfc.2019.02.002]

26 **Sowndhar Rajan B**, Manivasagam S, Dhanusu S, Chandrasekar N, Krishna K, Kalaiarasu LP, Babu AA, Vellaichamy E. Diet with high content of advanced glycation end products induces systemic inflammation and weight gain in experimental mice: Protective role of curcumin and gallic acid. *Food Chem Toxicol* 2018; **114**: 237-245 [PMID: 29432842 DOI: 10.1016/j.fct.2018.02.016]

27 **Small L**, Ehrlich A, Iversen J, Ashcroft SP, Trošt K, Moritz T, Hartmann B, Holst JJ, Treebak JT, Zierath JR, Barrès R. Comparative analysis of oral and intraperitoneal glucose tolerance tests in mice. *Mol Metab* 2022; **57**: 101440 [PMID: 35026435 DOI: 10.1016/j.molmet.2022.101440]

28 **Zhang L**, Ye K, Xiaokereti J, Ma M, Guo Y, Zhou X, Tang B. Histopathological substrate of the atrial myocardium in the

progression of obstructive sleep apnoea-related atrial fibrillation. *Sleep Breath* 2021; **25**: 807-818 [PMID: 33411188 DOI: 10.1007/s11325-020-02128-8]

29 **Wang J**, Li Z, Wang Y, Zhang J, Zhao W, Fu M, Han X, Zhou J, Ge J. *Qiliqiangxin* Enhances Cardiac Glucose Metabolism and Improves Diastolic Function in Spontaneously Hypertensive Rats. *Evid Based Complement Alternat Med* 2017; **2017**: 3197320 [PMID: 28706558 DOI: 10.1155/2017/3197320]

30 **Hellberg S**, Silvola JM, Kiugel M, Liljenbäck H, Metsälä O, Viljanen T, Metso J, Jauhiainen M, Saukko P, Nuutila P, Ylä-Herttuala S, Knuuti J, Roivainen A, Saraste A. Type 2 diabetes enhances arterial uptake of choline in atherosclerotic mice: an imaging study with positron emission tomography tracer ^{18}F-fluoromethylcholine. *Cardiovasc Diabetol* 2016; **15**: 26 [PMID: 26852231 DOI: 10.1186/s12933-016-0340-6]

31 **Sun Z**, Zhang L, Li L, Shao C, Liu J, Zhou M, Wang Z. Galectin-3 mediates cardiac remodeling caused by impaired glucose and lipid metabolism through inhibiting two pathways of activating Akt. *Am J Physiol Heart Circ Physiol* 2021; **320**: H364-H380 [PMID: 33275526 DOI: 10.1152/ajpheart.00523.2020]

32 **Sun Z**, Wang Z, Li L, Yan J, Shao C, Bao Z, Jing L, Pang Q, Geng Y, Zhang L. RAGE/galectin-3 yields intraplaque calcification transformation *via* sortilin. *Acta Diabetol* 2019; **56**: 457-472 [PMID: 30603868 DOI: 10.1007/s00592-018-1273-1]

33 **Sun Z**, Li L, Yan Z, Zhang L, Zang G, Qian Y, Wang Z. Circadian rhythm disorders elevate macrophages cytokines release and promote multiple tissues/organs dysfunction in mice. *Physiol Behav* 2022; **249**: 113772 [PMID: 35247442 DOI: 10.1016/j.physbeh.2022.113772]

34 **Chaanine AH**, Hajjar RJ. AKT signalling in the failing heart. *Eur J Heart Fail* 2011; **13**: 825-829 [PMID: 21724622 DOI: 10.1093/eurjhf/hfr080]

35 **Entezari M**, Hashemi D, Taheriazam A, Zabolian A, Mohammadi S, Fakhri F, Hashemi M, Hushmandi K, Ashrafizadeh M, Zarrabi A, Ertas YN, Mirzaei S, Samarghandian S. AMPK signaling in diabetes mellitus, insulin resistance and diabetic complications: A pre-clinical and clinical investigation. *Biomed Pharmacother* 2022; **146**: 112563 [PMID: 35062059 DOI: 10.1016/j.biopha.2021.112563]

36 **Olson LC**, Redden JT, Schwartz Z, Cohen DJ, McClure MJ. Advanced Glycation End-Products in Skeletal Muscle Aging. *Bioengineering (Basel)* 2021; **8** [PMID: 34821734 DOI: 10.3390/bioengineering8110168]

37 **Ajith TA**, Vinodkumar P. Advanced Glycation End Products: Association with the Pathogenesis of Diseases and the Current Therapeutic Advances. *Curr Clin Pharmacol* 2016; **11**: 118-127 [PMID: 27164965 DOI: 10.2174/1574884711666160511150028]

38 **Rabbani N**, Thornalley PJ. Advanced glycation end products in the pathogenesis of chronic kidney disease. *Kidney Int* 2018; **93**: 803-813 [PMID: 29477239 DOI: 10.1016/j.kint.2017.11.034]

39 **Wang J**, Cai W, Yu J, Liu H, He S, Zhu L, Xu J. Dietary Advanced Glycation End Products Shift the Gut Microbiota Composition and Induce Insulin Resistance in Mice. *Diabetes Metab Syndr Obes* 2022; **15**: 427-437 [PMID: 35210793 DOI: 10.2147/DMSO.S346411]

40 **Thornton K**, Merhi Z, Jindal S, Goldsammler M, Charron MJ, Buyuk E. Dietary Advanced Glycation End Products (AGEs) could alter ovarian function in mice. *Mol Cell Endocrinol* 2020; **510**: 110826 [PMID: 32339649 DOI: 10.1016/j.mce.2020.110826]

41 **Peppa M**, Mavroeidi I. Experimental Animal Studies Support the Role of Dietary Advanced Glycation End Products in Health and Disease. *Nutrients* 2021; **13** [PMID: 34684468 DOI: 10.3390/nu13103467]

42 **Chen G**. Dietary N-epsilon-carboxymethyllysine as for a major glycotoxin in foods: A review. *Compr Rev Food Sci Food Saf* 2021; **20**: 4931-4949 [PMID: 34378329 DOI: 10.1111/1541-4337.12817]

43 **Koyama AK**, Pavkov ME, Wu Y, Siegel KR. Is dietary intake of advanced glycation end products associated with mortality among adults with diabetes? *Nutr Metab Cardiovasc Dis* 2022; **32**: 1402-1409 [PMID: 35282981 DOI: 10.1016/j.numecd.2022.02.008]

44 **Linkens AM**, Houben AJ, Niessen PM, Wijckmans NE, de Goei EE, Van den Eynde MD, Scheijen JL, van den Waarenburg MP, Mari A, Berendschot TT, Streese L, Hanssen H, van Dongen MC, van Gool CC, Stehouwer CD, Eussen SJ, Schalkwijk CG. A 4-week high-AGE diet does not impair glucose metabolism and vascular function in obese individuals. *JCI Insight* 2022; **7** [PMID: 35133989 DOI: 10.1172/jci.insight.156950]

45 **Linkens AM**, Eussen SJ, Houben AJ, Kroon AA, Schram MT, Reesink KD, Dagnelie PC, Henry RM, van Greevenbroek M, Wesselius A, Stehouwer CD, Schalkwijk CG. Habitual Intake of Dietary Advanced Glycation End Products Is Not Associated with Arterial Stiffness of the Aorta and Carotid Artery in Adults: The Maastricht Study. *J Nutr* 2021; **151**: 1886-1893 [PMID: 33982103 DOI: 10.1093/jn/nxab097]

46 **Zhang Q**, Shao M, Zhang X, Wang Q, Guo D, Yang X, Li C, Wang Y. The Effect of Chinese Medicine on Lipid and Glucose Metabolism in Acute Myocardial Infarction Through PPARγ Pathway. *Front Pharmacol* 2018; **9**: 1209 [PMID: 30405421 DOI: 10.3389/fphar.2018.01209]

47 **Tuomainen T**, Tavi P. The role of cardiac energy metabolism in cardiac hypertrophy and failure. *Exp Cell Res* 2017; **360**: 12-18 [PMID: 28344054 DOI: 10.1016/j.yexcr.2017.03.052]

48 **Fillmore N**, Mori J, Lopaschuk GD. Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. *Br J Pharmacol* 2014; **171**: 2080-2090 [PMID: 24147975 DOI: 10.1111/bph.12475]

49 **RANDLE PJ**, GARLAND PB, HALES CN, NEWSHOLME EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963; **1**: 785-789 [PMID: 13990765 DOI: 10.1016/s0140-6736(63)91500-9]

50 **Feng H**, Wang X, Chen J, Cui J, Gao T, Gao Y, Zeng W. Nuclear Imaging of Glucose Metabolism: Beyond ^{18}F-FDG. *Contrast Media Mol Imaging* 2019; **2019**: 7954854 [PMID: 31049045 DOI: 10.1155/2019/7954854]

51 **Kircher M**, Lapa C. Novel Noninvasive Nuclear Medicine Imaging Techniques for Cardiac Inflammation. *Curr Cardiovasc Imaging Rep* 2017; **10**: 6 [PMID: 28357026 DOI: 10.1007/s12410-017-9400-x]

World Journal of Diabetes

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

World J Diabetes 2022 November 15; 13(11): 986-1000

DOI: 10.4239/wjd.v13.i11.986

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Observational Study

Risk factor analysis and clinical decision tree model construction for diabetic retinopathy in Western China

Yuan-Yuan Zhou, Tai-Cheng Zhou, Nan Chen, Guo-Zhong Zhou, Hong-Jian Zhou, Xing-Dong Li, Jin-Rui Wang, Chao-Fang Bai, Rong Long, Yu-Xin Xiong, Ying Yang

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

P-Reviewer: Alsaidan A, Saudi Arabia; Laddha UD, India

Received: June 13, 2022
Peer-review started: June 13, 2022
First decision: August 1, 2022
Revised: August 20, 2022
Accepted: October 27, 2022
Article in press: October 27, 2022
Published online: November 15, 2022

Yuan-Yuan Zhou, Hong-Jian Zhou, Xing-Dong Li, Department of Endocrinology and Metabolism, The Sixth Affiliated Hospital of Kunming Medical University, The People's Hospital of Yuxi City, Yuxi 653100, Yunnan Province, China

Tai-Cheng Zhou, Jin-Rui Wang, Chao-Fang Bai, Rong Long, Yu-Xin Xiong, Ying Yang, Department of Endocrinology and Metabolism, Affiliated Hospital of Yunnan University, The Second People's Hospital of Yunnan Province, Kunming 650021, Yunnan Province, China

Nan Chen, Guo-Zhong Zhou, Department of Endocrinology and Metabolism, The Frist People's Hospital of Anning City, Anning City 650300, Yunnan Province, China

Corresponding author: Ying Yang, PhD, Chief Doctor, Professor, Department of Endocrinology and Metabolism, Affiliated Hospital of Yunnan University, The Second People's Hospital of Yunnan Province, No. 176 Qingnian Road, Kunming 650021, Yunnan Province, China.
yangying2072@126.com

Abstract

BACKGROUND

Diabetic retinopathy (DR) is the driving force of blindness in patients with type 2 diabetes mellitus (T2DM). DR has a high prevalence and lacks effective therapeutic strategies, underscoring the need for early prevention and treatment. Yunnan province, located in the southwest plateau of China, has a high prevalence of DR and an underdeveloped economy.

AIM

To build a clinical prediction model that will enable early prevention and treatment of DR.

METHODS

In this cross-sectional study, 1654 Han population with T2DM were divided into groups without (n = 826) and with DR (n = 828) based on fundus photography. The DR group was further subdivided into non-proliferative DR (n = 403) and proliferative DR (n = 425) groups. A univariate analysis and logistic regression analysis were conducted and a clinical decision tree model was constructed.

RESULTS

Diabetes duration ≥ 10 years, female sex, standing- or supine systolic blood

pressure (SBP) ≥ 140 mmHg, and cholesterol ≥ 6.22 mmol/L were risk factors for DR in logistic regression analysis (odds ratio = 2.118, 1.520, 1.417, 1.881, and 1.591, respectively). A greater severity of chronic kidney disease (CKD) or hemoglobin A 1c increased the risk of DR in patients with T2DM. In the decision tree model, diabetes duration was the primary risk factor affecting the occurrence of DR in patients with T2DM, followed by CKD stage, supine SBP, standing SBP, and body mass index (BMI). DR classification outcomes were obtained by evaluating standing SBP or BMI according to the CKD stage for diabetes duration < 10 years and by evaluating CKD stage according to the supine SBP for diabetes duration ≥ 10 years.

CONCLUSION

Based on the simple and intuitive decision tree model constructed in this study, DR classification outcomes were easily obtained by evaluating diabetes duration, CKD stage, supine or standing SBP, and BMI.

Key Words: Diabetic retinopathy; Type 2 diabetes; Western China; Decision tree

Core Tip: Due to the underdeveloped economy and higher prevalence of diabetic retinopathy (DR), Yunnan province is facing a serious task of prevention. Based on a large sample of the Han population with type 2 diabetes mellitus in Yunnan province, this study constructed a cost-effective predictive model that may facilitate the timely and individualized estimation of DR risk.

Citation: Zhou YY, Zhou TC, Chen N, Zhou GZ, Zhou HJ, Li XD, Wang JR, Bai CF, Long R, Xiong YX, Yang Y. Risk factor analysis and clinical decision tree model construction for diabetic retinopathy in Western China. *World J Diabetes* 2022; 13(11): 986-1000

URL: https://www.wjgnet.com/1948-9358/full/v13/i11/986.htm

DOI: https://dx.doi.org/10.4239/wjd.v13.i11.986

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a common chronic disease, poses a severe threat to human health and quality of life. By 2045, an estimated 552 million people worldwide will suffer from T2DM[1]. Given the socio-economic developments over the past 40 years, China has become increasingly urbanized, with a growing aging population. Moreover, obesity and overweight caused by lifestyle changes contribute to the growing burden of T2DM in China[2]. As a major vascular complication of T2DM, diabetic retinopathy (DR) is currently largely responsible for blindness in the working-class[3,4].

The two major challenges associated with DR include the high disease prevalence and lack of effective treatments. The global prevalence of DR is 34.6%. As of 2011, 126.6 million people suffered from DR, and this number is estimated to reach 191.0 million in 2030 without effective and timely measures[5]. The concerning prevalence and severity of DR worldwide may be modulated by racial/ethnic disparities, socio-economic status, health care systems, lifestyles, research methods, and other factors[6]. Regarding Asian populations, the prevalence of DR varies according to the region. For example, the prevalence of DR is 20.1%[7], 25.7%[8], and 35.0%[9] in Chinese Singaporeans, Chinese Americans, and Taiwanese Chinese, respectively. Further, the prevalence of DR in inland areas of China is 23%. It is also higher in rural areas than in urban areas and in northern areas than in southern and eastern coastal areas[10-12].

Nevertheless, there is currently a paucity of effective treatments for DR. In addition to systematic interventions for controlling blood glucose levels, blood pressure, and blood lipid levels, several modern therapies have been developed, such as laser photocoagulation[13] and intravitreal injections of anti-vascular endothelial growth factor (VEGF) antibodies or glucocorticoids, which can delay the progress of proliferative DR (PDR)[14,15]. However, several side effects associated with these therapies should be noted. For instance, photocoagulation may cause potential retinal damage, anti-VEGF injections are associated with relapse after drug withdrawal, and glucocorticoid use contributes to cataracts and elevated intraocular pressure in a considerable number of patients. Moreover, intraocular injections may cause complications such as endophthalmitis, intraocular hemorrhage, vitreous hemorrhage, and even retinal detachment[14,15]. Therefore, the utilization of these therapeutic options in clinical practice should be based on systematic evaluation and strict indications.

The high prevalence of DR and lack of efficient therapeutic strategies are associated with reduced quality of life of patients and pose a substantial socio-economic burden on individuals, families, and the society[16]. According to an analysis of the pedigree of T2DM in Yunnan province, the prevalence of DR [17] approximates the national average[10]. Therefore, the active search for associated risk factors is a fundamental priority for the prevention of DR. In this regard, a decision tree model established using identified risk factors is a useful tool. Distinct from traditional statistical methods such as logistic regression analysis, decision trees are effective machine-learning algorithms that solve classification problems. This method obtains a set of effective classification rules through systematic learning of multiple attributes of samples with known classification results. When faced with new unknown samples, the choice of classification or characteristic attributes can be quickly obtained based on the set of rules extracted from the established decision tree[18-20]. Thus, a decision tree is a prediction model with a simple and intuitive flowchart structure that is particularly suitable for use in clinical practice. This study examined the risk factors associated with DR in the Han population with T2DM in Yunnan province and constructed a clinical decision tree model.

MATERIALS AND METHODS

Study subjects

Patients from the Han population with T2DM were enrolled from the Department of Endocrinology, Affiliated Hospital of Yunnan University. All patients fulfilled the Chinese Diabetes Association criteria for the diagnosis of T2DM[21]. The criteria for exclusion were as follows: (1) Age < 18 years; (2) positive islet autoantibodies [including islet cell autoantibodies and autoantibodies to glutamic acid decarboxylase-65, insulin, the tyrosine phosphatases islet antigen 2 (IA-2) and IA-2β, and zinc transporter 8]; (3) acute complications of diabetes mellitus (including diabetic ketoacidosis or diabetic hyperosmolar state); (4) severe hepatic damage; (5) malignant tumors; (6) acute or chronic infectious diseases; (7) other eye diseases (including glaucoma, retinal vascular occlusion, and ischemic optic neuropathy); and (8) pregnancy. Finally, 1654 patients with T2DM were enrolled.

Ethical principles

This study was approved by the Ethics Committee of Affiliated Hospital of Yunnan University (No. 2021062), and written informed consent was obtained from all participants according to the principles of the Helsinki Declaration.

This trial registration was registered at ChiCTR (ChiCTR2100041888; registration on January 9, 2021, http://www.chictr.org.cn/index.aspx).

Clinical information collection

Patient sex, age, diabetes duration, height, weight, waist circumference, hip circumference, waist-hip ratio (WHR), body mass index (BMI), and systolic blood pressure (SBP) and diastolic blood pressure (DBP) (both in the standing and supine positions) were recorded.

Laboratory assessments

All patients fasted at 22:00 the day before blood collection. At 8:00 the next day, 6 mL of venous blood was collected. Fasting blood glucose (Glu0), hemoglobin A 1c (HbA1c), serum creatinine (Scr), blood urea nitrogen (BUN), uric acid (UA), triglycerides (Trig), cholesterol (Chol), high-density lipoprotein Chol (HDL-C), and low-density lipoprotein Chol (LDL-C) were measured. Based on the formula eGFR (mL/min/1.73 m^2) = 175 × Scr (mg/dL) - 1.234 × age - 0.179 × (0.790 for women)[22], the estimated glomerular filtration rate (eGFR) was calculated.

Ophthalmological measurements

All patients underwent non-mydriatic fundus photography and were evaluated according to the international clinical grading standards for DR established by the American Academy of Ophthalmology[23].

Definitions

According to the DR Preferred Practice Pattern[23]. DR was clinically classified into two types—non-PDR (NPDR) and PDR; the latter was identified by neovascularization and preretinal or vitreous hemorrhage. To further study the effect of blood pressure-related indicators (SBP and DBP in both the standing and supine positions), obesity-related indicators (BMI and waist circumference), blood glucose-related indicators (Glu0 and HbA1c), blood lipid-related indicators (Chol, Trig, LDL-C, and HDL-C), and renal function related indicators (UA and eGFR) on DR, these indicators were defined according to relevant guidelines or expert consensus. Abnormal blood pressure was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg[24]. Overweight was defined as BMI ≥ 24 kg/m^2, obesity was defined as BMI ≥ 28 kg/m^2, and abdominal obesity was defined as waist circumference ≥ 90 cm in men

or ≥ 85 cm in women[25]. Well-controlled blood glucose level was defined as Glu0 ≤ 7.0 mmol/L or HbA1c < 7%, generally controlled blood glucose level was defined as 7% ≤ HbA1c < 8%, and poorly controlled blood glucose level was defined as Glu0 > 7.0 mmol/L or HbA1c ≥ 8%[21]. Dyslipidemia was defined as Chol ≥ 6.22 mmol/L, Trig ≥ 2.26 mmol/L, or LDL-C ≥ 4.14 mmol/L and/or HDL-C < 1.04 mmol/L[26]. Hyperuricemia was defined as fasting serum UA > 420 μmol/L[27]. Stages of chronic kidney disease (CKD) were determined by eGFR as follows: CKD stage 1 (G1), eGFR ≥ 90 mL/min; CKD stage 2 (G2), eGFR = 60-89 mL/min; CKD stage 3 (G3), eGFR = 30-59 mL/min; CKD stage 4 (G4), eGFR = 15-29 mL/minl and CKD stage 5 (G5), eGFR < 15 mL/min [22].

Statistical analysis

Based on fundus photography, participants were divided into groups without DR (WDR group, n = 826) or with DR (DR group, n = 828). The DR group was further classified into the NPDR (n = 403) and PDR (n = 425) groups according to severity. All statistical analyses were performed using SPSS (SPSS 20.0, IBM, United States). Differences between the WDR and DR groups or the NPDR and PDR groups were assessed (Table 1). For continuous variables, Welch's t-test was used for normal distributions, while the Mann-Whitney U test was used for skewed distributions. For categorical variables, the chi-square test was performed. Using DR or PDR as the dependent variable, logistic regression analysis was explored to analyze DR or PDR-related risk factors. Logistic regression analysis was performed using forward selection (likelihood ratio), with P < 0.05 as the entry criterion and P > 0.1 as the removal criterion. The decision tree method was performed using the chi-squared automatic interaction detector, with 70% participants as the training dataset, and the remaining 30% as the test dataset. The variable assignments used in both logistic regression analysis and decision tree model are presented in Table 2.

RESULTS

Baseline clinical characteristics

The baseline clinical characteristics of all participants are presented in Table 1.

Univariate analysis of DR-related risk factors

As shown in Table 1, the proportion of women, diabetes duration, supine SBP, standing SBP, supine DBP, Chol, BUN, UA, Scr, Glu0, and HbA1c were higher in the DR group than in the WDR group (all P values < 0.05). In contrast, the DR group had a lower BMI, hip circumference, and eGFR than the WDR group (all P values < 0.05).

Univariate analysis of PDR-related risk factors

The proportion of women, supine SBP, supine DBP, Chol, LDL-C, and BUN were significantly higher in the PDR group than in the NPDR group (all P values < 0.05). However, age, hip circumference, and eGFR were significantly lower in the PDR group than in the NPDR group (all P values < 0.05) (Table 1).

Logistic regression analysis of DR-related risk factors

Women had a 1.520 times higher risk of DR [95% confidence interval (CI): 1.218 to 1.897] compared to men (Figure 1). Patients with diabetes duration ≥ 10 years had a 2.118-fold higher risk of DR than those with diabetes duration < 10 years (95%CI: 1.661 to 2.700). The risk of DR in patients with standing SBP ≥ 140 mmHg was 1.417 times higher than that in patients with standing SBP < 140 mmHg (95%CI: 1.046 to 1.919). Compared to patients with supine SBP < 140 mmHg, those with supine SBP ≥ 140 mmHg had a 1.881-fold higher risk of DR (95%CI: 1.399 to 2.528). Compared to patients with normal Chol, those with Chol ≥ 6.22 mmol/L had a 1.591 times higher risk of DR (95%CI: 1.104 to 2.291). The risk of DR in patients with CKD stages G2, G3, G4, and G5 was 2.206 (95%CI: 1.678 to 2.899), 7.860 (95%CI: 4.573 to 13.512), 9.693 (95%CI: 3.255 to 28.862), and 20.691 (95%CI: 2.540 to 168.581) times higher than that in patients with CKD stage G1. In other words, a greater severity of CKD was associated with a higher risk of DR. The risk of DR in patients with 7% ≤ HbA1c < 8% or HbA1c ≥ 8% was 1.787 (95%CI: 1.198 to 2.664) and 3.073 (95%CI: 2.225 to 4.245) times higher than that in patients with HbA1c < 7%, indicating that worse control of HbA1c was associated with a higher risk of DR.

Logistic regression analysis of PDR-related risk factors

Compared to men, women had a 2.161-fold higher risk of progression to PDR (95%CI: 1.615 to 2.890) (Figure 2). Compared to patients with diabetes duration < 10 years, patients with diabetes duration ≥ 10 years had a 1.483 times higher risk of PDR (95%CI: 1.099 to 2.001). The risk of progression to PDR in patients with CKD stages G3 and G4 was 2.109 (95%CI: 1.362 to 3.266) and 2.290 (95%CI: 1.016 to 5.165) times higher than that in patients with CKD stage G1.

Decision tree modeling of DR-related risk factors

As shown in Figure 3, the importance of variables in the decision tree model was presented as a root-to-

Table 1 Univariate analysis of risk factors for diabetic retinopathy or proliferative diabetic retinopathy

Variable	WDR group (*n* = 826)	DR group (*n* = 828)	NPDR group (*n* = 403)	PDR group (*n* = 425)	*P* value[1]	*P* value[2]
Age (yr)	53.9 ± 10.3	54.9 ± 10.7	56.2 ± 10.9	53.6 ± 10.3	0.056	< 0.001
Sex (male/female)	487/339	424/404	238/165	186/239	0.002	< 0.001
Diabetes duration (yr)	6.9 ± 5.2	11.2 ± 6.8	10.9 ± 7.1	11.4 ± 6.4	< 0.001	0.221
Height (cm)	165.2 ± 8.3	162.5 ± 8.3	164.2 ± 8.5	160.8 ± 7.8	< 0.001	< 0.001
Weight (kg)	68.1 ± 11.7	64.2 ± 11.3	65.7 ± 11.5	62.8 ± 10.9	< 0.001	< 0.001
BMI (kg/m^2)	24.9 ± 3.3	24.3 ± 3.4	24.3 ± 3.3	24.2 ± 3.5	< 0.001	0.880
Waist circumference (cm)	90.8 ± 9.5	89.9 ± 10.0	90.2 ± 9.7	89.6 ± 10.3	0.060	0.399
Hip circumference (cm)	97.6 ± 7.6	95.9 ± 7.4	96.4 ± 7.2	95.4 ± 7.6	< 0.001	0.046
WHR	1.0 ± 0.5	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.284	0.092
Standing SBP (mmHg)	129.0 ± 16.9	136.4 ± 22.9	135.9 ± 22.6	136.9 ± 23.2	< 0.001	0.517
Standing DBP (mmHg)	83.4 ± 11.6	83.8 ± 13.4	83.5 ± 13.0	84.0 ± 13.7	0.501	0.578
Supine SBP (mmHg)	130.1 ± 16.3	141.0 ± 21.3	139.4 ± 20.6	142.4 ± 22.0	< 0.001	0.045
Supine DBP (mmHg)	82.6 ± 11.2	85.0 ± 12.3	83.9±12.0	86.1 ± 12.4	< 0.001	0.012
Glu0 (mmol/L)	9.4 ± 3.3	9.9 ± 3.7	10.0 ± 3.7	9.8 ± 3.7	0.008	0.647
HbA1c (%)	9.1 ± 2.5	9.8 ± 2.4	9.9 ± 2.5	9.7 ± 2.3	< 0.001	0.402
Chol (mmol/L)	4.4 ± 1.5	5.0 ± 1.5	4.8 ± 1.5	5.1 ± 1.4	< 0.001	0.013
Trig (mmol/L)	2.8 ± 2.3	2.6 ± 2.4	2.6 ± 2.5	2.6 ± 2.4	0.033	0.856
HDL-C (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	0.057	0.095
LDL-C (mmol/L)	2.9 ± 0.9	3.0 ± 1.0	2.9 ± 1.0	3.1 ± 1.1	0.101	0.004
BUN (μmol/L)	5.1 ± 1.7	6.6 ± 2.8	6.4 ± 2.8	6.8 ± 2.8	< 0.001	0.021
UA (μmol/L)	347.6 ± 103.4	359.4 ± 102.0	355.4 ± 106.4	363.2 ± 97.6	0.019	0.270
Scr (μmol/L)	69.8 ± 28.3	90.0 ± 61.8	86.0 ± 64.1	93.9 ± 59.4	< 0.001	0.068
eGFR (mL/min)	116.9 ± 37.3	98.3 ± 42.4	103.5 ± 40.9	93.3 ± 43.2	< 0.001	0.001

[1]WDR group *vs* DR group.
[2]NPDR group *vs* PDR group.
WDR: Without diabetic retinopathy; DR: Diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy; BMI: Body mass index; WHR: Waist-hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; Glu0: Fasting blood glucose; HbA1c: Glycated hemoglobin A1c; Chol: Cholesterol; Trig: Triglyceride; HDL-C: High-density lipoprotein Chol; LDL-C: Low-density lipoprotein Chol; BUN: Blood urea nitrogen; UA: Uric acid; Scr: Serum creatinine; eGFR: Estimated glomerular filtration rate.

leaf structure, with diabetes duration being the first variable or root node, followed by CKD stage, supine SBP, standing SBP, and BMI, in order of importance. As presented in Table 3, seven "if-then" rules summarized the path from the root node to each leaf node.

DISCUSSION

In this study, we attempted to reveal the DR-related risk factors in Han population with T2DM in Yunnan province and construct a predictive model for personalized DR risk assessment and early preventive effect.

Studies have reported that various factors modulate the effects of age on DR. Although sporadic cases have been reported, the onset of DR before puberty is extremely rare[28,29]. Researchers have suggested that patients with diabetes during adolescence are prone to develop serious vascular complications, including DR, compared to patients with diabetes after adolescence. This could be partly due to the characteristics of adolescent patients; for example, patients at this stage tend to be accompanied by dramatic hormone level fluctuations, and most patients present with type 1 diabetes tend to have relatively poor blood glucose self-management ability[30]. Therefore, this study focused on Han

Table 2 Variable assignment

Variable	Assignment
Sex	0 = male
	1 = female
Age	0 = age < 60 years
	1 = age ≥ 60 years
Diabetes duration	0 = duration < 10 years
	1 = duration ≥ 10 years
Blood pressure	0 = normal (SBP < 140 mmHg and/or DBP < 90 mmHg)
	1 = abnormal (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg)
BMI	0 = normal (BMI < 24 kg/m^2)
	1 = overweight (24 kg/m^2 ≤ BMI < 28 kg/m^2)
	2 = obesity (BMI ≥ 28 kg/m^2)
Waist circumference	0 = normal (male < 90 cm, female < 85 cm)
	1 = increased (male ≥ 90 cm, female ≥ 85 cm)
Glu0	0 = well controlled (Glu0 ≤ 7.0 mmol/L)
	1 = poorly controlled (Glu0 > 7.0 mmol/L)
HbA1c	0 = well controlled (HbA1c < 7%)
	1 = generally controlled (7% ≤ HbA1c < 8%)
	2 = poorly controlled (HbA1c ≥ 8%)
Blood lipids	0 = normal
	1 = dyslipidemia (met any of the following criteria: TG ≥ 2.26 mmol/L; TC ≥ 6.26 mmol/L; LDL ≥ 4.14 mmol/L; HDL < 1.04 mmol/L)
UA	0 = normal
	1 = hyperuricemia (UA > 420 μmmol/L)
CKD stage	0 = G1 (eGFR ≥ 90 mL/min)
	1 = G2 (eGFR = 60-89 mL/min)
	2 = G3 (eGFR = 30-59 mL/min)
	3 = G4 (eGFR = 15-29 mL/min)
	4 = G5 (eGFR < 15 mL/min)

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; Glu0: Fasting blood glucose; HbA1c: Glycated hemoglobin A1c; Trig: Triglyceride; Chol: Cholesterol; LDL-C: Low-density lipoprotein Chol; HDL-C: High-density lipoprotein Chol; UA: uric acid; CKD: Chronic kidney disease; G: Grade; eGFR: Estimated glomerular filtration rate.

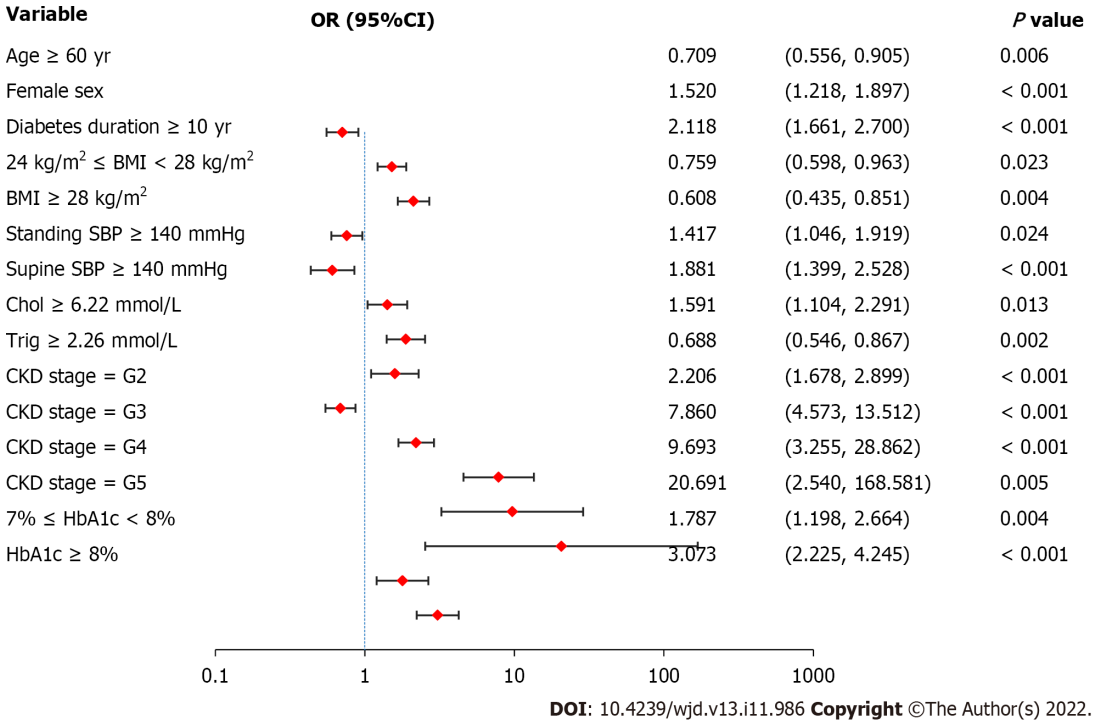
population with T2DM aged ≥ 18 years in Yunnan province to exclude the potential confounding effects of adolescence and type 1 diabetes on the results.

DR has emerged as the leading cause of blindness among 27-75 year olds worldwide[3,21]. A Chinese meta-analysis reported that the prevalence of DR in patients with T2DM was age related, increasing from 12.55% in adults aged 30-39 years to 20.44% in adults aged 60-69 years and decreasing to 11.22% in those aged ≥ 80 years[12]. Of the 1654 patients enrolled in this study, 33.49% and 16.56% patients with DR were < 60 years of age (n = 554) and ≥ 60 years of age (n = 274), respectively. This suggests that the peak of DR prevalence in Han population with T2DM in Yunnan province is concentrated in the population aged < 60 years, which accounts for the majority of the social labor force. In addition, univariate analysis (Table 1) revealed no significant difference in age between the DR and WDR groups (P = 0.056). However, overall age was dramatically lower in the PDR group than in the NPDR group (P < 0.001). As shown in Figures 1 and 2, age was not retained in the logistic regression equation. In conclusion, the correlation between age and DR is complex; this association depends on age stratification and may be affected by the degree of vision. This relationship warrants further exploration in future studies.

Table 3 "If-then" rules extracted from decision tree

Rule	If	Then
R1	Diabetes duration < 10 years, CKD stage = G1, standing SBP < 140 mmHg	Then: WDR
R2	Diabetes duration < 10 years, CKD stage = G1, standing SBP ≥ 140 mmHg	Then: DR
R3	Diabetes duration < 10 years, CKD stage = G2, BMI < 24 kg/m^2	Then: DR
R4	Diabetes duration < 10 years, CKD stage = G2, BMI ≥ 24 kg/m^2	Then: WDR
R5	Diabetes duration < 10 years, CKD stage = G3/G4/G5	Then: DR
R6	Diabetes duration ≥ 10 years, supine SBP < 140 mmHg, CKD staging = G1/G5	Then: WDR
R7	Diabetes duration ≥ 10 years, supine SBP < 140 mmHg, CKD staging = G2/G3/G4	Then: DR
R8	Diabetes duration ≥ 10 years, supine SBP ≥ 140 mmHg, CKD staging = G1/G2	Then: WDR
R9	Diabetes duration ≥ 10 years, supine SBP ≥ 140 mmHg, CKD staging = G3/G4/G5	Then: DR

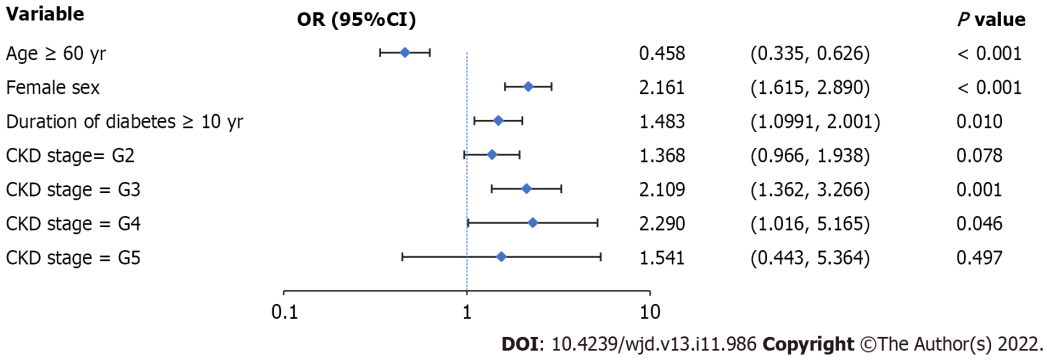
R: Rule; CKD: Chronic kidney disease; SBP: Systolic blood pressure; WDR: Without diabetic retinopathy; DR: Diabetic retinopathy.



DOI: 10.4239/wjd.v13.i11.986

Figure 1 Logistic regression analysis of diabetic retinopathy-related risk factors. Female sex, diabetes duration ≥ 10 years, standing systolic blood pressure (SBP) ≥ 140 mmHg, supine SBP ≥ 140 mmHg, cholesterol ≥ 6.22 mmol/L, greater severity of chronic kidney disease, and worse control of hemoglobin A 1c are associated with a higher risk of diabetic retinopathy. Values are shown using a base 10, logarithmic scale. DR: Diabetic retinopathy; OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; SBP: Systolic blood pressure; Chol: Cholesterol; Trig: Triglyceride; CKD: Chronic kidney disease; HbA1c: Glycated hemoglobin A1c.

The relationship between sex and DR is unclear. A study from Germany and Australia based on 120000 samples suggested that women are more likely to develop DR than men[31]. Similarly, studies in the United Kingdom and Japan have reported that women are more prone to visual impairments than men[32,33]. Other studies from the United States and India, however, have reported that the men have a higher risk of DR than women[6,34,35]. In particular, the United Kingdom Prospective Diabetes Study (UKPDS) has proposed that DR progression is associated with the male sex[36]. Therefore, it is necessary to further explore the correlation between sex and DR. Univariate analysis (Table 1) revealed that the proportion of women was significantly higher in the DR group than in the WDR group (P = 0.002). Moreover, the proportion of women was significantly higher in the PDR group than in the NPDR group (P < 0.001). Indeed, further logistic regression analysis (Figures 1 and 2) re-emphasizes the importance of female sex. These findings suggest that female sex not only is a risk factor for the development of DR in patients with T2DM but also contributes to the progression of DR to PDR, at least



DOI: 10.4239/wjd.v13.i11.986

Figure 2 Logistic regression analysis of proliferative diabetic retinopathy-related risk factors. Female sex, diabetes duration ≥ 10 years, and chronic kidney disease stage G3 or G4 are risk factors for the progression to proliferative diabetic retinopathy. Values are shown using a base 10, logarithmic scale. Abbreviations: PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; OR: Odds ratio; CI: Confidence interval; CKD: Chronic kidney disease.

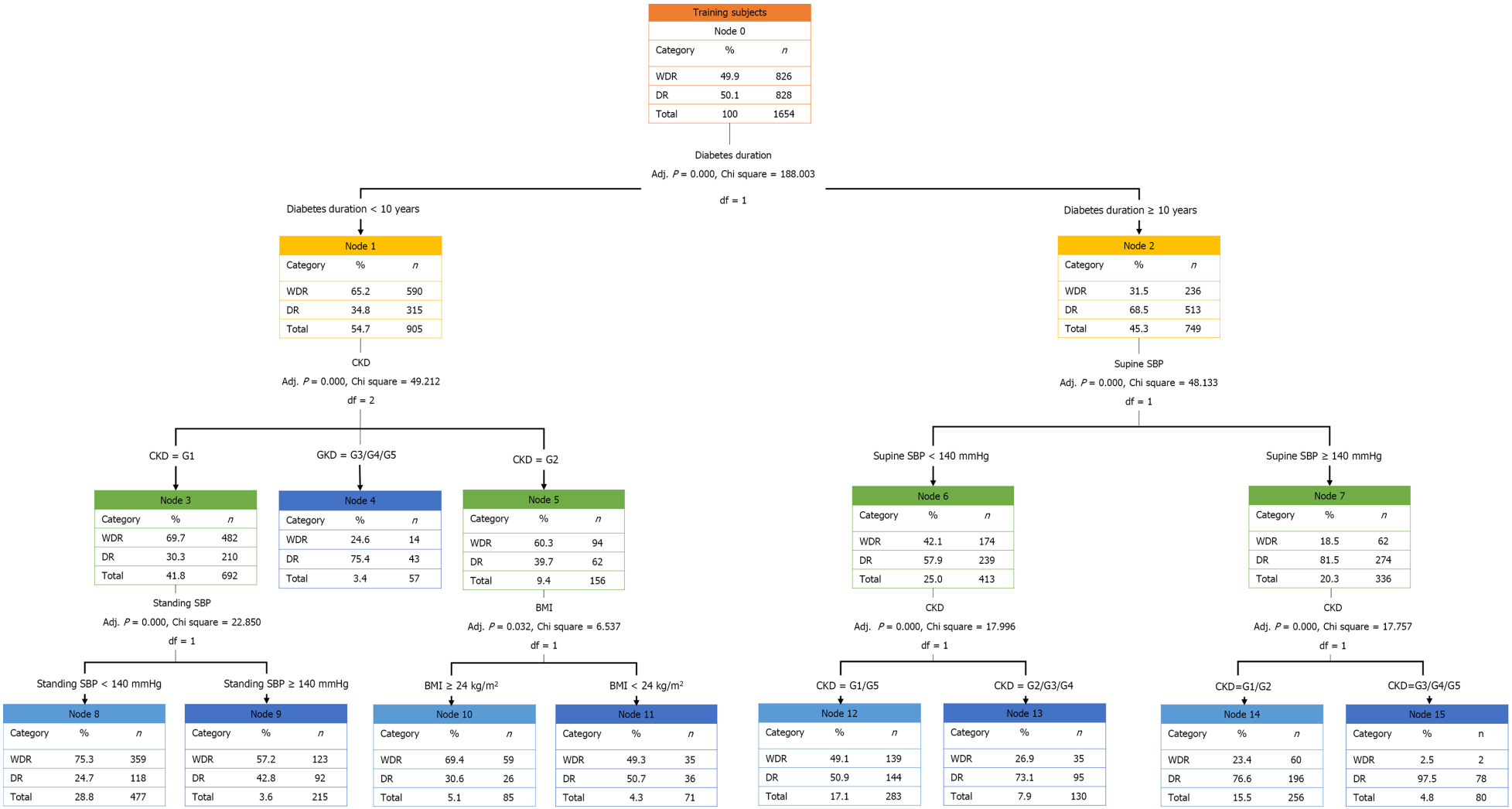
in Yunnan province.

It is well-established that the diabetes duration majorly affects the occurrence and progression of DR [23,37]. A case-control study in South Korea demonstrated that of 523 patients with T2DM, 44.9% developed DR, and 13.6% developed PDR. The average diabetes duration to mild NPDR, moderate-severe NPDR, and PDR was 14.8, 16.7, and 17.3 years, respectively[38]. Based on the Chinese population, a meta-analysis indicated that the prevalence of DR in patients newly diagnosed with diabetes and patients with a disease course of ≥ 10 years was 9.00% and 55.52%, respectively[12]. Univariate analysis (Table 1) revealed that the diabetes duration in the DR group was substantially longer than that in the WDR group ($P < 0.001$). In contrast, the diabetes duration did not differ significantly between the PDR and NPDR groups ($P = 0.221$). However, logistic regression analysis (Figures 1 and 2) revealed that a diabetes duration ≥ 10 years was an extremely risk for the occurrence and progression of DR. Crucially, the diabetes duration was classified as the root node of the DR decision tree model (Table 3 and Figure 3), emphasizing that the diabetes duration is critical in DR risk assessment.

In addition to the diabetes duration, good glycemic control is considered a key factor for reducing vascular complications of diabetes[39]. This study focused on two blood-glucose-related indicators, Glu0 and HbA1c. Compared with the transient characteristics of Glu0, HbA1c reflects the overall level of blood glucose control of patients in the prior 2 to 3 months. In this study, univariate analysis (Table 1) indicated that both Glu0 and HbA1c were substantially greater in the DR group than in the WDR group (all $P < 0.05$). As shown in Figure 1, HbA1c but not Glu0 was retained in the logistic regression equation. These findings suggest that poor HbA1c control is associated with a higher risk of DR. In conclusion, compared to Glu0, HbA1c, which reflects long-term glucose control levels, is more relevant for the prevention of DR. However, HbA1c did not negatively affect the progression of DR. Of note, large clinical studies such as the UKPDS[40,41] and the Diabetes Control and Complications Study[42] have demonstrated that early and intensive glucose control can reduce the occurrence and progression of diabetic vascular complications, including DR. However, good glycemic control is not equivalent to excessive control. Indeed, extensive evidence indicates that recurrent hypoglycemic episodes caused by excessive strict glycemic control with insulin are associated with the early deterioration of DR, but the underlying mechanisms are unclear[43-45].

Previous studies have demonstrated that hypertension is linked to the development and severity of DR[46,47]. Since patients with diabetes are prone to have complications of postural blood pressure changes[48,49], data on standing and supine blood pressure were collected simultaneously. Univariate analysis (Table 1) revealed that standing or supine SBP and supine DBP were significantly lower in the WDR group than in the DR group (all $P < 0.001$), but there was no obvious difference in standing DBP between the groups ($P = 0.501$). Although supine SBP and supine DBP were lower in the NPDR group than in the PDR group (all $P < 0.05$), no significant intergroup differences existed in standing SBP and standing DBP (all $P > 0.05$). Further logistic regression analysis (Figures 1 and 2) indicated that SBP had an effect on DR occurrence but not DR progression. In addition, supine SBP ≥ 140 mmHg was the leaf node of the decision tree model, second only to diabetes duration. Our results emphasize the detrimental effects of elevated SBP (especially supine SBP) on DR, suggesting that good blood pressure control is vital for the prevention of DR. Furthermore, the benefits of certain antihypertensive drugs, particularly angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, are not limited to lowering blood pressure[50,51] and may also benefit DR through neuroprotection[52,53], increasing insulin sensitivity[54], anti-inflammatory effects[55], and inhibiting the blood-eye barrier[56,57].

To date, the complex link between dyslipidemia and DR has remained controversial[10,47,58,59]. In this study, the occurrence and development of DR seemed to be more strongly affected by Chol than by



DOI: 10.4239/wjd.v13.i11.986

Figure 3 Training dataset of decision tree model for diabetic retinopathy. Based on the decision tree model constructed in this study, the diabetic retinopathy classification outcomes are obtained by evaluating standing systolic blood pressure (SBP) or body mass index according to the chronic kidney disease (CKD) stage for patients with a diabetes duration < 10 years and the evaluation of CKD stage according to the supine SBP for patients with a diabetes duration ≥ 10 years. WDR: Without diabetic retinopathy; DR: Diabetic retinopathy; CKD: Chronic kidney disease; SBP: Systolic blood pressure; BMI: Body mass index.

Trig, HDL-C, and LDL-C Univariate analysis (Table 1) revealed that Chol was significantly higher in the DR group than in the WDR group ($P < 0.001$) and significantly higher in the PDR group than in the NPDR group ($P = 0.013$). Moreover, as shown in Figures 1 and 2, Chol increased the risk of developing DR in patients with T2DM, but had no remarkable impact on DR progression. A recent meta-analysis revealed that lipid-lowering drugs exerted a protective effect on the progression of DR[60] but did not affect the deterioration of visual acuity or aggravation of hard exudate. Therefore, further large-scale clinical trials are urgently needed to substantiate the necessity of early application of lipid-lowering drugs in patients with DR.

In parallel with the tremendous rise in the global prevalence of obesity, the prevalence of obesity-related T2DM has also increased annually[2]. However, the relationship between obesity and DR has not been fully elucidated. Reports from Wisconsin illustrated that obesity (defined by BMI) was not independently implicated in the occurrence or progression of DR in patients with T2DM within 10 years [61]. Similarly, in the Hoorn study, WHR, but not BMI, was related to the occurrence of DR[62]. However, data based on Asian populations have provided the opposite conclusions. For example, based on a sample of 420 Asian patients with T2DM, Man *et al*[63] reported that BMI was negatively correlated with mild, moderate, and severe DR, but WHR was positively correlated with DR severity. Subsequently, a Korean study demonstrated that higher BMI, increased waist circumference, and higher body fat content (measured by dual-energy X-ray) were notably correlated with a lower risk of DR[64]. In this study, data regarding relevant body mass indicators were collected, including those on BMI, waist circumference, hip circumference, and WHR. Univariate analysis (Table 1) revealed that BMI and hip circumference in the DR group were remarkably lower than those in the WDR group (all $P < 0.001$), but waist circumference was did not differ across the groups ($P = 0.060$). Although the PDR group had a lower hip circumference than the NPDR group ($P = 0.046$), there were no significant intergroup differences in BMI and waist circumference ($P > 0.05$). In addition, an obvious difference in WHR was not noted among groups (all $P > 0.05$). Although the logistic regression model ultimately did not retain BMI, waist circumference, hip circumference, and WHR (Figures 1 and 2), the decision tree model (Table 3 and Figure 3) supported the protective effect of higher BMI for the assessment of DR risk. In general, although the relationship between these obesity-relevant indicators and DR is yet to be confirmed, it can be conjectured that excessively low BMI is not conducive to protection against DR in the Asian population.

Among BUN, Scr, and UA, eGFR calculated using Scr is the gold standard for CKD staging[65]. In addition, CKD staging is an essential approach to evaluating the severity of diabetic nephropathy in clinical practice. Univariate analysis (Table 1) indicated that eGFR was significantly lower in the DR group than in the WDR group ($P < 0.001$) and was significantly lower in the PDR group than in the NPDR group ($P = 0.001$). Furthermore, Figures 1 and 2 highlight the importance of CKD staging in the risk assessment of DR. In this regard, the risk of occurrence and progression of DR increased stepwise with each additional risk level of CKD staging. Notably, in the decision tree model (Table 3 and Figure 3), CKD staging was second only to diabetes duration for DR risk assessment. In particular, CKD staging is a key indicator of diabetic nephropathy, and the current results also suggest that DR is often comorbid with diabetic nephropathy. This highlights the need to simultaneously screen for diabetic nephropathy in patients with DR.

Differing from traditional statistical methods such as logistic regression analysis, decision trees are successfully employed in the field of medicine with its advantages in solving classification problems, that is, qualitatively judging the possibility of each risk factor at a specific level. Decision trees obtain a set of effective classification rules by systematically learning multiple attributes of the samples with known classification results. When faced with new unknown samples, the appropriate classification or characteristic attributes can be quickly obtained based on the set of rules extracted from the established decision tree[18-20]. In other words, three basic elements compose the decision tree: root node, internal node, and leaf node. The root node is the main feature attribute in the model, the internal node is the secondary attribute judgment based on the root node, and the leaf node is the final classification outcome of the model.

This study extended traditional statistical analysis by building a DR decision model using machine learning based on the attributes of T2DM samples. In the decision tree model, diabetes duration was demonstrated to primarily affect the occurrence of DR in patients with T2DM, namely, the root node. The extraction rules were interpreted as follows: for patients with diabetes duration < 10 years, if they met the criteria of: (1) CKD stage = G3/G4/G5; (2) CKD stage = G2 and BMI < 24 kg/m^2; or (3) CKD stage = G1 and standing SBP ≥ 140 mmHg, then DR was prone to occur. In contrast, for patients with diabetes duration ≥ 10 years, if they met the criteria of: (1) Supine SBP < 140 mmHg and CKD stage = G2/G3/G4; or (2) supine SBP ≥ 140 mmHg and CKD stage = G3/G4/G5, then DR was prone to occur. This model may assist clinicians in Yunnan province (particularly primary medical staff who lack relevant DR detection approaches such as ophthalmoscope) to make more effective clinical predictions of DR risk in patients with T2DM. Our decision tree model is simple and intuitive, highlighting its potential for application in clinical practice.

However, there are several limitations that should be noted. First of all, this study did not record in detail the medication of patients, especially the use of anti-diabetic, lipid-lowering and antihypertensive drugs. Secondly, Yunnan province is located in the western plateau of China, and its climate, cultural

and economic conditions are very different from those of plain areas. Therefore, these confounding factors should be included in the future to enhance the integrity and reliability of research conclusions.

CONCLUSION

Female sex, diabetes duration ≥ 10 years, standing or supine SBP ≥ 140 mmHg, Chol ≥ 6.22 mmol/L, deterioration of CKD stage, and HbA1c are key DR-related risk factors in the Han population with T2DM in Yunnan province. The concise and intuitive DR prediction model developed through machine learning in this study could help clinicians quickly predict DR outcomes based on patients' potential risk factors and conduct early individualized interventions.

ARTICLE HIGHLIGHTS

Research background

Yunnan province has a high prevalence of diabetic retinopathy (DR). Accordingly, it is of great significance to explore the DR-related factors and to construct an economic and intuitive clinical prediction model.

Research motivation

The research motivation is early intervention using the DR-related risk factors from the perspective of a predictive model to reduce the prevalence of DR in patients with type 2 diabetes mellitus (T2DM).

Research objectives

The research intends to establish a prediction model that allows clinically early prevention and treatment of DR.

Research methods

A total of 1654 Han population with T2DM were recruited in this study and were grouped in the without DR and DR groups. The DR group was further subgrouped according to the severity of DR. Then, univariate analysis, logistic regression analysis, and clinical decision tree models of clinical data were performed.

Research results

Based on the decision tree model constructed in this study, DR classification outcomes were obtained by evaluating diabetes duration followed by stages of chronic kidney disease, supine systolic blood pressure (SBP), standing SBP, and body mass index.

Research conclusions

Personalized interventions for DR-related risk factors based on a decision tree model may potentially reduce the prevalence of DR.

Research perspectives

In this study, patients with T2DM in Western China were taken as samples to analyze the influencing factors of DR and build a clinical prediction model. In the future, it is hoped that the prediction model can produce certain social and economic benefits in clinical practice. In addition, when comparing with other clinical studies on DR, we found some controversies, such as the impact of sex and body mass index on DR, which opened up a new direction for future research.

FOOTNOTES

Author contributions: Zhou YY contributed to the conception and design, acquisition of data or analysis and interpretation of data, and drafting the article or revising it critically for important intellectual content; Yang Y and Zhou TC were responsible for supervision, project administration, and funding acquisition; Chen N and Zhou GZ were responsible for literature and formal analysis; Wang JR, Bai CF, Long R, Xiong YX, Zhou HJ, and Li XD were responsible for patient recruitment and clinical data curation; all authors gave final approval of the version to be published.

Supported by the Natural Science Foundation of China, No. 82160159; Natural Science Foundation of Yunnan Province, No. 202101AY070001-199; Scientific Research Fund of Yunnan Education Department, No. 2021J0303; and Postgraduate Innovation Fund of Kunming Medical University, No. 2020D009.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Affiliated Hospital of Yunnan University (Approval No. 2021062).

Informed consent statement: Written informed consent was obtained from all participants.

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: Yuan-Yuan Zhou 0000-0003-2608-4866; Chao-Fang Bai 0000-0003-4724-6537; Ying Yang 0000-0001-5753-7106.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Whiting DR**, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011; **94**: 311-321 [PMID: 22079683 DOI: 10.1016/j.diabres.2011.10.029]
2 **The Lancet**. Obesity and diabetes in 2017: a new year. *Lancet* 2017; **389**: 1 [PMID: 28091363 DOI: 10.1016/S0140-6736(17)30004-1]
3 **American Diabetes Association**. 10. Microvascular Complications and Foot Care. *Diabetes Care* 2017; **40**: S88-S98 [PMID: 27979897 DOI: 10.2337/dc17-S013]
4 **Gyawali R**, Toomey M, Stapleton F, Dillon L, Zangerl B, Keay L, Jalbert I. Quality of 2019 American optometric association clinical practice guideline for diabetic eye care. *Ophthalmic Physiol Opt* 2021; **41**: 165-170 [PMID: 33210361 DOI: 10.1111/opo.12763]
5 **Ruta LM**, Magliano DJ, Lemesurier R, Taylor HR, Zimmet PZ, Shaw JE. Prevalence of diabetic retinopathy in Type 2 diabetes in developing and developed countries. *Diabet Med* 2013; **30**: 387-398 [PMID: 23331210 DOI: 10.1111/dme.12119]
6 **Zhang X**, Saaddine JB, Chou CF, Cotch MF, Cheng YJ, Geiss LS, Gregg EW, Albright AL, Klein BE, Klein R. Prevalence of diabetic retinopathy in the United States, 2005-2008. *JAMA* 2010; **304**: 649-656 [PMID: 20699456 DOI: 10.1001/jama.2010.1111]
7 **Chiang PP**, Lamoureux EL, Cheung CY, Sabanayagam C, Wong W, Tai ES, Lee J, Wong TY. Racial differences in the prevalence of diabetes but not diabetic retinopathy in a multi-ethnic Asian population. *Invest Ophthalmol Vis Sci* 2011; **52**: 7586-7592 [PMID: 21862647 DOI: 10.1167/iovs.11-7698]
8 **Wong TY**, Klein R, Islam FM, Cotch MF, Folsom AR, Klein BE, Sharrett AR, Shea S. Diabetic retinopathy in a multi-ethnic cohort in the United States. *Am J Ophthalmol* 2006; **141**: 446-455 [PMID: 16490489 DOI: 10.1016/j.ajo.2005.08.063]
9 **Chang C**, Lu F, Yang YC, Wu JS, Wu TJ, Chen MS, Chuang LM, Tai TY. Epidemiologic study of type 2 diabetes in Taiwan. *Diabetes Res Clin Pract* 2000; **50** Suppl 2: S49-S59 [PMID: 11024584 DOI: 10.1016/s0168-8227(00)00179-0]
10 **Liu L**, Wu X, Liu L, Geng J, Yuan Z, Shan Z, Chen L. Prevalence of diabetic retinopathy in mainland China: a meta-analysis. *PLoS One* 2012; **7**: e45264 [PMID: 23028893 DOI: 10.1371/journal.pone.0045264]
11 **Pan CW**, Wang S, Qian DJ, Xu C, Song E. Prevalence, Awareness, and Risk Factors of Diabetic Retinopathy among Adults with Known Type 2 Diabetes Mellitus in an Urban Community in China. *Ophthalmic Epidemiol* 2017; **24**: 188-194 [PMID: 28276757 DOI: 10.1080/09286586.2016.1264612]
12 **Song P**, Yu J, Chan KY, Theodoratou E, Rudan I. Prevalence, risk factors and burden of diabetic retinopathy in China: a systematic review and meta-analysis. *J Glob Health* 2018; **8**: 010803 [PMID: 29899983 DOI: 10.7189/jogh.08.010803]
13 Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. The Diabetic Retinopathy Study Research Group. *Ophthalmology* 1981; **88**: 583-600 [PMID: 7196564]
14 **Nurözler Tabakcı B**, Ünlü N. Corticosteroid Treatment in Diabetic Macular Edema. *Turk J Ophthalmol* 2017; **47**: 156-160 [PMID: 28630791 DOI: 10.4274/tjo.56338]
15 **Whitcup SM**, Cidlowski JA, Csaky KG, Ambati J. Pharmacology of Corticosteroids for Diabetic Macular Edema. *Invest Ophthalmol Vis Sci* 2018; **59**: 1-12 [PMID: 29297055 DOI: 10.1167/iovs.17-22259]

16 **Mazhar K**, Varma R, Choudhury F, McKean-Cowdin R, Shtir CJ, Azen SP; Los Angeles Latino Eye Study Group. Severity of diabetic retinopathy and health-related quality of life: the Los Angeles Latino Eye Study. *Ophthalmology* 2011; **118**: 649-655 [PMID: 21035872 DOI: 10.1016/j.ophtha.2010.08.003]

17 **Cha XP**. Analysis of the incidence of diabetic retinopathy in type 2 diabetes pedigree population in Yunnan. *Kunming Yike Daxue Xuebao* 2012; **12**: 33

18 **Ramezankhani A**, Hadavandi E, Pournik O, Shahrabi J, Azizi F, Hadaegh F. Decision tree-based modelling for identification of potential interactions between type 2 diabetes risk factors: a decade follow-up in a Middle East prospective cohort study. *BMJ Open* 2016; **6**: e013336 [PMID: 27909038 DOI: 10.1136/bmjopen-2016-013336]

19 **Park YM**, Lee BJ. Machine learning-based prediction model using clinico-pathologic factors for papillary thyroid carcinoma recurrence. *Sci Rep* 2021; **11**: 4948 [PMID: 33654166 DOI: 10.1038/s41598-021-84504-2]

20 **Ahn CH**, Lee C, Shim J, Kong SH, Kim SJ, Kim YH, Lee KE, Shin CS, Kim JH, Choi MH. Metabolic changes in serum steroids for diagnosing and subtyping Cushing's syndrome. *J Steroid Biochem Mol Biol* 2021; **210**: 105856 [PMID: 33647522 DOI: 10.1016/j.jsbmb.2021.105856]

21 **Chinese Elderly Type 2 Diabetes Prevention and Treatment of Clinical Guidelines Writing Group**; Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Society; Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Health Care Society; Geriatric Professional Committee of Beijing Medical Award Foundation; National Clinical Medical Research Center for Geriatric Diseases (PLA General Hospital). [Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition)]. *Zhonghua Nei Ke Za Zhi* 2022; **61**: 12-50 [PMID: 34979769 DOI: 10.3760/cma.j.cn112138-20211027-00751]

22 **Levey AS**, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2005; **67**: 2089-2100 [PMID: 15882252 DOI: 10.1111/j.1523-1755.2005.00365.x]

23 **Flaxel CJ**, Adelman RA, Bailey ST, Fawzi A, Lim JI, Vemulakonda GA, Ying GS. Diabetic Retinopathy Preferred Practice Pattern®. *Ophthalmology* 2020; **127**: P66-P145 [PMID: 31757498 DOI: 10.1016/j.ophtha.2019.09.025]

24 **Joint Committee for Guideline Revision**. 2018 Chinese Guidelines for Prevention and Treatment of Hypertension-A report of the Revision Committee of Chinese Guidelines for Prevention and Treatment of Hypertension. *J Geriatr Cardiol* 2019; **16**: 182-241 [PMID: 31080465 DOI: 10.11909/j.issn.1671-5411.2019.03.014]

25 **Zeng Q**, Li N, Pan XF, Chen L, Pan A. Clinical management and treatment of obesity in China. *Lancet Diabetes Endocrinol* 2021; **9**: 393-405 [PMID: 34022157 DOI: 10.1016/S2213-8587(21)00047-4]

26 **Joint committee for guideline revision**. 2016 Chinese guidelines for the management of dyslipidemia in adults. *J Geriatr Cardiol* 2018; **15**: 1-29 [PMID: 29434622 DOI: 10.11909/j.issn.1671-5411.2018.01.011]

27 **Multidisciplinary Expert Task Force on Hyperuricemia and Related Diseases**. Chinese Multidisciplinary Expert Consensus on the Diagnosis and Treatment of Hyperuricemia and Related Diseases. *Chin Med J (Engl)* 2017; **130**: 2473-2488 [PMID: 29052570 DOI: 10.4103/0366-6999.216416]

28 **Mameli C**, Invernizzi A, Bolchini A, Bedogni G, Giani E, Macedoni M, Zuccotti G, Preziosa C, Pellegrini M. Analysis of Retinal Perfusion in Children, Adolescents, and Young Adults with Type 1 Diabetes Using Optical Coherence Tomography Angiography. *J Diabetes Res* 2019; **2019**: 5410672 [PMID: 31205952 DOI: 10.1155/2019/5410672]

29 **Ibanez-Bruron MC**, Solebo AL, Cumberland PM, Rahi JS. Prevalence of diabetic retinopathy in children and young people living with diabetes: protocol for a systematic review. *BMJ Open* 2017; **7**: e018578 [PMID: 29146656 DOI: 10.1136/bmjopen-2017-018578]

30 **Cho YH**, Craig ME, Donaghue KC. Puberty as an accelerator for diabetes complications. *Pediatr Diabetes* 2014; **15**: 18-26 [PMID: 24443957 DOI: 10.1111/pedi.12112]

31 **Awa WL**, Fach E, Krakow D, Welp R, Kunder J, Voll A, Zeyfang A, Wagner C, Schütt M, Boehm B, de Souza M, Holl RW; DPV Initiative; German BMBF Competence Networks Diabetes mellitus and Obesity. Type 2 diabetes from pediatric to geriatric age: analysis of gender and obesity among 120,183 patients from the German/Austrian DPV database. *Eur J Endocrinol* 2012; **167**: 245-254 [PMID: 22645200 DOI: 10.1530/EJE-12-0143]

32 **Hayward LM**, Burden ML, Burden AC, Blackledge H, Raymond NT, Botha JL, Karwatowski WS, Duke T, Chang YF. What is the prevalence of visual impairment in the general and diabetic populations: are there ethnic and gender differences? *Diabet Med* 2002; **19**: 27-34 [PMID: 11869300 DOI: 10.1046/j.0742-3071.2001.00603.x]

33 **Kajiwara A**, Miyagawa H, Saruwatari J, Kita A, Sakata M, Kawata Y, Oniki K, Yoshida A, Jinnouchi H, Nakagawa K. Gender differences in the incidence and progression of diabetic retinopathy among Japanese patients with type 2 diabetes mellitus: a clinic-based retrospective longitudinal study. *Diabetes Res Clin Pract* 2014; **103**: e7-10 [PMID: 24503044 DOI: 10.1016/j.diabres.2013.12.043]

34 **Varma R**, Macias GL, Torres M, Klein R, Peña FY, Azen SP; Los Angeles Latino Eye Study Group. Biologic risk factors associated with diabetic retinopathy: the Los Angeles Latino Eye Study. *Ophthalmology* 2007; **114**: 1332-1340 [PMID: 17306879 DOI: 10.1016/j.ophtha.2006.10.023]

35 **Pradeepa R**, Anitha B, Mohan V, Ganesan A, Rema M. Risk factors for diabetic retinopathy in a South Indian Type 2 diabetic population--the Chennai Urban Rural Epidemiology Study (CURES) Eye Study 4. *Diabet Med* 2008; **25**: 536-542 [PMID: 18346159 DOI: 10.1111/j.1464-5491.2008.02423.x]

36 **Stratton IM**, Kohner EM, Aldington SJ, Turner RC, Holman RR, Manley SE, Matthews DR. UKPDS 50: risk factors for incidence and progression of retinopathy in Type II diabetes over 6 years from diagnosis. *Diabetologia* 2001; **44**: 156-163 [PMID: 11270671 DOI: 10.1007/s001250051594]

37 **Almutairi NM**, Alahmadi S, Alharbi M, Gotah S. The Association Between HbA1c and Other Biomarkers With the Prevalence and Severity of Diabetic Retinopathy. *Cureus* 2021; **13**: e12520 [PMID: 33564524 DOI: 10.7759/cureus.12520]

38 **Yun JS**, Lim TS, Cha SA, Ahn YB, Song KH, Choi JA, Kwon J, Jee D, Cho YK, Park YM, Ko SH. Clinical Course and Risk Factors of Diabetic Retinopathy in Patients with Type 2 Diabetes Mellitus in Korea. *Diabetes Metab J* 2016; **40**: 482-493 [PMID: 27766793 DOI: 10.4093/dmj.2016.40.6.482]

39 **Corcóstegui B**, Durán S, González-Albarrán MO, Hernández C, Ruiz-Moreno JM, Salvador J, Udaondo P, Simó R. Update

on Diagnosis and Treatment of Diabetic Retinopathy: A Consensus Guideline of the Working Group of Ocular Health (Spanish Society of Diabetes and Spanish Vitreous and Retina Society). *J Ophthalmol* 2017; **2017**: 8234186 [PMID: 28695003 DOI: 10.1155/2017/8234186]

40 **Holman RR**, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; **359**: 1577-1589 [PMID: 18784090 DOI: 10.1056/NEJMoa0806470]

41 **Chalmers J**, Cooper ME. UKPDS and the legacy effect. *N Engl J Med* 2008; **359**: 1618-1620 [PMID: 18843126 DOI: 10.1056/NEJMe0807625]

42 **Diabetes Control and Complications Trial Research Group**, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977-986 [PMID: 8366922 DOI: 10.1056/NEJM199309303291401]

43 **Zhang J**, Ma J, Zhou N, Zhang B, An J. Insulin use and risk of diabetic macular edema in diabetes mellitus: a systemic review and meta-analysis of observational studies. *Med Sci Monit* 2015; **21**: 929-936 [PMID: 25816765 DOI: 10.12659/MSM.892056]

44 **Kuo JZ**, Guo X, Klein R, Klein BE, Weinreb RN, Genter P, Hsiao FC, Goodarzi MO, Rotter JI, Chen YD, Ipp E. Association of fasting insulin and C peptide with diabetic retinopathy in Latinos with type 2 diabetes. *BMJ Open Diabetes Res Care* 2014; **2**: e000027 [PMID: 25452868 DOI: 10.1136/bmjdrc-2014-000027]

45 **Hu L**, Li DH. Relationship between modified homeostasis model assessment/correlative serum factors and diabetic retinopathy among type 2 diabetics with insulin therapy in Guangzhou, China. *Int J Ophthalmol* 2014; **7**: 463-468 [PMID: 24967192 DOI: 10.3980/j.issn.2222-3959.2014.03.14]

46 **Pang C**, Jia L, Jiang S, Liu W, Hou X, Zuo Y, Gu H, Bao Y, Wu Q, Xiang K, Gao X, Jia W. Determination of diabetic retinopathy prevalence and associated risk factors in Chinese diabetic and pre-diabetic subjects: Shanghai diabetic complications study. *Diabetes Metab Res Rev* 2012; **28**: 276-283 [PMID: 22139892 DOI: 10.1002/dmrr.1307]

47 **Liu Y**, Yang J, Tao L, Lv H, Jiang X, Zhang M, Li X. Risk factors of diabetic retinopathy and sight-threatening diabetic retinopathy: a cross-sectional study of 13 473 patients with type 2 diabetes mellitus in mainland China. *BMJ Open* 2017; **7**: e016280 [PMID: 28864696 DOI: 10.1136/bmjopen-2017-016280]

48 **de Boer IH**, Bangalore S, Benetos A, Davis AM, Michos ED, Muntner P, Rossing P, Zoungas S, Bakris G. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 1273-1284 [PMID: 28830958 DOI: 10.2337/dci17-0026]

49 **Krolewski AS**, Warram JH, Cupples A, Gorman CK, Szabo AJ, Christlieb AR. Hypertension, orthostatic hypotension and the microvascular complications of diabetes. *J Chronic Dis* 1985; **38**: 319-326 [PMID: 3998049 DOI: 10.1016/0021-9681(85)90078-5]

50 **Wang B**, Wang F, Zhang Y, Zhao SH, Zhao WJ, Yan SL, Wang YG. Effects of RAS inhibitors on diabetic retinopathy: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2015; **3**: 263-274 [PMID: 25660574 DOI: 10.1016/S2213-8587(14)70256-6]

51 **Mbwete GW**, Kilonzo KG, Shao ER, Chamba NG. Suboptimal Blood Pressure Control, Associated Factors, and Choice of Antihypertensive Drugs among Type 2 Diabetic Patients at KCMC, Tanzania. *J Diabetes Res* 2020; **2020**: 4376251 [PMID: 32775461 DOI: 10.1155/2020/4376251]

52 **Zafar S**, Sachdeva M, Frankfort BJ, Channa R. Retinal Neurodegeneration as an Early Manifestation of Diabetic Eye Disease and Potential Neuroprotective Therapies. *Curr Diab Rep* 2019; **19**: 17 [PMID: 30806815 DOI: 10.1007/s11892-019-1134-5]

53 **Narimatsu T**, Ozawa Y, Miyake S, Nagai N, Tsubota K. Angiotensin II type 1 receptor blockade suppresses light-induced neural damage in the mouse retina. *Free Radic Biol Med* 2014; **71**: 176-185 [PMID: 24662196 DOI: 10.1016/j.freeradbiomed.2014.03.020]

54 **Schupp M**, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. *Circulation* 2004; **109**: 2054-2057 [PMID: 15117841 DOI: 10.1161/01.CIR.0000127955.36250.65]

55 **Nagai N**, Izumi-Nagai K, Oike Y, Koto T, Satofuka S, Ozawa Y, Yamashiro K, Inoue M, Tsubota K, Umezawa K, Ishida S. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. *Invest Ophthalmol Vis Sci* 2007; **48**: 4342-4350 [PMID: 17724226 DOI: 10.1167/iovs.06-1473]

56 **Kim JH**, Kim JH, Yu YS, Cho CS, Kim KW. Blockade of angiotensin II attenuates VEGF-mediated blood-retinal barrier breakdown in diabetic retinopathy. *J Cereb Blood Flow Metab* 2009; **29**: 621-628 [PMID: 19107135 DOI: 10.1038/jcbfm.2008.154]

57 **Li Y**, Yan Z, Chaudhry K, Kazlauskas A. The Renin-Angiotensin-Aldosterone System (RAAS) Is One of the Effectors by Which Vascular Endothelial Growth Factor (VEGF)/Anti-VEGF Controls the Endothelial Cell Barrier. *Am J Pathol* 2020; **190**: 1971-1981 [PMID: 32590003 DOI: 10.1016/j.ajpath.2020.06.004]

58 **Liu L**, Wu J, Yue S, Geng J, Lian J, Teng W, Huang D, Chen L. Incidence Density and Risk Factors of Diabetic Retinopathy Within Type 2 Diabetes: A Five-Year Cohort Study in China (Report 1). *Int J Environ Res Public Health* 2015; **12**: 7899-7909 [PMID: 26184262 DOI: 10.3390/ijerph120707899]

59 **Du ZD**, Hu LT, Zhao GQ, Ma Y, Zhou ZY, Jiang T. Epidemiological characteristics and risk factors of diabetic retinopathy in type 2 diabetes mellitus in Shandong Peninsula of China. *Int J Ophthalmol* 2011; **4**: 202-206 [PMID: 22553643 DOI: 10.3980/j.issn.2222-3959.2011.02.20]

60 **Shi R**, Zhao L, Wang F, Liu F, Chen Z, Li R, Liu Y, Lin R. Effects of lipid-lowering agents on diabetic retinopathy: a Meta-analysis and systematic review. *Int J Ophthalmol* 2018; **11**: 287-295 [PMID: 29487821 DOI: 10.18240/ijo.2018.02.18]

61 **Klein R**, Klein BE, Moss SE. Is obesity related to microvascular and macrovascular complications in diabetes? *Arch Intern Med* 1997; **157**: 650-656 [PMID: 9080919]

62 **van Leiden HA**, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Polak BC. Risk factors for

incident retinopathy in a diabetic and nondiabetic population: the Hoorn study. *Arch Ophthalmol* 2003; **121**: 245-251 [PMID: 12583792 DOI: 10.1001/archopht.121.2.245]

63 **Man RE**, Sabanayagam C, Chiang PP, Li LJ, Noonan JE, Wang JJ, Wong TY, Cheung GC, Tan GS, Lamoureux EL. Differential Association of Generalized and Abdominal Obesity With Diabetic Retinopathy in Asian Patients With Type 2 Diabetes. *JAMA Ophthalmol* 2016; **134**: 251-257 [PMID: 26720805 DOI: 10.1001/jamaophthalmol.2015.5103]

64 **Hwang IC**, Bae JH, Kim JM. Relationship between body fat and diabetic retinopathy in patients with type 2 diabetes: a nationwide survey in Korea. *Eye (Lond)* 2019; **33**: 980-987 [PMID: 30760896 DOI: 10.1038/s41433-019-0352-z]

65 **Guo M**, Niu JY, Ye XW, Han XJ, Zha Y, Hong Y, Fang H, Gu Y. Evaluation of various equations for estimating renal function in elderly Chinese patients with type 2 diabetes mellitus. *Clin Interv Aging* 2017; **12**: 1661-1672 [PMID: 29070944 DOI: 10.2147/CIA.S140289]

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