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Preventing cardiovascular complications of type 2 diabetes

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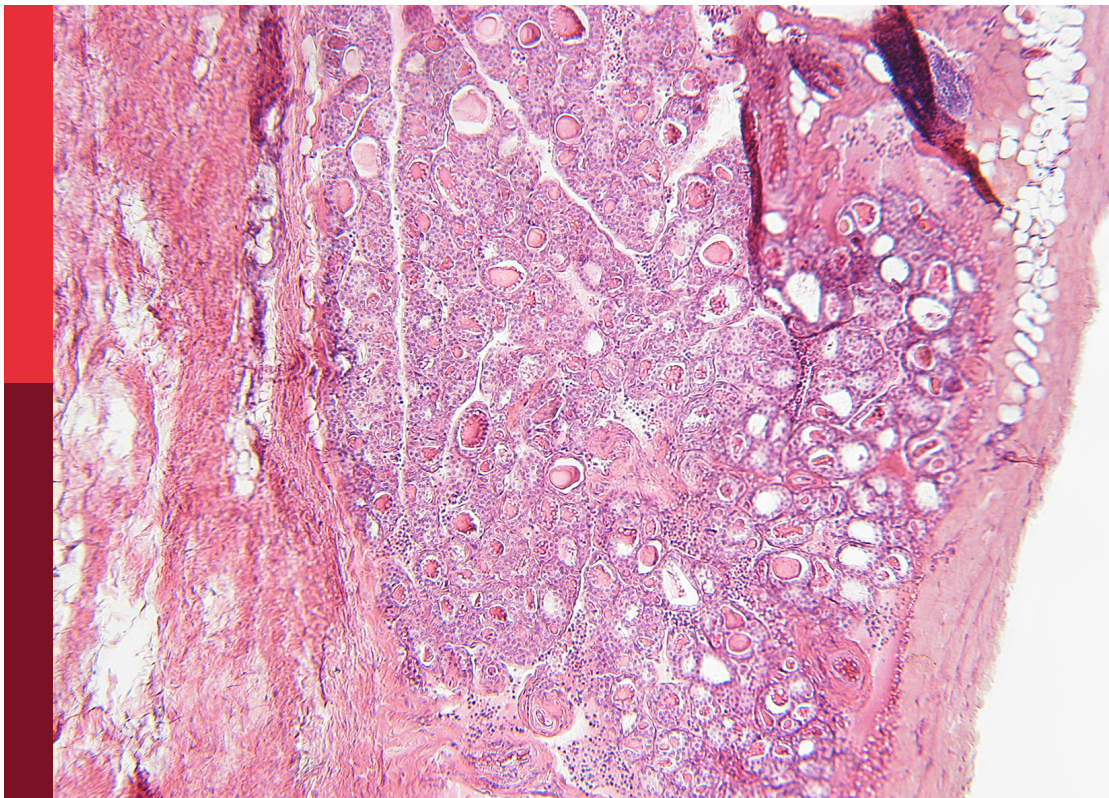

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Preventing cardiovascular complications of type 2 diabetes

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Editorial: Preventing cardiovascular complications of type 2 diabetes

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cardiovascular complications, type 2 diabetes, lifestyle modifications, medical management, community support, exercise

Editorial on the Research Topic
Preventing cardiovascular complications of type 2 diabetes

The global prevalence of type 2 diabetes (T2D) continues to rise at an alarming rate, bringing with it a myriad of health complications, the most critical of which are cardiovascular diseases (CVD) (1). As we grapple with this public health challenge, it becomes imperative to address the prevention of cardiovascular complications in individuals with T2D. These complications, ranging from myocardial infarction, stroke, heart failure, risk of cardiac rupture (Zu et al.) to peripheral artery disease, pose significant risks not only to the quality of life but also to overall mortality. Therefore, a comprehensive approach involving lifestyle changes, medical management, and community support is essential for mitigating these risks.

Unveiling hidden risks: early biomarkers for diabetes complications

Recent advances in biomarker research have opened new avenues for early detection and prevention of diabetes-related complications, potentially revolutionizing patient care and outcomes (2). The identification of useful biomarkers is crucial, as highlighted in this Research Topic. Chang et al. developed a novel nomogram with a simple graphical format for predicting the risk of cardiometabolic diseases which can be used by primary healthcare providers for predicting CVD and preventing their progression. Another intriguing potential approach for early diagnosis is the one explored by Tusongtuoheti et al., who employed interpretable machine learning models to predict the risk of subclinical atherosclerosis in patients with T2D in China. Interestingly, age, albumin, total protein,

total cholesterol, and serum creatinine have been identified as the top five contributing variables in this predictive model.

In the study by Cui et al., two datasets were integrated to identify differentially expressed genes (DEGs) between controls and type 2 diabetic cardiomyopathy cases. The team analyzed immune cell infiltration, constructed a gene co-expression network using weighted co-expression network analysis (WGCNA), and conducted clustering analysis. A diagnostic model for diabetic cardiomyopathy was subsequently developed using the least absolute shrinkage and selection operator (LASSO), leading to the identification of six potential biomarkers. This model was validated using additional datasets and cell line experiments.

Lifestyle modifications: the cornerstone of prevention

At the forefront of preventing cardiovascular complications in T2D is the implementation of lifestyle modifications encouraging to reduce sedentary time and break up sitting time with frequent activity breaks. Regular structured physical activity program stands out as one of the most effective strategies based on aerobic exercise, resistance training and combined exercises. As highlighted by Chen et al. in the published systematic review in this Research Topic, the impact of concurrent aerobic and resistance training enhances vascular health improving endothelial function and reduced arterial stiffness in individuals with T2D even if this kind of training may potentially exacerbate the vascular smooth muscle dysfunction. Further data from well-designed randomized clinical trials will undoubtedly be necessary to investigate this aspect. High-intensity interval training (HIIT) is also effective and has the added benefit of being very time-efficient. Rami et al.'s research on T2D rats demonstrated that HIIT could significantly mitigate pathological changes in heart tissue, such as hypertrophy, fibrosis, and apoptosis. In fact, HIIT improved molecular indices, including the reduction of B-catenin and c-Myc proteins and an increase in GSK3B and Bcl-2 proteins, suggesting a potential for HIIT to manage cardiovascular complications in diabetes. Furthermore, engaging in at least 150 minutes of moderate-intensity exercise per week can substantially improve cardiovascular health, help control blood sugar levels, and reduce body weight. Exercise enhances insulin sensitivity, lowers blood pressure, and improves lipid profiles, all of which are critical factors in reducing cardiovascular risk (3, 4).

A heart-healthy diet is equally crucial. Diets rich in fruits, vegetables, whole grains, lean proteins, and healthy fats, such as the Mediterranean diet, have been shown to lower cholesterol levels, reduce inflammation, and improve overall cardiovascular health. In the paper of Stelling-Férez et al. the effects of oleanolic acid (OA), a bioactive triterpenoid found in many plants, on endothelial cells from healthy and gestational diabetes-affected pregnancies was investigated. It demonstrated that OA reduces inflammation, decreases monocyte adhesion, enhances angiogenesis, and improves cell migration suggesting its potential on cardiovascular health as well as wound healing. Limiting the intake of processed foods, sugars, and unhealthy fats is essential in managing both diabetes and cardiovascular risk. Additionally, maintaining a healthy weight is vital, as obesity is a significant risk factor for both T2D and CVD. Even modest weight loss can lead to substantial improvements in glycemic control and a reduction in cardiovascular events.

Smoking cessation cannot be overstated in its importance. Smoking exacerbates cardiovascular risk and complicates diabetes management. Smokers with diabetes are at a higher risk of developing heart disease compared to non-smokers, making it imperative to promote smoking cessation programs as part of a comprehensive diabetes management plan.

Medical management: precision and personalization

To effectively implement personalized prevention strategies, it is crucial to identify specific risk profiles within the T2D population enabling healthcare providers to tailor interventions more precisely, potentially improving patient outcomes and resource allocation in the management of cardiovascular complications associated with T2D. In this Research Topic, Jiménez et al. revealed significant sex and age-related differences in cardiovascular event incidence and presentation among a large cohort of people living with T2D. The findings that men have higher risks for overall CVD, coronary heart disease, and peripheral artery disease, while women face greater risk of heart failure, alongside the observed age-related patterns in disease presentation, underscore the need for personalized approaches in clinical practice.

Effective medical management is fundamental in preventing cardiovascular complications. Regular monitoring and control of blood glucose levels, blood pressure, and cholesterol levels are crucial.

Glycemic control is the cornerstone of diabetes management, with medications such as metformin, sodium-glucose cotransporter 2 inhibitors (SGLT-2i), and Glucagon like peptide-1 (GLP-1) receptor agonists playing a pivotal role. These medications not only lower blood glucose but also provide cardiovascular benefits, including reduced risk of heart failure and improved kidney function. Notably, the network meta-analysis performed by Ghosal and Sinha published in this Research Topic examined the effectiveness of SGLT-2i in reducing cardiovascular death across 13 cardiovascular outcome trials. The analysis confirmed SGLT-2i as a class able to reduce CV death risk; empagliflozin emerged as the most effective agent overall and for people with atherosclerotic CVD, while dapagliflozin showed the best results for subjects affected by heart failure, finally providing valuable insights for tailoring treatment choices in different populations living with T2D and high cardiovascular risk.

Arterial hypertension is a common comorbidity in individuals with T2D and needs to be managed aggressively. In addition to the well-known damages caused by arterial hypertension, it appears

that people who simultaneously have T2D and hypertension exhibit significant thalamic changes compared to those without hypertension. This emphasizes the importance of managing hypertension in people living with T2D to prevent further brain damage (Cui et al.). Similarly, dyslipidemia should be addressed with statins or newer lipid-lowering agents (e.g. bempedoic acid, PCSK9 inhibitors) to reduce LDL cholesterol and improve cardiovascular outcomes. Moreover, even though He et al. did not find a causal relationship between serum uric acid levels and heart failure risk in individuals with T2D, suggesting that other factors may play a causal role in heart failure development in this population. In addition, uric acid can activate several pathophysiological mechanisms suggesting a possible causal role of uric acid in the genesis and progression of CVDs. Therefore, it is necessary to investigate and effectively treat hyperuricemia in patients with T2D.

Regular follow-ups with healthcare providers are essential to adjust treatment plans and ensure optimal management of all risk factors. Adherence to prescribed medications is critical, and healthcare providers must emphasize the importance of compliance and regular screenings for early detection of potential complications.

Recently, the investigation of genetic variability has garnered significant interest for its potential to identify subgroups of people living with T2D patients, who possess specific characteristics and may benefit from targeted drug therapies (5).

Psychosocial and community support: an integral component

Preventing cardiovascular complications in T2D extends beyond medical management and lifestyle changes. Community and psychosocial support are vital in the overall well-being and effective management of diabetes. Support groups, educational programs, and counseling services provide the necessary resources and encouragement for patients to adhere to lifestyle changes and medical regimens. Equally important, the impact of air pollution on CVD risk across the diabetes spectrum is significant, especially in the current context of increasing pollution levels. The review by Bonanni et al. emphasizes the need for healthcare and urban policies to limit exposure to fine particulate matter, as it correlates with elevated blood glucose and HbA1c levels, exacerbating CVD risks in people with T2D. This calls for preventive measures such as using portable air cleaners and other interventions to protect vulnerable populations. Another aspect of great importance to implement is the dissemination of scientific data by researchers and experts in the field, in order to encourage the population to adopt a healthy lifestyle and conscious food consumption.

Addressing the psychosocial aspects of diabetes, such as stress, depression, and anxiety, is equally important. These factors can negatively affect diabetes management and increase cardiovascular risk. Integrating mental health care into diabetes management plans can lead to better outcomes and enhance the quality of life for patients. Healthcare providers should screen for and address mental health issues as part of a comprehensive diabetes care strategy.

Conclusion: a call to action

Preventing cardiovascular complications in T2D requires a multifaceted approach. Lifestyle modifications, effective medical management, and robust community and psychosocial support are all critical components. By addressing these areas comprehensively, we can significantly reduce the burden of CVD in individuals with T2D, improve their quality of life, and extend their life expectancy.

Healthcare professionals, policymakers, and communities must collaborate to implement strategies that promote heart health and diabetes management. This includes creating supportive environments that encourage healthy lifestyles, providing access to effective medical treatments, and offering psychosocial support. By taking a proactive and holistic approach, we can make significant strides in preventing cardiovascular complications in T2D and ensuring that patients receive the comprehensive care they need to thrive.

Author contributions

MPAB: Conceptualization, Writing – original draft, Writing – review & editing. TP: Writing – original draft. KP: Writing – review & editing. CP: Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. (2021) 183:109119. doi: 10.1016/j.diabres.2021.109119

2. Baldassarre MPA, Pipino C, Pandolfi A, Consoli A, Di Pietro N, Formoso G. Old and new biomarkers associated with endothelial dysfunction in chronic hyperglycemia. *Oxid Med Cell Longev*. (2021) 2021:7887426. doi: 10.1155/2021/7887426

3. Reddy R, Wittenberg A, Castle JR, El Youssef J, Winters-Stone K, Gillingham M, et al. Effect of aerobic and resistance exercise on glycemic control in adults with type 1 diabetes. *Can J Diabetes*. (2019) 43:406–414.e1. doi: 10.1016/j.jcjd.2018.08.193

4. Kanaley JA, Colberg SR, Corcoran MH, Malin SK, Rodriguez NR, Crespo CJ, et al. Exercise/physical activity in individuals with type 2 diabetes: A consensus statement from the american college of sports medicine. *Med Sci Sports Exerc*. (2022) 54:353–68. doi: 10.1249/MSS.0000000000002800

5. Morieri ML, Pipino C, Doria AJ. Pharmacogenetics of cardiovascular prevention in diabetes: from precision medicine to identification of novel targetspers. *Med*. (2022) 12:1402. doi: 10.3390/jpm12091402

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Highlighting the novel effects of high-intensity interval training on some histopathological and molecular indices in the heart of type 2 diabetic rats

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Background: Type 2 diabetes is one of the most common metabolic diseases in recent years and has become an important risk factor for cardiovascular disorders. The first goal is to reduce type 2 diabetes, and in the case of cardiovascular disease, the second goal is to reduce and manage that disorder.

Materials and methods: The rats were divided into 4 groups: Healthy Control (n=8), Diabetes Control (n=8), Diabetes Training (n=8), and Healthy Training (n=8). The protocol consisted of 8 weeks of High-intensity interval (5 sessions per week), where the training started with 80% of the peak speed in the first week, and 10% was added to this speed every week. To measure the level of B-catenin, c-MYC, GSK3B, and Bcl-2 proteins using the western blot method, cardiac pathological changes were measured using hematoxylin and eosin staining, Masson's trichrome and PAS staining and apoptosis using the TUNEL method.

Findings: Histological results showed that diabetes causes significant pathological hypertrophy, fibrosis, and severe apoptosis in heart tissue. HIIT training significantly reduced pathological hypertrophy and fibrosis in heart tissue, and the rate of cardiomyocyte apoptosis was greatly reduced. This research showed that diabetes disorder increases the levels of B-catenin and c-Myc proteins and causes a decrease in the expression of GSK3B and Bcl-2 proteins. After eight weeks of HIIT training, the levels of B-catenin and c-Myc proteins decreased significantly, and the levels of GSK3B and Bcl-2 proteins increased.

Conclusion: This study showed that HIIT could be a suitable strategy to reduce cardiomyopathy in type 2 diabetic rats. However, it is suggested that in future studies, researchers should perform different intensities and exercises to promote exercise goals in type 2 diabetic cardiomyopathy.

KEYWORDS

high-intensity interval training, diabetic, cardiomyopathy, exercise, physical activity

Highlights

- Today, T2DM is known as the main factor in cardiovascular diseases, which can lead to cardiac disorders such as fibrosis, apoptosis, and oxidative stress in heart tissue.
- Different solutions and strategies have been proposed to reduce and treat cardiovascular disorders, one of the best "treatments without the cost" of exercise.
- HIIT can lead to the improvement of cardiomyopathy, including the reduction of cardiac myocytes' hypertrophy and the accumulation of collagen (fibrosis) in the heart's myocytes and around the arterial vessels.
- Also, HIIT improved the disorder of Purkinje fibers in the heart tissue of type 2 diabetic rats.
- In addition, the reduction of apoptosis after HIIT in type 2 diabetic rats in heart tissue was one of the prominent observations of the present study. Also, the reduction of β-catenin and c-Myc protein levels as a result of HIIT in type 2 diabetic rats indicated the positive effects of this exercise in improving the cardiomyopathy of T2DM.
- HIIT can be a suitable strategy to reduce and manage cardiomyopathy in people with T2DM, and to prove this, more future studies are needed to investigate signaling pathways and cardiac function.

1 Introduction

Type 2 diabetes (T2DM) is one of the most common metabolic disorders worldwide, which is caused by a combination of two main factors: defective insulin secretion by pancreatic beta cells and the inability of insulin-sensitive tissues to respond appropriately to insulin (1). According to the International Diabetes Federation (IDF), 9.3% (463 million) of adults worldwide had diabetes in 2019, and this figure is expected to increase to 10.2% (578 million) by 2030 to increase to 10.9 percent (700 million) by 2045 (2). The prevalence of T2DM among adults and young people has caused great concern, and people with T2DM in youth often have a more aggressive clinical course than people with T2 diabetes in adulthood (3).

With the prevalence of T2DM worldwide, its side effects and risks are more visible, and this has caused more concerns (3). One complication traditionally associated with T2DM, the risk of which has increased significantly, is cardiovascular disease (4, 5). Meanwhile, diabetic cardiomyopathy is one of the main and most important complications of T2DM; the pathogenesis and clinical features of diabetic cardiomyopathy has been well studied in the last decade, but effective approaches to prevent and treat this disease are limited (6). Diabetic cardiomyopathy is characterized by adverse structural changes (including cardiac hypertrophy and fibrosis), early diastolic dysfunction, and late systolic dysfunction (6, 7). Diabetic cardiomyopathy occurs because of impaired glucose, and lipid metabolism associated with diabetes, leading to increased oxidative stress and activation of multiple inflammatory pathways that mediate cellular and extracellular damage, pathological remodeling of the heart, and diastolic and systolic dysfunction (6). However, important signaling pathways are involved in cardiomyopathy, and targeting them can effectively treat it. Bcl-2 proteins are composed of anti- and pro-apoptotic members and play a key role in regulating apoptosis in the myocardium (8). Anti-apoptotic proteins have been shown to protect against various cardiac pathologies, while anti-apoptotic proteins contribute to heart disease (8). Previous studies have revealed that canonical Wnt/β-catenin/glycogen synthase kinase three beta (GSK3β) and c-Myc (Myc) pathways are widely engaged in regulating various biological processes and play critical roles in the pathogenesis of diabetic rats with myocardial injury (9–11). The inappropriate activation of the Wnt/β-catenin pathway resulting from β-catenin enhancer nuclear localization is also strongly associated with cardiomyopathy (11, 12). Also, with signaling changes in cardiomyopathy, reactive oxygen species (ROS) caused by metabolic disorders caused by excessive superoxide production by the mitochondrial electron transport chain during hyperglycemia can further worsen the disease (13). However, signaling pathways and oxidative stress changes have caused clinical studies to focus more on discovering therapeutic strategies for cardiomyopathy.

Looking at the history of the past, we can see that physical exercise is one of the effective and inexpensive medicines for treating or managing diseases (14). However, studies have focused specifically on the effect of physical exercise and T2DM treatment (15, 16). Different intensities of physical exercise have been investigated to understand the impact on diabetic patients better, but recently, high-intensity interval training (HIIT) has had a special place among researchers (17). Physical exercise appears to improve cardiovascular health in patients with T2DM, but its effects

on cardiac structure and function are unknown (17). However, HIIT is a potential treatment for modulating heart risk, cardiomyopathy, blood pressure, and blood glucose (17), but the reasons still need to be clearly understood. In confirmation of these findings, the study of Chavanelle et al. (2017) showed that HIIT has a more positive effect on blood glucose and heart structure changes in T2DM rats compared to moderate-intensity training (18).

Finally, with the emergence of HIIT as a potential treatment for T2DM and cardiomyopathy, many research gaps can still be filled with further studies. In this study, we are trying to determine whether HIIT positively affects these signaling pathways and the histology of the heart by examining the important signaling pathways in diabetic cardiomyopathy, oxidative stress indicators, and histopathological changes. Therefore, we will better understand the impact of HIIT by examining the signaling pathways.

2 Material and methods

2.1 Animals

We performed all procedures following the Guide for the Care and Use of Laboratory Animals, 8th edition (2011). The local ethics committee approved all procedures (Shahid Chamran University of Ahvaz: EE/1401.2.24.158669/scu.ac.ir). Eight-week-old rats were housed in individual cages with a natural light/dark cycle of 12 h:12 h and fed a standard diet. After one week of adaptation, 32 out of 40 rats were selected for the study after the running test, and the best runners were randomly divided into healthy control (n=8), diabetes control (n=8), healthy training (n=8) and diabetes training (n=8) groups (See Figure 1). All rats were dissected simultaneously, and all procedures and experiments were performed for all rats in accordance with each other.

2.2 Diabetes induction

Rats in the diabetes groups consumed a high-fat diet (HFD) containing 55% of energy from lard and soybean oil, 31% of carbohydrates, and 14% of protein for two months. The diet of healthy rats was equivalent to 10% fats, such as soybean oil, 76% carbohydrates, and 14% protein (19). After this period, the rats in the diabetes groups fasted for 12 hours. Then a 35 mg/kg dose of Streptozotocin (STZ) solution (Sigma, Germany) was injected intraperitoneally. Two weeks after STZ (Solutions needed to prepare STZ in Supplementary 1) injection, the blood glucose of the animals was measured through the tail vein of the rats using a glucometer (Roche Diagnostics K.K., Tokyo, Japan). Animals with

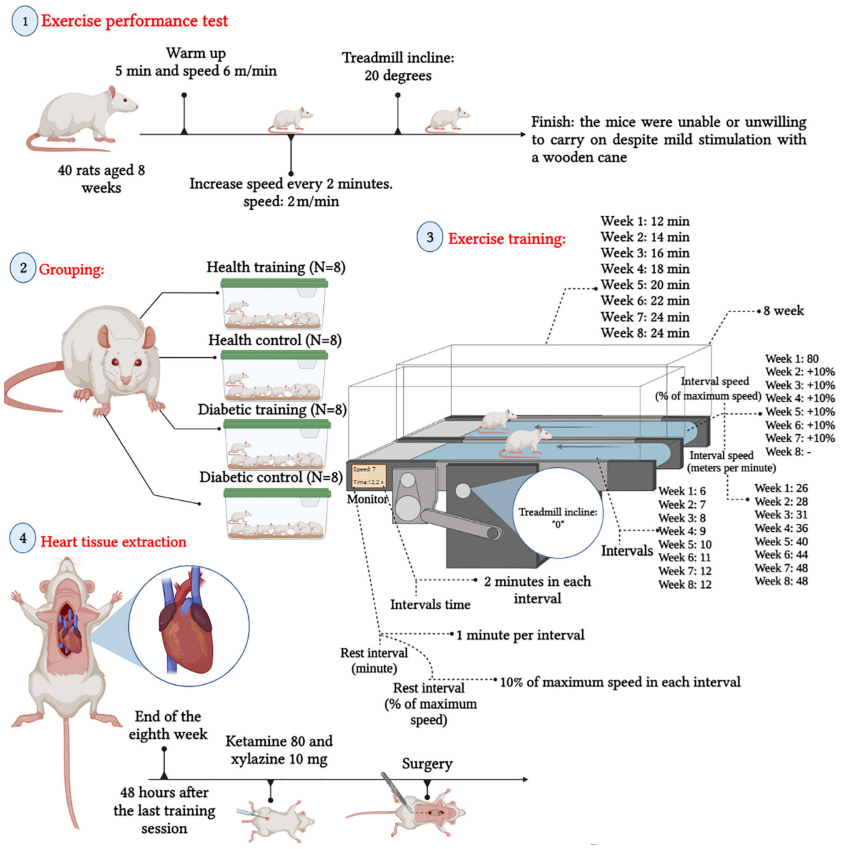

FIGURE 1
Schematic view training protocol and heart tissue extraction.

fasting blood glucose (FBG) higher than 210 mg/dl were considered diabetic and were included in the study (19). Rats in the control group were injected with an equivalent volume of citrate buffer.

2.3 Exercise performance test and exercise training

An exercise performance test was taken from the rats to formulate the training protocol. Before the start, the rats underwent a 2-week familiarization period with the treadmill. Then, Rats were first placed on a treadmill and warmed up for 5 minutes at a 6 m/min speed. Speed was then increased by steps of 2 m/min every 2 min until the rats were unable or unwilling to carry on despite mild stimulation with a wooden cane. In the whole length of the test, the slope was 20 degrees (18). After determining the intensity and speed of the rats, the training protocol began (20). The protocol consisted of 8 weeks of training (5 sessions per week), where the training started with 80% of the peak speed in the first week, and 10% was added to this speed every week. The speed stayed the same in the final two weeks to maintain the adaptations got. The training sessions comprised 6×2-minute training in the first week and continued to 12×2-minute training in the seventh and eighth weeks. After each training, a minute of active rest was performed at a speed of 10 m/min (See Figure 1).

2.4 Heart tissue extraction

At the end of the eighth week and 48 hours after the last training session, intraperitoneal injection of ketamine 80 and xylazine 10 mg anesthetized the rats. Then, the heart tissue was separated under sterile conditions and immediately transferred to a negative 70 freezer (model 88FD-2-93-A, Iran Madas Company).

2.5 Measurement of superoxide dismutase concentration

The measurement of superoxide dismutase (SOD) activity is based on the mechanism of inhibition of Nitrotetrazolium blue (NBT) decline by the xanthine-xanthine oxidase system as a superoxide producer. The optical absorption of each sample was read at 550 nm for 5 min every 30 s. To obtain the percentage of inhibition of NBT reduction by SOD enzyme, the formula corresponding to the Rendox kit was used (Rendox-UK). The activity of the enzyme was obtained by adjusting the percentage of inhibition to the standard curve and reported based on the international unit of protein (μmol/min.g tissue).

2.6 Measurement of malondialdehyde concentration

The concentration of Malondialdehyde (MDA) enzyme is based on photometric principles. The basis of this method is the formation of MDA-TBA complex between one molecule of malondialdehyde and two molecules of thiobarbituric acid. Thiobarbituric acid reactive substance (TBARS) was measured from supernatant. In summary, trichloroacetic acid and TBARS reagent were added to the supernatant, then the mixture was placed in an incubator at 100°C for 80 min. Afterwards, it was cooled on ice and centrifuged at RPM 1000 for 2 min. The optical absorption was read at 532nm. The TBARS results were expressed as MDA equivalents using by standard tetraethanoxypropane curve.

2.7 Antibodies

β-catenin (β-catenin (E-5): sc-7963, SANTA CRUZ), GSK-3β (GSK-3β (11B9): sc-81462, SANTA CRUZ), c-Myc (c-Myc Antibody (9E10): sc-40, SANTA CRUZ), Bcl-2 (Bcl-2 (C-2): sc-7382, SANTA CRUZ) and HPRT (HPRT Antibody (F-1): sc-376938, SANTA CRUZ).

2.8 Protein extraction and western blot analysis

To measure proteins, lysis buffer was first prepared (Preparation of lysis buffer in Supplementary File 1). Then, tissue samples were frozen in the -70 freezer to prepare tissue homogenate and western blot test. Also, the Bradford method was used to determine the amount of protein in the tissue homogenate. (Analysis method in Supplementary File 2). Then, in the next step, polyacrylamide gel electrophoresis with SDS was used (Procedure, buffers, and solutions required for SDS-PAGE and different steps of polyacrylamide gel electrophoresis with SDS in Supplementary File 3). After electrophoresis, blotting, blocking, incubation, and emergence were performed (Buffers and solutions, transfer steps (blotting), method, and blocking action by blocking buffer (blocking) required in Supplementary File 3).

Also, Hematoxylin-eosin (H & E) (To examine histological changes in cellular and structure details), TUNEL (Examining apoptotic changes), and Masson's trichrome and PAS staining (Examination of histopathological changes) were used to check changes (Staining and preparation method in Supplementary File 4).

2.9 Statistical analysis

Shapiro-Wilk test was used to check the normality of the data, and Levene's test was used to check the homogeneity of variances. A mixed ANOVA (composite analysis of variance) test was used to investigate changes in glucose and weight in different stages of exercise. In order to check the difference between the averages in the studied variables, a one-way ANOVA test was used, and in the next step, Tukey's test was used as a *post hoc* test. The significance level was also considered as $P<0.05$. Data were analyzed using SPSS version 25 software.

3 Results

In this research, we investigated the histopathological and histomorphometric changes in the heart tissue of rat T2DM following HIIT. The histological examination of the heart tissue of non-diabetic and diabetic rats following the HIIT was performed using light microscopy. To evaluate the effect of HIIT on the heart, myocardial tissue was stained with hematoxylin and eosin, Masson's trichrome, and PAS methods.

3.1 Changes in average weight and blood glucose

Figures 2, 3 show the changes in average weight and blood glucose in the weeks and stages of the training protocol. The results of the a mixed ANOVA test showed that the weight of the rats in the diabetes control and diabetes training groups increased significantly from the time of the start of training and high-fat diet to before STZ injection ($P<0.001$). Also, the weight of this rats showed a significant decrease in the last week and the end of the training protocol ($P<0.001$). After the end of the training, the weight of the rats in the diabetic control group was significantly lower than all other groups ($P<0.001$) (see Figure 2).

On the other hand, the statistical analysis showed that blood glucose in the diabetes control and diabetes training increased significantly one week after STZ injection ($P<0.001$). Nevertheless, at the end of the training protocol in the diabetes training group decreased significantly compared to the previous stage ($P<0.001$) (see Figure 3).

3.2 Histopathological changes, hypertrophy, Purkinje fibers and collagen area around arterial vessels of heart tissue

The results of H&E staining showed that the heart tissue of the healthy control group had a normal myofibril structure, which was observed in longitudinal and transverse sections (Figure 4, healthy control, A and B) with healthy nuclei located in the canter compared to the diabetes control group. In the diabetes control group, damaged and abnormal myofibrils, faded nuclei, increased collagen connective tissue, and hypertrophy of cardiomyocytes were observed in longitudinal and transverse sections (Figure 4, diabetes control, A and B). In the heart tissue of diabetes training rats, the damage of heart tissue myofibrils has decreased, cell nuclei have become clearer, collagen connective tissue has decreased, and hypertrophy caused by diabetes has significantly decreased in longitudinal and transverse sections (Figure 4, diabetes training, A and B). Also, this research showed that the average cross-sectional area of myocardial cells in the diabetes control group increased significantly compared to the healthy control group ($P<0.001$), and pathological hypertrophy was significantly controlled in the diabetes training group after performing the exercise protocol ($P<0.05$). The healthy training group also showed significant physiological hypertrophy after performing the exercise protocol ($P<0.05$) (Figure 4E).

The results of our study showed that in the diabetes control group, Purkinje fibers similar to cardiomyocyte cells were hypertrophied and their number increased significantly ($P<0.001$) (Figure 4C, diabetes control). HIIT in the diabetes training group significantly reduced the changes caused by diabetes in Purkinje cells ($P<0.001$) (Figure 4C, Diabetes Training). The number, length and width of Purkinje cells were not significantly different in healthy control and training groups (Figure 4F).

The results of H&E staining in heart tissue showed that the percentage of perivascular collagen area (PVCA) in the diabetes control group was significantly increased compared to the healthy control group ($P<0.001$) (Figures 4D, diabetes control). Also, the results of this study showed that after a period of HIIT exercise, the percentage of collagen area around the arterial vessels (PVCA) in the diabetes training group showed a significant decrease compared to the diabetic control group ($P<0.001$) (Figure 4D, Diabetes

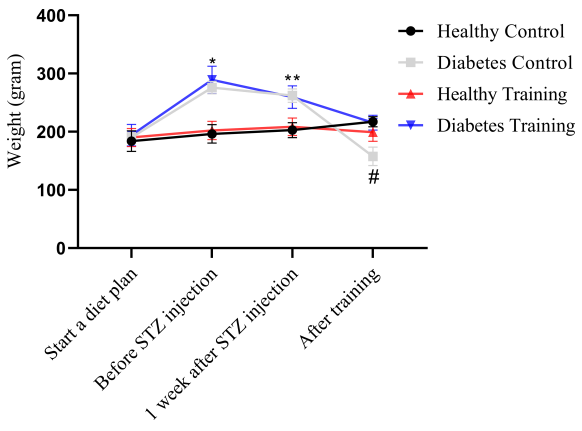

FIGURE 2
Weight comparison of different groups of rats participating in different stages of the training protocol. *Significant difference between diabetes control and diabetes training groups with healthy control and healthy training groups in the stage before STZ injection; **Significant difference between the diabetes control and diabetes training groups with the healthy control and healthy training groups at the stage of one week after STZ injection; #Significant difference between the diabetes control group and other groups in the phase after the end of the exercise; The significance level is $p<0.05$.

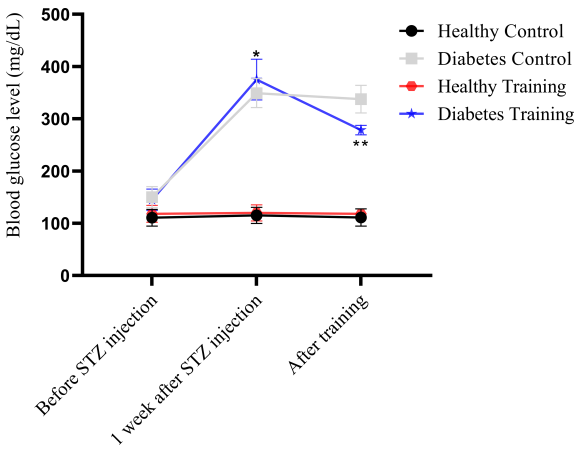

FIGURE 3
Blood glucose comparison of different groups of rats in different stages of the training protocol. *Significant difference in blood glucose in diabetes control and diabetes training groups with healthy control and healthy training groups at the stage of one week after STZ injection; **Significant difference between diabetes training group and other groups after the end of exercise; The significance level is p<0.05.

Training). There is no significant difference was observed in the collagen area around the arterial vessels in the healthy training and healthy control groups (Figure 4D).

3.3 Fibrosis changes of heart tissue

In the present study, we showed the area of cardiac tissue fibrosis by examining the extracellular matrix and collagen accumulation using PAS and Masson's trichrome staining. These areas are visible in the form of collagen scaffolds. This extracellular matrix and collagen accumulation can be seen in purple-red PAS staining and blue in Masson's trichrome staining (Figures 5A, 6A). The results of PAS and Masson's trichrome staining of the heart tissue of rats in diabetes groups show the presence of fibrosis in the heart tissue in the diabetes control group, which is obviously larger than the healthy control group (P<0.001) (Figures 5A, 6A). This issue supports the existence of diabetic cardiomyopathy in diabetic model rats with HFD diet combined with STZ injection. The results showed that a period of HIIT improved this pathological abnormality in diabetic hearts with a significant reduction in the area of cardiac tissue fibrosis in the diabetes training group (P<0.05) (Figures 5A, 6A). Normal amounts of collagen tissue can be seen in the healthy control and healthy training groups (Figures 5A, 6A). In addition, the results of Masson's trichrome staining in heart tissue showed that the percentage of perivascular collagen area (PVCA) in the diabetes control group was significantly increased compared to the healthy control group (P<0.001) (Figure 6B). Also, the results of this study showed that after a period of HIIT, the percentage of collagen area around the arterial vessels (PVCA) in the diabetes training group showed a significant decrease compared to the diabetes control group (P<0.001) (Figure 6B). The diagram of fibrosis changes in different groups is shown in Figure 5B.

3.4 Apoptosis rate of heart tissue

The results of the present research showed that TUNEL positive cells (apoptotic) were significantly more in the diabetes control group than in the healthy control group (P<0.001) (Figure 7). Also, the results of our research showed that the amount of TUNEL positive cells in the diabetes training group showed a significant decrease after eight weeks of HIIT activity (P<0.001) (Figure 7). There is no significant difference was observed in the TUNEL positive cells in the healthy control and healthy training groups (Figure 7).

3.5 Changes in the amount of proteins using the western blot method

The amounts of B-catenin, GSK3β, C-myc and Bcl-2 proteins in the heart tissue of rats in different groups are shown in Figure 8. The results showed that the content of B-catenin and C-myc proteins increased significantly in the diabetes control group compared to the healthy control group (P<0.001). This difference was also significant between the diabetes control and diabetes training groups (P<0.05), which shows that the amount of these two proteins in the diabetes training group has decreased significantly after HIIT. In addition, the content of GSK3β protein in the diabetes control group significantly decreased compared to the healthy control group (P<0.001), while the difference in the content of this protein in the diabetes training group

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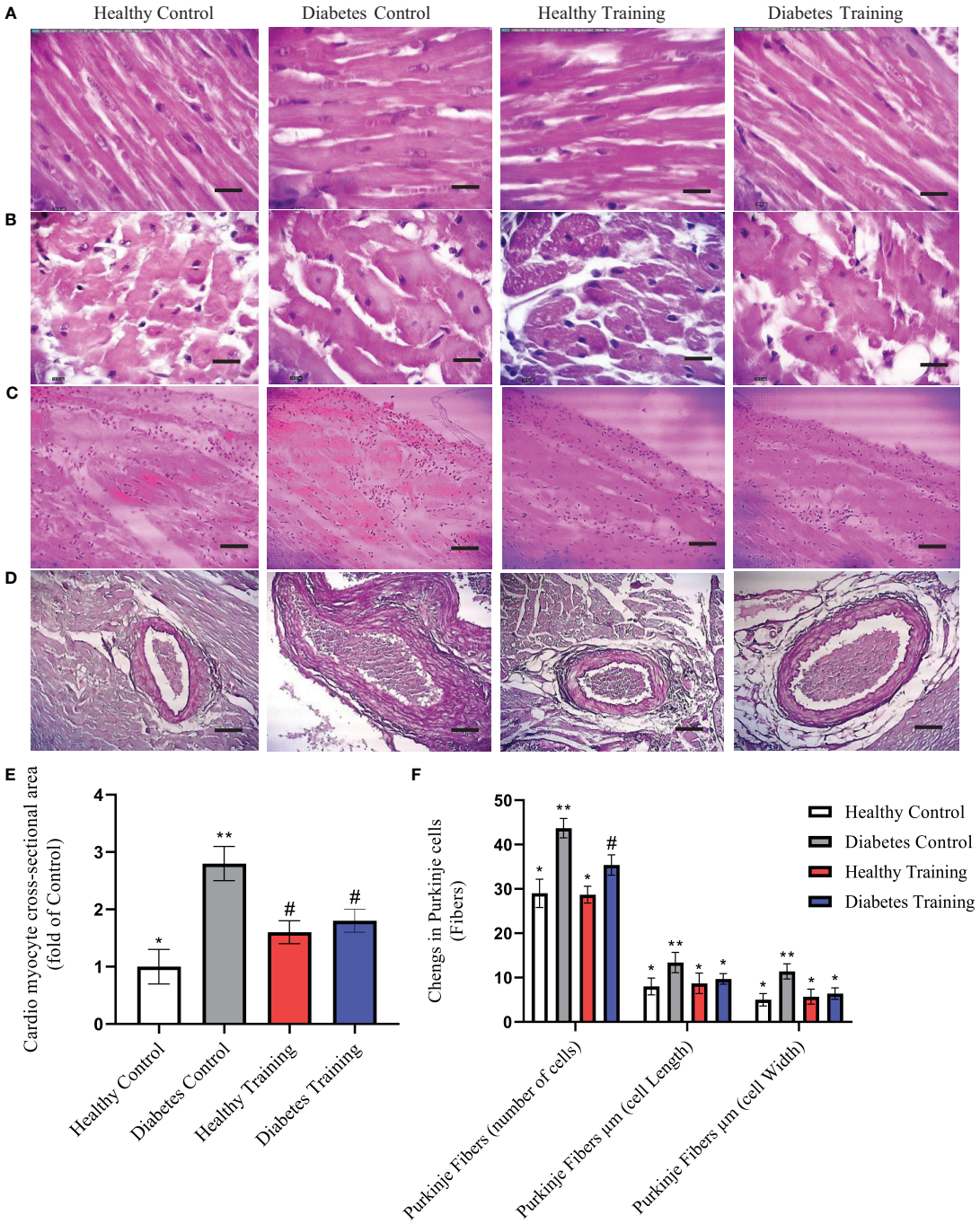

FIGURE 4
The results of Hematoxylin and Eosin (H&E)-stained cardiac tissue. **(A)** Longitudinal section, **(B)** cross-section, images represent ×40 magnification and scale bars represent 10 µm. **(C)** Purkinje cells (fibers) and **(D)** Collagen area around the arterial vessels, images represent ×10 magnification and scale bars represent 50 µm. **(E)** Diagram of cardio myocyte cross-sectional area. *Significant difference with all groups (P<0.05). **Significant difference with all groups (P<0.001). #Significant difference with diabetic control and healthy control groups (P<0.05). **(F)** Diagram of Purkinje cells (fibers), *Significant difference with diabetes control and diabetes training groups in Purkinje number cells and significant difference with diabetes control groups in Purkinje cell length and Purkinje cell width (P<0.001). **Significant difference with all groups (P<0.001). #Significant difference in diabetes training groups with all groups (P<0.001). Data are expressed as mean ± SEM.

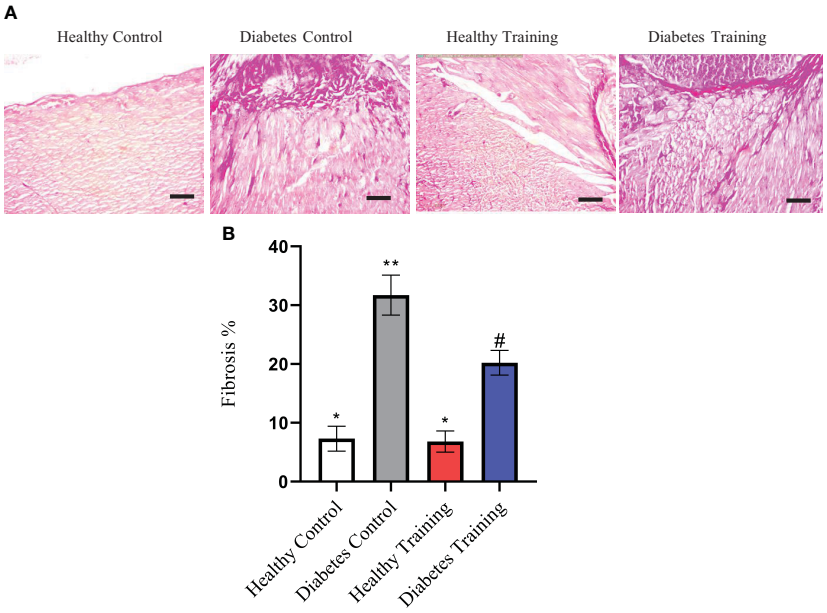

FIGURE 5
(A) The results of PAS staining of rat heart tissue in diabetic and non-diabetic groups following HIIT exercises to investigate the area of cardiac tissue fibrosis. **(B)** Diagram of cardiac tissue fibrosis. *Significant difference with diabetes control and diabetes training groups (P<0.001). All images represent ×10 magnification and scale bars represent 50 µm. **Significant difference with all groups (P<0.001). #Significant difference with all groups (P<0.05). Data are expressed as mean ± SEM.

compared to diabetes control group was not significant (P>0.05). The results of the present research also showed that the content of Bcl-2 protein in the diabetes control group was significantly reduced compared to the healthy control group (P<0.001), while in the diabetes training group, the content of this protein was significantly increased than the diabetes control group (P<0.05), the increase in protein content in the healthy training group compared to the healthy control group was also significant (P<0.05).

3.6 Antioxidant changes and oxidative stress in heart tissue

The results of the current research showed that the concentration of superoxide dismutase (SOD) as an antioxidant index in the diabetes control group was significantly reduced compared to the healthy control group, and in the diabetes training group, the concentration of SOD was significantly

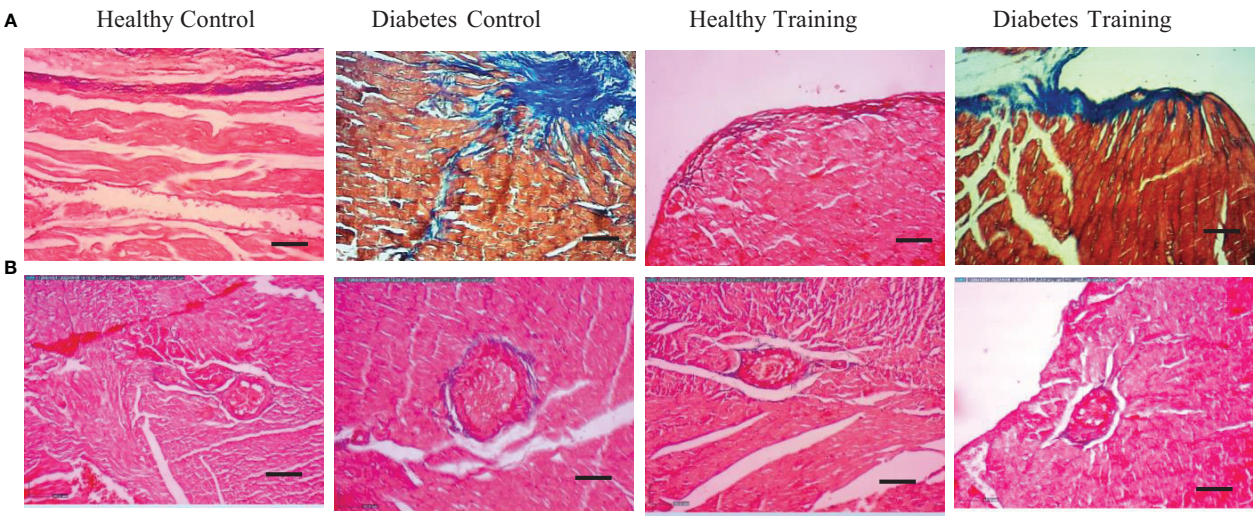

FIGURE 6
(A) The results of Masson's trichrome staining of rat heart tissue in diabetic and non-diabetic groups following HIIT exercises to investigate the area of cardiac tissue fibrosis. **(B)** Collagen area around the arterial vessels. All images represent ×10 magnification and scale bars represent 50 µm.

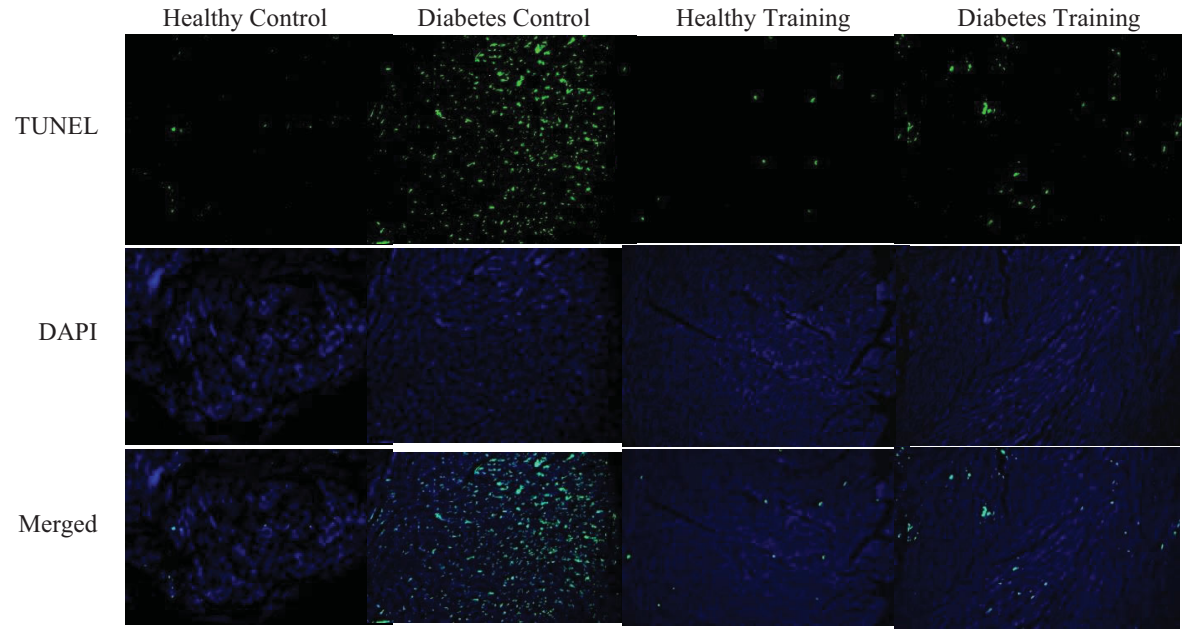

FIGURE 7
Investigating the apoptosis values of cardiac myocytes of rats in diabetic and non-diabetic groups following HIIT. Green dots show TUNEL positive cells. Fluorescent microscope images represent ×200 magnification.

increased compared to the diabetes control group (P<0.05). The increase in SOD concentration in the healthy training group was also significant compared to the healthy control group (P<0.001). The results of our study also showed that the concentration of malondialdehyde (MDA) as an index of oxidative stress increased significantly in the diabetes control group compared to the healthy control group (P<0.001). The reduction in MDA concentration in the diabetes training group compared to the diabetes control group was also significant (P<0.05) (Figure 9).

4 Discussion

In the present study, the results of blood glucose measurements showed that a period of HIIT reduces blood glucose levels in diabetic rats. In confirmation of these results, Cassidy et al. (21) also showed that 12 weeks of HIIT led to blood glucose control in people with T2DM by reducing HbA1c compared to the control group (21). Adams' study (2013) also showed that in T2DM patients, a 2-week HIIT program increased GLUT4 protein, a marker of insulin sensitivity, and decreased mean blood glucose 48-72 hours after exercise (22).

The results of our research showed that diabetes increases the cross-sectional area of cardiomyocytes in the heart tissue of rats. In line with the results of this research, Novoa et al. in 2017 (23), Wang et al. in 2019 (24), and Lu et al. in 2021 (25) stated that diabetes causes an increase in the cross-sectional area and thickness of cardiomyocytes in rats. Enlargement of the heart in response to stress, hypertension, myocardial injury, or neurohumoral overactivation is associated with cardiac dysfunction and is described as pathological hypertrophy (26).Cardiac function is initially preserved in pressure overload-induced cardiac hypertrophy, described as the adaptive phase; however, our study showed that HIIT significantly reduces the cross-sectional area of cardiomyocytes in the heart tissue of rats with T2DM. Our results are consistent with the studies of Yuan et al., 2020 and Nova et al., 2017 is in line with the report that HIIT can reduce pathological hypertrophy in the heart of T2DM rats (23, 27).

Our study showed that HIIT has significant positive effects on the regeneration of cardiac capacity in T2DM rats and can significantly reduce the fibrosis of the heart tissue of diabetic rats and the collagen area around the arterial vessels. Besides reducing the hypertrophy of cardiac myocytes, it reduces the accumulation of collagen (fibrosis) in the cardiac myocytes and around the arterial vessels. These results are consistent with the findings of Novoa et al. in 2017 (23), Chengji Ji et al. 2019 (28), and Marchini et al., 2020 (29), Lu et al., 2021 (25). The presence of interstitial fibrosis and fibrosis around arterial vessels is a common complication in diabetes and is usually considered an important factor in reducing contractility in the later stages (30). Previous studies reported a decrease in contractility and relaxation and an increase in left ventricular stiffness in STZ-treated diabetic rats. In diabetic models, hyperglycemia disrupts the dynamic balance of collagen synthesis and degradation in the myocardial extracellular matrix by promoting type I and III collagen deposition through converting cardiac fibroblasts to myofibroblasts (29). These results are in line with our observations in the diabetic group, which led to an increase in the level of cardiac tissue fibrosis in diabetic rats.

The results of our study regarding the Purkinje network of heart tissue showed that in the diabetes control group, Purkinje fibers

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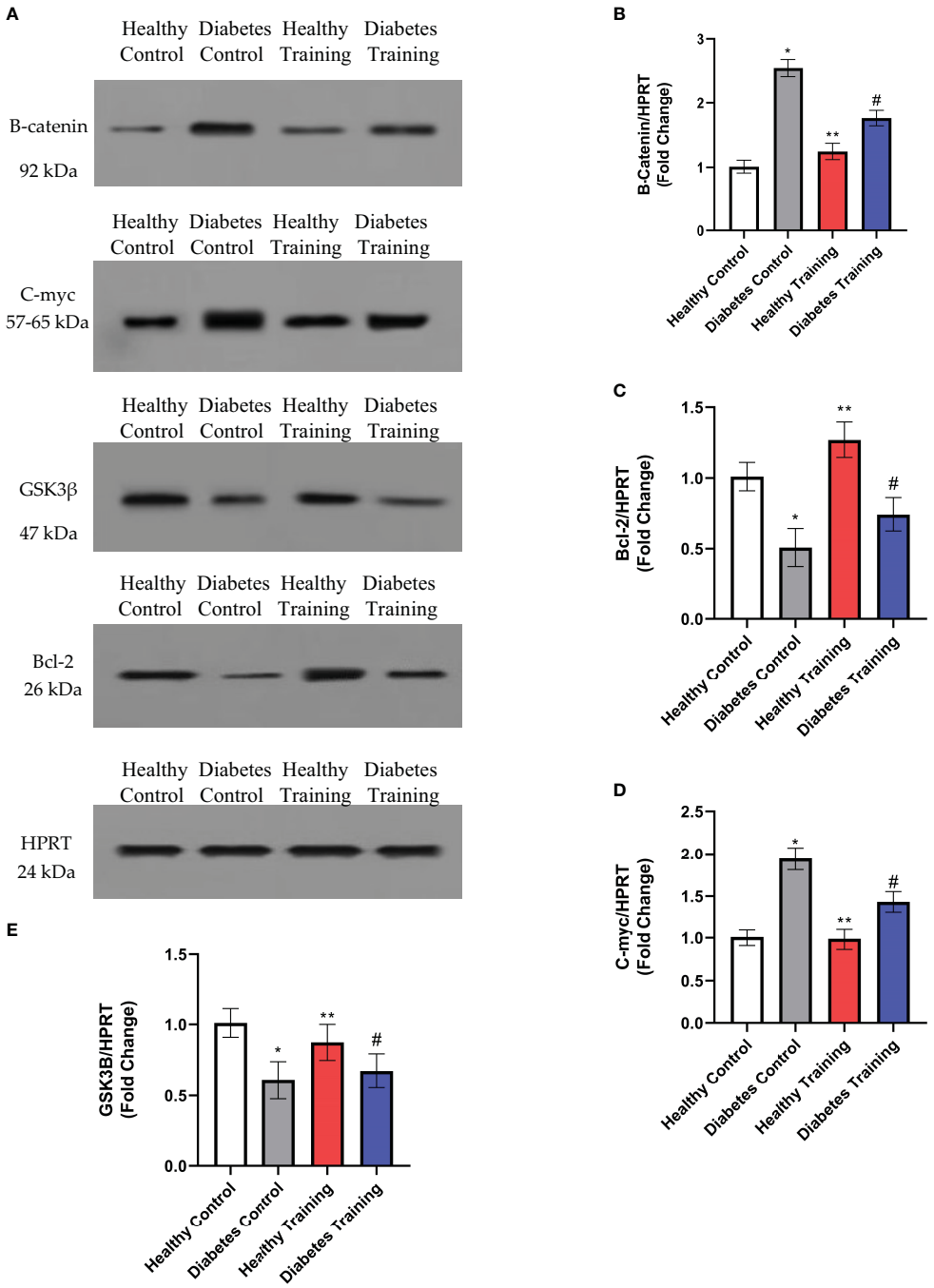

FIGURE 8
Evaluation of proteins content using western blot method. **(A)** The expression of B-catenin, C-myc, GSK3b and Bcl-2 proteins in the heart tissue of rats in different groups. **(B-E)** The relative ratio of proteins to HPRT levels were determined by image J. *Significant difference in Diabetes Control group with Healthy Control group (P<0.001). **Significant difference in Healthy Training group with all groups in Bcl-2 and GSK3β proteins and significant difference with Diabetes Control and Diabetes Training in B-catenin and C-myc proteins (P<0.05). #Significant difference in Diabetes Training group with all groups in B-catenin, C-myc and Bcl-2 proteins and significant difference with Health Control and Healthy Training in GSK3β protein (P<0.05). Data are expressed as mean ± SEM.

similar to cardiomyocyte cells were hypertrophied, and their number increased significantly. Kang, 2006 states that the normal distribution of Purkinje fibers in the myocardium is proportional to the mass of the heart (31). Cardiac hypertrophy caused by the hypertrophic growth of cardiac myocytes leads to the unbalanced distribution of Purkinje fibers in the regenerating heart (31). Therefore, the conduction of pacemaker potentials becomes problematic. Logantha et al. reported in 2021 (32) that in heart failure, there is extensive remodeling of the Purkinje network at the structural, molecular, and electrical levels, leading to dysfunction and an arrhythmic substrate. Also, therapeutic strategies that prevent heart dilatation may prevent the remodeling of Purkinje

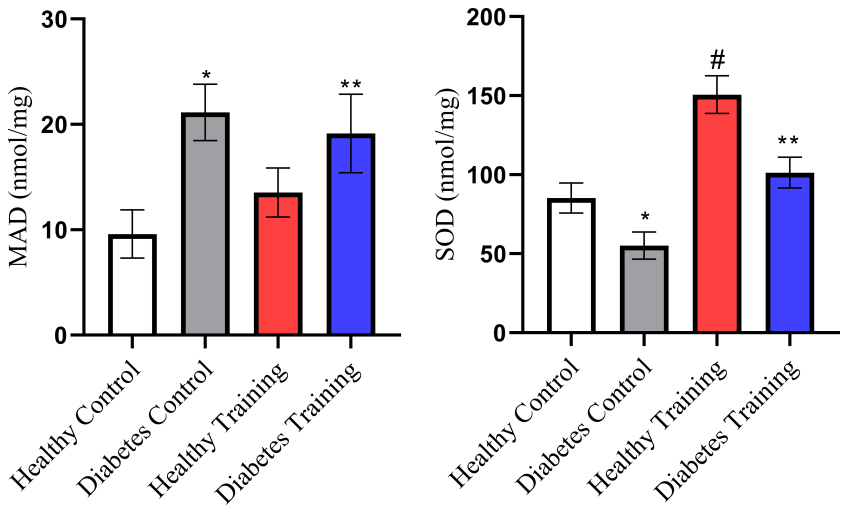

FIGURE 9
SOD and MDA changes in different groups. *Significant difference in Diabetes Control group with all groups (P<0.001). #Significant difference in Healthy Training group with all groups (P<0.001). **Significant difference in Diabetes Training group with Diabetes Control group (P<0.05). Data are expressed as mean ± SEM.

fibers and may be beneficial for patients with heart failure. Lanlua et al. also reported in 2012 that cardiac myocytes and Purkinje fibers hypertrophy in the heart of STZ-treated diabetic rats (33). In 2022, Chaturvedi et al. reported that in diabetes, Purkinje cell protein (PCP-4) is degraded by calpain-1, causing contractile dysfunction that can be reduced by exercise (34). Consistent with their results, our study showed that HIIT in diabetic rats significantly reduced diabetes-induced changes in Purkinje cells. However, the effects of different training interventions on the changes of Purkinje fibers seem ambiguous and need more studies.

This research showed that the amount of TUNEL positive cells (apoptotic) cells in the heart tissue of diabetic rats increased significantly. Our study's results align with the research results of Wu et al., 2017 (35), Xi et al., 2015 (36), Ren et al., 2020 (37), and Nova et al., 2017 (23). Previous studies have shown a close relationship between cardiac dysfunction and myocardial apoptosis and state that controlling myocardial apoptosis is crucial to improving cardiac function (38, 39). The increase in myocardial apoptosis causes the loss of contractile units and cardiac remodeling, which leads to cardiac dysfunction. Studies have shown that, at the cellular level, diabetes increases cardiac myocyte hypertrophy, interstitial fibrosis, and apoptosis (40). This heart damage process causes the production of active oxidative species (ROS) (40). Oxidative stress exists when the production of ROS exceeds its destruction by antioxidant systems (40).

The results of our study also indicate that the values of MDA as an index of oxidative stress in the hearts of diabetic rats increased, and the values of SOD as an antioxidant index decreased in the hearts of diabetic rats. One of the causes of diabetic cardiomyopathy (DCM) is apoptosis, caused by hyperglycemia and abnormal disorder in plasma glucose and insulin level. In fact, long-term hyperglycemia is one of the main causes of ROS formation, insulin resistance, and dysregulation of cytoplasmic calcium. This case makes heart muscle cells susceptible to cell death and ultimately causes changes in muscle contraction and heart failure (41).

Also, our research showed that the amount of TUNEL positive cells and, as a result, the amount of apoptosis in diabetic rats after eight weeks of HIIT showed a significant decrease. Consistent with the results of our study, the results of the study by Chengji and Xiajin (2019) (28), which investigated the effect of two types of low-intensity and HIIT on the apoptosis of heart tissue in diabetic rats, showed that HIIT was more effective in reducing apoptosis than low-intensity training. In contrast, the study by Novoa et al. (2017) (23) showed that, unexpectedly, the level of apoptosis was significantly increased in the diabetic training group; this is when the blood glucose level of their research samples was about 600 mg/dL. While in the diabetes induction protocol, blood glucose levels above 300 mg/dL have been considered as a criterion for diabetic samples, and it seems that they used high doses of alloxan to induce diabetes (42). The data from Novoa et al. (23) study showed that HIIT increases apoptosis in cardiomyocytes of diabetic rats. On the other hand, they showed that a HIIT program had a positive effect on cardiac regeneration, which was evident as a reduction in pathological hypertrophy caused by diabetes and a reduction in collagen deposition (fibrosis) in cardiac tissue, but HIIT increased apoptosis. In the study of Novoa et al. (2017) (23) the training program continued for only four weeks, which may not be the right time to create proper adaptations in intense training. Also, oxidative stress indices increased significantly after exercise in diabetic rats, which can explain the high levels of apoptosis in diabetic rats. On the other hand, it has been shown that HIIT can be an important factor in increased cell death. Based on the evidence, HIIT, besides creating physiological adaptations, may be associated with an ineffective antioxidant defense system of the body and lead to oxidative stress and cell damage (43). Whether HIIT (more than 85% VO2 max) is harmful or beneficial to the heart remains

controversial (44). This refers to an increase in energy consumption and a change in the metabolism of substances, which leads to a decrease in the efficiency of ATP synthesis (44). Tissue oxygenation and mitochondrial respiratory chain electron leakage are reduced, easily causing oxidative stress damage. Studies have shown that Wnt/β-catenin signaling is important in regulating cardiac function, but little information is available on its role in DCM (36). It has been shown that Wnt2 participates in early cardiogenesis and is upregulated following cardiac abnormalities (45). As the histopathology results of our study also showed, the DCM process is characterized by pathological hypertrophy of the heart, myocardial remodeling and dysfunction, and myocardial fibrosis, which in severe cases, can lead to heart failure (46). Previous studies have shown that inhibiting NF-κB activation or regulating GSK3β expression may, in turn, limit the transcription of NF-κB downstream factors and the Wnt/β-catenin/GSK3β pathways (47, 48). As a result, it improves the inflammatory response to myocardial damage in diabetic rats. NF-κB and Wnt/β-catenin signaling pathways are known as important regulators in cardiac pathophysiology (12). In the molecular part, our research showed that the amount of β-catenin and C-myc proteins increased significantly in diabetic rats compared to the control group. At the same time, the amounts of GSK3β and Bcl-2 proteins in diabetic rats show a significant decrease compared to the control group (49). C-Myc protein is an oncogenic transcription factor known to regulate cell proliferation, differentiation, and apoptosis as well as cell size (49) and is upregulated during experimental hypertrophy. Since activated Wnt/β-catenin signaling has been observed during DCM development, stabilization and increased levels of β-catenin and its downstream target genes may lead to myocardial injury (36). Stabilized β-catenin facilitates p53-mediated myocardial DNA damage and apoptosis under diabetic oxidative stress through c-Myc upregulation (36). Studies have also shown that systemic inhibition of GSK3β may cause abnormal hypertrophic growth of the heart, possibly leading to heart failure (50). Inhibition of Wnt signaling by activating GSK3β has also reduced the hypertrophic response (50). In another study, Xi et al. (36) found that inhibiting GSK3β stabilizes β-catenin, which may cause cardiac remodeling. Their study stated that the mRNA expression of GSK3β is decreased, and the phosphorylated form of GSK3β (P-GSK3β) is increased in diabetic rats. On the other hand, Al-Damry et al. (51) stated in 2018 that one of the anti-apoptotic mechanisms of AKT is to phosphorylate and thus inactivate GSK3β. They stated that the decreased level of P-GSK3β in diabetic rats reflects the activity of GSK3β. Their study stated that the p-GSK-3β protein level and the p-GSK-3β/GSK-3β ratio were increased in the sitagliptin-treated group, possibly attributed to the restored Akt activity. Al-Damry et al. reported that Akt inhibits GSK-3β activity under normal conditions by phosphorylation. However, in diabetes, the ability of Akt to phosphorylate GSK-3 is reduced, causing GSK3β to remain active and leading to cardiac apoptosis. In our study, the results show a decrease in GSK3β in diabetic rats, which is associated with widespread apoptosis in the heart tissue of rats. On the other hand, Pelozin et al. (52) reported in 2022 that GSK3β is a negative regulator of cardiac hypertrophy. They stated that endurance training decreased GSK3β and thus increased mTOR levels, which is an important factor in cardiac hypertrophy. Perhaps the reason for these contradictions in previous studies and the results of our research is the method used to induce diabetes and the high doses of STZ they used and not using high-fat diets and then injecting a low dose of STZ (42). It is also possible that HIIT, despite the significant increase in GSK3β levels, activating other signaling pathways such as IGF1/AKT/mTOR, is an important factor in achieving physiological hypertrophy and controlling pathological hypertrophy in our research results. However, other factors probably influence the expression and transcription of GSK3β, which were not investigated in this study and need further investigation in the future. One of the components of apoptosis is the increase of the pro-apoptotic protein Bax and the decrease of the anti-apoptotic protein Bcl-2, which causes heart damage due to exposure to oxidative stress. In 2020, Liu et al. (12) stated that the increase in Bax/Bcl-2 ratio in diabetes increases the apoptosis process through the activity of the P53 protein. In the present study, HIIT intervention has decreased the amount of β-catenin and c-Myc protein in diabetic rats. Studies have shown that β-catenin protein and the Wnt/β-catenin signaling pathway play an important role in the pathogenesis of diabetic cardiomyopathy, and inhibition of β-catenin and Wnt can reduce pathological hypertrophy and myocardial fibrosis. Also, in line with the current study's findings, Yang and et al. (14) showed in their study in 2017 that physical exercise at different intensities inhibits and reduces the expression of proteins of the Wnt/β-catenin signaling pathway in diabetic rats. Their study stated that inactivating the Wnt/β-catenin signaling pathway reduces fat synthesis, improves lipid metabolism, increases glucose absorption and use, prevents muscle atrophy, and ultimately improves insulin sensitivity. Also, the results of our research showed that the amount of anti-apoptotic protein Bcl-2 increased significantly after HIIT in healthy and diabetic groups. Quindry et al. (53) also pointed to the protective function of endurance training (70% of maximal oxygen consumption) in dealing with myocardial apoptosis in rats after ischemia-reperfusion injury. Consistent with the results of our study, Suri et al. (54) reported in 2021 that Bcl-2 gene expression increased in both young and aged rats following HIIT. These findings showed that physical exercise protects the heart against apoptosis and can be a useful strategy to prevent heart problems in people with diabetes. Physical exercise can partially prevent the release of mitochondrial cytochrome C by reducing ROS and preventing cardiac apoptosis (54).

One of the strengths of the present study is the examination of histological and molecular changes, in which we could show the details of the changes after HIIT. Also, investigating signaling pathways and different colorings was another strength of this study in proving the effectiveness of HIIT.

On the other hand, this study also had limitations and weaknesses. One of the weak points of the present study is the lack of cardiac function tests such as ECG due to the lack of

laboratory facilities. Therefore, it is suggested that in future studies, researchers examine the functional changes of the heart using echocardiography.

5 Conclusion

Although HIIT has been used in medical rehabilitation, the appropriate exercise protocol for preventing cardiac events is still debated. The various strategies used in previous studies also make it difficult to conduct a cross-comparative analysis among them. The results of our research have depicted only a part of the cellular, molecular and histological interactions in the heart tissue of rats suffering from diabetes. This study is not a decisive, solid, and comprehensive achievement regarding the events caused by exercise and its effects on samples with diabetes. However, what is certain is that investigating the effects of intense sports training on sick and healthy samples requires more research. By identifying other factors involved in the homeostasis of cardiomyocytes, researchers can achieve a non-pharmacological and stable strategy to help patients with diabetes and other diseases.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Shahid Chamran University of Ahvaz: EE/1401.2.24.158669/scu.ac.ir.

Author contributions

Idea, supervisor: MR, writing and editing: AAH, laboratory analysis, SR and edit writing: DAD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1175585/full#supplementary-material

References

1. Kurdiova T, Balaz M, Vician M, Maderova D, Vlcek M, Valkovic L, et al. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: *in vivo* and *in vitro* studies. *J Physiol* (2014) 592 (5):1091–107. doi: 10.1113/jphysiol.2013.264655

2. Su J, Wang H, Tian Y, Hu H, Gu W, Zhang T, et al. Impact of physical exercise intervention and PPARγ genetic polymorphisms on cardio-metabolic parameters among a Chinese youth population. *BMJ Open Sport Exercise Med* (2020) 6(1): e000681. doi: 10.1136/bmjsem-2019-000681

3. Yang X, Ni L, Sun J, Yuan X, Li D. Associations between rs3480 and rs16835198 gene polymorphisms of FNDC5 with type 2 diabetes mellitus susceptibility: a meta-analysis. *Front Endocrinol* (2022) 13:946982. doi: 10.3389/fendo.2022.946982

4. Jang DK, Choi JH, Paik WH, Ryu JK, Kim Y-T, Han K-D, et al. Risk of cardiovascular disease and mortality in patients with diabetes and acute pancreatitis history: a nationwide cohort study. *Sci Rep* (2022) 12(1):18730. doi: 10.1038/s41598-022-21852-7

5. Young MF, Valaris S, Wrann CD. A role for FNDC5/Irisin in the beneficial effects of exercise on the brain and in neurodegenerative diseases. *Prog Cardiovasc Dis* (2019) 62(2):172–8. doi: 10.1016/j.pcad.2019.02.007

6. Fain JN, Company JM, Booth FW, Laughlin MH, Padilla J, Jenkins NT, et al. Exercise training does not increase muscle FNDC5 protein or mRNA expression in pigs. *Metabolism* (2013) 62(10):1503–11. doi: 10.1016/j.metabol.2013.05.021

7. Agha-Alinejad H, Ahmadi Hekmatikar AH, Ruhee RT, Shamsi MM, Rahmati M, Khoramipour K, et al. A guide to different intensities of exercise, vaccination, and sports nutrition in the course of preparing elite athletes for the management of upper respiratory infections during the COVID-19 pandemic: a narrative review. *Int J Environ Res Public Health* (2022) 19(3). doi: 10.3390/ijerph19031888

8. Whillier S. Exercise and insulin resistance. *Phys Exercise Hum Health* (2020), 137–50. doi: 10.1007/978-981-15-1792-1_9

9. Dawson K, Aflaki M, Nattel S. Role of the wnt-frizzled system in cardiac pathophysiology: a rapidly developing, poorly understood area with enormous potential. *J Physiol* (2013) 591(6):1409–32. doi: 10.1113/jphysiol.2012.235382

10. Liu P, Su J, Song X, Wang S. Activation of nuclear β-catenin/c-Myc axis promotes oxidative stress injury in streptozotocin-induced diabetic cardiomyopathy. *Biochem Biophys Res Commun* (2017) 493(4):1573–80. doi: 10.1016/j.bbrc.2017.10.027

11. Zhang M, Ye M. Hydrogen sulfide attenuates high glucose-induced myocardial injury in rat cardiomyocytes by suppressing wnt/beta-catenin pathway. *Curr Med sci* (2019) 39(6):938–46. doi: 10.1007/s11596-019-2120-5

12. Liu JJ, Shentu LM, Ma N, Wang LY, Zhang GM, Sun Y, et al. Inhibition of NF-κB and wnt/β-catenin/GSK3β signaling pathways ameliorates cardiomyocyte hypertrophy and fibrosis in streptozotocin (STZ)-induced type 1 diabetic rats. *Curr Med Sci* (2020) 40(1):35–47. doi: 10.1007/s11596-020-2144-x

13. Dayan CM, Besser RE, Oram RA, Hagopian W, Vatish M, Bendor-Samuel O, et al. Preventing type 1 diabetes in childhood. *Science* (2021) 373(6554):506–10. doi: 10.1126/science.abi4742

14. Yang Q, Wang W-w, Ma P, Ma Z-X, Hao M, Adelusi TI, et al. Swimming training alleviated insulin resistance through Wnt3a/β-catenin signaling in type 2 diabetic rats. *Iran J Basic Med Sci* (2017) 20(11):1220. doi: 10.22038/IJBMS.2017.9473

15. Dotzert MS, Murray MR, McDonald MW, Olver TD, Velenosi TJ, Hennop A, et al. Metabolomic response of skeletal muscle to aerobic exercise training in insulin resistant type 1 diabetic rats. *Sci Rep* (2016) 6(1):26379. doi: 10.1038/srep26379

16. Farhani F, Shahrbanian S, Auais M, Hekmatikar AHA, Suzuki K. Effects of aerobic training on brain plasticity in patients with mild cognitive impairment: a systematic review of randomized controlled trials. *Brain Sci* (2022) 12(6):732. doi: 10.3390/brainsci12060732

17. Hall KE, McDonald MW, Grisé KN, Campos OA, Noble EG, Melling CJ. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced type 1 diabetic rodents. *Metabolism* (2013) 62(10):1485–94. doi: 10.1016/j.metabol.2013.05.012

18. Liu Y, Liu C, Lu M-L, Tang F-T, Hou X-W, Yang J, et al. Vibration exercise decreases insulin resistance and modulates the insulin signaling pathway in a type 2 diabetic rat model. *Int J Clin Exp Med* (2015) 8(8):13136.

19. de Bem GF, Costa CA, Santos IB, Cristino Cordeiro V, de Carvalho LCRM, de Souza MAV, et al. Antidiabetic effect of euterpe oleracea mart.(açaí) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: a positive interaction. *PloS One* (2018) 13(6):e0199207. doi: 10.1371/journal.pone.0199207

20. Thomas C, Bishop D, Moore-Morris T, Mercier J. Effects of high-intensity training on MCT1, MCT4, and NBC expressions in rat skeletal muscles: influence of chronic metabolic alkalosis. *Am J Physiol-Endocrinol Metab* (2007) 293(4):E916–E22. doi: 10.1152/ajpendo.00164.2007

21. Cassidy S, Vaidya V, Houghton D, Zalewski P, Seferovic JP, Hallsworth K, et al. Unsupervised high-intensity interval training improves glycaemic control but not cardiovascular autonomic function in type 2 diabetes patients: a randomised controlled trial. *Diabetes Vasc Dis Res* (2019) 16(1):69–76. doi: 10.1177/1479164118816223

22. Adams OP. The impact of brief high-intensity exercise on blood glucose levels. *Diab Metab syndr obes: Targets Ther* (2013), 113–22. doi: 10.2147/DMSO.S29222

23. Novoa U, Arauna D, Moran M, Nuñez M, Zagmutt S, Saldivia S, et al. High-intensity exercise reduces cardiac fibrosis and hypertrophy but does not restore the nitroso-redox imbalance in diabetic cardiomyopathy. *Oxid Med Cell Longev* (2017) 2017. doi: 10.1155/2017/7921363

24. Wang X, Pan J, Liu D, Zhang M, Li X, Tian J, et al. Nicorandil alleviates apoptosis in diabetic cardiomyopathy through PI3K/Akt pathway. *J Cell Mol Med* (2019) 23(8):5349–59. doi: 10.1111/jcmm.14413

25. Lu Z, Xu Y, Song Y, Bíró I, Gu Y. A mixed comparisons of different intensities and types of physical exercise in patients with diseases related to oxidative stress: a systematic review and network meta-analysis. *Front Physiol* (2021) 12. doi: 10.3389/fphys.2021.700055

26. Hill JA, Olson EN. Cardiac plasticity. *N Engl J Med* (2008) 358(13):1370–80. doi: 10.1056/NEJMra072139

27. Yuan G, Qin Y, Peng P. High-intensity interval training for treating pathological cardiac hypertrophy in spontaneously hypertensive rats: effects and mechanisms. *Chin J Tissue Eng Res* (2020) 24(23):3708.

28. Chengji W, Xianjin F. Exercise protects against diabetic cardiomyopathy by the inhibition of the endoplasmic reticulum stress pathway in rats. *J Cell Physiol* (2019) 234 (2):1682–8. doi: 10.1002/jcp.27038

29. Marchini GS, Cestari IN, Salemi VM, Irigoyen MC, Arnold A, Kakoi A, et al. Early changes in myocyte contractility and cardiac function in streptozotocin-induced type 1 diabetes in rats. *PloS One* (2020) 15(8):e0237305. doi: 10.1371/journal.pone.0237305

30. Bulani Y, Sharma SS. Argatroban attenuates diabetic cardiomyopathy in rats by reducing fibrosis, inflammation, apoptosis, and protease-activated receptor expression. *Cardiovasc Drugs Ther* (2017) 31(3):255–67. doi: 10.1007/s10557-017-6732-3

31. Kang YJ. Cardiac hypertrophy: a risk factor for QT-prolongation and cardiac sudden death. *Toxicol Pathol* (2006) 34(1):58–66. doi: 10.1080/01926230500419421

32. Logantha SJR, Cai XJ, Yanni J, Jones CB, Stephenson RS, Stuart L, et al. Remodeling of the purkinje network in congestive heart failure in the rabbit. *Circul: Heart Fail* (2021) 14(7):e007505. doi: 10.1161/CIRCHEARTFAILURE.120.007505

33. Lanlua P, Chotimol P, Sricharoenvej S, Baimai S, Piyawinijwong S. Cardiac ultrastructure in streptozotocin-induced diabetic rats. (2012).

34. Chaturvedi P, Kalani A, Chaturvedi P, Kalani K, Verma VK, Tyagi SC. Exercise mitigates calpain induced purkinje cell loss in diabetes. *Life Sci* (2022) 308:120982. doi: 10.1016/j.lfs.2022.120982

35. Wu Z, Huang A, Yan J, Liu B, Liu Q, Zhang J, et al. Resveratrol ameliorates cardiac dysfunction by inhibiting apoptosis *via* the PI3K/Akt/FoxO3a pathway in a rat model of diabetic cardiomyopathy. *J Cardiovasc Pharmacol* (2017) 70(3):184–93. doi: 10.1097/FJC.0000000000000504

36. Xi X-H, Wang Y, Li J, Wang F-W, Tian G-H, Yin M-S, et al. Activation of wnt/β-catenin/GSK3β signaling during the development of diabetic cardiomyopathy. *Cardiovasc Pathol* (2015) 24(3):179–86. doi: 10.1016/j.carpath.2014.12.002

37. Ren BC, Zhang YF, Liu SS, Cheng XJ, Yang X, Cui XG, et al. Curcumin alleviates oxidative stress and inhibits apoptosis in diabetic cardiomyopathy *via* Sirt1-Foxo1 and PI3K-akt signalling pathways. *J Cell Mol Med* (2020) 24(21):12355–67. doi: 10.1111/jcmm.15725

38. Sadi G, Pektaş MB, Koca HB, Tosun M, Koca T. Resveratrol improves hepatic insulin signaling and reduces the inflammatory response in streptozotocin-induced diabetes. *Gene* (2015) 570(2):213–20. doi: 10.1016/j.gene.2015.06.019

39. Gencoglu H, Tuzcu M, Hayirli A, Sahin K. Protective effects of resveratrol against streptozotocin-induced diabetes in rats by modulation of visfatin/sirtuin-1 pathway and glucose transporters. *Int J Food Sci Nutr* (2015) 66(3):314–20. doi: 10.3109/09637486.2014.1003534

40. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* (2005) 115(3):500–8. doi: 10.1172/JCI200524408

41. de Las Heras N, Lahera V. Relevance of mitochondrial dysfunction in heart disease associated with insulin resistance conditions. *Pflügers Archiv-European J Physiol* (2022) 474(1):21–31. doi: 10.1007/s00424-021-02638-8

42. Khoramipour K, Hekmatikar AA, Sotvan H. An overview of fatmax and MFO in exercise. *Razi J Med Sci* (2020) 27:49–59.

43. Wang L, Lavier J, Hua W, Wang Y, Gong L, Wei H, et al. High-intensity interval training and moderate-intensity continuous training attenuate oxidative damage and promote myokine response in the skeletal muscle of ApoE KO mice on high-fat diet. *Antioxidants* (2021) 10(7):992. doi: 10.3390/antiox10070992

44. Heinonen I, Sorop O, de Beer VJ, Duncker DJ, Merkus D. What can we learn about treating heart failure from the heart's response to acute exercise? focus on the coronary microcirculation. *J Appl Physiol* (2015) 119(8):934–43. doi: 10.1152/japplphysiol.00053.2015

45. Aisagbonhi O, Rai M, Ryzhov S, Atria N, Feoktistov I, Hatzopoulos AK. Experimental myocardial infarction triggers canonical wnt signaling and endothelial-to-mesenchymal transition. *Dis Models Mech* (2011) 4(4):469–83. doi: 10.1242/dmm.006510

46. Wei H, Qu H, Wang H, Ji B, Ding Y, Liu D, et al. 1, 25-Dihydroxyvitamin-D3 prevents the development of diabetic cardiomyopathy in type 1 diabetic rats by enhancing autophagy *via* inhibiting the β-catenin/TCF4/GSK-3β/mTOR pathway. *J Steroid Biochem Mol Biol* (2017) 168:71–90. doi: 10.1016/j.jsbmb.2017.02.007

47. Tsai S-J, Huang C-S, Mong M-C, Kam W-Y, Huang H-Y, Yin M-C. Anti-inflammatory and antifibrotic effects of naringenin in diabetic mice. *J Agric Food Chem* (2012) 60(1):514–21. doi: 10.1021/jf203259h

48. Martinez A, Castro A, Dorronsoro I, Alonso M. Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med Res Rev* (2002) 22(4):373–84. doi: 10.1002/med.10011

49. Swanberg SE, Delany ME. Differential expression of genes associated with telomere length homeostasis and oncogenesis in an avian model. *Mech Ageing Dev* (2005) 126(10):1060–70. doi: 10.1016/j.mad.2005.03.022

50. Sugden PH, Fuller SJ, Weiss S, Clerk A. Glycogen synthase kinase 3 (GSK3) in the heart: a point of integration in hypertrophic signalling and a therapeutic target? a critical analysis. *Br J Pharmacol* (2008) 153(S1):S137–S53. doi: 10.1038/sj.bjp.0707659

51. Al-Damry NT, Attia HA, Al-Rasheed NM, Al-Rasheed NM, Mohamad RA, Al-Amin MA, et al. Sitagliptin attenuates myocardial apoptosis *via* activating LKB-1/AMPK/Akt pathway and suppressing the activity of GSK-3β and p38α/MAPK in a rat model of diabetic cardiomyopathy. *Biomed Pharmacother* (2018) 107:347–58. doi: 10.1016/j.biopha.2018.07.126

52. Pelozin BR, Soci UP, Gomes JL, Oliveira EM, Fernandes T. mTOR signaling-related microRNAs as cardiac hypertrophy modulators in high-volume endurance training. *J Appl Physiol* (2022) 132(1):126–39. doi: 10.1152/japplphysiol.00881.2020

53. Quindry JC, Miller L, McGinnis G, Kliszczewicz B, Irwin JM, Landram M, et al. Ischemia reperfusion injury, KATP channels, and exercise-induced cardioprotection against apoptosis. *J Appl Physiol* (2012) 113(3):498–506. doi: 10.1152/japplphysiol.00957.2011

54. Soori R, Ghram A, Zare Shahneh M, Choobineh S, Costa PB, Voltarelli FA. Effects of high intensity interval training and aging on cardiac muscle apoptosis markers in C57BL/6 mice. *Sport Sci Health* (2021) 17(1):173–9. doi: 10.1007/s11332-020-00670-2

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Exploring the comparative cardiovascular death benefits of sodium–glucose cotransporter 2 inhibitors in type 2 diabetes: a frequentist and Bayesian network meta-analysis-based scoring

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Background and aims: Cardiovascular death (CV death) is the most objective component of the primary or secondary endpoint in cardiovascular outcome trials (CVOTs) conducted with sodium–glucose cotransporter 2 inhibitors (SGLT-2is). CV death is often incorporated into primary composite outcomes. It is combined with major adverse cardiovascular events (MACEs) in trials with atherosclerotic cardiovascular disease (ASCVD) at baseline and with hospitalization due to heart failure (hHF) in trials with heart failure at baseline. Unlike the primary composites, CV death reduction by itself demonstrated significant variations among the CVOTs with SGLT-2is. Moreover, the impact of the individual agents within the SGLT-2i group on the reduction in CV death has not been explored objectively. This network meta-analysis was undertaken to construct a hierarchy based on indirect pairwise comparisons and rankings among the individual agents within SGLT-2is.

Methods: A Cochrane library-based web search yielded 13 randomized controlled trials for analysis. Stata/BE 17.0 and RStudio 2022.07.1 Build 554 software were used to conduct a frequentist and Bayesian network meta-analysis. The effect size was assessed based on the risk ratio (RR). Ranking of the individual agents was performed with a frequentist approach (P-score and a multidimensional scaling [MDS] rank system) and a Bayesian ranking (surface under the cumulative ranking [SUCRA]).

Results: Regarding the overall data, SGLT-2is reduced the CV death risk by 12% (RR: 0.88, 95% CI 0.80–0.96). All three scoring methods resulted in empagliflozin scoring the highest. There was a 15% RR reduction in CV death (95% CI 0.71–1.02) in the ASCVD and multiple cardiovascular risk factor (MRF) groups and an 11% RR reduction in the HF group, with empagliflozin ranking the highest in the former group and dapagliflozin in the latter.

Conclusions: Empagliflozin ranked the highest compared to the other SGLT-2is in the overall population and the trials including type 2 diabetes (T2D) patients with ASCVD or MRF at baseline, while dapagliflozin ranked the highest in the trials of patients with HF at baseline.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022381556, identifier CRD42022381556.

KEYWORDS

SGLT-2is, network meta-analysis, CV death, type 2 diabetes, ranking

1 Introduction

The cardiorenal benefits associated with sodium–glucose cotransporter 2 inhibitors (SGLT-2is) are well established (1). Practically all major diabetes, cardiac, and nephrology guidelines recommend SGLT-2is as the first-line drug for the prevention of atherosclerotic cardiovascular disease (ASCVD), heart failure, or progression of diabetic kidney disease in conjunction with good metabolic control (2–4). The initial studies of SGLT-2is were conducted on patients with type 2 diabetes (T2D) and either established atherosclerotic cardiovascular disease (eASCVD) or multiple cardiovascular (CV) risk factors (MRFs) (5–7). The primary endpoint in all these trials was a composite 3-point major adverse cardiovascular event (MACE) comprising cardiovascular death (CV death), non-fatal myocardial infarction (NFMI), and non-fatal stroke (NFS), except for the DECLARE-TIMI 58 trial, where a coprimary endpoint in the form of CV death or hospitalization due to heart failure (hHF) was introduced (6). Due to the differences in the types of populations recruited in these studies, the results were heterogeneous, with MACE and CV death benefits in the EMPAREG Outcomes trial, a MACE benefit in CANVAS, and CV death or hHF coprimary in the DECLARE-TIMI 58 trial. Considering the significant contribution of hHF and renal benefits in these trials, all subsequent trials utilized CV death, hHF, or a renal composite as the primary outcome (8–14). In all these trials, the primary outcome endpoint was achieved. As one of the most objective endpoints, CV death featured in all these studies as part of the primary or key secondary outcome.

The results from all the above-mentioned trials resulted in the American Diabetes Association (ADA)/European Association for the Study of Diabetes (EASD) 2022 guidelines recommending either empagliflozin or canagliflozin as the SGLT-2i of choice for patients with ASCVD or MRF and any of the three (empagliflozin, dapagliflozin, or canagliflozin) for patients with T2D and heart failure or diabetic kidney disease (DKD) (2). Although group specific, these recommendations do not clarify the issues related to the within-group choice of agents. In the absence of head-to-head comparison between the agents, it is incumbent upon the physician to choose among these medications, often arbitrarily.

This network meta-analysis was designed to explore the within-group differences of CV death benefits associated with SGLT-2is in patients with T2D. CV death was chosen over composite endpoints and other stand-alone endpoints because of its objective nature, consistent reporting across the trials, and being the most controversial outcome.

This meta-analysis was designed following the PICO question format (shown below):

P (patient population) = Patients diagnosed with T2D.

I (intervention) = Received drugs belonging to the SGLT-2i group.

C (control group) = Compared to placebo.

O (outcome) = The primary aim was to determine whether there was a hierarchical choice when selecting one of the agents from the intervention arm (I) as far as a reduction in CV death was concerned.

2 Materials and methods

This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Our review protocol was prospectively registered (15).

2.1 Search strategy and eligibility criteria

This network meta-analysis was conducted using a predetermined protocol and was reported in accordance with the PRISMA statement. An electronic database search was conducted by the authors (SG and BS) using the Cochrane Library without any limitations on date or language. The search for relevant abstracts presented at major congresses was reviewed manually. The search headings included the following terms [("type 2 diabetes" {MeSH}, OR "T2D") OR ("sodium glucose cotransporter 2 inhibitors" {MeSH}, OR "SGLT-2i" OR "empagliflozin" OR "dapagliflozin", OR "canagliflozin", OR "ertugliflozin", OR "sotagliflozin")] AND

["cardiovascular death", or "CV death"]. The full search strategy is detailed in Figure 1.

2.2 Study selection and eligibility criteria

Having performed the preliminary web search, the screened citations were manually sorted by the authors based on the PICO search criteria and the prespecified eligibility criteria. The key eligibility criteria for positive selection included phase 3 randomized controlled trials (RCTs) with the following:

- Patients with T2D.
- Age of the patients ≥18 years.
- Placebo as the comparator arm.
- Studies clearly mentioning the primary outcome of interest that conformed to our intervention requirements: MACE, CV death or hHF, renal composite of CV death as the primary endpoint, and CV death as part of the primary endpoint component or as a key secondary endpoint.

Exclusion criteria:

- Other types of diabetes.
- Acute decompensated metabolic, cardiac, or renal disorders.
- Non-randomized controlled trials.
- Age <18 years.
- Active comparator arm.
- Doses of SGLT-2is used in dose-finding studies. For example, 2.5 mg of dapagliflozin.
- Direct comparison between different doses of SGLT-2is. For example, 100 versus 300 mg of canagliflozin.

2.3 Data extraction

Both authors (SG and BS) conducted the database search and literature screening independently and compared the output. After the removal of the duplicate citations and those not conforming to the

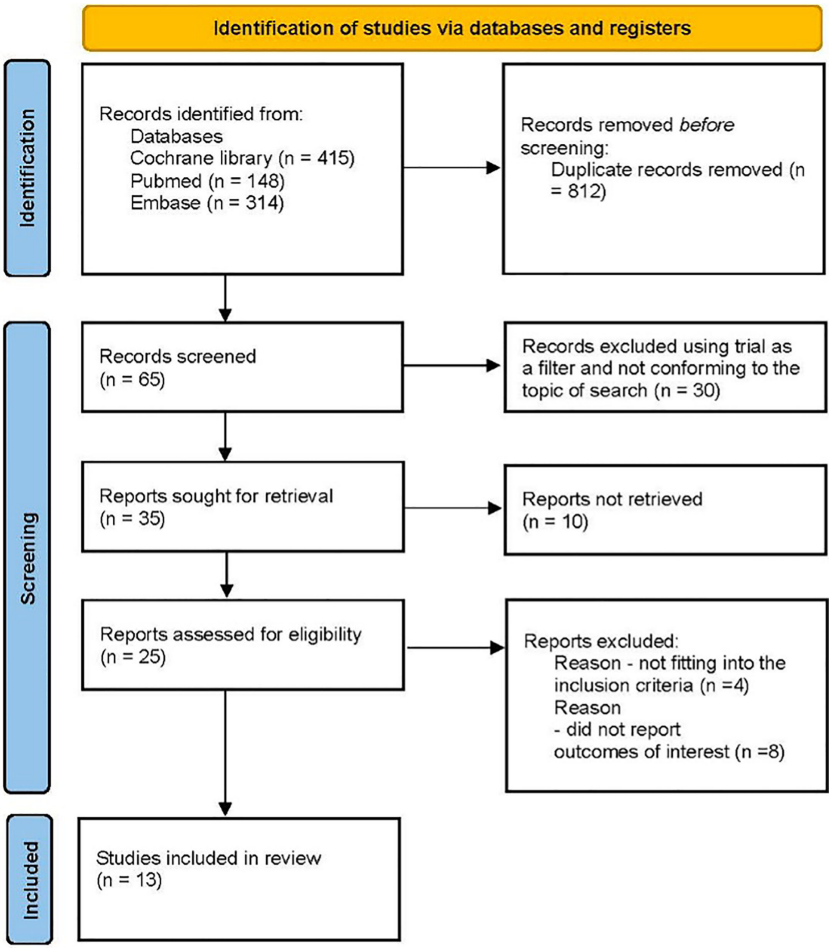

FIGURE 1
Study selection criteria.

predetermined inclusion criteria after screening the titles and abstracts, the full-text articles were screened manually. Any disagreement between the authors was settled with mutual agreement.

2.4 Risk of bias

The Cochrane Collaboration Risk of Bias 2.0 algorithm was used to assess and report the bias associated with the individual studies (Supplementary Figure A (2)). The citations were assessed by all the authors, and any dispute was resolved with consensus. Publication bias was assessed qualitatively using a funnel plot and quantitatively using Peter's method (Supplementary Figure A (1)).

2.5 Statistical analysis

The primary objective was to create a hierarchical model to assist the concerned physician in choosing among the different approved SGLT-2is for patients with T2D to reduce their risk of CV death.

Two software programs were used for performing the analysis and preparing the graphical data. The RStudio 2022.07.1 Build 554 software was used to perform the Bayesian network analysis. Stata/BE 17.0 software was used to conduct the pairwise meta-analysis. In addition, multidimensional scaling (MDS) ranking was performed with STATA. With *a priori* power calculation assuming a minimal difference in effect size (risk ratio (RR)) of 10%, from at least 10 studies with 5,000 participants in each arm, an alpha of 0.05, and a moderate degree of heterogeneity, the estimated power of this meta-analysis was 100% (Supplementary Figure A (3)).

The planned statistical analysis included the following steps:

a. We planned to perform a baseline meta-analysis using the overall (empagliflozin, dapagliflozin, and canagliflozin) database and a subgroup analysis using the individual agents. The primary aim was to identify the quantum of benefit as evident from the effect size, along with an estimation of the precision interval indicating the accuracy of the measurement and the prediction interval indicating the presence of heterogeneity (if any). The degree of overlap of the precision intervals while comparing the individual agents versus placebo was noted.
b. The second step would involve conducting a pairwise network meta-analysis using the overall data and then with the individual subgroups (ASCVD or MRF and those with HF).
c. The third step included non-reshaped scoring using the frequentist approach (P scores) and reshaped scoring using both a frequentist (MSD rank score) and Bayesian (surface under the cumulative ranking [SUCRA]) approach (Supplementary Appendix B–D). This strategy was planned to be applied to the overall population and subsequently to both the subgroups.

The rationale behind including the different scoring systems for assessment was based on the consensus that a P score (without reshaping of data) creates a rank using the effect size as the quantum of benefit, while both the SUCRA and MDS ranking considers the precision interval in addition to the effect size differential. Any discrepancy between the rankings would not allow us to reach a definitive conclusion and hence would be discarded. The top ranking was considered significant if there was concordance between the pairwise comparisons and among all three scoring methods. This strategy would be extremely conservative, and any agent able to overcome this significant burden would be considered for discussion.

3 Results

3.1 Baseline characteristics of the studies

An electronic database search yielded 13 citations that were included in the network meta-analysis (5–14, 16–18). There were 74,804 patients included in the analysis, with 40,691 in the intervention arm (SGLT-2is) compared to 34,113 in the placebo arm. The mean age of the participants ranged between 62 and 71 years. The primary endpoint was 3-P MACE in four trials (EMPAREG, DECLARE TIMI-58, VERTIS-CV, and CANVAS). DECLARE TIMI-58 had a coprimary endpoint (CV death or hHF) analyzed alongside MACE. CV death or hHF was the primary endpoint in seven trials (EMPEROR-Preserved, EMPEROR-Reduced, DECLARE TIMI-58, DAPA-HF, DELIVER, SOLOIST-WHF, and SCORED). Three trials (EMPA-Kidney, DAPA-CKD, and CREDENCE) had renal composite or CV death as the primary endpoint. CV death was part of the primary component in nine trials (EMPAREG, EMPEROR-Preserved, EMPEROR-Reduced, DECLARE TIMI-58, DAPA-CKD, DAPA-HF). DELIVER, CANVAS, and CREDENCE), while it was part of the secondary outcomes in four trials (EMPA-Kidney, VERTIS-CV, SOLOIST-WHF, and SCORED) (Table 1).

3.2 Baseline meta-analysis including subgroup analysis

The pooled data from all 13 citations demonstrated a 12% relative risk (RR) reduction compared to the placebo with a 95% precision interval of 0.80–0.96. However, there was significant heterogeneity in the outcome, as evidenced by a prediction interval ranging between 0.69 and 1.12 (Supplementary Figure A [4: a(i)]). A sensitivity analysis was performed using the leave-one-study strategy to explore whether a particular study skewed the pooled effect size. In view of the similarity of the effect size, precision interval, and prediction interval of the sensitivity analysis, we went ahead with the subgroup analysis (Supplementary Material E).

The p-value for interaction was not significant ($p = 0.85$) in the agent-type subgroup analysis. The RR reduction was the highest (20%, 95% CI 0.57–1.14) in the empagliflozin arm and the lowest in

the canagliflozin arm (7%, 95% CI 0.12–7.35) (Supplementary Figure A [4: a(ii)]).

When the inclusion criteria were T2D with established ASCVD or MRF (renal insufficiency included as a CVD risk factor), there was a 15% RR reduction in CV death compared to placebo with a precision interval of 0.71–1.02. The agent-specific subgroup analysis resulted in a maximum 35% RR (95% CI 0.30–1.39) reduction in CV death with empagliflozin and a minimum of 7% with canagliflozin (95% CI 0.12–7.35). The p-value of interaction between the subgroups was significant at $p < 0.01$ (Supplementary Figure A [4: b(i) & (ii)]).

Including trials with HF as the primary inclusion criteria, there was an 11% RR reduction in CV death compared to placebo (95% CI 0.83–0.96). There was significant heterogeneity associated with the outcomes (prediction interval 0.77–1.03). The agent-type subgroup analysis resulted in a maximum 17% RR reduction with dapagliflozin (95% CI 0.58–1.20) and a minimum effect size of 5% with empagliflozin (95% CI 0.65–1.35). The p-value for subgroup interactions was significant (<0.01) (Supplementary Figure A [4: c(i) & (ii)]).

3.3 The network meta-analysis and scoring

All of the included studies had placebo as the comparative arm, and hence, no loop was formed. The network plot had free nodes (Supplementary Figure A [5]).

3.3.1 Overall data

The indirect pairwise comparison demonstrated a consistent trend in favor of empagliflozin (Supplementary Figure A [6 (a)]).

The frequentist P-score constructed based on the effect size (RR reduction) reflected the pattern encountered in the baseline meta-analysis, with empagliflozin scoring the highest (0.895) among the SGLT-2is as far as a reduction in CV death is concerned. The reshaped frequentist MDS rank scores and the Bayesian SUCRA scores were consistent with the P-score, with empagliflozin scoring the highest (Figure 2).

The within-design heterogeneity was significant (Q: 19.84, df: 8, $p = 0.01$).

3.3.2 ASCVD or MRF

Empagliflozin demonstrated a clear trend favoring it over dapagliflozin (MD 1.45, 95% CI 1.00–2.120) and canagliflozin (MD 1.46, 95% CI 1.01–2.11) (Supplementary Figure A [6 (b)]).

All three scoring systems indicated a clear benefit associated with empagliflozin over all its counterparts. There was a large difference between empagliflozin and the agent ranking second irrespective of the method used (Figure 3).

The within-design heterogeneity was not significant (Q: 2.03, df: 3, $p = 0.56$).

3.3.3 HF

There was no clear trend visible in the comparison versus placebo or the indirect pairwise comparisons (Supplementary Figure A [6 (c)]).

However, all three scoring systems were consistent in designating dapagliflozin as the agent securing the highest rank (Figure 4).

The within-design heterogeneity was not significant (Q: 0.24, df: 2, $p = 0.88$).

4 Discussion

The cardiorenal benefits of SGLT-2is and GLP1-RAs have revolutionized the management strategy of T2D. The cardiovascular benefits associated with SGLT-2is can be broadly divided into benefits related to the atherosclerotic process or the pumping action of the heart (19). The earlier trials (EMPAREG Outcomes and CANVAS) documented MACE benefits, while all of the recent trials (EMPEROR-Preserved, EMPEROR-Reduced, DAPA-HF, and DELIVER) targeted heart failure as the outcome of prime importance. One of the consistent components of the primary endpoint, MACE, or the heart failure composite was CV death. Being the most objective and

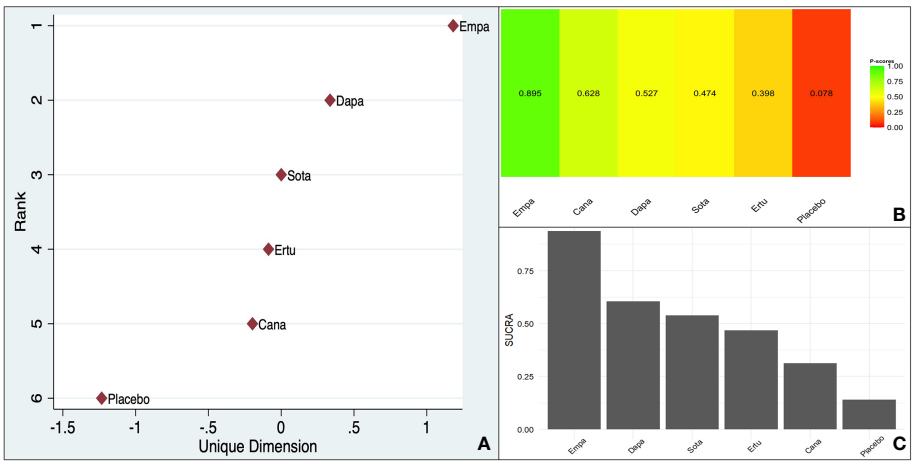

FIGURE 2
Ranking of different SGLT-2is (Overall data - CV death reduction in the pooled population including ASCVD/MRF & HF): **(A)**. MDS rank score (frequentist), **(B)**. P-score (frequentist), **(C)**. SUCRA (Bayesian).

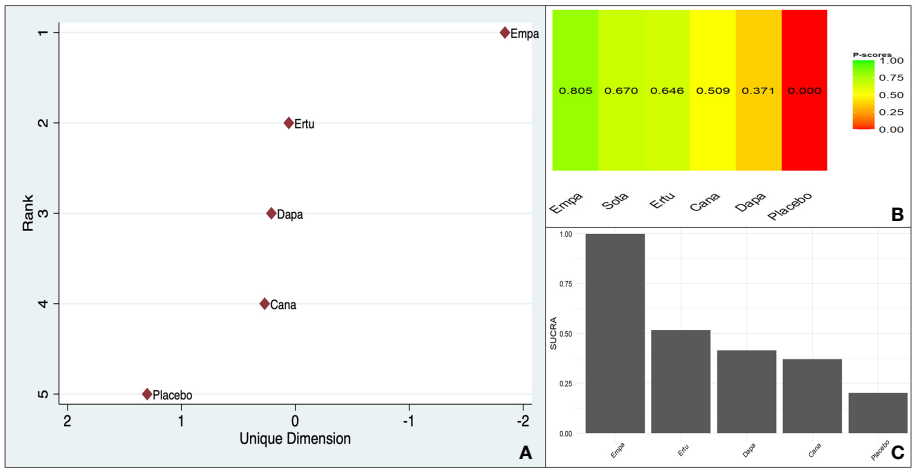

FIGURE 3
Ranking of CV death benefit of different SGLT-2is taking ASCVD or MRF as the baseline characteristic: **(A)**. MDS rank score (frequentist), **(B)**. P-score (frequentist), **(C)**. SUCRA (Bayesian).

clearly defined outcome, CV death reduction has been at the center of academic controversy (20). The only cardiovascular outcome trial (CVOT) to demonstrate CV death benefit as a part of a pre-adjudicated, prespecified primary endpoint component was the EMPAREG Outcomes trial. As a result, empagliflozin was assigned a class IB recommendation as an agent preventing CV death in patients with T2D by the 2019 European Society of Cardiology (ESC) guidelines (3). This outcome was not replicated in any of the subsequent trials recruiting patients with ASCVD or MRF. In contrast, all heart failure trials with SGLT-2is were successful in achieving the primary endpoint of CV death or hHF. However, the stand-alone endpoint of CV death reduction was comparable to a placebo in all the heart failure trials, except for DAPA-HF. Although there was an 18% difference in CV death reduction compared to placebo with dapagliflozin, the outcome was exploratory due to alpha spending.

There are no real controversies, as the primary composite outcome benefits are concerned with the use of SGLT-2is by T2D patients with CV risk (ASCVD as well as HF). However, the CV death benefit effect size is heterogeneous and needs to be explored further.

A recent network meta-analysis (NMA) suggested that empagliflozin had the highest SUCRA-based rank in terms of the reduction in mortality (21). However, that analysis included dose-finding studies that used both standardized doses and doses lower than the clinically approved doses, thus confounding the results. In addition, the distinction between patients with ASCVD/MRF and HF was not explored in this analysis.

This network meta-analysis was undertaken to confirm the findings from a previous meta-analysis (CV death benefits associated with SGLT-2is) with standardized doses and to explore the unchartered area of CV death benefits for T2D patients with ASCVD/MRF or HF.

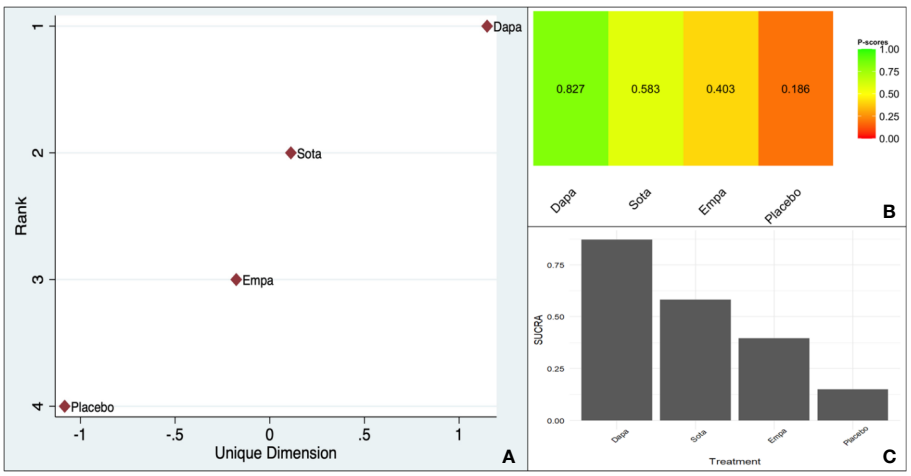

FIGURE 4
Ranking of CV death benefit of different SGLT-2is taking HF as the baseline characteristic: **(A)**. MDS rank score (frequentist), **(B)**. P-score (frequentist), **(C)**. SUCRA (Bayesian).

TABLE 1 Baseline characteristics of the studies included for analysis.

Study	Year	Intervention	Intervention/ placebo (n)	Mean age	Study duration (years)	ASCVD/MRF (%) Intervention arm (entire cohort)	HF (%)	Primary endpoint	CV death status
EMPA KIDNEY (10)	2022	Empagliflozin	1,525/1,515	63.9 ± 13.9	2.0	26.1/73.9	NR	Kidney disease progression or cardiovascular death	Other secondary outcomes
EMPAREG (5)	2015	Empagliflozin	4,645/2,323	63.0 ± 8.6)	3.1	99.5/0.5	9.9	3-P MACE	Primary component
EMPEROR PRESERVED (8)	2021	Empagliflozin	1,466/1,472	70.9 ± 9.0	2.1	36/64	100 (heart failure with preserved ejection fraction [HFpEF])	CV death or hHF	Primary component
EMPEROR REDUCED (9)	2022	Empagliflozin	927/929	67.6 ± 11.6	1.3	52.8/47.2	100 (heart failure with reduced ejection fraction [HFrEF])	CV death or hospitalization due to worsening HF	Primary component
DECLARE TIMI-58 (6)	2019	Dapagliflozin	8,582/8,578	63.9 + 6.8	4.2	40.5/59.5	9.9	3-P MACE and CV death or hHF	Primary component
DAPA-HF (11)	2019	Dapagliflozin	1,075/1,064	66.2 + 11.0	1.5	55.5/36.1 [unknown: 8.4]	100 (HFrEF)	CV death or hospitalization due to worsening HF	Primary component
DAPA-CKD (13)	2020	Dapagliflozin	1,455/1,451	61.8 ± 12.1	2.4	37.8/62.2	10.9	Renal composite or CV death	Primary component
DELIVER (12)		Dapagliflozin	1,578/1,572		2.3	NR	100 (HFpEF)	CV death or hospitalization due to worsening HF	Primary component
CANVAS (7)	2017	Canagliflozin	5,795/4,347	63.2 + 83	3.6	64.8/35.2	13.9	3-P MACE	Primary component
CREDENCE (14)	2019	Canagliflozin	2,202/2,199	62.9 ± 9.2	2.6	50.5/49.5	14.9	Renal composite or CV death	Primary component
VERTIS-CV (16)	2020	Ertugliflozin	5,499/2,747	64.4 ± 8.1	3.5	100	23.4	3-P MACE	Key secondary outcome
SOLOIST-WHF (17)	2121	Sotagliflozin	608/614	69 (median)	0.75	NR [ASCVD was an exclusion criterion]	79.1	CV death or hospitalization due to worsening HF	Secondary endpoint
SCORED (18)	2021	Sotagliflozin	5,292/5,292	69 (Median)	1.3	11.5/88.5	31	CV death or hospitalization due to worsening HF	Major secondary endpoint

4.1 Findings from our NMA

The baseline pooled meta-analysis indicated a 12% RR reduction (95% CI 0.80–0.96) in CV death compared to placebo, with a non-significant p-value for interaction. However, there was significant heterogeneity, indicating additional factors responsible for the outcome benefit. There was a 15% RR (95% CI 0.71–1.02) and an 11% RR reduction (95% CI 0.83–0.96) in the ASCVD/MRF

and HF subgroups, respectively. Empagliflozin scored the highest on the frequentist P score (0.895), in the MDS ranking, and the Bayesian SUCRA scoring of the overall data, with significant within-study design heterogeneity (p = 0.01).

Empagliflozin ranked the highest in the ASCVD and MRF subgroups in all three-ranking systems with non-significant within-study heterogeneity (p = 0.56). This was in accordance with the trend observed from the indirect pairwise meta-analysis.

The picture was less clear in the pairwise network meta-analysis regarding the preferred agent in the HF subgroup. All the three scores were, however, consistent, indicating the superiority of dapagliflozin over all its counterparts with non-significant within-design heterogeneity (p = 0.88).

To summarize, the overall analysis was in favor of SGLT-2is as a group consistent with a recent meta-analysis. However, this conclusion was diluted by the presence of significant heterogeneity (prediction interval 0.69–1.12). Regarding the agent that performed the best in the ranking, our analysis mirrored that of Jiang et al., with empagliflozin emerging as the superior choice. The additional aspect that emerged from our analysis was that dapagliflozin scored the highest in the HF subgroup and empagliflozin scored the highest in the ASCVD and MRF subgroups.

4.2 Limitations and strengths

One of the primary limitations of this analysis is the lack of access to individual patient data. The entire analysis was conducted based on published pooled analysis. The design of the indirect pairwise comparison between agents invariably leads to inflation of the confidence interval, which could have led to an underestimation of the pooled effect size. Although the scoring system seems to provide a sense of hierarchy, it is by no means a substitute for a well-conducted head-to-head comparative study. The significant heterogeneity associated with both the overall analysis and the subgroups makes it extremely difficult to identify the use of SGLT-2is as the sole reason for the CV death benefits. The frequent coexistence of ASCVD and HF makes it impossible to choose a single agent in view of empagliflozin being favored for ASCVD and MRF and dapagliflozin for HF. The heterogeneity in the overall data could be related to clinical and biochemical parameters in addition to the differences between the agents used. This network meta-analysis explored the differences between different SGLT-2is as a covariate explaining the heterogeneity of CV death outcomes. However, there is a possibility that other clinical and biochemical parameters could also explain this heterogeneity. To avoid the issue of multiplicity and its associated correction, a single parameter was used as the covariate.

The main strength of this analysis is the very large amount of pooled data included in the analysis. In addition, the inclusion of RCTs as well as a large preanalytical power was an additional strength. Despite significant heterogeneity of the outcome benefit, we cannot deny the role of SGLT-2is in CV death reduction. The additional contributive factors need to be explored. In the absence of planned studies evaluating the comparative effectiveness of SGLT-2is for CV death reduction, a network meta-analysis and scoring seems to be the best available option.

The difference between empagliflozin and dapagliflozin cannot be explained by molecular structure or differences between their pharmacodynamics and pharmacokinetics. A head-to-head 52-week prospective trial found greater glycated hemoglobin (HbA1C) lowering with empagliflozin compared to dapagliflozin but did not find any difference as far as other cardio-metabolic parameters were concerned (22). The only plausible explanation could be related to trial design. With the EMPAREG Outcomes trial recruiting T2D patients with eASCVD in contrast to a mixed population of eASCVD and MRF, the CV death benefits became skewed toward empagliflozin. We could find a very similar trend in the VERTIS-CV trial, where despite not meeting the primary endpoint (MACE), the exploratory endpoint of CV death had a positive trend compared to DECLARE TIMI-58 and CANVAS program.

In the HF trials, CV death as a stand-alone endpoint was exploratory in both the empagliflozin and dapagliflozin trials. The scoring favoring dapagliflozin was a direct result of the positive trend from the DAPA-HF trial. Once again, the differences can only be explained based on the baseline trial designs. Homogeneity of the baseline characteristics across these trials could have resulted in a different inference.

5 Conclusion

SGLT-2is is associated with an impressive reduction in CV death in patients with T2D compared to standard of care. Empagliflozin ranked the highest in T2D patients with ASCVD or MRF, and dapagliflozin ranked the highest in T2D patients with HF.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

SG and BS conceptualized the study. The meta-analysis was conducted by SG. BS cross validated the data and prepared the manuscript. SG and BS conducted an expanded literature review to contextualize the results from the meta-analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1168755/full#supplementary-material

References

1. Gao Y, Feng S, Wen Y, Tang T, Wang B, Liu B. Cardiorenal protection of SGLT2 inhibitors–perspectives from metabolic reprogramming. *eBioMedicine* (2022) 83:104215. doi: 10.1016/j.ebiom.2022.104215

2. Davies M, Aroda V, Collins B, Gabbay R, Green J, Maruthur N, et al. Management of hyperglycemia in type 2 diabetes, 2022. A consensus report by the American diabetes association (ADA) and the European association for the study of diabetes (EASD). *Diabetes Care* (2022) 45(11):2753–86. doi: 10.2337/dci22-0034

3. Cosentino C, Grant P, Aboyans V, Bailey C, Ceriello A, Delgado V, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the task force for diabetes, pre-diabetes, and cardiovascular diseases of the European society of cardiology (ESC) and the European association for the study of diabetes (EASD). *Eur Heart J* (2020) 41(2):255–323. doi: 10.1093/eurheartj/ehz486

4. Boer I, Khunti K, Sadusky T, Tuttle K, Neumiller J, Rhee C, et al. Diabetes management in chronic kidney disease: a consensus report by the American diabetes association (ADA) and kidney disease: improving global outcomes (KDIGO). *Diabetes Care* (2022) 45(12):3075–90. doi: 10.2337/dci22-0027

5. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. EMPA-REG outcome investigators. empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* (2015) 373:2117–28. doi: 10.1056/nejmoa1504720

6. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* (2019) 380:347–57. doi: 10.1056/NEJMoa1812389

7. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925

8. Anker S, Butler J, Filippatos G, Ferreira J, Bocchi E, Bohm M, et al. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med* (2021) 385:1451–61. doi: 10.1056/NEJMoa2107038

9. Packer M, Anker S, Butler J, Filippatos G, Pocock S, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med* (2020) 383:1413–24. doi: 10.1056/NEJMoa2022190

10. Herrington W, Staplin N, Wanner C, Green J, Hauske S, Emberson J, et al. Empagliflozin in patients with chronic kidney disease. *N Engl J Med* (2022) 388(2):117–27. doi: 10.1056/NEJMoa2204233

11. McMurray J, Solomon S, Inzucchi S, Kober L, Kosiborod M, Martinez F, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med* (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303

12. Solomon S, McMurray JJB, Clagget B, Boer R, DeMets D, Hernandez A, et al. Dapagliflozin in heart failure with mildly reduced or preserved ejection fraction. *N Engl J Med* (2022) 387:1089–98. doi: 10.1056/NEJMoa2206286

13. Heerspink H, Stefansson B, Correa-Rotter R, Chertow G, Greene T, Hou F, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med* (2020) 383:1436–46. doi: 10.1056/NEJMoa2024816

14. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HLJ, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med* (2019) 380:2295–306. doi: 10.1056/nejmoa1811744

15. Ghosal S, Sinha B. *Exploring the comparative cardiovascular death (CV) benefits of sodium glucose co-transporter 2 inhibitors (SGLT-2is) in type 2 diabetes: a frequentist and Bayesian network meta-analysis-based scoring*. PROSPERO (2022). Available at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022381556.

16. Cnnon CP, Pratley R, Dogogo-Jack S, Mancuso J, Huyck S, Masiukiewicz U, et al. Cardiovascular outcomes with ertugliflozin in type 2 diabetes. *N Engl J Med* (2020) 383:1425–35. doi: 10.1056/NEJMoa2004967

17. Bhatt D, Szarek M, Steg G, Cannon C, Leiter L, McGuire D, et al. Sotagliflozin in patients with diabetes and recent worsening heart failure. *N Engl J Med* (2021) 384:117–28. doi: 10.1056/NEJMoa2030183

18. Bhatt D, Szarek M, Pitt B, Cannon C, Leiter L, McGuire D, et al. Sotagliflozin in patients with diabetes and chronic kidney disease. *N Engl J Med* (2021) 384:129–39. doi: 10.1056/nejmoa2030186

19. Verma S, Jüni P, Mazer CD. Pump, pipes, and filter: do SGLT2 inhibitors cover it all? *Lancet* (2019) 393(10166):3–5. doi: 10.1016/S0140-6736(18)32824-1

20. Giorgino F, Vora J, Fenici P, Solini A. Cardiovascular protection with sodium-glucose co-transporter-2 inhibitors in type 2 diabetes: does it apply to all patients? *Diabetes Obes Metab* (2020) 22(9):1481–95. doi: 10.1111/dom.14055

21. Jiang Y, Yang P, Fu L, Sun L, Shen W, Wu Q. Comparative cardiovascular outcomes of SGLT2 inhibitors in type 2 diabetes mellitus: a network meta-analysis of randomized controlled trials. *Front Endocrinol* (2022) 13:802992. doi: 10.3389/fendo.2022.802992

22. Ku EJ, Lee DH, Jeon HJ, Oh TK. Empagliflozin versus dapagliflozin in patients with type 2 diabetes inadequately controlled with metformin, glimepiride and dipeptidyl peptide 4 inhibitors: a 52-week prospective observational study. *Diabetes Res Clin Pract* (2019) 151:65–73. doi: 10.1016/j.diabres.2019.04.008

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Effects of concurrent aerobic and resistance training on vascular health in type 2 diabetes: a systematic review and meta-analysis

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Objective: To determine the impacts of concurrent aerobic and resistance training on vascular structure (IMT) and function (PWV, FMD, NMD) in type 2 diabetes (T2D).

Methods: The electronic databases PubMed, Web of Science Core Collection, Cochrane Library, Embase, Scopus, CINAHL, and SPORTDiscus were systematically searched for articles on "type 2 diabetes" and "concurrent training" published from inception to August 2, 2022. We included randomized controlled trials that examined the effects of concurrent training versus passive controls on IMT, PWV, FMD and NMD in T2D.

Results: Ten studies were eligible, including a total of 361 participants. For IMT, concurrent training showed a slight decrease by 0.05 mm (95% CI −0.11 to 0.01, $p > 0.05$). concurrent training induced an overall significant improvement in FMD by 1.47% (95% CI 0.15 to 2.79, $p < 0.05$) and PWV by 0.66 m/s (95% CI −0.89 to −0.43, $p < 0.01$) in type 2 diabetics. However, concurrent training seemed to exaggerate the impaired NMD (WMD = −2.30%, 95% CI −4.02 to −0.58, $p < 0.05$).

Conclusions: Concurrent training is an effective method to improve endothelial function and artery stiffness in T2D. However, within 24 weeks concurrent training exacerbates vascular smooth muscle dysfunction. More research is needed to explore whether longer and/or higher-intensity concurrent training interventions could enhance the vascular structure and smooth muscle function in this population.

Systematic review registration: www.crd.york.ac.uk/PROSPERO/, identifier CRD42022350604.

KEYWORDS

concurrent training, aerobic and resistance training, type 2 diabetes, vascular structure, vascular function

1 Introduction

The global incidence of diabetes is 537 million adults (20–79 years) in 2021, of which 90–95% is type 2 diabetes (T2D) (1). T2D is a chronic condition characterized by persistent hyperglycemia and glucose intolerance. This disease has grown up to be an extremely serious public health problem in the world due to its high mortality, high disability rate and high morbidity. Over time, the hyperglycemia seen in T2D can elevate the risk of both macro- and microvascular complications, resulting in dysfunction across the spectrum of the heart, kidneys, eyes, and vasculature (2), underscoring the imperative of treating T2D to reduce complications, especially those related to micro- and macrovascular disease, intertwined with impaired vascular structure and function.

With the onset of T2D, subclinical manifestations of cardiovascular pathology occur, which in turn may lead to artery stenosis (3). Intima-media thickness (IMT) is an established index of structural change in the artery and increased in T2D (4). Vascular elasticity is an indicator of vascular function. Pulse wave velocity (PWV) is well established arterial stiffness measurement. Patients with T2D have increased PWV when compared with nondiabetic patients. Accelerated arterial stiffness is believed to be related to structural changes within the media of the arterial wall caused by abnormal calcification (5). Impaired endothelial function, long before symptoms, seems to occur in macro- and microvascular in T2D most extensively studied being decreased NO bioavailability (6, 7). Among the subclinical markers, flow-mediated dilation (FMD) measurement is considered the gold standard of endothelial function (8). Simultaneously, vascular smooth muscle (VSM) cells, integral to vascular physiology, exhibit functional impairment along with intricate shifts in the intracellular biomolecular milieu within the context of T2D (9). Nitroglycerin-mediated vasodilation (NMD) is an index of VSM (endothelial-independent) function. Nitrates cause smooth muscle relaxation once they enter the VSM cell through a series of processes that include the bioconversion of nitrate to nitric oxide, stimulation of soluble guanylate cyclase, production of cyclic guanosine monophosphate, and a reduction in cytosolic calcium levels (10). Arterial stiffness is affected not only by structural changes but also by functional variables of the artery (11, 12). The components of impaired vascular structure and function are all associated with cardiovascular mortality and are important markers for evaluating early atherosclerosis development (13). Therefore, interventions to manage and ameliorate vascular health are highly recommended for T2D.

Exercise can effectively reduce the risk of cardiovascular and all-cause mortality and is encouraged by guidelines for T2D. Aerobic exercise (AE) and resistance training (RT) are currently recommended as effective treatments in diabetes management. In this regard, it is well-established that AE and RT induce specific adaptations in vascular homeostasis. The most common explanation for the beneficial effects of AT on the vascular system is that exercise depresses the sympathetic nervous system and the renin-angiotensin system, as well as increases shear stress (14). The improvement of vascular health by RT may be associated with a reduction in risk factors affecting cardiovascular diseases such as body fat mass, HbA1c levels, blood pressure, and inflammatory mediators (15). Concurrent training (CT), combining AE and RT simultaneously in the same training period, is a popular training strategy to impact overall health. Increasing studies have investigated a focus on the outcomes of CT in T2D for the distinct adaptations and health benefits (16, 17). Randomized controlled trials (RCTs) demonstrated that CT showed a higher reduction of hemoglobin A1c, inflammatory markers and fasting glucose than AE or RT alone (18, 19). Furthermore, CT is considered the first choice to improve vascular health (20, 21). However, available studies on this topic in T2D have shown inconsistent and controversial findings. Specifically, some evidence indicated that CT induces beneficial effects on vascular structure and function (17, 22–25), while other studies revealed that CT does not affect vascular function (24–30). Therefore, there is a need for pooled data and meta-analysis to clarify the efficacy of CT for vascular health to draw more comprehensive and robust conclusions so that useful guidelines for T2D can be generated.

Therefore, this systematic review aimed to examine the effects of CT on vascular structure, vascular stiffness, endothelial and VSM function in patients with T2D by incorporating the latest evidence and indicators, and comparison of inter-group differences to test whether CT is beneficial to the vascular complications of type 2 diabetics.

2 Materials and methods

This systematic review and meta-analysis was conducted using Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (31) and registered with PROSPERO (Registration ID CRD42022350604), an international prospective registry for systematic reviews.

2.1 Search strategy and study selection

A systematic literature search for relevant studies published was conducted in the electronic databases Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Scopus, PubMed (including MEDLINE), Cumulative Index to Nursing and Allied Health Literature (CINAHL), SPORTDiscus, US Registry of clinical trials (www.clinicaltrials.gov) from inceptions until August 2022. Potentially relevant key terms (and synonyms searched for by the MeSH database) were collected through experts' opinions, literature review and included in the electronic databases in different combinations using a Boolean search strategy with the operators "AND", "OR" and "NOT": *strength training, resistance training, endurance training, aerobic exercise, vascular function, vascular structure, type 2 diabetes, and concurrent training*

The search syntax can be found in the Electronic Supplementary Material (see Supplementary Table S1). We additionally track the references of included articles and relevant systematic reviews and meta-analyses to identify potential studies.

2.2 Eligibility criteria

Studies eligible for inclusion in this meta-analysis are required to fulfil the following formal criteria: peer-reviewed original research, and full-text availability. In addition, we followed a PICOS (population, intervention, comparator, outcome, study design) approach to select eligibility for inclusion (Table 1). Two authors (S.C. and Y.C.) reviewed the titles, abstracts, and/or full-texts for each of the articles identified by the literature search after the removal of duplicates, aiming to determine the eligibility for this meta-analysis. During the study selection process, discrepancies were resolved by discussion with a third author (K.Z. or H.S.).

2.3 Risk of bias and quality assessment

Two investigators (S.C. and Y.C.) independently assessed the risk of bias in eligible studies using the Cochrane risk-of-bias tool (RoB2) (32). The tool assesses bias in five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of the reported result. Each domain was scored as low risk, some concern, or high risk. The summary score for each study was evaluated as follows (1): low risk of bias, is that all domains were low risk; or (2) some concerns, if ≥ 1 domain was some concerns, but not to be at a high risk of bias for any domain; and (3) high risk of bias, if ≥ 1 domain was at high risk or some concerns for multiple domains in a manner that substantially lowers confidence in the result. Any disagreements that arose were resolved through discussion with a third investigator (K.Z or H.S.).

2.4 Data extraction

Two researchers (SC and YC) performed the search and reviewed the results. The source (name of the first author and year of publication), participant characteristics (age, sex, number), training variables (intervention duration, frequency, session, intensity, and total length of intervention) and main outcomes were extracted. In the case of no agreement regarding data extraction, a third author (K.Z.) was consulted for clarification. To compute effect size, baseline and follow-up means and standard deviations (SDs) for measures of vascular structure or function of both the intervention and control groups were extracted. The extracted data were coded as outlined in Supplementary Table S2.

For each outcome, two reviewers (S.C. and Y.C.) used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology to assess the certainty of the evidence as described elsewhere (33). GRADE ratings for each outcome started at 'high' due to the randomized controlled trial (RCT) design. Downgrading was determined by the factors of risk of bias, inconsistency, indirectness, impression and publication bias (34).

2.5 Data synthesis and statistical analysis

For studies reporting interquartile ranges, the standard deviations were obtained using the methods described in Cochrane Handbook for Systematic Reviews (31). For a trial consisting of 2 CT intervention groups, two subgroups were pooled into a single group using the formulae recommended by the Cochrane Collaboration recommendations (31), to avoid bias due to overweighting of individual trials.

The weighted mean differences (WMDs) with 95% CIs were calculated using fixed-effect models, which could provide more conservative results than random-effects models (31). Statistical heterogeneity was evaluated using heterogeneity chi-squared (χ^2) and I^2 values. The level of heterogeneity was interpreted according to guidelines from the Cochrane Collaboration: I^2 values of 25%, 50% and 75% for low, moderate, and high heterogeneity, respectively (35). In addition, publication bias was assessed by generating funnel plots and conducting Egger's test (36). All the analyses were conducted using Stata v17.0 (STATA Corp., College Station, TX). A 2-side $p < 0.05$ was considered statistically significant unless otherwise indicated.

3 Results

The results of the systematic search process are illustrated in Figure 1. After screening study titles and eliminating duplicates, 14,139 potentially eligible studies were identified. Following the

TABLE 1 Study selection.

Category	Inclusion criteria	Exclusion criteria
Population	T2D, irrespective of sex and level of physical activity	Individuals with major macrovascular complications (coronary artery disease, cerebral vascular disease) or microvascular complications (e.g., nephropathy, neuropathy, retinopathy)
Intervention	Concurrent training interventions (i.e., a combination of AE and RT)	Single-mode training interventions (e.g., single-mode AE or RT); intervention duration ≤ 4 weeks
Comparator	Passive control group	Absence of a control group, active controls
Outcome	Measures of vascular structure and/or function (i.e., IMT, PWV, FMD, NMD)	No reported measures of vascular function and/or structure
Study design	RCTs	Non-RCTs

T2D type 2 diabetes, AE aerobic exercise, RT resistance training, IMT intima-media thickness, PWV pulse wave velocity, FMD flow-mediated dilation, NMD nitrate-mediated dilation, RCT randomized controlled trial.

abstract examination, 222 studies remained. After reviewing the full texts, 212 studies were excluded. Finally, 10 studies were eligible for inclusion in this meta-analysis.

3.1 Description of the included studies

The 10 eligible studies included an overall sample size of 361 participants, with a mean age ranging from 17 to 65 years (mean 55.84 ± 5.64 years). Out of the included studies, 193 participants received the training intervention and 183 served as control, as one study was a crossover trial (n = 15).

The characteristics of the included studies are summarized in Table 2. 2 out of the 10 studies provided data for the effect of CT on vasculature structure (17, 22), 5 studies on artery stiffness (22, 26–29), 4 studies on vascular endothelial function (23–25, 37). 3 studies investigated the effects of CT on VSM function (24, 25, 30). The total length of intervention ranged from 6 to 96 weeks, and training frequency varied from 3 to 6 sessions per week, with CT sessions lasting from 33.1 min to 70 min.

The approaches for assessing vascular structure (IMT), arterial stiffness (PWV), vascular endothelial function (FMD) and VSM function (NMD) were well described in each individual study except the one by Okada et al. (25). Carotid intima-media thickness was calculated as the average of both sides or single side using carotid ultrasound. PWV is an established metric for assessing vascular stiffness and is measured by different methods which include aortic arch-popliteal PWV (ap PWV), brachial-ankle PWV (ba PWV), carotid-femoral PWV (cf PWV), carotid-radial PWV (cr PWV) and carotid-distal posterior tibial PWV (cd PWV). These values reflect properties of central/aortic and peripheral arterial stiffness for upper and lower limb. Fasting participants were requested to assess FMD through ultrasound, a process wherein the release of NO is prompted by post-ischemic reactive hyperemia, leading to endothelium-dependent vasodilation. A rapid inflation/deflation pneumatic cuff was placed at the forearm in FMD measurement procedures and inflated to 200–250 mmHg to occlude blood flow for 270–300 s. Brachial artery images of diameter were obtained continuously 30 s before and 2–5 min after cuff deflation. After FMD measured, brachial artery diameters were again assessed by sublingual administration of nitroglycerin (0.4–2.5 mg) to calculate NMD using the same methods described for FMD.

3.2 Risk of bias assessment and quality of evidence assessment

The risk of bias estimate is provided in Figure 2. Overall, two studies presented an overall low risk of bias across the five domains (17, 22). Only one RCT showed an overall high risk of bias (29). The

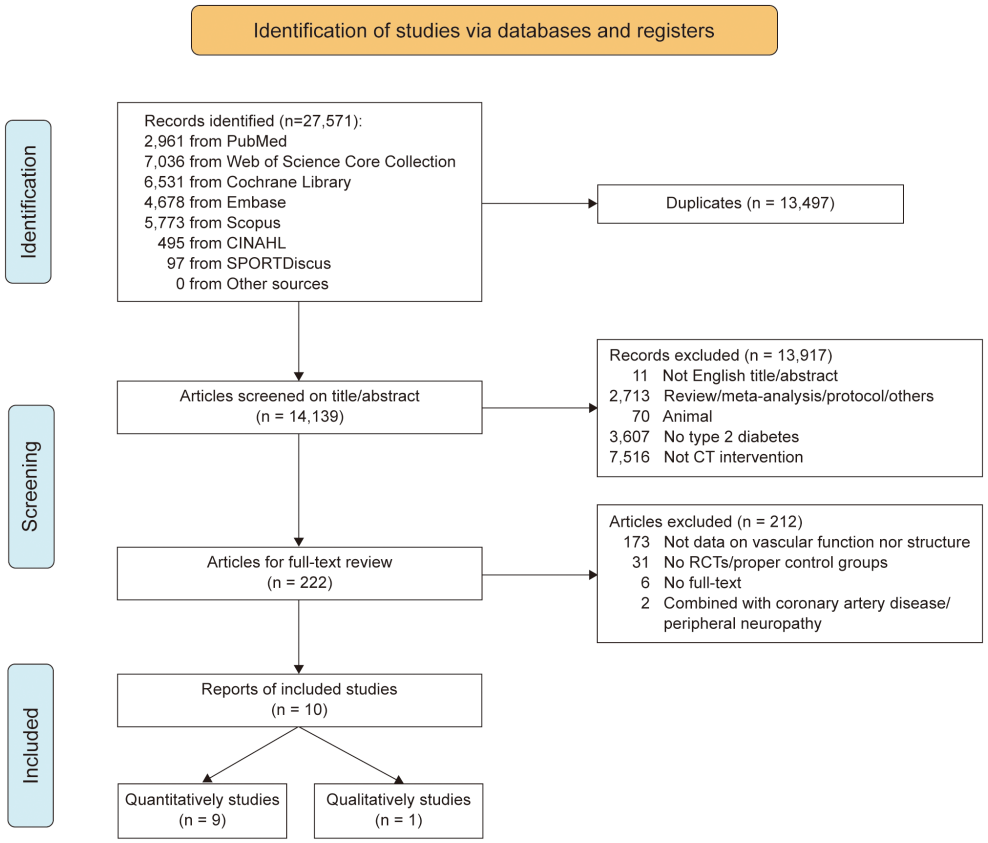

FIGURE 1
PRISM flow chart illustrating the different phases of the search and study selection. *PRISMA* Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

TABLE 2 Characteristics of the included studies.

References	Training moderator variables					Comp	Group	N	Age, year	BMI, kg/m^2	SBP, mmHg	DBP, mmHg	FBG, mmol/L	HbA_{1c}, %	VO_{2peak}, ml/kg/min
	idur	tfre	sdur	Intensity RT	Intensity AT										
Maiorana et al[†] (24).,	8	3	60	Moderate	Moderate-to-high	FMD, NMD	CT	15	52.0 ± 2.0	NA	NA	NA	12.0 ± 1.94	8.5 ± 1.55	23.1 ± 4.65
Maiorana et al[†] (24),	8	3	60	Moderate	Moderate-to-high	FMD, NMD	Con	15	52.0 ± 2.0	NA	NA	NA	12.0 ± 1.94	8.5 ± 1.55	23.1 ± 4.65
Loimaala et al. (26),	52	4	≥60	Moderate	Moderate-to-high	ap PWV	CT	24	53.6 ± 6.2	29.3 ± 3.7	142.0 ± 17.0	NA	NA	8.2 ± 2.1	31.9 ± 5.1
Loimaala et al. (26),	52	4	≥60	Moderate	Moderate-to-high	ap PWV	Con	25	54.0 ± 5.0	29.8 ± 3.6	145.0 ± 14.0	NA	NA	8.0 ± 1.3	32.6 ± 6.4
Loimaala et al. (27),	96	4	≥60	Moderate	Moderate-to-high	ap PWV	CT	24	53.6 ± 6.2	29.8 ± 3.7	142 ± 13.72	83.0 ± 12.7	NA	8.2 ± 2.1	31.9 ± 5.1
Loimaala et al. (27),	96	4	≥60	Moderate	Moderate-to-high	ap PWV	Con	24	54.0 ± 5.0	29.3 ± 3.6	145 ± 13.72	86.0 ± 11.5	NA	8.0 ± 0.3	32.6 ± 6.4
Okada et al. (25),	12	3-5	60	NA	NA	FMD, NMD	CT	21	61.9 ± 8.6	25.7 ± 3.2	129.0 ± 21.6	74.6 ± 11.6	7.7 ± 2.0	8.5 ± 1.8	22.4 ± 3.2
Okada et al. (25),	12	3-5	60	NA	NA	FMD, NMD	Con	17	64.5 ± 5.9	24.5 ± 2.9	126.6 ± 16.8	73.8 ± 11.8	8.2 ± 1.6	7.9 ± 1.1	22.3 ± 3.7
Lee et al. (28),	6	5-6	60-70	Light	Moderate	ba PWV	CT	23	50.4 ± 7.6	28.6 ± 3.4	125.1 ± 14.0	80.4 ± 10.1	8.1 ± 1.7	7.7 ± 0.7	NA
Lee et al. (28),	6	5-6	60-70	Light	Moderate	ba PWV	Con	12	48.5 ± 7.9	28.1 ± 3.2	131.2 ± 7.8	86.3 ± 4.9	8.8 ± 2.6	8.0 ± 0.9	NA
Barone Gibbs et al. (30),	26	3	>45	Moderate-to-high	light	FMD, NMD	CT	49	58.0 ± 5.0	32.3 ± 5.3	127.0 ± 13.0	72.0 ± 8.0	NA	6.6 ± 1.5	22.7 ± 5.9
Barone Gibbs et al. (30),	26	3	>45	Moderate-to-high	light	FMD, NMD	Con	63	56.0 ± 6.0	33.5 ± 4.3	126.0 ± 13.0	70.0 ± 9.0	NA	6.6 ± 1.4	22.4 ± 5.3
Dobrosielski et al. (29),	26	3	>45	Moderate-to-high	light	cf PWV	CT	70	57.0 ± 6.0	33.0 ± 5.0	126.9 ± 13.4	72.4 ± 9.2	7.6 ± 0.7	6.6 ± 1.7	21.9 ± 5.9
Dobrosielski et al. (29),	26	3	>45	Moderate-to-high	light	cf PWV	Con	70	56.0 ± 6.0	33.6 ± 4.2	126.5 ± 15.9	71.1 ± 9.2	8.3 ± 0.9	6.7 ± 1.7	22.1 ± 5.0
Kadoglou et al. (17),	26	4	45	Moderate-to-high	Light-to-moderate	IMT	CT	22	57.9 ± 6.5	31.9 ± 2.9	138.0 ± 16.0	82.0 ± 12.0	11.2 ± 2.9	8.2 ± 1.0	23.7 ± 5.6
Kadoglou et al. (17),	26	4	45	Moderate-to-high	Light-to-moderate	IMT	Con	24	57.9 ± 7.2	32.1 ± 3.0	135.0 ± 15.0	81.0 ± 11.0	9.9 ± 2.0	7.8 ± 0.8	22.9 ± 3.5
Naylor et al. (23),	12	3	60	Moderate-to-high	Light-to-moderate	FMD	CT	8	17.3 ± 2.3	36.1 ± 11.0	NA	NA	NA	8.8 ± 2.8	25.7 ± 6.8
Naylor et al. (23),	12	3	60	Moderate-to-high	Light-to-moderate	FMD	Con	5	15.3 ± 1.8	30.0 ± 4.9	NA	NA	NA	6.6 ± 0.5	29.9 ± 6.0
Magalhães et al. (22),	52	3	33.1 ± 6.4	Moderate-to-high	moderate	IMT, cf PWV, cd PWV, cr PWV	CT (HIIT)	25	56.7 ± 8.3	30.1 ± 5.7	142.2 ± 18.3	82.6 ± 10.3	NA	4.9 ± 1.2[a]	27.1 ± 6.3
Magalhães et al. (22),	52	3	45.0 ± 7.1	Moderate	moderate	IMT, cf PWV, cd PWV, cr PWV	CT (MCT)	28	59.7 ± 6.5	31.11 ± 5.0	139.9 ± 13.5	82.0 ± 8.8	NA	5.3 ± 2.3[a]	24.1 ± 3.2
Magalhães et al. (22),	52	3	33.1 ± 6.4	Moderate-	moderate	IMT, cf PWV, cd PWV, cr PWV	Con	27	59.0 ± 8.1	30.7 ± 5.0	136.8 ± 13.4	81.2 ± 10.5	NA	5.0 ± 2.1[a]	25.9 ± 5.5

idur intervention duration (weeks), tfre training frequency (sessions/week), sdur session duration (min), N number of participants, FBG fasting blood glucose, HbA_{1c} hemoglobin A1c, VO_{2peak} peak oxygen consumption. NA, not applicable.

[†]Crossover trial.

[a]Skewed value are presented as median ± inter quartile range.

remaining 7 studies were classified as of some concerns (23–28, 37). Results from the risk of bias assessment using funnel plot asymmetry are displayed in Supplementary Figures 1A–D. Egger's test of the intercept provided no evidence for funnel plot asymmetry and potential publication bias (all $p > 0.05$).

The GRADE system was used to assess the quality of the evidence. The evaluation results are presented in Supplementary Table S3. In Summary, the quality of the evidence was rated as moderate for IMT, PWV, FMD, and NMD due to the small sample size.

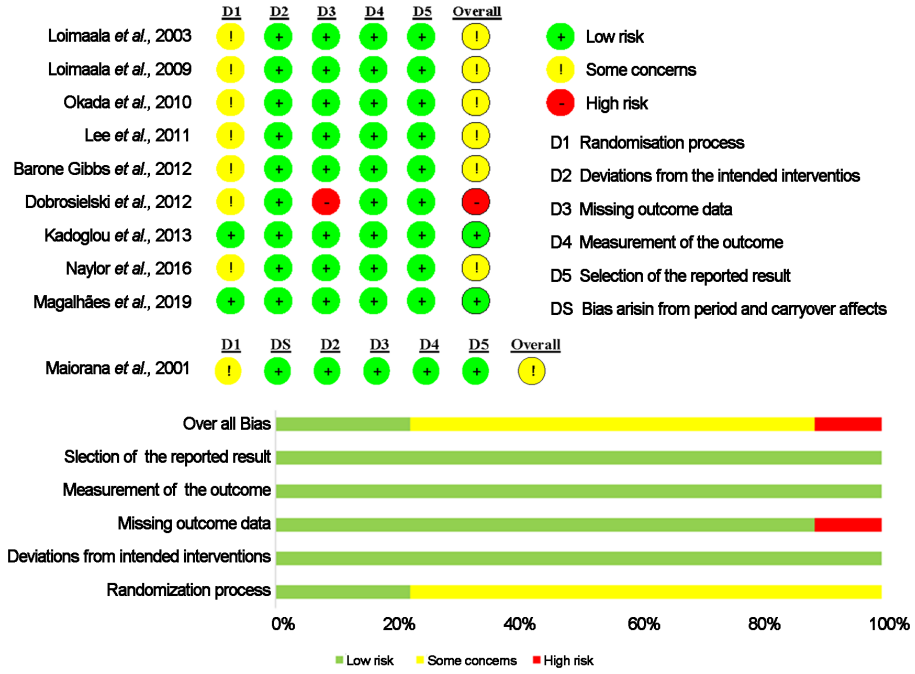

FIGURE 2
Risk of bias assessment.

3.3 Meta-analysis

3.3.1 Effects of CT on vascular structure in T2D

Two studies enrolling 97 patients made comparison between the effect of CT on IMT (17, 22). The result of meta-analysis showed that CT could not significantly influence the vascular structure (IMT). The pooled effect size was not significant (WMD = −0.05 95% CI −0.11 to 0.01, p = 0.11) and without the heterogeneity (I^2 = 0%, p = 0.82; Figure 3A).

3.3.2 Effects of CT on vascular function in T2D

Arterial stiffness (PWV), vascular endothelial function (FMD) and smooth muscle function (NMD) were included in 8 RCTs (22, 23, 25–30). As the study by Maiorana et al. was a crossover trial, it would be analyzed in the discussion (38). Seven RCTs enrolling 249 subjects from five studies were analyzed with PWV (22, 26–29). There was an efficacy decrease (WMD = −0.66, 95% CI −0.89 to −0.43, p < 0.001) and low heterogeneity (I^2 = 0%, p = 0.71; Figure 3B) in PWV. Both FMD and NMD were expressed as percentage changes from baseline to maximal dilation. Three studies assessed FMD enrolling 163 T2D patients were included (23, 25, 30). After data pooling, the result of our meta-analysis showed that CT led to a significant increase in FMD by 1.49% (95% CI 0.15% to 2.83%, p = 0.03) without heterogeneity (I^2 = 0%, p = 0.42; Figure 3C). For NMD, two trials enrolling 150 patients with T2D were included (25, 30). Meta-analysis demonstrated that CT could significantly influence the NMD. The pooled effect size was significant (WMD = −2.37%, 95% CI −4.22% to −0.53%, p = 0.01) and with low heterogeneity (I^2 = 23.65%, p = 0.25; Figure 3D).

4 Discussion

Our meta-analysis revealed that vascular function adaptation in response to CT might precede structural remodeling in T2D patients. Here, we showed that CT tended to improve the vascular structure (IMT) and significantly enhanced vascular function (PWV, FMD) of T2D patients. However, impaired VSM cell function (NMD) was not alleviated following CT.

In diabetes, thickening and stiffening are early markers of vascular damage (4). Firstly, we investigated the influence of CT on the vascular structure. Results showed that CT tended to reduce IMT by 0.05 mm in T2D patients. IMT has been identified to be strongly and positively associated with the progression of atherosclerosis (4). For every 1mm increase in carotid artery IMT, the risk of diabetic retinopathy increases by 2.9 times (39), but IMT varies according to exercise type and intensity. Kadoglou et al. (17) reported that AE considerably attenuated IMT progression in T2D patients, while CT did not confer any change. However, Magalhães et al. (22) showed that high-intensity AE combined with RT improves IMT more than low-intensity AE combined with RT. This finding is consistent with investigations conducted on obese subjects, where neither aerobic nor anaerobic exercises for 12 weeks yielded noteworthy alterations in IMT (40). Conversely, IMT exhibited a reduction over the same duration of CT (41). Long-term exercise training, on the one hand, alters the shear stress of blood vessels by modulating local blood flow and increases the sensitivity of ion channels in endothelial cells (14). On the other hand, it enhances endogenous antioxidant defense, alters the underlying wall structure, and induces vasodilation, thereby bolstering NO bioavailability (6).

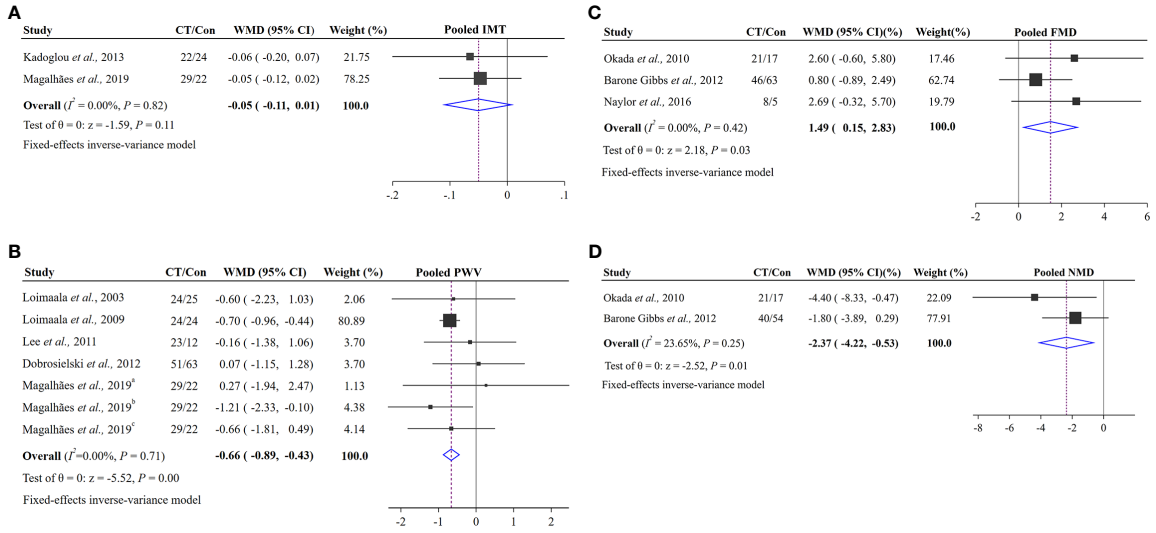

FIGURE 3
Pooled effects of CT groups vs. control groups on changes in vascular structure and function: **(A)** vascular structure index (IMT); **(B)** vascular function index (PWV); **(C)** vascular function index (FMD); **(D)** vascular function index (NMD). The squares represent the mean difference for each trial. The diamond represents the pooled mean difference across all trials. [a] represents cf PWV, [b] represents cd PWV, [c] represents cr PWV.

A meta-analysis of 17 longitudinal studies (42) found that each 1m/s increase in resting aortic stiffness corresponds to a 14%, 15%, and 15% increase in the risk of CVD events, CVD mortality, and all-cause mortality, respectively, adjusting for traditional CVD risk factors. Our results showed that arterial stiffness decreased by 0.66 m/s in T2D patients following CT, which is much more than thiazolidinediones (43). On the one hand, this decrease may be related to a function of structural changes in the arteries. Exercise inhibits the development of advanced glycation end products, reduces glucose forms cross-links with collagen proteins within the arteries, and therefore, restores the important balance between elastin and collagen (44). Exercise reduces the generation of reactive oxygen species and inflammation, further promoting the restoration of the structural and functional integrity of vascular wall (44). Alternatively, it could be related to the amelioration of endothelial dysfunction. Endothelial dysfunction can contribute to arterial stiffness in diabetic hypertensive individuals (45). Vasoconstriction in healthy subjects can shift the pressure load bearing toward elastin, unloading stiff collagen. But in individuals with T2DM who have impaired arterial function, vasoconstriction presumably leads to an increase in functional stiffness (46). FMD is a predictor of arterial stiffening (8). Interestingly, a recent meta-analysis of aerobic exercise plus machine-assisted RT failed to provide significant improvement in PWV (0.54 m/s) (47), suggesting that the disturbance of long-term RE to vascular health varies according to population, exercise intensity and frequency. Here, we found that CT had little structural impact, and then we hypothesized that this change in PWV might be related to an increase in endothelial function. It has also been suggested that diabetes-related endothelial dysfunction precedes changes in morphology and structural vessels (48). The increase in FMD (1.49%) observed in T2D subjects might be indicative of restoration of conduit artery endothelial function since a similar extent of FMD impairment has been repeatedly observed in T2D subjects compared with control non-diabetic subjects (45). Accordingly, in addition to the previously reported beneficial effect of AE or RT on FMD in T2D, CT can be considered a valuable strategy to improve endothelial function in this population (21, 49). Moreover, a meta-analysis of 14 prospective studies including 5,547 subjects demonstrated that a 1% increase in FMD was associated with a 13% reduction in the incidence of cardiovascular events (50), thus the 1.47% increase in FMD could indicate a significant effect of exercise interventions on cardiovascular health in T2D patients. Interestingly, as the exercise duration increased, the trend for FMD amelioration was suppressed, which was consistent with the previous review (51). However, as there are fewer included studies, long-term prospective studies are required to determine whether the endothelial function-enhancing effect of exercise is preserved over time and related to a reduced risk of atherosclerosis and CVD in T2D.

In addition to the endothelium, VSM needs to be considered as a potential cause of vascular dysfunction in T2D (52). As T2D progresses, both endothelium-dependent and endothelium-independent vasodilation impairments coexist. However, the pooled effect showed an exacerbating VSM dysfunction (2.37% reduction) in T2D following CT intervention, which was inconsistent with our previous hypothesis. A previous meta-analysis found that exercise, including CT, AT and RT, had no effect on the NMD of T2D for comparing exercise and control groups (51). Furthermore, the time-dependent adaptation in vascular function of exercise in T2D was observed by monitoring FMD and NMD 2-weekly for 8 weeks of exercise. It was found that there was a tendency for FMD to increase and NMD to decrease with time-course, but no significant differences. However, when corrected for endothelium-independent dilation, the FMD/NMD increased across the exercise training programme, resulting in a time-dependent improvement in diabetic endothelial function (53). Some research also supports the notion that exercise-induced adaptions are more

pronounced in the endothelial function in arteries compared with VSM function (24, 51). Short-term vigorous exercise exacerbated VSM dysfunction, and reduction in impaired NMD only occurred when exercise intervention lasted longer than 12 weeks (54). With increasing duration, the improvement becomes more evident, which is consistent with our findings that 24 weeks of CT significantly improved the impaired NMD compared with 12 weeks of CT. Nevertheless, this endothelium-independent dysfunction has to be interpreted with caution, given that the endothelium-dependent response includes, at least in part, the function of VSM. The effect of exercise on VSM function may involve the following mechanisms. The mechanical stress stimulation caused by skeletal muscle contraction on blood vessels is related to the type and intensity of exercise, resulting in differences in blood flow shear stress (14), and thereby inducing different degrees of NO release (6). The response to shear stress and the degree of muscle fiber recruitment may be the key to the effectiveness of training. In addition, exercise training, especially RT combined with high-intensity interval aerobics, thickens skeletal muscle capillary thinning due to capillary hemodynamic changes, alters endothelial and VSM cell phenotypes, and enhances glycemic control (19). It is conceivable that longer and/or more intense exercise training programmes could enhance VSM function in T2D patients.

Our results were based on studies that investigated the effect of CT on vascular health, with no emphasis on the mechanistic aspects (e.g., critical pathways of VSM cells signal transduction). Of note, the current research on potential physiological mechanisms has become a hot spot in the field of sports science. As the rate of exercise prescription in the field of chronic disease intervention rises, our findings showed that improvements in vascular function preceded structural remodeling following CT, highlighting the importance of CT in the overall population of T2D. These improvements include the enhancement of endothelial function and reduction of arterial stiffness, but the effective improvement of impaired NMD and remodeling of the vascular structure requires longer intervention.

Despite the effects of CT on structure and vascular function in T2D having been comprehensively investigated, some limitations should be considered when interpreting the results of the analysis. Firstly, only ten studies met the inclusion criteria, one of which was not eligible for the conducted analyses due to a crossover randomized trial (24). These were limited by small sample sizes, for fewer than 50 participants should not be powerful enough. Secondly, few studies reported exercise adherence, which can mask the actual effects of exercise and widen the differences among studies. Finally, some variances existed in the measurements' parts for PWV. Due to the small sample size, all results of PWV were pooled in our review, so this needs to be considered when applying the results.

5 Conclusion

Although individual trials on the effects of CT on vascular function in T2D subjects have shown partially conflicting results, the current meta-analysis suggests that CT is an effective method to improve vascular function (including vascular endothelial function and artery stiffness). Increases of this magnitude would be expected to substantially restore the impaired vascular function commonly found in T2D. Although the relationship between vascular structure and endothelial function in the progression of T2D is still not well understood, the effect of CT on vascular endothelial function precedes structural changes. Future research will be needed to clarify whether longer and/or more intense CT interventions may improve vascular health overall in patients with T2D.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SC, YC, KZ, HS, MD and LW made substantial contributions to conception and design; YC designed the study; SC, YC, MD and LW collected the data, SC and YC interpreted the data and wrote the first draft of the manuscript; KZ analyzed the data and contributed to the results; HS reviewed/edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1216962/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Funnel plots of publication bias in IMT, PWV, FMD and NMD. se standard error, WMD weighted mean difference.

References

1. Federation ID. *Idf Diabetes Atlas 2021* (2021). Available at: https://diabetesatlas.org/atlas/tenth-edition/.

2. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* (2013) 93(1):137–88. doi: 10.1152/physrev.00045.2011

3. Suryavanshi SV, Kulkarni YA. Nf-Kβ: A potential target in the management of vascular complications of diabetes. *Front Pharmacol* (2017) 8:798. doi: 10.3389/fphar.2017.00798

4. Lorenz MW, Price JF, Robertson C, Bots ML, Polak JF, Poppert H, et al. Carotid intima-media thickness progression and risk of vascular events in people with diabetes: results from the prog-imt collaboration. *Diabetes Care* (2015) 38(10):1921–9. doi: 10.2337/dc14-2732

5. Fernández-Alvarez V, Linares Sánchez M, López Alvarez F, Suárez Nieto C, Mäkitie AA, Olsen KD, et al. Evaluation of intima-media thickness and arterial stiffness as early ultrasound biomarkers of carotid artery atherosclerosis. *Cardiol Ther* (2022) 11(2):231–47. doi: 10.1007/s40119-022-00261-x

6. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* (1992) 340(8828):1111–5. doi: 10.1016/0140-6736(92)93147-f

7. Evans BR, Yerly A, van der Vorst EPC, Baumgartner I, Bernhard SM, Schindewolf M, et al. Inflammatory mediators in atherosclerotic vascular remodeling. *Front Cardiovasc Med* (2022) 9:868934. doi: 10.3389/fcvm.2022.868934

8. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: A systematic review. *Hypertension* (2009) 54(6):1328–36. doi: 10.1161/hypertensionaha.109.137653

9. Murugan DD, Md Zain Z, Choy KW, Zamakshshari NH, Choong MJ, Lim YM, et al. Edible bird's nest protects against hyperglycemia-induced oxidative stress and endothelial dysfunction. *Front Pharmacol* (2019) 10:1624. doi: 10.3389/fphar.2019.01624

10. Ignarro LJ, Lippton H, Edwards JC, Baricos WH, Hyman AL, Kadowitz PJ, et al. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Ther* (1981) 218(3):739–49.

11. Goto K, Kitazono T. Endothelium-dependent hyperpolarization (Edh) in diabetes: mechanistic insights and therapeutic implications. *Int J Mol Sci* (2019) 20(15):3737. doi: 10.3390/ijms20153737

12. Wakasugi S, Mita T, Katakami N, Okada Y, Yoshii H, Osonoi T, et al. Associations between continuous glucose monitoring-derived metrics and arterial stiffness in Japanese patients with type 2 diabetes. *Cardiovasc Diabetol* (2021) 20(1):15. doi: 10.1186/s12933-020-01194-2

13. Peñín-Grandes S, Martín-Hernández J, Valenzuela PL, López-Ortiz S, Pinto-Fraga J, Solá LDR, et al. Exercise and the hallmarks of peripheral arterial disease. *Atherosclerosis* (2022) 350:41–50. doi: 10.1016/j.atherosclerosis.2022.04.025

14. Laughlin MH, Newcomer SC, Bender SB. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol (1985)* (2008) 104(3):588. doi: 10.1152/japplphysiol.01096.2007

15. Isath A, Howard CE, VIrani SS, Wang Z, Lavie CJ, Naidu SS, et al. Strength training and cardiovascular health: A meta-analysis. *Prog Cardiovasc Dis* (2022) 73:85–7. doi: 10.1016/j.pcad.2022.05.001

16. Zhao X, He Q, Zeng Y, Cheng L. Effectiveness of combined exercise in people with type 2 diabetes and concurrent overweight/obesity: A systematic review and meta-analysis. *BMJ Open* (2021) 11(10):e046252. doi: 10.1136/bmjopen-2020-046252

17. Kadoglou NP, Fotiadis G, Kapelouzou A, Kostakis A, Liapis CD, Vrabas IS. The differential anti-inflammatory effects of exercise modalities and their association with early carotid atherosclerosis progression in patients with type 2 diabetes. *Diabetes Med* (2013) 30(2):e41–50. doi: 10.1111/dme.12055

18. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, et al. Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutr Metab Cardiovasc Dis* (2010) 20(8):608–17. doi: 10.1016/j.numecd.2009.04.015

19. Olver TD, Laughlin MH. Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes. *Am J Physiol Heart Circ Physiol* (2016) 310(3):H337–50. doi: 10.1152/ajpheart.00440.2015

20. Li Y, Hanssen H, Cordes M, Rossmeissl A, Endes S, Schmidt-Trucksäss A. Aerobic, resistance and combined exercise training on arterial stiffness in normotensive and hypertensive adults: A review. *Eur J Sport Sci* (2015) 15(5):443–57. doi: 10.1080/17461391.2014.955129

21. Qiu S, Cai X, Yin H, Sun Z, Zugel M, Steinacker JM, et al. Exercise training and endothelial function in patients with type 2 diabetes: A meta-analysis. *Cardiovasc Diabetol* (2018) 17(1):64. doi: 10.1186/s12933-018-0711-2

22. Magalhães JP, Melo X, Correia IR, Ribeiro RT, Raposo J, Dores H, et al. Effects of combined training with different intensities on vascular health in patients with type 2 diabetes: A 1-year randomized controlled trial. *Cardiovasc Diabetol* (2019) 18(1):34. doi: 10.1186/s12933-019-0840-2

23. Naylor LH, Davis EA, Kalic RJ, ParaMalingam N, Abraham MB, Jones TW, et al. Exercise training improves vascular function in adolescents with type 2 diabetes. *Physiol Rep* (2016) 4(4):e12713. doi: 10.14814/phy2.12713

24. Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, et al. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* (2001) 38(3):860–6. doi: 10.1016/s0735-1097(01)01439-5

25. Okada S, Hiuge A, Makino H, Nagumo A, Takaki H, Konishi H, et al. Effect of exercise intervention on endothelial function and incidence of cardiovascular disease in patients with type 2 diabetes. *J Atheroscler Thromb* (2010) 17(8):828–33. doi: 10.5551/jat.3798

26. Loimaala A, Huikuri HV, Kööbi T, Rinne M, Nenonen A, Vuori I, et al. Exercise training improves baroreflex sensitivity in type 2 diabetes. *Diabetes* (2003) 52(7):1837–42. doi: 10.2337/diabetes.52.7.1837

27. Loimaala A, Groundstroem K, Rinne M, Nenonen A, Huhtala H, Parkkari J, et al. Effect of long-term endurance and strength training on metabolic control and arterial elasticity in patients with type 2 diabetes mellitus. *Am J Cardiol* (2009) 103(7):972–7. doi: 10.1016/j.amjcard.2008.12.026

28. Lee DH, Lee SH, An K, Moon JY, Kim SH, Choi YJ. Effects of 6 weeks of lifestyle modification including combined exercise program on the risk of metabolic parameters and macrovascular complications in type 2 diabetic patients. *korean J Obes* (2011) 20(3):147–59. doi: 10.7570/kjo.2011.20.3.147

29. Dobrosielski DA, Gibbs BB, Ouyang P, Bonekamp S, Clark JM, Wang NY, et al. Effect of exercise on blood pressure in type 2 diabetes: A randomized controlled trial. *J Gen Intern Med* (2012) 27(11):1453–9. doi: 10.1007/s11606-012-2103-8

30. Barone Gibbs B, Dobrosielski DA, Bonekamp S, Stewart KJ, Clark JM. A randomized trial of exercise for blood pressure reduction in type 2 diabetes: effect on flow-mediated dilation and circulating biomarkers of endothelial function. *Atherosclerosis* (2012) 224(2):446–53. doi: 10.1016/j.atherosclerosis.2012.07.035

31. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. (editors). *Cochrane Handbook for Systematic Reviews of Interventions. 2nd Edition*. (Chichester (UK): John Wiley & Sons) (2019).

32. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. Rob 2: A revised tool for assessing risk of bias in randomised trials. *BMJ* (2019) 366:l4898. doi: 10.1136/bmj.l4898

33. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. Grade: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* (2008) 336(7650):924–6. doi: 10.1136/bmj.39489.470347.AD

34. Guyatt GH, Oxman AD, Vist G, Kunz R, Brozek J, Alonsocoello P, et al. Grade guidelines: 4. Rating the quality of evidence–study limitations (Risk of bias). *J Clin Epidemiol* (2011) 64(4):407–15. doi: 10.1016/j.jclinepi.2010.07.017

35. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557

36. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* (2000) 56(2):455–63. doi: 10.1111/j.0006-341x.2000.00455.x

37. Barone Gibbs B, Dobrosielski DA, Althouse AD, Stewart KJ. The effect of exercise training on ankle-brachial index in type 2 diabetes. *Atherosclerosis* (2013) 230(1):125–30. doi: 10.1016/j.atherosclerosis.2013.07.002

38. Stedman MR, Curtin F, Elbourne DR, Kesselheim AS, Brookhart MA. Meta-analyses involving cross-over trials: methodological issues. *Int J Epidemiol* (2011) 40(6):1732–4. doi: 10.1093/ije/dyp345

39. Rema M, Mohan V, Deepa R, Ravikumar R. Association of carotid intima-media thickness and arterial stiffness with diabetic retinopathy: the chennai urban rural epidemiology study (Cures-2). *Diabetes Care* (2004) 27(8):1962–7. doi: 10.2337/diacare.27.8.1962

40. Horner K, Kuk JL, Barinas-Mitchell E, Drant S, DeGroff C, Lee S. Effect of aerobic versus resistance exercise on pulse wave velocity, intima media thickness and left ventricular mass in obese adolescents. *Pediatr Exerc Sci* (2015) 27(4):494–502. doi: 10.1123/pes.2015-0067

41. Kim DK, Jee JH, Park WH. Effects of aerobic and resistance exercise training on carotid intima-media thickness in abdominal obese women. *Metab Syndr Relat Disord* (2021) 19(4):200–4. doi: 10.1089/met.2020.0118

42. Charalambos Vlachopoulos, Konstantinos Aznaouridis, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness a systematic review and meta-analysis. *J Am Coll Cardiol* (2010) 55(13):1318–27. doi: 10.1016/j.jacc.2009.10.061

43. Webb DR, Davies MJ, Gray LJ, Abrams KR, Srinivasan B, Das S, et al. Searching for the right outcome? A systematic review and meta-analysis of controlled trials using carotid intima-media thickness or pulse wave velocity to infer antiatherogenic properties of thiazolidinediones. *Diabetes Obes Metab* (2010) 12(2):124–32. doi: 10.1111/j.1463-1326.2009.01122.x

44. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J* (2013) 34(31):2436–43. doi: 10.1093/eurheartj/eht149

45. Bruno RM, Penno G, Daniele G, Pucci L, Lucchesi D, Stea F, et al. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. *Diabetologia* (2012) 55(6):1847–55. doi: 10.1007/s00125-012-2517-1

46. Lacolley P, Regnault V, Segers P, Laurent S. Vascular smooth muscle cells and arterial stiffening: relevance in development, aging, and disease. *Physiol Rev* (2017) 97 (4):1555–617. doi: 10.1152/physrev.00003.2017

47. Guo X, Guo S, Zhang H, Li Z. Does aerobic plus machine-assisted resistance training improve vascular function in type 2 diabetes? A systematic review and meta-analysis of randomized controlled trials with trial sequential analysis. *J Clin Med* (2022) 11(15):4257. doi: 10.3390/jcm11154257

48. Rahman S, Rahman T, Ismail AA, Rashid AR. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. *Diabetes Obes Metab* (2007) 9(6):767–80. doi: 10.1111/j.1463-1326.2006.00655.x

49. Silva J, Menêses AL, Parmenter BJ, Ritti-Dias RM, Farah BQ. Effects of resistance training on endothelial function: A systematic review and meta-analysis. *Atherosclerosis* (2021) 333:91–9. doi: 10.1016/j.atherosclerosis.2021.07.009

50. van Sloten TT, Henry RM, Dekker JM, Nijpels G, Unger T, Schram MT, et al. Endothelial dysfunction plays a key role in increasing cardiovascular risk in type 2 diabetes: the Hoorn study. *Hypertension* (2014) 64(6):1299–305. doi: 10.1161/hypertensionaha.114.04221

51. Montero D, Walther G, Benamo E, Perez-Martin A, Vinet A. Effects of exercise training on arterial function in type 2 diabetes mellitus: A systematic review and meta-analysis. *Sports Med* (2013) 43(11):1191–9. doi: 10.1007/s40279-013-0085-2

52. Montero D, Walther G, Perez-Martin A, Vicente-Salar N, Roche E, Vinet A. Vascular smooth muscle function in type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetologia* (2013) 56(10):2122–33. doi: 10.1007/s00125-013-2974-1

53. Schreuder THA, Green DJ, Nyakayiru J, Hopman MTE, Thijssen DHJ. Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls. *Eur J Appl Physiol* (2015) 115(1):187–96. doi: 10.1007/s00421-014-3006-7

54. Liu Y, Sun Z, Chen T, Yang C. Does exercise training improve the function of vascular smooth muscle? A systematic review and meta-analysis. *Res Sports Med* (2022) 30(6):577–92. doi: 10.1080/15438627.2021.1917408

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Effects of hypertension on subcortical nucleus morphological alternations in patients with type 2 diabetes

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Objectives: Type 2 diabetes mellitus(T2DM) and hypertension(HTN) are common comorbidities, and known to affect the brain. However, little is known about the effects of the coexisting HTN on brain in T2DM patients. So we aim to investigate the impact of HTN on the subcortical nucleus morphological alternations in T2DM patients.

Materials & methods: This work was registered by the clinicaltrials.gov (grant number NCT03564431). We recruited a total of 92 participants, comprising 36 only T2DM patients, 28 T2DM patients with HTN(T2DMH) and 28 healthy controls(HCs) in our study. All clinical indicators were assessed and brain image data was collected for each participant. Voxel-based morphometry (VBM), automatic volume and vertex-based shape analyses were used to determine the subcortical nucleus alternations from each participant's 3D-T1 brain images and evaluate the relationship between the alternations and clinical indicators.

Results: T2DMH patients exhibited volumetric reduction and morphological alterations in thalamus compared to T2DM patients, whereas T2DM patients did not demonstrate any significant subcortical alterations compared to HCs. Furthermore, negative correlations have been found between thalamic alternations and the duration of HTN in T2DMH patients.

Conclusion: Our results revealed that HTN may exacerbate subcortical nucleus alternations in T2DM patients, which highlighted the importance of HTN management in T2DM patients to prevent further damage to the brain health.

KEYWORDS

type 2 diabetes mellitus, hypertension, volume analysis, shape analysis, subcortical nucleus

1 Introduction

Diabetes mellitus (DM) is a chronic and non-infectious disease that has seen a significant increase in incidence over recent years, with the International Diabetes Federation (IDF) reporting a 16% rise in the number of DM patients over the past two years (1). Alongside the classical clinical symptoms commonly associated with DM, including polydipsia, polyuria and weight loss, additional studies on brain health have revealed clinical manifestations of cognitive decline in individuals with DM, such as reduced executive function or decision-making ability. This cognitive impairment is particularly prevalent among individuals with T2DM, which constitutes around 90% of all DM cases (2).

In fact, abnormal brain structures are often responsible for cognitive impairment with clinical symptoms. Prior investigations have observed volume reduction in multiple cortical and/or subcortical regions in individuals with T2DM (3, 4). Although some studies have reported inconsistent findings, most have cited atrophy in the prefrontal cortex (5, 6) and medial temporal cortex, particularly in the hippocampus (6, 7). Furthermore, functional magnetic resonance imaging (fMRI) studies have uncovered a significant decrease in functional connections within the hippocampus of T2DM patients (8). Thus, both structural and functional abnormalities in these brain regions may play a role in the development and manifestation of cognitive impairment among T2DM patients.

Currently, the potential mechanisms underlying the subcortical gray matter alterations in T2DM patients are gradually becoming clear. Longitudinal studies with autopsy confirmation have established a link between T2DM and cardiovascular and cerebrovascular disease (CVD) (9), while vascular risk factors have been identified as important contributors to brain defects in T2DM patients (10, 11). HTN, in particular, has emerged as a significant factor, with approximately 75% of self-reported T2DM patients being afflicted by this condition (12). Indeed, HTN has been shown to independently affect brain structures, and the longer duration of HTN is associated with the smaller whole brain volumes (13). Moreover, a longitudinal study of middle-aged HTN patients has revealed thinning of the insular, frontal and temporal cortex in those with long-term exposure to this condition (14). In young HTN patients, cumulative systolic blood pressure (SBP) exposure has been negatively correlated with the morphological changes in subcortical regions such as the putamen, nucleus accumbens, pallidum, and thalamus (15). Importantly, structural abnormalities in several brain regions observed in HTN patients, including the frontal temporal cortex and basal nucleus, are also evident in T2DM patients. However, it remains unclear whether these defects are caused by HTN, T2DM, or their related complications. Furthermore, most previous T2DM studies have failed to regulate these factors, resulting in potentially biased outcomes. Only a recent study has examined the effect of HTN on cortex alterations in T2DM patients, confirming that coexisting HTN accelerates the reduction in cortex thickness, with a significant association between thinner cortex and cognitive decline in these patients (16). However, cognitive impairment may not be restricted to defects in the cortex alone, with other studies identifying associations between cognition and subcortical nucleus defects such as the thalamus (17), caudate nucleus (18), putamen (19), and hippocampus (20, 21). We, therefore, suspect that coexisting HTN may also impact subcortical nucleus, which could have implications for the cognitive function of T2DM patients.

Our study aims to investigate whether concurrent HTN exacerbates subcortical nuclei abnormalities in T2DM patients. VBM, automatic volume and vertex-based analyses (22) will be utilized to precisely locate and visualize subcortical nuclei alternations. Furthermore, we will evaluate the correlation between clinical features and subcortical nuclei structural abnormalities using neuropsychological tests and relevant clinical indicators.

2 Materials and methods

2.1 Participants

This was a prospective study, and the ethics committee of the First Affiliated Hospital of Kunming Medical University had approved the study protocol. In this study, 28 T2DM patients and 36 T2DM patients with HTN were recruited by the Department of Endocrinology at the First Affiliated Hospital of Kunming Medical University between June 2021 and March 2022. According to the 2010 edition of American Diabetes Association guidelines for diagnosis and treatment, T2DM was diagnosed by two experienced endocrinologists. The diagnosis of HTN was based on the criteria of 1999 World Health Organization-International Society of Hypertension Guidelines for the management of HTN. Blood pressure (BP) was recorded as the average of all measurements collected by a 24-hour ambulatory BP monitor. The exclusion criteria for T2DM were as follow: (a) Secondary diabetes and chronic diabetic complications such as clinical diabetic nephropathy, proliferative diabetic retinopathy, painful or symptomatic diabetic neuropathy (b) Mental disorders such as depression, epilepsy, Parkinson's disease or schizophrenia (c) Previous central nervous system (CNS) injuries such as cerebral infarction, cerebral hemorrhage, brain tumor and brain trauma (d) Abuse of alcohol, drug addiction or other psychoactive agents. Besides, a total of 28 healthy controls (HCs), matched for age, gender and number of years of education, were recruited at the same time. They were also interviewed to affirm that there was no history of psychiatric illness, brain injury or drug abuse. All the participants were right-handed and provided signed informed consent.

All subjects' blood samples were collected in the morning after fasting more than 10 hours overnight. General demographic and biomedical data of each participant were recorded by standard measurement method before magnetic resonance imaging.

The sample size was defined on the basis of results of a "*Post hoc*" power analysis, computed with G Power 3.1 [Parameters: effect size f(U) = 0.4; α err prob = 0.05; power (1-β err prob) = 0.93]. And output of the analysis revealed that a group sample size of at least 28 patients would have a 93% power to detect such a difference as statistically significant at a level (α) of 0.05 in the present study.

2.2 Neuropsychological assessments

All participants underwent a series of neuropsychological tests, including Mini Mental-Status Examination (MMSE) (23), Digital Symbol Substitution Test (DSST) (24), Hamilton Depression Scale (HAMD) (25) and Hamilton Anxiety Scale (HAMA) (26). The MMSE was used to assess general cognitive function of each participant. The DSST was involved in assessing the advanced cognitive functions, including executive function, attention and information processing speed. The HAMD and HAMA were used to evaluate the depression and anxiety of each subject. All neuropsychological tests were administered to all participants by the same and experienced psychiatric professional.

2.3 Data acquisition

All participants were scanned on a 3T Trio MRI system (GE Discovery 750w 3.0T) equipped with a 32-channel phase-array head coil. All participants were requested to remain calm, keep their eyes closed and avoid any movement during the image acquisition. Axial T1/T2-weighted images and T2 fluid attenuated inversion recovery (T2-FLAIR) images were performed to eliminate significant structural abnormalities of each subject. A high-resolution 3D fast-spoiled gradient recalled acquisition (FSPGR) sequence was acquired with the following parameters: rotation time (TR)/echo time (TE): 8.7/3.2ms, slice thickness: 1.0 mm, field of view (FOV): 256 mm × 256 mm, matrix size: 256 × 256, flip angle: 12°, slice number: 160 with no gap, and scan duration: 4 minutes 23 seconds. All sections were acquired parallel to the anterior–posterior commissure line.

2.4 Voxel-based morphometry analysis

Structural data of all subjects were processed by FMRIB Software Library (FSL, version5.0, http://www.fmrib.ox.ac.uk/fsl). A voxel-based morphometry (VBM) analysis in FSL was simplified to four steps: Brain Extraction, Tissue Segmentation, Spatial Normalization, Modulation and Smoothing. In the third step, we chose the non-linear registration instead of the linear registration to normalize our data. Especially, in the fourth step, we chose the 3mm sigma of isotropic Gaussian kernel for the smoothing of all grey matter images. Other steps were performed with the default options.

2.5 Subcortical grey matter volumetric analysis

Each subject's 3D-FSPGR images was automatically segmented to the amygdala, accumbens, caudate, pallidum, putamen nucleus, hippocampus and thalamus by FMRIB's Integrated Registration and Segmentation Tool (FIRST) (22), part of the FSL. FIRST was a deformable Active Appearance Model (AAM) based on a Bayesian framework, significantly different from VBM, it depended directly on the geometry/location of the structure boundary, not on the classification or smoothing extents of the tissue type. Because the quality of the automatic segmentation would affect the subsequent vertex-based analysis and statistical results, each step should be carefully examined, and corrected if necessary.

Meanwhile, the original volume of each subcortical nucleus would be obtained automatically at this stage. In order to eliminate the difference in head size among subjects, the brain image extracted from the single whole-head input data, and non-linear registered to the MNI152 space, which used for normalization of head size (27).

2.6 Shape analysis of subcortical grey matter

Vertex-based shape analysis was performed after the automatic segmentation. In this stage, the first step was to perform two-stage linear registration of the training data to achieve a more robust and accurate pre-alignment of the subcortical structure. Then a probabilistic math model was trained to collect the shape and intensity parameters of the training data. When applied to the new data, the math model is registered to the native space using the inverse transform, and then vertex analysis was performed by carrying out a multivariate test on the three-dimensional coordinates of corresponding vertices (22). Each vertex was analyzed independently to check the difference in the three-dimensional parameters. These differences are visualized by subsequent statistical analysis.

2.7 Statistical analysis

All demographic, biomedical and neuropsychological data tests were processed in SPSS, version 26.0 (SPSS, Chicago, IL, USA). An unpaired two-sample t-test was performed to continuous variables in this study. Moreover, a chi-square test was using for categorical variables and a nonparametric Mann–Whitney U test for non-normally distributed variables. All tests were 2-sided, and a $p < 0.05$ was considered statistically significant. Continuous variables were expressed as mean ± standard deviation (SD), and as medians ± interquartile ranges (IQR) for other variables.

In this study, the effect of T2DM and T2DMH on the subcortical nucleus was evaluated separately. Firstly, in order to access the volume difference between the T2DM and HCs, an analysis of covariance (ANCOVA) was conducted for the normalized volume data of each structure (28). Age, gender and the duration of DM were regarded as covariates and a $p < 0.05$ was considered statistically significant. Meanwhile, VBM and vertex-based analyses were respectively used to access the morphometry and shape difference of each structure between the T2DM and HCs in FSL. Similarly, age, gender and the duration of DM were regarded as covariates again. Clusters were identified using a threshold-free cluster enhancement (TFCE) by running 5000 random permutations. The cluster-wise threshold was setting at p

< 0.05 with a family wise error (FWE) correction. Subsequently, the same step applied between the T2DM and T2DMH.

Correlations between the volume/shape difference and the cognitive impairment or related clinical characteristics were performed with a partial correlation analysis. The volume difference-based correlation analysis was accessed in SPSS. Different from the volume analysis, the shape difference -based correlation analysis was calculated in Permutation Analysis of Linear Model (PALM, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM) (28), which was a tool that allowed inference using permutation methods, offering a number of features not available in other analysis software. ***p*** value was still corrected by FWE.

3 Results

3.1 Demographic, biomedical and neuropsychological characteristics

The demographic biomedical and neuropsychological characteristics of all subjects were shown in Table 1. There was no significant difference in demographic characteristics between groups (***p*** >0.05) except for a higher average age in the T2DMH than the T2DM (p = 0.005). However, in the comparison of biological and neuropsychiatric characteristics, multiple indicators were statistically different among groups. Compared with the HCs, several

TABLE 1 Characteristics of study participants.

Characteristics	HCs (n=28)	T2DM (n=36)	T2DMH (n=28)	T2DM-HCs		T2DMH-T2DM	
				t/χ2	*P*	t/χ2	*P*
Demographic characteristics							
Age, mean (SD),years	50.46 (6.374)	49.19 (5.879)	54.39 (7.87)	-0.826	0.412	2.920	**0.005***
Sex (Male/Female)	26/10	15/13	18/10	2.38	0.189	0.462	0.341
Education level, mean (SD),year	10.86 (3.932)	11.00 (3.381)	11.14 (2.84)	0.156	0.876	0.180	0.858
BMI, mean (SD), kg/m^2	23.70 (2.52)	24.27 (2.56)	25.15 (2.19)	0.893	0.376	1.336	0.187
Hand (left/right)	0/28	0/36	0/28	–	–	–	–
Diabetes-related clinical characteristics							
Time since diagnosis of T2DM, mean (SD), years	–	5.31 (5.21)	8.43 (6.59)	–	–	2.117	**0.038***
HbA_{1c},mean (SD), % (mmol/mol)	–	9.13 (2.44)	8.59 (1.97)	–	–	-0.966	0.338
FPG, mean (SD), mmol/l	4.87 (0.37)	7.63 (3.41)	6.93 (3.03)	4.823	**<0.001***	-0.854	0.357
FI, mean (SD), pmol/l	10.93 (7.70)	7.64 (3.80)	11.92 (4.94)	-2.244	**0.028***	3.929	**<0.001***
HOMA-IR, mean (SD)	2.43 (2.05)	2.67 (1.85)	4.04 (2.92)	0.498	0.620	2.170	**0.036***
HOMA-beta, mean (SD),%	162.19 (74.56)	55.97 (44.07)	85.08 (102.58)	-7.109	**<0.001***	1.533	0.130
LnHOMA-IR	0.75 (0.43)	0.71 (0.85)	1.16 (0.65)	-0.251	0.803	2.294	**0.025***
LnHOMA-beta	5.01 (0.39)	3.71 (0.84)	4.30 (0.66)	-8.215	**<0.001***	3.082	**0.003***
Blood Pressure							
Time since diagnosis of HTN, mean (SD), years	–	–	6.79 (5.36)	–	–	–	–
Systolic, mean (SD), mmHg	117.18 (12.51)	114.83 (12.79)	132.25 (13.29)	-0.735	0.465	5.313	**<0.001***
Diastolic, mean (SD), mmHg	77.36 (9.67)	74.75 (7.65)	82.46 (7.60)	-1.205	0.233	4.013	**<0.001***
Vascular risks							
Your 10-year risk (%)	9.55 (7.35)	18.29 (9.93)	34.23 (16.79)	4.05	**<0.001***	4.453	**<0.001***
Vascular age, year	54.21 (10.08)	66.58 (12.75)	80.86 (6.08)	4.209	**<0.001***	5.909	**<0.001***
IMT, mean (SD), mm	–	0.87 (0.17)	0.94 (0.22)	–	–		
Lipid profiles							
TC, mean (SD), mmol/l	4.82 (0.67)	4.60 (1.06)	4.29 (0.92)	-0.95	0.346	-1.216	0.229
TG, mean (SD), mmol/l	1.61 (0.77)	2.59 (1.82)	4.60 (1.06)	2.926	**0.005***	-0.173	0.863
LDL-c, mean (SD), mmol/l	1.35 (0.56)	2.90 (0.85)	2.68 (0.72)	8.722	**<0.001***	-1.087	0.281

(Continued)

TABLE 1 Continued

Characteristics	HCs (n=28)	T2DM (n=36)	T2DMH (n=28)	T2DM-HCs		T2DMH-T2DM	
				t/χ2	P	t/χ2	P
HDL-c, mean (SD), mmol/l	3.27 (0.93)	1.04 (0.25)	1.02 (0.27)	-12.407	**<0.001***	-0.245	0.808
Renal function							
BUN, mean (SD), mmol/l	4.52 (0.97)	4.41 (1.06)	4.54 (1.15)	-0.411	0.683	0.437	0.663
Cr, mean (SD), μmol/L	73.81 (12.43)	71.02 (16.57)	70.05 (9.93)	-0.744	0.460	-0.275	0.784
UA, mean (SD), μmol/L	308.12 (85.09)	328.89 (64.07)	345.68 (92.14)	1.115	0.269	-0.859	0.394
UAER, mean (SD),mg/24h	–	35.27 (38.06)	88.78 (235.31)	–	–	1.345	0.184
UPCR, mean (SD), mg/24h	–	51.72 (101.43)	206.38 (804.05)	–	–	1.145	0.257
Cognitive assessments							
MMSE, mean (SD)	27.11 (3.25)	29.28 (2.59)	28.71 (3.37)	2.892	**0.006***	-0.757	0.452
DSST, mean (SD)	45.86 (21.43)	32.18 (10.27)	29.39 (11.41)	-3.111	**0.004***	-1.026	0.309
HAMD, mean (SD)	3.89 (3.93)	3.81 (3.27)	4.11 (2.99)	-0.097	0.923	0.380	0.705
HAMA, mean (SD)	3.54 (4.33)	4.61 (3.24)	4.82 (2.67)	1.137	0.260	0.278	0.782

HCs, healthy controls; T2DM, Type 2 diabetes mellitus; T2DMH, T2DM patients with hypertension; BMI, body mass index; H bA1c, the levels of Hemoglobin A1c; FPG, Fasting plasma glucose; FI, Fasting insulin; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-beta, homeostatic model assessment of insulin Beta-cell function index; LnHOMA-IR and LnHOMA-beta were the natural logarithms of HOMA-IR and HOMA-beta. TC, Total cholesterol; TG, Triacylglycerol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; IMT, Intima-media thickness; BUN, blood urea nitrogen; Cr, Creatinine; UA, Uric acid; UAER, Urinary albumin excretion rates; UPCR, Urine protein to creatinine ratio; MMSE, Mini-Mental State Examination; DSST, Digit Symbol Substitution Test; HAMD, Hamilton depression scale; HAMA, Hamilton Anxiety Scale. *: P value was less than 0.05, which is statistically significant.
The symbol "-" represents parameters that were not applicable or no significance for measurement in the column.
The bold values represent P-values with statistical differences.

characteristics including Your 10-year risk ($p < 0.001$), vascular age ($p < 0.001$), TG ($p = 0.009$), FPG ($p < 0.001$) and MMSE ($p = 0.004$) were significantly increased in the T2DM and several other indicators including ALT($p = 0.025$), AST($p < 0.001$), HDL($p < 0.001$), INS(0) ($p = 0.028$), HOMA beta($p < 0.001$) and DSST($p = 0.001$) were significantly decreased in the T2DM. Moreover, multiple indicators including the duration of DM ($p = 0.038$), Your 10-year risk ($p < 0.001$), vascular age ($p < 0.001$), SBP ($p < 0.001$), DBP ($p < 0.001$), FI ($p < 0.001$) and HOMA-IR ($p = 0.036$) were observed with a higher level in the T2DMH than the T2DM. No decreased characteristics were observed in the T2DMH contrast to the T2DM.

3.2 Voxel-based morphometry analysis

Among T2DM, T2DMH and HC groups, there was no significant alternations in grey matter found by VBM analysis, at the threshold of $p < 0.05$(FWE-corrected).

3.3 Volumetric analysis of subcortical gray matter

Statistical results for normalized volumes of the 14 subcortical nucleus between groups had been showed in Table 2. An ANCOVA revealed that there was no significant difference was found in all subcortical nucleus volume between the T2DM patients and HCs. However, compare to T2DM, the volume of right thalamus in T2DMH patients showed a significant reduction (F= 4.555, p=0.037). Meanwhile, correlation analysis showed that the reduction of right thalamic volume in T2DMH patients was negatively correlated with the duration of HTN (p =0.002, r=0.40). Table 3.

3.4 Shape analysis of subcortical gray matter

Vertex-based shape analysis revealed significant regional shape deformation on the medial, dorsal aspects of the left thalamus ($cluster_a$, voxels=87, p=0.036, FWE-corrected, x=-9, y=-5, z=11) and the medial, dorsal, ventral aspects of the right thalamus ($cluster_b$, voxels=283, p=0.014, FWE-corrected, x=13, y=-33, z=0; $cluster_c$, voxels=287, p=0.008, FWE-corrected, x=14, y=-8, z=14), which were shown in Figure 1. No significant difference was found in the subcortical structures between the T2DM and HCs ($p > 0.05$, FWE-corrected). Meanwhile, there were significant negative correlations of the duration of HTN with regional shape deformation of the left and right thalamus (left thalamus, $cluster_a$, voxels=87, p=0.002, FWE-corrected, x=-11, y=-5, z=13; right thalamus, $cluster_b$, voxels=181, p=0.003, FWE-corrected, x=12, y=-33, z=1; $cluster_c$, voxels=262 p=0.003, FWE-corrected, x=14, y=-6, z=12, r=0.46), which were shown in Figure 2 and Table 3.

4 Discussion

In this study, we exploited the VBM, automatic volume and vertex-based shape analyses to determine the subcortical nucleus abnormalities in T2DM and T2DMH patients. Our results showed that there were no significant alterations in the subcortical nucleus

TABLE 2 Normalized subcortical grey matter structural volumes of study participants (mm^3).

Structures	HCs (n=28)	T2DM (n=36)	T2DMH (n=28)	T2DM-HCs		T2DMH-T2DM	
				F	*P*	F	*P*
Left							
Accu	462.75 ± 108.702	483.44 ± 89.395	456.64 ± 92.206	0.179	0.674	0.316	0.576
Amyg	1073.21 ± 245.512	1122.22 ± 217.149	1158.93 ± 278.196	0.013	0.910	0.733	0.396
Caud	3351.39 ± 379.624	3401.75 ± 389.437	3286.96 ± 423.778	0.011	0.917	0.374	0.543
Hipp	3439.61 ± 364.640	3589.72 ± 408.713	3531.11 ± 589.248	0.877	0.353	0.033	0.856
Pall	1846.71 ± 409.993	1820.33 ± 252.956	1808.89 ± 324.462	0.037	0.847	0.479	0.452
Puta	4955.29 ± 604.757	5039.08 ± 560.724	4836.39 ± 540.755	0.056	0.814	0.197	0.659
Thal	7822.11 ± 702.907	7997.22 ± 639.488	7584.11 ± 675.678	0.082	0.775	1.322	0.255
Right							
Accu	363.82 ± 97.604	354.64 ± 89.126	344.82 ± 93.281	0.212	0.647	0.004	0.947
Amyg	1104.25 ± 383.398	1072.28 ± 197.825	1147.50 ± 241.613	0.853	0.359	0.631	0.430
Caud	3499.61 ± 372.473	3543.17 ± 420.635	3419.25 ± 397.934	0.156	0.694	0.410	0.524
Hipp	3584.71 ± 428.424	3753.08 ± 391.165	3759.43 ± 496.421	1.145	0.289	0.782	0.380
Pall	1851.96 ± 339.667	1804.14 ± 201.035	1843.57 ± 337.670	0.403	0.528	0.001	0.978
Puta	4909.64 ± 554.494	4960.89 ± 489.745	4761.68 ± 508.780	0.172	0.680	0.187	0.667
Thal	7556.61 ± 632.588	7775.56 ± 535.139	7305.82 ± 641.500	0.672	0.416	3.632	**0.042***

Accu, Accumbens; Amyg, Amygdala; Caud, Caudate; Hipp, Hippocampus; Pall, Pallidum; Puta, Putamen; Thal, Thalamus
*: P value was less than 0.05, which was statistically significant.
The bold values represent P-values with statistical differences.

of T2DM patients compared with HCs. However, we observed a volume reduction in the right thalamus and the regional shape deformation in the bilateral thalamus of T2DMH patients when comparing T2DM patients. A vertex-based shape analysis revealed additional abnormalities of the thalamus in T2DMH patients than the VBM and automatic volume analysis. Furthermore, we found negative correlations between all the volume/shape alternations in the thalamus and the duration of HTN.

The present study did not show any significant subcortical nucleus defects in T2DM patients when compared to HCs, which differed from the consistent findings in previous studies that emphasized the hippocampal atrophy (6, 7). We hypothesized that blood pressure control could be the primary factor explaining our results. A recent study found that the presence of HTN in T2DM patients contributed to a further reduction in cortical thickness (16). Additionally, HTN alone had been linked to an increased risk of hippocampal atrophy (29). It was evident that in most previous T2DM studies, blood pressure was not adequately controlled in all participants, which may have contributed to inconsistent results. Therefore, obtaining a more reliable conclusion may necessitate balancing the blood pressure profile in future studies. Secondly, recent evidence suggested a compensatory mechanism within the hippocampus that may compensate for structural defects in the early stage of T2DM or before cognitive impairment develops (30). Meanwhile, similar studies also have confirmed that hippocampal atrophy was more likely to occur in T2DM patients with cognitive impairment (31). Thus, based on our present data, we speculate that the compensation mechanism may have played a role in this study and may explain why hippocampal defects

TABLE 3 Correlation results for the subcortical GM in volume and shape analysis.

Measures	cluster	r/R^2	*P*
Correlation with the duration of HTN			
The volume of right thalamus	–	-0.40	**0.002***
The shape of left thalamus	87	-0.43	**0.002***
The shape of right thalamus	181	-0.46	**0.003***
	262	-0.46	**0.003***

*: P value was less than 0.05, which was statistically significant.
The symbol "-" represents parameters that were not applicable or no significance for measurement in the column.
The bold values represent P-values with statistical differences.

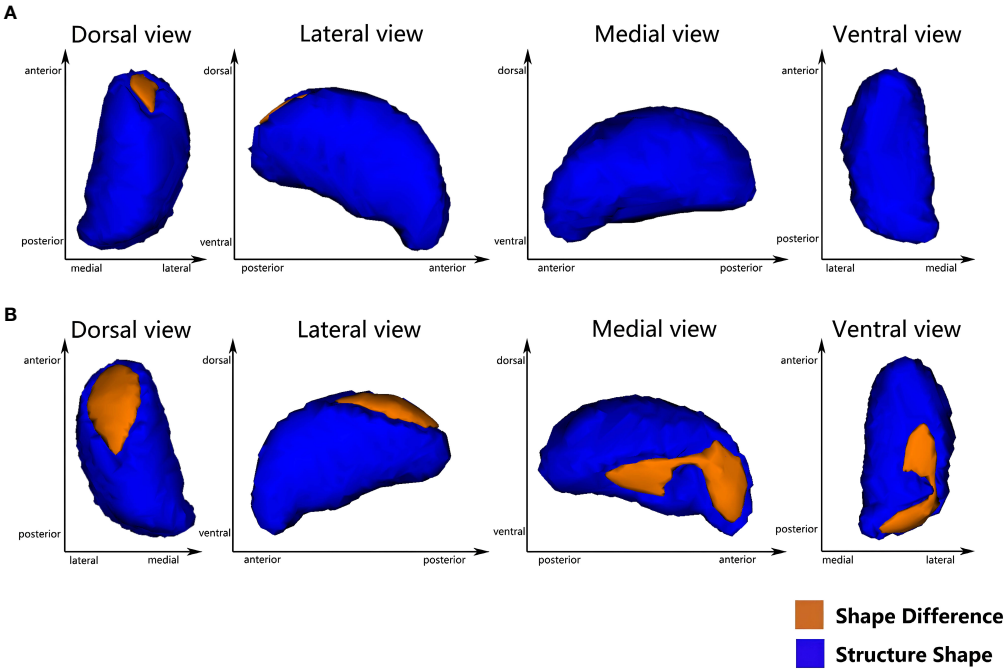

FIGURE 1
Vertex-wise comparison between T2DMH and T2DM shows significant regional shape deformation on the dorsal and medial aspects of the left thalamus **(A)**, and the dorsal, medial and ventral aspects of the right thalamus **(B)** (FWE-corrected, $p<0.05$).

were not observed. It is necessary to conduct more longitudinal studies with larger sample sizes to support our hypothesis in the future.

An important finding of this study was that T2DMH patients exhibited a volume reduction in right thalamus and the regional deformation in the bilateral thalamus compared to T2DM patients. Obviously, the vertex-based shape analysis could unveil nuances that VBM remained elusive, owing to the robust mathematical framework afforded by FIRST (22). Thalamus was considered to be an important

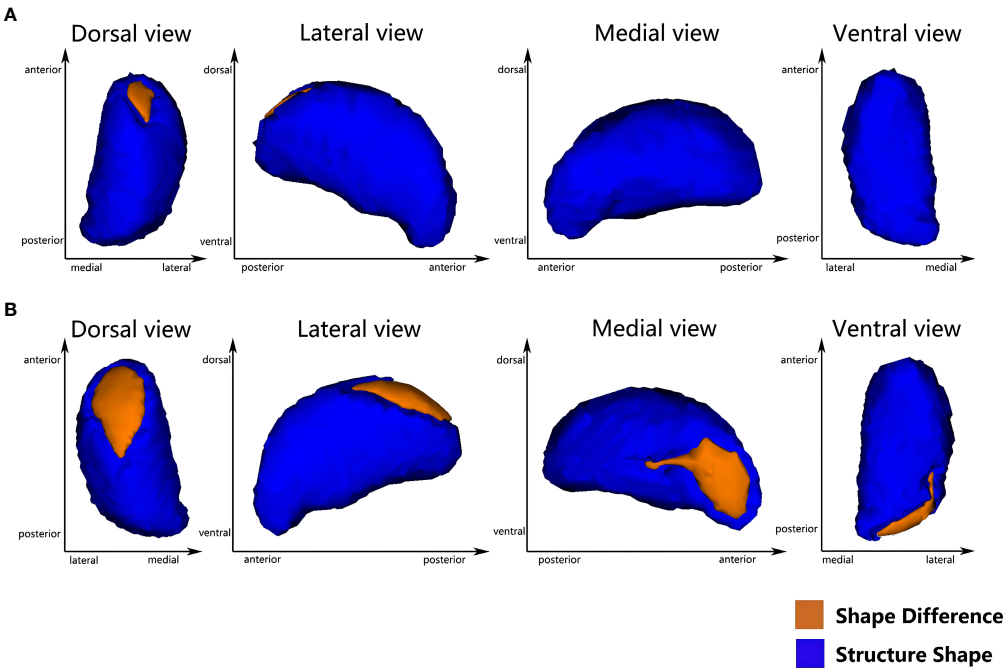

FIGURE 2
Vertex-wise comparison shows a significant negative correlation between the duration of HTN and the regional shape deformations on the dorsal and medial side of the bilateral thalamus in T2DMH patients **(A)** the left thalamus; **(B)** the right thalamus) (FWE-corrected, p <0.05).

structure for the generation and transmission of high-level neural activities, all kinds of sensory conduction of the whole body except for the sensation of smell were transformed in the thalamus, and then projected to the cerebral cortex to produce specific sensation (32). In addition, thalamus was thought to be associated with learning memory and executive function (33). So the atrophy of the thalamus in T2DMH patients may imply a loss of neurons, which may result in increased risks of sensory or cognitive decline. In fact, the thalamus may have shown the metabolic or functional defects before the obvious structural abnormalities. A previous Magnetic Resonance Spectroscopy (MRS) research found that the N-Acetyl-Aspartate/Creatin (NAA/Cr) of the bilateral thalamus was reduced in HTN patients (34). As a unique metabolite of neurons, the reduction of the NAA level meant that the activity of neurons was reduced, and it was related to the cognitive level. Combined with our research, it showed that HTN was an important risk factor for thalamus defects in T2DM. Although the exact physiological or pathological mechanism of the thalamus defects which was dominated by HTN in T2DM patients was still unclear, some robust researches had shown that the oxidative stress dominated the vascular damage in both HTN and T2DM, including large blood vessels and microcirculation (35, 36). The oxygen free radicals which were produced in the process of oxidative stress have been proved to be an important factor which led to the thalamus degeneration (37). Therefore, we speculated that the oxygen free radicals may enter the thalamus through the microcirculation and accumulate constantly, thus these overloaded oxygen free radicals created an environment which was unfavorable to the survival of thalamic cells. Over time, a neurodegeneration may occur in thalamus which was exposed to this environment. In addition, inflammatory factors related to the oxidative stress may also participate in the pathology of thalamus defects (38). These assumptions may provide some insights for explaining thalamus defects, more rigorous and direct evidence was needed to show the relationship between the oxidative stress and thalamus defects of T2DMH in the future.

Furthermore, our study revealed that the morphological deformation in T2DMH patients were predominantly localized in the medial and dorsal regions of the left thalamus, as well as the medial, dorsal, and ventral regions of the right thalamus. It is worth noting that the dorsal and medial thalamus had been shown to be structurally and functionally connected to the prefrontal and temporal cortex, while the ventral thalamus was associated with the parietal cortex (32). Previous research has suggested that subcortical nucleus neural degeneration was frequently a secondary effect of defects in the cortex regions which were synaptically associated with corresponding subcortical nucleus (39). Therefore, we proposed that the deformation observed in the dorsal, medial, and ventral regions of the thalamus may be associated with structural defects in the corresponding cortex regions. Previous researches on T2DM patients that did not control their blood pressure had already observed defects in the prefrontal (5, 16), temporal, and parietal cortex (40). Nevertheless, our current study suggested that the presence of HTN may be a crucial factor in contributing to cortex defects in T2DM patients. It has been reported that HTN may impair the blood supply system of cerebral cortex by altering cerebral vascular microcirculation hemodynamics (35), which may lead to the prolonged cortical ischemia and cortex atrophy (41). An arterial spin labeling (ASL) study had revealed that the decrease of cerebral blood flow in HTN patients were primarily concentrated in the frontal, temporal, and parietal lobes (42). Therefore, we speculated that the prefrontal, temporal, and parietal cortex atrophy may lead to the neural degeneration in the dorsal, medial, and ventral regions of the thalamus via a cortical-subcortical synapse connection. Currently, we hypothesize that concurrent HTN may exacerbate brain damage and participate in cortex defects by influencing the thalamus deformation in T2DMH patients.

Moreover, another important finding in our study was the thalamic volume reduction and deformation were negatively correlated with the duration of HTN in T2DMH patients, which suggested that the longer the duration of HTN, the more significant subcortical nucleus defects in T2DMH patients. Our finding was consistent with previous studies that had shown a correlation between the duration of HTN and brain volume (13). Meanwhile, a longitudinal study of HTN patients had emphasized the relationship between the duration of HTN and the incidence of T2DM (43). This indicated that the influence of HTN on T2DM may exist before the confirmed diagnosis of T2DM. Integrating these findings suggested that the effects of HTN on T2DM had increased gradually with the duration of HTN. Effective interventions, such as blood pressure screening and follow-up treatment for early T2DM patients, were necessary to reduce the potential threat of HTN to brain health.

For cognition, we found that the MMSE score of T2DM group was higher than that of HCs, and the score was higher in the T2DMH group compared to the T2DM group. The outcome, at first glance, appeared paradoxical. However, a more nuanced understanding emerged when we turned our attention to those participants endowed with the levels of education. There is a higher education level of T2DM and T2DMH participants in two controls. In this context, the facile nature of the MMSE test appeared to veil the cognitive decline evident in these patients, as their scores harmoniously nestled within the bounds of normalcy. In addition, our study unveiled the potency of the DSST test in discerning cognitive decline in the T2DM patients. However, the DSST scores exhibited a convergence between the T2DM and T2DMH. This result may imply that the DSST, despite its utility as an indicator of glycemic control-related cognition, might not possess the requisite sensitivity to detect the concomitant presence of HTN. This intriguing observation prompts consideration that the absence of correlation between thalamus alterations and DSST scores in T2DMH patients could be attributed to this potential limitation.

Although the current study did not establish a clear relationship between subcortical nucleus defects and cognitive impairment in T2DMH patients, animal models had demonstrated the impact of the mediodorsal thalamus-prefrontal cortex loop on cognition (44, 45). According to this framework, the mediodorsal thalamus dominated the cortex representation under different behavioral conditions, and was associated with cognitive impairment. We speculated that an insufficient sample size and indistinguishable cognitive scores between T2DM and T2DMH patients in this study may limit us to describe the accurate relationship between subcortical nucleus defects and cognitive impairment in T2DMH patients. Meanwhile, it was noting that these animal studies had also confirmed the findings of past decades brain imaging researches which revealed that prefrontal cortex may more strongly associated with cognition (46). For example, this relationship was proved in different cohort population involving

depression, schizophrenia, and Alzheimer's disease (47–49). Given the evidence from these robust studies, it was plausible to postulate that while subcortical nucleus defects may exert some influence on cognition, the relationship between cortical defects and cognitive impairment in T2DMH patients appeared to be more straightforward.

The present study had some limitations that should be acknowledged. Firstly, this study was a horizontal study with a relatively small sample size, so the conclusion cannot be extended to the general population. Secondly, the use of medications for T2DM or T2DMH subjects could potentially impact our findings to some extent. Meanwhile, this study employed the MMSE and DSST, two simple cognitive function screening scales, to evaluate the cognition of all participants. Future researches should consider more comprehensive cognitive evaluation methods to investigate the potential relationship between subcortical nucleus defects and cognition in T2DMH patients. Lastly, although we speculated on the relationship between cortex and subcortical nucleus defects in T2DMH based on related literature, we did not separately analyze the cortex in the current study, and thus could not further explore this relationship.

5 Conclusion

In summary, our study suggested that HTN may exacerbated brain damage in T2DM patients and may potentially threaten their cognitive abilities. Thus, early interventions such as an effective blood pressure control could mitigate extra brain damage in T2DMH patients. Regrettably, our findings did not yield conclusive evidence to establish a direct association between subcortical nucleus deficits and cognition in T2DMH patients. Further longitudinal researches or more comprehensive cognition investigations may provide valuable insights into this inquiry.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethics committee of the First Affiliated Hospital of Kunming Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LS and YL designed the study and experiments. FC, Z-QO, Y-ZZ, and B-BL contributed to the drafting of the manuscript. FC and YL contributed to the data analysis. YL and X-JS edited the manuscript. Y-ZZ and YL acquired the magnetic resonance images. YZ, H-YG, W-LJ, TZ, X-JS, and DH contributed to the clinical studies and data acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

2. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* (2018) 14:88–98. doi: 10.1038/nrendo.2017.151

3. van Harten B, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain imaging in patients with diabetes: a systematic review. *Diabetes Care* (2006) 29:2539–48. doi: 10.2337/dc06-1637

4. Jongen C, Biessels GJ. Structural brain imaging in diabetes: a methodological perspective. *Eur J Pharmacol* (2008) 585:208–18. doi: 10.1016/j.ejphar.2007.11.085

5. Ajilore O, Narr K, Rosenthal J, Pham D, Hamilton L, Watari K, et al. Regional cortical gray matter thickness differences associated with type 2 diabetes and major depression. *Psychiatry Res* (2010) 184:63–70. doi: 10.1016/j.pscychresns.2010.07.003

6. Brundel M, van den Heuvel M, de Bresser J, Kappelle LJ, Biessels GJ. Cerebral cortical thickness in patients with type 2 diabetes. *J Neurol Sci* (2010) 299:126–30. doi: 10.1016/j.jns.2010.08.048

7. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, et al. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* (2003) 46:1604–10. doi: 10.1007/s00125-003-1235-0

8. Musen G, Jacobson AM, Bolo NR, Simonson DC, Shenton ME, McCartney RL, et al. Resting-state brain functional connectivity is altered in type 2 diabetes. *Diabetes* (2012) 61:2375–9. doi: 10.2337/db11-1669

9. Chornenkyy Y, Wang WX, Wei A, Nelson PT. Alzheimer's disease and type 2 diabetes mellitus are distinct diseases with potential overlapping metabolic dysfunction upstream of observed cognitive decline. *Brain Pathol* (2019) 29:3–17. doi: 10.1111/bpa.12655

10. Bernardes G, IJzerman RG, Ten Kulve JS, Barkhof F, Diamant M, Veltman DJ, et al. Cortical and subcortical gray matter structural alterations in normoglycemic obese and type 2 diabetes patients: relationship with adiposity, glucose, and insulin. *Metab Brain Dis* (2018) 33:1211–22. doi: 10.1007/s11011-018-0223-5

11. Jung CH, Mok JO. Recent updates on vascular complications in patients with type 2 diabetes mellitus. *Endocrinol Metab (Seoul)* (2020) 35:260–71. doi: 10.3803/EnM.2020.35.2.260

12. Colosia AD, Palencia R, Khan S. Prevalence of hypertension and obesity in patients with type 2 diabetes mellitus in observational studies: a systematic literature review. *Diabetes Metab Syndr Obes* (2013) 6:327–38. doi: 10.2147/DMSO.S51325

13. Gianaros PJ, Greer PJ, Ryan CM, Jennings JR. Higher blood pressure predicts lower regional grey matter volume: Consequences on short-term information processing. *Neuroimage* (2006) 31:754–65. doi: 10.1016/j.neuroimage.2006.01.003

14. Vuorinen M, Kåreholt I, Julkunen V, Spulber G, Niskanen E, Paajanen T, et al. Changes in vascular factors 28 years from midlife and late-life cortical thickness. *Neurobiol Aging* (2013) 34:100–9. doi: 10.1016/j.neurobiolaging.2012.07.014

15. Jenkins LM, Garner CR, Kurian S, Higgins JP, Parrish TB, Sedaghat S, et al. Cumulative blood pressure exposure, basal ganglia, and thalamic morphology in midlife. *Hypertension* (2020) 75:1289–95. doi: 10.1161/HYPERTENSIONAHA.120.14678

16. Shi L, Cheng Y, Xu Y, Shen Z, Lu Y, Zhou C, et al. Effects of hypertension on cerebral cortical thickness alterations in patients with type 2 diabetes. *Diabetes Res Clin Pract* (2019) 157:107872. doi: 10.1016/j.diabres.2019.107872

17. Matías-Guiu JA, Cortés-Martínez A, Montero P, Pytel V, Moreno-Ramos T, Jorquera M, et al. Identification of cortical and subcortical correlates of cognitive performance in multiple sclerosis using voxel-based morphometry. *Front Neurol* (2018) 9:920. doi: 10.3389/fneur.2018.00920

18. Woollard SM, Olwenyi OA, Dutta D, Dave RS, Mathews S, Gorantla S, et al. Preliminary studies on immune response and viral pathogenesis of zika virus in rhesus macaques. *Pathogens* (2018) 7(3):70. doi: 10.3390/pathogens7030070

19. de Jong LW, van der Hiele K, Veer IM, Houwing JJ, Westendorp RG, Bollen EL, et al. Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. *Brain* (2008) 131:3277–85. doi: 10.1093/brain/awn278

20. Rodriguez-Porcel F, Espay AJ, Carecchio M. Parkinson disease in Gaucher disease. *J Clin Mov Disord* (2017) 4:7. doi: 10.1186/s40734-017-0054-2

21. Rosso AL, Verghese J, Metti AL, Boudreau RM, Aizenstein HJ, Kritchevsky S, et al. Slowing gait and risk for cognitive impairment: The hippocampus as a shared neural substrate. *Neurology* (2017) 89:336–42. doi: 10.1212/WNL.0000000000004153

22. Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* (2011) 56:907–22. doi: 10.1016/j.neuroimage.2011.02.046

23. Pangman VC, Sloan J, Guse L. An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. *Appl Nurs Res* (2000) 13:209–13. doi: 10.1053/apnr.2000.9231

24. Lafont S, Marin-Lamellet C, Paire-Ficout L, Thomas-Anterion C, Laurent B, Fabrigoule C. The Wechsler Digit Symbol Substitution Test as the best indicator of the risk of impaired driving in Alzheimer disease and normal aging. *Dement Geriatr Cognit Disord* (2010) 29:154–63. doi: 10.1159/000264631

25. Williams JB. Standardizing the Hamilton Depression Rating Scale: past, present, and future. *Eur Arch Psychiatry Clin Neurosci* (2001) 251(Suppl 2):II6–12. doi: 10.1007/BF03035120

26. Hamilton M. The assessment of anxiety states by rating. *Br J Med Psychol* (1959) 32:50–5. doi: 10.1111/j.2044-8341.1959.tb00467.x

27. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* (2002) 17:825–41. doi: 10.1006/nimg.2002.1132

28. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage* (2014) 92:381–97. doi: 10.1016/j.neuroimage.2014.01.060

29. Fiford CM, Nicholas JM, Biessels GJ, Lane CA, Cardoso MJ, Barnes J. High blood pressure predicts hippocampal atrophy rate in cognitively impaired elders. *Alzheimers Dement (Amst)* (2020) 12:e12035. doi: 10.1002/dad2.12035

30. Fang F, Lai MY, Huang JJ, Kang M, Ma MM, Li KA, et al. Compensatory hippocampal connectivity in young adults with early-stage type 2 diabetes. *J Clin Endocrinol Metab* (2019) 104:3025–38. doi: 10.1210/jc.2018-02319

31. Li M, Huang L, Yang D, Luo C, Qin R, Zhang B, et al. Atrophy patterns of hippocampal subfields in T2DM patients with cognitive impairment. *Endocrine* (2020) 68:536–48. doi: 10.1007/s12020-020-02249-w

32. Behrens TE, Johansen-Berg H, Woolrich MW, Smith SM, Wheeler-Kingshott CA, Boulby PA, et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci* (2003) 6:750–7. doi: 10.1038/nn1075

33. Sweeney-Reed CM, Buentjen L, Voges J, Schmitt FC, Zaehle T, Kam JWY, et al. The role of the anterior nuclei of the thalamus in human memory processing. *Neurosci Biobehav Rev* (2021) 126:146–58. doi: 10.1016/j.neubiorev.2021.02.046

34. Cao Z, Ye BD, Shen ZW, Cheng XF, Yang ZX, Liu YY, et al. 2D-1H proton magnetic resonance spectroscopic imaging study on brain metabolite alterations in patients with diabetic hypertension. *Mol Med Rep* (2015) 11:4232–8. doi: 10.3892/mmr.2015.3305

35. Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. *Can J Cardiol* (2018) 34:575–84. doi: 10.1016/j.cjca.2017.12.005

36. Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. *Cardiovasc Diabetol* (2018) 17:57. doi: 10.1186/s12933-018-0703-2

37. He M, Xing S, Yang B, Zhao L, Hua H, Liang Z, et al. Ebselen attenuates oxidative DNA damage and enhances its repair activity in the thalamus after focal cortical infarction in hypertensive rats. *Brain Res* (2007) 1181:83–92. doi: 10.1016/j.brainres.2007.08.072

38. Zhang Y, Xing S, Zhang J, Li J, Li C, Pei Z, et al. Reduction of β-amyloid deposits by γ-secretase inhibitor is associated with the attenuation of secondary damage in the ipsilateral thalamus and sensory functional improvement after focal cortical infarction in hypertensive rats. *J Cereb Blood Flow Metab* (2011) 31:572–9. doi: 10.1038/jcbfm.2010.127

39. Zhang J, Zhang Y, Xing S, Liang Z, Zeng J. Secondary neurodegeneration in remote regions after focal cerebral infarction: a new target for stroke management. *Stroke* (2012) 43:1700–5. doi: 10.1161/STROKEAHA.111.632448

40. Yau PL, Kluger A, Borod JC, Convit A. Neural substrates of verbal memory impairments in adults with type 2 diabetes mellitus. *J Clin Exp Neuropsychol* (2014) 36:74–87. doi: 10.1080/13803395.2013.869310

41. Jouvent E, Viswanathan A, Chabriat H. Cerebral atrophy in cerebrovascular disorders. *J Neuroimaging* (2010) 20:213–8. doi: 10.1111/j.1552-6569.2009.00370.x

42. Hajjar I, Zhao P, Alsop D, Novak V. Hypertension and cerebral vasoreactivity: a continuous arterial spin labeling magnetic resonance imaging study. *Hypertension* (2010) 56:859–64. doi: 10.1161/HYPERTENSIONAHA.110.160002

43. Chen Y, Ma J, Lu D, Fang Y. The risk factors of type 2 diabetes in hypertensive subjects. *Front Endocrinol (Lausanne)* (2022) 13:901614. doi: 10.3389/fendo.2022.901614

44. Bolkan SS, Stujenske JM, Parnaudeau S, Spellman TJ, Rauffenbart C, Abbas AI, et al. Thalamic projections sustain prefrontal activity during working memory maintenance. *Nat Neurosci* (2017) 20:987–96. doi: 10.1038/nn.4568

45. Schmitt LI, Wimmer RD, Nakajima M, Happ M, Mofakham S, Halassa MM. Thalamic amplification of cortical connectivity sustains attentional control. *Nature* (2017) 545:219–23. doi: 10.1038/nature22073

46. Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* (2001) 24:167–202. doi: 10.1146/annurev.neuro.24.1.167

47. Ingvar DH, Franzén G. AbnorMalities of cerebral blood flow distribution in patients with chronic schizophrenia. *Acta Psychiatr Scand* (1974) 50:425–62. doi: 10.1111/j.1600-0447.1974.tb09707.x

48. Weinberger DR, Berman KF. Prefrontal function in schizophrenia: confounds and controversies. *Philos Trans R Soc Lond B Biol Sci* (1996) 351:1495–503. doi: 10.1098/rstb.1996.0135

49. Price RB, Duman R. Neuroplasticity in cognitive and psychological mechanisms of depression: an integrative model. *Mol Psychiatry* (2020) 25:530–43. doi: 10.1038/s41380-019-0615-x

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Risk of cardiac rupture among elderly patients with diabetes presenting with first acute myocardial infarction

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Objective: We aimed to analyze the risk of cardiac rupture (CR) in aged diabetic patients with acute ST-segment elevated myocardial infarction (STEMI) who were followed up for one month, and analyze its independent risk factors.

Methods: A total of 3063 aged patients with first onset STEMI admitted to Beijing Anzhen Hospital from January 2001 to December 2020 were retrospectively included. There were 2020 patients without diabetes mellitus (DM) and 1043 patients with DM. We used propensity scores matching (PSM) method to balance baseline exposure factors between patients with or without DM, and all were divided the DM group (1043 cases) and the non-DM group (1043 cases) after the PSM. The primary outcome was CR (the composite rate of papillary muscle rupture, ventricular septum perforation, free wall rupture), which was diagnosed based on clinical manifestations and/or echocardiographic findings. Kaplan-meier survival analyses and log-rank test was used to evaluate the risk of CR between the two groups, and *Cox* regression analysis was used to evaluate the independent risk factors for CR.

Results: After PSM, the baseline clinical data were similar between the DM and non-DM group (all $P>0.05$). However, level of glycated hemoglobin was significantly higher in the DM group ($P<0.05$). During 1 month of follow-up, there were 55 (2.64%) cases of CR, most occurred within 48h after admission (40 cases). Among the 55 cases, 11(0.53%) had papillary muscle rupture, 18(0.86%) had ventricular septum perforation, and 26(1.25%) had free wall rupture. Kaplan-meier survival analyses detected that the DM group was associated with significantly increased risk of CR (3.36% *vs.* 1.92%, *HR*=1.532, 95% *CI*: 1.054-2.346, $P=0.030$), ventricular septum perforation (1.05% *vs.* 0.67%, *HR*=1.464, 95% *CI*: 1.021-2.099, $P=0.038$) and free wall rupture (1.63% *vs.* 0.86%, *HR*=1.861, 95% *CI*: 1.074-3.225, $P=0.027$) than those in the non-DM group. Among the 2031 aged STEMI patients without CR, 144 cases (6.90%, 144/2086) died; and among the 55 patients with CR, 37 cases (1.77%, 37/2086) died due to CR. Therefore, twenty percent (20.44%, 37/181) of death was due to CR. Multivariate *Cox* regression analysis indicated that DM (*HR*=1.532, 95%*CI*: 1.054-2.346), age (*HR*=1.390, 95%*CI*: 1.079-1.791), female (*HR*=1.183, 95%*CI*: 1.049-1.334), troponin I (*HR*=1.364, 95%*CI*: 1.108-1.679), brain natriuretic peptide (*HR*=1.512, 95%*CI*: 1.069-2.139), revascularization (*HR*=0.827, 95%*CI*: 0.731-0.936) and β-

receptor blocker (*HR*=0.849, 95%*CI*: 0.760-0.948) were independent risk factors of CR (all *P*<0.05).

Conclusion: DM as well as a few other factors, are independent determinants of CR. CR is not a rare event among the aged STEMI patients and twenty percent of deaths are due to CR. However, large sample-sized studies are warranted to confirm these findings.

KEYWORDS

acute ST-segment elevated myocardial infarction, cardiac rupture, advanced age, diabetes, risk factor

Introduction

Cardiac rupture (CR) has become a major clinical problem in patients with acute ST-segment elevated myocardial infarction (STEMI) (1). CR secondary to STEMI is one of the main causes of early mortality, and its in-hospital mortality is more than 50% (2, 3). CR usually manifests as sudden death, and patients without sudden death also involve multiple organ ischemic injury (4). Therefore, CR is a catastrophic complication of STEMI, with an incidence of 1% - 2% (5–7). In the past 40 years, the incidence of CR has generally shown a downward trend. The widespread use of thrombolytic therapy has reduced the mortality by 40% (8). Since the early 1990s, with the development of primary percutaneous coronary intervention (PCI), the short-term and long-term mortality of STEMI has further decreased (9, 10). In the past 20 years, primary PCI has become the preferred reperfusion strategy, and the focus of of STEMI has also shifted from the choice of reperfusion methods to the improvement of medical quality. These progress has also further reduced the incidence of CR (7, 11).

However, the improvement of medical quality in STEMI was not improve the clinical prognosis of patients with CR. Recently, the use of mechanical circulatory support has increased, new interventional therapies have been applied, and surgical techniques have been improved in the treatment of CR, but the mortality remains high (11–13). Recent studies in our center showed that the in-hospital mortality rate of ventricular free wall rupture is still as high as 90% (4).

Whether diabetes affects the risk of CR in elderly STEMI patients remains controversial. The occurrence of CR is affected by many factors, including general conditions, clinical manifestations, laboratory tests and treatment. Old age, female, first myocardial infarction and infarcted myocardial area are risk factors for CR, while reperfusion, early usage of β receptor blockers, angiotensin converting enzyme inhibitor (ACEI)/angiotensin II receptor blocker (ARB) are protective factors of CR. Based on the analysis of 6712 STEMI patients in our research center, we found the neutrophil count ≥ 9×10^9/L was an independent risk factor for ventricular free wall rupture, but DM was not an risk factor for CR. Diabetes is a common complication among elderly STEMI patients, which will significantly increase the risk of adverse cardiovascular events (14). Recently, we retrospectively analyzed a total of 10284 patients with STEMI admitted to Beijing Anzhen Hospital from January 2012 to March 2015. Among them, 81 were diagnosed with CR, including 67 cases of acute left ventricular free wall rupture and 14 cases of ventricular septum perforation. Although patients with CR were associated with higher levels of fasting blood glucose, the incidence rate of DM was similar between the two groups. Logistic regression analysis did not detect any significant relationship between DM and CR, and those with advanced age, recurrent myocardial infarction, low systolic blood pressure, left anterior descending artery disease, decreased hemoglobin, low total protein, and high blood magnesium were prone to have CR(all *P*<0.05) (15). However, patients with diabetes can not only affect wound healing after trauma, but also affect the effect of coronary artery reperfusion, which is strongly related to the occurrence of CR (16). Therefore, whether DM was an independent risk factor of CR remains inconclusive.

We aimed to analyze the risk of CR in aged diabetic patients with STEMI who admitted to Beijing Anzhen Hospital from January 2013 to December 2020 and were followed up for one month, and also evaluate the association between DM and CR.

Methods

Patients

This is a retrospective cohort study. A total of 3063 aged patients with STEMI who were consecutively admitted to Beijing Anzhen Hospital from January 2013 to December 2020 were included. There were 2020 patients without DM and 1043 patients with DM. According to the propensity score matching method, all were divided into the DM group (n=1043) and the non-DM group (n=1043).

Inclusion criteria: (1) aged 60-87 years old; (2) diagnosed as STEMI based on clinical manifestations, myocardial enzymes (CK-MB, cTNI/cTNT) and ECG results (17); (3) had complete clinical data and laboratory examination data; (4) had complete echocardiographic findings performed in-hospital and within one-month follow-up after discharge; (5) with the permission of patient.

Exclusion criteria: (1) had old myocardial infarction; (2) had congenital heart disease before admission; (3) combined with aortic dissection; (4) had severe lung, liver and kidney function failure; (5) had serious infection; (6) had malignant tumor, with expected survival time of less than 1 year; (7) had unclear echocardiographic findings; (8) had incomplete clinical data and/or prognosis.

This study meets the requirements of medical ethics and has been approved by the ethics committee of Beijing Anzhen Hospital (ethics number: KS2022068).

Data collection

We searched the HIS electronic medical record system database of our hospital and extract patients' data recorded within 24h after admission, including basic demograpic information (age, sex, body mass index [BMI], current smoking), past medical history (duration of diabetes, hypertension, hyperlipidemia, transient ischemic attack or stroke, atrial fibrillation, peripheral artery disease, chronic kidney disease), hemodynamic parameters (heart rate and blood pressure at admission), and blood biochemical test performed within 24h after admission (fasting blood glucose [FBG], glycated hemoglobin [HbA1c], hemoglobin, white blood cell [WBC] count, platelet count, serum creatinine, urea nitrogen, brain natriuretic peptide [BNP], cardiac troponin I [TNI]), transthoracic echocardiographic findings (left ventricular ejection fraction [LVEF], left ventricular end-systolic diameter [LVESd], left ventricular end-diastolic diameter [LVEDd]), coronary angiographic findings (left main artery or three branch lesions, Gensini score), revascularization method (thrombolysis, percutaneous coronary intervention, coronary artery bypass grafting) and drug treatment (dual antiplatelet therapy, warfarin, new oral anticoagulant [NOAC], ACEI, ARB, angiotensin receptor neprilysin inhibitor (ARNI), β-receptor blockers, statins).

Gensini score

The patients' coronary artery stenosis was judged according to the Gensini score. All patients underwent quantitative coronary angiography (QCA) or CT angiography coronary angiography (CTA). The results of QCA or CTA were independently analyzed and judged by two professional doctors. The degrees of luminal stenosis <25%, 25%——<50%, 50%——<75%, 75%——<90%, 90%——<99% and 99%——100% were regarded as 1, 2, 4, 8, 16 and 32 points, respectively. Finally, according to the degree of stenosis and the stenosis coefficient, the patient's Gensini score was calculated (18).

Diagnosis of CR

Diagnosis of CR was based on clinical manifestations and/or echocardiographic findings. Papillary muscle rupture was suggested by physical examination with a holo-systolic murmur across the precordium, and a transthoracic echocardiogram revealed severe mitral regurgitation, and a transesophageal echocardiogram detected complete disruption of the mitral leaflet, chordal apparatus, and/or the papillary muscle (19). Ventricular free wall rupture was diagnosed by the presence of echo-signal free space of the free wall or presence of pericardial effusion, and Color Doppler showed blood flow shunt between the ventricle and the pericardium when patients developed sudden onset of electro-mechanic dissociation (characterized by cardiogenic shock, conscious disturbance, and pulseless electric activity) (19). Ventricular septum rupture was suggested by physical examination with strong cardiac murmur and diagnosed by echocardiography with presence of echo signal-free pace of the ventricular septum and Color Doppler showed blood flow signal across the ventricular septum (19).

Main outcome and follow-up

All patients were followed up within one month after admission. The primary outcome was CR (the composite rate of papillary muscle rupture, ventricular septum perforation and free wall rupture), which was diagnosed based on clinical manifestations and/or echocardiographic findings (19).

Propensity score matching

The matching ratio is 1:1, and the caliper value is 0.2. The patients' age, female, smoking, hypertension, chronic renal disease, heart rate, diastolic blood pressure, FBG, hemoglobin, WBC count, TNI, BNP, LVEF, Gensini score, revascularization and ACEI/ARB/ARNI were the covariates, and whether CR occurred was the dependent variable.

Statistical analyses

Descriptive characteristics were summarized as the mean ± standard deviation, medians with interquartile ranges (IQRs), and frequencies with percentages (%), when applicable. Comparisons of continuous variables were analyzed by using t tests, and comparisons of categorical variables were analyzed by using the chi-squared test or Fisher's exact test. The *Cox* proportional hazards regression model was utilized to analyze the relationship between DM and risk of CR. Multiple potential confounders in this study were also considered, including age, female sex, TNI, BNP, and revascularization. Using the log-rank test, the cumulative incidence of CR was computed by using the Kaplan-Meier survival curve.

All of the statistical analyses were performed by using IBM SPSS software (version 23.0, SPSS Inc., Chicago, IL) and associated packages. Two-tailed *P* values <0.05 were considered to be statistically significant.

Results

Baseline clinical data before PSM

As shown in Table 1, before PSM, there were 3063 aged STEMI patients, 1573 were women (51.4%), with an average age of 75.1 ±

9.6 years. There were significant differences between the two groups in age, female ratio, BMI, rates of current smoker, peripheral artery disease, heart rate, systolic blood pressure, diastolic blod pressure, fasting blood glucose, HbA1c, hemoglobin, serum creatinine, TNI, BNP, Gensini score, LVEF, LVESd, LVEDd, revascularization rate and the application rate of NOAC, β-receptor blocker, ACEI/ARB/ARNI and statin (all $P<0.05$). Other data were similar between the two groups, and the differences were not statistically significant ($P>0.05$).

Baseline clinical data after PSM

As shown in Table 2, after PSM, there are 2086 aged STEMI patients, including 1006 females (48.2%), with an average age of 74.8 ± 8.7 years. The baseline clinical data were similar between the DM and non-DM group (all $P>0.05$). However, level of HbA1c was significantly higher in the DM group compared with that in the non-DM group ($P<0.05$).

Rate of CR during follow-up

There were 55 (2.64%) aged STEMI patients with CR, most (40 cases, 72.7%) occurred within 48h after admission, and the median occurrence time was 24h after admission (5h, 96h).

Among the 55 cases of CR, there were 11 cases (0.53%) of papillary muscle rupture, 18 cases (0.86%) of ventricular septal perforation, and 26 cases (1.25%) of ventricular free wall rupture (Table 3).

Kaplan-meier survival analysis and log-rank test results showed that the incidence of overall CR (3.36% *vs.* 1.92%, *HR*=1.532, 95% *CI*: 1.054-2.346, $P=0.030$) (Figure 1) in the DM group was significantly higher than that in the non-DM group. Additionally,

TABLE 1 Baseline clinical data between the two groups before PSM.

Characteristic	Non-DM group (n=2020)	DM group (n=1043)	t/x^2 value	*P* value
Age(year)	75.3 ± 6.5	74.7 ± 8.8	2.137	0.033
Female(%)	1068(38.9%)	505(48.4%)	5.460	0.019
Body mass index(kg/m^2)	25.1 ± 3.7	25.5 ± 4.8	2.554	0.011
Current smoker(%)	852(34.2%)	398(38.2%)	4.599	0.032
Duration of diabetes(year)	–	12.1 ± 5.6	–	
Hypertension(%)	531(23.9%)	277(26.6%)	0.026	0.872
Hyperlipidemia(%)	728(37.7%)	349(33.5%)	2.006	0.157
TIA/stroke(%)	156(11.8%)	90(8.6%)	0.765	0.382
Atrial fibrillation(%)	301(14.5%)	139(13.3%)	1.385	0.239
Peripheral artery disease(%)	236(12.4%)	150(14.4%)	4.547	0.033
Chronic kidney disease(%)	286(13.9%)	158(15.1%)	0.544	0.461
Heart rate(bpm)	85.3 ± 10.1	82.7 ± 12.4	6.235	<0.001
Systolic blood pressure(mmHg)	110.7 ± 9.3	113.3 ± 11.4	6.776	<0.001
Didastolic blood pressure(mmHg)	71.4 ± 8.6	72.4 ± 9.5	2.941	0.003
Fasting blood glucose(mmol/L)	6.94 ± 1.62	7.98 ± 3.05	12.325	<0.001
HbA1c(%)	5.42 ± 0.76	6.91 ± 1.40	38.169	<0.001
Hemoglobin(g/dL)	11.0 ± 1.7	11.2 ± 2.5	2.612	0.009
White blood cell(×10^9/L)	8.94 ± 2.81	10.5 ± 2.9	14.401	<0.001
Platelet count(×10^9/L)	225.4 ± 28.7	222.8 ± 30.5	2.325	0.020
Serum creatinine(mg/dL)	1.31 ± 0.23	1.37 ± 0.38	5.428	<0.001
Troponin I(ng/ml)	12.7 ± 4.3	14.1 ± 6.0	8.645	<0.001
Brain natriuretic peptide(mmol/L)	360.4 ± 55.4	371.2 ± 95.1	3.965	<0.001
Left main/Three artery(%)	279(12.4%)	149(14.3%)	0.129	0.720

(Continued)

TABLE 1 Continued

Characteristic	Non-DM group (n=2020)	DM group (n=1043)	t/x^2 value	*P* value
Gensini score	75.2 ± 20.1	76.0 ± 22.5	1.002	0.317
Left ventricular ejection fraction(%)	50.5 ± 4.8	48.5 ± 6.8	9.431	<0.001
LV end-systolic diameter(mm)	38.0 ± 6.0	38.6 ± 7.1	2.460	0.014
LV end-diastolic diameter(mm)	48.7 ± 5.3	49.4 ± 6.4	3.222	0.001
Revascularization(%)	1415(73.4%)	785(75.3%)	9.241	0.002
Dual antiplatelet therapy(%)	1064(52.1%)	512(49.1%)	3.537	0.060
Wafarin(%)	271(13.4%)	150(14.4%)	0.541	0.462
NOAC(%)	116(5.0%)	87(8.3%)	7.507	0.006
β-receptor blocker(%)	340(18.4%)	126(12.1%)	12.037	0.001
ACEI/ARB/ARNI(%)	281(23.6%)	176(16.9%)	4.759	0.029
Statin(%)	817(44.7%)	507(48.6%)	18.682	<0.001

TIA, transient ischemic stroke; HbA1c, glycated hemoglobin; NOAC, new oral anticoagulant; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor.

TABLE 2 Baseline clinical data between the two groups after PSM.

Characteristic	Non-DM group (n=1043)	DM group (n=1043)	t/x^2 value	*P* value
Age(year)	74.9 ± 8.4	74.7 ± 8.8	0.531	0.596
Female(%)	501(48.0%)	505(48.4%)	0.031	0.861
Body mass index(kg/m^2)	25.4 ± 4.7	25.5 ± 4.8	0.481	0.631
Current smoker(%)	392(37.6%)	398(38.2%)	0.073	0.787
Duration of diabetes(year)	–	12.1 ± 5.6	–	
Hypertension(%)	263(25.2%)	277(26.6%)	0.489	0.484
Hyperlipidemia(%)	346(33.2%)	349(33.5%)	0.019	0.889
TIA/stroke(%)	83(8.0%)	90(8.6%)	0.310	0.578
Atrial fibrillation(%)	131(12.6%)	139(13.3%)	0.272	0.602
Peripheral artery disease(%)	122(11.7%)	150(14.4%)	0.289	0.591
Chronic kidney disease(%)	147(14.1%)	158(15.1%)	0.465	0.495
Heart rate(bpm)	82.4 ± 11.3	82.7 ± 12.4	0.578	0.564
Systolic blood pressure(mmHg)	112.5 ± 12.7	113.3 ± 11.4	1.514	0.130
Didastolic blood pressure(mmHg)	72.0 ± 10.3	72.4 ± 9.5	0.922	0.357
Fasting blood glucose(mmol/L)	7.91 ± 2.89	7.98 ± 3.05	0.538	0.591
HbA1c(%)	5.23 ± 0.68	6.91 ± 1.40	34.861	<0.001
Hemoglobin(g/dL)	11.0 ± 2.8	11.2 ± 2.5	1.721	0.085
White blood cell(×10^9/L)	10.7 ± 2.4	10.5 ± 2.9	1.716	0.086
Platelet count(×10^9/L)	221.5 ± 34.7	222.8 ± 30.5	0.909	0.364
Serum creatinine(mg/dL)	1.34 ± 0.45	1.37 ± 0.38	1.645	0.101
Troponin I(ng/ml)	13.7 ± 5.5	14.1 ± 6.0	1.738	0.082
Brain natriuretic peptide(mmol/L)	364.2 ± 75.3	371.2 ± 95.1	1.864	0.063

(Continued)

TABLE 2 Continued

Characteristic	Non-DM group (n=1043)	DM group (n=1043)	t/x^2 value	*P* value
Left main/Three artery(%)	140(13.4%)	149(14.3%)	0.325	0.568
Gensini score	75.2 ± 20.7	76.0 ± 22.5	0.845	0.398
Left ventricular ejection fraction(%)	49.0 ± 5.5	48.5 ± 6.8	1.846	0.065
LV end-systolic diameter(mm)	38.1 ± 6.3	38.6 ± 7.1	1.701	0.089
LV end-diastolic diameter(mm)	49.0 ± 5.9	49.4 ± 6.4	1.477	0.139
Revascularization(%)	779(74.7%)	785(75.3%)	0.092	0.762
Dual antiplatelet therapy(%)	504(48.3%)	512(49.1%)	0.123	0.726
Wafarin(%)	141(13.5%)	150(14.4%)	0.324	0.570
NOAC(%)	95(9.1%)	87(8.3%)	0.385	0.535
β-receptor blocker(%)	151(14.5%)	126(12.1%)	1.647	0.199
ACEI/ARB/ARNI(%)	163(15.6%)	176(16.9%)	0.595	0.440
Statin(%)	494(47.4%)	507(48.6%)	0.325	0.569

TIA, transient ischemic stroke; HbA1c, glycated hemoglobin; NOAC, new oral anticoagulant; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor.

the incidences of ventricular septum perforation (1.05% *vs.* 0.67%, *HR*=1.464, 95% *CI*: 1.021-2.099, *P*=0.038) and free wall rupture (1.63% *vs.* 0.86%, *HR*=1.861, 95% *CI*: 1.074-3.225, *P*=0.027) were significantly increased in the DM group than those in the non-DM. However, the incidence of papillary muscle rupture were similar between the two groups (0.67% *vs.* 0.38%, *HR*=1.315, 95% *CI*: 0.982-1.761, *P*=0.066).

Survival of patients with STEMI

Among the 2031 aged STEMI patients without CR, 144 cases (6.90%, 144/2086) died; and among the 55 patients with CR, 37 cases (1.77%, 37/2086) died due to CR. Therefore, twenty percent (20.44%, 37/181) of death was due to CR. Of the 11 patients with papillary muscle rupture, 4 cases were completely ruptured, complicated with acute left heart failure, and died after rescue. The remaining 7 cases underwent valve and chordae tendineae repair, and survived after 1 month follow-up. Meanwhile, of the 18 patients with ventricular septum perforation, 5 died due to cardiogenic shock; the remaining 13 patients underwent surgical repair. However, 10 survived and 3 died after operation. Of the 26 patients with free wall rupture, only 1 patient underwent surgery and survived, and the remaining 25 patients died. Therefore, among the 55 patients with CR, 37 cases (67.3%) died after a follow-up of 1 month, most of them had free wall rupture (25 cases) or ventricular septum perforation (8 cases). Therefore, among the 55 cases of CR, only 8 cases (32.7%) survived, and all received cardiac surgery.

Risk factors of CR

The results of univariate cox regression analysis (Table 4) showed that diabetes, age, female, chronic renal disease, heart rate, fasting blood glucose, HbA1c, TNI, WBC count, BNP, serum creatinine, revascularization, β-receptor blocker, ACEI/ARB/ARNI were important risk factors of CR (*P*<0.05).

Discussion

At present, although the incidence of CR is significantly reduced, it is still an important cause of death in elderly patients with STEMI. In our study, among the aged patients with STEMI, 55 (2.64%) had CR, which was slightly higher than previous studies. It may be related to the aged patients included in this study. In

TABLE 3 Rate of CR between the two groups after PSM.

prognosis	Non-DM group (n=1043)	DM group (n=1043)	*Log-rank* x^2 value	*P* value
Papillary muscle rupture(%)	4(0.38%)	7(0.67%)	1.638	0.066
Ventricular septum perforation(%)	7(0.67%)	11(1.05%)	2.073	0.038
Free wall rupture(%)	9(0.86%)	17(1.63%)	2.285	0.027
All events (%)	20(1.92%)	35(3.36%)	2.617	0.030

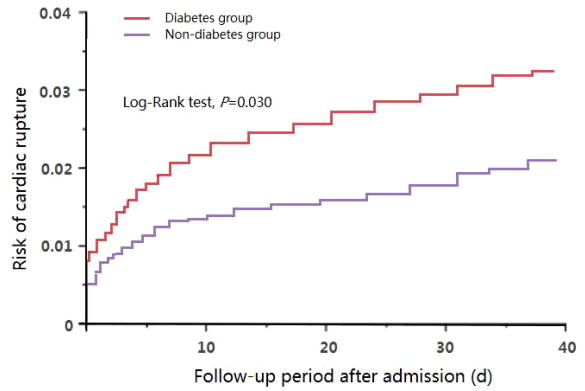

FIGURE 1
Rate of CRbetween the two groups.

addition, previous epidemiological studies have detected that CR usually occurred within one week, mostly within 3-5 days after STEMI. Similarly, our study also confirmed that in aged patients with STEMI, CR mostly occurred within 48h after admission. Therefore, in clinical practice, we should also pay attention to the high risk of CR in aged patients with STEMI.

Whether DM was an independent risk factor of CR has been controversial (11, 12). In the APEX-STEMI study, 5745 patients with STEMI were included, with a median age of 68 years. The researchers found 52 cases (0.91%) of CR during follow-up, including 30 cases (0.52%) of free wall rupture, 15 cases (0.26%) of papillary muscle rupture, and 10 cases (0.17%) of ventricular septum perforation. However, they found that there was no significant difference in the incidence of DM betwee patients with or without CR (P>0.05). Additionally, multivariate *logistic* regression analyses did not detect any significant association between DM and the risk of CR (P>0.05) (13). However, in another study which enrolled 148881 aged patients (67-71 years) with first-onset STEMI, all were divided into the CR group (n=408, with free wall rupture) and the control group (n=148473, without CR). They found that the occurrence of DM history was significantly lower than that in the control group (18% *vs.* 24%), and DM was a protective factor of ventricular free wall rupture (*OR*=0.67,95%*CI*: 0.52-0.87) (14). Meanwhile, for patients with papillary muscle rupture or ventricular septum perforation, diabetes is more rare (15, 16), and several published studies did not detect the association between DM and the risk of papillary muscle rupture or ventricular septum perforation (P>0.05). Therefore, Whether DM was a risk factor of CR in aged patients with STEMI is still inconclusive.

We confirmed that DM was associated with increased risk of CR. In our study, 2086 aged patients with first-onset STEMI were included, and 55 patients (2.64%) had CR during 1 month follow-up. We found that DM was associated with significantly increased risk of CR (3.36% *vs.* 1.92%, *HR*=1.532, 95% *CI*: 1.054-2.346, P=0.030). Furthermore, the risk of ventricular septum perforation (1.05% *vs.* 0.67%, *HR*=1.464, 95% *CI*: 1.021-2.099, P=0.038) and free wall rupture (1.63% *vs.* 0.86%, *HR*=1.861, 95% *CI*: 1.074-3.225, P=0.027) were also marked higher in the DM group than those in

TABLE 4 Univariate and multivariate Cox regression analyses.

Factors	Univariate			Multivariate		
	HR	95%CI	P value	HR	95%CI	P value
Age	1.432	1.105-1.856	0.007	1.390	1.079-1.791	0.011
Female	1.219	1.063-1.398	0.005	1.183	1.049-1.334	0.006
Chronic kidney disease	1.464	1.093-1.961	0.011	–		
Heart rate	1.218	1.052-1.410	0.008	–		
Fasting blood glucose	1.317	1.061-1.635	0.013	–		
HbA1c	1.255	1.037-1.519	0.020	–		
Cardiac troponin I	1.416	1.142-1.756	0.002	1.364	1.108-1.679	0.003
White blood cell	1.303	1.033-1.644	0.026	–		
Brain natriuretic peptide	1.615	1.108-2.354	0.013	1.512	1.069-2.139	0.019
Serum creatinine	1.481	1.016-2.159	0.041	–		
Revascularization	0.794	0.664-0.950	0.016	0.827	0.731-0.936	0.003
β-receptor blocker(%)	0.834	0.712-0.977	0.024	0.849	0.760-0.948	0.004
ACEI/ARB/ARNI(%)	0.947	0.905-0.991	0.019	–		
Diabetes	1.482	1.079-2.036	0.015	1.532	1.054-2.346	0.030

HbA1c, glycated hemoglobin; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ARNI, angiotensin receptor neprilysin inhibitor.
Multivariate Cox regression analyses (Table 4) detected that DM (HR=1.532, 95%CI: 1.054-2.346), age (HR=1.390, 95%CI: 1.079-1.791), female (HR=1.183, 95%CI: 1.049-1.334), TNI (HR=1.364, 95%CI: 1.108-1.679), BNP (HR=1.512, 95%CI: 1.069-2.139), revascularization (HR=0.827, 95%CI: 0.731-0.936), β-receptor blocker (HR=0.849, 95%CI: 0.760-0.948) were independent risk factors of CR (all P<0.05).

the non-DM group. However, the incidence of papillary muscle rupture were similar between the two groups (0.67% *vs.* 0.38%, *HR*=1.315, 95% *CI*: 0.982-1.761, *P*=0.066). Therefore, DM as well as a few other factors, were independent determinants of CR.

There were several strengths in our study. First, our study's sample size was relatively large. We enrolled 3063 aged patients with STEMI who were consecutively admitted to our hospital from January 2013 to December 2020. There were 2020 patients without DM (non-DM group) and 1043 patients with DM (DM group). Second, the baseline clinical data was comparable between those with or wihout DM. Previously, there was no study to specifically compare the risk of CR between the DM and non-DM groups, and there were significant differences in baseline clinical data between those with or without DM. In our study, using the PSM method, patients were divided into the DM group (n=1043) and the non-DM group (n=1043), and the baseline clinical data were almost all comparable between the DM and non-DM group, except the level of HbA1c. Third, only aged patients with first-onset STEMI were included, and those were the high-risk patients for developing CR. Fourth, all patients were followed up for 1 month after admission. Most previous studies only reported the risk of CR occurred in-hospital and many CR cases would be missed. However, large sample-sized studies are warranted to confirm these findings.

Diabetes involved multiple cardiac repair mechanisms after STEMI. Myocardial repair consisted of several stages: (1) early repair stage (within 72h in the early stage); (2) late repair and proliferation stage (within 72h-10d in the early stage); (3) maturation of proliferative tissue and ventricular remodeling stage (lasting for several months) (19, 20). CR mainly occurred in the early and late repair stage, that is, within 7d after the occurrence of STEMI (21). In the first 72h after MI, the myocardial cells in the infarct focus die, leading to the myocardial thinning in this area. At the same time, the release of dead cells in the infarct focus and the lysis of extracellular matrix could generate a large number of risk related molecular signals, which can be captured by the pattern recognition receptor on the immune cells, thus causing the activation of immune cells and infiltration into the infarct focus (22). The infiltrating immune cells not only engulf the cell debris in the infarcted area, but also release various enzymes, further degrading the extracellular matrix and making the myocardium more fragile, and eventually leading to myocardial damage (23). This aseptic inflammatory process can be well controlled by subsequent anti-inflammatory and proliferative processes, and recent studies have found that this processes were closely related to a class of specialized proresolving mediators (SPMs) (24–26). Previous studies have reported that SPM can inhibit the infiltration of pro-inflammatory polylobulated neutrophils into tissues, promote macrophage differentiation towards an anti-inflammatory phenotype, and enhance their ability to phagocytose apoptotic neutrophils. In addition, it had the function of regulating the transition of T cells from an activated state to a regulated state (27). However, in patients with DM, serum SPM and SPM related synthase 15-LOX-1 were significantly lower. In addition, among the DM patients, the inflammatory digestion process in the infarct focus was damaged, which led to the expansion of the ischemic focus (28). Moreover, the content of pro-inflammatory M1 phenotype macrophages was significantly increased in DM patients, and further increased the risk of CR development (29–31).

Many other factors were also associated with the occurrence of CR. Previous studies confirmed that age, medical history, clinical manifestations, biochemical test findings, and treatment (32–34). Qian et al. (34) designed a clinical risk scoring system for predicting CR in STEMI patients. Seven factors were associated with the risk of CR, including age, gender, heart rate, myocardial infarction site, hemoglobin count, white blood cell count, and admission time. Meanwhile, early use of ACEI/ARB and β-receptor blockers could help prevent cardiac rupture. Revascularization, especially the primary PCI should be carried out as soon as possible to minimize the occurrence of CR (35, 36). Similar to previous findings, we found that age (HR=1.390, 95%CI: 1.079-1.791), female (HR=1.183, 95%CI: 1.049-1.334), troponin I (HR=1.364, 95%CI: 1.108-1.679), BNP (HR=1.512, 95%CI: 1.069-2.139), revascularization (HR=0.827, 95%CI: 0.731-0.936) and β-receptor blocker (HR=0.849, 95%CI: 0.760-0.948) were also strongly related to the occurrence of CR (all $P<0.05$). Therefore, considering the extremely high risk of mortality with CR, it is necessary to further confirm its predictive factors, in order to prevent and interven early and timely (37, 38).

Limitations

There are several limitations in our study. (1) It was conducted in a single center, and the patients's characteristics maybe different from other centers. (2) Our study's sample size was not large enough. The risk of CR is relatively low. Therefore, larger sample-sized studies are warranted to confirm the association between DM and CR. (3) CR was diagnosed by echocardiography. However, echocardiography examination is operator dependent, and several patients' follow-up visits were conducted in non-teaching hospital. Therefore, some CR cases might be missed. (4) The follow-up period was relatively short. Meanwhile, follow-up mainly relied on outpatient services, and some therapeutic information could have been missed, which might affect the incidence of CR (39). Therefore, high qualified studies are needed to confirm these findings.

Conclusions

DM as well as a few other factors, are independent determinants of CR. CR is not a rare event among the aged STEMI patients. Meanwhile, twenty percent (20.44%, 37/181) of deaths are due to CR. However, large sample-sized studies are warranted to confirm these findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Beijing Anzhen Hospital Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because This was a retrospective study.

Author contributions

XZ conducted this study and wrote the paper. YJ, YZ and PL analyzed the data. HG designed this study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. National Center for Cardiovascular Disease. *China cardiovascular health and disease report 2021[M]*. Beijing: Science Press (2022).

2. Figueras J, Alcalde O, Barrabés JA, Serra V, Alguersuari J, Cortadellas J, et al. Changes in hospital mortality rates in 425 patients with acute ST-elevation myocardial infarction and CRover a 30-year period. *Circulation* (2008) 118(25):2783–9. doi: 10.1161/CIRCULATIONAHA.108.776690

3. López-Sendón J, Gurfinkel EP, Lopez de Sa E, Agnelli G, Gore JM, Steg PG, et al. Factors related to heart rupture in acute coronary syndromes in the Global Registry of Acute Coronary Events. *Eur Heart J* (2010) 31(12):1449–56. doi: 10.1093/eurheartj/ehq061

4. Gong W, Shi H, Yan M, Yan Y, Wang X, Li S, et al. Clinical manifestation, timing course, precipitating factors, and protective factors of ventricular free wall rupture following ST-segment elevation myocardial infarction. *Int Heart J* (2020) 61(4):651–7. doi: 10.1536/ihj.19-541

5. Gao XM, White DA, Dart AM, Du XJ. Post-infarct cardiac rupture: recent insights on pathogenesis and therapeutic interventions. *Pharmacol Ther* (2012) 134(2):156–79. doi: 10.1016/j.pharmthera.2011.12.010

6. Damluji AA, van Diepen S, Katz JN, Menon V, Tamis-Holland JE, Bakitas M, et al. Mechanical complications of acute myocardial infarction: A scientific statement from the American Heart Association. *Circulation* (2021) 144(2):e16–35. doi: 10.1161/CIR.0000000000000985

7. Honda S, Asaumi Y, Yamane T, Nagai T, Miyagi T, Noguchi T, et al. Trends in the clinical and pathological characteristics of CRin patients with acute myocardial infarction over 35 years. *J Am Heart Assoc* (2014) 3(5):e000984. doi: 10.1161/JAHA.114.000984

8. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2 [J]. *Lancet* (1988) 2(8607):349–60. doi: 10.1016/S0140-6736(88)92833-4

9. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. *Lancet* (2003) 361(9351):13–20. doi: 10.1016/S0140-6736(03)12113-7

10. Grines CL, Browne KF, Marco J, Rothbaum D, Stone GW, O'Keefe J, et al. A comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction. The Primary Angioplasty in Myocardial Infarction Study Group. *N Engl J Med* (1993) 328(10):673–9. doi: 10.1056/NEJM199303113281001

11. Puerto E, Viana-Tejedor A, Martínez-Sellés M, Domínguez-Pérez L, Moreno G, Martín-Asenjo R, et al. Temporal trends in mechanical complications of acute myocardial infarction in the elderly. *J Am Coll Cardiol* (2018) 72(9):959–66. doi: 10.1016/j.jacc.2018.06.031

12. Goldsweig AM, Wang Y, Forrest JK, Cleman MW, Minges KE, Mangi AA, et al. Ventricular septal rupture complicating acute myocardial infarction: incidence, treatment, and outcomes among medicare beneficiaries 1999-2014. *Catheter Cardiovasc Interv* (2018) 92(6):1104–15. doi: 10.1002/ccd.27576

13. Jones BM, Kapadia SR, Smedira NG, Robich M, Tuzcu EM, Menon V, et al. Ventricular septal rupture complicating acute myocardial infarction: a contemporary review. *Eur Heart J* (2014) 35(31):2060–8. doi: 10.1093/eurheartj/ehu248

14. Fu W, Dong R, Zheng JB, Zhang K, Mu JS. Risk factors of early death and long-term outcomes in myocardial infarction complicated with ventricular septal rupture. *Chin J Geriatr* (2022) 41(5):517–22. doi: 10.3760/cma.j.issn.0254-9026.2022.05.003

15. Liu ZJ, Miao HT, Nie SP. Risk factors of CRin patients with acute myocardial infarction. *Chin J Cardiol* (2016) 44(10):862–7. doi: 10.3760/cma.j.issn.0253-3758.2016.10.007

16. Miao HT, Zhang M, Liu ZJ, Cheng J, Chen ZS, Nie SP. Clinical characteristics and prognosis of patients with acute myocardial infarction complicated with different parts of heart rupture. *Chin Crit Care Med* (2016) 28(12):1080–5. doi: 10.3760/cma.j.issn.2095-4352.2016.12.003

17. Emergency Medical Branch of Chinese Medical Doctor Association, Cardiovascular Epidemiology Branch of Chinese Medical Association and Laboratory Medicine Branch of Chinese Medical Association. Emergency rapid diagnosis and treatment of guidelines acute coronary syndrome. *Chin J Emerg Med* (2016) 25(4):397–404. doi: 10.3760/cma.j.issn.1671-0282.2016.04.002

18. Rampidis GP, Benetos G, Benz DC, Giannopoulos AA, Buechel RR. A guide for Gensini Score calculation. *Atherosclerosis* (2019) 287:181–3. doi: 10.1016/j.atherosclerosis.2019.05.012

19. Lu Q, Liu P, Huo JH, Wang YN, Ma AQ, Yuan ZY, et al. Cardiac rupture complicating acute myocardial infarction: the clinical features from an observational study and animal experiment. *BMC Cardiovasc Disord* (2020) 20(1):409. doi: 10.1186/s12872-020-01683-y

20. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ Res* (2016) 119(1):91–112. doi: 10.1161/circresaha.116.303577

21. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation* (2000) 101(25):2981–8. doi: 10.1161/01.cir.101.25.2981

22. Gong FF, Vaitenas I, Malaisrie SC, Maganti K. Mechanical complications of acute myocardial infarction: A review. *JAMA Cardiol* (2021) 6(3):341–9. doi: 10.1001/jamacardio.2020.3690

23. Timmers L, Pasterkamp G, de Hoog VC, et al. The innate immune response in reperfused myocardium. *Cardiovasc Res* (2012) 94(2):276–83. doi: 10.1093/cvr/cvs018

24. Hess A, Borchert T, Ross TL, et al. Characterizing the transition from immune response to tissue repair after myocardial infarction by multiparametric imaging. *Basic Res Cardiol* (2022) 117(1):14. doi: 10.1007/s00395-022-00922-x

25. Fosshaug LE, Colas RA, Anstensrud AK, Gregersen I, Nymo S, Sagen EL, et al. Early increase of specialized pro-resolving lipid mediators in patients with ST-elevation

myocardial infarction. *EBioMedicine* (2019) 46:264–73. doi: 10.1016/j.ebiom.2019.07.024

26. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfSTEMIly of mediators[J]. *J Clin Invest* (2018) 128(7):2657–69. doi: 10.1172/jci97943

27. Chiurchiù V, Leuti A, Dalli J, Jacobsson A, Battistini L, Maccarrone M, et al. Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. *Sci Transl Med* (2016) 8(353):353ra111. doi: 10.1126/scitranslmed.aaf7483

28. Tang X, Liu L, Miao Z, Zhang J, Cai X, Zhao BQ, et al. Resolution of inflammation is disturbed in acute ischemic stroke with diabetes mellitus and rescued by resolvin D2 treatment. *Free Radic Biol Med* (2022) 188:194–205. doi: 10.1016/j.freeradbiomed.2022.06.231

29. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des* (2008) 14 (12):1225–30. doi: 10.2174/138161208784246153

30. Okonkwo UA, DiPietro LA. Diabetes and wound angiogenesis. *Int J Mol Sci* (2017) 18(7):1419. doi: 10.3390/ijms18071419

31. Sawaya AP, Stone RC, Brooks SR, Pastar I, Jozic I, Hasneen K, et al. Deregulated immune cell recruitment orchestrated by FOXM1 impairs human diabetic wound healing. *Nat Commun* (2020) 11(1):4678. doi: 10.1038/s41467-020-18276-0

32. Al-Masawa ME, Alshawsh MA, Ng CY, Ng AMH, Foo JB, Vijakumaran U, et al. Efficacy and safety of small extracellular vesicle interventions in wound healing and skin regeneration: A systematic review and meta-analysis of animal studies. *Theranostics* (2022) 12(15):6455–508. doi: 10.7150/thno.73436

33. Oualha D, Ben Abderrahim S, Ben Abdeljelil N, BelHadj M, Ben Jomâa S, Saadi S, et al. CRduring acute myocardial infarction : Autopsy study (2004-2020). *Ann Cardiol Angeiol (Paris)* (2023) 72(3):101601. doi: 10.1016/j.ancard.2023.101601

34. Qian G, Jin RJ, Fu ZH, Yang YQ, Su HL, Dong W, et al. Development and validation of clinical risk score to predict the CRin patients with STEMI. *Am J Emerg Med* (2017) 35(4):589–93. doi: 10.1016/j.ajem.2016.12.033

35. David TE. Post-infarction ventricular septal rupture. *Ann Cardiothorac Surg* (2022) 11(3):261–7. doi: 10.21037/acs-2021-STEMI-111

36. Simsek B, Kostantinis S, Karacsonyi J, Brilakis ES. Can we predict CRin patients with ST-segment elevation myocardial infarction? *J Thorac Dis* (2022) 14(7):2451–3. doi: 10.21037/jtd-22-655

37. De Lazzari M, Cipriani A, Cecere A, Niero A, De Gaspari M, Giorgi B, et al. CRin acute myocardial infarction: a cardiac magnetic resonance study. *Eur Heart J Cardiovasc Imaging* (2023), jead088. doi: 10.1093/ehjci/jead088

38. Xu Z, Li Y, Zhang R, Liu Y, Liu H, Yu J, et al. Risk factors for CRafter acute ST-segment elevation myocardial infarction during the percutaneous coronary intervention era: a retrospective case-control study. *J Thorac Dis* (2022) 14(4):1256–66. doi: 10.21037/jtd-22-394

39. Yousef S, Sultan I, VonVille HM, Kahru K, Arnaoutakis GJ. Surgical management for CRof acute myocardial infarction: a systematic review of long-term outcomes. *Ann Cardiothorac Surg* (2022) 11(3):239–51. doi: 10.21037/acs-2021-STEMI-20

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Serum uric acid levels and risk of cardiovascular disease in type 2 diabetes: results from a cross-sectional study and Mendelian randomization analysis

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Aims: Serum uric acid (SUA) levels have been previously linked to a higher risk of cardiovascular disease (CVD) in individuals with type 2 diabetes (T2D) according to various observational studies. However, whether this association is causally linked or simply influenced by confounding factors is unclear. Therefore, this study utilized Mendelian randomization (MR) analysis to explore the causality between SUA levels and the risk of CVD in individuals with T2D.

Methods: Our study cohort consisted of 5723 participants who were diagnosed with T2D in the National Health and Nutrition Examination Survey (NHANES) from 1999-2018. The study assessed the association between SUA levels and the risk of CVD using a multivariable logistic regression model. To further examine causality between SUA levels and CVD, a two-sample MR study was conducted utilizing genetic data from genome-wide association studies (GWAS) involving over 140,000 individuals. The main MR analysis employed the inverse-variance-weighted (IVW) method. Additionally, several sensitivity analyses were performed to evaluate the robustness and pleiotropy of the results.

Results: In the cross-sectional study, after multivariable adjustment, participants with SUA levels >6.7 mg/dL exhibited odds ratios (ORs) of 1.51 (95% CI: 1.01-2.26, p=0.049) for heart failure, 1.02 (95% CI: 0.69-1.50, p=0.937) for coronary heart disease, 1.36 (95% CI: 0.78-2.38, p=0.285) for angina, and 1.22 (95% CI: 0.80-1.85, p=0.355) for myocardial infarction when compared to participants with SUA levels ≤ 4.6 mg/dL. However, in the IVW analysis, no causality between SUA levels and the risk of heart failure was observed (OR = 1.03, 95% CI: 0.97-1.09, p =

0.293). The secondary analysis yielded similar results (OR = 1.05, 95% CI: 0.96-1.14, p = 0.299). The sensitivity analyses further supported our primary findings.

Conclusion: Based on the MR study, we did not find supporting evidence for a causal association between SUA levels and the risk of heart failure.

KEYWORDS

T2D, serum uric acid, cardiovascular disease, Mendelian randomization, heart failure

1 Introduction

Diabetes mellitus affects approximately one in every 10.5 adults globally, with an estimated 90% of cases being T2D. This makes it a critical public health challenge of the 21st century (1, 2). T2D is typically associated with metabolic disorders, progressive insulin resistance and hyperglycemia (3). Prolonged hyperglycemia significantly increases the risk of microvascular and macrovascular complications, potentially resulting in premature mortality or disability (4, 5). Cardiovascular complications account for the most frequently occurring adverse events and remain the primary cause of mortality in this population (6). Up to 50% of patients may succumb to such complications, underscoring the need for robust prevention of CVD in T2D (7). Unfortunately, the prevention and treatment of CVD among individuals with diabetes may not present an optimistic outlook. Some large prospective cohort studies suggest that tight glucose control may not effectively mitigate the risk of cardiovascular complications and mortality (8). Even some hypoglycemic drugs may increase the risk of CVD (9). Therefore, it is crucial to identify effective intervention factors that can significantly reduce the risk of CVD. Uric acid, which remains a highly debated variable, has captured the attention of researchers.

Uric acid is considered a biologically inactive waste product resulting from purine metabolism, and it was identified as the cause of gout (10). Many observational studies have established a notable association between SUA levels and several cardiovascular conditions, leading to uric acid being recognized as a potential risk factor for CVD (11, 12). Nonetheless, the associations remain controversial. Some experts have contended that SUA may not be a substantial risk factor for CVD (13). One problem with determining the link between SUA and CVD is that elevated levels of SUA often coincide with identified cardiovascular risk factors, including hypertension, metabolic syndrome, and renal disease. Given that cardiovascular risk factors tend to be more prevalent in individuals with T2D, this population may be more susceptible to developing hyperuricemia due to hyperinsulinemia and chronic kidney disease (CKD) (14). There appears to be an accelerated progression of CVD in individuals with T2D, so it is particularly important to investigate the relationship between SUA levels and CVD risk in T2D. However, due to the limited research specifically addressing this issue in individuals with T2D, further studies are necessary to comprehensively understand the association between SUA levels and CVD in this specific group.

To assess the impact of SUA levels on CVD risk in individuals with T2D, we conducted an initial cross-sectional study and selected 5723 participants with T2D in the NHANES, which provides a large and nationally representative dataset that encompasses the U.S. population. Furthermore, we employed MR analysis to evaluate the causal correlation between SUA levels and the risk of CVD. MR is a statistical method that leverages genetic variants strongly associated with the exposure of interest to estimate a nonconfounded causal association between that exposure and an outcome (15). One of the key advantages of MR analysis is its ability to mitigate biases arising from reverse causality and uncontrolled confounders, since genetic variants are stable over time and are not susceptible to influence from environmental or other factors (16).

2 Materials and methods

2.1 Research design

The NHANES program is conducted in the U.S. by the National Center for Health Statistics of the Centers for Disease Control and Prevention. It originated in the early 1960s when a series of surveys were initiated to examine various health topics (17). In 1999, the survey transitioned into a continuous program, which adapted its focus to address emerging health and nutrition needs. An important characteristic of the NHANES program is its integration of both interviews and physical examinations. The interview component takes place in participants' homes, followed by a standardized physical examination and laboratory tests (18). The comprehensive methodology for data collection and the data in our study are publicly available on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/default.aspx).

In this cohort study, we analyzed data from participants aged 18 or older with T2D from 10 cycles of NHANES from 1999-2018. Diabetes was defined based on self-reported physician diagnosis. Out of the initial 6699 participants who met the diagnostic criteria

Abbreviations: SUA, serum uric acid; T2D, type 2 Diabetes; CVD, cardiovascular disease; NHANES, National Health and Nutrition Examination Survey; MR, Mendelian randomization; OR, odds ratio; GWAS, genome-wide association studies; IVW, inverse-variance-weighted; T1D, type 1 Diabetes; CKD, chronic kidney disease.

for diabetes, we excluded individuals who had type 1 diabetes (n=17) and excluded individuals who had missing data on SUA, BMI, HbA1c levels, and age at diagnosis (n=959). Ultimately, 5723 participants were included in our study.

2.2 Exposure serum uric acid measurement

SUA measurements were obtained from different laboratory instruments across multiple NHANES cycles. In NHANES 1999-2000, SUA was measured using a Hitachi Model 704 multichannel analyser. In NHANES 2001-2007, a Beckman Synchron LX20 instrument was used for SUA measurements. In NHANES 2008-2016, a Beckman UniCel DxC800 Synchron analyser was used for SUA measurements. Finally, in NHANES 2017-2018, SUA measurements were obtained using a Roche Cobas 6000 analyser. Previous studies have reported combining SUA data from multiple NHANES cycles for analysis (19, 20).

2.3 Assessment of covariates

Participants' height (cm) and weight (kg) were measured to ascertain BMI. BMI is computed by dividing an individual's weight in kilograms by the square of their height in meters. The classification is as follows: normal range (BMI<25 kg/m^2), overweight (25 kg/m^2<BMI<30 kg/m^2), and obesity (BMI ≥ 30 kg/m^2) (21). Hypertension is diagnosed when an individual's systolic blood pressure is equal to or exceeds 140 mmHg and/or their diastolic blood pressure is equal to or exceeds 90 mmHg (22). Detailed laboratory methods for measuring HbA1c, lipid profiles, ALT, AST, creatinine, blood urea nitrogen (BUN), fasting insulin (FI), and fasting plasma glucose (FPG) are provided in the NHANES documentation. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated by FI (μU/mL) * FPG (mmol/L)/22.5 (23).

Chronic kidney disease was determined by an estimated glomerular filtration rate of less than 60 mL/min/1.73 cm^2 (24), calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (25). Participants were categorized into three groups based on their smoking status: nonsmokers, former smokers, and current smokers. Educational level was calculated by the highest grade or the highest degree obtained. Physically active levels were determined using survey questionnaires. The physically active level was calculated by low-intensity, moderate-intensity or vigorous sports and fitness programs for more than 10 minutes per week (26).

2.4 Prescription medications

Participants were asked to provide information on any prescription medications they had taken in the 30 days leading up to the examination. Additionally, they were requested to bring their medication bottles to the examination for verification. Information regarding medications (uric acid-lowering medications and hypoglycemic medications) was then recorded accordingly.

2.5 Statistical analysis

All statistical analyses were performed using Rtools, version 4.2, taking into account the complex survey design of NHANES. We applied appropriate weighting for each analysis to ensure that our results accurately reflected the nationally representative estimates. Categorical variables are presented as numbers and weighted proportions, and continuous variables are presented as weighted means ± standard deviations (SDs). To examine the associations between variables, multivariable logistic regression models were employed to calculate ORs with 95% confidence intervals (95% CIs). Participants were categorized into four groups based on their SUA levels. To detect differences across the four groups, two different statistical tests were employed based on the variable type. Multivariable models were constructed to account for potential confounders. The proportional hazard assumption was assessed for all models to ensure that there were no violations. Stratified analyses were conducted within specific subgroups, including age, sex, race or ethnicity (white and nonwhite), duration of diabetes (<5 years, 5-10 years, ≥10 years), BMI (<25, 25-30, ≥30 kg/m^2), hypertension (yes or no), smoking (never, ever, or current), alcohol intake in drinks per day (0, 1 to ≤2, >2), physical activity (low intensity, moderate intensity, high intensity), hyperlipidemia (yes or no), CKD (yes or no), HbA1c level (<7.0% or ≥7.0%), and diabetes treatment (insulin, insulin + oral medications, oral medications, no pharmacotherapy). Additionally, the study conducted several sensitivity analyses to assess the robustness of the main findings. First, to mitigate the potential impact of reverse causality, participants who were taking uric acid-lowering medications at the baseline examination were excluded. Second, the associations were evaluated after excluding individuals who were taking SGLT-2 inhibitors, as these medications have been shown to reduce cardiovascular risk (27). Third, participants with a history of CKD (eGFR<60 mL/min/1.73cm^2) were excluded from the primary analyses. A p value < 0.05 (two-tailed) was considered statistically significant.

3 Mendelian randomization study

3.1 Data sources and study participants

Summary-level GWAS data for heart failure were obtained from two sources. The primary analysis was conducted by Sonia Shah et al. and included 47,309 heart failure cases and 930,014 controls. In this study, participants were diagnosed with heart failure without specific criteria based on left ventricular ejection fraction (LVEF), encompassing cases with both reduced and preserved heart function. Controls in this study comprised individuals without a diagnosis of heart failure (28). The secondary analysis used data from the FinnGen study, which comprised 19,676 heart failure cases

and 272,371 controls of European descent. Heart failure diagnoses in the FinnGen data were based on the International Classification of Diseases code R7 (29). For more detailed information, the following link can be accessed: gs://finngen-public-data-r7/summary_stats/finngen_R7_I9_HEARTFAIL.gz.

3.2 Genetic instrument selection

Genetic instruments for SUA levels were obtained from a GWAS conducted by the Global Urate Genetics Consortium (GUGC). This GWAS included >140,000 individuals of European ancestry and identified and replicated genome-wide loci associated with SUA levels (30). The study further analyzed replicated and genome-wide significant uric acid-related SNPs in other populations. Specifically, this included 8,340 individuals of Indian descent, 5,820 African Americans, and 15,286 Japanese individuals. By examining these diverse populations, the study aimed to assess the broader applicability of the SNP associations beyond individuals of European ancestry.

3.3 Statistical analysis

To explore potential causal associations, we employed IVW of MR as our primary analysis (15). This method allowed us to estimate the effect of a 1-SD increase in SUA exposure on the risk of CVD. The IVW approach assumes no horizontal pleiotropy. To address the potential impact of horizontal pleiotropy, we conducted several sensitivity analyses using the MendelianRandomization package in R (31). These analyses included the weighted median, MREgger, simple mode, and Mendelian randomization pleiotropy RESidual sum and outlier (MR-PRESSO). In the IVW and MR−Egger analyses, we employed the "random" model to account for potential heterogeneity among the genetic instruments. Additionally, we utilized the "penalized" parameter to penalize variants with heterogeneous causal estimates (32). These sensitivity analyses helped to address potential biases arising from horizontal pleiotropy and provided additional insights into the robustness of our results.

4 Results

4.1 Characteristics of the participants at baseline

We identified 6699 participants who had been diagnosed with diabetes. Of these, 976 participants with missing SUA, BMI, HbA1c or age at diagnosis and participants with T1D were excluded. A total of 5723 participants were included in the study. The characteristics of the study sample are presented in Table 1. Participants had a mean age of 61.9 (± 13.2) years and were predominantly male (51.3%). Participants with the lowest SUA levels had a mean age of 56.4 (± 13.8) years, with a mean BMI of 30.8 (± 6.9) kg/m^2, and 60.0% were women. Among this group, 20.3% had retinopathy, 7.3% had chronic kidney disease, 7.7% had tumors, and 61.9% had hypertension. Among participants with the highest SUA levels, the mean age was 62.5 (± 12.9) years, the mean BMI was 34.8 (± 8.0) kg/m^2, and 41.1% were women. Additionally, 22.7% of participants had retinopathy, 40.7% had chronic kidney disease, 11.7% had tumors, and 84.5% had hypertension, indicating the presence of higher diabetes comorbidities. Furthermore, participants with the lowest uric acid levels displayed higher levels of fasting glucose, HbA1c, estimated GFR, and HDL. Conversely, participants with the highest SUA levels exhibited higher levels of BUN, creatinine, and urinary albumin, suggesting the presence of renal dysfunction.

4.2 Serum uric acid and cardiovascular disease

Without adjusting for variables, compared to participants with the lowest SUA levels, the ORs were 2.96 (95% CI, 2.09-4.18; P<0.001) for heart failure, 1.97 (95% CI, 1.46-2.65; P<0.001) for coronary heart disease, 2.04 (95% CI, 1.32-3.13; P=0.001) for angina/angina pectoris, and 1.92 (95% CI, 1.38-2.66; P<0.001) for myocardial infarction in the highest SUA levels. After adjusting for multiple variables, compared to participants with the lowest SUA levels, the ORs for the highest SUA levels were 1.51 (95% CI, 1.01-2.26; P=0.049) for heart failure, 1.02 (95% CI, 0.69-1.50; P=0.937) for coronary heart disease, 1.36 (95% CI, 0.78-2.38; P=0.285) for angina/angina pectoris, and 1.22 (95% CI, 0.80-1.85; P=0.355) for myocardial infarction (Table 2).

The sensitivity analyses excluded participants who were diagnosed with gout and took uric acid-lowering medicines at the baseline examination (Supplementary Table 1). In addition, participants who took SGLT2 inhibitors at baseline (Supplementary Table 2) and participants with CKD (eGFR <60 mL/min/1.73 cm^2) at baseline (Supplementary Table 3) were also excluded from the analysis. The results did not show substantial changes in the associations across the different sensitivity analysis models.

There was an interaction between SUA and race and HbA1c and the risk of heart failure (P <0.05 for interaction). In the subgroup analysis for non-Hispanic white individuals to the group 1 (<4.6 mg/dL), the ORs for heart failure were 0.76 (95% CI, 0.44-1.29) in group 3 (5.6-6.7 mg/dL) and 1.17 (95% CI, 0.69-1.97) in group 4 (>6.7 mg/dL). In the subgroup analysis involving other racial groups compared to group 1 (<4.6 mg/dL), the ORs for heart failure were 1.73 (95% CI, 1.13-2.65) in group 3 (5.6-6.7 mg/dL) and 2.86 (95% CI, 1.72-4.77) in group 4 (>6.7 mg/dL). In the subgroup analysis of participants with HbA1c <7.0%, compared to group 1 (<4.6 mg/dL), the ORs for heart failure were 1.14 (95% CI, 0.80-1.62) in group 3 (5.6-6.7 mg/dL) and 2.17 (95% CI, 1.57-3.03) in group 4 (>6.7 mg/dL). Additionally, in the subgroup analysis of participants with HbA1c ≥ 7.0%, compared to group 1 (<4.6 mg/dL), the ORs for heart failure were 2.00 (95% CI, 1.38-2.91) in group 3 (5.6-6.7 mg/dL) and 4.18 (95% CI, 2.98-5.94) in group 4 (>6.7 mg/dL). No significant interactions were observed between sex, age, BMI, hypertension, smoking, alcohol consumption, physical activity, or CKD categories and the risk of heart failure (Table 3).

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TABLE 1 Basic characteristics of participants according to uric acid status in NHANES 1999-2018.

Characteristics	Uric acid status				
	≤4.6 (n=1,525)	4.7-5.5 (n=1,388)	5.6-6.7 (n=1,471)	>6.7 (n=1,339)	*P*
Age, mean (SD)	56.4 (13.8)	59.9 (13.3)	60.1 (13.5)	62.5 (12.9)	<0.001
BMI, mean (SD)	30.8 (6.9)	32.0 (7.1)	33.6 (7.5)	34.8 (8.0)	<0.001
Gender, n (%)					
Male	595 (40.0)	694 (48.9)	845 (56.5)	801 (58.9)	<0.001
Female	930 (60.0)	694 (51.1)	626 (43.5)	538 (41.1)	
Weight status, kg/m^2					
<25 kg/m^2	309 (19.7)	228 (14.9)	161 (7.9)	120 (7.8)	<0.001
25 to <30 kg/m^2	512 (30.6)	451 (28.7)	435 (26.5)	330 (22.4)	
≥30 kg/m^2	704 (49.6)	709 (56.4)	875 (65.6)	889 (69.8)	
Race/ethnicity					
Mexican American	434 (12.3)	315 (9.6)	294 (9.2)	140 (4.2)	<0.001
Other Hispanic	176 (8.1)	129 (5.1)	128 (5.8)	83 (4.0)	
Non-Hispanic White	488 (59.7)	488 (62.4)	521 (61.9)	543 (64.7)	
Non-Hispanic Black	289 (12.1)	324 (13.5)	389 (15.2)	450 (18.1)	
Other Race	138 (7.8)	132 (9.5)	139 (7.9)	123 (9.0)	
Education level, n (%)					
High school or less	952 (50.5)	841 (53.1)	857 (49.2)	796 (50.4)	0.706
Some college	368 (31.2)	338 (27.7)	397 (32.5)	363 (30.8)	
College graduate	205 (18.3)	209 (19.2)	217 (18.3)	180 (18.7)	
Insurance, n (%)					
Any insurance	1264 (86.3)	1190 (88.9)	1303 (90.7)	1224 (92.0)	0.029
Uninsured	256 (13.3)	193 (10.7)	165 (9.2)	113 (7.7)	
Smoking status, n (%)					
Never	801 (50.9)	719 (50.0)	730 (49.2)	583 (44.9)	<0.001
Ever	434 (28.6)	448 (33.7)	514 (33.7)	579 (43.7)	
Current	290 (20.5)	221 (16.3)	227 (17.1)	177 (11.4)	
Alcohol, n (%)					
0 drinks/day	857 (49.5)	764 (47.6)	785 (47.8)	740 (50.2)	0.840
1-2 drinks/day	460 (36.5)	440 (39.6)	490 (38.4)	419 (35.9)	
>2 drinks/day	208 (14.1)	184 (12.8)	196 (13.9)	180 (13.8)	
Physical activity, n (%)					
Low intensity	786 (45.5)	713 (47.4)	779 (52.5)	764 (54.3)	0.014
Moderate-intensity	405 (28.6)	386 (29.0)	365 (24.3)	320 (24.8)	
High-intensity	334 (25.9)	289 (23.6)	327 (23.2)	255 (20.9)	
Comorbidities, n (%)					
Retinopathy	321 (20.3)	279 (18.1)	315 (19.4)	329 (22.7)	0.247
Chronic kidney disease	119 (7.3)	195 (12.4)	342 (19.8)	592 (40.7)	<0.001

(Continued)

TABLE 1 Continued

Characteristics	Uric acid status				
	≤4.6 (n=1,525)	4.7-5.5 (n=1,388)	5.6-6.7 (n=1,471)	>6.7 (n=1,339)	*P*
Tumor	125 (7.7)	135(9.1)	121 (6.8)	161 (11.7)	0.004
Hypentension	991 (61.9)	979 (67.1)	1144 (76.7)	1129 (84.5)	<0.001
Diabetes mellitus treatment					<0.001
Insulin	229 (15.0)	140 (10.1)	169 (11.5)	198 (14.8)	
Insulin+oral medications	226 (14.8)	178 (12.8)	184 (12.5)	216 (16.1)	
Oral medications	826 (54.2)	839 (60.4)	890 (60.5)	753 (56.2)	
No pharmacotherapy	244 (16.0)	231 (16.6)	228 (15.5)	172 (12.8)	
Age at diagnosis, mean(SD)	46.5 (15.5)	50.1 (15.3)	50.6 (14.7)	51.9 (14.9)	0.122
Duration of diabetes, mean(SD)	12.2 (11.2)	11.7(11.8)	12.6 (12.2)	12.5 (12.3)	0.414
Biochemical profile, mean (SD)					
Glucose (mmol/L)	10.0 (4.3)	8.8 (3.5)	8.6 (3.4)	8.2 (3.1)	<0.001
HbA1c (%)	8.0 (2.1)	7.4 (1.7)	7.2 (1.7)	7.2 (1.5)	<0.001
HOMA-IR	9.0 (14.9)	7.2 (9.2)	10.1 (17.2)	10.3 (17.7)	<0.001
C-reactive protein (mg/dL)	2.1 (5.3)	2.6 (6.9)	2.4 (5.8)	3.6 (11.5)	0.009
ALT (U/I)	25.6 (20.0)	25.8 (26.2)	26.9 (19.0)	27.3 (41.8)	0.510
AST (U/I)	24.6 (19.5)	25.3 (23.5)	26.1 (14.2)	27.3 (23.3)	0.114
eGFR (ml/min/1.73 m^2)	93.5 (22.4)	85.5 (22.7)	81.1 (24.2)	69.2 (26.5)	<0.001
Cholesterol (mmol/L)	4.9 (1.4)	4.8 (1.2)	4.8 (1.2)	4.7 (1.3)	0.245
Triglycerides (mmol/L)	2.2 (2.9)	2.3 (1.8)	2.4 (2.5)	2.4(2.1)	0.214
LDL-Cholesterol (mmol/L)	2.7 (1.0)	2.7 (0.9)	2.7 (1.0)	2.5 (0.9)	0.280
HDL-Cholesterol (mmol/L)	1.3 (0.4)	1.2 (0.4)	1.2(0.3)	1.2 (0.3)	<0.001
Blood urea nitrogen (mg/dl)	5.0 (2.1)	5.5 (2.2)	5.9 (2.5)	7.6 (3.8)	<0.001
Blood creatinine (mg/dl)	0.9 (0.7)	0.9 (0.7)	1.0 (0.7)	1.2 (0.6)	<0.001
Uric acid (mg/dl)	3.9 (0.6)	5.1 (0.3)	6.1 (0.3)	7.9 (1.1)	<0.001
Urinary albumin	82.7 (749.9)	88.5 (474.5)	152.5 (592.3)	268.1(964.8)	<0.001
Urine creatinine	104.3 (67.4)	111.93 (69.6)	120.67 (73.0)	121.4 (73.0)	<0.001

NHANES, National Health and Nutrition Examination Survey; ALT, Alamine aminotransferase; AST, Aspartate aminotransferase; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low density lipoprotein; n, numbers of subjects; SD, standard deviation; %, weighted percentage.

4.3 Mendelian randomization study

Following application of the selection criteria for SNPs, we identified a total of 20 SNPs from the Shah et al. dataset and 44 SNPs from the FinnGen dataset that met the criteria for analyzing the effects of SUA on heart failure. Detailed descriptions of the selected SNPs can be found in Supplemental Tables 4, 5.

In the primary analysis using the IVW model, there was no suggestion of a causal association between a 1-SD change in levels of SUA and the risk of heart failure (OR: 1.05, 95% CI: 0.97-1.14, p=0.241). The sensitivity analyses utilizing different methods, including weighted median (OR: 0.97, 95% CI: 0.91~1.03, p=0.332), simple mode (OR: 1.24, 95% CI: 1.00~1.53, p=0.058), and weighted mode (OR: 0.96, 95% CI: 0.91~1.02, p=0.232), provided consistent results. The MR–Egger regression test showed the presence of some unbalanced horizontal pleiotropy (P intercept =0.002). The Q test revealed no evidence of heterogeneity in the effect of SUA variants on the risk of heart failure (P Cochran's Q =0.784). We confirmed this causality between SUA and the risk of heart failure by utilizing the FinnGen database, which yielded comparable results (OR: 1.05, 95% CI: 0.96-1.14, p=0.299) (Table 4).

In our analysis, we did not find a causal relationship between SUA and the risk of coronary artery disease (OR: 1.03, 95% CI: 0.98-1.08, p=0.235), angina (OR: 1.00, 95% CI: 0.99-1.01, p=0.414), or myocardial infarction (OR: 1.00, 95% CI: 0.99-1.01, p=0.744). The

TABLE 2 OR (95% CIs) for cardiovascular disease according to uric acid status among participants in NHANES 1999-2018 (n=5,723).

	Group 1 (<4.6)	Group 2 (4.7-5.5)		Group 3 (5.6-6.7)		Group 4 (>6.7)	
	OR (95% CI)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Nonadjusted							
Heart failure	1.00 (reference)	1.00 (0.69, 1.44)	0.999	1.37 (0.96, 1.96)	0.082	2.96 (2.09, 4.18)	<0.001
Coronary heart disease	1.00 (reference)	1.31 (0.91, 1.87)	0.144	1.47 (1.09, 1.99)	0.013	1.97 (1.46, 2.65)	<0.001
Angina/angina pectoris	1.00 (reference)	1.18 (0.76, 1.84)	0.450	1.41 (0.98, 2.04)	0.068	2.04 (1.32, 3.13)	0.001
Myocardial infarction	1.00 (reference)	1.16 (0.82, 1.63)	0.404	1.35 (0.97, 1.87)	0.080	1.92 (1.38, 2.66)	<0.001
Multivariable-adjusted [a]							
Heart failure	1.00 (reference)	0.79 (0.53, 1.18)	0.249	0.95 (0.65, 1.41)	0.812	1.51 (1.01, 2.26)	0.049
Coronary heart disease	1.00 (reference)	1.03 (0.71, 1.51)	0.862	1.06 (0.76, 1.48)	0.715	1.02 (0.69, 1.50)	0.937
Angina/angina pectoris	1.00 (reference)	1.00 (0.60, 1.64)	0.992	1.11 (0.75, 1.64)	0.611	1.36 (0.78, 2.38)	0.285
Myocardial infarction	1.00 (reference)	0.99 (0.69, 1.43)	0.960	1.07 (0.74, 1.56)	0.705	1.22 (0.80, 1.85)	0.355

NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; CI, confidence interval; n, the number.
[a]Data were adjusted for age, sex, race or ethnicity, education level, body mass index, smoking status, drinking status, hypentension, physical activity, total cholesterol, triglycerides, HbA1C, high-density lipoprotein, blood urea nitrogen, and blood creatinine.

TABLE 3 Stratified analyses of the associations (OR, 95% CIs) between uric acid status and cardiovascular disease among participants in NHANES 1999-2018 (n=5,723).

	Group 1 (<4.6)	Group 2 (4.7-5.5)	Group 3 (5.6-6.7)	Group 4 (>6.7)	*P* for interaction
	OR (95% CI) [a]	OR (95% CI) [a]	OR (95% CI) [a]	OR (95% CI) [a]	
Age, years					0.291
<60	1.00 (reference)	0.87 (0.40, 1.92)	1.37 (0.72, 2.62)	1.74 (0.73, 4.15)	
≥60	1.00 (reference)	0.76 (0.48, 1.19)	0.84 (0.52, 1.37)	1.41 (0.91, 2.20)	
Gender					0.725
Male	1.00 (reference)	0.70 (0.39, 1.25)	0.85 (0.51, 1.44)	1.4 (0.81, 2.41)	
Female	1.00 (reference)	0.84 (0.48, 1.46)	0.96 (0.52, 1.77)	1.43 (0.8, 2.56)	
Race					0.034
Non-Hispanic White	1.00 (reference)	0.69 (0.40, 1.18)	0.76 (0.44, 1.29)	1.17 (0.69, 1.97)	
Other Race	1.00 (reference)	1.12 (0.67, 1.88)	1.73 (1.13, 2.65)	2.86 (1.72, 4.77)	
Weight status, kg/m^2					0.536
<25	1.00 (reference)	1.95 (0.71, 5.32)	1.28 (0.33, 4.94)	2.41 (0.59, 9.91)	
25 to <30	1.00 (reference)	0.68 (0.35, 1.34)	0.85 (0.45, 1.62)	1.25 (0.64, 2.43)	
≥30	1.00 (reference)	0.69 (0.39, 1.22)	0.97 (0.57, 1.64)	1.45 (0.85, 2.49)	
Hypertension					0.099
Yes	1.00 (reference)	3.69 (1.40, 9.75)	3.21 (1.20, 8.54)	6.98 (2.45, 19.87)	
No	1.00 (reference)	0.63 (0.41, 0.98)	0.79 (0.52, 1.19)	1.18 (0.76, 1.84)	
Smoking status					0.883
Never	1.00 (reference)	0.84 (0.46, 1.55)	1.04 (0.55, 1.94)	1.94 (1.08, 3.48)	
Ever	1.00 (reference)	0.85 (0.44, 1.66)	1.06 (0.55, 2.07)	1.12 (0.59, 2.12)	
Current	1.00 (reference)	0.63 (0.27, 1.50)	0.65 (0.29, 1.47)	2.36 (0.92, 6.02)	

(Continued)

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TABLE 3 Continued

	Group 1 (<4.6)	Group 2 (4.7-5.5)	Group 3 (5.6-6.7)	Group 4 (>6.7)	*P* for interaction
	OR (95% CI) [a]	OR (95% CI) [a]	OR (95% CI) [a]	OR (95% CI) [a]	
Physical activity					0.675
Low intensity	1.00 (reference)	0.94 (0.59, 1.50)	1.08 (0.73, 1.60)	1.50 (0.96, 2.35)	
Moderate intensity	1.00 (reference)	0.59 (0.23, 1.55)	0.99 (0.39, 2.53)	1.28 (0.45, 3.66)	
High intensity	1.00 (reference)	0.54 (0.20, 1.49)	0.59 (0.22, 1.56)	1.66 (0.61, 4.55)	
Duration of diabetes, year					0.656
<5Y	1.00 (reference)	0.86 (0.46, 1.58)	1.45 (0.85, 2.52)	2.66 (1.63, 4.48)	
5-10Y	1.00 (reference)	1.49 (0.77, 2.96)	1.77 (0.94, 3.43)	3.89 (2.19, 7.24)	
≥10Y	1.00 (reference)	0.95 (0.65, 1.37)	1.58 (1.13, 2.20)	3.22 (2.38, 4.41)	
HbA1c, %					0.011
< 7.0	1.00 (reference)	0.83 (0.57, 1.23)	1.14 (0.80, 1.62)	2.17 (1.57, 3.03)	
≥ 7.0	1.00 (reference)	1.11 (0.73, 1.69)	2.00 (1.38, 2.91)	4.18 (2.98, 5.94)	
Diabetes treatment					0.411
Insulin	1.00 (reference)	1.00 (0.50, 1.92)	2.03 (1.17, 3.57)	3.78 (2.30, 6.36)	
Insulin+oral medications	1.00 (reference)	1.38 (0.75, 2.52)	1.20 (0.65, 2.22)	2.47 (1.47, 4.26)	
Oral medications	1.00 (reference)	0.90 (0.58, 1.37)	1.77 (1.23, 2.58)	2.96 (2.09, 4.26)	
No pharmacotherapy	1.00 (reference)	1.42 (0.67, 3.05)	1.16 (0.53, 2.56)	4.22 (2.19, 8.58)	
Alcohol (drinks/day)					0.281
0	1.00 (reference)	0.84 (0.56, 1.26)	1.36 (0.91, 2.04)	1.81 (1.14, 2.86)	
1 to ≤2	1.00 (reference)	0.66 (0.30, 1.46)	0.52 (0.22, 1.22)	1.28 (0.58, 2.79)	
>2	1.00 (reference)	0.97 (0.23, 4.17)	0.65 (0.19, 2.23)	0.71 (0.19, 2.64)	
Chronic kidney disease					0.093
Yes	1.00 (reference)	2.08 (1.09, 3.96)	1.23 (0.65, 2.32)	1.69 (0.93, 3.07)	
No	1.00 (reference)	0.54 (0.33, 0.88)	0.84 (0.52, 1.36)	1.48 (0.88, 2.47)	

NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; CI, confidence interval; n, the number.
[a]Data were adjusted for age, sex, race or ethnicity, education level, body mass index, smoking status, drinking status, hypentension, physical activity, total cholesterol, triglycerides, HbA1C, high-density lipoprotein, blood urea nitrogen, and blood creatinine.

consistency of these results across different methods was demonstrated and can be seen in Supplementary Table 6. The leave-one-out analyses, depicted in Figure 1, did not identify any single nucleotide polymorphisms (SNPs) that strongly influenced the estimates. Additionally, the results from the forest scatter plots (Supplement Figure S1) and scatter plots (Supplement Figure S2) further support the findings from the main analysis, showing no causal relationship between SUA levels and these cardiovascular outcomes.

5 Discussion

This study employed a cross-sectional study utilizing data from the NHANES. Additionally, a two-sample MR analysis with summary data from GWAS was conducted to explore the potential association between SUA levels and the risks of CVD in individuals with T2D. Our observational research findings revealed that higher SUA levels were associated with an increased risk of heart failure. However, no significant associations were observed between SUA levels and coronary heart disease, angina pectoris, or myocardial infarction. Nevertheless, the MR analysis indicated that the association between SUA levels and heart failure may not be causal.

Although numerous studies have explored the relationship between SUA levels and the risk of CVD, a definitive conclusion has yet to be reached. Some epidemiological studies have analyzed the link between SUA levels and CVD, revealing suggestive evidence of an association (33, 34). However, a systematic review examining the existing evidence on the associations between SUA levels and several health outcomes found that the evidence for a definitive role of SUA levels in CVD outcomes was not compelling. While there was highly suggestive evidence for associations between SUA levels and CVD, it is important to acknowledge that a significant

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TABLE 4 MR results for association between serum urate concentrations and heart failure.

Exposure	Outcome	No.SNP	Methods	OR	95% CI	*P*	Horizontal pleiotropy *P* for Egger intercept	Heterogeneity *P* for Cochran's Q	*P* for MR PRESSO global test
Urate	Heart failure/ GWAS	20	IVW	1.03	0.97-1.09	0.293		0.784	
			WM	0.97	0.91-1.03	0.332			
			MR–Egger	0.90	0.82-0.98	0.023	0.002		
			Simple mode	1.24	1.00-1.53	0.058			
			Weighted mode	0.96	0.91-1.02	0.232			
			MR-PRESSO	1.08	1.00-1.17	0.079			0.072
	Heart failure/ FinnGen	44	IVW	1.05	0.96-1.14	0.299		0.205	
			WM	1.04	0.94-1.17	0.432			
			MR–Egger	0.95	0.81-1.11	0.509	0.136		
			Simple mode	1.08	0.97-1.21	0.057			
			Weighted mode	1.05	0.96-1.02	0.302			
			MR-PRESSO	1.05	0.96-1.14	0.305			0.591

MR, mendelian randomization; IVW, inverse-variance weighted, WM, weighted median; MR-PRESSO, MR pleiotropy residual sum and outlier.

proportion of the meta-analyses exhibited substantial heterogeneity ($I^2 > 50\%$). This suggests that caution is needed when interpreting these associations (11).

Although observational studies cannot establish a causality between SUA levels and CVD in individuals, the use of uric acid-lowering medications, such as oxypurinol and allopurinol, in patients with symptomatic heart failure has also generated conflicting findings. For example, one large RCT did not find any improvements in clinical outcomes with oxypurinol, and the EXACT-HF trial found no significant differences in clinical status, heart failure scores, or 6-minute walk distances between patients with symptomatic heart failure who received allopurinol or placebo, despite reductions in SUA levels with allopurinol (35, 36). However, two small double-blind placebo-controlled RCTs reported potential benefits of allopurinol in improving endothelial function in patients with heart failure (37). Furthermore, an additional small RCT suggested that the beneficial effect of allopurinol on endothelial function in patients with heart failure may be dependent on the dosage administered (38). Overall, it is important to note that the current body of research has not yet provided conclusive evidence on the impact of SUA levels on CVD. Further investigation is necessary to gain a comprehensive understanding of the potential relationship between SUA levels and CVD in the general or diabetes population.

Compared to the general population, individuals with diabetes exhibit specific characteristics. In our study, we identified a notable observed relationship between elevated SUA levels and an increased risk of heart failure, coronary heart disease, angina, and myocardial infarction in individuals with T2D without adjusting for variables. However, once we accounted for multiple confounding variables, the previously observed association between SUA levels and CVD became less conclusive, and only heart failure remained significantly correlated with SUA levels. These findings highlight the importance of accounting for potential confounding factors when examining the relationship between SUA levels and CVD outcomes. In individuals with T2D, hypertension and CKD may serve as relevant confounding factors that can contribute to elevated SUA levels. Considering these factors is essential in accurately assessing the impact of SUA levels on CVD outcomes in individuals with T2D and increasing the risk of CVD and other adverse outcomes. In our study, only 7.3% of T2D patients with the lowest SUA levels had chronic kidney disease, and 61.9% had hypertension. However, at the highest SUA levels, 40.7% of T2D patients had chronic kidney disease, and 84.5% had hypertension.

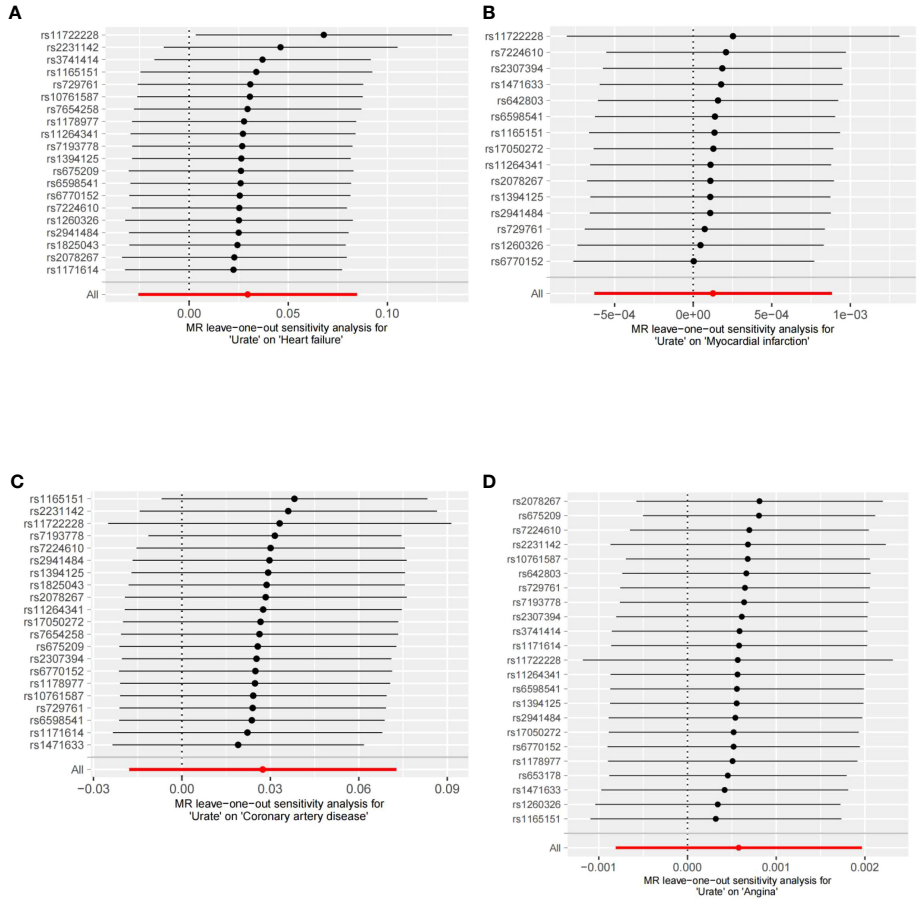

FIGURE 1
Leave-one-out analyses of the association between SUA traits and CVD. **(A)** Leave-one-out analyses of the association between SUA traits and heart failure. **(B)** Leave-one-out analyses of the association between SUA traits and myocardial infarction. **(C)** Leave-one-out analyses of the association between SUA traits and coronary artery disease. **(D)** Leave-one-out analyses of the association between SUA traits and angina.

However, our subgroup analysis revealed an interaction between SUA and race and HbA1c. Specifically, among non-Hispanic white individuals, increasing SUA levels were not associated with the risk of heart failure, whereas other racial groups experienced an elevated risk of heart failure as SUA increased. In the subgroup with HbA1c<7.0%, the risk of heart failure only showed an increase in the highest SUA group. However, in the subgroup with HbA1c>7.0%, the risk of heart failure was elevated even at lower uric acid concentrations. Although age, sex, hypertension, and chronic kidney disease are known to influence uric acid levels in the general or diabetes population, research examining specific subgroups of individuals with diabetes suggests that these factors may not have an impact on the interaction between uric acid and heart failure risk. These findings suggest that race and HbA1c may be important factors to consider in the assessment of the relationship between SUA and heart failure risk in the U.S. diabetes population.

One potential explanation for the observed association between SUA levels and heart failure is the possibility of residual confounding. There may be other factors besides SUA that contribute to heart failure risk, such as dietary factors, hypoglycemic medications, duration of diabetes or other comorbidities. To mitigate the effects of confounding factors, a two-sample MR analysis was performed to evaluate the potential causal relationship between SUA levels and CVD. After conducting both primary and sensitivity MR analyses, we found no evidence to support a causal relationship between genetically predicted SUA levels and CVD. However, our observational study did suggest a potential association between uric acid levels and heart failure. Our finding aligns with a few studies that have shown a lack of evidence linking increased SUA levels directly to heart failure in patients with T2D. Furthermore, this study contributes to the literature on this subject by using genetic variations associated with SUA levels as instrumental variables, which provides stronger evidence for a lack of causal relationship.

The current study possesses several notable strengths, including the use of a large sample size with multiple CVD outcomes and adjustment for relevant confounders. Additionally, the Mendelian randomization analysis provided robust evidence of the causality between SUA levels and heart failure risk. However, there are several limitations within the scope of the current study. To enhance the statistical power and broaden the scope of our study, we combined

NHANES data, but our study might still lack sufficient statistical power to detect small or subtle effects. Additionally, we recognize that some unmeasured or inadequate variables might have differed between groups and could have influenced the observed outcomes. Furthermore, the possibility of T1D cannot be entirely excluded from our study. The NHANES database has provided explicit diagnoses of T1D and T2D since 2013. There are limitations to Mendelian randomization analyses, as they were not able to completely rule out the pleiotropy of SNP levels. Urate transporters are mostly associated with UA underexcretion than UA hyperproduction (such as URAT1, SLC2A9, GLUT9) (39). Therefore, the variability and diversity of urate transporters may have different effects on uric acid concentration. However, in this study, we did not examine relevant SNPs related to urate transporters. This limitation emphasizes the importance of future research in exploring the association between genetic variations in urate transporters and uric acid concentration. Additionally, there can be differences in SNPs among different racial and ethnic groups. These genetic variations can differ among various ethnic and geographic groups due to differences in population history, migration patterns, and genetic admixture. Therefore, it is important to consider these potential differences when studying the impact of SNPs on health outcomes in different populations. Mendelian analysis is based on the genetic pattern of a single gene, considering its influence on a specific trait (40). However, many traits are determined by multiple genes and environmental factors, neglecting these complexities. Mendelian analysis primarily focuses on the direct impact of genotypes on traits, overlooking the role of gene expression and regulation. In fact, the same genotype may exhibit different phenotypes in different individuals, which is associated with gene expression under different environmental conditions.

In conclusion, the current study provides evidence to suggest that elevated SUA levels may increase the risk of heart failure in individuals with T2D and related comorbidities but not necessarily other types of CVD. However, the MR analysis did not support a causal relationship between SUA levels and heart failure risk, indicating that other factors may play a causal role in heart failure development among those with T2D. Further research is needed to identify these factors and investigate their potential interactions with SUA levels.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The survey protocols of the NHANES were approved by the Research Ethics Review Board of the National Center for Health Statistics (https://www.cdc.gov/nchs/nhanes/irba98.htm), and all survey participants provided informed consent.

Author contributions

YH and JF had full access to all of the data in the study, takes responsibility for the integrity of the data and the accuracy of the data analysis, and wrote the paper. YH and JF contributed equally to this work. QW, BZ and YZ: provided continuous support and conceptual advice for this study. DH and DZ: analyzed the data and discussed the results. JY: designed the research, provided continuous support and conceptual advice for this study, is the guarantor of this work, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Supervision: JY. All authors contributed to the articles and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1251451/full#supplementary-material

References

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* (2018) 14(2):88–98. doi: 10.1038/nrendo.2017.151

2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

3. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia* (2018) 61(1):21–8. doi: 10.1007/s00125-017-4390-4

4. Wong E, Backholer K, Gearon E, Harding J, Freak-Poli R, Stevenson C, et al. Diabetes and risk of physical disability in adults: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* (2013) 1(2):106–14. doi: 10.1016/S2213-8587(13)70046-9

5. Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol* (2022) 18(9):525–39. doi: 10.1038/s41574-022-00690-7

6. Mosenzon O, Alguwaihes A, Leon JLA, Bayram F, Darmon P, Davis TME, et al. CAPTURE: a multinational, cross-sectional study of cardiovascular disease prevalence in adults with type 2 diabetes across 13 countries. *Cardiovasc Diabetol* (2021) 20(1):154. doi: 10.1186/s12933-021-01344-0

7. Yun JS, Ko SH. Current trends in epidemiology of cardiovascular disease and cardiovascular risk management in type 2 diabetes. *Metabolism* (2021) 123:154838. doi: 10.1016/j.metabol.2021.154838

8. Boussageon R, Bejan-Angoulvant T, Saadatian-Elahi M, Lafont S, Bergeonneau C, Kassaï B, et al. Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials. *BMJ* (2011) 343:d4169. doi: 10.1136/bmj.d4169

9. Udell JA, Cavender MA, Bhatt DL, Chatterjee S, Farkouh ME, Scirica BM. Glucose-lowering drugs or strategies and cardiovascular outcomes in patients with or at risk for type 2 diabetes: a meta-analysis of randomised controlled trials. *Lancet Diabetes Endocrinol* (2015) 3(5):356–66. doi: 10.1016/S2213-8587(15)00044-3

10. Garrod AB. Observations on certain pathological conditions of the blood and urine, in gout, rheumatism, and Bright's disease. *Med Chir Trans* (1848) 31:83–97. doi: 10.1177/095952874803100109

11. Li X, Meng X, Timofeeva M, Tzoulaki I, Tsilidis KK, Ioannidis JP, et al. Serum uric acid levels and multiple health outcomes: umbrella review of evidence from observational studies, randomised controlled trials, and Mendelian randomisation studies. *BMJ* (2017) 357:j2376. doi: 10.1136/bmj.j2376

12. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* (2008) 359(17):1811–21. doi: 10.1056/NEJMra0800885

13. Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann Intern Med* (1999) 131(1):7–13. doi: 10.7326/0003-4819-131-1-199907060-00003

14. Fralick M, Chen SK, Patorno E, Kim SC. Assessing the risk for gout with sodium-glucose cotransporter-2 inhibitors in patients with type 2 diabetes: A population-based cohort study. *Ann Intern Med* (2020) 172(3):186–94. doi: 10.7326/M19-2610

15. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res* (2017) 26(5):2333–55. doi: 10.1177/0962280215597579

16. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol* (2016) 27 (11):3253–65. doi: 10.1681/ASN.2016010098

17. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA* (2011) 305(24):2532–9. doi: 10.1001/jama.2011.861

18. Ruhl CE, Menke A, Cowie CC, Everhart JE. Relationship of hepatitis C virus infection with diabetes in the U.S. population. *Hepatology* (2014) 60(4):1139–49. doi: 10.1002/hep.27047

19. Mazidi M, Katsiki N, Mikhailidis DP, Banach MLipid and Blood Pressure Meta-Analysis Collaboration (LBPMC) Group. Associations of serum uric acid with total and cause-specific mortality: Findings from individuals and pooling prospective studies. *Atherosclerosis* (2020) 296:49–58. doi: 10.1016/j.atherosclerosis.2019.07.019

20. Li B, Chen L, Hu X, Tan T, Yang J, Bao W, et al. Association of serum uric acid with all-cause and cardiovascular mortality in diabetes. *Diabetes Care* (2023) 46 (2):425–33. doi: 10.2337/dc22-1339

21. Park SJ, Ha KH, Kim DJ. Body mass index and cardiovascular outcomes in patients with acute coronary syndrome by diabetes status: the obesity paradox in a Korean national cohort study. *Cardiovasc Diabetol* (2020) 19(1):191. doi: 10.1186/s12933-020-00997-7

22. Messerli FH, Williams B, Ritz E. Essential hypertension. *Lancet (London England)* (2007) 370(9587):591–603. doi: 10.1016/S0140-6736(07)61299-9

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) 28(7):412–9. doi: 10.1007/BF00280883

24. Kalantar-Zadeh K, Jafar TH, Nitsch D, Neuen BL, Perkovic V. Chronic kidney disease. *Lancet* (2021) 398(10302):786–802. doi: 10.1016/S0140-6736(21)00519-5

25. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* (2009) 150 (9):604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

26. Liu Y, Geng T, Wan Z, Lu Q, Zhang X, Qiu Z, et al. Associations of serum folate and vitamin B12 levels with cardiovascular disease mortality among patients with type 2 diabetes. *JAMA Netw Open* (2022) 5(1):e2146124. doi: 10.1001/jamanetworkopen.2021.46124

27. Palmer SC, Tendal B, Mustafa RA, Vandvik PO, Li S, Hao Q, et al. Sodium-glucose cotransporter protein-2 (SGLT-2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists for type 2 diabetes: systematic review and network meta-analysis of randomised controlled trials. *BMJ* (2021) 372:m4573. doi: 10.1136/bmj.m4573

28. Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* (2020) 11(1):163. doi: 10.1038/s41467-019-13690-5

29. FinnGen. *{FinnGen}. Documentation of R7 release* (2022). Available at: https://finngen.gitbook.io/documentation/v/r7/.

30. Köttgen A, Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet* (2013) 45(2):145–54. doi: 10.1038/ng.2500

31. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *Int J Epidemiol* (2017) 46(6):1734–9. doi: 10.1093/ije/dyx034

32. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* (2018) 50(5):693–8. doi: 10.1038/s41588-018-0099-7

33. Saito Y, Tanaka A, Node K, Kobayashi Y. Uric acid and cardiovascular disease: A clinical review. *J Cardiol* (2021) 78(1):51–7. doi: 10.1016/j.jjcc.2020.12.013

34. Yanai H, Adachi H, Hakoshima M, Katsuyama H. Molecular biological and clinical understanding of the pathophysiology and treatments of hyperuricemia and its association with metabolic syndrome, cardiovascular diseases and chronic kidney disease. *Int J Mol Sci* (2021) 22(17):9221. doi: 10.3390/ijms22179221

35. Hare JM, Mangal B, Brown J, Fisher C Jr, Freudenberger R, Colucci WS, et al. Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study. *J Am Coll Cardiol* (2008) 51(24):2301–9. doi: 10.1016/j.jacc.2008.01.068

36. Givertz MM, Anstrom KJ, Redfield MM, Deswal A, Haddad H, Butler J, et al. Effects of xanthine oxidase inhibition in hyperuricemic heart failure patients: the xanthine oxidase inhibition for hyperuricemic heart failure patients (EXACT-HF) study. *Circulation* (2015) 131(20):1763–71. doi: 10.1161/CIRCULATIONAHA.114.014536

37. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation* (2002) 106(2):221–6. doi: 10.1161/01.CIR.0000022140.61460.1D

38. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* (2006) 114(23):2508–16. doi: 10.1161/CIRCULATIONAHA.106.651117

39. So A, Thorens B. Uric acid transport and disease. *J Clin Invest* (2010) 120 (6):1791–9. doi: 10.1172/JCI42344

40. Smith GD, Ebrahim S. Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* (2003) 32(1):1–22. doi: 10.1093/ije/dyg070

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Oleanolic acid rescues critical features of umbilical vein endothelial cells permanently affected by hyperglycemia

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Skin wound healing is a physiological process that involves several cell types. Among them, endothelial cells are required for inflammation resolution and neo-angiogenesis, both necessary for tissue restoration after injury. Primary human umbilical vein endothelial cells (C-HUVECs) are derived from the umbilical cord. When women develop gestational diabetes, chronic exposure to hyperglycemia induces epigenetic modifications in these cells (GD-HUVECs), leading to a permanent pro-inflammatory phenotype and impaired angiogenesis in contrast to control cells. Oleanolic acid (OA) is a bioactive triterpenoid known for its epithelial cell migration promotion stimulation and higher tensile strength of wounds. However, the potentially anti-inflammatory and pro-angiogenic properties of OA are still under investigation. We tested OA on C- and GD-HUVECs under inflammatory conditions induced by low levels of the inflammatory cytokine TNF-α. Reduced expression of adhesion molecules *VCAM1*, *ICAM1*, and *SELE* was obtained in OA-pre-treated C- and GD-HUVECs. Additionally, protein VCAM1 levels were also decreased by OA. Coherently, monocyte adhesion assays showed that a lower number of monocytes adhered to GD-HUVEC endothelium under OA pre-treatment when compared to untreated ones. It is noteworthy that OA improved angiogenesis parameters in both phenotypes, being especially remarkable in the case of GD-HUVECs, since OA strongly rescued their poor tube formation behavior. Moreover, endothelial cell migration was improved in C- and GD-HUVECs in scratch assays, an effect that was further confirmed by focal adhesion (FA) remodeling, revealed by paxillin staining on immunocytochemistry assays. Altogether, these results suggest that OA could be an emergent wound healing agent due to its capacity to rescue endothelial malfunction caused by hyperglycemia.

KEYWORDS

oleanolic acid, endothelial cells, angiogenesis, inflammation, chronic hyperglycemia, adhesion molecules

Introduction

During wound healing, epithelial cell migration is a crucial process to close and repair the skin barrier (1, 2). In pathological conditions, a physiologic impairment that halts wound healing may occur. Therefore, new treatments that could enhance or accelerate cell migration are currently of great interest. Oleanolic acid (OA), a bioactive triterpenoid present in a wide variety of plants, has shown promising effects on wound healing due to its cell migration activity on epithelial cells (3–7). Thus, OA activates epidermal growth factor receptor (EGFR), enabling a complex MAP kinase system, which in turn triggers c-Jun phosphorylation and overexpression, a key transcription factor that enhances a gene expression cell migration program (7, 8). Besides these molecular effects, OA also changes elements of the epithelial cell architecture. OA promotes the assemble–disassemble turnover of focal adhesions (FAs) together with the actin and paxillin remodeling, a dynamic state that is evidence of cell migration (7, 9–11).

The findings of effects of OA on epithelial cells encouraged us to explore the role of this bioactive compound on other wound healing players, closely related to epithelial cells and their migration. In an acute wound, to reach wound closure, sequential phases must occur with the involvement of different cell types. The proliferative phase and the remodeling phase are critical for a correct wound resolution (12). These two stages need to take place after a correct inflammation mitigation in the wound, which is produced after skin injury and defines the inflammatory phase (13). At this point, in the injured blood vessels of the wound, endothelial cells respond by expressing adhesion molecules on the endothelium surface in order to facilitate the recruitment of immune cells to the wound site. In particular, they recruit immune cells that are known for their reparative properties, such as M2 macrophages and type T lymphocytes, which release anti-inflammatory cytokines to modulate the wound inflammation milieu (14). The adequate resolution of this phase allows the wound to progress into the subsequent proliferative and remodeling phases, including angiogenesis and tissue regeneration. During angiogenesis, endothelial cells proliferate, migrate, and form a tube for the correct supply of nutrients, oxygen, and growth factors to the newly formed wound bed (15, 16). Briefly, angiogenesis is critical and occurs with cell migration during the proliferative and remodeling phases, supporting skin reepithelization.

However, when the wound is subjected to continuous inflammation, the other stages come to a halt, resulting in a chronic, non-healing wound that may progress to an ulcer (17, 18). Indeed, there are many causes that trigger this condition, including trauma, burns, infections, or underlying chronic diseases such as diabetes (19). In fact, diabetes is one of the leading causes of impaired wound healing, and represents a complex issue due to its socioeconomic impact and the elevated number of patients (20, 21). For instance, one of its most severe complications is diabetic foot ulcer (DFU), in which the patient's ulcer shows poor reepithelization and vascularization, leading to the amputation of the limb (20).

Diabetic ulcers display an excessive inflammatory response and deficient angiogenesis due to endothelium malfunction, which causes delayed healing and uncontrolled scar tissue formation (22–24). Thus, the use of *in vitro* cell models that can mimic endothelium diabetic features seems very relevant to studying possible strategies or agents that help rescue endothelial cells from this condition, and eventually restore their regular function. This is the case of human umbilical cord vein endothelial cells (HUVECs) exposed to hyperglycemia during pregnancy in mothers affected by gestational diabetes (GD) (25). Interestingly enough, this unique endothelial cell model (GD-HUVECs) displays an altered phenotype that has been exhaustively studied and described (26). Although regular primary HUVECs are a well-known *in vitro* model to study the process and molecular mechanisms related to inflammation and neo-angiogenesis (27, 28), GD-HUVECs are permanently damaged by hyperglycemia, thus showing a senescent pro-inflammatory phenotype that leads to endothelial dysfunction (26). Therefore, GD-HUVECs are a suitable model to study and to try to rescue an endothelium that is affected by diabetic ulcers and causes either a delay or even a halt on wound healing. Indeed, previous studies have shown that OA attenuates adhesion molecule overexpression under inflammation stimuli in C-HUVECs (29, 30). Nevertheless, it might be very interesting to carry out these studies on GD-HUVECs, which are endothelial cells experiencing a pathologic condition.

In this article, we have investigated the effects of OA on C-HUVECs and GD-HUVECs. Our results show that OA attenuates inflammatory responses, improves migration, and favors tube formation in both types of cells. Furthermore, these aspects are especially relevant in GD-HUVECs.

Materials and methods

HUVEC isolation and culture

All procedures adhered to the ethical standards of the Institutional Committee on Human Experimentation (reference number 1879/09COET) and to the principles of the Declaration of Helsinki. The protocol used was approved by the Institutional Review Board and informed consent was signed by every participating subject. Primary endothelial cells were collected from umbilical cord veins (HUVECs) of newborns delivered between the 36th and the 40th gestational week at the Hospital of Chieti and Pescara (Italy) from randomly selected Caucasian mothers affected by GD or not (control, C) following previously published methods (31). Briefly, veins of the umbilical cords were immediately collected after delivery, cannulated and perfused with 1 mg/mL collagenase 1A at 37°C. Obtained HUVECs were isolated in a base medium composed of DMEM/M199 (1:1) supplemented with 1% L-glutamine, 1% penicillin/streptomycin, and 20% fetal bovine serum (FBS) (all from Biowest, Nuaillé, France). Then, the cell suspension was centrifuged at 1,200 rpm for 10 min, and the cell pellet was re-suspended in HUVEC base medium and plated on 1.5% gelatin-coated (Sigma-Aldrich, St Louis, MO, USA) tissue culture flasks. HUVECs were confirmed by the presence of specific markers such as von Willebrand factor, CD31 and CD34 positive, together with the induced expression of cell adhesion molecules

ICAM1, VCAM1, and E-selectin, and cytokines IL-6 and IL-8 under pro-inflammatory stimuli, as well as the formation of cord-like structures on Matrigel (25, 32). For all experiments, the cells were used *in vitro* between the 3rd and 5th passage, never exceeding the 5th passage. The HUVECs selected for the assays were grown on 1.5% gelatin-coated tissue culture plates in HUVEC complete medium: low-glucose (1 g/L) DMEM and M199 medium (ratio 1:1), supplemented with 10 µg/mL heparin (Sigma-Aldrich, St Louis, MO, USA), 50 µg/mL endothelial cell growth factor (ECGF), 20% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. All experiments were performed, at least, in technical triplicate, using three different cellular strains (n = 3) of C- and GD-HUVECs.

Oleanolic acid preparation

OA (purity > 97%) (Merck, Darmstadt, Germany) was solubilized to a 25 mM final concentration in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St Louis, MO, USA). Assay concentrations are indicated for each experiment in figure legends. MTT assays were performed in C- and GD-HUVECs prior to functional assays, in order to optimize the OA/DMSO effect (see Supplementary Figure 1). In all the assays, DMSO concentration never exceeded 1% to avoid cytotoxic effects.

RNA extraction and quantitative PCR

C- and GD-HUVECs were seeded in 5-cm-diameter Petri dishes coated with 1.5% gelatin in HUVEC complete medium. When cells reached sub-confluence (60%), cells were pre-treated for 24 h with OA or DMSO (basal condition) in HUVEC complete medium with 10% FBS. After this, a 2-h serum-starvation period was established in HUVEC serum-starvation medium: low-glucose (1 g/L) DMEM with 0.1% FBS, supplemented with 10 µg/mL heparin, 50 µg/mL endothelial cell growth factor (ECGF), 0.3% bovine serum albumin (BSA, from Sigma-Aldrich, St Louis, MO, USA), 1% penicillin/streptomycin, and 1% L-glutamine. After serum starvation, cells were treated with TNF-α at 1 ng/mL, using this concentration for subsequent assays as well (28, 33, 34). Then, cells were incubated for 2, 6, and 24 h to induce the gene expression of adhesion molecules: vascular cell adhesion molecule 1, *VCAM1*, intercellular adhesion molecule 1, *ICAM1*, and, E-selectin, *SELE*. At the times indicated above, RNA was extracted using the RNeasy-mini system (Qiagen, Venlo, The Netherlands). Usually, 800 ng of RNA from independent samples was retro-transcribed using iScript reagents (Bio-Rad, Hercules, CA, USA). The obtained cDNA was used for quantitative PCR (qPCR) using the SYBR premix ex Taq kit (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) according to the manufacturer's protocol. The primers used for the analyzed genes related to inflammation are indicated in Table 1. For gene expression analysis, qPCR cycles were normalized with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene expression according to the $2^{-\Delta\Delta Ct}$ method (35). The experiment was carried out on four different strains for C-HUVECs and four different strains for GD-HUVECs, each in technical triplicate. Analyzed data represent mean ± SEM.

MTT assay

The effects of increasing concentrations of OA on C-HUVEC and GD-HUVEC viability were assessed with the 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) method (36). C- and GD-HUVECs were seeded in 96-well microplates, 2×10^4 cells/cm^2 (approximately 6500 cells per well), coated with 1.5% gelatin in HUVEC complete medium. When cells reached sub-confluence (80%), a 24-h serum-starvation period was established in HUVEC serum-starvation medium (0.1% FBS). After this, cells were treated with vehicle control DMSO or OA, as indicated in Supplementary Figure 1, in 0.5% FBS media. After 24-h incubation, 20 µL of MTT 5 mg/mL in PBS was added to each well. Plates were incubated for 3 h at 37 °C and finally the absorbance at 540 nm was detected by a microplate reader (SpectraMAX 190, Molecular Devices, Sunnyvale, CA, USA).

Western blot

C- and GD-HUVECs were seeded in 5-cm-diameter Petri dishes coated with 1.5% gelatin in HUVEC complete medium. When cells reached sub-confluence (60%), cells were pre-treated for 24 h with OA or DMSO (basal) in HUVEC complete medium with 10% FBS. After this, a 2-h serum-starvation period (0.1% FBS) was established in HUVEC serum-starvation medium. Then, cells were treated with TNF-α (1 ng/mL) for 1, 3, 6, or 24 h to induce inflammation. At the indicated times, cells were collected, washed twice with cold PBS, and lysed with 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1.2 mM $MgCl_2$, 0.5%, Nonidet p-40, 1 mM DTT, 25 mM NaF, and 25 mM β-glycerophosphate supplemented

TABLE 1 Different primers used to study the expression of several genes.

Gene name (GeneCards)/ Primer name	Primer sequence 5′-3′
GAPDH Fwd	AGCTCAGGCCTCAAGACCTT
GAPDH Rev	AAGAAGATGCGGCTGACTGT
ICAM1 Fwd	ACCATCTACAGCTTTCCG (Sigma KiCqStart)
ICAM1 Rev	TCACACTTCACTGTCACC (Sigma KiCqStart)
SELE Fwd	GAGAATTCACCTACAAGTCC (Sigma KiCqStart)
SELE Rev	AGGCTTGAACATTTTACCAC (Sigma KiCqStart)
VCAM1 (mix Fwd/Rev)	Proprietary sequence (Qiagen QuantiTect®) QT00018347

GADPH, glyceraldehyde-3-phosphate dehydrogenase; ICAM1, intercellular adhesion molecule 1; SELE, E-Selectin; VCAM1, vascular cell adhesion molecule 1.

with phosphatase inhibitor cocktails (I and II) and protease inhibitors (all from Sigma-Aldrich, St Louis, MO, USA). Total protein amount of all samples was measured and normalized by Bradford assay (37) (Sigma-Aldrich, St Louis, MO, USA). Samples were analyzed by SDS-PAGE followed by Western blot using the indicated antibodies (see the *Antibodies* section). Blots were revealed by using horseradish peroxidase substrate (ECL) (GE Healthcare, GE, Little Chalfont, United Kingdom) and images were taken with a ChemiDoc MP (Bio-Rad, Hercules, CA, USA). To quantify Western blot protein bands, pictures in 8-bit format were processed in ImageJ software. In every picture, a lane was established for each sample. In each lane, only the band with the specific size (kDa) of the protein of interest was quantified. For each total protein and its phosphorylated form, each band's intensity peak was plotted, and subsequently, the area under the plot was measured by using "Wand tool" of ImageJ to finally obtain pixel intensity value. In order to normalize data, obtained intensity values were referred to those of the unphosphorylated form of the protein (total) or a loading control protein (β-actin) if the unphosphorylated form was undetectable (non-available antibody for the unphosphorylated form).

Monocyte-HUVEC adhesion assay

C- and GD-HUVECs were seeded in six-well plates (200,000 cells/well) coated with 1.5% gelatin in HUVEC complete medium until they reached 60% confluence. At this time, cells were pre-treated for 24 h with 20 μM OA in HUVEC complete medium with 10% FBS. When confluent, a 2-h serum-starvation period was established washing and adding HUVEC serum starvation media. After this, cells were stimulated with TNF-α (1 ng/mL) for 16 h. The U937 monocyte cell line (European Collection of Authenticated Cell Cultures, ECACC) was used to evaluate the adhesion to C- and GD-HUVEC monolayers, as previously described (28, 33, 34). Briefly, the medium was removed from each HUVEC well, cells were gently washed with DMEM, and a suspension with 1 million monocytes was added to each well. Plates were incubated for 20 min, with gentle shaking at room temperature. Finally, to remove non-adhered monocytes, HUVECs were gently washed and fixed with 1% paraformaldehyde. To identify the number of adherent monocytes for each tested strain, 12 counts were performed for every experimental condition (by using at least three different randomly selected high-power fields, at 10× magnification) using Paula Nuc microscope (Leica Microsystems, Wetzlar, Germany). Images were acquired by using Paula software version 1.2.2. For this experiment, four different strains of both C-HUVECs and GD-HUVECs were used.

Matrigel tube formation assay

C- and GD-HUVECs were seeded on 12-well plates coated with growth factor-reduced basement membrane matrix gel, known as Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) all in 10% FBS HUVEC complete medium. A number of 1.4×10^5 cells/well was the suitable amount for the assay. After plating, cells were incubated for 15 min at 37°C to induce cellular adhesion to Matrigel. Then, 20 μM OA and DMSO equivalent volume as control were ready to add to cells. After 6 h, representative images were taken using a Paula Nuc microscope (Leica Microsystems, Wetzlar, Germany). Images were processed and measured by ImageJ software. In this software, "Angiogenesis Analyzer" plugin (38) was used to analyze key neo-angiogenesis markers: number of isolated segments, total length of isolated branches, number of master segments, number of meshes, number of nodes, number of segments, number of master junctions, total length of branches, and total length. The data presented are the data gathered from four C-HUVEC strains and four GD-HUVEC strains.

Wound healing scratch assay

C- and GD-HUVECs were grown in 24-well plates coated with 1.5% gelatin until they reached 100% confluence in HUVEC complete medium. At this point, a serum-starvation period was performed in 1% FBS HUVEC serum starvation medium for 24 h. Cells were scratched using a sterile p-40 μL pipette tip and then the resulting wounds were gently washed with free-FBS DMEM low glucose to remove released cells. Treatments were performed in the plates by adding DMSO and 20 μM OA in 0.5% FBS media. Additionally, 20% FBS was added as a positive control. After 12 h, the assay was stopped by fixing the cells with 4% formaldehyde (Applichem GmbH, Darmstadt, Germany) in PBS (Biowest, Nuaillé, France) for 10 min. Finally, cells were washed twice with PBS. Pictures were taken at 10× magnification using an optical microscope equipped with a digital camera (Motic Optic AE31, Motic Spain, Barcelona, Spain). Areas in the wounds at 0 h and 12 h were measured by ImageJ software. The initial cell area (0 h) was subtracted from the final cell area (12 h) and plotted in a graph as migration percentage (39).

Focal adhesion quantification assay

C- and GD-HUVECs were grown on round-glass coverslips coated with 1.5% gelatin until they were sub-confluent (60%) in HUVEC complete medium. At this time, cells were washed with serum-deprived medium and then treated with 20 μM OA and DMSO equivalent volume (basal) in 0.1% FBS HUVEC starvation medium. After 24-h incubation, coverslips were fixed with 4% formaldehyde (Applichem GmbH, Darmstadt, Germany) in PBS (Biowest, Nuaillé, France) for 10 min and washed twice with PBS. Then, cells were permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) in PBS for 10 min. For immunostaining, a 30-min blocking was performed in PBS solution with 10% FBS, 5% skim milk (Beckton Dickinson, Franklin Lakes, NJ, USA), 0.3% bovine serum albumin (BSA, Sigma-Aldrich, St Louis, MO, USA) and 0.1% Triton X-100. Subsequently, cells were incubated for 1 h with anti-paxillin antibody, diluted in the above-mentioned blocking solution without skim milk. Proper fluorescent-labeled secondary

antibodies (see the *Antibodies* section) were co-incubated for 30 min with Alexa Fluor 594 conjugated phalloidin (Molecular Probes, Thermo Fisher Scientific, Waltham, MA, USA) and Hoechst 33258 (Fluka, Biochemika, Sigma-Aldrich, St Louis, MO, USA) to reveal actin cytoskeleton and nuclei, respectively. Once the immunostaining was completed, representative pictures were acquired with a confocal microscope at 40x magnification (LSM 510 META from ZEISS, Jena, Germany). The setting of images was performed using Zeiss Efficient Navigation (ZEN) interface software (ZEISS, Jena, Germany). The "Z stack" ZEN tool was used in order to observe deep cytoskeleton structures (paxillin), taking picture slices along the *Z* axis. Then, picture slices were merged by the "Maximum intensity projection" ZEN tool. FA quantification was carried out as previously described by using CLAHE and Log3D macros for ImageJ (40). Essentially, FAs were quantified from paxillin-stained acquired pictures. We used four different replicates for each condition. Specifically, cell filopodia were selected as regions of interest (ROIs) and the resulting areas (containing FAs) were considered for further analysis. A number of five filopodia were considered from each picture. Then, the number of FAs were calculated in each filopodia by using the previously mentioned macros. The obtained number was divided by the total filopodia area to determine FA density in the cells.

Antibodies

The following commercial primary antibodies were used: 1:1,000 anti-phospho-NF-κB (Cell Signaling Technology, Danvers, MA, USA); 1:1,000 anti-NF-kB and 1:1,000 anti-VCAM1 (Abcam, Cambridge, United Kingdom); 1:200 anti-paxillin (Santa Cruz Biotechnology, Heidelberg, Germany); and 1:4,000 anti-β-actin (Sigma-Aldrich, St Louis, MO, USA). Secondary antibodies were as follows: 1:1,000 anti-rabbit IgG Horseradish peroxidase linked F(ab')2 I fragment (from donkey) (GE Healthcare, GE, Little Chalfont, United Kingdom); 1:3,000 anti-mouse IgG1 (BD Pharmingen, Beckton Dickinson, Franklin Lakes, NJ, USA); and 1:400 Alexa Fluor 488 conjugated anti-mouse (from donkey) (Thermo Fisher Scientific, Rockford, IL, USA).

Statistical analysis

The gathered data were represented and analyzed using GraphPad Prism v7 software. Classical statistical parameters were calculated and statistical tests were performed with a 95% confidence interval. Consequently, in each test, *p*-values lower than 0.05 were considered to be statistically significant. At the figure legends, asterisks indicate statistically significant differences between assay conditions (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, and ****$p < 0.0001$). Data were analyzed by a one-way ANOVA test, comparing the mean of each condition with the mean of every other condition. Subsequently, a Tukey's multiple comparisons test was performed. *p*-values lower than 0.05 indicate statistically significant differences between the means of conditions.

Results

Oleanolic acid attenuates adhesion molecule overexpression induced by TNF-α in C- and GD-HUVEC

Adhesion molecule expression on endothelial cell surface is needed for immune cell recruitment to endothelial surface and, finally, migration to the inflammation source at the wound (41). However, an uncontrolled recruitment triggers endothelium dysfunction (42). Adhesion molecule gene expression, which is upregulated in endothelial cells in response to the pro-inflammatory cytokine TNF-α, was tested on HUVECs. Both C- and GD-HUVECs were treated with 20 μM OA and then TNF-α stimulated for the indicated times. On the whole, no gene expression differences were detected after 24 h pre-treatment with OA (Supplementary Figure 1) (0 h). Generally speaking, adhesion molecule expression in untreated C- and GD-HUVECs showed a strong response by TNF-α at 2 and 6 h in all genes tested. However, beginning with the *VCAM1* gene (Figure 1A), a patent attenuation with OA was detected at 2 and 6 h in both C- and GD-HUVECs. Strikingly, this reduction was even more significant in GD-HUVECs at 6 h. Regarding *ICAM1* (Figure 1B), this OA-dependent decrease was less patent but remained significant, mostly at 6 h, in C- and GD-HUVECs. The expression of the third adhesion molecule tested *SELE* (Figure 1C) also showed a strong attenuation with OA, which was more patent in the GD phenotype. At 24 h, in both types of endothelial cells and both conditions, all adhesion molecules showed a drop in their expression; therefore, we could not see any statistically significant differences.

Subsequently, we studied VCAM1 total protein amount in C- and GD-HUVECs using the same experimental design (Figure 2). At basal conditions, total VCAM1 protein levels were detected in both cell lines after 3 h of TNF-α stimulation, showing its highest level at 6 h, whereas 24 h later, the levels plummeted. To begin with, GD-HUVECs showed higher protein levels than control ones. Interestingly, C- and GD-HUVEC lysates with OA pre-treatment showed significantly lower total VCAM1 protein levels than control, which suggested a strong OA attenuation on the TNF-α stimulation. Endothelial cell stimulation with TNF-α induces the expression of adhesion molecules through the participation of nuclear factor-κB (NF-κB) that is phosphorylated at Ser 536 and then translocates to the nucleus where it activates the expression of *VCAM1*, among others (43–45). However, when Ser 536 phosphorylated NF-κB was assayed in response to TNF-α stimulation, no significant differences were found between 20 μM OA treated and non-treated C- or GD-HUVECs. Only a slight decrease of phospho-NF-κB level was noticed in C-HUVECs after 24 h OA stimulation and only in the TNF-α sample.

All these data suggest that OA attenuates the expression of VCAM1 protein and of the *VCAM1, SELE*, and *ICAM1* genes in response to TNF-α in HUVECs regardless of its glucose-affected condition.

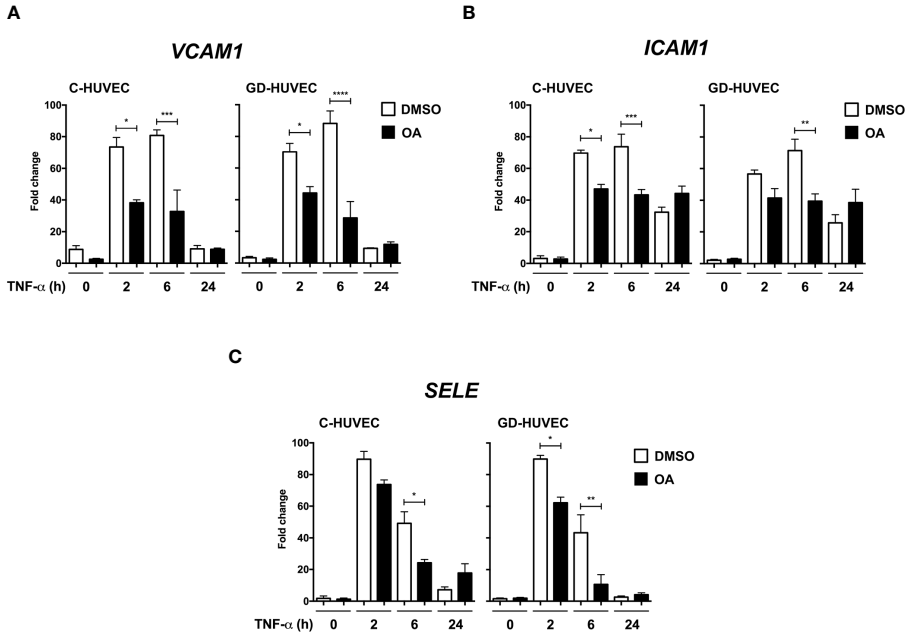

FIGURE 1
Oleanolic acid reduces the expression of adhesion molecule genes induced by TNF-α. Gene expression analysis of **(A)** *VCAM-1*, **(B)** *ICAM-1*, and **(C)** *SELE* in C- and GD-HUVECs pre-treated 24 h with 20 μM OA (black) or DMSO equivalent volume (white, DMSO). After pre-treatments, cells were stimulated with TNF-α at 2, 6, and 24 h. Histograms represent mRNA relative expression of each gene (normalized with GAPDH expression) for both C- and GD-HUVECs. Each condition represents the mean ± SEM using four different strains for C-HUVECs and four other different strains for GD-HUVECs. Asterisks indicate statistically significant differences between the selected conditions according to a one-way ANOVA statistical analysis (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ and ****$p < 0.0001$).

Oleanolic acid reduces the number of monocytes adhered to GD-HUVECs

Given the attenuation effect of OA on adhesion molecule expression, a monocyte adhesion assay was conducted on C- and GD-HUVEC monolayers (Figure 3). HUVECs pre-treated or not with OA were subjected to 16 h TNF-α, to study whether the OA effect on adhesion molecules correlated with the number of monocytes adhered to their surface. When C- and GD-HUVECs were stimulated with TNF-α, there was a clear increase in the number of adhered monocytes, which was slightly higher on GD-HUVECs (Figure 3). Interestingly, OA pre-treatment decreased the number of monocytes in both C- and GD-HUVECs (Figure 3) before and after treatment with TNF-α.

Overall, this functional assay reveals less monocyte–endothelial interaction triggered by TNF-α in both C- and GD-HUVECs when the cells are previously treated with OA.

Oleanolic acid improves neo-angiogenesis in GD-HUVECs

Matrigel tube formation assay with HUVEC is a well-established and informative test to evaluate the angiogenesis function of endothelial cells *in vitro* (28, 38, 46). C- and GD-HUVECs were seeded in Matrigel and treated with OA for 6 h. Representative pictures indicated a greater network complexity in both HUVEC phenotypes under OA conditions (Figure 4A); however, the possible effects of OA on the GD-HUVEC versus the C-HUVEC were difficult to interpret. To gain more knowledge about angiogenesis features with OA, nine key parameters related to this meshed network were measured (Figure 4B). The number of isolated segments were higher in GD- compared to C-HUVEC, as it was reduced with OA. Similarly, total length of isolated branches was reduced with OA in GD-HUVEC. In addition, the number of master segments, which were affected in GD-HUVEC, was ameliorated with OA. Finally, both the number of master junctions and total length of branches were deficient in GD-HUVEC when compared to C-HUVEC. In all cases, the presence of OA produced and improvement of the parameters. Finally, the number of nodes, the number of segments and total length, all representing the complexity of the network, were all increased by OA in both C- and GD-HUVEC, but showed a more powerful effect on GD-HUVEC.

Altogether, GD-HUVEC exhibited poor performance for most of the measured tube-formation parameters. Generally, the quantification of all these parameters suggested that OA generally improved angiogenesis, with clear healing tendencies for the GD-HUVECs.

Oleanolic acid enhances C- and GD-HUVEC migration

Cell migration, a crucial process in wound healing to restore skin integrity, is enhanced by OA in epithelial cells (7, 8).

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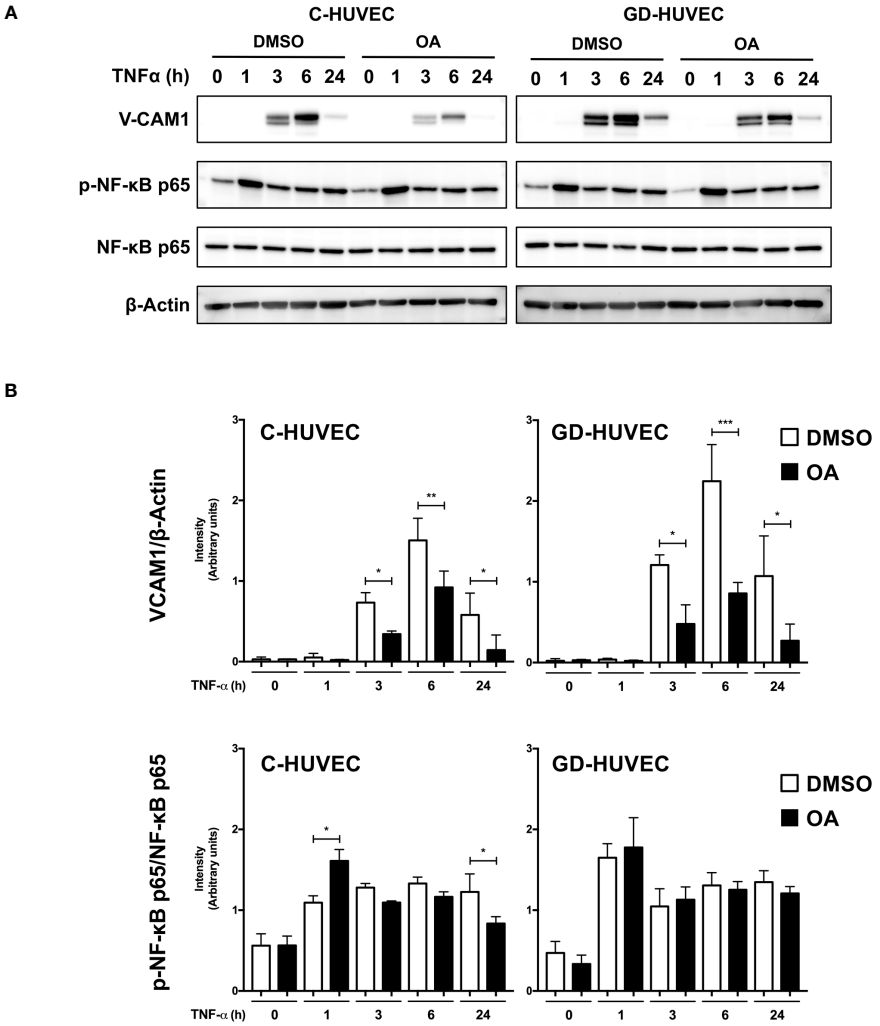

FIGURE 2
Oleanolic acid reduces total VCAM1 protein expression in C- and GD-HUVECs induced by TNF-α. **(A)** Total protein extracts from sub-confluent C- and GD-HUVECs pre-treated with 20 μM OA or DMSO equivalent volume, and then stimulated with TNF-α at 1, 3, 6, and 24 h. These extracts were assayed at these times targeting the following: VCAM1, phospho-NF-κB, and NF-κB. β-Actin was used as a loading control. A representative experiment is shown. **(B)** Column bar graphs represent intensity values of each protein assayed by Western blot, by collecting the data of four C-HUVEC and four GD-HUVEC strains. Intensity values were quantified and gathered by ImageJ software. Asterisks indicate statistically significant differences between the selected conditions according to a one-way ANOVA statistical analysis: (*$p < 0.05$, **$p < 0.005$, and ***$p < 0.001$).

Interestingly enough, migration also contributes to the organization and formation of new vessels (47). Therefore, we performed scratch assays on confluent C- and GD-HUVECs to see whether OA could also have this effect on endothelial cells. C- and GD-HUVECs were scratched and allowed to migrate for 12 h in the presence of OA (Figure 5A). Strikingly, OA activity promoted the migration of both C- and GD-HUVECs from the wound edges, since the wound gap area was surrounded with endothelial cells. To better comprehend the level of this promoting effect of OA, cell migration was quantified measuring the resulting areas of the wounds (Figure 5B). Thus, the obtained migration percentages in both C- and GD-HUVECs were clearly significant between basal condition and OA. Interestingly, 20% FBS was used as a positive control of cell migration, but the resulting migration with GD-HUVEC under this condition was significantly lower than with OA.

Given these results with wound healing scratch assays on HUVECs, OA showed cell migration promoting effects on endothelial cells that could probably enhance the wound healing process together with neo-angiogenesis.

Oleanolic acid increases focal adhesion number in C- and GD-HUVECs and their dynamization

It is known that OA-triggered molecular effects on cell migration include the role of cell architecture, by dynamizing actin cytoskeleton and FA remodeling (9, 48, 49). We performed immunocytochemistry assays in sub-confluent C- and GD-HUVECs targeting actin fibbers (F-actin) and paxillin to reveal

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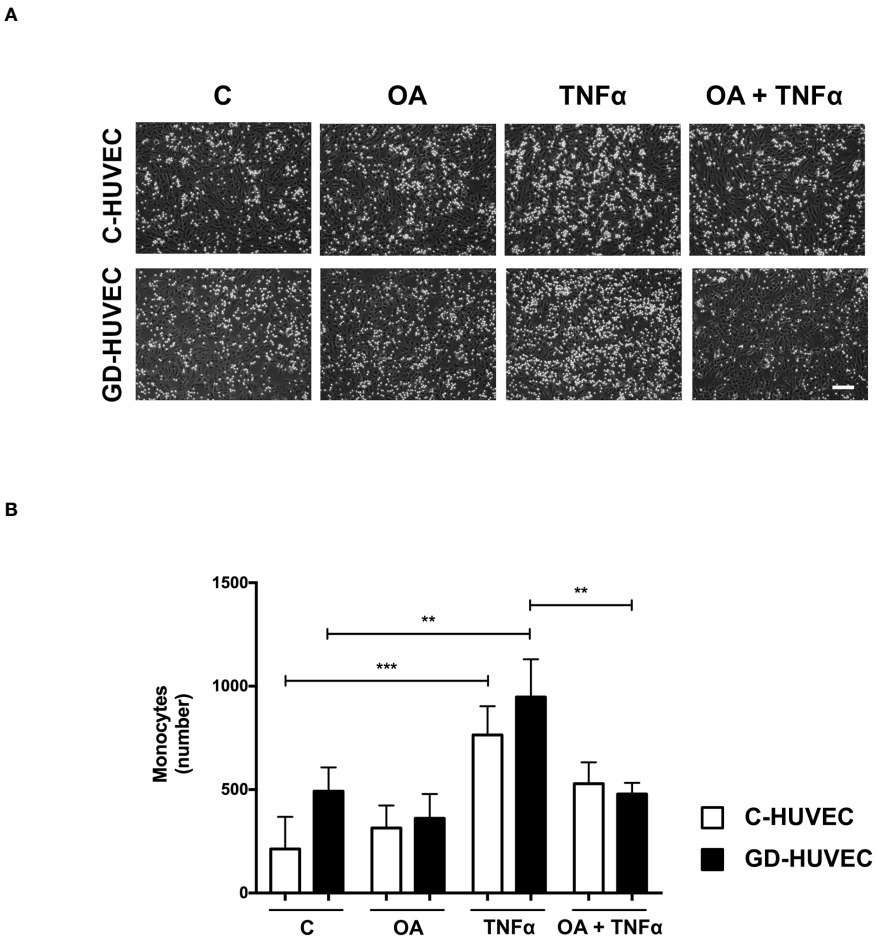

FIGURE 3
Oleanolic acid reduces the number of monocytes adhered to C- and GD-HUVECs. **(A)** For monocyte adhesion experiment, C- and GD-HUVEC monolayers were left untreated unless otherwise indicated (OA), where they were pre-treated with 20 μM OA for 24 h. Subsequently, TNF-α was added for 16 h to either untreated or OA pre-treated HUVECs (TNF-α and OA+TNF-α conditions). At this point, monocytes were added and the experiment was completed. Representative pictures of C- and GD-HUVECs are shown for each condition. **(B)** Graph represents the number of adhered U937 monocytes in each field of 12 fields. Each condition represents the mean ± SEM obtained from the data collection of four different strains for both C-HUVECs and GD-HUVECs. Asterisks indicate statistically significant differences between the selected conditions according to a one-way ANOVA statistical analysis (*$p < 0.05$, **$p < 0.001$). The scale bar indicates 100 μm.

FAs. With regard to cell morphology, untreated C- and GD-HUVECs exhibited a morphology related to a stressed condition due to the low amount of FBS (0.1%), with no apparent FA-like structures (Figure 6A). By contrast, C- and GD-HUVECs treated with OA displayed filopodia and lamellipodia garnished with FAs. Actin fibers were also modified by OA presence, because they were encompassing the newly formed filopodia and lamellipodia in response to OA. Interestingly and in line with this, the quantified FA density, revealed by paxillin staining, exhibited a significant increase in OA-treated C- and GD-HUVECs versus untreated ones (Figure 6B). This increase was even more significant in GD-HUVECs than in C-HUVECs.

Actin fiber and FA data revealed that HUVECs changed their cell architecture during OA-stimulated cell migration, thus suggesting a high dynamization of the migration-related machinery in both C- and GD-HUVECs.

Discussion

The results of this study provide intriguing insights into the effects of OA treatment on C- and GD-HUVECs in the context of inflammation and angiogenesis.

Plant-derived bioactive compounds present in various dietary sources have been widely studied for their significant effects to rescue endothelial cell function (50–52). Their antioxidant, anti-inflammatory, vasodilatory, angiogenic, and protective properties collectively contribute to the preservation of vascular health and the prevention of endothelial dysfunction-associated diseases (53–55). Indeed, a large number of these bioactive molecules or peptides modulate the signaling pathway of nuclear factor-kappa B (NF-κB), which is needed for adhesion molecules and pro-inflammatory cytokine expression (43, 56). For instance, studies have shown that carotenoids lycopene and β-carotene have anti-inflammatory

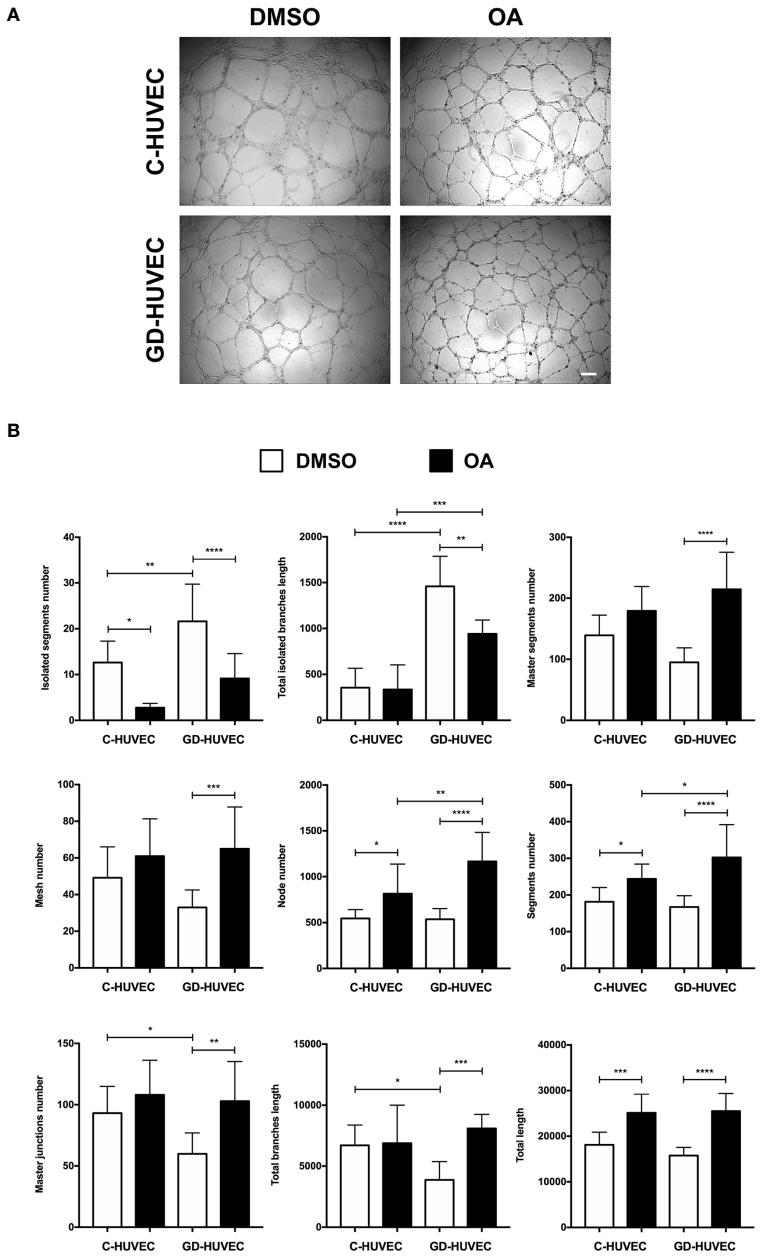

FIGURE 4
Effect of OA on tube-like structure formation capacity on Matrigel. Tube-like structure formation ability on Matrigel after 6-h treatment with 20 μM OA; a DMSO equivalent volume was added as control condition. **(A)** Representative pictures of C- and GD-HUVECs for both experimental conditions. Scale bar indicates 200 μm. **(B)** Graphs representing multiple angiogenic parameters analyzed: number of isolated segments, total length of isolated branches, number of master segments, number of meshes, number of nodes, number of segments, number of master junctions, total length of branches, and total length. Each bar in the plot represents the mean ± SEM using three different strains for C-HUVECs and also three for GD-HUVECs. Asterisks indicate statistically significant differences between the selected conditions according to a one-way ANOVA statistical analysis (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, and ****$p < 0.0001$).

effects on both C- and GD-HUVECs (31, 33). Indeed, the addition of these carotenoids under TNF-α stimulation show less monocyte–endothelial cell interaction, enhanced by less ICAM1 and VCAM1 membrane exposure and total expression. All these effects depend on the attenuation that these carotenoids have on NF-κB phosphorylation and translocation to the cell nucleus (31, 33). In fact, the effects of pentacyclic triterpenes, OA, and its isomers ursolic acid (UA) and maslinic acid (MA) are similar to carotenoids and have been addressed *in vitro* by using regular HUVEC phenotype. Thus, these studies showed attenuation effects on adhesion molecule expression under inflammation conditions (29, 30, 57). However, there are no studies so far on the effects of OA on hyperglycemia-modified cells (GD-HUVECs), which have remarkably impaired functionality. Moreover, the concentration of serum used in those assays was not always clarified, a factor that is critical to properly study OA effects *in vitro*, since serum proteins

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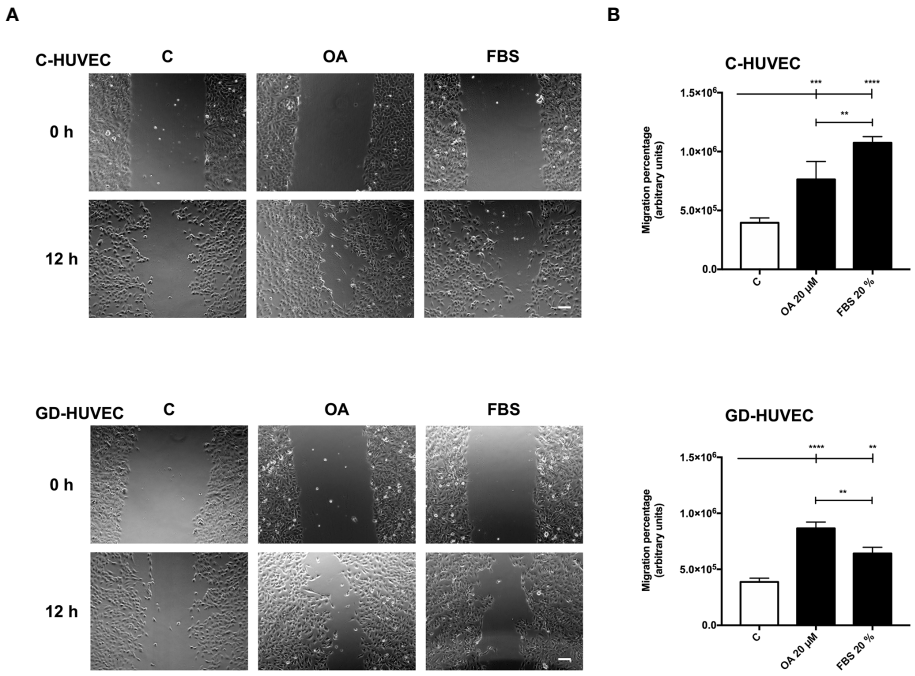

FIGURE 5
Oleanolic acid induces C- and GD-HUVEC migration in wound healing scratch assays. Confluent C- and GD-HUVECs were scratched with a pipette tip and allowed to migrate for 12 h. **(A)** Representative images of the wound healing assay with cell migration under basal conditions (control, C), compared to those with 20 µM OA after 24-h treatment. A condition with 20% FBS was added as positive-migration control. Scale bar indicates 200 µm. **(B)** Graphs represent C- and GD-HUVEC migration as the difference between areas at 0 h and 12 h in each condition, named as migration percentage. Asterisks indicate statistically significant differences between conditions according to a one-way ANOVA statistical analysis (**$p < 0.005$, ***$p < 0.001$, and ****$p < 0.0001$).

buffer OA activity and modify its optimal concentration of use (7, 8). Indeed, OA effects and bioavailability depend on the final OA concentration, the cell type used, and the serum concentration. High concentrations of OA produce cytotoxic and antiproliferative effects, while low concentrations do not produce any beneficial effect on cells (8, 58, 59). This is the reason why, in the present study, an MTT assay was conducted with C- and GD-HUVECs under the lowest possible serum concentration (0.5% FBS). In this way, a 20 µM OA concentration was established seeking a compromise between the optimal effects of OA and the abolition of the serum buffer effect, together with cell viability compatibility.

Furthermore, it should be noted that OA treatments, followed by TNF-α induction, should be performed at longer incubation times to unravel OA ameliorative effects on inflammation (30). Indeed, the highest expression of adhesion molecules *ICAM1*, *VCAM1*, and *SELE* in C- and GD-HUVECs was detected at 2 h and 6 h after TNF-α addition, thus showing a clear inflammatory profile. Strikingly, pre-treating the same endothelial cells with OA before TNF-α clearly attenuated adhesion molecule overexpression by the cytokine, especially on *VCAM1* and *SELE*. In addition to this, the preventive effect produced by OA was even more patent in GD-HUVECs, probably because of their senescent phenotype and endothelial dysfunction (26). Interestingly enough, in the case of *ICAM1*, at 24 h, gene expression levels were not fully abrogated by OA in C- and GD-HUVECs. This could be explained by other functions of this integrin, since controlled levels of ICAM1 on the cell surface are needed during wound healing to promote endothelial cell migration, thus leading to neo-angiogenesis (60). In particular, *VCAM1* showed the strongest attenuation by OA in C- and GD-HUVECs, and also showed decreased protein amount. However, we saw a window transient effect of TNF-α, since VCAM1 levels decreased at 24 h. Despite this, we still observed the mitigating effect of OA on VCAM1 protein levels. It should be highlighted that *in vitro* assays have this limitation, because, in a tissue with chronic inflammation, we would see sustained high levels of VCAM1 and other adhesion molecules due to the constant production of TNF-α and other pro-inflammatory cytokines (61). VCAM1 is endothelium-specific and this TNF-α inducible molecule is necessary for monocyte extravasation (44). NF-κB activation by its phosphorylation on the p65 subunit is required to promote adhesion molecule expression in the cell nucleus, such as *VCAM1*, among others (43). Although we observed lower VCAM1 protein levels with OA pre-treatment, we did not observe any differences on phospho-NF-κB p65. In contrast, in a similar set of experiments, the precondition of HUVECs with amniotic membrane was able to reduce the levels of phosphorylation of phospho-Ser-536 NF-κB p65 in response to TNF-α, which was coherent with an attenuation of the NF-κB p65 nuclear translocation and a reduction of the expression of *VCAM1* (28). Thus, our results have to be explained by the fact that the OA attenuation effect on VCAM1 could be due to different molecular mechanisms or also to the way it is synthesized. In fact, it has been

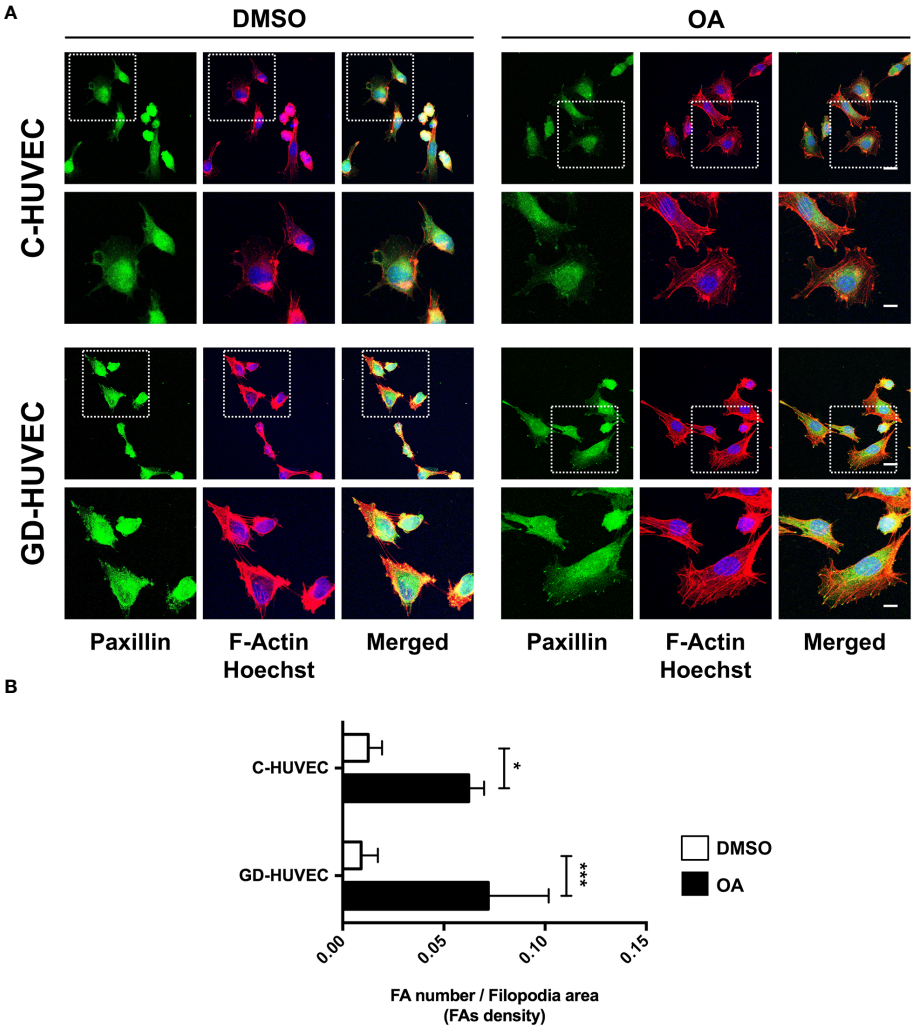

FIGURE 6
Oleanolic acid triggers focal adhesion remodeling in C- and GD-HUVECs revealed by paxillin. **(A)** Sub-confluent C- and GD-HUVECs were treated for 24 h with 20 μM OA and DMSO equivalent volume. Cells were immunostained with specific antibodies against paxillin. Co-staining with phalloidin and Hoechst-33258 was used to show actin cytoskeleton and nuclei, respectively. Paxillin: green. Actin fibers (F-Actin): red. Nuclei: blue. Images obtained with a confocal microscope at 63× magnification and their corresponding insets for a detailed view of paxillin structures. This experiment was repeated at least three times. 63× picture scale bar indicates 25 μm. Inset scale bar indicates 5 μm. **(B)** Column bar graphs show the quantification of the density of FAs (as FA number per filopodia area). Asterisks indicate statistically significant differences between conditions according to a one-way ANOVA statistical analysis (*$p < 0.05$ and ***$p < 0.001$).

shown that UA, an OA isomer, blocks VCAM1 traffic to the membrane (62). Another possibility could be that the amount of anchored-membrane VCAM1 is regulated by proteases, where specifically TNF-α converting enzyme (TACE/ADAM17) proteolyzes this molecule and releases it to the extracellular medium (44). Therefore, OA could be enhancing TACE/ADAM17 protease activity on VCAM1, thus decreasing its protein levels in endothelial cells. However, a complicated regulation must be involved, since the expression of *VCAM1* is effectively attenuated by the presence of OA. A more plausible, although uncertain, mechanism of regulation could be related to something different from NF-κB transcription factor or even its phosphorylation at Ser 536 residue. Further research is necessary to better clarify the mechanism behind *VCAM1* regulation in this context.

An excessive release of TNF-α in chronic inflammation conditions produces the overexpression of adhesion molecules on the endothelium surface. As a consequence, the uncontrolled adhesion and transmigration of immune cells occur, thus triggering endothelial cell apoptosis (42). E-selectin acts at the first steps of monocyte recruiting to produce their tethering and rolling (63). Then, integrins ICAM1 and VCAM1 secure the adhesion and allow monocyte extravasation to the injured wound (64). In a chronic wound, the high recruitment of monocytes leads to an uncontrolled population of M1 macrophages in the wound, which have hyperinflammatory, reduced phagocytic activity, and increase oxidative stress (14). By

contrast, a regular recruitment of monocyte population swings toward M2 macrophages, with anti-inflammatory, regenerative, and tissue remodeling properties, all in line with a healing wound (14). OA effects regarding monocyte adhesion are strongly coherent with the observed changes of adhesion molecule levels. In the context of either chronic or diabetic wounds, OA-reduced levels of VCAM1 on endothelial cells surely imply a better inflammation resolution.

Diabetes negatively affects angiogenesis (22, 24). Considering tube formation assays, GD-HUVEC exhibited a poorer tube formation; for instance, the number and length of isolated segments in basal condition was higher in GD-HUVEC. Strikingly, OA treatment clearly ameliorated this impairment in GD-HUVECs and, although more lightly, also in C-HUVECs. Coherently, positive features such as the number of master segments, the number of meshes, and the length of branches were significantly improved by OA only in GD-HUVECs. Indeed, this could be explained by the GD phenotype, which, in contrast to C-HUVEC, showed poorer performance for these parameters in basal conditions. This behaviour strongly suggests that OA restores GD-HUVEC to a more regular angiogenic phenotype, but does not intrinsically affect C-HUVEC's capability of achieving a full network. Overall, the network complexities achieved for both types of cells were higher with OA, as reflected by the observed incremented number of nodes, branches, master junctions, and the total length of the networks. These changes indicate that OA enhances all aspects of the complexity of the vascular network, which may have a positive impact on tissue regeneration in a complex healing wound milieu. Nonetheless, a good line of research could be testing the effects of OA on more complex systems, because tube formation assays on Matrigel do not compile/integrate endothelial cell interaction with other cell types, as happens during neo-angiogenesis in a real wound. Therefore, a 3D co-culture of endothelial cells with both primary fibroblasts and keratinocytes, which exhibit features more similar to natural skin, could be considered a good option to further assess the effects of OA on wound healing (65). Moreover, there are well-established *in vivo* angiogenesis assays that can unravel potential OA effects; for instance, one of the best is chorionallantoic membrane assays in chicken embryo, which are widely used in vascular biology (66). Regarding the potential molecular effects behind OA angiogenesis promotion, we would like to conduct future experiments in order to study the effects of OA on the stimulation of VEGFR-2, given its importance in angiogenesis (15, 67, 68), and due to the fact that OA has been directly involved in the activation of the similar function and structure receptor: EGFR (7, 8).

Cell migration is carried out by endothelial cells together with proliferation to enhance angiogenesis and vasculogenesis (69). According to the data of the scratch assays, OA was also capable of enhancing this process in both C- and GD-HUVECs to the same extent. However, FBS stimulation was unable to match the levels achieved by the OA stimulation for the GD phenotype. These data indicate that OA, but not FBS, rescues, in GD-HUVEC, an impaired migration mechanism resistant to the serum rescue. Thus, OA may trigger a particular molecular mechanism that is revealed only in the GD-HUVEC-impaired cells. Indeed, the quantification of FA density upon treatment with OA, detected by paxillin immunostaining, was stronger in GD-HUVECs than in C-HUVECs. Overall, the collected data of FAs strongly suggest that, generally, OA promotes a better endothelial cell movement to manage migration in both GD- and C-HUVECs. OA also contributes positively to angiogenesis by cell migration promotion, which is a favorable condition for tissue repair in a wound healing context (69).

Our findings suggest a clear OA ability to rescue the altered features of an endothelium affected by high blood sugar levels, which correlate with impaired metabolism and inflammation (25, 42, 70). It is well-known that, in order to ameliorate these processes, signaling pathways depending on the activation of G protein-coupled receptors (GPCRs) take place to protect cells from injury and malfunction. Concretely, the Takeda G protein-coupled receptor (TGR5), also known as Gpbbar1, is a transmembrane-type bile acid receptor that has been found to regulate a large number of specific molecular pathways (71, 72). Interestingly, TGR5 modulates inflammation by decreasing adhesion molecule expression in endothelial cells and blocking pro-inflammatory cytokines released by immune cells (71). Moreover, this receptor is also linked to tyrosine kinase receptor (RTK) transactivation by second messengers (73, 74). Strikingly, some evidence points out the interaction between OA, which has a similar chemical structure to bile acids, and TGR5, with OA behaving as a clear agonist of TGR5 (75). For these reasons, it is remarkable to suggest that probably all OA promotion and modulation effects related to monocyte adhesion, angiogenesis, and cell migration on C- and GD-HUVECs could be related to the interaction between TGR5 and OA. Thus, more research is needed to decipher this molecular mechanism, which may solve some of the conundrums revealed in our data. In addition, other mechanisms may be involved under OA effects regarding regulatory non-coding RNA expression, as microRNAs (miRs) have recently gained prominence due to their role in regulating several essential processes in endothelial cells (76, 77). For instance, it is shown that miR-4432 controls the expression of fibroblast growth factor binding protein 1 (FGFBP1), which is needed to preserve endothelial barrier function in the brain (78). On top of that, other studies reported that low expression levels of miR-145 and miR-885 cause thrombotic risk and mortality in COVID-19 patients; thus, the expression of these miRs in endothelial cells is critical to prevent a prothrombotic condition during the infection (79). Therefore, further studies focusing on OA's contribution to these miR expressions in endothelial cells seem very pertinent.

To sum up, this study sheds some light on the multifaceted effects of OA on inflammation, angiogenesis, and migration in C- and GD-HUVECs (Figure 7). The findings underline the potential of OA as a therapeutic agent for restoring vascular function and ameliorating inflammation excess in diabetic wounds. However,

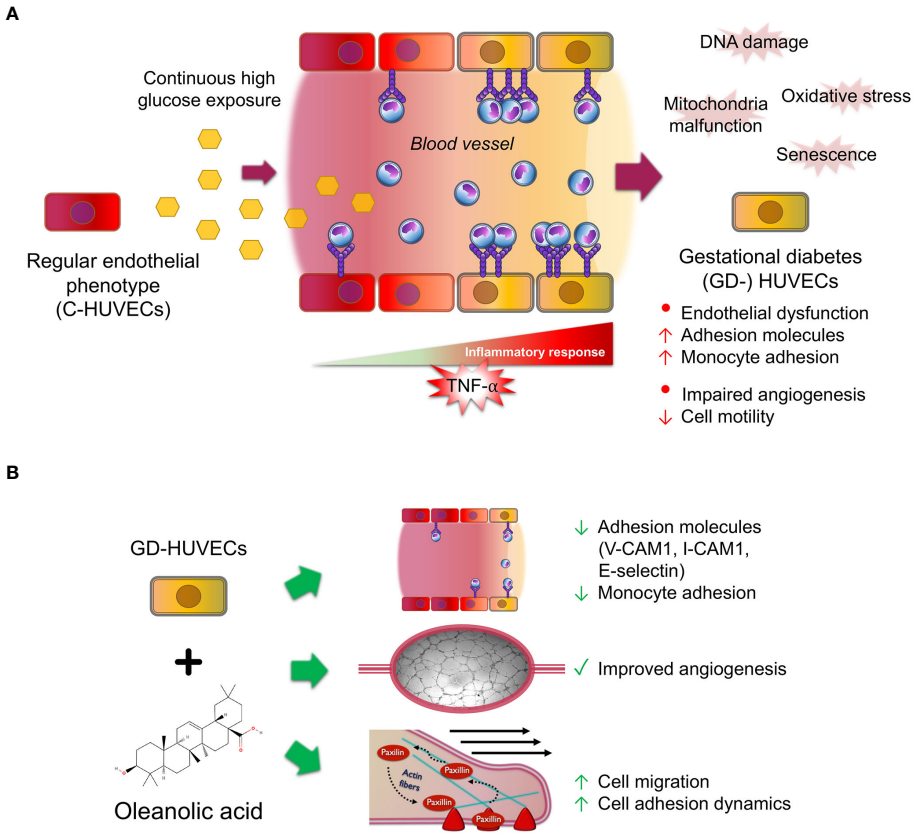

FIGURE 7
Oleanolic acid rescues multi-affected GD-HUVEC features caused by high blood glucose levels. **(A)** When regular endothelial cells (C-HUVECs) are exposed to a continuous high glucose exposure in blood vessels, several changes on their phenotype occur. These cells undergo the effects of high oxidative stress and damages on their DNA and mitochondria malfunction, thus triggering cellular senescence. As a result, these cells have an excessive and uncontrolled inflammation response when stimulated by pro-inflammatory cytokines such as TNF-α, triggering high adhesion molecule exposure on endothelium surface, subsequently displaying a high recruitment of circulating monocytes, and resulting in endothelial dysfunction. Moreover, these cells have an aberrant and limp tube formation (angiogenesis). **(B)** Strikingly, OA pre-treatment in GD-HUVECs before TNF-α addition attenuates key adhesion molecule overexpression of VCAM1, ICAM1, and E-selectin, resulting in less adhesion of the monocytes. Furthermore, OA displayed promotion effects on GD-HUVECs by restoring the impaired angiogenesis. Moreover, cell migration, a crucial process for angiogenesis, is also promoted by OA because it increases endothelial cell migration and adhesion dynamics by focal adhesion formation.

further research is needed to unravel the precise underlying molecular mechanisms driving these effects in order to evaluate the translational potential of OA clinical treatments for the management of complex wounds.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Institutional Committee on Human Experimentation (reference number 1879/09COET). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JS-F: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. IC: Data curation, Investigation, Methodology, Writing – review & editing. AP: Conceptualization, Data curation, Funding acquisition, Supervision, Validation, Writing – review & editing. JG: Funding acquisition, Project administration, Supervision, Writing – review & editing. CP: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – review & editing. FN: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1308606/full#supplementary-material

References

1. Vicente-Manzanares M, Horwitz AR. Cell migration: an overview. *Methods Mol Biol* (2011) 769:1–24. doi: 10.1007/978-1-61779-207-6_1

2. Trepat X, Chen Z, Jacobson K. Cell migration. *Compr Physiol* (2012) 2(4):2369–92. doi: 10.1002/cphy.c110012

3. Moura-Letts G, Villegas LF, Marcalo A, Vaisberg AJ, Hammond GB. *In vivo* wound-healing activity of oleanolic acid derived from the acid hydrolysis of Anredera diffusa. *J Nat Prod* (2006) 69(6):978–9. doi: 10.1021/np0601152

4. Pollier J, Goossens A. Oleanolic acid. *Phytochemistry* (2012) 77:10–5. doi: 10.1016/j.phytochem.2011.12.022

5. Ayeleso TB, Matumba MG, Mukwevho E. Oleanolic acid and its derivatives: biological activities and therapeutic potential in chronic diseases. *Molecules* (2017) 22 (11). doi: 10.3390/molecules22111915

6. Kuonen R, Weissenstein U, Urech K, Kunz M, Hostanska K, Estko M, et al. Effects of lipophilic extract of viscum album L. and oleanolic acid on migratory activity of NIH/3T3 fibroblasts and on haCat keratinocytes. *Evid Based Complement Alternat Med* (2013) 2013:718105. doi: 10.1155/2013/718105

7. Stelling-Ferez J, Gabaldon JA, Nicolas FJ. Oleanolic acid stimulation of cell migration involves a biphasic signaling mechanism. *Sci Rep* (2022) 12(1):15065. doi: 10.1038/s41598-022-17553-w

8. Bernabe-Garcia A, Armero-Barranco D, Liarte S, Ruzafa-Martinez M, Ramos-Morcillo AJ, Nicolas FJ. Oleanolic acid induces migration in Mv1Lu and MDA-MB-231 epithelial cells involving EGF receptor and MAP kinases activation. *PloS One* (2017) 12 (2):e0172574. doi: 10.1371/journal.pone.0172574

9. Turner CE. Paxillin and focal adhesion signalling. *Nat Cell Biol* (2000) 2(12): E231–6. doi: 10.1038/35046659

10. Schaller MD. Paxillin: a focal adhesion-associated adaptor protein. *Oncogene* (2001) 20(44):6459–72. doi: 10.1038/sj.onc.1204786

11. Schaks M, Giannone G, Rottner K. Actin dynamics in cell migration. *Essays Biochem* (2019) 63(5):483–95. doi: 10.1042/EBC20190015

12. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med* (1999) 341 (10):738–46. doi: 10.1056/NEJM199909023411006

13. Childs DR, Murthy AS. Overview of wound healing and management. *Surg Clin North Am* (2017) 97(1):189–207. doi: 10.1016/j.suc.2016.08.013

14. Aitcheson SM, Frentiu FD, Hurn SE, Edwards K, Murray RZ. Skin wound healing: normal macrophage function and macrophage dysfunction in diabetic wounds. *Molecules* (2021) 26(16):4917. doi: 10.3390/molecules26164917

15. Tonnesen MG, Feng X, Clark RA. Angiogenesis in wound healing. *J Investig Dermatol Symp Proc* (2000) 5(1):40–6. doi: 10.1046/j.1087-0024.2000.00014.x

16. Guerra A, Belinha J, Jorge RN. Modelling skin wound healing angiogenesis: A review. *J Theor Biol* (2018) 459:1–17. doi: 10.1016/j.jtbi.2018.09.020

17. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. *Clin Dermatol* (2007) 25(1):19–25. doi: 10.1016/j.clindermatol.2006.12.005

18. Qing C. The molecular biology in wound healing & non-healing wound. *Chin J Traumatol.* (2017) 20(4):189–93. doi: 10.1016/j.cjtee.2017.06.001

19. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, et al. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen.* (2003) 11 Suppl 1:S1–28. doi: 10.1046/j.1524-475X.11.s2.1.x

20. Greenhalgh DG. Wound healing and diabetes mellitus. *Clin Plast Surg* (2003) 30 (1):37–45. doi: 10.1016/S0094-1298(02)00066-4

21. Ferreira MC, Tuma P Jr., Carvalho VF, Kamamoto F. Complex wounds. *Clinics (Sao Paulo).* (2006) 61(6):571–8. doi: 10.1590/S1807-59322006000600014

22. Burgess JL, Wyant WA, Abdo Abujamra B, Kirsner RS, Jozic I. Diabetic wound-healing science. *Medicina (Kaunas).* (2021) 57(10):1072. doi: 10.3390/medicina57101072

23. Stachura A, Khanna I, Krysiak P, Paskal W, Wlodarski P. Wound healing impairment in type 2 diabetes model of leptin-deficient mice-A mechanistic systematic review. *Int J Mol Sci* (2022) 23(15):8621. doi: 10.3390/ijms23158621

24. Okonkwo UA, DiPietro LA. Diabetes and wound angiogenesis. *Int J Mol Sci* (2017) 18(7):1419. doi: 10.3390/ijms18071419

25. Di Fulvio P, Pandolfi A, Formoso G, Di Silvestre S, Di Tomo P, Giardinelli A, et al. Features of endothelial dysfunction in umbilical cord vessels of women with gestational diabetes. *Nutr Metab Cardiovasc Dis* (2014) 24(12):1337–45. doi: 10.1016/j.numecd.2014.06.005

26. Di Tomo P, Alessio N, Falone S, Pietrangelo L, Lanuti P, Cordone V, et al. Endothelial cells from umbilical cord of women affected by gestational diabetes: A suitable in *vitro* model to study mechanisms of early vascular senescence in diabetes. *FASEB J* (2021) 35(6):e21662. doi: 10.1096/fj.202002072RR

27. Medina-Leyte DJ, Domínguez-Pérez M, Mercado I, Villarreal-Molina MT, Jacobo-Albavera L. Use of human umbilical vein endothelial cells (HUVEC) as a model to study cardiovascular disease: A review. *Appl Sci* (2020) 10(3):938. doi: 10.3390/app10030938

28. Pipino C, Bernabe-Garcia A, Cappellacci I, Stelling-Ferez J, Di Tomo P, Santalucia M, et al. Effect of the human amniotic membrane on the umbilical vein endothelial cells of gestational diabetic mothers: new insight on inflammation and angiogenesis. *Front Bioeng Biotechnol* (2022) 10:854845. doi: 10.3389/fbioe.2022.854845

29. Lee W, Yang EJ, Ku SK, Song KS, Bae JS. Anti-inflammatory effects of oleanolic acid on LPS-induced inflammation in *vitro* and in vivo. *Inflammation* (2013) 36(1):94–102. doi: 10.1007/s10753-012-9523-9

30. Takada K, Nakane T, Masuda K, Ishii H. Ursolic acid and oleanolic acid, members of pentacyclic triterpenoid acids, suppress TNF-alpha-induced E-selectin

expression by cultured umbilical vein endothelial cells. *Phytomedicine* (2010) 17 (14):1114–9. doi: 10.1016/j.phymed.2010.04.006

31. Ucci M, Di Tomo P, Tritschler F, Cordone VGP, Lanuti P, Bologna G, et al. Anti-inflammatory role of carotenoids in endothelial cells derived from umbilical cord of women affected by gestational diabetes mellitus. *Oxid Med Cell Longev* (2019) 2019:8184656. doi: 10.1155/2019/8184656

32. Unger RE, Krump-Konvalinkova V, Peters K, Kirkpatrick CJ. *In vitro* expression of the endothelial phenotype: comparative study of primary isolated cells and cell lines, including the novel cell line HPMEC-ST1.6R. *Microvasc Res* (2002) 64(3):384–97. doi: 10.1006/mvre.2002.2434

33. Di Tomo P, Canali R, Ciavardelli D, Di Silvestre S, De Marco A, Giardinelli A, et al. beta-Carotene and lycopene affect endothelial response to TNF-alpha reducing nitro-oxidative stress and interaction with monocytes. *Mol Nutr Food Res* (2012) 56 (2):217–27. doi: 10.1002/mnfr.201100500

34. Di Tomo P, Di Silvestre S, Cordone VGP, Giardinelli A, Faricelli B, Pipino C, et al. Centella Asiatica and Lipoic Acid, or a combination thereof, inhibit monocyte adhesion to endothelial cells from umbilical cords of gestational diabetic women. *Nutrition Metab Cardiovasc Dis* (2015) 25(7):659–66. doi: 10.1016/j.numecd.2015.04.002

35. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* (2001) 25 (4):402–8. doi: 10.1006/meth.2001.1262

36. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc* (2018) 2018(6). doi: 10.1101/pdb.prot095505

37. Ernst O, Zor T. Linearization of the bradford protein assay. *J Vis Exp* (2010) 38). doi: 10.3791/1918-v

38. Carpentier G, Berndt S, Ferratge S, Rasband W, Cuendet M, Uzan G, et al. Angiogenesis Analyzer for ImageJ — A comparative morphometric analysis of "Endothelial Tube Formation Assay" and "Fibrin Bead Assay". *Sci Rep* (2020) 10 (1):11568. doi: 10.1038/s41598-020-67289-8

39. Liarte S, Bernabe-Garcia A, Armero-Barranco D, Nicolas FJ. Microscopy based methods for the assessment of epithelial cell migration during *in vitro* wound healing. *J Vis Exp* (2018) 131). doi: 10.3791/56799

40. Horzum U, Ozdil B, Pesen-Okvur D. Step-by-step quantitative analysis of focal adhesions. *MethodsX* (2014) 1:56–9. doi: 10.1016/j.mex.2014.06.004

41. Reglero-Real N, Colom B, Bodkin JV, Nourshargh S. Endothelial cell junctional adhesion molecules: role and regulation of expression in inflammation. *Arterioscler Thromb Vasc Biol* (2016) 36(10):2048–57. doi: 10.1161/ATVBAHA.116.307610

42. Theofilis P, Sagris M, Oikonomou E, Antonopoulos AS, Siasos G, Tsioufis C, et al. Inflammatory mechanisms contributing to endothelial dysfunction. *Biomedicines* (2021) 9(7):781. doi: 10.3390/biomedicines9070781

43. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* (2009) 1(6):a001651. doi: 10.1101/cshperspect.a001651

44. Troncoso MF, Ortiz-Quintero J, Garrido-Moreno V, Sanhueza-Olivares F, Guerrero-Moncayo A, Chiong M, et al. VCAM-1 as a predictor biomarker in cardiovascular disease. *Biochim Biophys Acta Mol Basis Dis* (2021) 1867(9):166170. doi: 10.1016/j.bbadis.2021.166170

45. Sakurai H, Suzuki S, Kawasaki N, Nakano H, Okazaki T, Chino A, et al. Tumor Necrosis Factor-α-induced IKK Phosphorylation of NF-κB p65 on Serine 536 Is Mediated through the TRAF2, TRAF5, and TAK1 Signaling Pathway*. *J Biol Chem* (2003) 278(38):36916–23. doi: 10.1074/jbc.M301598200

46. Flores AI, Pipino C, Jerman UD, Liarte S, Gindraux F, Kreft ME, et al. Perinatal derivatives: How to best characterize their multimodal functions in vitro. *Part C: Inflammation angiogenesis Wound healing. Front Bioeng Biotechnol* (2022) 10:965006. doi: 10.3389/fbioe.2022.965006

47. Lamalice L, Le Boeuf F, Huot J. Endothelial cell migration during angiogenesis. *Circ Res* (2007) 100(6):782–94. doi: 10.1161/01.RES.0000259593.07661.1e

48. Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: in command and control of cell motility. *Nat Rev Mol Cell Biol* (2005) 6(1):56–68. doi: 10.1038/nrm1549

49. Hu YL, Lu S, Szeto KW, Sun J, Wang Y, Lasheras JC, et al. FAK and paxillin dynamics at focal adhesions in the protrusions of migrating cells. *Sci Rep* (2014) 4:6024. doi: 10.1038/srep06024

50. Duffy SJ, Keaney JF Jr., Holbrook M, Gokce N, Swerdloff PL, Frei B, et al. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation* (2001) 104(2):151–6. doi: 10.1161/01.CIR.104.2.151

51. Jia Z, Babu PV, Si H, Nallasamy P, Zhu H, Zhen W, et al. Genistein inhibits TNF-alpha-induced endothelial inflammation through the protein kinase pathway A and improves vascular inflammation in C57BL/6 mice. *Int J Cardiol* (2013) 168(3):2637–45. doi: 10.1016/j.ijcard.2013.03.035

52. Ried K, Frank OR, Stocks NP. Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. *Eur J Clin Nutr* (2013) 67(1):64–70. doi: 10.1038/ejcn.2012.178

53. Kanitkar M, Gokhale K, Galande S, Bhonde RR. Novel role of curcumin in the prevention of cytokine-induced islet death in *vitro* and diabetogenesis in vivo. *Br J Pharmacol* (2008) 155(5):702–13. doi: 10.1038/bjp.2008.311

54. Bagul PK, Middela H, Matapally S, Padiya R, Bastia T, MadhuSudana K, et al. Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. *Pharmacol Res* (2012) 66(3):260–8. doi: 10.1016/j.phrs.2012.05.003

55. Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM. Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. *Can J Cardiol* (2015) 31(5):631–41. doi: 10.1016/j.cjca.2015.02.008

56. Martinez-Sanchez SM, Perez-Sanchez H, Antonio Gabaldon J, Abellan-Aleman J, Montoro-Garcia S. Multifunctional peptides from spanish dry-cured pork ham: endothelial responses and molecular modeling studies. *Int J Mol Sci* (2019) 20(17):4204. doi: 10.3390/ijms20174204

57. Hsuan CF, Hsu HF, Tseng WK, Lee TL, Wei YF, Hsu KL, et al. Glossogyne tenuifolia Extract Inhibits TNF-alpha-Induced Expression of Adhesion Molecules in Human Umbilical Vein Endothelial Cells *via* Blocking the NF-kB Signaling Pathway. *Molecules* (2015) 20(9):16908–23. doi: 10.3390/molecules200916908

58. He Y, Liu X, Huang M, Wei Z, Zhang M, He M, et al. Oleanolic acid inhibits the migration and invasion of hepatocellular carcinoma cells by promoting microRNA-122 expression. *Pharmazie* (2021) 76(9):422–7. doi: 10.1691/ph.2021.1366

59. Liu J, Ban H, Liu Y, Ni J. The expression and significance of AKR1B10 in laryngeal squamous cell carcinoma. *Sci Rep* (2021) 11(1):18228. doi: 10.1038/s41598-021-97648-y

60. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol* (2020) 108(3):787–99. doi: 10.1002/JLB.2MR0220-549R

61. Hong YK, Chang YH, Lin YC, Chen B, Guevara BEK, Hsu CK. Inflammation in wound healing and pathological scarring. *Adv Wound Care (New Rochelle).* (2023) 12 (5):288–300. doi: 10.1089/wound.2021.0161

62. Mitsuda S, Yokomichi T, Yokoigawa J, Kataoka T. Ursolic acid, a natural pentacyclic triterpenoid, inhibits intracellular trafficking of proteins and induces accumulation of intercellular adhesion molecule-1 linked to high-mannose-type glycans in the endoplasmic reticulum. *FEBS Open Bio.* (2014) 4:229–39. doi: 10.1016/j.fob.2014.02.009

63. Silva M, Videira PA, Sackstein R. E-selectin ligands in the human mononuclear phagocyte system: implications for infection, inflammation, and immunotherapy. *Front Immunol* (2017) 8:1878. doi: 10.3389/fimmu.2017.01878

64. Medrano-Bosch M, Simon-Codina B, Jimenez W, Edelman ER, Melgar-Lesmes P. Monocyte-endothelial cell interactions in vascular and tissue remodeling. *Front Immunol* (2023) 14:1196033. doi: 10.3389/fimmu.2023.1196033

65. Ribatti D, Nico B, Crivellato E. Morphological and molecular aspects of physiological vascular morphogenesis. *Angiogenesis* (2009) 12(2):101–11. doi: 10.1007/s10456-008-9125-1

66. Nowak-Sliwinska P, Alitalo K, Allen E, Anisimov A, Aplin AC, Auerbach R, et al. Consensus guidelines for the use and interpretation of angiogenesis assays. *Angiogenesis* (2018) 21(3):425–532. doi: 10.1007/s10456-018-9613-x

67. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* (2004) 56 (4):549–80. doi: 10.1124/pr.56.4.3

68. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and development. *Cell* (2019) 176(6):1248–64. doi: 10.1016/j.cell.2019.01.021

69. Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res* (2012) 49 (1):35–43. doi: 10.1159/000339613

70. Muller MM, Griesmacher A. Markers of endothelial dysfunction. *Clin Chem Lab Med* (2000) 38(2):77–85. doi: 10.1515/CCLM.2000.013

71. Pols T, Eggink H, Soeters M. TGR5 ligands as potential therapeutics in inflammatory diseases. *Int J Interferon Cytokine Mediator Res* (2014) 2014(6):27–38. doi: 10.2147/IJICMR.S40102

72. Guo C, Chen WD, Wang YD. TGR5, not only a metabolic regulator. *Front Physiol* (2016) 7:646. doi: 10.3389/fphys.2016.00646

73. Yasuda H, Hirata S, Inoue K, Mashima H, Ohnishi H, Yoshiba M. Involvement of membrane-type bile acid receptor M-BAR/TGR5 in bile acid-induced activation of epidermal growth factor receptor and mitogen-activated protein kinases in gastric carcinoma cells. *Biochem Biophys Res Commun* (2007) 354(1):154–9. doi: 10.1016/j.bbrc.2006.12.168

74. Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* (2001) 22(7):368–76. doi: 10.1016/S0165-6147(00)01678-3

75. Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, et al. Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. *Biochem Biophys Res Commun* (2007) 362(4):793–8. doi: 10.1016/j.bbrc.2007.06.130

76. Kir D, Schnettler E, Modi S, Ramakrishnan S. Regulation of angiogenesis by microRNAs in cardiovascular diseases. *Angiogenesis* (2018) 21(4):699–710. doi: 10.1007/s10456-018-9632-7

77. Felicetta A, Condorelli G. RNA binding protein and microRNA control of endothelial cell function. *Cardiovasc Res* (2019) 115(12):1690–1. doi: 10.1093/cvr/cvz144

78. Avvisato R, Mone P, Jankauskas SS, Varzideh F, Kansakar U, Gambardella J, et al. miR-4432 targets FGFBP1 in human endothelial cells. *Biol (Basel)* (2023) 12 (3):459. doi: 10.3390/biology12030459

79. Gambardella J, Kansakar U, Sardu C, Messina V, Jankauskas SS, Marfella R, et al. Exosomal miR-145 and miR-885 Regulate Thrombosis in COVID-19. *J Pharmacol Exp Ther* (2023) 384(1):109–15. doi: 10.1124/jpet.122.001209

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A novel nomogram for predicting cardiometabolic diseases from modifiable risks in middle-aged adults-implication for health education

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Background: Middle-aged adults often overlook critical modifiable risk factors that contribute to the emergence of cardiometabolic diseases (CMDs), including hypertension and diabetes. Many CMDs can be alleviated by addressing these modifiable risks. However, there has been insufficient research focused on rural adults with lower levels of health literacy in this regard. The aim of this study was to explore and develop an intuitive assessment tool for predicting cardiometabolic diseases (CMDs), which can be used for health education with adults of low health literacy.

Methods: This was a community-based, cross-sectional study. A structured questionnaire on health-promoting habits, smoking, sleep, and physiological biomarkers was obtained via community health screening in the coastal region of Yunlin County, Taiwan. Multivariate logistic regression was used to screen for significant variables in the nomogram construction. Analysis with nonlinear restricted cubic spline was performed.

Results: A total of 712 participants (60.9% females) aged 40–64 years, with middle school level or lower education, were included. The average age was 55.6 years (SD=7.3), and 220 individuals (31%) had CMDs. Multivariate logistic regression analysis revealed that age, lower scores of vegetables, fruit, water, and exercise (VFWE), smoking history, sleep deprivation, and being overweight were significantly associated with CMDs. The model incorporating these modifiable risk factors demonstrated good discriminatory ability, as indicated by an area under the receiver operating characteristic curve of 0.75 (0.73–0.76). A predictive nomogram was developed that presented modifiable risk factors in a simple graphical format to facilitate the prediction of CMDs.

Conclusions: This study highlights a high prevalence of CMDs among middle-aged adults, along with the disregard for important risk factors that could be

modified. The developed nomogram could be a practical and effective tool for community health education to enhance health literacy and prevent the progression of CMDs.

KEYWORDS

middle-aged, modifiable risks, health-promoting habits, cardiometabolic disease, nomogram, health education

Introduction

Middle-aged adults, typically aged between 40 and 64 years, are often called the "sandwich generation." The term arises from the dual responsibility of caring for children and aging parents (1). As they navigate these roles, they face work demands and societal obligations. However, studies have indicated that healthy behaviors tend to decline in middle adulthood, increasing the risk of developing cardiometabolic diseases (CMDs), e.g., hypertension, diabetes, hyperlipidemia, and coronary heart disease (2, 3). For example, Sakib et al. (3) observed an increase in the prevalence of multimorbidity from 29.7% in the 45–49-year-old age group to 52% in individuals aged 60–64 years.

Empirical evidence has consistently demonstrated a strong association between CMDs, the occurrence of cerebrovascular disease (stroke), and cardiovascular events. CMDs are major contributors to global mortality, accounting for 32% of all deaths worldwide, with heart attack and stroke being the primary causes, representing 85% of CMD-related deaths. These acute events typically arise from blockages that impede blood flow to the heart or brain (4, 5). In Taiwan, CMDs account for a significant proportion of mortality and are the leading cause of cancer-related deaths. Stroke has consistently been ranked as the second leading cause of death in Taiwan for over three decades, imposing substantial burdens on affected families and society (6).

The existing literature highlights that most CMDs can be prevented by addressing lifestyle interventions and avoiding behavioral risk factors such as excessive alcohol consumption, tobacco use, unhealthy diet, physical inactivity, and obesity (7–9). A recent paradigm shift advocated by the American Heart Association, known as "life's essential 8 (LE-8)," emphasizes the promotion of cardiovascular health (10, 11). LE-8 comprises five core health habits: maintaining a healthy diet, engaging in regular physical activity, avoiding nicotine exposure, and achieving an adequate body mass index and sleep health. It includes three ideal physiological indicators: levels of blood lipids, blood glucose, and blood pressure (4, 10). Literature indicates that lifestyle modifications, such as regular exercise, a healthy diet, smoking cessation, sufficient sleep quality, and weight management, can effectively prevent stroke, heart attacks, and the progression of CMDs and promote longevity (12–14).

Previous studies have established the pathways of CMDs and the dysregulation of systemic inflammation and metabolism, as well as their effects on atherosclerotic plaques and insulin resistance (15–17). Adopting primary prevention strategies can help mitigate accelerated inflammation associated with CMDs. These strategies include avoiding smoking, reducing alcohol consumption, engaging in physical activity, consuming adequate amounts of vegetables and fruits, ensuring sufficient water intake (>2000 mL/day), and maintaining a healthy weight (18–21). Although CMDs are a leading cause of death globally, research in this area has primarily focused on older adults, with limited studies examining and establishing easily understandable assessment tools for middle-aged individuals with CMDs, particularly those with lower health literacy. Therefore, this study aimed to investigate the association between modifiable risk factors and CMDs and to develop a nomogram format that provides an intuitive understanding of risk prediction for CMDs among rural adults.

Materials and methods

Design and population

In collaboration with a local hospital and health center, a community-based cross-sectional study was conducted between March and December 2022 in five townships located along the western coast of Yunlin County, Taiwan. Convenience sampling was used for participant selection. Before commencing the study, the institutional review board of the research ethics committee (IRB no: 202000109B0C102) and the approval of inform consent from each participant were obtained. The study selected community middle-aged adults who met the following criteria: (1) aged between 40 and 64 years and educational level ≦middle school (2), capable of communicating in either Mandarin or Taiwanese for one-on-one interviews, and (3) willing to sign the consent form. Participants who were unable to complete the questionnaires, those who independently walked to the community activity center, or individuals with incomplete laboratory data were excluded from the analysis.

Measurement

Demographics and cardiometabolic diseases were assessed: (a) age, sex, education, marital status, living arrangement (alone, living with family, or living with friends), and health history of comorbidities diagnosed by a physician. Participants with hypertension, diabetes, hyperlipidemia, coronary heart disease, or stroke were classified as having CMDs; (b) height (cm) and body weight (kg) were measured while wearing light clothing and no shoes.

Cardiometabolic risk factors were assessed based on the previous reports (2, 10), including (a) control blood pressure (BP): the cutoff point of systolic/diastolic blood pressure (SBP/DBP) was >130/85 mmHg; (b) blood glucose control: We used glycated hemoglobin (HbA1C) ≥6% as the cutoff point of inadequate control level of blood glucose; (c) blood lipids control: serum triglyceride levels (TG) >150 (mg/dL) were classified as inadequate control.

Modifiable risk factors were assessed with a cluster of four health-promoting habits and three health-related behaviors that were based on the American Heart Association recommendations of LE-8 (2, 10) and previous studies (22–24).

(A)Health-promoting habits (including vegetables, fruits, water intake, and exercise, VFWE): Participants were asked to scale the frequency of healthy eating of vegetables, fruits, water intake, and practicing regular exercise on a four-point Likert scale, recorded as never (0), seldom (1), usually (2), and always (3). Do you (a) consume vegetables (V) ≥3 servings (1.5 bowls) per day; (b) consume fruit (F) 2 servings (1 bowl) per day; (c) consume water (W) ≥2000 mL per day; (d) practice regular exercise (E) ≥3 times, each for 30 min a week or 150 min per week with moderate sweating? The total score ranged from 0–12, with higher scores indicating better health-promoting habits.

(B)Ever-smoker: Participants were asked, "Do you smoke cigarettes or use e-cigarettes in recent months? with responses of "no: never" and "yes: former/current user."

(C)Sleep deprivation: Based on the literature (25), participants were asked to answer this question: Currently or during the past week, have you experienced sleep distress or difficulty falling asleep? The responses were 0=never, 1=slightly, 2=ordinarily, 3=quite often, and 4=most often. Sleep deprivation was categorized as "no" (score 0–1) and "yes" (score 2–4).

(D)Healthy weight: Body mass index (BMI) was calculated using the standard formula (kg/m^2), and BMI >24 was classified as overweight.

Statistical analysis

Continuous variables were presented as mean (± SD); categorical variables were presented as percentages. Exceptions to blood pressure, blood glucose, and blood lipid levels were multicollinear with CMDs. We attempted to establish an understandable and predictive model between modifiable risk factors and age associated with CMDs. The correlations between CMDs and age, VFWE, smoking, sleep deprivation, and BMI were examined using a binary logistic regression model. Restricted cubic spline (RCS) analysis was performed to estimate the relationship between VFWE points and CMDs outcomes, adjusting for age, cigarette smoking, sleep deprivation, BMI, BP, HbA1C, and TG. We fitted a smooth continuous curve of adjusted odds ratio (OR) by RCS analysis with 95% confidence intervals (CI) across VFWE points, allowing for cubic form changes at the knot point. Thus, two linear relationships were generated (Figure 1). To test for the nonlinearity of the RCS curves, we checked the nonlinear p-value using Wald statistics. The p=0.03 implied that VFWE have a nonlinear relationship with the CMDs.

Furthermore, we used SiZer, an open-source R package that fits a degree 1 spline with one knot point using two-line piecewise-linear models. This package can check the critical (knot) point of the VFsWE, i.e., when the first derivative of a smooth continuous curve is significantly negative, possibly zero, or significantly positive across a range of smoothing bandwidths. The knot point is determined as follows:

$y_i = \beta_0 + \beta_1 x_i + e_i$when $x_i \leq \alpha$ and

$y_i = \beta_0 + \beta_1 x_i + \beta_2 (x_i - \alpha) + e_i$ when $x_i > \alpha$ where α is the knot point.

Because VFWE=3.95 has been reported as a knossst point, 4 was chosen for a categosrical split. Finally, to build an easy-to-use tool for predicting CMDs risk, each category of the five variables in the nomogram was given a point by representing ORs to a 0–100 "points" scale, and total points were calculated by summing these points. All statistical analyses were performed using R programming language (version 4.2.0) for Windows.

Results

Demographic characteristics and univariate analysis of modifiable risk factors associated with cardiometabolic diseases

A total of 712 participants aged 40–64 years who completed the community health screening were enrolled, of whom 434 (60.9%) were female and had attained education levels ≦middle school. Most of the participants (91%) lived with family or friends, 83.7% were married, the mean age was 55.6 (SD=7.3) years, and 220 (31%) participants were categorized as having CMDs. Table 1 shows that among the participants with CMDs, 25% reported being ever smokers, 53% had sleep deprivation, 72% had BMI>24, 63% had SBP/DBP>130/85 (mmHg), 63% had HbA1C≥6%, and 41% had TG>150 (mg/dL). Regardless of age, adults with CMDs had significantly lower VFWE scores ($p<0.05$), were ever-smokers ($p<0.05$), had sleep deprivation ($p<0.01$), were overweight ($p<0.01$), and had abnormal blood pressure ($p<0.01$), HbA1C ($p<0.01$), and TG ($p<0.01$).

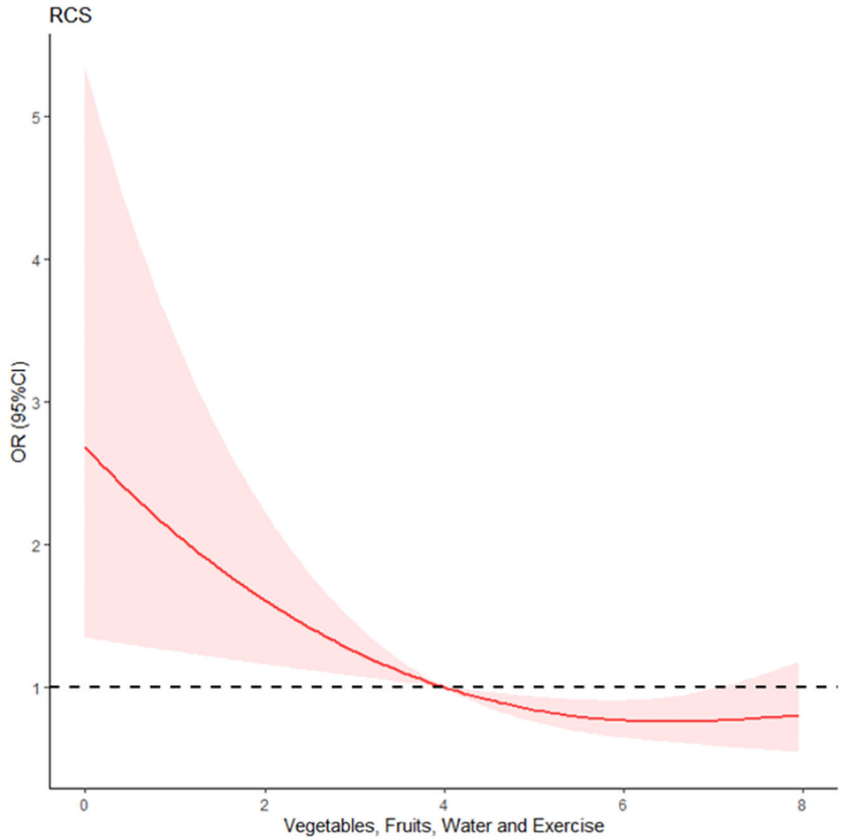

FIGURE 1
Non-linear correlation between VFWE (vegetables, fruits, water, and exercise) and cardiometabolic diseases. RCS, Restricted cubic spline of VFWE and CMDs.

TABLE 1 Univariate analysis of demographic characteristics and potential modifiable factors associated with CMDs (N=712).

Variables	Cardiometabolic diseases (N %)		χ^2/t	*p*
	No (n=492, 69%)	Yes (n=220, 31%)		
Age (years)	54.72±7.66	57.80±6.02	5.27	<0.01
VFWE (0–12)[1]	6.92±3.17	6.60±3.47	2.14	0.03
Ever-smoker			4.79	0.03
No	404 (82)	165 (75)		
Yes	88 (18)	55 (25)		
Sleep deprivation			14.25	<0.01
No	307 (62)	104 (47)		
Yes	185 (38)	116 (53)		
Body mass index (kg/m^2)			21.14	<0.01
≦24	226 (46)	61 (28)		
24	265 (54)	159 (72)		
SBP/DBP (mmHg)[2]			13.74	<0.01
≦130/85	254 (52)	81 (37)		
130/85	236 (48)	139 (63)		

(Continued)

TABLE 1 Continued

Variables	Cardiometabolic diseases (N %)		χ^2/t	p
	No (n=492, 69%)	Yes (n=220, 31%)		
HbA1C (%)[3]			25.33	<0.01
<6	281 (57)	81 (37)		
6	210 (43)	139 (63)		
Triglyceride (mg/dL)			11.55	<0.01
≦150	354 (72)	130 (59)		
150	138 (28)	90 (41)		

[1]VFWE, vegetables, fruits, water, exercise; [2] SBP/DBP, systolic/diastolic blood pressure; [3] Glycated hemoglobin.

Modifiable risk factors associated with CMDs

We observed a nonlinear association between VFWE and CMDs (p<0.05) in the RCS analysis (Figure 1), indicating a nonlinear relationship between VFWE and CMDs. We used SiZer, an open-ssource R package that fits a degree 1 spline with one knot point using two-line piecewise-linear models. The knot point (VFWE=3.95) with the maximum likelihood in the nonlinearity models in the RCS analysis was found. Therefore, a VFWE of four was chosen for the categorical split. We conducted a multivariate binary logistic regression analysis of modifiable risk factors associated with CMDs, and the results are presented in Table 2. In Model 1, all modifiable risk factors and age groups were included. Except for ever-smokers (p>0.05), the lower scores for health-promoting habits (VFWE, p<0.01), older age (p<0.001), sleep deprivation (p<0.001), BMI >24 (p<0.001), HbA1C ≥6% (p<0.01), and TG >150 (mg/dL) (p=0.05) were significantly associated with the log-odds of CMDs.

In Model 2, after adjustment with blood pressure, blood glucose, and blood lipids, the result showed that exception age, a lower score of VFWE (OR=0.34, 95% CI=0.17–0.69), ever-smoker (OR=1.53, 95% CI=1.02–2.32), sleep deprivation (OR=1.84, 95% CI=1.31–2.60) and overweight (OR=2.38, 95% CI=1.64–3.40) were significantly associated with CMDs.

Construction of CMDs prediction nomogram

To predict modifiable risk factors for CMDs, we constructed a nomogram based on five variables (age, VFWE, ever-smoker status, sleep deprivation, and BMI) that were significant in **Model 2**. The points for each variable are listed in the right column of Table 2. By summing the points for the five variables, we derived the total number of points that changed from 185 to 412. The nomogram shows the modifiable risks of CMDs based on total points (Figure 2). For example, the risk of CMDs was lower than approximately 20% for those below 255 points and higher than approximately 65% for those with over 350 points. Additionally, if the logistic regression model predicts a probability of 0.827 for case number 134, it indicates an 82.7% likelihood that CMDs will occur based on the provided input variables (BMI greater than 24, smoking status, sleep deprivation, age of 61, and a VFWE score of 0).

TABLE 2 Logistic regression of modifiable risk factors and age associated with cardiometabolic diseases.

Variables	Model 1			Model 2					
	Beta	SE	p	Beta	SE	p	OR	95% CI	Point
Age (years)	0.06	0.01	<0.001	0.06	0.15	<0.001	2.12	1.57-2.86	0-78
VFWE (4-0)[1]	-0.28	0.10	<0.01	-1.08	0.36	<0.01	0.34	0.17-0.69	44-100
Ever-smoker (1= yes)	0.30	0.21	0.16	0.43	0.21	0.04	1.53	1.02-2.32	47, 68
Sleep deprivation (1= yes)	0.59	0.18	<0.001	0.61	0.17	<0.001	1.84	1.31-2.60	47, 77
BMI (1 >24 kg/m^2) [2]	0.67	0.19	<0.001	0.86	0.18	<0.001	2.38	1.64-3.40	47, 89
SBP/DBP (1>130/85 mmHg)[3]	0.37	0.18	0.06						
HbA1C (1 ≥ 6 %)[4]	0.53	0.18	<0.01						
TG (1 > 150 mg/dL)[5]	0.37	0.19	0.05						

[1]VFWE, vegetables, fruits, water, exercise; [2]BMI, body mass index; [3]SBP/DBP, systolic/diastolic blood pressure; [4]HbA1C, glycated hemoglobin; [5]TG, triglyceride.

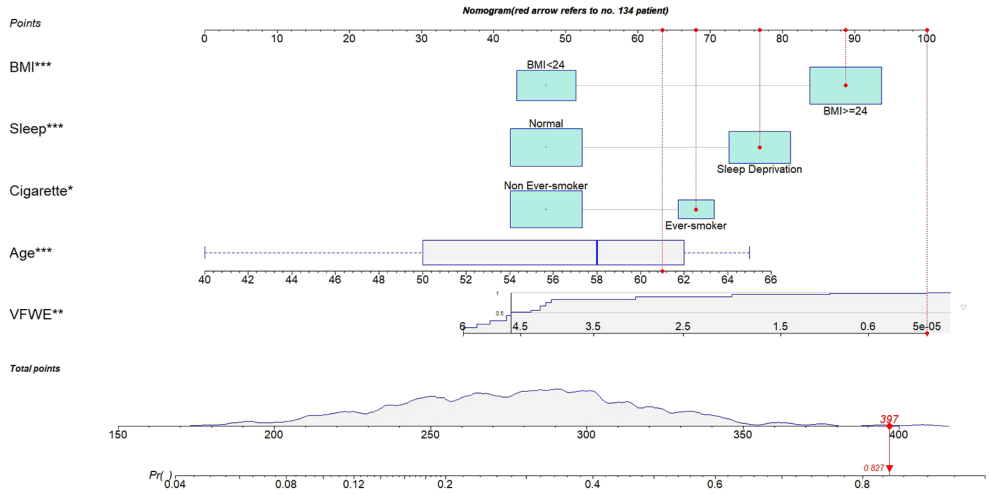

FIGURE 2
The nomogram was used to predict CMDs probability. A specific resident (case no 134) was shown to illustrate how to use the nomogram. This case of no 134 who had a BMI greater than 24, smoker, sleep deprivation, age of 61 and the score of VFWE was 0. The specific resident's point corresponding to each covariate was presented at the top, and the total points were obtained from the sum of the points corresponding to each variable by a red dot. Once we obtained values from the 5 variables, the subject can be intuitively mapped onto the nomogram. From nomogram, we observed that the total points of case no 134 was 397, and the corresponding probability of CMDs was 0.827. BMI: body mass index; CMDs: cardiometabolic diseases; VFWE: vegetable, fruit, water, and exercise.

Risk stratification

The discriminatory performance and cutoff probability of risk stratification are commonly quantified by measuring the area under the receiver operating characteristic curve (AUC). Overall, the discriminatory performance of the full model revealed an AUC of 0.75 (0.73–0.76, p<0.001) (Figure 3), indicating the suitability of this model in identifying participants with high risks of CMDs. Currently, there is a lack of research examining the efficacy of utilizing only health habits for the prevention and treatment of CMDs. Typically, the evaluation of CMDs relies on indirect functional and physiological assessments, historically presenting challenges attributed to technical limitations. Nevertheless, healthcare providers can proficiently leverage health habits to enhance cardiometabolic health literacy and to provide guidance for therapies aimed at the prevention and treatment of CMDs. The prevailing approach in investigating this association utilized BMI categories as the predictor variable. In their meta-analysis, Darbandi (26) integrated 38 cross-sectional and 2 cohort studies, encompassing a participant range of 105 to 137,256 individuals aged 18 or older. The collective AUC for BMI was 0.66 (95% CI, 0.63–0.69), which is comparatively lower than the AUC of 0.75 (95% CI, 0.73–0.76) observed in our study.

In order to determine the optimal cutoff point for the ROC curve, one commonly used method is the Youden index (27). The calculation formula for the Youden index involves finding the maximum value of sensitivity plus specificity minus 1. Consequently, this method aligns with the maximum sum of sensitivity and specificity. Figure 4 provides a gauge of CMDs assessment, which illustrates the risk stratification based on the nomogram model by thresholding with the optimal cutoff predicted risk (the point-to-probability nomogram in Figure 2 demonstrate that a probability value of 0.35 corresponds to a total of 290 points on the nomogram). The two risk intervals were defined as low- and high-risk groups for CMDs that were more understandable for health education. In our data, approximately 62.8% of the participants were classified into the low-risk group and 37.2% into the high-risk group.

Discussion

This study yielded four important findings. First, nearly one-third of middle-aged rural adults reported having CMDs. Second, adults with CMDs have a high prevalence of modifiable risk factors, such as adopting few health-promoting habits, smoking, being overweight, sleep deprivation, and abnormal physiological biomarkers. Third, results suggest that significant reductions in CMDs can be achieved by encouraging individuals to start consuming vegetables and fruits, paying attention to hydration, and initiating physical activity without needing to reach a high frequency at which it becomes difficult to implement. Fourth, a novel nomogram with a simple graphical format for predicting the risk of CMDs was established, which can be used by primary healthcare providers to increase health literacy and prevent the progression of CMDs.

The present study showed that many participants reported knowing about the diagnosis of CMDs. However, many did not adopt health-promoting habits, such as eating adequate amounts of vegetables, fruits, drinking adequate amounts of water, and practicing regular exercise. Additionally, many of their cardiometabolic biomarkers remained at abnormal levels, such as body mass index, blood pressure, blood glucose, and blood lipids. Adults with known CMDs remain less likely to adopt health-

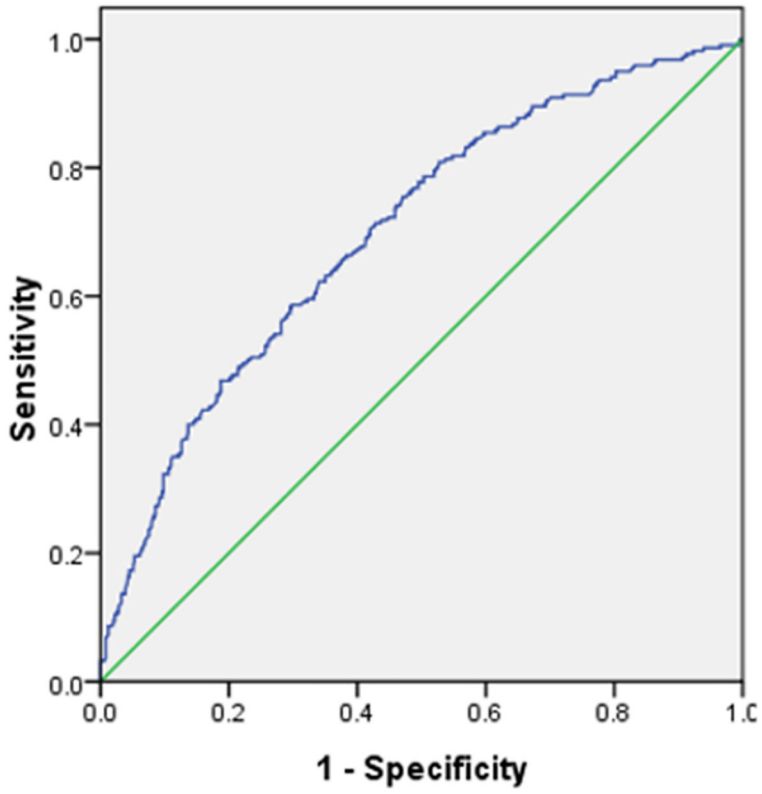

FIGURE 3
Assessing the discrimination of a fitted logistic model, via the ROC curve.

promoting habits or LE 8 (2, 10). Possible reasons might be the low health literacy about cardiometabolic health and the limited quality of health resources provided by healthcare providers in rural areas. Further studies should consider enhancing cardiometabolic health literacy through innovative strategies and providing feasible continued education related to the new paradigm of cardiometabolic health for local healthcare providers.

Not surprisingly, these findings were consistent with those of previous studies. For example, in the United States, Lloyd-Jones et al. (10) pointed out that based on the LE8, the prevalence of ideal cardiovascular health is very low in all age groups (<1%) and 11% among the middle-aged group. In the United Kingdom, Stefan et al. (28) also stated that among the 20 leading global risk factors for years of life lost, high blood pressure, BMI, and blood glucose levels

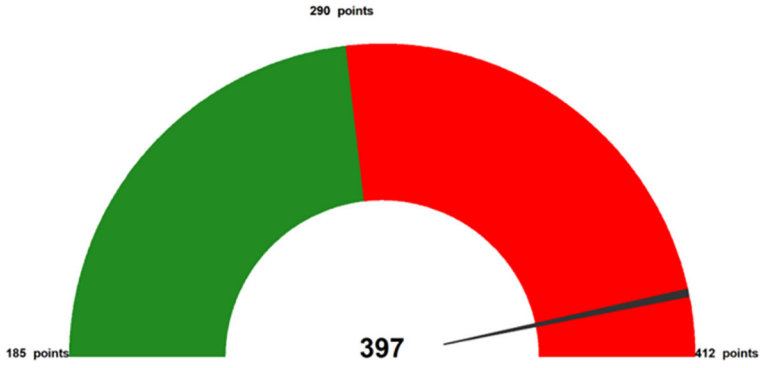

FIGURE 4
Example (case no. 134) as the representative of total points from nomogram. The above figure offers a specific case of how to represent a subject's CMDs assessment with the five variables. The gauge at the top corresponds to the subject's total point (which can range from 185–412 points, 290 points is optimal cutoff), with higher total point shown toward the right (a "full tank" of CMDs) in red. Thus, identifying VFWE and the other modifiable risks on which the subject can judge to near a full tank of CMDs. The total points of predicting the risk of CMDs are the sum of the 5 variables.

were the top risk factors for CMDs. Furthermore, previous studies have reported that most cardiometabolic events, such as myocardial infarction and stroke, can be prevented by adopting a healthy lifestyle and managing known risk factors (2, 8, 11). Hence, providing comprehensive, evidence-based behavioral counseling in primary care settings is a recommended first-line approach for promoting healthy behaviors and preventing worsening cardiovascular outcomes in adults with cardiovascular risks, especially in middle age (2). Thus, initiating a simple assessment tool to increase health literacy and prevent the progression of cardiometabolic diseases is an important strategy.

Promoting health literacy is a crucial approach within primary healthcare settings. Traditional educational manuals frequently play a significant role in the prevention and treatment of cardiometabolic diseases. Individuals with limited educational backgrounds may find understanding and identifying information in text to be abstract and challenging. As a result, healthcare providers, especially those dealing with demanding work schedules, carry a considerable workload. The present study's strength is the application of a nomogram format to establish a simple, evidence-based model for predicting the risk of CMDs. We found that a novel nomogram with a simple graphical format to predict the risk of CMDs was easy to determine and offered each middle-aged adult to represent their CMD risk score with the sum of five variables. This research presents an innovative nomogram featuring a simple graphical format, aiming to enhance visual comprehension for individuals with limited health literacy or those living in rural areas. Furthermore, there is an expectation that this tool will substantially decrease the time needed for health education.

Healthcare providers can also combine the gauge reflecting each adult's total points (ranging from 185 to 412 points). Further studies can apply this nomogram tool and gauge figures in an interesting board game or serious game to assess the total points for each middle-aged adult's health-related lifestyle and quickly reflect the value of changing modifiable risks to prevent the progression of CMDs.

Limitations

This study had some limitations. First, its cross-sectional design limits the causal relationship between modifiable risk factors and cardiometabolic diseases. Hence, a prospective design is necessary for future research. Second, we conducted this study in only one county and used nonrandom sampling, which might limit the generalizability of these findings. Third, health-promoting habits were based on self-report and recall bias, which may have resulted in inaccurate estimations. Fourth, the total points of the nomogram in this study ranged from 185 to 412, which is not a clinically convenient value. Simplification of these values will rely on a larger sample size in the future.

Conclusions

Managing the progression of CMDs is difficult but important. A high prevalence of CMDs and many neglect-modifiable risk factors have been found among middle-aged rural adults. A novel nomogram with a simple graphical format was established to predict the risk of CMDs, which healthcare providers can easily use it to increase cardiometabolic health literacy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by institutional review board of the Chang Gung Memorial Hospital Foundation (IRB no: 202000109B0C102). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

C-HC: Methodology, Software, Writing – original draft. M-YC: Conceptualization, Methodology, Project administration, Writing – original draft. M-SL: Conceptualization, Data curation, Investigation, Writing – original draft. Y-CL: Data curation, Investigation, Writing – original draft. T-JH: Conceptualization, Investigation, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Orenstein GA, Lewis L. *Erikson's stages of psychosocial development*. StatPearls. Treasure Island (FL: StatPearls Publishing (2022).

2. Laddu D, Ma J, Kaar J, Ozemek C, Durant RW, Campbell T, et al. Health behavior change programs in primary care and community practices for cardiovascular disease prevention and risk factor management among midlife and older adults: a scientific statement from the American Heart Association. *Circulation* (2021) 144(24):e533–49. doi: 10.1161/CIR.0000000000001026

3. Sakib MN, Shooshtari S, St John P, Menec V. The prevalence of multimorbidity and associations with lifestyle factors among middle-aged Canadians: an analysis of Canadian longitudinal study on aging data. *BMC Public Health* (2019) 19(1):243. doi: 10.1186/s12889-019-6567-x

4. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2020 update: A report from the American Heart Association. *Circulation* (2020) 141:e139–596. doi: 10.1161/CIR.0000000000000757

5. World Health Organization. *Cardiovascular diseases*. Available at: https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1. (Accessed 25 November 2023).

6. Health Promotion Administration, HPA. *Cardiovascular diseases* . Available at: https://www.hpa.gov.tw/Search/GoogleSearch.aspx?queryString=cardiometabolic+diseasese (Accessed 8 August 2023).

7. Mu X, Liu S, Fu M, Luo M, Ding D, Chen L, et al. Associations of physical activity intensity with incident cardiovascular diseases and mortality among 366,566 UK adults. *Int J Behav Nutr Phys Act* (2022) 19(1):151. doi: 10.1186/s12966-022-01393-y

8. Ioachimescu OC. From to : A journey from life's simple 7 to life's essential 8 and beyond. *J Am Heart Assoc* (2022) 11(21):e027658. doi: 10.1161/JAHA.122.027658

9. Zhang Y, Yang Y, Huang Q, Zhang Q, Li M, Wu Y. The effectiveness of lifestyle interventions for diabetes remission on patients with type 2 diabetes mellitus: A systematic review and meta-analysis. *Worldviews Evid Based Nurs* (2023) 20(1):64–78. doi: 10.1111/wvn.12608

10. Lloyd-Jones DM, Allen NB, Anderson CAM, Black T, Brewer LC, Foraker RE, et al. Life's essential 8: updating and enhancing the American Heart Association's construct of cardiovascular health: a presidential advisory from the American Heart Association. *Circulation* (2022) 146(5):e18–43. doi: 10.1161/CIR.0000000000001078

11. Mok Y, Sang Y, Ballew SH, Rebholz CM, Rosamond WD, Heiss G, et al. American Heart Association's life's simple 7 at middle age and prognosis after myocardial infarction in later life. *J Am Heart Assoc* (2018) 7(4):e007658. doi: 10.1161/JAHA.117.007658

12. Duncan MS, Vasan RS, Xanthakis V. Trajectories of blood lipid concentrations over the adult life course and risk of cardiovascular disease and all-cause mortality: Observations from the Framingham study over 35 years. *J Am Heart Assoc* (2019) 8 (11):e011433. doi: 10.1161/JAHA.118.011433

13. Lobelo F, Young DR, Sallis R, Garber MD, Billinger SA, Duperly J, et al. Routine assessment and promotion of physical activity in healthcare settings: a scientific statement from the American Heart Association. *Circulation* (2018) 137(18):e495-e522. doi: 10.1161/CIR.0000000000000559

14. Nicholson K, Rodrigues R, Anderson KK, Wilk P, Guaiana G, Stranges S. Sleep behaviours and multimorbidity occurrence in middle-aged and older adults: findings from the Canadian longitudinal study on aging (CLSA). *Sleep Med* (2020) 75:156–62. doi: 10.1016/j.sleep.2020.07.002

15. Arnett DK, Blumenthal RS, Albert A, Buroker AB, Goldberger ZD, Hahn EJ. ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American college of Cardiology/American heart association task force on clinical practice guidelines. *Circulation* (2019) 140:e596–646. doi: 10.1161/CIR.0000000000000678

16. Chang CH, Weng HH, Lin YC, Lin CN, Huang TJ, Chen MY. Association between serum carcinoembryonic antigen and cardiometabolic risks: Implication for cardiometabolic prevention. *Front Endocrinol (Lausanne)* (2023) 14:1113178. doi: 10.3389/fendo.2023.1113178

17. Koelwyn GJ, Aboumsallem JP, Moore KJ, de Boer RA. Reverse cardio-oncology: Exploring the effects of cardiovascular disease on cancer pathogenesis. *J Mol Cell Cardiol* (2022) 163:1–8. doi: 10.1016/j.yjmcc.2021.09.008

18. Eizenberg Y, Grossman E, Tanne D, Koton S. Admission hydration status and ischemic stroke outcome-experience from a national registry of hospitalized stroke patients. *J Clin Med* (2022) 10(15):3292. doi: 10.3390/jcm10153292

19. Cortés-Vicente E, Guisado-Alonso D, Delgado-Mederos R, Camps-Renom P, Prats-Sánchez L, Martínez-Domeño A, et al. Frequency, risk factors, and prognosis of dehydration in acute stroke. *Front Neurol* (2019) 10:305. doi: 10.3389/fneur.2019.00305

20. Ahmad S, Moorthy M, Demler OV, Hu FB, Ridker PM, Chasman DI. Assessment of risk factors and biomarkers associated with risk of cardiovascular disease among women consuming a Mediterranean diet. *JAMA Netw Open* (2018) 1 (8):e185708. doi: 10.1001/jamanetworkopen.2018.5708

21. Owolabi MO, Thrift AG, Mahal A. Primary stroke prevention worldwide: Translating evidence into action. *Lancet Public Health* (2022) 7:e74–85. doi: 10.1016/S2468-2667(21)00230-9

22. Guo YW, Chang SH, Huang CH, Chen MY. A parsimonious and comprehensive screening tool for adult health behaviors. *Public Health Nurs* (2022) 39(5):965–72. doi: 10.1111/phn.13085

23. Lee DH, Rezende LFM, Joh HK, Ferrari G, Rey-Lopez JP, Rimm EB, et al. Long-term leisure-time physical activity intensity and all-cause and cause-specific mortality: a prospective cohort of US adults. *Circulation* (2022) 146(7):523–34. doi: 10.1161/CIRCULATIONAHA.121.058162

24. Vriz O, Mos L, Palatini P. Leisure-time physical activity has a more favourable impact on carotid artery stiffness than vigorous physical activity in hypertensive human beings. *J Clin Med* (2022) 11(18):5303. doi: 10.3390/jcm11185303

25. Wu CY, Lee JI, Lee MB, Liao SC, Chang CM, Chen HC, et al. Predictive validity of a five-item symptom checklist to screen psychiatric morbidity and suicide ideation in general population and psychiatric settings. *J Formos Med Assoc* (2016) 115(6):395–403. doi: 10.1016/j.jfma.2015.05.004

26. Darbandi M, Pasdar Y, Moradi S, Mohamed HJJ, Hamzeh B, Salimi Y. Discriminatory capacity of anthropometric indices for cardiovascular disease in adults: a systematic review and meta-analysis. *Prev Chronic. Dis* (2020) 17:E131. doi: 10.5888/pcd17.200112

27. Youden WJ. Index for rating diagnostic tests. *Cancer* (1950) 3(1):32–5. doi: 10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3

28. Stefan N, Schulze MB. Metabolic health and cardiometabolic risk clusters: implications for prediction, prevention, and treatment. *Lancet Diabetes Endocrinol* (2023) S2213-8587(23):00086–4. doi: 10.1016/S2213-8587(23)00086-4

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Identification of important modules and biomarkers in diabetic cardiomyopathy based on WGCNA and LASSO analysis

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Background: Diabetic cardiomyopathy (DCM) lacks specific and sensitive biomarkers, and its diagnosis remains a challenge. Therefore, there is an urgent need to develop useful biomarkers to help diagnose and evaluate the prognosis of DCM. This study aims to find specific diagnostic markers for diabetic cardiomyopathy.

Methods: Two datasets (GSE106180 and GSE161827) from the GEO database were integrated to identify differentially expressed genes (DEGs) between control and type 2 diabetic cardiomyopathy. We assessed the infiltration of immune cells and used weighted coexpression network analysis (WGCNA) to construct the gene coexpression network. Then we performed a clustering analysis. Finally, a diagnostic model was built by the least absolute shrinkage and selection operator (LASSO).

Results: A total of 3066 DEGs in the GSE106180 and GSE161827 datasets. There were differences in immune cell infiltration. According to gene significance (GS) > 0.2 and module membership (MM) > 0.8, 41 yellow Module genes and 1474 turquoise Module genes were selected. Hub genes were mainly related to the "proteasomal protein catabolic process", "mitochondrial matrix" and "protein processing in endoplasmic reticulum" pathways. LASSO was used to construct a diagnostic model composed of OXCT1, CACNA2D2, BCL7B, EGLN3, GABARAP, and ACADSB and verified it in the GSE163060 and GSE175988 datasets with AUCs of 0.9333 (95% CI: 0.7801-1) and 0.96 (95% CI: 0.8861-1), respectively. H9C2 cells were verified, and the results were similar to the bioinformatics analysis.

Conclusion: We constructed a diagnostic model of DCM, and OXCT1, CACNA2D2, BCL7B, EGLN3, GABARAP, and ACADSB were potential biomarkers, which may provide new insights for improving the ability of early diagnosis and treatment of diabetic cardiomyopathy.

KEYWORDS

diabetic cardiomyopathy, WGCNA, LASSO, biomarkers, diagnosis

1 Introduction

Diabetic cardiomyopathy (DCM) is a pathophysiological condition induced by diabetes mellitus (DM) that can lead to heart failure (HF). The initial stage of DCM is characterized by extensive hypertrophy of the heart and moderate fibrosis, leading to defects in systolic and diastolic function (1). Research has suggested that persistent hyperglycemia, insulin resistance, abnormal insulin signal transduction, impaired glucose metabolism, abnormal free fatty acid uptake, oxidative stress, increased aldosterone renin-angiotensin-aldosterone system activity, heart inflammation, and abnormal mitochondrial functions are the key determinants of the cycle and biochemical changes in disease (2). Earlier intervention in hyperglycemia can effectively maintain the normal metabolic function of the myocardium, protect myocardial tissue and preserve cardiac pump function.

The diagnosis of DCM remains a challenge, especially in asymptomatic patients. At present, the main method to detect asymptomatic DCM is echocardiography, which shows cardiac hypertrophy and dysfunction, and excludes other causes (3). However, echocardiographic features of the heart lack specificity. Biopsy is the gold standard of diagnosis, and is subject to complex procedures and high risks. In addition, specificity and sensitivity biomarkers for DCM are lacking (4). Therefore, there is an urgent need to develop useful biomarkers to help diagnose and assess the prognosis of DCM in this situation.

Weighted gene coexpression network analysis (WGCNA) is a systems biology method used to describe the correlation patterns between genes. WGCNA can be used to find clusters of highly correlated genes, aggregate such clusters with modular trait genes or in-module hub genes, and calculate module membership metrics. It can also be used to identify candidate biomarkers or therapeutic targets (5, 6). The least absolute shrinkage and selection operator (LASSO) generates a more refined model by constructing a penalty function and setting some regression coefficients to zero. Therefore, the advantage of subset shrinkage is retained, with high predictive value (7), which can improve the accuracy and predictive value of key genes identified from microarray and high-throughput data (8). In this study, WGCNA and LASSO regression were used to screen and verify the biological diagnostic markers of DCM to reduce the difficulty of screening and diagnosing DCM.

2 Materials and methods

2.1 Data source

We searched the database for myocardial sample data of mice with a high-fat diet combined with a short-term intraperitoneal injection of STZ-induced type 2 diabetes and fed a high-fat diet for more than 16 weeks, showing changes in cardiac function. As previously mentioned, a high-fat diet in mice combined with intraperitoneal injection of STZ resulted in a phenotype similar to insulin resistance and glucose intolerance used to simulate type 2 diabetes (9). After feeding a high-fat diet for more than 16 weeks, the mice developed cardiac structural dysfunction, impaired cardiac energy response, and other manifestations, and we believe that they developed myocardial damage in type 2 diabetes (10). The myocardial gene expression datasets (GSE106180, GSE161827) of the DCM group and control group were selected from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) on the platform GPL19057 [Illumina NextSeq 500 (Mus musculus)].

2.2 Differential gene screening

We performed a homologous gene conversion with the R package "biomaRt" (11), eliminating genes that cannot translate into human genes. Log2 (x+1) processing was performed on count data and the "normalizeBetweenArrays" algorithm was used to remove the batch effect (12). Then we used the "limma" package in R to screen differentially expressed genes (DEGs). $P<0.05$ was set as the threshold value for DEG identification.

2.3 Evaluating immune cell infiltration

The genes of different types of immune cells were obtained (13). The infiltration levels of 28 immune cells were calculated using the "GSVA" R package single sample gene concentration analysis (ssGSEA) algorithm.Immune cell infiltration was compared between the DCM group and the control group. $P<0.05$ was considered to indicate differential infiltration of immune cells.

2.4 WGCNA network construction and module identification

We used WGCNA to construct gene coexpression networks and explored modules highly associated with diabetic cardiomyopathy (5). The cluster tree was constructed to test outliers. Soft threshold power was selected through network topology analysis. Then the adjacency relation was calculated, and transformed into a topology overlapping matrix (TOM). We calculated the corresponding differential degree and generated a hierarchical cluster tree of genes. Modules with similar expressions are identified and merged by dynamic tree cutting. Genes in the most important modules associated with clinical characteristics were identified for further analysis. Gene significance (GS) and module membership (MM) were used to quantitatively analyze the module genes with the highest correlation with traits. MM > 0.8 and GS> 0.2 were set as the hub gene criteria. Selected hub genes were included for further analysis.

2.5 Functional and pathway enrichment analysis

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed using the R package "clusterProfiler" (14). We used the string (V11.5, Swiss) database (string-db.org/) to build a

protein-protein interaction (PPI) network and used Cytoscape Software (V3.8.2) to calculate degree scores and construct a gene association map. The minimum needed interaction score was set at 0.900 (highest confidence).

2.6 Screening the diagnostic markers

We searched DCM-related genes in the GeneCards Database (www.genecards.org/). Then a custom Venn diagram was drawn through the web (bioinformatics.psb.ugent.be/webtools/Venn/) to screen for the common key genes between the DCM genes, DEGs and module genes. We built LASSO models to identify key genes. The "Glmnet" package in R software was used to establish the LASSO model to identify key genes. The minimum lambda value is then used as a reference to determine the best parameter. The expression values of the selected genes were weighted using regression coefficients from LASSO analysis. The final model generated by the optimal lambda values was analyzed and the regression coefficients for each gene were calculated. After removing the gene with a coefficient of 0, we used the remaining coefficients to build a diagnostic model. The regression coefficient of hub genes was determined according to the following formula:

$$\text{index} = ExpGene1 \times Coef1 + ExpGene2 \times Coef2 + \ldots + ExpGenen \times Coefn$$

"Exp" refers to the expression value of a gene and "Coef" refers to the regression coefficient of the gene." The pROC" package of R software was used to evaluate the stability and sensitivity of the LASSO model in recognizing DCM by ROC curve analysis (15).

2.7 Module validation and efficacy evaluation

The accuracy and validity of the model were verified by data sets of DCM mouse myocardial tissue (GSE163060) and human peripheral blood mononuclear cells (PBMCs) (GSE175988). Then we used ROC curves and AUC (Area Under Curve) to evaluate the ability to distinguish DCM from the control group with the "pROC" R package.

2.8 Cell culture and treatment

H9c2 cells were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences and cultured with DMEM containing 10% FBS and 1% penicillin/streptomycin at 37° C in a 5% CO2 atmosphere. We induced DCM *in vitro* by culturing H9C2 cells in 33 mmol/l glucose for 24 h.

2.9 Real-time quantitative PCR analysis

The total RNA of H9c2 cells was collected according to the instructions of the TRIzol reagent (Solarbio, China), and cDNA was synthesized with the reverse transcription reagent (Yeasen, China). RT-qPCR with SYBR Green detection chemistry was performed on a Roche LightCycler 96 system with the following primers (Table 1).

2.10 Statistical analysis

Data analysis was performed using R4.1.0 and GraphPad Prism 8. A T-test was used to compare normally distributed data, while the Wilcoxon test was used to compare nonnormally distributed data between the control group and the DCM group. $P<0.05$ was considered statistically significant.

3 Results

3.1 Workflow

The flow chart was shown in Figure 1. First, we compared the myocardial genes of the DCM group and the control group in the GSE106180 and GSE161827 datasets to screen out DEGs. Then we used WGCNA to construct a coexpression network and search the gene modules with the strongest correlation. Hub genes were screened by GS and MS, and we used GO analysis, KEGG analysis, and PPI network analysis to identify them. The intersection of DEGs, module key genes, and DCM was thought of as the key genes, and LASSO analysis was conducted to construct a prediction model. The ROC curve was used to test the accuracy and sensitivity of the model.

TABLE 1 Primers used for RT-qPCR amplification.

Primers	Forward	Reverse
Cacna2d2	GCCTGTGACCTTGGACTTC	CTGATCTGCTTGTGGCCTT
Bcl7b	CATCGAGAAAGTGCGGAAA	CGGAGGGAAAACCATTAGG
Egln3	TCTGTGAGCGAGATGCC	GGAAGTTGTCCAGGTAGCA
Gabarap	CCAGGAACACCATGAAGAA	GGATGCCAAGGAAGGAG
Acadsb	CCCTATGTTTCGCACCTC	CAACTTCAATGCCCATCA
Oxct1	ACATTCACCTTCCCCACA	TCCTGTTTGCCGTCTCA

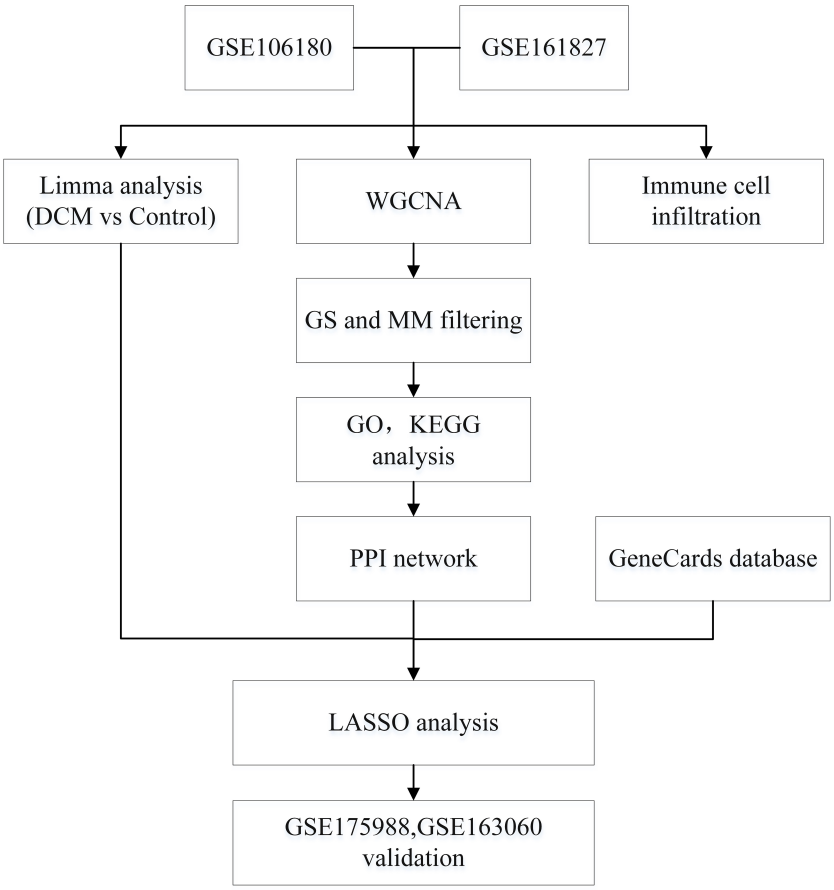

FIGURE 1
Study flowchart. Sequencing data from diabetic cardiomyopathy and controls in the GSE106180 and GSE161728 datasets were analyzed by bioinformatics to identify early potential biomarkers of diabetic cardiomyopathy. DCM, diabetic cardiomyopathy; WGCNA, weighted gene coexpression network analysis; GS, gene significance; MM, Module membership; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein-protein interaction; LASSO, the least absolute shrinkage and selection operator.

3.2 Identification of DEGs

To compare the difference between the DCM group and the control group, we performed differential gene expression analysis. Sixteen samples were obtained from the GSE106180 and GSE161827 gene datasets, including 8 control myocardial samples and 8 diabetic myocardial samples. After adjustment, the median, upper and lower quartiles, and maximum and minimum values of genes in the 16 samples were roughly similar (Figure 2A). According to $P< 0.05$, 3066 DEGs were screened, among which 367 genes were upregulated and 2699 genes were downregulated. The first three upregulated genes with minimum p values were ALDH1A2, ADAM23, and MS4A1, while the downregulated genes with minimum p values were RRAGB, BCKDHB, and TMEM120A (Figure 2B; Table 2). The DEG heat map shows the first 25 upregulated genes and 25 downregulated genes with minimum p values (Figure 2C).

3.3 Immune cell infiltration

Heatmaps showed differences in immune cell infiltration between the DCM group and the control group (Figure 3A). The violin diagram showed that MDSCs, activated CD4 T cells, effector memory CD8 T cells, type 1 T helper cell, type 17 T helper cells, natural killer cells, regulatory T cells, immature dendritic cells, neutrophils, activated B cells, and immature B cells were higher than those of the control group. In contrast, the cell infiltration of activated CD8 T cells and central memory CD4 T cells in the DCM group were less than that in the control group (Figure 3B).

3.4 DCM-associated module

We performed WGCNA on genes in the dataset to reveal the key modules most relevant to DCM. We chose 12 as the soft thresholding power, while 0.85 was used as the correlation coefficient threshold (Figures 4A, B, C). After combining similar modules, we identified a total of seven modules, each shown in a different color (Figure 4D; Table 3). The yellow module with 392 genes [correlation coefficient = −0.68, P=0.004] and the turquoise module with 6471 genes [correlation coefficient =−0.63, P=0.008] are the most correlated with DCM and are the fundamental modules of DCM. According to MM> 0.8 and GS> 0.2, a total of 41 yellow module genes (Figure 4E) and 1474 turquoise module genes (Figure 4F) were included for further analysis.

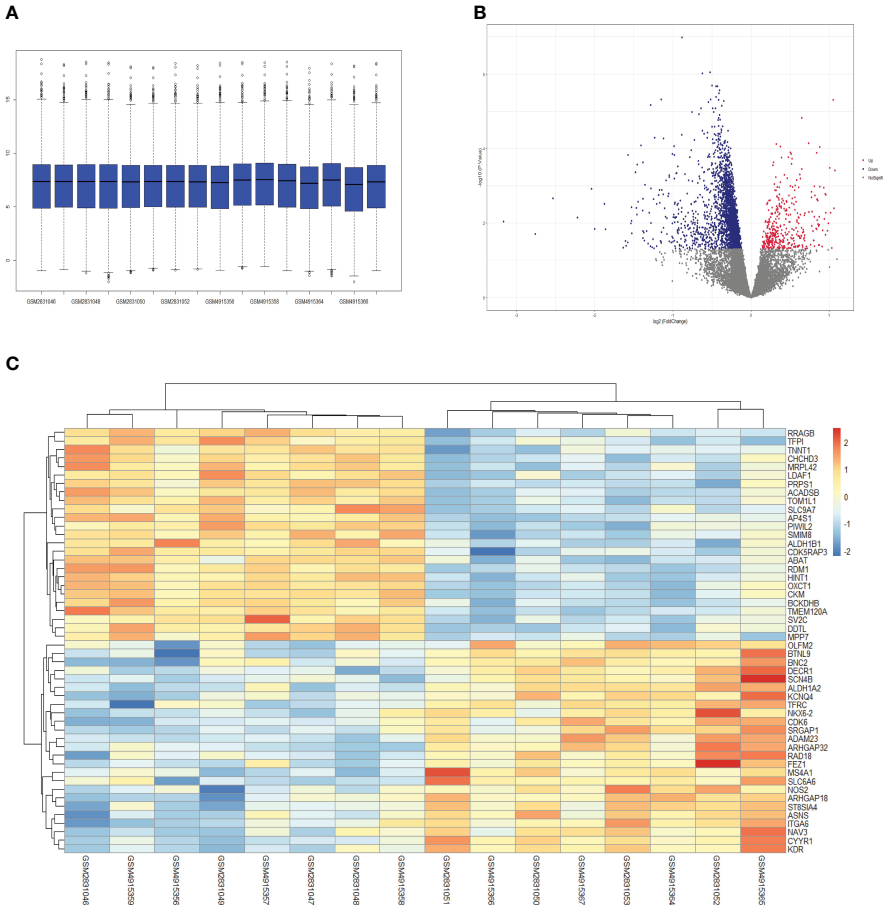

FIGURE 2
Visualization of differentially expressed genes. The red dots represent genes that are significantly upregulated and the blue dots represent significantly downregulated genes. **(A)** Box plot map **(B)** Volcano plot and **(C)** heatmap of the first 25 upregulated genes and 25 downregulated genes with minimum p values.

TABLE 2 Top 5 upregulated and downregulated genes with the most significant differences.

Genes	Regulate	logFC	P value
ALDH1A2	Up	1.050	4.92E-06
ADAM23	Up	0.648	1.51E-05
MS4A1	Up	0.736	7.23E-05
CYYR1	Up	0.323	7.58E-05
KDR	Up	0.373	8.78E-05
RRAGB	Down	-0.883	1.04E-07
BCKDHB	Down	-0.524	9.05E-07
TMEM120A	Down	-0.623	9.69E-07
OXCT1	Down	-0.501	2.04E-06
CKM	Down	-0.449	2.12E-06

3.5 Functional correlation analysis of genes in the key module

A total of 1515 hub genes of the yellow module and turquoise module were divided into the following categories: biological process (BP) (Figure 5A; Table 4), cellular component (CC) (Figure 5B), and molecular function (MF) (Figure 5C). The GO analysis results showed that the hub genes were mainly related to "proteasomal protein catabolic process" (BP, GO:0010498, adjusted P-value = 7.79E-26), "mitochondrial matrix" (CC, GO:0005759, adjusted P value = 1.72E-21), and "translation regulator activity" (MF, GO:0045182, adjusted P value = 1.11E-16), etc. KEGG pathway enrichment analysis showed that hub genes were mainly enriched in "protein processing in endoplasmic reticulum" (hsa04141, adjusted P value = 4.47E-11), "Parkinson's disease" (hsa05012, adjusted P value = 7.11E-10) and "citrate cycle" (hsa00020, adjusted P value = 4.27E-09) (Figure 5D; Table 5). PPI analysis showed the 100 genes with the highest score, and the top 3 genes were RPS9, FTSJ3, and PPP2CA (Figure 5E).

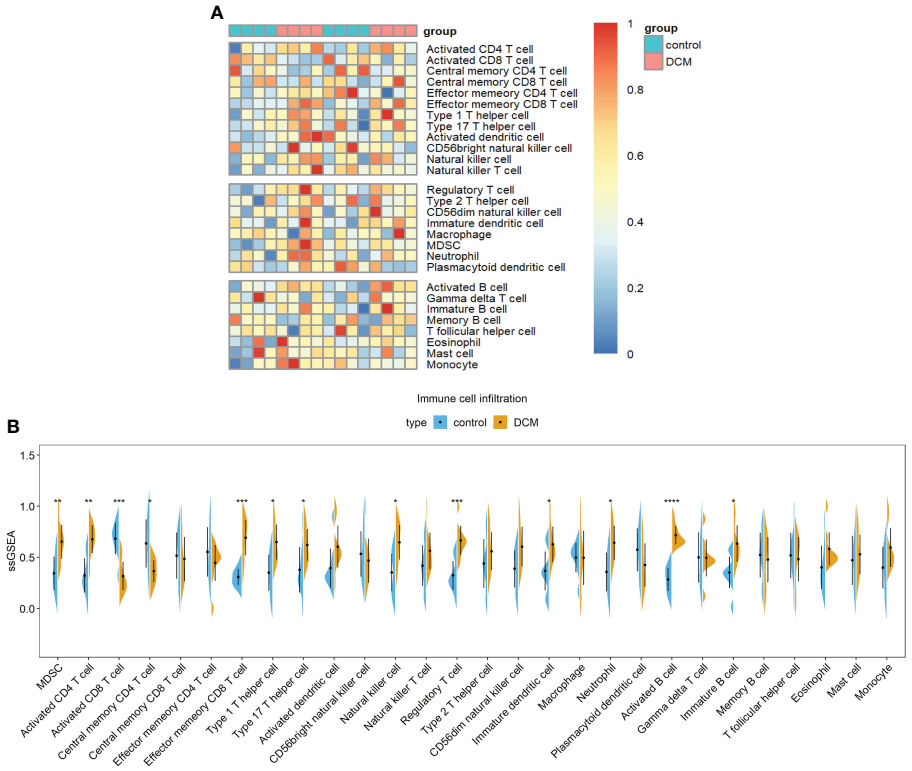

FIGURE 3
Immune cell infiltration.**(A)** heatmap **(B)** violin plot of immune cell infiltration. *p<0.05; **p<0.01; ***p<0.001. ****p<0.0001. DCM, diabetic cardiomyopathy.

3.6 Screening the diagnostic markers

We searched the GeneCards database for diabetic cardiomyopathy and found 4360 related genes. There were 323 genes included in all of the module hub genes and DEGs and the GeneCards database at the same time (Figure 6A). Then, we established a LASSO model with these selected genes based on the value of lambda (minλ= 0.00457) (Figures 6B, C). LASSO regression analysis was further performed, and nonzero regression coefficients were used to construct gene characteristics identified with six genes (OXCT1, CACNA2D2, BCL7B, EGLN3, GABARAP, and ACADSB). In addition, these six genes were identified based on model indices according to the following formula:

$$\text{index} = \text{ExpOXCT1} \times (-0.9471179) + \text{ExpCACNA2D2} \times (-0.4471940) + \text{ExpBCL7B} \times (-1.6038937) + \text{ExpEGLN3} \times (2.5024612) + \text{ExpGABARAP} \times (-2.1876917) + \text{ExpACADSB} \times (-8.4851697).$$

"Exp" refers to the expression value of a gene after logarithmic conversion and coefficient refers to the non-zero coefficient calculated by the model. The DCM group tended to have higher index scores than the control group. Therefore, the calculation of the gene expression index can help diagnose DCM. In addition, we evaluated the accuracy of the LASSO model by ROC curves (Figure 6D), suggesting that these genes could be used as potential biomarkers for further testing of DCM. Box plots illustrate the expression trends of OXCT1 (Figure 6E), CACNA2D2 (Figure 6F), EGLN3 (Figure 6G), GABARAP (Figure 6H), BCL7B(Figure 6I), and ACADSB (Figure 6J) align with the predictions.

3.7 Validating the diagnostic markers in public datasets

A dataset containing DCM and control mouse myocardial tissue (GSE163060) was performed for human homologous gene transformation and data normalization to verify the accuracy and efficiency of the model. The results showed that the index value of the DCM group was significantly higher than that of the control group (P<0.001, Figure 7A). Roc curve showed that the overall AUC was 1.00 (Table 6). GABARAP (AUC=0.88), EGLN3 (AUC=0.86), OXCT1 (AUC=0.76), CACNA2D2 (AUC=0.68), ACADSB (AUC=0.60) had high prediction efficiency. BCL7B (AUC=0.32) was poor. The human blood PBMC cell dataset (GSE175988) was also used for verification. The index value of the DCM group was significantly higher than that of the control group (P<0.001, Figure 7B), and the AUC of the model was 0.94. CACNA2D2 (AUC=0.87), ACADSB (AUC=0.79), and GABARAP (AUC=0.75) had better prediction effects. OXCT1 (AUC=0.48), EGLN3 (AUC=0.38), BCL7B (AUC=0.32) was poor. There was no statistical difference in the hub genes in the mouse myocardial tissue (GSE163060) dataset, which may be related to the small sample size (Figure 7C). The expressions of ACADSB,

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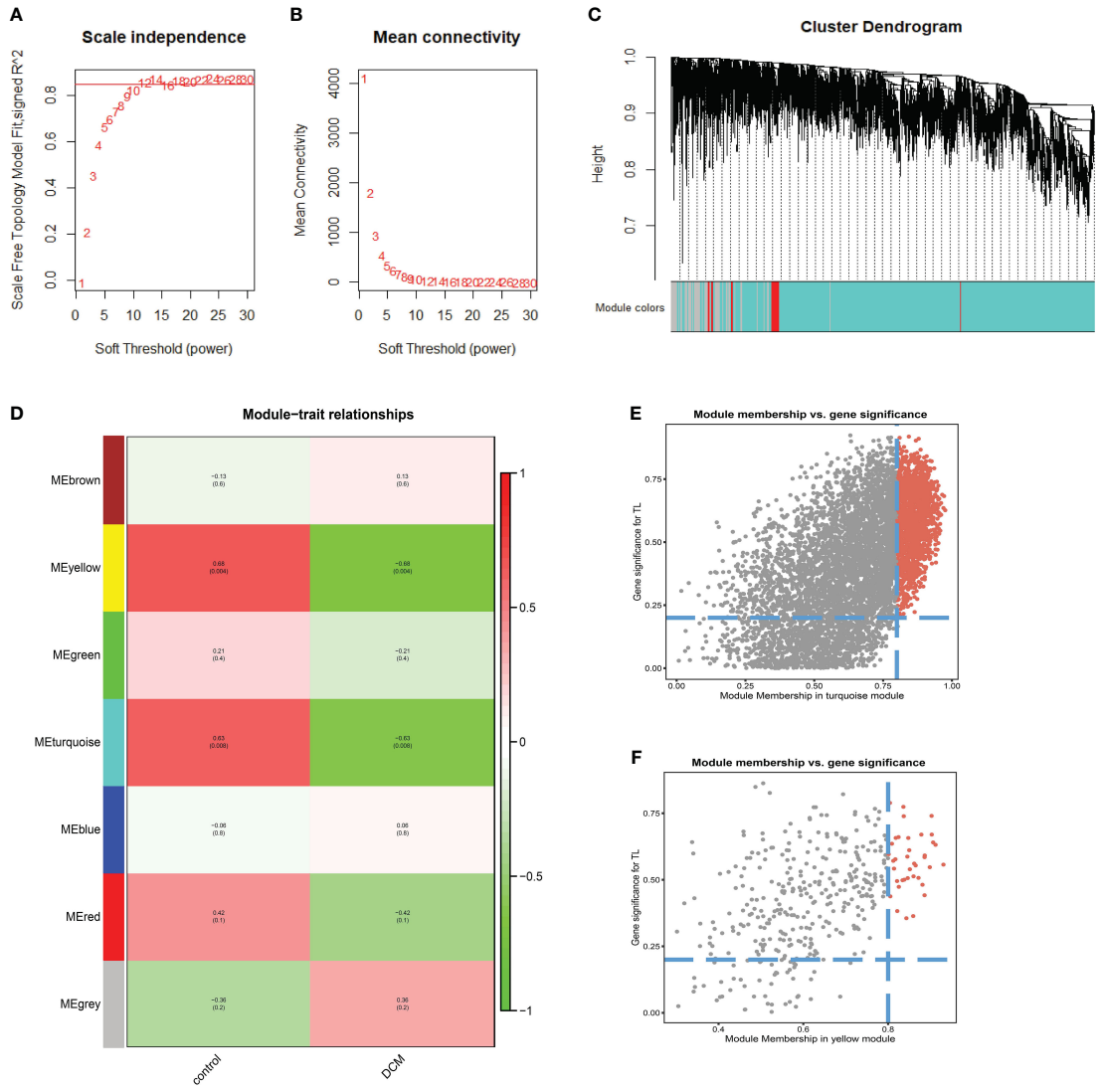

FIGURE 4
Results of weighted gene coexpression network analysis. **(A)** Scale-free fitting index (β) of soft threshold abilities **(B)** Average connectivity of soft threshold abilities **(C)** Cluster dendrogram **(D)** Module-trait relationships. Red represents a positive correlation with clinical traits, green represents a negative correlation with clinical traits **(E)** Correlation between module membership and gene significance in turquoise modules. **(F)** Correlation between module membership and gene significance in yellow modules. DCM, diabetic cardiomyopathy.

TABLE 3 The characteristics of different modules.

Model	Count	Coefficient	P value
brown	619	0.13	0.633
yellow	392	0.68	0.004
green	255	0.21	0.442
turquoise	6471	0.63	0.008
blue	3788	0.06	0.826
red	176	0.42	0.107
grey	1869	0.36	0.170

GABARAP, and CACNA2D2 in the DCM group were significantly lower than those in the control group in the human blood PBMC cell dataset (GSE175988, Figure 7D).

3.8 Validating the diagnostic markers in H9C2 cell

The expression of the hub gene in H9C2 cells was verified, and the results showed that the expression of Acadsb, Oxct1, Bcl7b, Gabarap, and Cacna2d2 was decreased and the expression of Egln3 was increased in cardiomyocytes after high glucose treatment (Figure 8), which was similar to the previous results.

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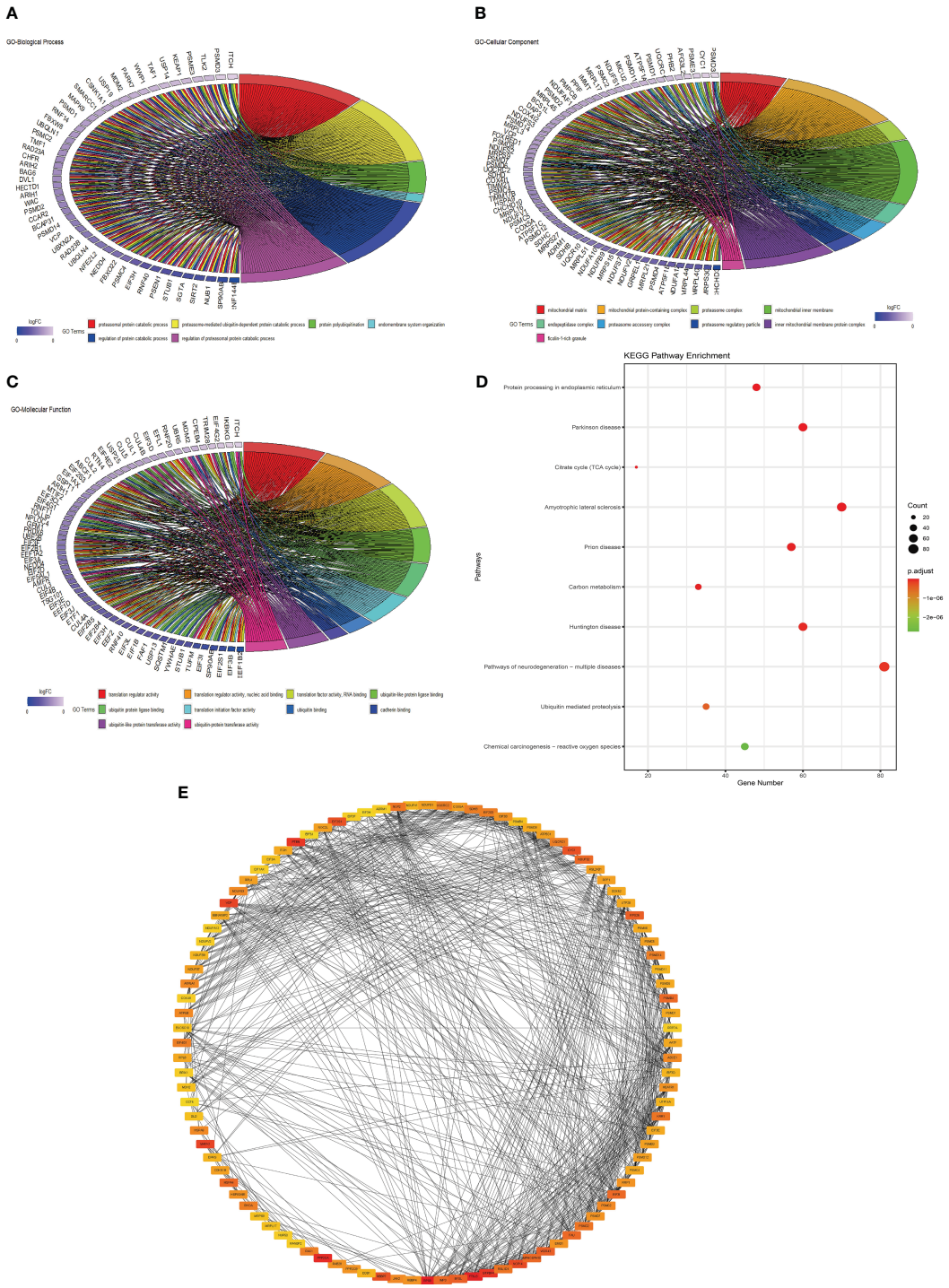

FIGURE 5
GO analysis, KEGG analysis, and PPI network of the hub genes. **(A)** Biological process; **(B)** cellular component; **(C)** molecular function;**(D)** KEGG pathway enrichment analysis; **(E)** top 100 gene network. KEGG, Kyoto Encyclopedia of Genes and Genomes.

4 Discussion

Diabetic cardiomyopathy is a pathophysiological condition caused by diabetes that can lead to heart failure. In a series of cardiac pathologies, such as myocardial ischemia and heart failure, infiltrating immune cells are an important factor in the occurrence and development of myocardial injury and left ventricular dysfunction (16). Diabetic cardiomyopathy has attracted the attention of cardiologists and endocrinologists at home and abroad, but the diagnosis of diabetic cardiomyopathy is still a challenge, especially for asymptomatic patients. The pathogenesis, progression, and therapeutic targets of diabetic cardiomyopathy remain unknown. Therefore, it is of great significance to study the regulatory mechanisms and key targets of DCM for early prevention and treatment.

TABLE 4 Top 5 GO enriched by the yellow and turquoise module hub genes.

ONTOLOGY	ID	Description	Count	P.adjust
BP	GO:0106106	proteasomal protein catabolic process	116	7.79E-26
BP	GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	105	1.79E-24
BP	GO:0045333	cellular respiration	55	1.54E-15
BP	GO:0015980	energy derivation by oxidation of organic compounds	68	4.96E-15
BP	GO:0048193	Golgi vesicle transport	78	1.86E-13
CC	GO:0005759	mitochondrial matrix	106	1.72E-21
CC	GO:0098798	mitochondrial protein-containing complex	61	1.36E-12
CC	GO:0000151	ubiquitin ligase complex	63	4.82E-12
CC	GO:0005743	proteasome complex	26	2.31E-11
CC	GO:1905369	mitochondrial inner membrane	86	4.53E-11
MF	GO:0045182	translation regulator activity	49	1.11E-16
MF	GO:0090079	translation regulator activity, nucleic acid binding	41	3.58E-15
MF	GO:0008135	translation factor activity, RNA binding	35	2.07E-14
MF	GO:0044389	ubiquitin-like protein ligase binding	65	3.09E-10
MF	GO:0031625	ubiquitin protein ligase binding	62	3.54E-10

Our study selected specimens from the early stage of diabetic cardiomyopathy and found that the genes related to DCM are mainly enriched in “proteasomal protein catabolic process”, “mitochondrial matrix”, “translation regulator activity”, “protein processing in endoplasmic reticulum” and” citrate cycle” indicating that the occurrence and development of DCM are closely related to myocardial cell metabolism. Studies have shown that in patients with diabetes, the utilization rate of heart substrates is reduced, which may be due to the inhibition of fatty acid accumulation on glycolysis (17). Mitochondrial dysfunction is another cause of DCM and HF development. In general, approximately 90% of intracellular ATP production in cardiomyopathy is produced by mitochondrial oxidative phosphorylation. However, in T2DM patients, mitochondria produce ATP by oxidation of free fatty acids instead of glucose, which in turn leads to increased mitochondrial ROS production and decreased oxidative phosphorylation (17, 18). Heart failure in glycolysis and the changes in mitochondrial oxidative metabolism are due to the change in key enzymes involved in the metabolic pathway of transcription, and the NAD redox state (NAD and nicotinamide adenine dinucleotide level) and the change in metabolite signaling. Epigenetic changes after these signals will help translation control coding energy metabolism enzyme-encoding gene expression (19). Therefore, improving the energy metabolism pathway of diabetic cardiomyopathy is a new treatment direction.

TABLE 5 Top 10 KEGG pathways enriched by the yellow and turquoise module hub genes.

ID	Description	Count	P.adjust
hsa04141	Protein processing in endoplasmic reticulum	48	4.47E-11
hsa05012	Parkinson disease	60	7.11E-10
hsa00020	Citrate cycle (TCA cycle)	17	4.27E-9
hsa05014	Amyotrophic lateral sclerosis	70	1.44E-8
hsa05020	Prion disease	57	2.51E-8
hsa01200	Carbon metabolism	33	2.79E-8
hsa05016	Huntington disease	60	8.22E-8
hsa05022	Pathways of neurodegeneration - multiple diseases	81	1.23E-7
hsa04120	Ubiquitin mediated proteolysis	35	4.69E-7
hsa05208	Chemical carcinogenesis - reactive oxygen species	45	2.75E-6

After WGCNA and LASSO analysis, we found diagnostic and predictive biomarkers of type 2 DCM, including OXCT1, CACNA2D2, BCL7B, EGLN3, GABARAP, and ACADSB. However, the relationship and mechanism between these genes and DCM have not been fully confirmed. OXCT1 is a member of the OXCT1 gene family that encodes 3-oxyacid coenzyme A-transferase. This coding protein is a homologous dimer mitochondrial matrix enzyme that plays a core role in the catabolism of ketone bodies by catalyzing the reversible transfer of coenzyme A from succinyl-CoA to acatalectic acid (20). OXCT1 plays an important role in heart failure and diabetes (21, 22). Studies have shown that hyperglycemia reduces the expression of Bdh1 and Oxct1 in the hearts of mice. BDH1 and OXCT1 are also inhibited in the failing heart of diabetic patients but not in nondiabetic patients (23), which is similar to our findings. Chronic elevation of circulating ketone inhibits the development of inflammatory heart failure (22). Therefore, OXCT1 may be an important diagnostic and therapeutic target for diabetic

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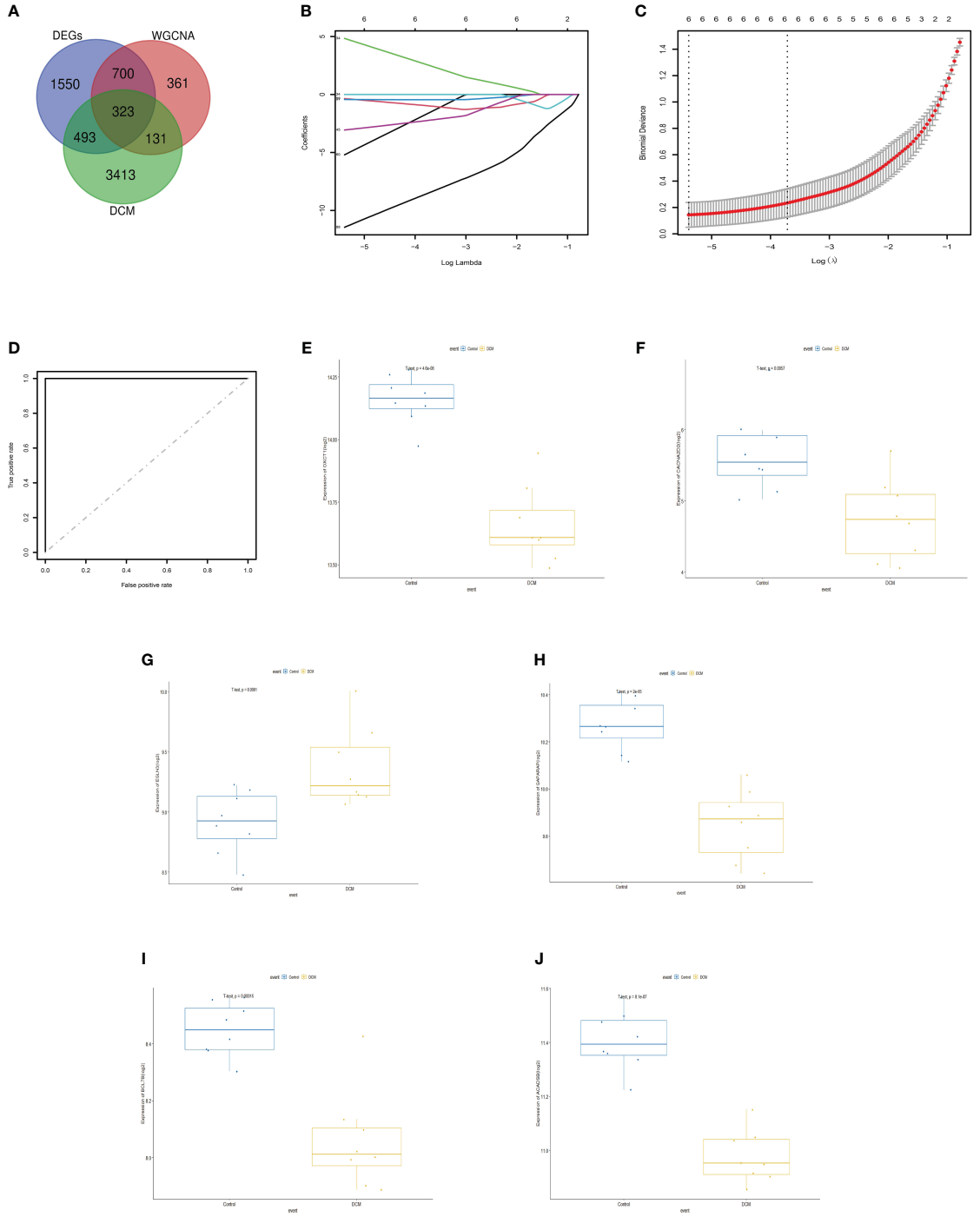

FIGURE 6
Establishment of a model for predicting DCM and its verification. **(A)** Venn diagrams; **(B)** LASSO model; **(C)**binomial deviance; **(D)** ROC curve analysis of GSE106180 and GSE161827; **(E)** Expression of OXCT1; **(F)** expression of CACNA2D2; **(G)** expression of EGLN3; **(H)** expression of GABARAP; **(I)** expression of BCL7B; **(J)** expression of ACADSB. DCM, diabetic cardiomyopathy.WGCNA, weighted gene coexpression network analysis.DEGs, differentially expressed genes.

cardiomyopathy. CACNA2D2 encodes a subunit of the voltage-dependent calcium channel complex, plays an important role in complex assembly and membrane localization, and regulates calcium current and channel dynamics. Studies have shown that high glucose induces mitochondrial fission through the Orai1 calcium channel and participates in diabetic cardiomyopathy hypertrophy (24). Calcium channel signal transduction is linked to mitochondrial function. Calcium overload leads to decreased mitochondrial membrane potential and affects cell function (25). BCL7B has some roles in maintaining nuclear structure and is

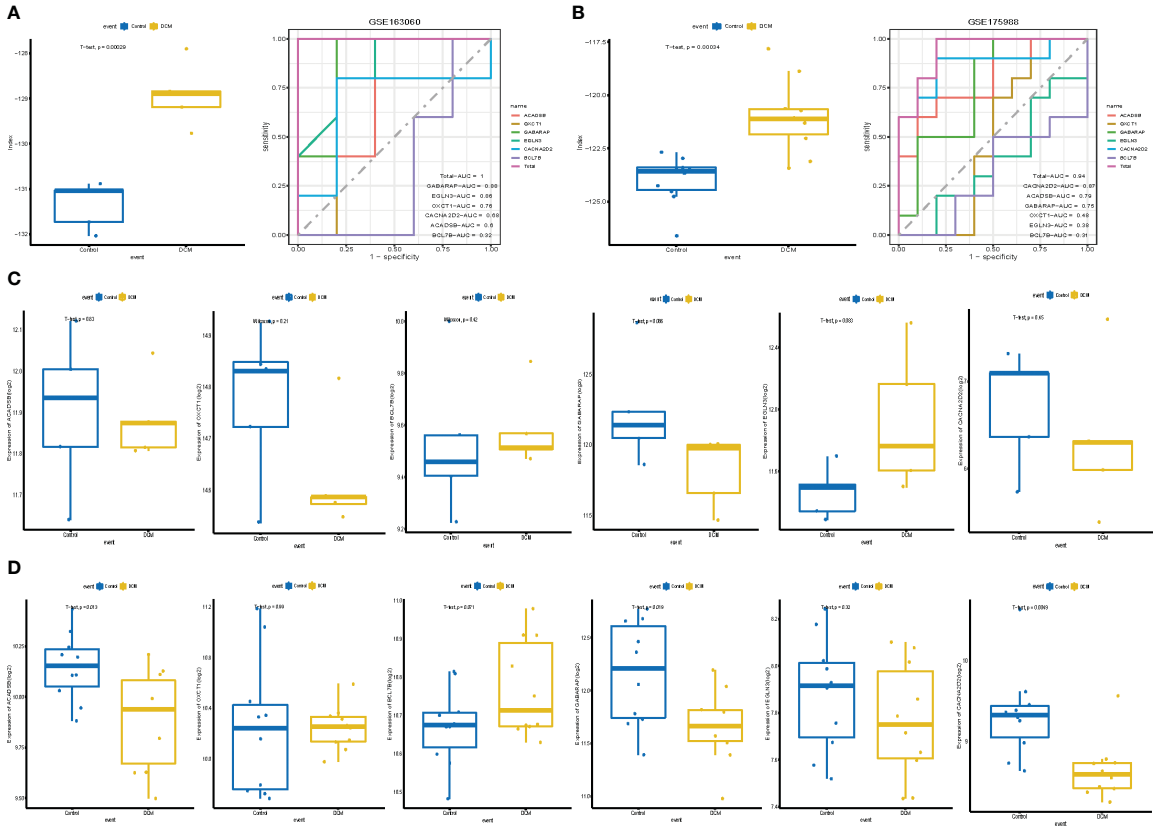

FIGURE 7
Validation of the module in Public datasets. **(A)** Box plot and roc curve of the model in GSE163060; **(B)** Box plot and roc curve of the model in GSE175988; **(C)** Box plot of the hub genes in GSE163060. **(D)** Box plot of the hub genes in GSE175988. DCM, diabetic cardiomyopathy.

TABLE 6 Validation of the hub gene in datasets GSE163060 and GSE175988.

Dateset	Genes	AUC	95%CI
GSE163060	Total	1.000	1.000-1.000
GSE163060	ACADSB	0.600	0.189-1.000
GSE163060	OXCT1	0.760	0.372-1.000
GSE163060	GABARAP	0.880	0.626-1.000
GSE163060	EGLN3	0.860	0.618-1.000
GSE163060	CACNA2D2	0.680	0.265-1.000
GSE163060	BCL7B	0.320	0.000-0.735
GSE175988	Total	0.940	0.841-1.000
GSE175988	ACADSB	0.790	0.586-0.994
GSE175988	OXCT1	0.480	0.190-0.770
GSE175988	GABARAP	0.750	0.519-0.981
GSE175988	EGLN3	0.380	0.121-0.639
GSE175988	CACNA2D2	0.870	0.697-1.000
GSE175988	BCL7B	0.310	0.066-0.554

involved in the regulation of multiple pathways, including Wnt and apoptosis (26). EGLN3 participates in the activation of cysteine-type endopeptidase activity during apoptosis. A study by Xia et al. showed that inhibition of EGLN3 ameliorates cardiac dysfunction in diabetic cardiomyopathy (27). The presence of GABARAP is very important for Golgi body morphology. In addition, studies have shown that it is involved in autophagy and may play an important role in diabetes (28, 29). ACADSB, a member of the acyl-CoA dehydrogenase family, catalyzes the dehydrogenation of acyl-CoA derivatives in fatty acid or branched amino acid metabolism. It plays an important role in the energy homeostasis of pathophysiological processes (30).

The hub genes in multiple datasets and cardiomyocytes were verified, and the results all showed that the hub genes had good diagnostic efficacy. Notably, similar results were obtained in the human blood PBMC dataset. So, the relevant genes could be detected in the blood PBMC, which significantly reduces the difficulty of detection compared to traditional biopsies. DCM lacks specific diagnostic criteria. As an invasive examination, myocardial biopsy has disadvantages such as high risk and complicated procedures. In addition, when DCM develops to a certain stage the heart enlarges significantly and the heart function declines, myocardial biopsy is not suitable (31). In this case, the

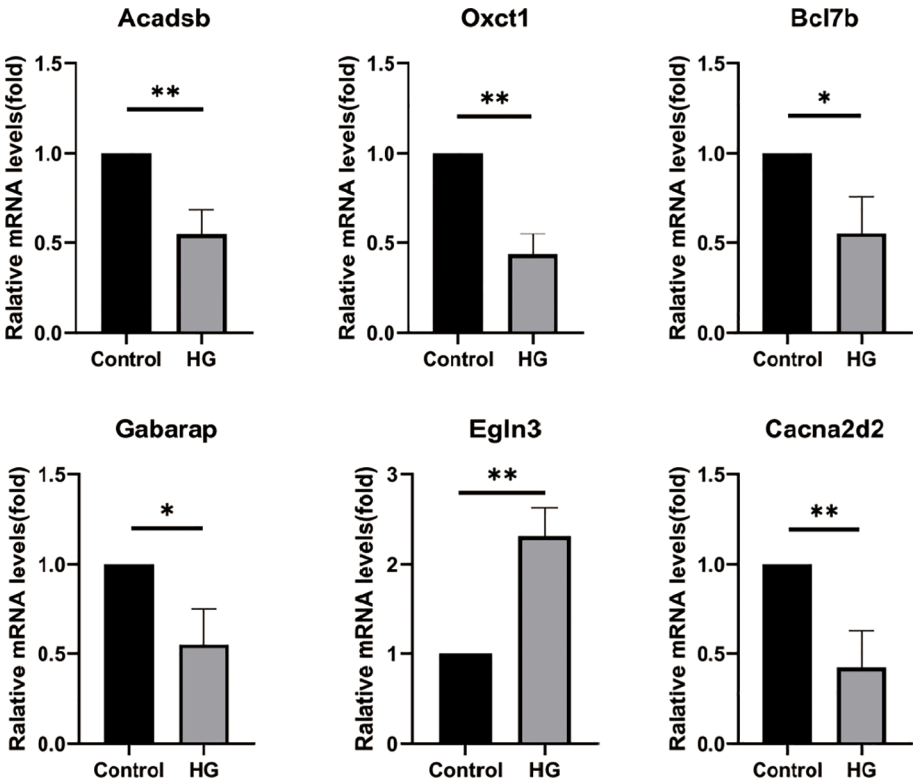

FIGURE 8
The mRNA levels of Acadsb, Oxct1, Bcl7b, Gabarap, Egln3, Cacna2d2 in H9C2 cell. HG, high glucose concentration. *P<0.05,**P<0.01.

detection of blood PBMC has the advantages of convenience, low risk, and small damage. ROC curves showed that GABARAP, EGLN3, OXCT1, CACNA2D2, and ACADSB had high detection accuracy in myocardial tissue. CACNA2D2, ACADSB, and GABARAP are more accurate in human blood PBMCs. When the economic budget is insufficient to carry out multi-gene sequencing, genes with higher diagnostic efficiency can be preferentially selected for detection by RT-qPCR. At present, the diagnosis of DCM is mainly based on myocardial biopsy, combined with a variety of detection methods. RT-qPCR is relatively cheap and therefore more suitable for screening in the population.

In addition, our study found changes in immune infiltration in cardiomyocytes in diabetic cardiomyopathy. A large number of studies have shown that white blood cells and their subgroups, such as neutrophils, monocytes, and lymphocytes, are involved in inflammation and play an important role in the occurrence and progression of cardiovascular diseases (32). Th and Treg subgroups are involved in inflammation, insulin resistance, and vascular changes in diabetes (33). Compared with healthy controls, neutrophils promote the secretion of cytokines and growth factors in diabetic patients, which helps neutrophils further migrate to inflammatory sites and promote the production of reactive oxygen species and apoptosis (29). In T2DM, Treg cells can inhibit Th1, Th2, and Th17 responses through various pathways, such as inhibiting cytokine secretion, regulating the microenvironment, and changing the expression of surface receptors to improve insulin resistance (34). B lymphocytes are antigen-presenting cells and autoantibody secretions. B-cell-deficient mice showed less inflammation and improved glucose tolerance (35). Further study of these mechanisms may contribute to the search and development of new therapeutic targets.

The strength of this study lies in the development of a diagnostic and predictive model for diabetic cardiomyopathy. The results were verified in datasets and cardiomyocytes. The model was also found to have a good predictive effect in the PBMC datasets, which means that the diagnosis of DCM can be made by blood, making detection more convenient.

Our study also has some limitations. Since it is difficult to obtain cardiac tissue samples from diabetic cardiomyopathy patients, this study only analyzed the heart tissue of diabetic cardiomyopathy animals. Second, the public datasets we analyzed did not have the results of cardiac function and pathological tests. Therefore, we cannot compare the results of this study with other models. In addition, we did not verify the diagnostic efficiency of the genes involved in PBMC in the population. Therefore, large-scale clinical studies are needed to further validate the model in the future.

5 Conclusion

We found diagnostic and predictive biomarkers of type 2 DCM consisting of OXCT1, CACNA2D2, BCL7B, EGLN3, GABARAP, and ACADSB, which may reduce the difficulty of early prediction of diabetic cardiomyopathy, and lay a foundation for prospective

research. Our analyses provide a reference for the detection of promising biomarkers and therapeutic targets and for improving the ability to diagnose and treat patients with DCM.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

Conceptualization, MC, and HW. Methodology, YA. Validation, HW, YA, and YL. Data curation, HW, and YL. Writing—original draft preparation. MC. Writing—review and editing, LW and XQ. Visualization, MC. Supervision, LW. Project administration, XQ. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res* (2018) 122(4):624–38. doi: 10.1161/CIRCRESAHA.117.311586

2. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia* (2018) 61(1):21–8. doi: 10.1007/s00125-017-4390-4

3. Paolillo S, Marsico F, Prastaro M, Renga F, Esposito L, De Martino F, et al. Diabetic cardiomyopathy: definition, diagnosis, and therapeutic implications. *Heart Fail Clin* (2019) 15(3):341–7. doi: 10.1016/j.hfc.2019.02.003

4. Yin Z, Chen C. Biological functions and clinical prospects of extracellular non-coding RNAs in diabetic cardiomyopathy: an updated review. *J Cardiovasc Transl Res* (2022) 15(3):469–76. doi: 10.1007/s12265-022-10217-0

5. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf* (2008) 9:559. doi: 10.1186/1471-2105-9-559

6. Wen Z, Wu L, Wang L, Ou Q, Ma H, Wu Q, et al. Comprehensive genetic analysis of tuberculosis and identification of candidate biomarkers. *Front Genet* (2022) 13:832739. doi: 10.3389/fgene.2022.832739

7. Robert T. Regression shrinkage and selection via the lasso. *R Statist. Soc B* (1996) 58:267–88. doi: 10.1111/j.2517-6161.1996.tb02080.x

8. Yu T, Huang Z, Pu Z. Identification of potential diagnostic biomarkers and biological pathways in hypertrophic cardiomyopathy based on bioinformatics analysis. *Genes (Basel)* (2022) 13(3):530. doi: 10.3390/genes13030530

9. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res* (2005) 52(4):313–20. doi: 10.1016/j.phrs.2005.05.004

10. Croteau D, Luptak I, Chambers JM, Hobai I, Panagia M, Pimentel DR, et al. Effects of sodium-glucose linked transporter 2 inhibition with ertugliflozin on mitochondrial function, energetics, and metabolic gene expression in the presence and absence of diabetes mellitus in mice. *J Am Heart Assoc* (2021) 10(13):e019995. doi: 10.1161/JAHA.120.019995

11. Drost HG, Paszkowski J. Biomartr: genomic data retrieval with R. *Bioinformatics* (2017) 33(8):1216–7. doi: 10.1093/bioinformatics/btw821

12. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* (2015) 43(7):e47. doi: 10.1093/nar/gkv007

13. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep* (2017) 18(1):248–62. doi: 10.1016/j.celrep.2016.12.019

14. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* (2012) 16(5):284–7. doi: 10.1089/omi.2011.0118

15. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinf* (2011) 12:77. doi: 10.1186/1471-2105-12-77

16. He A, Fang W, Zhao K, Wang Y, Li J, Yang C, et al. Mast cell-deficiency protects mice from streptozotocin-induced diabetic cardiomyopathy. *Transl Res* (2019) 208:1–14. doi: 10.1016/j.trsl.2019.01.005

17. Jankauskas SS, Kansakar U, Varzideh F, Wilson S, Mone P, Lombardi A, et al. Heart failure in diabetes. *Metabolism* (2021) 125:154910. doi: 10.1016/j.metabol.2021.154910

18. Gambardella J, Lombardi A, Santulli G. Metabolic flexibility of mitochondria plays a key role in balancing glucose and fatty acid metabolism in the diabetic heart. *Diabetes* (2020) 69(10):2054–7. doi: 10.2337/dbi20-0024

19. Lopaschuk GD, Karwi QG, Tian R, Wende AR, Abel ED. Cardiac energy metabolism in heart failure. *Circ Res* (2021) 128(10):1487–513. doi: 10.1161/CIRCRESAHA.121.318241

20. Cotter DG, d'Avignon DA, Wentz AE, Weber ML, Crawford PA. Obligate role for ketone body oxidation in neonatal metabolic homeostasis. *J Biol Chem* (2011) 286(9):6902–10. doi: 10.1074/jbc.M110.192369

21. Al Batran R, Gopal K, Capozzi ME, Chahade JJ, Saleme B, Tabatabaei-Dakhili SA, et al. Pimozide alleviates hyperglycemia in diet-induced obesity by inhibiting

skeletal muscle ketone oxidation. *Cell Metab* (2020) 31(5):909–19. doi: 10.1016/j.cmet.2020.03.017

22. Byrne NJ, Soni S, Takahara S, Ferdaoussi M, Al Batran R, Darwesh AM, et al. Chronically elevating circulating ketones can reduce cardiac inflammation and blunt the development of heart failure. *Circ Heart Fail* (2020) 13(6):e006573. doi: 10.1161/CIRCHEARTFAILURE.119.006573

23. Brahma MK, Ha CM, Pepin ME, Mia S, Sun Z, Chatham JC, et al. Increased glucose availability attenuates myocardial ketone body utilization. *J Am Heart Assoc* (2020) 9(15):e013039. doi: 10.1161/JAHA.119.013039

24. Wu QR, Zheng DL, Liu PM, Yang H, Li LA, Kuang SJ, et al. High glucose induces Drp1-mediated mitochondrial fission via the Orai1 calcium channel to participate in diabetic cardiomyocyte hypertrophy. *Cell Death Dis* (2021) 12(2):216. doi: 10.1038/s41419-021-03502-4

25. Wang J, Tang Z, Zhang Y, Qiu C, Zhu L, Zhao N, et al. Matrine alleviates AGEs-induced cardiac dysfunctions by attenuating calcium overload via reducing ryanodine receptor 2 activity. *Eur J Pharmacol* (2019) 842:118–24. doi: 10.1016/j.ejphar.2018.10.010

26. Uehara T, Kage-Nakadai E, Yoshina S, Imae R, Mitani S. The tumor suppressor BCL7B functions in the wnt signaling pathway. *PloS Genet* (2015) 11(1):e1004921. doi: 10.1371/journal.pgen.1004921

27. Xia Y, Gong L, Liu H, Luo B, Li B, Li R, et al. Inhibition of prolyl hydroxylase 3 ameliorates cardiac dysfunction in diabetic cardiomyopathy. *Mol Cell Endocrinol* (2015) 403:21–9. doi: 10.1016/j.mce.2015.01.014

28. Sanwald JL, Dobner J, Simons IM, Poschmann G, Stühler K, Üffing A, et al. Lack of GABARAP-type proteins is accompanied by altered golgi morphology and surfaceome composition. *Int J Mol Sci* (2020) 22(1):85. doi: 10.3390/ijms22010085

29. Hui KK, Tanaka M. Autophagy links MTOR and GABA signaling in the brain. *Autophagy* (2019) 10):1848–9. doi: 10.1080/15548627.2019.1637643

30. Wu Y, Zhang X, Wei X, Feng H, Hu B, Deng Z, et al. A mitochondrial dysfunction and oxidative stress pathway-based prognostic signature for clear cell renal cell carcinoma. *Oxid Med Cell Longev* (2021) 2021:9939331. doi: 10.1155/2021/9939331

31. Francis R, Lewis C. Myocardial biopsy: techniques and indications. *Heart* (2018) 104(11):950–8. doi: 10.1136/heartjnl-2017-311382

32. Bajpai A, Tilley DG. The role of leukocytes in diabetic cardiomyopathy. *Front Physiol* (2018) 9:1547. doi: 10.3389/fphys.2018.01547

33. Tian CJ, Zhang JH, Liu J, Ma Z, Zhen Z. Ryanodine receptor and immune-related molecules in diabetic cardiomyopathy. *ESC Heart Fail* (2021) 8(4):2637–46. doi: 10.1002/ehf2.13431

34. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci Transl Med* (2015) 7(315):315ra189. doi: 10.1126/scitranslmed.aad4134

35. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med* (2011) 17(5):610–7. doi: 10.1038/nm.2353

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Predicting the risk of subclinical atherosclerosis based on interpretable machine models in a Chinese T2DM population

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Background: Cardiovascular disease (CVD) has emerged as a global public health concern. Identifying and preventing subclinical atherosclerosis (SCAS), an early indicator of CVD, is critical for improving cardiovascular outcomes. This study aimed to construct interpretable machine learning models for predicting SCAS risk in type 2 diabetes mellitus (T2DM) patients.

Methods: This study included 3084 T2DM individuals who received health care at Zhenhai Lianhua Hospital, Ningbo, China, from January 2018 to December 2022. The least absolute shrinkage and selection operator combined with random forest-recursive feature elimination were used to screen for characteristic variables. Linear discriminant analysis, logistic regression, Naive Bayes, random forest, support vector machine, and extreme gradient boosting were employed in constructing risk prediction models for SCAS in T2DM patients. The area under the receiver operating characteristic curve (AUC) was employed to assess the predictive capacity of the model through 10-fold cross-validation. Additionally, the SHapley Additive exPlanations were utilized to interpret the best-performing model.

Results: The percentage of SCAS was 38.46% (n=1186) in the study population. Fourteen variables, including age, white blood cell count, and basophil count, were identified as independent risk factors for SCAS. Nine predictors, including age, albumin, and total protein, were screened for the construction of risk prediction models. After validation, the random forest model exhibited the best clinical predictive value in the training set with an AUC of 0.729 (95% CI: 0.709-0.749), and it also demonstrated good predictive value in the internal validation set [AUC: 0.715 (95% CI: 0.688-0.742)]. The model interpretation revealed that age, albumin, total protein, total cholesterol, and serum creatinine were the top five variables contributing to the prediction model.

Conclusion: The construction of SCAS risk models based on the Chinese T2DM population contributes to its early prevention and intervention, which would reduce the incidence of adverse cardiovascular prognostic events.

KEYWORDS

subclinical atherosclerosis, type 2 diabetes mellitus, independent risk factors, interpretable machine learning, prediction model

1 Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance and relative insulin deficiency. In recent years, the prevalence of T2DM has increased steadily, which has become a serious public health issue. Updated estimates for 2021 showed that about 10.5% of the global population had T2DM, a prediction that this figure would increase to 12.2% by 2045 (1). Cardiovascular disease (CVD) is the leading cause of death and disability in T2DM (2, 3). Studies have shown that the risk of CVD in patients with T2DM is two to four times higher than in individuals without diabetes (4, 5). Atherosclerosis (AS), the predominant pathophysiologic process in CVD, may begin early in life and remain latent and asymptomatic for extended periods before progressing to advanced stages. Subclinical atherosclerosis (SCAS) serves as an early indicator of atherosclerotic burden, and its timely recognition can help slow down or prevent the progression to CVD (6). Therefore, the early identification and effective management of SCAS in individuals with T2DM are crucial strategies to mitigate progression to overt CVD, thereby improving life expectancy and quality.

Diagnostic methods for SCAS include angiography, intravascular ultrasound, carotid ultrasound (CUS), computed tomography (CT), and magnetic resonance imaging. Measuring carotid intima-media thickness (CIMT) and coronary artery calcification (CAC) using CUS and CT has become the mainstay for assessing SCAS, owing to their noninvasive and easily accessible nature (7, 8). However, large-scale use of CUS and CT could inevitably lead to the waste of medical resources and increased costs. Thus, establishing an assessment tool capable of screening individuals at high risk for SCAS without the need for imaging examinations is of great significance.

In recent years, artificial intelligence (AI) and machine learning (ML) have increasingly been utilized in the healthcare field (9). Several studies currently employ ML methods to research SCAS. For example, Sánchez-Cabo et al. (10) developed a SCAS risk prediction model for young asymptomatic individuals using four ML algorithms, demonstrating good clinical predictive value with an area under the receiver operating characteristic curve (AUC) of 0.890. Additionally, Núñez et al. (11) used ML methods to identify circulating proteins that can predict SCAS, also showing good clinical predictive value with an AUC of 0.730. However, there are few reports on the risk prediction models for SCAS in T2DM patients. The purpose of this study was to establish SCAS risk prediction models based on interpretable machine learning algorithms, contributing to the early identification of SCAS and guiding appropriate prevention and interventions.

2 Methods

2.1 Participants

This study enrolled 3140 T2DM individuals who had sought medical care through outpatient visits, inpatient admissions, and routine physical examinations at Zhenhai Lianhua Hospital in Ningbo, China, from January 2018 to December 2022. The sample size for this study adhered to the rule of 10 events per variable (12). The demographic data, comorbidities, complications, and biochemical parameters were obtained by questionnaires and laboratory tests. Inclusion criteria: participants aged ≥ 18 years who either self-report T2DM, are undergoing pharmacological treatment for T2DM, or meet the diagnostic criteria of T2DM. These criteria include fasting blood glucose (FBG) levels of ≥ 7.0 mmol/L, 2-hour blood glucose levels of ≥ 11.1 mmol/L, or a glycated hemoglobin level of ≥ 6.5% (13). Exclusion criteria: individuals with other forms of diabetes mellitus, concurrent coronary heart disease or cerebral infarction, acute complications related to diabetes mellitus, malignant tumors, severe liver and kidney function abnormalities, or pregnancy. SCAS was defined as CIMT > 1.0 mm and/or the presence of plaque without clinical manifestations (14). Data with more than 20% missing were excluded (n=56), and those with less than 20% were filled by multiple interpolations (Supplementary Figure 1). Ultimately, 3084 T2DM patients were included in this study. The study's flow diagram is depicted in Figure 1.

2.2 Clinical baseline data

Participants' general characteristics include gender, age, body mass index, and blood pressure (both systolic and diastolic measurements). Blood cell counts comprise white blood cell count (WBC), neutrophil count, eosinophil count, basophil count (BASO), lymphocyte count (LYC), red blood cell count, hemoglobin, red blood cell distribution width, mean red blood cell volume (MCV), platelet count, platelet distribution width (PDW), and mean platelet volume (MPV). Biochemical indicators encompass total cholesterol (TC), triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), FBG, total protein (TP), albumin (ALB), aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase (GGT), serum uric acid (SUA), and serum creatinine (SCR).

2.3 Statistical analysis

Kolmogorov-Smirnov assessed sample distribution normality. Normal continuous variables were expressed as means (standard deviation, SD), non-normal continuous variables as median (interquartile range, IQR), and categorical variables as frequency (percentage, %). Between-group analyses involved independent samples t-tests for normal continuous variables, Mann-Whitney U tests for non-normal continuous variables, and chi-square tests for categorical variables. Box plots were used to elucidate the relationship between various metabolic parameters [including atherogenic index of plasma (AIP), Castelli risk index (CRI), metabolic score for insulin resistance (METS-IR), and triglyceride-glucose (TyG) index] and SCAS. The formulas for these parameters were calculated as follows: AIP = Log(TG/HDL); CRI = TC/HDL; METS-IR = Ln((2 * FBG + TG) * BMI)/(Ln (HDL)); TyG = Ln[(TG * FBG)/2]. Multivariate logistic regression

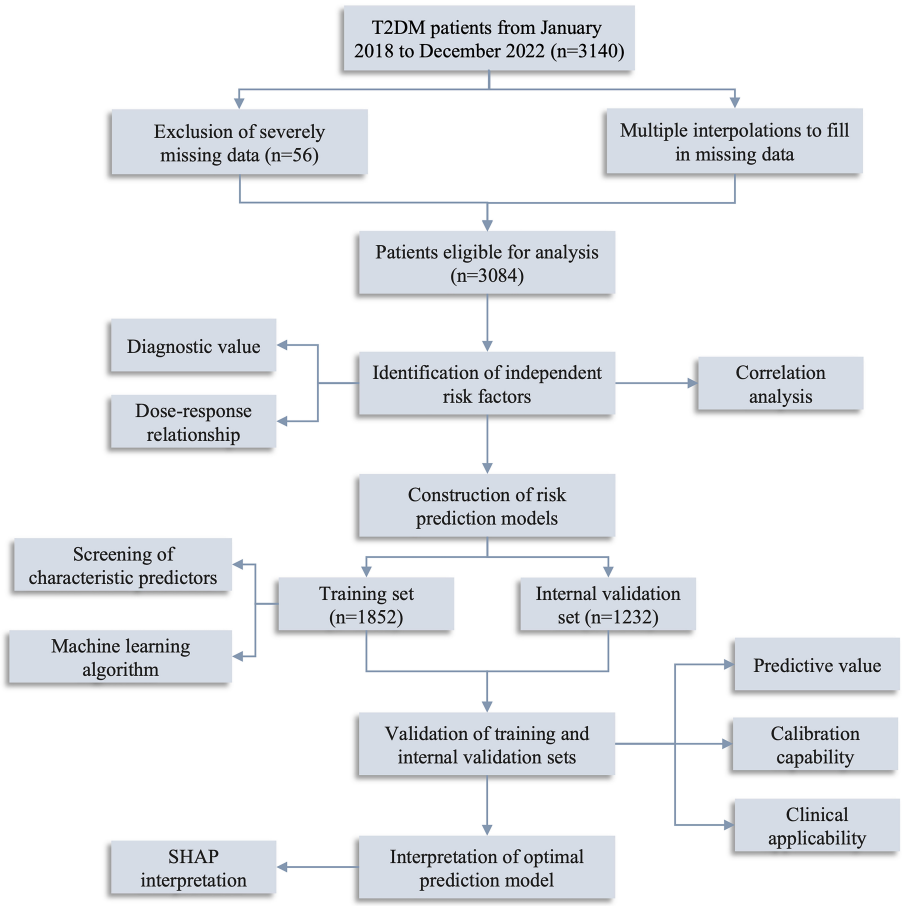

FIGURE 1
Flow diagram of the study. T2DM, type 2 diabetes mellitus; SHAP, Shapley Additive exPlanations.

identified independent risk factors for SCAS. Restricted cubic spline was employed to analyze the dose-response relationship betweent AIP and SCAS.

Least absolute shrinkage and selection operator (LASSO) combined with random forest-recursive feature elimination (RF-RFE) were used to screen for characteristic variables. Six ML methods, including linear discriminant analysis (LDA), logistic regression (LR), Naive Bayes (NB), random forest (RF), support vector machine (SVM), and extreme gradient boosting (XGboost), were used to model construction. The primary parameters used to evaluate the effectiveness of risk prediction models included accuracy, sensitivity, specificity, precision, recall, and the F1 score. AUC was utilized to assess the models' predictive ability. Calibration curves and the Brier score were used to assess calibration capability, while decision curve analysis (DCA) was employed to evaluate clinical applicability. Additionally, the Shapley Additive exPlanations (SHAP) was used to interpret the best predictive model.

All statistical analyses were conducted using Python (https://www.python.org/, version: 3.9.0) and R (https://cran.r-project.org/, version: 4.1.3). All tests were two-sided and $P < 0.05$ was deemed statistically significant.

3 Results

3.1 Clinical baseline information of the study population

A total of 3084 participants were enrolled in this study, comprising 1898 individuals with T2DM without SCAS, and 1186 individuals with T2DM with SCAS. The percentage of SCAS in the T2DM population was found to be as high as 38.46%. The median age of participants was 56 years (IQR: 49-61). Participants in the SCAS group were older, with a median age of 58 years (IQR: 53-62), compared to 54 years (IQR: 46-60) in the control group. The male proportion was similar in both groups (74.6% in the SCAS group vs. 73.8% in the control group, $P > 0.05$). Additionally, statistically significant differences were observed between the groups in terms of routine blood tests, lipid and glucose levels, and liver and kidney function ($P < 0.05$). The baseline clinical characteristics of the study population are presented in Table 1.

The AIP, CRI, METS-IR, and TyG index are metabolism-related parameters commonly used in the diagnosis and risk assessment of metabolism-related diseases (15–18). The current study showed that three metabolism-related parameters, including

TABLE 1 Univariate analysis of subclinical atherosclerosis.

	Overall	Normal	SCAS	*P*-value
N	3084	1898	1186	
Sex (male), %	2291 (74.3)	1416 (74.6)	875 (73.8)	0.639
Age, years	56.00 (49.00, 61.00)	54.00 (46.00, 60.00)	58.00 (53.00, 62.00)	<0.001
BMI, kg/m2	24.62 (22.72, 26.99)	24.69 (22.77, 26.99)	24.54 (22.68, 26.96)	0.209
SBP, mmHg	133.69 (17.48)	133.43 (16.70)	134.10 (18.65)	0.298
DBP, mmHg	81.00 (73.00, 89.00)	81.00 (74.00, 89.00)	80.00 (72.00, 89.00)	0.009
WBC, 10^9/L	6.54 (1.73)	6.48 (1.67)	6.63 (1.81)	0.026
NEU, 10^9/L	3.70 (2.97, 4.60)	3.60 (2.90, 4.50)	3.80 (3.00, 4.81)	<0.001
EOS, 10^9/L	0.11 (0.07, 0.19)	0.11 (0.07, 0.19)	0.12 (0.07, 0.20)	0.123
BASO, 10^9/L	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.02 (0.02, 0.03)	<0.001
LYC, 10^9/L	2.00 (1.60, 2.50)	2.09 (1.60, 2.50)	1.92 (1.55, 2.40)	<0.001
RBC, 10^{12}/L	4.88 (0.52)	4.93 (0.51)	4.79 (0.53)	<0.001
HB, g/L	150.00 (139.00, 159.00)	151.00 (140.00, 160.00)	148.00 (137.00, 158.00)	<0.001
RDW, %	12.50 (12.20, 12.90)	12.50 (12.20, 12.90)	12.60 (12.20, 13.00)	0.003
MCV, fL	91.00 (88.60, 94.00)	91.00 (88.10, 93.80)	91.90 (89.00, 94.90)	<0.001
PLT, 10^9/L	226.93 (58.27)	227.49 (58.41)	226.04 (58.06)	0.499
PDW, %	15.00 (12.50, 16.30)	14.10 (12.30, 16.20)	15.90 (12.90, 16.30)	<0.001
MPV, fL	10.59 (1.15)	10.70 (1.15)	10.41 (1.13)	<0.001
TC, mmol/L	5.00 (1.16)	4.97 (1.07)	5.05 (1.30)	0.057
TG, mmol/L	1.50 (1.06, 2.21)	1.48 (1.04, 2.22)	1.54 (1.11, 2.20)	0.090
HDL, mmol/L	1.14 (0.95, 1.39)	1.16 (0.95, 1.44)	1.11 (0.96, 1.33)	0.001
LDL, mmol/L	2.82 (0.88)	2.80 (0.82)	2.85 (0.96)	0.081
FBG, mmol/L	6.80 (6.19, 8.35)	6.71 (6.18, 8.21)	6.98 (6.21, 8.61)	0.013
TP, g/L	73.00 (68.70, 76.60)	73.70 (70.10, 76.90)	71.50 (66.60, 75.50)	<0.001
ALB, g/L	44.90 (42.27, 46.60)	45.35 (43.10, 46.90)	44.00 (40.90, 46.00)	<0.001
AST, IU/L	23.00 (18.00, 29.00)	23.00 (18.00, 29.00)	23.00 (18.00, 30.00)	0.780
ALT, IU/L	24.00 (17.00, 38.00)	25.00 (17.00, 39.00)	23.00 (16.00, 37.00)	0.058
GGT, U/L	31.00 (21.00, 52.00)	30.00 (20.00, 51.00)	33.00 (22.00, 54.00)	0.008
SUA, μmol/L	357.19 (96.25)	354.05 (95.07)	362.22 (97.95)	0.022
SCR, μmol/L	66.00 (56.00, 76.00)	64.45 (55.10, 74.38)	68.00 (58.00, 79.00)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count; NEU, neutrophil count; EOC, eosinophil count; BASO, basophil count; LYC, lymphocyte count; RBC, red blood cell count; HB, hemoglobin; RDW, red blood cell distribution width; MCV, mean red blood cell volume; PLT, platelet count; PDW, platelet distribution width; MPV, mean platelet volume; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting blood glucose; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; SUA, serum uric acid; SCR, serum creatinine.

AIP, CRI, and TyG, were significantly higher in the SCAS group than in the control group ($P < 0.05$) (Figure 2).

3.2 Independent risk factors

Nineteen potential risk factors associated with SCAS were initially screened by univariate analysis ($P < 0.05$) (Table 1). To ensure the accuracy and credibility of the findings, we calculated the variance inflation factor (VIF) for each variable and considered to exhibit lower multicollinearity when their VIF was below 10 (Supplementary Figure 2). Afterward, we performed stepwise backward logistic regression analysis with the Akaike information criterion to filter and remove multicollinear variables. Ultimately, fifteen variables were included in the multivariate logistic regression analysis, and the final fourteen variables such as Age, WBC, BASO, and LYC ($P < 0.05$) were identified as independent risk factors for SCAS (Figure 3).

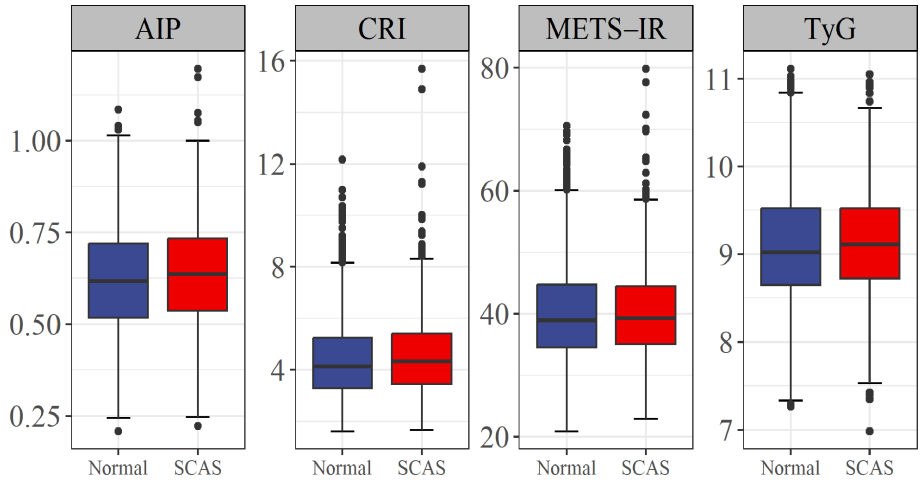

FIGURE 2
Association of four metabolism-related parameters with risk of SCAS. AIP, atherogenic index of plasma; CRI, Castelli risk index; TyG, triglyceride-glucose; METS-IR, metabolic score for insulin resistance; SCAS, subclinical atherosclerosis.

Based on the independent risk factors, we proceeded to explore the correlation between the variables (Figure 4). From the correlation analysis, we observed a negative correlation between AIP and Age (r = -0.24, $P < 0.01$), MCV (r = -0.13, $P < 0.01$), and HDL (r = -0.69, $P < 0.01$). Additionally, positive correlations were observed between AIP and WBC (r = 0.14, $P < 0.01$), GGT (r = 0.28, $P < 0.01$), and SUA (r = 0.27, $P < 0.01$).

To further assess the clinical applicability of AIP, we conducted a diagnostic experiment and a dose-response relationship study. The result of the diagnostic experiment (Figure 5A) revealed that although AIP holds promise as a potential biomarker for SCAS, its diagnostic value was moderate (AUC: 0.535). The dose-response relationship (Figure 5B) demonstrated a linear correlation between AIP and the risk of SCAS prevalence (P-overall < 0.001, P-non-linear = 0.319), with a significant increase in risk observed when AIP was greater than 0.625.

3.3 Construction of risk prediction models

The study population was divided into training and internal validation sets at a 6:4 ratio. The basic characteristics of the participants in the two sets did not differ (Table 2). LASSO enables a data dimensionality reduction algorithm that screens feature predictors by constructing a penalty function that compresses regression coefficients to zero (19). RF-RFE is a

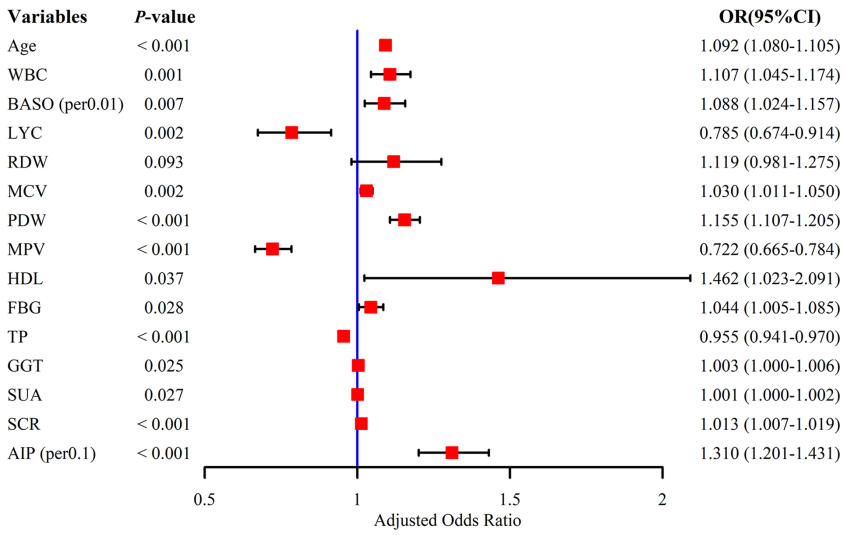

FIGURE 3
Multivariate logistic regression analysis of subclinical atherosclerosis. WBC, white blood cell count; BASO, basophil count; LYC, lymphocyte count; RDW, red blood cell distribution width; MCV, mean red blood cell volume; PDW, platelet distribution width; MPV, mean platelet volume; HDL, high-density lipoprotein; FBG, fasting blood glucose; TP, total protein; GGT, gamma-glutamyl transpeptidase; SUA, serum uric acid; SCR, serum creatinine; AIP, atherogenic index of plasma.

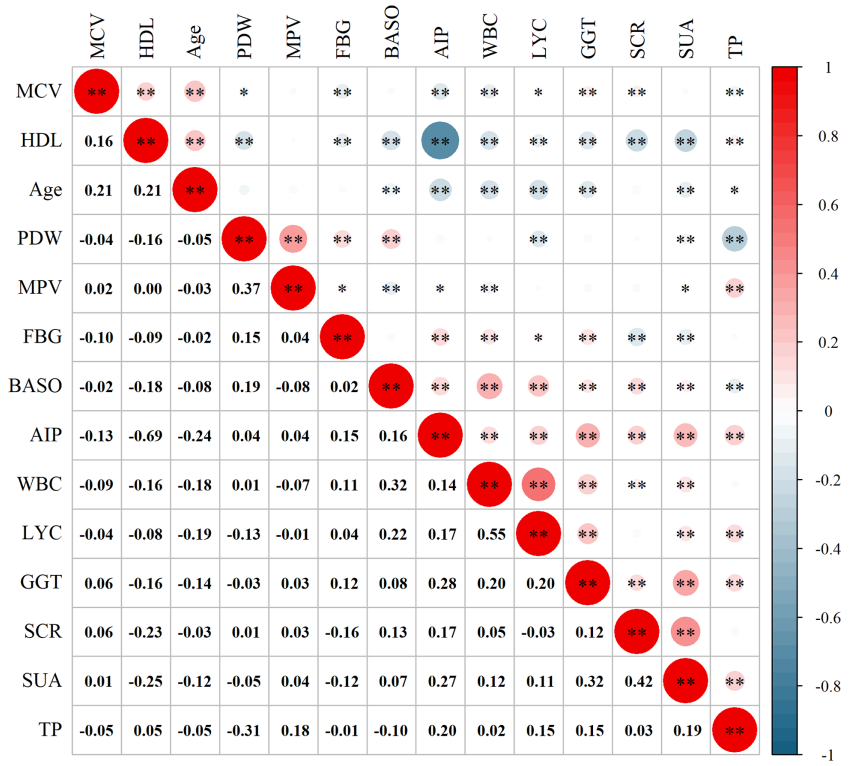

FIGURE 4
Correlation analysis between the variables. MCV, mean red blood cell volume; HDL, high-density lipoprotein; PDW, platelet distribution width; MPV, mean platelet volume; FBG, fasting blood glucose; BASO, basophil count; AIP, atherogenic index of plasma; WBC, white blood cell count; LYC, lymphocyte count; GGT, gamma-glutamyl transpeptidase; SCR, serum creatinine; SUA, serum uric acid; TP, total protein. *$P < 0.05$; **$P < 0.01$.

recursive backward feature elimination method that evaluates the importance of variables and progressively removes the least important ones, ultimately screening the optimal number of features (20). In the training set, LASSO combined with RF-RFE was applied to screen the most characteristic variables for SCAS (Figures 6A, B). Subsequently, the common variables screened by both algorithms were selected as predictors for constructing the SCAS risk prediction models, which included Age, FBG, TC, HDL, LDL, TP, ALB, SUA, and SCR (Figure 6C). To determine the optimal risk prediction model, six machine learning algorithms, namely LDA, LR, NB, RF, SVM, and XGboost, were employed to construct risk prediction models.

3.4 Validation of risk prediction models

Within the training set, 10-fold cross-validation was employed to evaluate the predictive value of the models and showed that the

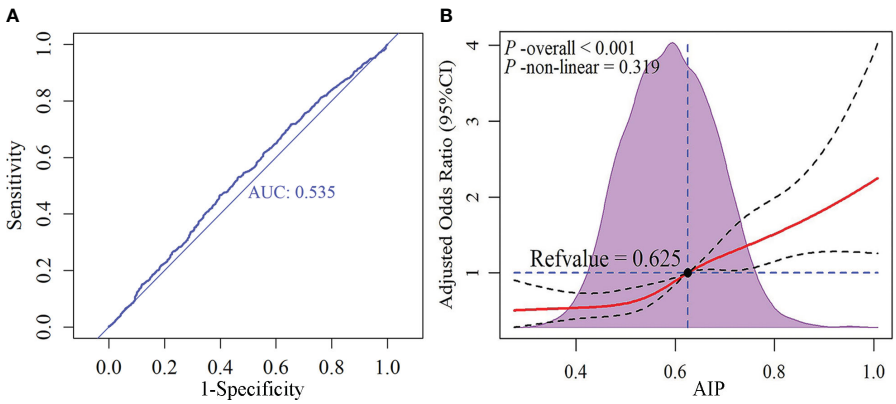

FIGURE 5
Receiver operating characteristic (ROC) curve and dose-response relationship between AIP and subclinical atherosclerosis. **(A)** ROC curve; **(B)** Dose-response relationship. AIP, atherogenic index of plasma.

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TABLE 2 Characteristics of participants in different sets.

	Training set	Internal validation set	*P*-value
N	1852	1232	
Sex (male), %	1367 (73.8)	924 (75.0)	0.486
Age, years	56.00 (49.00, 61.00)	55.00 (50.00, 61.00)	0.406
BMI, kg/m^2	24.70 (22.81, 26.99)	24.56 (22.65, 26.95)	0.132
SBP, mmHg	133.83 (17.23)	133.48 (17.84)	0.590
DBP, mmHg	81.00 (73.00, 90.00)	81.00 (73.00, 89.00)	0.750
WBC, $10^9/L$	6.52 (1.73)	6.57 (1.72)	0.473
NEU, $10^9/L$	3.70 (2.96, 4.60)	3.70 (2.99, 4.67)	0.765
EOS, $10^9/L$	0.11 (0.07, 0.19)	0.11 (0.07, 0.19)	0.417
BASO, $10^9/L$	0.02 (0.01, 0.03)	0.02 (0.01, 0.03)	0.579
LYC, $10^9/L$	2.00 (1.60, 2.44)	2.00 (1.60, 2.50)	0.354
RBC, $10^{12}/L$	4.86 (0.53)	4.90 (0.52)	0.043
HB, g/L	150.00 (138.00, 159.00)	150.00 (139.00, 159.25)	0.124
RDW, %	12.60 (12.20, 13.00)	12.50 (12.20, 12.90)	0.113
MCV, fL	91.00 (88.80, 94.00)	91.00 (88.40, 94.00)	0.909
PLT, $10^9/L$	226.23 (59.25)	227.99 (56.77)	0.413
PDW, %	15.20 (12.50, 16.30)	14.60 (12.50, 16.30)	0.409
MPV, fL	10.59 (1.17)	10.58 (1.12)	0.726
TC, mmol/L	4.99 (1.16)	5.02 (1.17)	0.552
TG, mmol/L	1.50 (1.08, 2.20)	1.49 (1.03, 2.22)	0.288
HDL, mmol/L	1.14 (0.95, 1.39)	1.14 (0.97, 1.40)	0.254
LDL, mmol/L	2.81 (0.88)	2.83 (0.88)	0.622
FBG, mmol/L	6.78 (6.18, 8.36)	6.84 (6.20, 8.31)	0.433
TP, g/L	72.90 (68.60, 76.60)	73.00 (68.80, 76.50)	0.870
ALB, g/L	44.90 (42.30, 46.60)	44.85 (42.20, 46.52)	0.818
AST, IU/L	23.00 (18.00, 29.00)	23.00 (18.00, 30.00)	0.216
ALT, IU/L	24.00 (17.00, 37.00)	24.00 (17.00, 40.00)	0.098
GGT, U/L	31.00 (21.00, 50.00)	31.00 (21.00, 54.00)	0.319
SUA, μmol/L	356.67 (94.60)	357.97 (98.72)	0.714
SCR, μmol/L	65.90 (56.00, 76.00)	66.00 (56.00, 76.43)	0.864
AIP	0.63 (0.14)	0.62 (0.14)	0.432
CRI	4.24 (3.36, 5.31)	4.15 (3.30, 5.32)	0.379
TyG	9.12 (0.64)	9.09 (0.65)	0.295
METS-IR	39.32 (34.83, 44.89)	38.62 (34.54, 44.24)	0.058

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count; NEU, neutrophil count; EOC, eosinophil count; BASO, basophil count; LYC, lymphocyte count; RBC, red blood cell count; HB, hemoglobin; RDW, red blood cell distribution width; MCV, mean red blood cell volume; PLT, platelet count; PDW, platelet distribution width; MPV, mean platelet volume; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting blood glucose; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; SUA, serum uric acid; SCR, serum creatinine; AIP, atherogenic index of plasma; CRI, Castelli risk index; TyG, triglyceride-glucose; METS-IR, metabolic score for insulin resistance.

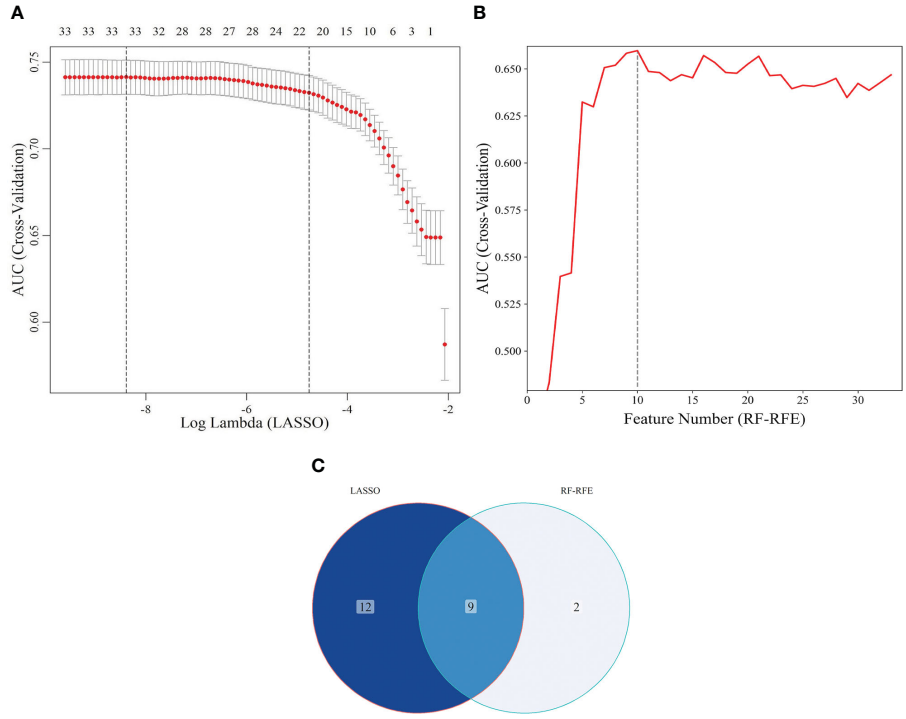

FIGURE 6
Screening of characteristic predictors. **(A)** Characteristic variables screening based on LASSO (lambda: 1SE); **(B)** Characteristic variables screening based on RF-RFE; **(C)** LASSO combined RF-RFE. LASSO, least absolute shrinkage and selection operator; SE, standard error; RF-RFE, random forest-recursive feature elimination.

RF model had the best clinical predictive value [AUC: 0.729 (95% CI: 0.709-0.749)], followed by the SVM model [AUC: 0.720 (0.705-0.735)](Figure 7A). In the internal validation set, the RF model also demonstrated a good clinical predictive value [AUC: 0.715 (95% CI: 0.688-0.742)](Figure 7B). Furthermore, a comprehensive comparison of other clinical performance parameters, such as sensitivity and specificity, was conducted among the prediction models (Table 3). From the table, we observed that the RF model exhibits excellent performance in various parameters in the training set. The confusion matrix of the six machine learning models in the training set is shown in Figure 8.

The calibration curve visually displays the fit of the risk prediction models. As shown in Figure 9, except for the XGboost and NB models, the predicted values of the other models closely match the theoretical values, demonstrating good clinical calibration.

DCA was used to assess the clinical applicability of predictive models by showing the relationship between risks and benefits corresponding to different decision-making. In the training set, all

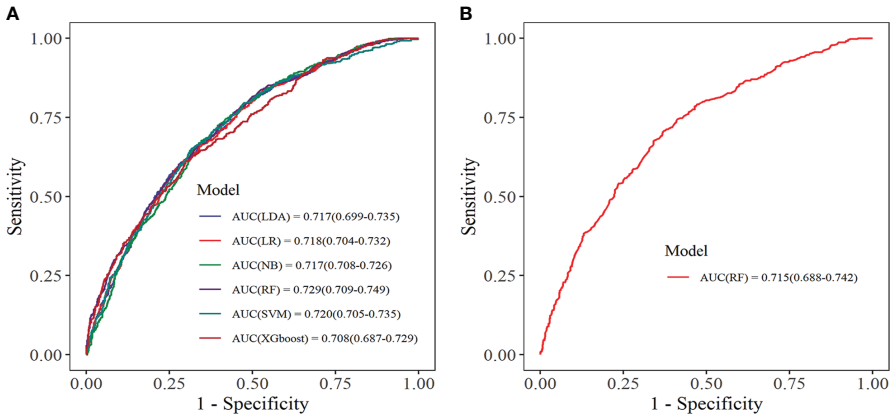

FIGURE 7
Receiver operating characteristic curve. **(A)** Training set; **(B)** Internal validation set. LDA, linear discriminant analysis; LR, logistic regression; NB, Naive Bayes; RF, random forest; SVM, support vector machine; XGboost, extreme gradient boosting.

TABLE 3 Performance parameters of six machine learning prediction models in the training set.

Model	Accuracy	Sensitivity	Specificity	Precision	Recall	F1
LDA	0.676 (0.654-0.697)	0.407	0.842	0.613	0.407	0.489
LR	0.677 (0.655-0.698)	0.421	0.834	0.610	0.421	0.498
NB	0.664 (0.642-0.685)	0.442	0.800	0.577	0.442	0.500
RF	0.681 (0.659-0.702)	0.445	0.826	0.612	0.445	0.515
SVM	0.670 (0.648-0.691)	0.399	0.836	0.600	0.399	0.480
XGboost	0.678 (0.656-0.699)	0.425	0.834	0.612	0.425	0.502

LDA, linear discriminant analysis; LR, logistic regression; NB, Naive Bayes; RF, random forest; SVM, support vector machine; XGboost, extreme gradient boosting.

six ML models showed good clinical applicability (Figure 10A). Further, we calculated the risk threshold probability for the RF prediction model in the internal validation set, which showed that the RF model was clinically beneficial in the range of 2%-70% (Figure 10B).

3.5 Interpretation of risk prediction model

Based on the aforementioned analysis, we found that the RF prediction model demonstrated outstanding performance in both the training and internal validation sets, with the highest clinical predictive value observed in the training set [AUC: 0.729 (95% CI: 0.709-0.749)] and outperformed others in terms of accuracy, sensitivity, recall, and F1 score. Therefore, we have selected the RF model as the optimal prediction model for further model interpretation. SHAP interpretation is currently an emerging and the most commonly used method for interpreting predictive models in the field of ML, which interprets the model by computing the "contribution value" (Shapley values) of each characteristic predictor (21). Figure 11A depicts the contribution degree of the characteristic predictors to the prediction model, with the top five variables being Age, ALB, TP, TC, and SCR. Moreover, we observed that higher values of Age, TC, and SCR correspond to higher SHAP values and increased disease risk, whereas higher values of ALB and TP result in smaller SHAP values and reduced disease risk (Figure 11B).

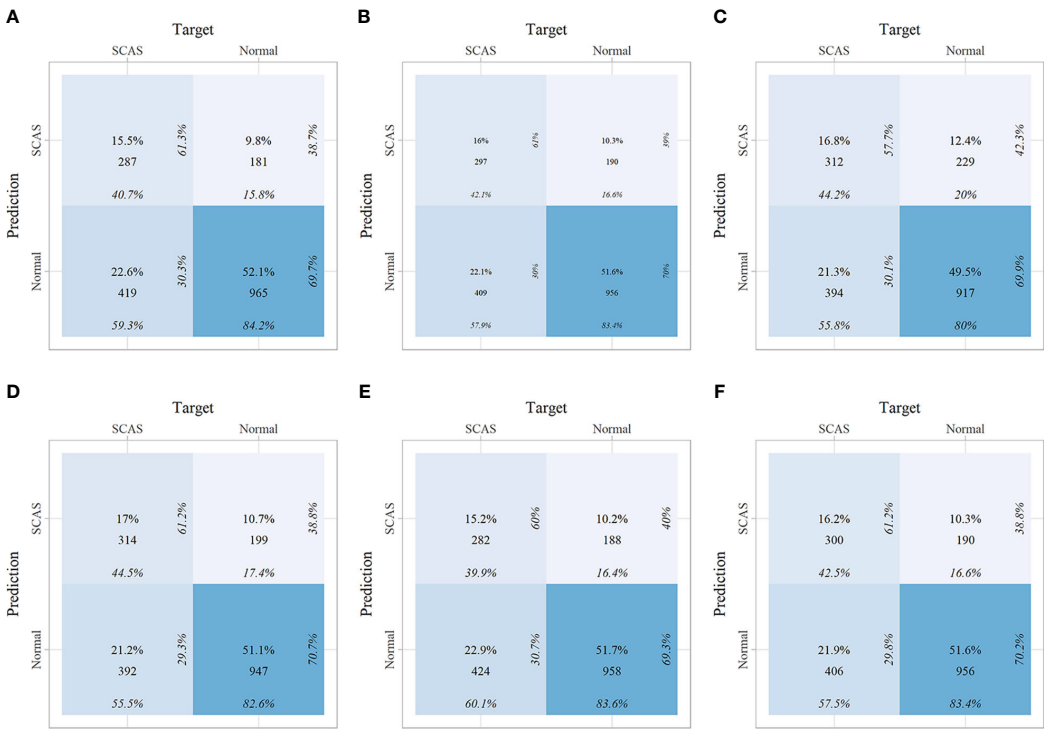

FIGURE 8
The confusion matrix of the six machine learning models in the training set. **(A)** Linear discriminant analysis; **(B)** Logistic regression; **(C)** Naive Bayes; **(D)** Random forest; **(E)** Support vector machine; **(F)** Extreme gradient boosting.

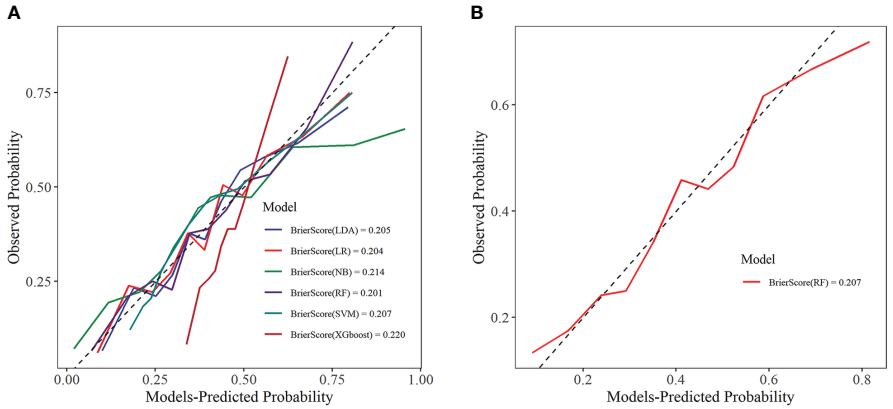

FIGURE 9
Calibration curve. **(A)** Training set; **(B)** Internal validation set. LDA, linear discriminant analysis; LR, logistic regression; NB, Naive Bayes; RF, random forest; SVM, support vector machine; XGboost, extreme gradient boosting.

4 Discussion

This study included a total of 3084 T2DM individuals, of whom 1186 had SCAS. Multivariate logistic regression analysis identified 14 variables, such as Age, WBC, BASO, and LYC ($P < 0.05$) as independent risk factors for SCAS in T2DM patients. LASSO combined with RF-RFE algorithms revealed nine characteristic variables, including Age, FBG, TC, HDL, LDL, TP, ALB, SUA, and SCR, as predictors for the SCAS risk model. Six ML models were developed and validated for clinical performance. Ultimately, the RF model exhibited the highest clinical predictive value in the training set [AUC: 0.729 (0.709-0.749)] and outperformed in accuracy, sensitivity, recall, and F1 score. The SHAP interpretation of the RF model revealed that Age, ALB, TP, TC, and SCR were the top five variables that made the most significant contributions to the predictive model.

In this study, the percentage of SCAS in the T2DM population was 38.46%, lower than the 43.68% reported by Hashimoto et al. in a Japanese T2DM population (22), which might be related to the region and sample size. Multiple studies have demonstrated an association between the TyG index and the incidence of CVD, coronary artery stenosis, stroke, and AS (23, 24). A meta-analysis has revealed that an elevated TyG index is associated with SCAS and arterial stiffness in the adult population (25). Notably, the I-Lan Longitudinal Aging Study identified an association between the TyG index and SCAS in non-diabetic individuals, but not in those with diabetes (26). Consistent with this finding, our study also found no significant statistical association between the TyG index and SCAS in the T2DM population. AIP has emerged as a novel predictive biomarker for CVD. Associations have been identified between elevated AIP levels and increased incidences of CAC and AS (27, 28). In this study, we observed that for every 0.1 unit increase in AIP, the risk of SCAS increased by 0.31-fold [OR: 1.310 (1.201-1.401)]. However, the receiver operating characteristic curve indicated an average diagnostic value for AIP (AUC: 0.535).

Age, PDW, MPV, SUA, and GGT were observed as independent risk factors for SCAS, consistent with previous studies (29–33). Inflammation-related markers such as WBC, BASO, and LYC, were also found to be independent risk factors for SCAS. Long-term studies

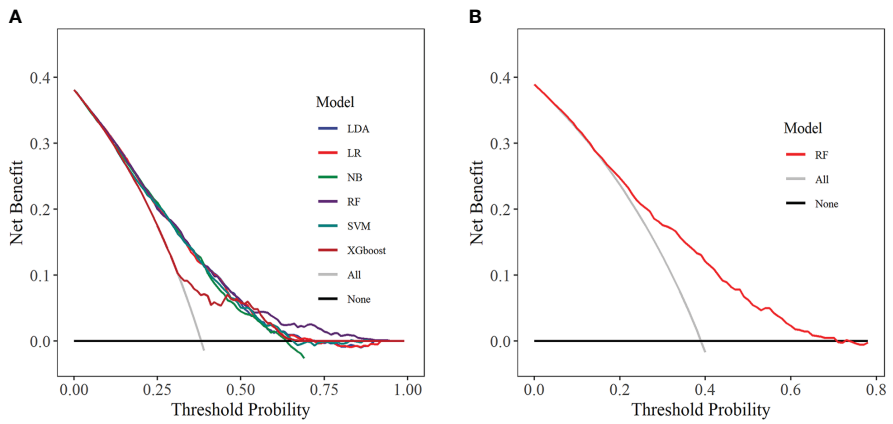

FIGURE 10
Decision curve analysis. **(A)** Training set; **(B)** Internal validation set. LDA, linear discriminant analysis; LR, logistic regression; NB, Naive Bayes; RF, random forest; SVM, support vector machine; XGboost, extreme gradient boosting.

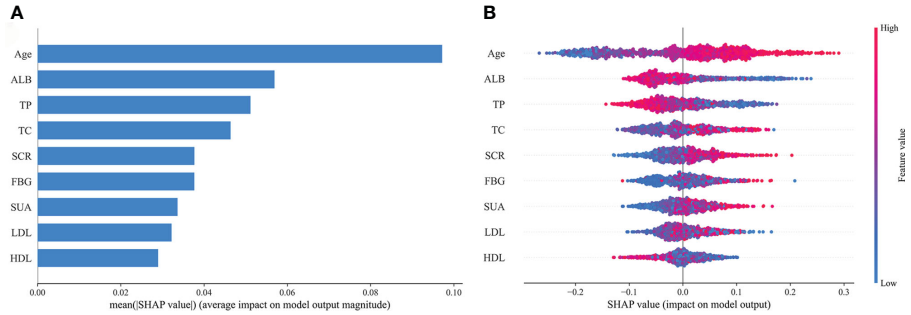

FIGURE 11
Feature importance of random forest model. **(A)** The importance ranking of the features according to the mean absolute SHAP value; **(B)** The effect of features on the outcome of the model. ALB, albumin; TP, total protein; TC, total cholesterol; SCR, serum creatinine; FBG, fasting blood glucose; SUA, serum uric acid; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SHAP, Shapley Additive exPlanation.

have shown that AS has a complex pathogenesis, primarily attributed to lipoprotein retention in the arterial wall and chronic inflammation (34, 35). Hyperglycemia leads to increased inflammasome activity, upregulated nucleotide-binding oligomerization domain-like receptor 3, and ultimately elevated pro-inflammatory interleukin1β and interleukin 18 levels (36). Our study further confirms that SCAS in T2DM is a chronic inflammatory condition. Dyslipidemia is a well-established independent risk factor for CVD. In our study, we observed that HDL is an independent risk factor for SCAS. While early research consistently demonstrated an inverse correlation between HDL levels and CVD risk (37, 38), more recent studies have unveiled a non-linear, U-shaped relationship, with very high HDL levels associated with cardiovascular mortality (39, 40).

Optimizing approaches for early diagnosis of SCAS and providing earlier and more precise interventions are crucial to reducing adverse cardiovascular events. Currently, CUS and CT examinations are the primary methods for screening SCAS, but massive generalization inevitably leads to the wastage of medical resources and increased costs, particularly in low-income countries with limited resources. In recent years, with the growing demand for high-quality healthcare, AI has become a powerful tool in clinical medicine. ML, as a branch of AI, was able to analyze large datasets, find complex patterns, and generate insights that contribute to early disease diagnosis, drug discovery, and risk prediction (41, 42). For instance, a study based on electronic health records used ML to generate an in-silico marker for coronary artery disease (CAD) that can non-invasively quantify AS and risk of death on a continuous spectrum, and identify underdiagnosed individuals (43). In addition, Ninomiya et al. (44) developed ML models to predict 5-year all-cause mortality in patients with CAD and assessed ML's benefit in guiding decision-making between percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). The results showed that the hybrid gradient boosting model was the most effective for predicting 5-year all-cause mortality (C-indexes of 0.78) and that ML is feasible and effective for identifying individuals who benefit from CABG or PCI. In this study, we have developed risk prediction models for SCAS in T2DM patients based on interpretable machine learning methods that could contribute to the early identification of high-risk individuals.

Our study carries significant clinical importance. This might be one of the initial studies to perform SCAS risk prediction in the T2DM population using interpretable ML methods. As a chronic condition, SCAS is challenging to reverse once it develops, emphasizing the effectiveness of early prevention over active treatment. This prediction model enables the identification of high-risk individuals with SCAS within the T2DM population, providing a valuable advantage for early disease prevention. Moreover, the prediction model could bring benefits not only to medically underdeveloped regions but also to inform the clinical decisions of physicians, thus contributing to the optimization of healthcare resources.

This study has certain unavoidable limitations. Firstly, the study population was limited to a specific region, which might impact the generalizability of the prediction model. Secondly, the collection of clinical data lacked comprehensiveness, which may have led to the omission of potential predictors. Thirdly, the risk prediction model has only undergone validation using internal datasets, necessitating further validation with external datasets. In future studies, we will conduct a long-term follow-up study and collaborate with multiple centers to further revise and improve the model.

5 Conclusions

In summary, the development, validation, and interpretation of the SCAS risk prediction model in a Chinese T2DM population has significant implications for the reduction and prevention of adverse cardiovascular events.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study protocol adhered to the Declaration of Helsinki and received approval from the Ethics Committee of the Affiliated Hospital of Medical School, Ningbo University, (In March 2023,

renamed as The First Affiliated Hospital of Ningbo University), Ningbo, China (KY20220607). Informed consent was obtained from all participants, and the study data were anonymized.

Author contributions

XT: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. YS: Data curation, Formal analysis, Methodology, Writing – review & editing. GH: Conceptualization, Data curation, Methodology, Validation, Visualization, Writing – review & editing. YM: Conceptualization, Data curation, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1332982/full#supplementary-material

References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

2. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovasc Diabetol* (2018) 17:83. doi: 10.1186/s12933-018-0728-6

3. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. Heart disease and stroke statistics-2023 update: A report from the American heart association. *Circulation* (2023) 147:e93–e621. doi: 10.1161/cir.0000000000001123

4. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *Lancet* (2010) 375:2215–22. doi: 10.1016/s0140-6736(10)60484-9

5. Gregg EW, Sattar N, Ali MK. The changing face of diabetes complications. *Lancet Diabetes Endocrinol* (2016) 4:537–47. doi: 10.1016/s2213-8587(16)30010-9

6. Singh SS, Pilkerton CS, Shrader CD Jr., Frisbee SJ. Subclinical atherosclerosis, cardiovascular health, and disease risk: Is there a case for the Cardiovascular Health Index in the primary prevention population? *BMC Public Health* (2018) 18:429. doi: 10.1186/s12889-018-5263-6

7. Zaid M, Fujiyoshi A, Kadota A, Abbott RD, Miura K. Coronary artery calcium and carotid artery intima media thickness and plaque: Clinical use in need of clarification. *J Atheroscler Thromb* (2017) 24:227–39. doi: 10.5551/jat.RV16005

8. Jeevarethinam A, Venuraju S, Dumo A, Ruano S, Mehta VS, Rosenthal M, et al. Relationship between carotid atherosclerosis and coronary artery calcification in asymptomatic diabetic patients: A prospective multicenter study. *Clin Cardiol* (2017) 40:752–8. doi: 10.1002/clc.22727

9. Prakash S, Balaji JN, Joshi A, Surapaneni KM. Ethical conundrums in the application of artificial intelligence (AI) in healthcare-A scoping review of reviews. *J Pers Med* (2022) 12:1914. doi: 10.3390/jpm12111914

10. Sánchez-Cabo F, Rossello X, Fuster V, Benito F, Manzano JP, Silla JC, et al. Machine learning improves cardiovascular risk definition for young, asymptomatic individuals. *J Am Coll Cardiol* (2020) 76:1674–85. doi: 10.1016/j.jacc.2020.08.017

11. Núñez E, Fuster V, Gómez-Serrano M, Valdivielso JM, Fernández-Alvira JM, Martínez-López D, et al. Unbiased plasma proteomics discovery of biomarkers for improved detection of subclinical atherosclerosis. *EBioMedicine* (2022) 76:103874. doi: 10.1016/j.ebiom.2022.103874

12. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* (1996) 49:1373–9. doi: 10.1016/s0895-4356(96)00236-3

13. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. Addendum. 2. Classification and diagnosis of diabetes: Standards of care in diabetes-2023. *Diabetes Care* (2023) 46:S19–40. doi: 10.2337/dc23-ad08

14. Wu L, Qian L, Zhang L, Zhang J, Zhou J, Li Y, et al. Fibroblast growth factor 21 is related to atherosclerosis independent of nonalcoholic fatty liver disease and predicts atherosclerotic cardiovascular events. *J Am Heart Assoc* (2020) 9:e015226. doi: 10.1161/jaha.119.015226

15. Lioy B, Webb RJ, Amirabdollahian F. The association between the atherogenic index of plasma and cardiometabolic risk factors: A review. *Healthcare (Basel)* (2023) 11:966. doi: 10.3390/healthcare11070966

16. Mahdavi-Roshan M, Mozafarihashjin M, Shoaibinobarian N, Ghorbani Z, Salari A, Savarrakhsh A, et al. Evaluating the use of novel atherogenicity indices and insulin resistance surrogate markers in predicting the risk of coronary artery disease: A case –control investigation with comparison to traditional biomarkers. *Lipids Health Dis* (2022) 21:126. doi: 10.1186/s12944-022-01732-9

17. Zhang X, Liu F, Li W, Zhang J, Zhang T, Yu X, et al. Metabolic score for insulin resistance (METS-IR) predicts adverse cardiovascular events in patients with type 2 diabetes and ischemic cardiomyopathy. *Diabetes Metab Syndr Obes* (2023) 16:1283–95. doi: 10.2147/dmso.S404878

18. Thai PV, Tien HA, Van Minh H, Valensi P. Triglyceride glucose index for the detection of asymptomatic coronary artery stenosis in patients with type 2 diabetes. *Cardiovasc Diabetol* (2020) 19:137. doi: 10.1186/s12933-020-01108-2

19. Zheng Z, Si Z, Wang X, Meng R, Wang H, Zhao Z, et al. Risk prediction for the development of hyperuricemia: Model development using an occupational health examination dataset. *Int J Environ Res Public Health* (2023) 20:3411. doi: 10.3390/ijerph20043411

20. Zhou L, Wang Q, Yin P, Xing W, Wu Z, Chen S, et al. Serum metabolomics reveals the deregulation of fatty acids metabolism in hepatocellular carcinoma and chronic liver diseases. *Anal Bioanal Chem* (2012) 403:203–13. doi: 10.1007/s00216-012-5782-4

21. Linardatos P, Papastefanopoulos V, Kotsiantis S. Explainable AI: A review of machine learning interpretability methods. *Entropy (Basel)* (2020) 23:18. doi: 10.3390/e23010018

22. Hashimoto Y, Takahashi F, Okamura T, Osaka T, Okada H, Senmaru T, et al. Relationship between serum creatinine to cystatin C ratio and subclinical atherosclerosis in patients with type 2 diabetes. *BMJ Open Diabetes Res Care* (2022) 10:e002910. doi: 10.1136/bmjdrc-2022-002910

23. Li H, Jiang Y, Su X, Meng Z. The triglyceride glucose index was U-shape associated with all-cause mortality in population with cardiovascular diseases. *Diabetol Metab Syndr* (2023) 15:181. doi: 10.1186/s13098-023-01153-3

24. da Silva A, Caldas APS, Hermsdorff HHM, Bersch-Ferreira ÂC, Torreglosa CR, Weber B, et al. Triglyceride-glucose index is associated with symptomatic coronary artery disease in patients in secondary care. *Cardiovasc Diabetol* (2019) 18:89. doi: 10.1186/s12933-019-0893-2

25. Sajdeya O, Beran A, Mhanna M, Alharbi A, Burmeister C, Abuhelwa Z, et al. Triglyceride glucose index for the prediction of subclinical atherosclerosis and arterial stiffness: A meta-analysis of 37,780 individuals. *Curr Probl Cardiol* (2022) 47:101390. doi: 10.1016/j.cpcardiol.2022.101390

26. Lu YW, Chang CC, Chou RH, Tsai YL, Liu LK, Chen LK, et al. Gender difference in the association between TyG index and subclinical atherosclerosis: Results from the I-Lan Longitudinal Aging Study. *Cardiovasc Diabetol* (2021) 20:206. doi: 10.1186/s12933-021-01391-7

27. Nam JS, Kim MK, Nam JY, Park K, Kang S, Ahn CW, et al. Association between atherogenic index of plasma and coronary artery calcification progression in Korean adults. *Lipids Health Dis* (2020) 19:157. doi: 10.1186/s12944-020-01317-4

28. Huang Q, Liu Z, Wei M, Huang Q, Feng J, Liu Z, et al. The atherogenic index of plasma and carotid atherosclerosis in a community population: A population-based cohort study in China. *Cardiovasc Diabetol* (2023) 22:125. doi: 10.1186/s12933-023-01977-3

29. van den Munckhof ICL, Jones H, Hopman MTE, de Graaf J, Nyakayiru J, van Dijk B, et al. Relation between age and carotid artery intima-medial thickness: a systematic review. *Clin Cardiol* (2018) 41:698–704. doi: 10.1002/clc.22934

30. Lappegård J, Ellingsen TS, Vik A, Skjelbakken T, Brox J, Mathiesen EB, et al. Red cell distribution width and carotid atherosclerosis progression. *Tromsø Study. Thromb Haemost* (2015) 113:649–54. doi: 10.1160/th14-07-0606

31. Adam G, Kocak E, Reşorlu M. Evaluation of platelet distribution width and mean platelet volume in patients with carotid artery stenosis: Author's reply. *Angiology* (2015) 66:380. doi: 10.1177/0003319714565169

32. Gao Y, Xu B, Yang Y, Zhang M, Yu T, Zhang Q, et al. Association between serum uric acid and carotid intima-media thickness in different fasting blood glucose patterns: A case-control study. *Front Endocrinol (Lausanne)* (2022) 13:899241. doi: 10.3389/fendo.2022.899241

33. Kim YG, Park GM, Lee SB, Yang DH, Kang JW, Lim TH, et al. Association of gamma-glutamyl transferase with subclinical coronary atherosclerosis and cardiac outcomes in non-alcoholics. *Sci Rep* (2020) 10:17994. doi: 10.1038/s41598-020-75078-6

34. Wojtasińska A, Frąk W, Lisińska W, Sapeda N, Młynarska E, Rysz J, et al. Novel insights into the molecular mechanisms of atherosclerosis. *Int J Mol Sci* (2023) 24:13434. doi: 10.3390/ijms241713434

35. Morrison AM, Sullivan AE, Aday AW. Atherosclerotic disease: Pathogenesis and approaches to management. *Med Clin North Am* (2023) 107:793–805. doi: 10.1016/j.mcna.2023.04.004

36. Alfadul H, Sabico S, Ansari MGA, Alnaami AM, Amer OE, Hussain SD, et al. Differences and associations of NLRP3 inflammasome levels with interleukins 1α, 1β, 33 and 37 in adults with prediabetes and type 2 diabetes mellitus. *Biomedicines* (2023) 11:1315. doi: 10.3390/biomedicines11051315

37. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. *Four prospective Am Stud Circulation* (1989) 79:8–15. doi: 10.1161/01.CIR.79.1.8

38. Ko DT, Alter DA, Guo H, Koh M, Lau G, Austin PC, et al. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions: The CANHEART study. *J Am Coll Cardiol* (2016) 68:2073–83. doi: 10.1016/j.jacc.2016.08.038

39. Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J* (2017) 38:2478–86. doi: 10.1093/eurheartj/ehx163

40. Liu C, Dhindsa D, Almuwaqqat Z, Ko YA, Mehta A, Alkhoder AA, et al. Association between high-density lipoprotein cholesterol levels and adverse cardiovascular outcomes in high-risk populations. *JAMA Cardiol* (2022) 7:672–80. doi: 10.1001/jamacardio.2022.0912

41. Kumar Y, Koul A, Singla R, Ijaz MF. Artificial intelligence in disease diagnosis: A systematic literature review, synthesizing framework and future research agenda. *J Ambient Intell Humaniz Comput* (2023) 14:8459–86. doi: 10.1007/s12652-021-03612-z

42. Krishnan G, Singh S, Pathania M, Gosavi S, Abhishek S, Parchani A, et al. Artificial intelligence in clinical medicine: Catalyzing a sustainable global healthcare paradigm. *Front Artif Intell* (2023) 6:1227091. doi: 10.3389/frai.2023.1227091

43. Forrest IS, Petrazzini BO, Duffy Á, Park JK, Marquez-Luna C, Jordan DM, et al. Machine learning-based marker for coronary artery disease: Derivation and validation in two longitudinal cohorts. *Lancet* (2023) 401:215–25. doi: 10.1016/s0140-6736(22)02079-7

44. Ninomiya K, Kageyama S, Shiomi H, Kotoku N, Masuda S, Revaiah PC, et al. Can machine learning aid the selection of percutaneous vs surgical revascularization? *J Am Coll Cardiol* (2023) 82:2113–24. doi: 10.1016/j.jacc.2023.09.818

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A review of air pollution as a driver of cardiovascular disease risk across the diabetes spectrum

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The prevalence of diabetes is estimated to reach almost 630 million cases worldwide by the year 2045; of current and projected cases, over 90% are type 2 diabetes. Air pollution exposure has been implicated in the onset and progression of diabetes. Increased exposure to fine particulate matter air pollution ($PM_{2.5}$) is associated with increases in blood glucose and glycated hemoglobin (HbA1c) across the glycemic spectrum, including normoglycemia, prediabetes, and all forms of diabetes. Air pollution exposure is a driver of cardiovascular disease onset and exacerbation and can increase cardiovascular risk among those with diabetes. In this review, we summarize the literature describing the relationships between air pollution exposure, diabetes and cardiovascular disease, highlighting how airborne pollutants can disrupt glucose homeostasis. We discuss how air pollution and diabetes, via shared mechanisms leading to endothelial dysfunction, drive increased cardiovascular disease risk. We identify portable air cleaners as potentially useful tools to prevent adverse cardiovascular outcomes due to air pollution exposure across the diabetes spectrum, while emphasizing the need for further study in this particular population. Given the enormity of the health and financial impacts of air pollution exposure on patients with diabetes, a greater understanding of the interventions to reduce cardiovascular risk in this population is needed.

KEYWORDS

air pollution, cardiovascular risk, environmental exposure, inflammation, oxidative stress, particulate matter, prevention

1 Introduction

Since antiquity, physicians have suspected that air quality could alter human health. Indeed, the Hippocratic Corpus details the importance of clean air, and the philosopher Seneca noted the deleterious health effects of Rome's contaminated air (1). Research in the past few decades has implicated air pollution in the development of non-communicable

diseases, with a strong body of observational and experimental studies establishing a link between air pollution and cardiovascular disease (CVD), encompassing coronary heart disease, heart failure, stroke, peripheral artery disease, and hypertension (2). For example, airborne co-pollutants have been observed to increase hospital admissions for CVD (3, 4). More recently, evidence has implicated air pollution in the onset and progression of type 2 diabetes mellitus (hereafter referred to as diabetes), a widely recognized and significant cardiovascular risk factor (5, 6). Converging lines of evidence in a growing body of literature support the notion that air pollution, especially fine particulate matter ($PM_{2.5}$), can markedly exacerbate CVD risk in patients with diabetes and prediabetes, referred to as the "diabetes spectrum" in this review.

Globally, exposure to air pollution is the fourth leading risk factor for early death and the fourth leading modifiable risk factor for cardiovascular disease (CVD) (7, 8). In the US, exposure to fine particulate matter ($PM_{2.5}$) has been estimated to result in 8.2 million healthy life-years lost annually from diabetes (9). The magnitude of this ongoing and ubiquitous risk factor for diabetes and CVD would be difficult to overstate. Yet, the problem remains absent from most discussions of risk in health education (10–12). As such, in this review we aim to summarize this existing evidence supporting the relationship between air pollution, diabetes and CVD (Figure 1), including the biological mechanisms underlying this phenomenon. Furthermore, this review will discuss potential interventions to reduce air pollution exposure among patients with diabetes and barriers to effective implementation of such interventions. Lastly, this review will identify gaps in the current research landscape and suggest future directions.

2 Scope of the problem

2.1 Diabetes and CVD

Globally, an estimated 536 million adults have diabetes, either diagnosed or undiagnosed, a number that will increase to 783 million by 2045, driven by expanding populations in middle-income countries (13). In the United States, approximately 37.3 million people have diagnosed or undiagnosed diabetes, with this number expected to increase to over 54.9 million by 2030 (14, 15). Similarly, impaired glucose tolerance has a global prevalence of 464 million that is projected to increase to 638 million by 2045 (16). Whereas in the United States, 96 million adults have prediabetes, estimated to increase to 107 million in 2030 (14, 15). The projected increase in diabetes and prediabetes is partially driven by an aging population in conjunction with climbing obesity rates; increased body mass index (BMI) is strongly related to increased diabetes risk (17). The annual total cost of diagnosed diabetes in the United States is estimated at $327 billion, taking into account healthcare utilization and lost productivity (18). These data make evident the pressing need to identify ways to minimize the incidence and progression of diabetes spectrum disorders, including attention to emerging modifiable risk factors such as environmental exposures.

Like diabetes, CVD is on the rise worldwide, with ischemic heart disease now the second leading cause of morbidity and mortality globally (19). In the United States, nearly 128 million adults live with at least one manifestation of CVD, and 928,741 deaths were attributed to CVD in 2020 alone (20). This heavy burden of CVD in the United States comes at a substantial price, necessitating $407 billion in direct and indirect costs in 2018 (20). By 2060, an estimated 234 million Americans will have CVD, with racial and ethnic minorities bearing the majority of this increased burden (21). As these statistics demonstrate, CVD is and will remain a tremendous problem that will require a multimodal approach to prevention and treatment.

These two chronic conditions separately affect massive populations worldwide with substantial economic and quality of life impact. However, we know there is significant interplay between diabetes and CVD. A long history of prospective cohort studies dating back to the first Framingham Heart Study has established diabetes as a major risk factor for CVD (22). In 2010, the Emerging Risk Factors Collaboration published a meta-analysis of 102 prospective cohort studies, concluding that diabetes confers an

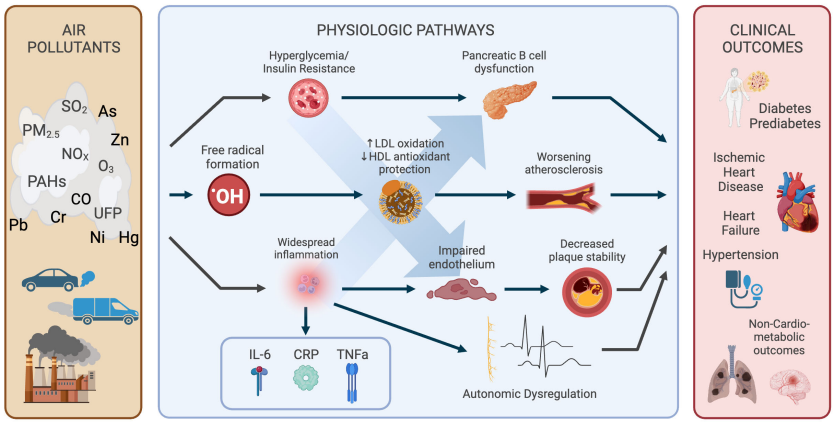

FIGURE 1
Relationships between air pollution, diabetes and CVD. Multiple physiologic pathways are affected by exposures and drive numerous subclinical and clinical outcomes. Created with BioRender.com.

approximate 2-fold risk increase for coronary heart disease (95% CI [1.83, 2.19]), with similar risk increases for ischemic (2.27 [1.95, 2.65]) and hemorrhagic stroke (1.84 [1.59, 2.13]) (23). Subsequently, a competing risks analysis using data from 12 Spanish prospective cohorts followed for a median of 10 years found that diabetes increased cumulative risk of cardiovascular death by 1.5-2.5% in both men and women (24). In addition, diabetes appears to not only confer its own risk, but also to accelerate the age-related increase in CVD. A retrospective cohort study found that adults with diabetes develop a high risk of CVD on average 14.6 years sooner versus their counterparts without diabetes (25).

In contrast with older thinking that diabetes increases disease risk only beyond a certain threshold of HbA1c, it appears that CVD risk increases across the continuum of glucose intolerance. An international prospective cohort study of nearly 19,000 adults without diabetes at baseline found that the risk of incident CV events increased by 1.16 [1.11, 1.22] per 1 mmol/L increase in fasting plasma glucose (26). These findings suggest that glucose intolerance should be considered along a continuum, similar to blood pressure (27). There is also evidence linking insulin resistance to CVD. For example, in a prospective cohort study of elderly men in Sweden, insulin resistance was associated with an increased risk of developing congestive heart failure over 7-12 years of follow-up (HR 1.44, 95% CI 1.08-1.93 per 1-SD increase in oral glucose tolerance test glucose level) (28). After 20 years of follow-up in the same cohort, insulin resistance at age 50 was associated with left ventricular dysfunction at age 70 (29). Further supporting a link between diabetes and heart failure, a Swedish cohort study of over 270,000 adults demonstrated that, even with other risk factors in target ranges, patients with diabetes still had an excess risk for hospitalization due to heart failure (HR 1.45 [1.34, 1.57]) (30). Given the significant CVD risk that increases across the diabetes spectrum, developing personal and public strategies to mitigate glucose intolerance at every stage is paramount to preventing excess morbidity and mortality worldwide.

3 Air pollution as a risk factor

3.1 Background on air pollution

The World Health Organization (WHO) has defined air pollution as "contamination of the indoor or outdoor environment by any chemical, physical, or biological agent that modifies the natural characteristics of the atmosphere" (31). Air pollution is a heterogeneous mixture of particles and gases, much of which is anthropogenic in origin. Nitrogen oxides (NO_x), including nitrogen dioxide (NO_2), and carbon monoxide (CO) are generated by fossil fuel combustion, with traffic as a major source. Sulfur dioxide (SO_2) is generated by fossil fuel combustion for heating homes and generating power (32). Ozone (O_3) forms in reactions between light and various compounds, including CO and NO_x. Particulate matter (PM) air pollution is composed of sulfates, nitrogen oxides, ammonia, sodium chloride, black carbon, mineral dust, organic compounds, and products of incomplete combustion of petroleum. PM is generated from many sources, including traffic, industrial, construction, fires, and trash burning, and is typically described in terms of particle size. Coarse PM (PM_{10}) is defined as PM with a diameter between 2.5 and 10 μm and fine PM ($PM_{2.5}$) with a diameter less than 2.5 μm; ultrafine PM is PM smaller than 0.1 μm ($PM_{0.1}$). Most studies examine the effects of $PM_{2.5}$, since this is the most widely available data. Thus, the most conclusive effects are seen for this particular pollutant.

3.2 Epidemiologic studies on air pollution and diabetes

As early as 1967, researchers probed Public Health Service data to investigate the relationship between air quality and diabetes death rate in urban populations across the US (33). Since then, the body of research on air pollution and diabetes has expanded, especially in the past two decades. This research is summarized in Table 1. A 2002 ecological study demonstrated a significant positive correlation between industrial air emissions and diabetes prevalence by state in the US ($r = 0.54$, $p = 5.7x\ 10^{-5}$) (34). Another ecological study in 2010 conducted a county-level analysis across the US, showing a 1% increase in county diabetes prevalence per 10 $\mu g/m^3$ per average county increase in $PM_{2.5}$ ($\beta = 0.81$ [0.48, 1.07], $p < 0.001$) (35). Many observational studies over the following decade supported the early ecological findings. A cross-sectional analysis of a Swiss cohort study showed a positive association between 10-year average PM_{10} and NO_2 exposure and diabetes prevalence, even at levels below the World Health Organization air quality guidelines (OR: 1.40 [1.17 – 1.67], 1.19 [1.03 – 1.38] per 10 $\mu g/m^3$ increase in pollutant, respectively) (45). A cross-sectional study of 69,000 adults in China without a prior history of diabetes demonstrated that for each standard deviation increase in 3-year average concentration of $PM_{2.5}$ there were increased odds of diagnosed diabetes (OR: 1.04 [1.01, 1.07]) by fasting blood (46). Another cross-sectional study of 11,847 adults in China found that annual average $PM_{2.5}$ exposure was associated with diabetes prevalence (PR: 1.14 [1.08, 1.20] for a 41.1 $\mu g/m^3$ increase in $PM_{2.5}$), with a greater effect seen in subjects who were male, smoking, elderly, or had high BMI or less education (38).

The findings from these cross-sectional studies have been recapitulated in prospective data with mixed results. Particulate matter-associated diabetes incidence was investigated in a prospective cohort study of over 61,000 elderly Hong Kong residents without diabetes at baseline followed from 1998 to 2010. The analysis showed an increased risk of incident diabetes (HR: 1.15 [1.05, 1.25]) per 3.2 $\mu g/m^3$ increase in average annual $PM_{2.5}$ exposure (39). A Canadian cohort study followed 62,000 adults without diabetes in Ontario for up to 15 years, during which time a 10 $\mu g/m^3$ increase in average $PM_{2.5}$ exposure was associated with an increased risk of incident diabetes (HR 1.11 [1.02, 1.21]) (47). A Danish prospective cohort study from 1993 until 2013 found that annual average $PM_{2.5}$ was significantly associated with increased diabetes incidence (HR: 1.11 [1.02, 1.22]), especially in patients with obesity (40). Additionally, a 16-year-long cohort study of women in Germany without diabetes at baseline demonstrated a 15% [4%,

TABLE 1 Studies of links between air pollution and DM incidence and prevalence.

First Author (Year)	Design	Location	Population/Health Data Source	Pollutants	Relevant Outcomes
Lockwood (2002) (34)	Ecological; state-level	United States	Behavioral Risk Factor Surveillance System, 184,450 respondents	State industrial air pollutant releases	DM prevalence; linear regression $r = 0.54$, $p < 0.0001$
Pearson et al. (2010) (35)	Ecological; county-level	United States	CDC DM statistics, nationwide	Annual $PM_{2.5}$	1% increase in DM prevalence for 10 $\mu g/m^3$ increase in $PM_{2.5}$, $\beta = 0.81$, $p < 0.001$
Eze et al. (2014) (36)	Cross-sectional	Switzerland	SAPALDIA cohort, 6,392 adults	10-year PM_{10}, NO_2	DM prevalence per 10 $\mu g/m^3$ increase in: PM_{10} OR 1.40, NO_2 1.19
Li et al. (2023) (37)	Cross-sectional	Southwest China	CMEC cohort, 69,210 adults	3-year $PM_{2.5}$, BC, NH_4^+, NO_3^-, OM, soil	DM prevalence at follow-up per SD increase in: $PM_{2.5}$ OR 1.08, BC 1.07, NO_3^- 1.08, OM 1.09, soil 1.09; Nonsignificant positive association for NH_4^+
Liu et al. (2016) (38)	Cross-sectional	China	CHARLS cohort, 11,847 adults	Annual $PM_{2.5}$	DM prevalence per IQR increase in $PM_{2.5}$ PR 1.14
Qiu et al. (2018) (39)	Prospective cohort	Hong Kong	EHS cohort, 61,447 older adults	Annual $PM_{2.5}$ for 10 years	DM prevalence per IQR increase in $PM_{2.5}$ OR 1.06 DM incidence per IQR increase in $PM_{2.5}$ HR 1.15
Chen et al. (2013) (14)	Prospective cohort	Ontario	National Population Health Survey and Canadian Community Health Survey, 62,012 adults, no baseline DM	6-year $PM_{2.5}$	DM incidence per 10 $\mu g/m^3$ increase in $PM_{2.5}$ HR 1.11
Hansen et al. (2016) (40)	Prospective cohort	Denmark	Danish Nurse Cohort, 24,174 female nurses, no baseline DM	Annual $PM_{2.5}$, PM_{10}, NO_2, NO_x for 15 years	DM incidence per IQR increase in: $PM_{2.5}$ HR 1.11. Nonsignificant positive associations for NO_2, NO_x
Krämer et al. (2010) (41)	Prospective cohort	Germany	SALIA cohort, 1,775 adult women, no baseline DM	Annual PM_{10}, NO_2, Traffic-related PM for 16 years	DM incidence per IQR increase in: traffic-related PM HR 1.15, NO_2 HR 1.34. Nonsignificant positive association for PM_{10}
Puett et al. (2011) (42)	Prospective cohort	United States	NHS and HPFS cohorts, 89,460 adults, no baseline DM	Annual PM_{10}, $PM_{2.5}$, $PM_{10\text{-}2.5}$	DM incidence per IQR increase in pollutant. Nonsignificant positive associations for all pollutants.
Eze et al. (2014) (36)	Prospective cohort	Switzerland	SAPALDIA cohort, 2,631 adults, no baseline DM	Annual NO_2 at beginning and end of 9-years	DM incidence per IQR increase in NO_2, nonsignificant negative association
Coogan et al. (2016) (43)	Prospective cohort	United States	BWHS cohort, 43,003 black women, no baseline DM	Annual NO_2 for 10 years	DM incidence per IQR increase in NO_2, nonsignificant negative association
Andersen et al. (2012) (44)	Prospective cohort	Denmark	Danish DCR cohort, 51,818 middle-aged adults, no baseline DM	35-year NO_2	DM incidence per IQR increase in NO_2, nonsignificant positive association

Outcomes column reports results for fully adjusted models when applicable. Means reported for significant results.

27%] increase in the risk of incident diabetes per 1 interquartile range increase in traffic-related PM (a composite of particles derived from traffic) and NO_2 exposure, but no significant risk increase due to PM_{10} exposure (41). In contrast, an analysis of participants in the Nurses' Health Study and the Health Professionals Follow-Up Study found no significant association between 1-year average $PM_{2.5}$ or PM_{10} and incident diabetes, although the direction of effect was weakly positive (1.03 [0.96, 1.10] and 1.04 [0.99, 1.09] for $PM_{2.5}$ and PM_{10}, respectively) (42). Differences across these studies may be due to varying exposure assessments or particle compositions, or due to differences in cohort characteristics (e.g., diet, BMI). Thus, despite conflicting results, the majority of studies support an association between particulate matter air pollution exposure and diabetes incidence.

Similar to particulate matter, there are differing reports on associations between NO_2 exposure and diabetes. NO_2 exposure

was studied in a prospective study of 2,631 Swiss adults without baseline diabetes followed from 2002 to 2011; results showed no significant association between average annual NO_2 exposure and diabetes incidence (RR: 0.87 [0.60, 1.22]). However, few incident diabetes cases in the cohort may have diminished the ability to detect an effect (36). A similar negative finding for NO_2 was found in a prospective cohort of African American women residing in US cities (HR: 0.90 [0.82 – 1.00] for a 9.7 ppb increase in NO_2 in the fully adjusted model); this negative result was purported to be due to confounding by socioeconomic status (SES) given the inverse correlation between neighborhood SES and NO_2 in this cohort (43). Among women in the Nurses' Health Study, there was, however, a significant association between increased proximity to a roadway and developing diabetes (HR: 1.14 [1.03, 1.27] for living < 50 m vs. ≥00 m from a roadway). Because motor vehicles are a major generator of NO_2, and proximity to roadways has been used as a surrogate marker for exposure, the authors suggest this result may support a link between NO_2 and diabetes in females (42). Similar to the Nurses' Health Study results, a Danish prospective cohort study using a national public register found a nonsignificant but positive association between NO_2 exposure and confirmed diabetes (HR: 1.04 [1.00, 1.08] per 4.9 $\mu g/m^3$ increase in average NO_2), with the strongest associations in women and subjects with elevated waist-to-hip ratio (44). A cross-sectional study of respiratory clinic patients in two Canadian cities found a positive association between NO_2 exposure and diabetes diagnosis but only for women (OR: 1.04 [1.00, 1.08]) (48). A comparable cross-sectional study in the Netherlands showed a non-significant association between increasing levels of NO_2 exposure and diabetes diagnosis, with the direction of effect stronger in women (OR: 1.48 [1.07, 2.04]) (49). Given that air pollution is a complex mixture of particles and gases, it is challenging to interpret studies of the health effects of individual component pollutants. Regardless, evidence continues to mount in support of the association between increasing air pollution exposure and increases in incident and prevalent diabetes.

3.2.1 Short-term effects of air pollution on diabetes and glucose homeostasis

In addition to the literature supporting associations between air pollution exposure and incidence of diabetes, there is growing literature showing worsening of glucose metabolism with air pollution exposure for patients that already have a diabetes diagnosis. Furthermore, short-term exposures to air pollution, over days to weeks, seem to cause dysregulated glucose homeostasis, even among those without diabetes. The following studies examining short-term air pollution exposures and diabetes are summarized in Table 2.

A cross-sectional study of 2,840 patients with diabetes hospitalized from 2013-2016 in Chongqing, China, investigated the impact of short-term, 15-day average air pollution exposure on length of stay and cost of admission. The study authors found a positive correlation between a 10 $\mu g/m^3$ increase in sulfur dioxide (SO_2) and carbon monoxide (CO) exposure and prolonged length of stay, increased by 0.487 days [0.318, 0.656] and 0.013 days [0.003, 0.022], respectively, with a concordant increase in the cost of hospitalization (50). In Israel, a retrospective study between 2001-2012 of over 1 million fasting blood glucose tests from approximately 130,000 patients found a significant positive association between fasting blood glucose and 24-72 hour averages for NO_2 and SO_2 in all patients regardless of diabetes status. A 6.36 ppb increase in NO_2 was associated with a 0.40% [0.31%, 0.50%] increase in fasting glucose in patients without diabetes, 0.56% [0.40%, 0.71%] in those with prediabetes, and 1.08% [0.86%, 1.29%] in those with diabetes.; for a 1.17 ppb increase in SO_2 fasting glucose increased by 0.29% [0.22%, 0.36%], 0.20% [0.10%, 0.31%], 0.33% [0.14%, 0.52%], in these same groups (51). A similar retrospective study in the same Israeli population found that 12-week average PM_{10} and $PM_{2.5}$ exposure was associated with increased fasting blood glucose in all patients (0.30% [0.153%, 0.452%]; 0.02% [-0.12%, 0.18%], respectively) and this increase was more pronounced in those with diabetes (0.57% increase [0.29%, 0.85%], 0.41% increase [0.12%, 0.69%]). Also, HbA1c increases were found in patients with diabetes (3.58% [1.03%, 6.20%]; 2.93% [0.35%, 5.59%] for PM_{10} and $PM_{2.5}$ respectively). The 1-7 day average PM_{10} and $PM_{2.5}$ exposure windows had no or negligible association with fasting blood glucose and HbA1c (55).

Prospective data have corroborated the retrospective data suggesting short-term effects of air pollution on blood glucose. A German prospective cohort study between 2000-2008 of 7,108 adults without diabetes at baseline evaluated short-term associations between air pollution exposure and fasting blood glucose levels and HbA1c. Increases in 28-day average accumulation mode particle number (PN_{AM}, PM between 0.1-1μm in aerodynamic diameter) and $PM_{2.5}$ concentrations were both positively associated with increasing blood glucose (0.64 mg/dL [0.07, 1.21] per 2,142.3 n/mL increase and 0.91 mg/dL [0.38, 1.44] per 5.7 $\mu g/m^3$ increase, respectively) (52). In the US Framingham Heart Study, increased 7-day moving average BC and NO_x exposures were positively associated with higher fasting glucose among adults without diabetes. In contrast, an increased short-term O_3 exposure was inversely associated with blood glucose (exact numbers not provided by the study authors) (56). A prospective cohort study of approximately 28,000 adults in China followed from 2006-2008 found that a 100 $\mu g/m^3$ increase in the 4-day average of NO_2, SO_2, or PM_{10} exposure was associated with elevated fasting blood glucose (0.53 mmol/L [0.42, 0.65], 0.17 mmol/L [0.15, 0.19], 0.11 mmol/L [0.07, 0.15], respectively), with increased elevations among female, elderly, or overweight subjects (53). A recent study of 2 large Indian cities (Chennai and Delhi) found that a 10 $\mu g/m^3$ difference in 1-month average exposure to $PM_{2.5}$ was associated with a 0.40 mg/dL increase in fasting plasma glucose (95% CI 0.22 to 0.58) and 0.021 unit increase in HbA1c (95% CI 0.009 to 0.032) (57).

Some studies have been conducted on short-term air pollution exposure and glucose metabolism using the homeostasis model assessment of insulin resistance (HOMA-IR). In one study, 25 adults without diabetes who resided in rural Michigan were exposed to urban ambient air for 4-5 hours per day for 5 days. HOMA-IR was measured before, during, and after the air pollution intervention. A positive correlation was found between

TABLE 2 Studies of short-term effects of air pollution on glucose homeostasis.

First Author (Year)	Design	Location	Population/ Health Data Source	Pollutants	Relevant Outcomes
Li et al. (2018) (50)	Cross-sectional	China	Xinqiao Hospital, 2,840 hospitalized DM patients	15-day SO_2, CO, NO_2	LOS per 10 μg/m^3 rise in: SO_2 0.487 days increase, CO 0.013 days increase, NO_2 0.359 days decrease
Yitshak-Sade et al. (2015) (51)	Retrospective cohort	Israel	Clalit Health Services, 131,882 adult members	24–72-hour SO_2, NO_2	FBG increase: per IQR increase in: NO_2 in healthy adults 0.40%, prediabetes 0.56%, diabetes 1.08%; SO_2 in healthy adults 0.29%, prediabetes 0.20%, diabetes 0.33%
Yitshak-Sade et al. (2015) (51)	Retrospective cohort	Israel	Clalit Health Services, 73,117 adult members	1-7-day and 12-week PM_{10}, $PM_{2.5}$	Overall FBG increase per IQR increase in: 12-week PM_{10} 0.30%. Nonsignificant positive association for 12-week $PM_{2.5}$ and negligible associations for 1-7-day PM_{10} or $PM_{2.5}$. In people with diabetes, HbA1c increase per IQR increase in: 12-week PM_{10} 3.58%, $PM_{2.5}$ 2.93%
Lucht et al. (2018) (52)	Prospective cohort	Germany	HNR study cohort, 7,108 adults, no baseline DM	28-day PN_{AM}, $PM_{2.5}$	FBG increase per IQR increase in: PN_{AM} 0.64 mg/dL, $PM_{2.5}$ 0.91 mg/dL
Li et al. (2018) (50)	Prospective cohort	United States	Framingham cohorts, 5,958 adults, no baseline DM	1-3-7-day BC, NO_x, $PM_{2.5}$, O_3, SO_4^{2-}	FBG increase: 7-day BC approx. 0.5%, 7-day NO_x approx. 0.5%. Other results nonsignificant or neglibible
Chen et al. (2016) (53)	Prospective cohort	China	Kailuan cohort, 27,685 adults	4-day NO_2, SO_2, PM_{10}	FBG increase per 100 μg/m^3 increase in: NO_2 0.53 mmol/L, SO_2 0.17 mmol/L, PM_{10} 0.11 mmol/L
Brook et al. (2013) (54)	Experimental	United States	25 adults, no DM	5 days $PM_{2.5}$ from ambient urban air, 4-5-hr/d	HOMA-IR increase per 10 μg/m^3 increase in $PM_{2.5}$ 0.7
Chen et al. (2016) (53)	Cross-sectional	United States	BetaGene cohort, 1,023 adult Mexican Americans, personal or family history of GDM	37-40-days $PM_{2.5}$, NO_2	HOMA-IR increase for $PM_{2.5}$ β = 6.99 p = 0.002, NO_2 β = 6.63, p = 0.009
Li et al. (2018) (50)	Randomized, double-blind, crossover trial	China	55 college students, no DM	9-day $PM_{2.5}$ during trial	HOMA-IR increase per 10 μg/m^3 increase in $PM_{2.5}$ approx. 10%

Outcomes column reports results for fully adjusted models when applicable. Means reported for significant results.

each 10 μg/m^3 increase in measured $PM_{2.5}$ exposure and study subjects' HOMA-IR (+0.7 [0.1, 1.3]). A 3.5 μg/m^3 increase in $PM_{2.5}$ was associated with worsening HOMA-IR (+0.25 [0.04, 0.46], indicating potential adverse effects even at low concentrations of $PM_{2.5}$ (54). A cross-sectional analysis of Mexican American women with a personal or family history of gestational diabetes but without diabetes at the time of the study revealed that up to 40 days of daily $PM_{2.5}$ exposure and up to 37 days of daily NO_2 exposure were associated with increased HOMA-IR (β = 6.99, p = 0.002 for $PM_{2.5}$; β= 6.63, p = 0.009 for NO_2). However, no significant associations were found for O_3 exposure (58). Last, a clinical trial testing 48 hours of portable air cleaner (PAC) intervention in healthy college students in China showed an approximately 10% increase in HOMA-IR per 10 μg/m3 increase in $PM_{2.5}$ (exact numbers not provided) (59).

In summary, the collective evidence supports short-term associations between air pollution exposure and fasting glucose and dysregulated glucose metabolism evidenced by HOMA-IR, but not HbA1c.

3.2.2 Long-term effects of air pollution on diabetes and glucose metabolism

Long-term exposures to air pollution also have been shown to affect glucose homeostasis. Studies examining this phenomenon have been summarized in Table 3. Cross-sectional analyses of a Chinese cohort found 1-year average $PM_{2.5}$ to be positively associated with both elevated fasting glucose (0.26 mmol/L increase [0.19, 0.32]) and HbA1c (0.08% increase [0.06%, 0.10%]) for a large, 41.1 μg/m^3 increase in $PM_{2.5}$ (38). Furthermore, a secondary analysis of a Taiwanese cohort found 1-year average $PM_{2.5}$, PM_{10}, O_3, and NO_2 to be positively associated with fasting glucose and HbA1c; a 20.42 μg/m^3 increase in $PM_{2.5}$ was associated with 34.6 mg/dL [16.5, 52.7] increase in fasting glucose and 2.1% [1.5, 2.7] increase in HbA1c (60). The air pollution exposures experienced by this cohort were substantially above the WHO guidelines. A subsequent cross-sectional study of 2,895 adults in the Dunkirk and Lille areas of France, regions with relatively low concentrations of air pollution, found that HbA1c was 0.044% higher [0.021%, 0.067%] with a 2 μg/m^3 increase in annual mean

TABLE 3 Studies of long-term effects of air pollution on glucose homeostasis.

First Author (Year)	Design	Location	Population/ Health Data Source	Pollutants	Relevant Outcomes
Liu et al. (2016) (38)	Cross-sectional	China	CHARLS cohort, 11,847 adults	Annual $PM_{2.5}$	FBG increase per 41.1 μg/m^3 increase in $PM_{2.5}$ 0.26 mmol/L. HbA1c increase per 41.1 μg/m^3 increase in $PM_{2.5}$ 0.08%
Chuang et al. (2011) (60)	Cross-sectional	Taiwan	SEBAS sample, 1,023 older adults	Annual PM_{10}, $PM_{2.5}$, O_3, NO_2, SO_2	FBG increase per IQR increase in: PM_{10} 22.88 mg/dL, $PM_{2.5}$ 36.55 mg/dL, O_3 21.10 mg/dL, NO_2 17.03 mg/dL, SO_2 positive but nonsignificant. HbA1c increase per IQR increase in: PM_{10} 1.40%, $PM_{2.5}$ 2.24%, NO_2, O_3, and SO_2 positive but nonsignificant
Riant et al. (2018) (61)	Cross-sectional	France	ELISABET survey, 2,895 adults	Annual PM_{10}, NO_2	HbA1c increase per 2 μg/m^3 increase in PM_{10} 0.044%, per 5 μg/m^3 increase in NO_2 0.031%. Nonsignificant associations for FBG
Lucht et al. (2018) (52)	Prospective cohort	Germany	HNR study cohort, 7,108 adults, no baseline DM	91-day PN_{AM}, $PM_{2.5}$, PM_{10}, NO_2	FBG increase per IQR increase in: PN_{AM} 0.67 mg/dL, $PM_{2.5}$ 0.81 mg/dL. HbA1c increase per IQR increase in: PN_{AM} 0.09%, $PM_{2.5}$ 0.07%, PM_{10} 0.04%. No association for NO_2
Brook et al. (2013) (62)	Prospective cohort	Canada	Canadian census mortality follow-up study, 2.1 million adults	5-year $PM_{2.5}$	Diabetes related mortality per 10 μg/m^3 increase in $PM_{2.5}$ HR 1.49
Hwang et al. (2022) (63)	Cross-sectional	South Korea	Seoul National University Health Examination Center, 4,251 adults	Annual PM_{10} exposure	HOMA-IR increase per 11 μg/m^3 increase in PM_{10} 14%
Honda et al. (2017) (64)	Prospective cohort	United States	NSHAP cohort, 4,121 older adults	2-year $PM_{2.5}$ NO_2	In people with diabetes, HbA1c increase per IQR increase in: $PM_{2.5}$ 1.8%, NO_2 2.0%
Khafaie et al. (2018) (65)	Cross-sectional	India	WellGen study cohort, 1,213 young adults with DM	1-year PM_{10}	HOMA-IR increase per 43.83 μg/m^3 increase in $PM_{2.5}$ 4.89%
Chen et al. (2016) (53)	Cross-sectional	United States	BetaGene cohort, 1,023 adult Mexican Americans, personal or family history of GDM	Annual $PM_{2.5}$, NO_2, O_3	HOMA-IR increase for $PM_{2.5}$ β = 5.81 p = 0.016, no association for NO_2 or O_3
Wolf et al. (2016) (66)	Cross-sectional	Germany	KORA study, 2,944 adults	2-year $PM_{2.5}$, NO_2	HOMA-IR increase per IQR increase in: $PM_{2.5}$ 15.6%, NO_2 19.2%. Insulin increase per IQR increase in: $PM_{2.5}$ 14.5%, NO_2 17.2%

Outcomes column reports results for fully adjusted models when applicable. Means reported for significant results.

PM_{10} and 0.031% higher [0.010%, 0.053%] with a 5 μg/m^3 increase in annual mean NO_2. However, neither NO_2 nor PM_{10} were significantly associated with diabetes prevalence, likely due to a low number of patients with diabetes in the study sample. Moreover, neither pollutant had an association with fasting blood glucose (61).

Large prospective cohort studies repeatedly demonstrate the long-term effects of air pollution exposure on diabetes outcomes. A German prospective cohort study without baseline diabetes demonstrated that 91-day average exposure to PN_{AM} and $PM_{2.5}$ was associated with increased random blood glucose and, more strongly, with increased HbA1c. There was a 0.67 mg/dL [0.10, 1.24] and 0.81 mg/dL [0.05, 1.58] increase in random blood glucose (adjusted for time since last meal) per interquartile range (IQR) increase in PN_{AM} (1,352.7 n/mL) and $PM_{2.5}$ (4.0 μg/m^3), respectively. In this German study, HbA1c increased by 0.09% [0.07, 0.11] and 0.07% [0.04, 0.10] per IQR increase of each pollutant, respectively) (52). A census data analysis of 2.1 million randomly selected Canadian adults, followed from 1991 to 2001, found that a 10 μg/m^3 increase in average $PM_{2.5}$ exposure over a 5-year period was associated with a hazard ratio of 1.49 [1.37, 1.62] for diabetes-related mortality. This association was consistent across subgroups of age, sex, education, income, community, and at low concentrations of $PM_{2.5}$ (<5 μg/m^3). The risk of diabetes-related mortality was most pronounced in participants with lower SES as well as aboriginal ancestry (62). Similar effects on HbA1c have been observed in U.S. cohort studies. For example, in a probability sample of U.S. adults with diabetes over 57 years of age (n= 4121) followed from 2005 to 2011, a 3.7 μg/m^3 increase in 2-year moving average $PM_{2.5}$ was associated with an increase in HbA1c of 1.8% ± .6% (p<0.01). In

subjects without diabetes, a significant positive association with HbA1c was found for NO_2 exposure (0.8% ± .2%, p<0.01) (64). The prospective cohort study from Chennai and Delhi showed that a 1-year increase in $PM_{2.5}$ exposure of 10 μg/m^3 was associated with increased HR for incident diabetes (1.22 [1.09, 1.36]), with similar significant estimates for 1.5-year and 2-year exposures as well (57).

In addition to the long-term associations with HbA1c, long-term exposures have been associated with measures of insulin resistance. A cross-sectional study of long-term air pollution exposure in Korea found that the relationship between HOMA-IR and PM_{10} observed in studies of short-term air pollution exposures retained significance even with rigorous adjustment for visceral adiposity, with a dose-dependent increase in HOMA-IR by 14% [8%, 21%] for men and 14% [7%, 21%] for women, per 11 μg/m^3 increase in PM_{10} (63). Other cross-sectional studies have found similar associations with HOMA-IR. A cross-sectional study investigating the effect of PM_{10} exposure in young-onset (before age 46) patients with diabetes at a clinic in India found that a 43.83 μg/m^3 increase in 1-year average PM_{10} exposure was associated with increased HOMA-IR of 4.89% [0.59%, 9.37%], with a significantly greater effect in female and patients with obesity (65). In the cross-sectional analysis of Mexican American women discussed previously, annual $PM_{2.5}$ exposure was associated with increased HOMA-IR (beta coefficient 5.81, p = 0.016), without significant associations for annual NO_2 or O_3 exposures (58). A German prospective cohort study of nearly 3,000 adults with and without diabetes/prediabetes found that a 7.9 μg/m^3 increase in 2-year average $PM_{2.5}$ exposure was associated with increased HOMA-IR (15.6% [4.0%, 28.6%]) and insulin (14.5% [3.6%, 26.5%]). In contrast, an 11.9 μg/m^3 increase in 2-year average NO_2 exposure was associated with increases in HOMA-IR by 19.2% [7.7%, 31.6%], insulin by 17.2% [6.6%, 29.0%], glucose by 1.7% [0.1%, 3.3%], and leptin by 15.3% [6.8%, 24.5%]. However, there was no association between either pollutant and HbA1c (66).

While effect sizes have varied across cohorts and pollutants, the directionality of the relationships between long-term air pollution exposures and HbA1c remains generally consistent. If short-term air pollution exposure induces hyperglycemia, then we would expect increases in medium- and long-term exposure to have the effect of raising HbA1c. As expected, most studies to date support that months-long exposures are more strongly associated with HbA1c, indicating a potential cumulative effect of shorter-term air pollution exposure.

3.3 Diabetes may confer increased vulnerability to the cardiovascular effects of air pollution

Patients with diabetes appear to be more vulnerable to the vasculotoxic effects of air pollution exposure. Studies examining this potential predisposition have been summarized in Table 4. A cross-sectional analysis using Illinois Medicare data from 1988-1994 found that a 10 μg/m^3 increase in ambient PM_{10} exposure in the 24 hours prior to admission was associated with a 2.01% [1.40%, 2.62%] increase in hospital admission for CVD in patients with diabetes, a two-fold higher increase in CVD hospitalizations than that observed for adults without diabetes (67). A case-crossover study examining emergency department visits in Atlanta reported increased odds of visits for dysrhythmia with increasing NO_2 exposure for people with diabetes (OR 1.158 [1.046, 1.282]) compared to people without diabetes (1.014 [0.988, 1.040]; p<0.05 for regression coefficient difference between diabetes vs. no diabetes) (72). However, this study did not find significant associations for other pollutants such as PM_{10} and O_3, perhaps due to estimating air pollution exposures using central monitors rather than patient residential addresses.

In addition to clinical outcomes, exposure to air pollutants has also been associated with subclinical effects. In 2005, a cross-sectional study of 270 adults in Boston found that, among subjects with diabetes, $PM_{2.5}$ exposure was associated with a 7.6% decrease in nitroglycerin-mediated vascular reactivity [-12.8%, -2.1%], while black carbon exposure was associated with a 10.7% decrease in flow-mediated reactivity [-17.3, -3.5]. However, there was no such association among subjects without diabetes (68). That same year, a cross-sectional study of participants in the Veterans Administration Normative Aging Study observed that the association between $PM_{2.5}$ exposure and reduced heart rate variability (HRV) was more pronounced in participants with diabetes. Indeed, the percent change in the standard deviation of normal-to-normal intervals (a measure of HRV) due to $PM_{2.5}$ exposure, although not significant, was nearly 4-fold higher in participants with diabetes (-16.6% [-36.3, 9.2] compared to -4.7% [-11.4%, 2.6%]) (69). A double-blind, crossover exposure study of 17 never-smoker adults with diabetes found that 2 hours of controlled exposure to $PM_{0.1}$ reduced heart rate variability (p = 0.014) and also increased average heart rate by approximately 8 beats per minute over a day after the exposure (74). These data point to the synergistic interaction between diabetes and air pollution in driving CVD.

Not all studies support an interaction between diabetes and air pollution. A case-crossover study examining emergency department visits for acute coronary syndrome in Utah found little difference in the $PM_{2.5}$ risk estimate for people with diabetes compared to those without (71). A similar case-crossover study of death records from 20 cities in the United States found no significant effect modification of the PM_{10}-CVD death association by diabetes status, although the point estimate for the association between PM_{10} and all-cause mortality was higher for people with diabetes compared to those without (70). Moreover, in an analysis of 22 years of follow up in the American Cancer Society Cancer Prevention Study II cohort, people with diabetes had a higher risk of CVD mortality at both high (HR 2.4 [2.3, 2.5]) and low $PM_{2.5}$ exposure (2.2 [2.1, 2.3]) compared to people without diabetes. However, when comparing high to low $PM_{2.5}$ exposure, the CVD mortality risk increase was similar in both groups, and formal tests of interaction between diabetes status and $PM_{2.5}$ exposure were nonsignificant (73).

3.4 Environmental inequities contribute to unequal diabetes and cardiovascular disease risk

Consistently, research in the United States has shown that racial/ethnic minority communities, and individuals with low education and

TABLE 4 Studies examining the increased CVD risk for people with diabetes exposed to air pollution.

First Author (Year)	Design	Location	Population/Health Data Source	Pollutants	Relevant Outcomes
Zanobetti & Schwartz (2000) (67)	Cross-sectional	IL, USA	Medicare claims data, years 1988-1994	24-hr PM_{10}	2.01% greater hospital admissions for CVD in people with diabetes per 10 $\mu g/m^3$ increase in PM_{10} vs. 0.94% greater CVD admissions for people without diabetes.
O'Neill et al. (2005) (68)	Cross-sectional	MA, USA	Boston-area residents, 270 adults	24-hr $PM_{2.5}$, BC	7.6% decrease in nitroglycerin-mediated vascular reactivity and 10.7% decrease in flow-mediated reactivity in people with diabetes. Null findings for people without diabetes.
Park et al. (2005) (69)	Cross-sectional	MA, USA	VA Normative Aging study, 603 adults	4-24-48-hr $PM_{2.5}$, PN, BC, O_3, NO_2, SO_2, CO	Nonsignificant but marked trend toward reduced HRV in people with diabetes compared to people without diabetes.
Zeka et al. (2006) (70)	Case-crossover	United States	National Center for Health Statistics, 1,896,306 deaths	PM_{10} 0-3d before death	No significant effect modification of PM_{10}-CVD death association by diabetes status.
Pope et al. (2006) (71)	Case-crossover	UT, USA	Intermountain Health Collaborative Study, 12,865 adults	PM_{10}, $PM_{2.5}$	Similar $PM_{2.5}$ risk estimates for people with vs. without diabetes.
Peel et al. (2007) (72)	Case-crossover	GA, USA	Emergency department data, 4,407,535 visits	24-hr PM_{10}, 8-hr O_3, 1-hr NO_2, SO_2, CO	For increasing NO_2 exposure, 15.6% greater odds of visit for dysrhythmia in people with diabetes compared to 1.4% greater odds in people without diabetes.
Pope et al. (2014) (73)	Prospective cohort	United States	American Cancer Society Cancer Prevention Study II, 669,049 adults with varying diabetes status at baseline	Monthly $PM_{2.5}$	People with both diabetes and >75th percentile for exposure to $PM_{2.5}$ at highest risk of CVD death. No evidence of interaction between diabetes status and $PM_{2.5}$ exposure.
Vora et al. (2014) (74)	Double-blind, randomized, crossover trial	NY, USA	19 adults with diabetes	$PM_{0.1}$ vs. filtered air	8 beats/min greater heart rate in $PM_{0.1}$ condition.

income, have greater diabetes prevalence (75) and mortality (76). Racial/ethnic minorities also develop diabetes at lower BMIs compared to white people, and the strength of the association between BMI and diabetes is weaker in racial/ethnic minorities, highlighting the complexity of factors that may contribute to this disproportionate burden (77). Recent work in the US and limited research in Asia and Africa has shown that low-SES communities are subject to higher air pollution exposures (78). In the United States, black and Hispanic minority groups are disproportionately exposed to the air pollution generated by the white majority (79). This disproportionate exposure translates into a greater burden of death due to air pollution. A retrospective cohort study of US Veterans Administration patients found that excess death due to $PM_{2.5}$ exposure was disproportionately borne by black patients (55.2 deaths per 100,000 [50.5, 60.6]) compared to nonblack patients (51.0 [46.4, 56.1]), as well as by patients living in low SES counties (65.3 [56.2, 75.4]) compared to those living in high SES counties (46.1 [42.3, 50.4]). Notably, 99% of these excess deaths were due to $PM_{2.5}$ concentrations below the US Environmental Protection Agency (EPA) recommended limit of 12$\mu g/m^3$ (80). These findings are put into historical context when considering that historically redlined neighborhoods face greater $PM_{2.5}$ and NO_2 exposures compared to other communities in the same cities. Even within redlined neighborhoods, racial and ethnic disparities in air pollution exposure persist (81). Thus, multiple traditional and non-traditional risk factors are disproportionately concentrated in minority communities and may act in concert to further widen health disparities.

3.5 Inconsistencies in the data

As noted previously in this review, some studies have not detected an association between air pollution and incident or prevalent diabetes. Moreover, associations between long-term air pollution exposure and HbA1c were, though not entirely consistent, generally positive. Results for gaseous pollutants appear to be more heterogeneous than particulates; however, since there are correlations between these pollutants, it can be challenging to parse out outcomes for individual components in epidemiological studies. Some associations appear to be only in, or stronger in, subgroups; additionally, some effects appear to be attenuated by other factors such as medications (45). Regional, cultural, gender, socioeconomic or other differences in work or lifestyle can influence how people spend time in different geographical areas, thereby changing both pollution sources and exposures. Given the temporal and spatial heterogeneity of air pollution concentration and composition, accurate and precise exposures are extremely difficult to assess making some degree of variation in these results unsurprising. Lastly, although experimental studies suggested that having diabetes can exacerbate the cardiovascular derangements induced by air pollution exposure, and epidemiologic observations often reported greater associations between air pollution and death for people with diabetes, the evidence of an effect modification on air pollution-CVD death by diabetes status or an air pollution-diabetes interaction on CVD death remains lacking. The lack of detectable effect modification could be due to inaccurate reporting

on surveys and death records. Alternatively, the majority of the effect of air pollution on CVD death may be attributable to air pollution promoting a cardiometabolic disease state. Thus, some of this effect could be lost when stratifying by diabetes status (73).

3.6 A word on the exposome

Although this review is concerned with the health effects of air pollution exposure, it is important to note that such exposure does not occur in a vacuum. Instead, air pollution exposure often co-occurs with a variety of other environmental exposures, such as noise pollution, nighttime light, and temperature, especially in urban areas (82). Originally conceived as a complement to the genome (83), the term "exposome" aims to capture the host of biological responses to the myriad environmental exposures experienced throughout the life course. In recent years, there has been a growing call to consider each environmental exposure in context of the entire exposome. Much of the prior observational analysis has focused on single exposures in isolation. To completely understand the health implications of environmental exposures, including air pollution, experts have argued in favor of advanced analytic methods that consider diverse, simultaneous exposures, their interactions, and their measurable biological effects (84). Such analytic methods will need to venture beyond standard univariate and multivariate regression models to tease out the likely non-linear effects of a multitude of co-occurring exposures (85). Taking a page from studies of genetic associations, a novel method known as environment-wide association study might identify the effects of mixtures of exposures (86). Future observational studies examining the air pollution, diabetes, and CVD link should take into account the exposome concept to more accurately reflect the reality of how people experience these exposures.

3.7 The global variability of air pollution and its health implications

It is worth noting that although air pollution is experienced by nearly all people globally, the burden of air pollution varies by region. Over the past two decades, average airborne $PM_{2.5}$ concentrations have declined in North America, Europe, and East Asia, whereas the opposite has occurred in the Middle East, Africa, and South Asia (87, 88). Despite this increase in ambient PM concentration in the Middle East and North Africa, morbidity and mortality rates due to air pollution have decreased in these regions, which might be due to a declining rate of indoor fuel burning (89). Nevertheless, ambient air pollution remains an urgent public health concern, with approximately 22% of deaths due to ischemic heart disease and 21% of deaths due to diabetes attributed to air pollution in the Middle East and North Africa (89).

Among global regions, substantial differences have been noted in the magnitude of the association between higher PM exposure and increased mortality (90). Such differences are likely due to a variety of factors. Countries vary in the relative contributions of traffic, industry, and biomass burning to the generation of PM (91). These pollution sources differ in the exact chemical composition PM (92), which might explain the global differences in mortality risk due to PM exposure. Moreover, indoor burning of biomass fuel, such as wood, crops, and manure, for heating and cooking can drastically worsen indoor air quality (93). The greater indoor burning of such fuels in lower- and middle-income countries (LMIC) could alter the observed association between outdoor air pollution and mortality while also placing people in these countries at higher risk (90). As discussed previously in this review, LMIC face a greater projected increase in prediabetes and diabetes compared to high-income countries. Many of these countries also face trends of worsening air quality. Evidently, the combined epidemics of air pollution exposure and diabetes represent an urgent threat to global public health.

3.8 Conclusions from epidemiological data

Overall, the studies to date indicate that $PM_{2.5}$, PM_{10}, PN_{AM}, black carbon, SO_2, and CO may have deleterious effects on glucose homeostasis in the short- and long-term. Consistently, $PM_{2.5}$ had the most consistently demonstrated effect on glucose across multiple populations. The inconsistent results for NO_2 may be due to low diabetes event rate in the study subjects and/or low overall levels of NO_2 leading to small effect size. Acute air pollution exposure is more strongly associated with increased fasting blood glucose, whereas chronic exposure has a stronger association with worsened HbA1c. Susceptible subgroups demonstrate stronger effects of air pollution on glucose metabolism as well as diabetes prevalence and incidence among those with overweight and obesity. Consequently, air pollution appears to have stronger effects on adverse CV outcomes among those with dysregulated glucose homeostasis.

While there is a growing body of work in this field, most epidemiological studies to date have been conducted in populations residing in the US, Canada, western Europe, and East Asia. Most of these regions have experienced improvements in air pollution levels in recent decades, while LMIC have experienced an increase in morbidity and mortality due to air pollution (94). With diabetes prevalence on the rise worldwide, more studies are needed to investigate the effects of worsening pollution on the metabolic health of people living in LMIC. Results from wealthier countries cannot be extrapolated to LMIC, given the known differences in air pollution exposures and population characteristics between these regions.

4 Shared mechanisms

While human epidemiological studies can identify associations, mechanistic evidence is important to define the affected biological pathways. Such data can assist in identifying susceptibility factors, specific pollutants to target with regulation, or molecular targets for pharmaceutical interventions. To date, mechanistic studies in this field include known exposure studies in cell lines and in animal models, exposure chamber studies, and natural experiments with

humans. In this section, we will review these types of studies and summarize the identified mechanisms that underlie the associations between air pollution, dysregulated glucose metabolism, and increased CVD risk.

4.1 Mechanisms in animal studies

There are multiple convening pathways by which air pollution, diabetes, and cardiovascular disease interact (Figure 2). Much of the data to date suggests two primary culprits are inflammation and oxidative stress, themselves intertwined, which often form self-perpetuating feedback loops.

4.1.1 Inflammation in *animal models*

4.1.1.1 Hypothalamic inflammation, diabetes, and air pollution

Inflammation plays a dominant role in the development and progression of diabetes (95, 96). Hypothalamic inflammation is proposed to be a major driver of disorders of glucose homeostasis due to its role in regulating energy intake and expenditure via insulin and leptin (97). Animal models show that recurrent hypothalamic inflammation, via diet-induced increases in TNFα expression (98–100), leads to a dysregulated body weight set-point, driving increased energy intake and decreased energy expenditure. These behaviors serve to increase adiposity that further increases inflammation (101). These appear to be independent, yet synergistic, effects on inflammation. In fact, a study in a mouse model of diabetes and prediabetes suggested that differences in hypothalamic inflammation could be to blame for the observed variation in the onset and progression of prediabetes to diabetes within the group of mice (102).

The hypothalamus appears to be susceptible to the inflammatory effects of particulate matter pollution. A mouse model was exposed to $PM_{2.5}$ or clean air, and inhibitor of nuclear factor kappa-B kinase subunit beta (IKK2), an NF-kB inhibitor that interferes with inflammatory signal transduction. Pollution-exposed mice treated with cerebral IKK2 demonstrated an attenuation of the insulin resistance shown in pollution-exposed mice not treated with IKK2; they also had evidence of inhibition of hepatic gluconeogenesis enzymes. Overall, this suggests that PM likely increases the production of these enzymes in part via inflammatory signaling through NF-kB (103). In a separate study, mice were fed a normal chow diet and exposed to $PM_{2.5}$ or filtered air. After five days of $PM_{2.5}$ exposure, there was chemical and histologic evidence of a heightened inflammatory response within the hypothalamus, with accompanying food-seeking, exercise-avoidant behavior changes, and adipose gain. After exposure to $PM_{2.5}$ for twelve weeks, the mice developed increased toll-like receptor 4 (TLR4) and Ikbke (related to NF-kB) expression, leptin and insulin resistance, and a worsening of their energy homeostasis and development of frank obesity. In this study, knockdown of TLR4 and Ikbke completely attenuated the effects of $PM_{2.5}$ exposure on leptin and insulin (104). Together, these suggest that hypothalamic inflammation could lie along a potential causal pathway between air pollution exposure and dysregulated glucose homeostasis.

4.1.1.2 Inflammation, vascular disease, and air pollution

In addition to the hypothalamic inflammation, air pollution has been shown to exacerbate inflammation systemically. In airway epithelial cells and macrophages, O_3 exposure has been shown to induce the production of inflammatory cytokines, interleukin-6 (IL-6), and interleukin-8 (IL-8) (105). Animal experiments show that

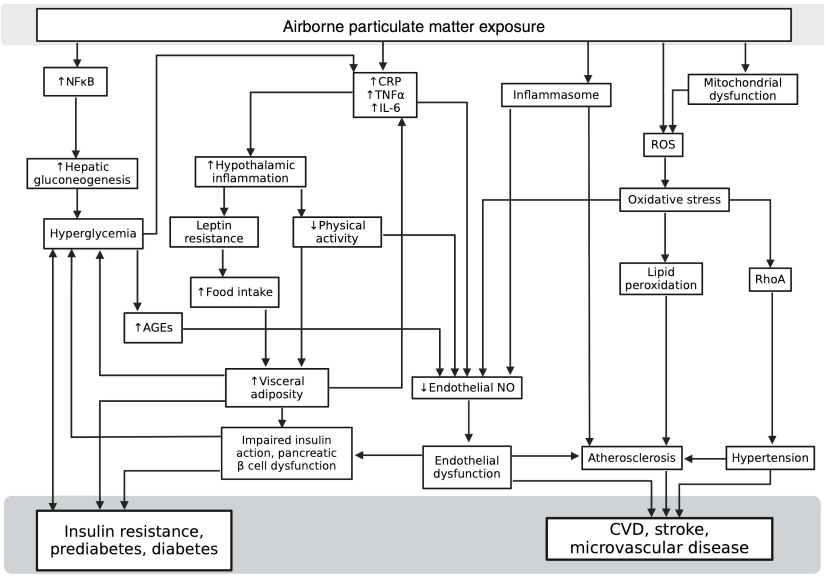

FIGURE 2
Multiple overlapping mechanisms by which air pollution, diabetes, and cardiovascular disease interact. (AGEs, advanced glycosylation end products; CRP, C-reactive Protein; CVD, cardiovascular disease; IL-6, Interleukin-6; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; RhoA, Ras homolog family member A; ROS, reactive oxygen species; TNFα, tumor necrosis factor alpha). Created with BioRender.com.

excess glucose and triglycerides cause inflamed adipose tissue to secrete adipokines, driving insulin resistance and pancreatic β cell exhaustion, thereby exacerbating nutrient excess and leading to further inflammation (106). In a C57BL/6 mouse model, male mice fed a high-fat diet were randomly assigned to $PM_{2.5}$ exposure or clean, filtered air. Compared to the control, clean air group, the mice in the exposed group developed elevated insulin resistance, increased visceral fat, and increased adipose inflammation (107). Furthermore, the exposed mice exhibited decreased vascular relaxation in response to insulin and acetylcholine, indicating insulin resistance (107). In mice, co-exposure to SO_2, NO_2, and $PM_{2.5}$ increased circulating levels of the inflammatory molecules TNF-α, IL-6, and cyclooxygenase-2, while also dose-dependently increasing endothelin-1 and decreasing endothelial nitric oxide synthase, which reflect impaired endothelial function (108). This finding is consistent with prior animal research that implicates inflammatory cytokines in the impairment of vascular tone (109, 110).

Air pollution exposure may also potentiate atherosclerosis progression. $PM_{2.5}$ exposure in mouse models accelerates atherosclerosis and increases inflammation compared to filtered air, with stronger effects in mice on a high-fat diet (111, 112). Notably, inflammasome activation plays a central role in this process (113). These results demonstrate the indirect effects of air pollution on vascular function. Interestingly, inhaled nanoparticles in rats accumulate in areas of vascular inflammation, including atherosclerotic plaques, suggesting the direct effects of PM exposure on vascular tissue may also be relevant. Together, these studies support that particulate matter pollution can accumulate in, and worsen the inflammation of, adipose and vascular tissue, potentially worsening already impaired vascular and endothelial function (114). Overall, animal experiments to date suggest that air pollution exposure may independently promote the development and worsening of both diabetes and atherosclerosis with a central role for inflammation in each of these processes.

4.1.2 Oxidative *stress*

Separate from inflammation, air pollution exposure induces oxidative stress, which refers to the state of imbalance in reactive oxygen species (ROS) and antioxidant mechanisms such that ROS may induce damage to cellular structures or other biomolecules of importance (115). $PM_{2.5}$ and $PM_{0.1}$ were shown to accumulate in mitochondria, causing damage to the mitochondria and possibly potentiating the effect of ROS (37). Transition metals, present in $PM_{2.5}$ and $PM_{0.1}$, generate ROS at the particle surface, causing oxidative stress and mitochondrial damage (37). Furthermore, polycyclic aromatic hydrocarbons, quinones, and peroxyacetyl nitrate found in the organic carbon fraction of PM are potent inducers of oxidative stress (116, 117). Even O_3, when dissolved in plasma, or serum, or saline, generates H_2O_2 (118).

Multiple studies *in vivo* and *in vitro* have shown that oxidative stress drives many of the vascular complications of diabetes (119). In particular, hyperglycemia appears to promote mitochondrial generation of superoxide (120), while interfering with this production attenuates the damaging effects of hyperglycemia on the endothelium (121). ROS also produce nitrotyrosine, which has been shown to accumulate in necrotic and apoptotic cardiac myocytes from patients with diabetes and from a rat model of diabetes (122). Generation of superoxide by NADPH oxidase also appears to play a significant role in the micro- and macro-vascular complications of diabetes (123).

Oxidative stress is extensively implicated in the development of CVD (124) and has been shown in animal models to play a key role in mediating the cardiovascular effects of air pollution exposure (125). After 10 weeks of exposure to 14.1 $\mu g/m^3$ $PM_{2.5}$ and $PM_{0.1}$, rats had increased superoxide concentrations in their aortas with signs of oxidative stress due to both impaired endothelial nitric oxide synthase and impaired hepatic synthetic function. In these rats, ROS generation from both $PM_{2.5}$ and $PM_{0.1}$ activated RhoA, a known mediator of vasoconstriction and acute hypertension. RhoA activation correlated with an increased mean arterial pressure of the rats exposed to the PM versus control (126). Compared to clean air controls, rats exposed to concentrated PM for 5 hours had twice the amount of oxidative stress in cardiac tissue (127).

4.1.3 Conclusions on mechanisms from animal studies

Numerous studies show increases in both inflammation and oxidative stress. The specific vascular impact of air pollution-related oxidative stress in animal models of diabetes has not been extensively studied. However, evaluating the evidence discussed in this section, it is reasonable to hypothesize that air pollution can exacerbate vascular complications of diabetes via increased oxidative stress. Air pollution-induced inflammation and diabetes may then synergistically exacerbate cardiac and vascular dysfunction, providing plausible causal explanations for the links between air pollution, diabetes, and cardiovascular disease in epidemiological studies.

4.2 Mechanisms in human studies

Human studies are limited in the ability to test exposures and outcomes ethically. Some experimental exposure studies in healthy volunteers have investigated the molecular mechanisms underpinning the adverse health effects associated with air pollution exposure. There have also been some epidemiological studies with molecular testing that is suggestive of mechanisms. While limited, these studies can confirm animal studies and validate these pathways in humans.

4.2.1 Inflammation in *humans*

4.2.1.1 Inflammation and diabetes

Inflammation appears to play a role in the pathogenesis of diabetes. Studies investigating this relationship have been outlined in Table 5. Multiple nested case-control studies have investigated the role of inflammatory cytokines in the development of diabetes. In the Women's Health Study, there was an increased risk of developing diabetes for those in the highest vs. lowest quartile of baseline IL-6 (RR: 2.3 [0.9, 5.6]) and CRP (RR: 4.2 [1.5 – 12.0])

(128). Similarly, in the EPIC-Potsdam study, IL-6 and CRP were not only significantly correlated with HbA1c (0.099, p = 0.019, and 0.1, p = 0.017, respectively), but also with odds of developing diabetes (OR: 2.57 [1.24 – 5.47] and 1.9 [1.2 – 3.2], respectively) in models adjusting for traditional diabetes risk factors and HbA1c (129). A nested case-control analysis within a prospective study of 3,842 Swiss adults without baseline diabetes followed for 5.5 years on average showed that diabetes risk increased with highest vs. lowest quartile of baseline IL-6 (OR 1.58 [1.02 – 2.45]) and CRP (OR 4.63 [2.85 – 7.53]) (130). Finally, the Cardiovascular Health Study in the United States showed that having baseline CRP in the highest quartile was associated with increased odds of developing diabetes (OR 2.03 [1.44 - 2.86]) versus the lowest quartile (131). Related to metabolism and inflammation, the Framingham Heart Study showed a positive correlation between exposure to $PM_{2.5}$ and SO_4^{2-} with adipokines adiponectin and resistin, respectively.[1]

The hypothalamic inflammation linked with obesity and diabetes in mouse models recapitulates in humans with multiple magnetic resonance imaging (MRI) studies (100, 132–135). However, while growing evidence implicates hypothalamic inflammation in abnormal glucose homeostasis in humans, we are aware of no human studies investigating this as a direct result of air pollution exposure. Such investigation, using quantitative MRI methods similar to the other studies in this section, may confirm the air pollution and hypothalamic inflammation link observed in animal studies.

4.2.1.2 Inflammation, atherosclerosis, and air pollution

There is a well-known association between inflammation and clinical atherosclerosis (136, 137). Multiple recent reviews (138–142), as well as a meta-analysis (143), discuss the associations between inflammatory biomarkers and air pollution exposure. The most commonly studied inflammatory biomarkers include CRP, IL-6, and TNF-α, but others demonstrate associations with pollution exposure. Literature exploring air pollution promoting CVD via inflammation has been listed in Table 6. Experimental exposure studies in healthy volunteers have shown increases in biomarkers of inflammation with controlled exposure to urban air pollution (147, 148), but not wood smoke (149), indicating the importance of pollution composition. Gene expression studies show increased activation of anti-inflammatory pathways that support inflammation as a mediator for air pollution exposure-related adverse effects (150). A study of traffic-related air pollution exposure in adolescents and young adults with type 1 diabetes showed increases in IL-6 and CRP as well (151).

Several recent studies have investigated the role of inflammation as a mediator of air pollution-linked CVD risk. Inflammation and CVD biomarkers were examined in a cross-sectional analysis of 6,103 participants with and without CVD in a Swiss cohort. A 5 $\mu g/m^3$ increase in annual mean $PM_{2.5}$ exposure was associated with increased ceruloplasmin (β = 0.1328 [0.0898, 0.1757]) alpha-1-antitrypsin (β = 0.105 [0.0564, 0.1537]), Lp-PLA_2 (β = 0.085 [0.0303, 0.1397]), neutrophil-leukocyte ratio (0.074 [0.0054, 0.14]), C3 (β = 0.1618 [0.1302, 0.2071]), haptoglobin (β = 0.0981 [0.0075, 0.1886]), and orosomucoid (β = 0.205 [0.1505, 0.2595]) (144).

Increased exposure to metals from measured $PM_{2.5}$ is associated with increased inflammatory biomarker sCD36, which in turn has a significant mediating effect on the association of these metals with pulse pressure, providing further evidence that inflammation appears to occur upstream of CVD in the context of $PM_{2.5}$ exposure. No significant associations were found for total $PM_{2.5}$, highlighting that the individual components of $PM_{2.5}$ likely have differential effects on inflammation and oxidative stress in humans, which should prompt further investigation (146).

TABLE 5 Cited literature regarding the link between inflammation and diabetes in humans.

First Author (Year)	Design	Location	Population/ Health Data Source	Biomarkers	Relevant Outcomes
Pradhan et al. (2001) (128)	Prospective, nested case-control	United States	Women's Health Study, 188 cases, 362 controls	IL-6, CRP	Diabetes incidence RR 7.5 for IL-6, 15.7 for CRP
Spranger et al. (2003) (129)	Prospective, nested case-control	United States	EPIC-Potsdam, 192 cases, 384 controls	IL-1β, IL-6, TNF-α, CRP	Diabetes incidence OR 2.57 for IL-6, 1.9 for CRP. No association for TNF-α, IL-1β
Marques-Vidal et al. (2012) (130)	Prospective	Switzerland	CoLaus Study, 3,842 adults, no baseline DM	IL-1β, IL-6, TNF-α, CRP	Diabetes incidence, unadjusted OR 1.58 for IL-6, 4.63 for CRP. No significant associations for any biomarker after adjustment.
Barzilay et al. (2001) (131)	Prospective	United States	Cardiovascular Health Study, 4,481 older adults	CRP, WBC, platelets, fibrinogen, factor VIIIc, albumin	Diabetes incidence OR 2.03 for CRP, no associations for other biomarkers
Li et al. (2018) (50)	Prospective cohort	United States	Framingham cohorts, 5,958 adults, no baseline DM	adiponectin, resistin, leptin	Positive association between 7-day $PM_{2.5}$ and adiponectin, 7-day SO_4^{2-} and resistin; negative association between 7-day NO_x and adiponectin
Krämer et al. (2010) (41)	Prospective cohort	Germany	SALIA cohort, 1,775 adult women, no baseline DM	C3c	C3c significantly associated with PM_{10} and diabetes incidence, HR 1.12 per 10 mg/dL increase

Outcomes column reports results for fully adjusted models when applicable. Means reported for significant results.

TABLE 6 Cited studies investigating the impact of air pollution driving CVD via inflammation.

First Author (Year)	Design	Location	Population/Health Data Source	Pollutants	Relevant Outcomes
Azzouz et al. (2022) (144)	Cross-sectional	Sweden	Malmö Diet and Cancer, Cardiovascular Subcohort, 6,103 adults	Annual $PM_{2.5}$, PM_{10}, NO_x	$PM_{2.5}$ and PM_{10} associated with increased ceruloplasmin, alpha-1-antitrypsin. $PM_{2.5}$ associated with Lp-PLA_2, NLR, C3, haptoglobin, orosomucoid. No associations for NO_x
Abohashem et al. (2021) (145)	Retrospective cohort	United States	Massachusetts General Hospital, 503 adults	Annual 24-hr $PM_{2.5}$	$PM_{2.5}$ associated with bone marrow and splenic activity, arterial inflammation, and MACE (HR 1.404)
Brook et al. (2008) (48)	Randomized, double-blind, crossover	Canada	31 healthy adults	2-hr exposure to $PM_{2.5}$, O_3	$PM_{2.5}$ and O_3 increased WBC, decreased FMD. $PM_{2.5}$ increased neutrophils and diastolic BP.
Zhang et al. (2023) (146)	Panel	China	45 healthy college students	1-, 2-, 3-day $PM_{2.5}$ and metal fractions	Association between metal fractions and sCD36, CRP, and pulse pressure.

Outcomes column reports results for fully adjusted models when applicable. Means reported for significant results.

Vascular inflammation is seen in subclinical atherosclerosis using PET/MRI hybrid imaging techniques (152), implying that inflammation has a role early in the development of the disease. Inhalable particles likely have direct local effects on arterial disease and indirect systemic effects. For example, when human volunteers at risk for stroke were exposed to inhalable gold nanoparticles, these particles were then detected in their diseased carotid arteries (153). Advanced imaging techniques have been implemented to investigate a direct link between PM, inflammation, arterial damage, and CVD outcomes in humans (145), supporting likely direct vascular inflammatory effects of air pollution exposure. These studies suggest air pollutants may act via both systemic and direct vascular inflammatory actions to promote CVD, corroborating the epidemiological studies and animal models. Additional research may more fully elucidate which specific components of pollutants may act on which pathways to identify potential targets for intervention.

4.2.2 Oxidative *stress* in *humans*

The effect of air pollution on oxidative stress, demonstrated robustly in animal models, has had inconsistent evidence in humans, likely because measuring oxidized DNA and lipids in humans can be a technological challenge (154, 155). However, a meta-analysis of studies examining oxidized DNA and lipids in subjects exposed to air pollution that had minimal measurement error demonstrated a consistent association between $PM_{2.5}$ and these measures of oxidative stress (156).

In particular, a study in healthy adults and adults with diabetes found that inducing labile blood glucose via clamp resulted in elevations of the markers of oxidative stress plasma 3-nitrotyrosine and PGF2α, a decrease in NO synthesis, as well as impaired endothelial function in the presence of vasodilating agents (157). Furthermore, peroxynitrite, generated by the reaction of superoxide and endothelial NO, has been detected at elevated concentrations (158) and shown to induce platelet damage in the blood of patients with diabetes (159).

4.2.3 Inflammation and oxidative stress promote endothelial dysfunction

Endothelial dysfunction mainly refers to the impairment of endothelium-mediated relaxation of vascular tone. Human studies have consistently reported an association between traditional cardiovascular risk factors and endothelial dysfunction (160). Furthermore, endothelial dysfunction can predict progression and long-term outcomes of coronary heart disease (161).

Inflammation is known to promote endothelial dysfunction in humans (110, 162) in a variety of disease states, including obesity and diabetes (163). This link between inflammation and endothelial dysfunction appears to be present in the context of air pollution exposure. In a study of healthy volunteers with experimental exposure to $PM_{2.5}$, higher TNF-α just after air pollution exposure was associated with poorer endothelial function a day later, suggesting that endothelial dysfunction seen with air pollution may be mediated by an inflammatory cascade that begins acutely during and after exposure, even in people that are free of diagnosed disease (164). Research in animals has demonstrated that the production of ROS by inflammatory cytokines, as well as the formation of advanced glycation end products (AGEs), alter the availability of endothelial NO, leading to endothelial dysfunction (110). ROS occurs in hypertension, hyperlipidemia, and diabetes, providing a likely explanation for the link between these traditional risk factors and endothelial dysfunction (165). The mechanistic inclusion of AGEs in this pathway is of special interest, as diabetic hyperglycemia promotes the endogenous production of AGE by irreversibly glycating tissue proteins and lipids (166).

Recently, endothelial dysfunction of arterioles and microvessels was demonstrated to predict the development and progression of diabetes in a German prospective cohort study of 15,000 adults without baseline diabetes or prediabetes (167). Mechanistically, microvascular endothelial dysfunction may impair insulin action in skeletal muscle and favor blood flow to nonnutritive tissues, thereby promoting hyperglycemia (168). Moreover, experiments in animal models have demonstrated

that microvascular disease in pancreatic ß cells may drive the pathogenesis of diabetes (169, 170).

Lastly, accumulating evidence has demonstrated a link between air pollution and endothelial dysfunction mediated by inflammation and oxidative stress (171). Taken together, it appears that endothelial dysfunction may partly explain the air pollution-diabetes link while also providing a mechanism for the accelerated development of CVD in people with diabetes exposed to air pollution.

4.3 Conclusions from mechanistic data

Systemic inflammation and oxidative stress, converging at endothelial dysfunction, represent common mechanisms whereby air pollution induces glucose dysregulation and exacerbates CVD risk. Due to its role in the onset and progression of both diseases, air pollution represents a critical target in promoting the health of the public and individuals. The next section will explore interventions directed toward mitigating the effects of inflammation and oxidative stress.

5 An eye toward prevention

Despite inconsistencies in the observational literature, the overall balance of evidence across all the tiers of evidence quality supports a deleterious effect of short- and long-term air pollution exposure on metabolic health. Given that the global population will remain exposed to air pollution, for which a completely benign dose has yet to be established, efforts have been made to counter these adverse effects. This section will detail the research concerning such interventions, with close attention paid to barriers to effective implementation.

5.1 Interventions to reduce air pollution as driver of CVD risk in people with diabetes

There is a burgeoning body of literature demonstrating that the use of portable air cleaners (PAC), which are known to reduce $PM_{2.5}$ exposure, can reduce serum concentrations of CRP, IL-6, and TNFα. An overview of this research can be found in a recent systematic review and meta-analysis written by our group (172). PACs may also reduce blood pressure (173, 174). Given the importance of inflammation in CVD progression in diabetes, interventions to attenuate the upregulation of these pathways are appealing. However, most of the trials that intervened with PACs were conducted in healthy volunteers for short periods of time and in very controlled settings. Functioning similarly to PACs, a series of trials that used N95 respirators on participants in China demonstrated benefits on systolic blood pressure, HRV, and IL-1 (175).

The testing of interventions to ameliorate the adverse effects of air pollution exposure on glucose metabolism has been limited. A randomized, double-blind crossover trial in 55 healthy college students residing in Shanghai placed sham or real air purifiers in participants' dormitories for 1 week, followed by a 17-day washout period, then 1 week of the alternate treatment. The investigators found that serum glucose, glucose-6-phosphate, insulin, and HOMA-IR were lower during the real air purification period compared to the sham period (59).

Diet patterns and nutritional supplementation with antioxidants or vitamins have been examined for their potential to protect against the adverse cardiometabolic effects of air pollution. Numerous studies have investigated the anti-inflammatory effects of dark chocolate (176–181). Supplementation with L-arginine has been shown to mitigate air pollution-related blood pressure increases among adults with hypertension (182), while in adults with diabetes, L-arginine was shown to improve glucose control, blood pressure, and forearm blood flow (183). Vitamin E has also been studied and shown *in vitro* to reduce inflammatory biomarker expression after PM2.5 exposure to endothelial cells (184) and to reduce oxidative stress in humans with occupational exposures to air pollutants (185, 186). In a cross-sectional study of 47,000 adults, those in the highest quartile of compliance to the Dietary approaches to stop hypertension (DASH) diet had no significantly increased risk of $PM_{2.5}$-associated hypertension. In contrast, the lowest quartile had significantly increased risk (OR: 1.20 [1.10, 1.30]) (187). While generally low-risk, none of these studies were adequate to conclusively recommend specific dietary interventions for protection against the adverse cardiometabolic effects of air pollution. However, they are suggestive of potential options that warrant further investigation.

5.2 Other interventions to reduce the cardiovascular harms of air pollution

Although the literature investigating interventions to reduce the cardiovascular harms of air pollution exposure in persons with diabetes may be limited, there are additional interventions that are low-risk and readily accessible. These other interventions may provide protection against air pollution exposure or mitigate its harms, despite a weaker evidence base compared to PACs.

First, observational evidence suggests that central air conditioning might mitigate the adverse cardiovascular effects of PM exposure (188–191), even though the filters commonly used in air conditioning systems are less efficient at removing airborne particulate matter compared to HEPA filters. Thus, people with prediabetes or diabetes could be encouraged to use central air conditioning, if accessible and affordable, instead of electric fans and especially instead of opening windows for indoor temperature regulation. The results of a few experimental studies also support the use of in-vehicle air conditioning to reduce air pollution exposure while driving (192–194).

Second, the use of cigarettes and other combustible tobacco products indoors generates smoke that reduces indoor air quality (195, 196). Residue from cigarette smoke can adhere to indoor surfaces, creating thirdhand smoke that may continue to harm health after a smoking session has ended (197). Furthermore, although the health effects of electronic cigarettes are still under active investigation, electronic cigarette vapors contain some of the same pollutants as tobacco smoke (198) and therefore may also worsen air quality when used indoors. Tobacco product and

electronic cigarette cessation should be strongly encouraged in all people, especially those with diabetes. However, if a person with diabetes is unable to quit, they should be counseled to avoid smoking indoors.

Other practical advice includes limiting outdoor activities during periods of poor air quality. The Air Quality Index, an easily understandable scale that describes outdoor air quality (199), is available on the internet for many cities around the world, especially in North America, East Asia, and Europe. People with diabetes can be advised to regularly check the air quality index (AQI) for their location and adjust activity accordingly. Keeping windows closed can also mitigate exposure to poor outdoor air quality, as can the avoidance of walking beside roads with heavy traffic.

5.3 Policy implications and public health initiatives for prevention

Given the worldwide contributions of traffic, industry, and biomass burning to the generation of ambient PM, policies that address these sources would reduce the ambient air pollution in urban environments (91). Furthermore, policies aimed at improving capture of industrial air pollution, developing more efficient industrial and agricultural systems, promoting electrification of motor vehicles, decreasing meat consumption, and reducing carbon emissions have been identified as feasible ways to improve global air quality within the next few decades if sufficient political will is generated (200).

Regular use of screening tests such as HbA1c and fasting plasma glucose alone do not appear sufficient to identify all people at risk for diabetes and its complications (201), therefore, diabetes prevention would likely benefit from population-level interventions. Cross-sectional evidence suggests that policies and public health initiatives that aim to improve the walkability of urban spaces and access to green space should be pursued (202). Although enhancing access to healthy food might theoretically reduce the population risk for diabetes (203), the evidence supporting such an initiative is limited due to relatively few studies and heterogenous measures of the food environment (202, 204).

5.4 Prevention conclusions

Overall, there is a dearth of data on individual-level interventions to prevent PM-related CVD in people with diabetes. PACs have the most robust experimental evidence to support their use to lower blood pressure, reduce inflammation, and potentially improve glucose control. However, whether PACs can reduce the macrovascular or microvascular complications of diabetes is unknown. Efforts are ongoing to regulate pollutant concentrations on a societal level, but more research is needed to identify susceptible subgroups and effective interventions for them. Avoiding traffic exposure, closing windows, and using air conditioning at home and in vehicles are commonsense actions unsuited for a clinical trial. Thus, data on these preventive strategies are limited (205). However, that should not preclude recommending these low-risk interventions, particularly for those at increased risk.

6 Summary of key points

- Air pollution exposure, especially fine particulate matter, is known to increase the risk of incident CVD and worsened CVD outcomes. There is no known safe dose of air pollution.
- Air pollution exposure increases the risk of incident diabetes and prediabetes in diverse populations and perturbs glucose homeostasis.*
- Prediabetes and diabetes confer an increased susceptibility to the cardiovascular harms of air pollution exposure.
- Air pollution exposure promotes local and systemic inflammation, which exacerbates atherosclerosis progression as well as endothelial dysfunction. In animal experiments, air pollution contributes to ROS formation and excess oxidative stress, as well as hypothalamic inflammation which may promote excess nutrient intake and resultant diabetes.
- Inflammation and oxidative stress are associated with dysregulated glucose metabolism in humans and animals. Hyperglycemia promotes further oxidative stress and inflammation, which may explain the progression from prediabetes to diabetes as well as the well-known increased CVD risk observed in people with diabetes.
- * Mechanistic evidence supports the role of inflammation, oxidative stress, and hyperglycemia in the development of endothelial dysfunction. Air pollution and diabetes are both associated with endothelial dysfunction, which has been shown to predict CVD outcomes and incident diabetes.
- The study of interventions in people with diabetes to reduce the CVD risk due to air pollution has been limited. Some evidence points to the potential usefulness of portable air cleaners. Suggestive evidence supports further research into the effects of certain dietary and nutritional supplement interventions.

7 Conclusion

The importance of minimizing the impact of air pollution on a global scale cannot be overstated. The impact of air pollution on driving both the development of diabetes and exacerbating CVD risk in patients with diabetes is a topic that needs more research to reach a complete understanding of the interactions and mechanisms at play. Although there is heightened awareness of the adverse health effects of air pollution, further study on preventive strategies in people across the spectrum of dysregulated glucose homeostasis is greatly needed. An improved understanding of the mechanisms by which air pollution, diabetes, and cardiovascular disease interact would hasten the development of interventions to minimize the risks of exposure and slow disease progression. Furthermore, insights from this would greatly benefit a range of parties, including individuals

concerned about their risks, healthcare providers wanting to provide optimal care and recommendations, and governments aiming to promote public health.

Author contributions

LJB: Writing – original draft, Writing – review & editing, Visualization. SW: Writing – original draft, Writing – review & editing, Conceptualization, Visualization, Supervision. CL: Visualization, Writing – original draft. JA: Conceptualization, Writing – review & editing. JN: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Brimblecombe P. History of urban air pollution. In: Fenger J, Hertel O, Palmgren F, editors. *Urban air pollution — European aspects*. Dordrecht, Netherlands: Springer (1998). p. 7–20.

2. de Bont J, Jaganathan S, Dahlquist M, Persson A, Stafoggia M, Ljungman P. Ambient air pollution and cardiovascular diseases: an umbrella review of systematic reviews and meta-analyses. *J Intern Med.* (2022) 291:779–800. doi: 10.1111/joim.13467

3. Dastoorpoor M, Sekhavatpour Z, Masoumi K, Mohammadi MJ, Aghababaeian H, Khanjani N, et al. Air pollution and hospital admissions for cardiovascular diseases in ahvaz, Iran. *Sci Total Environ.* (2019) 652:1318–30. doi: 10.1016/j.scitotenv.2018.10.285

4. Momtazan M, Geravandi S, Rastegarimehr B, Valipour A, Ranjbarzadeh A, Yari AR, et al. An investigation of particulate matter and relevant cardiovascular risks in abadan and khorramshahr in 2014–2016. *Toxin Rev.* (2019) 38:290–7. doi: 10.1080/15569543.2018.1463266

5. Xu JQ MS, Kochanek KD, Arias E. Statistics NCfH. *Mortality in the United States, 2021*. Hyattsville, MD, USA: National Center for Health Statistics (2022). doi: 10.15620/cdc:122516

6. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. Acc/Aha guideline on the primary prevention of cardiovascular disease: executive summary: A report of the american college of cardiology/American heart association task force on clinical practice guidelines. *J Am Coll Cardiol.* (2019) 74:1376–414. doi: 10.1016/j.jacc.2019.03.009

7. Murray CJL, Aravkin AY, Zheng P, Abbafati C, Abbas KM, Abbasi-Kangevari M, et al. Global burden of 87 risk factors in 204 countries and territories, 19902019: A systematic analysis for the global burden of disease study 2019. *Lancet.* (2020) 396:1223–49. doi: 10.1016/S0140-6736(20)30752-2

8. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the gbd 2019 study. *J Am Coll Cardiol.* (2020) 76:2982–3021. doi: 10.1016/j.jacc.2020.11.010

9. Bowe B, Xie Y, Li T, Yan Y, Xian H, Al-Aly Z. The 2016 global and national burden of diabetes mellitus attributable to pm(2.5) air pollution. *Lancet Planet Health.* (2018) 2:e301–e12. doi: 10.1016/S2542-5196(18)30140-2

10. Schenk M, Popp SM, Neale AV, Demers RY. Environmental medicine content in medical school curricula. *Acad Med.* (1996) 71:499–501. doi: 10.1097/00001888-199605000-00022

11. Graber DR, Musham C, Bellack JP, Holmes D. Environmental health in medical school curricula: views of academic deans. *J Occup Environ Med.* (1995) 37:807–11. doi: 10.1097/00043764-199507000-00009

12. Gehle KS, Crawford JL, Hatcher MT. Integrating environmental health into medical education. *Am J Prev Med.* (2011) 41:S296–301. doi: 10.1016/j.amepre.2011.06.007

13. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. Idf diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract.* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

14. Centers for Disease Control and Prevention Diabetes Statistics. *National diabetes statistics report* . Available online at: https://www.cdc.gov/diabetes/data/statistics-report/index.html (Accessed August 15, 2023).

15. Rowley WR, Bezold C, Arikan Y, Byrne E, Krohe S. Diabetes 2030: insights from yesterday, today, and future trends. *Popul Health Manag.* (2017) 20:6–12. doi: 10.1089/pop.2015.0181

16. Rooney MR, Fang M, Ogurtsova K, Ozkan B, Echouffo-Tcheugui JB, Boyko EJ, et al. Global prevalence of prediabetes. *Diabetes Care.* (2023) 46:1388–94. doi: 10.2337/dc22-2376

17. Menke A, Rust KF, Fradkin J, Cheng YJ, Cowie CC. Associations between trends in race/ethnicity, aging, and body mass index with diabetes prevalence in the United States: A series of cross-sectional studies. *Ann Intern Med.* (2014) 161:328–35. doi: 10.7326/M14-0286

18. American Diabetes A. Economic costs of diabetes in the U.S. In 2017. *Diabetes Care.* (2018) 41:917–28. doi: 10.2337/dci18-0007

19. Diseases Injuries Collaborators GBD. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: A systematic analysis for the global burden of disease study 2019. *Lancet.* (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9

20. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. Heart disease and stroke statistics-2023 update: A report from the american heart association. *Circulation.* (2023) 147:e93–e621. doi: 10.1161/CIR.0000000000001123

21. Mohebi R, Chen C, Ibrahim NE, McCarthy CP, Gaggin HK, Singer DE, et al. Cardiovascular disease projections in the United States based on the 2020 census estimates. *J Am Coll Cardiol.* (2022) 80:565–78. doi: 10.1016/j.jacc.2022.05.033

22. Diabetes mellitus: A major risk factor for cardiovascular disease. A joint editorial statement by the american diabetes association; the national heart, lung, and blood institute; the juvenile diabetes foundation international; the national institute of diabetes and digestive and kidney diseases; and the american heart association. *Circulation.* (1999) 100:1132–3. doi: 10.1161/01.CIR.100.10.1132

23. Emerging Risk Factors C, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *Lancet.* (2010) 375:2215–22. doi: 10.1016/S0140-6736(10)60484-9

24. Baena-Diez JM, Penafiel J, Subirana I, Ramos R, Elosua R, Marin-Ibanez A, et al. Risk of cause-specific death in individuals with diabetes: A competing risks analysis. *Diabetes Care.* (2016) 39:1987–95. doi: 10.2337/dc16-0614

25. Booth GL, Kapral MK, Fung K, Tu JV. Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study. *Lancet.* (2006) 368:29–36. doi: 10.1016/S0140-6736(06)68967-8

26. Anand SS, Dagenais GR, Mohan V, Diaz R, Probstfield J, Freeman R, et al. Glucose levels are associated with cardiovascular disease and death in an international cohort of normal glycaemic and dysglycaemic men and women: the epidream cohort study. *Eur J Prev Cardiol*. (2012) 19:755–64. doi: 10.1177/1741826711409327

27. Dal Canto E, Ceriello A, Ryden L, Ferrini M, Hansen TB, Schnell O, et al. Diabetes as a cardiovascular risk factor: an overview of global trends of macro and micro vascular complications. *Eur J Prev Cardiol*. (2019) 26:25–32. doi: 10.1177/2047487319878371

28. Ingelsson E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. *JAMA*. (2005) 294:334–41. doi: 10.1001/jama.294.3.334

29. Arnlov J, Lind L, Zethelius B, Andren B, Hales CN, Vessby B, et al. Several factors associated with the insulin resistance syndrome are predictors of left ventricular systolic dysfunction in a male population after 20 years of follow-up. *Am Heart J*. (2001) 142:720–4. doi: 10.1067/mhj.2001.116957

30. Rawshani A, Rawshani A, Franzén S, Sattar N, Eliasson B, Svensson A-M, et al. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. *New Engl J Med*. (2018) 379:633–44. doi: 10.1056/NEJMoa1800256

31. Organization WH. *Air pollution* (2023). Available online at: https://www.who.int/health-topics/air-pollution#tab=tab_2 (Accessed August 28, 2023).

32. Organization WH. *Air quality and health* (2023). Available online at: https://www.who.int/teams/environment-climate-change-and-health/air-quality-and-health/health-impacts/types-of-pollutants (Accessed August 28, 2023).

33. Hickey RJ, Schoff EP, Clelland RC. Relationship between air pollution and certain chronic disease death rates. *Multivariate Stat Stud Arch Environ Health*. (1967) 15:728–38. doi: 10.1080/00039896.1967.10664990

34. Lockwood AH. Diabetes and air pollution. *Diabetes Care*. (2002) 25:1487–8. doi: 10.2337/diacare.25.8.1487

35. Pearson JF, Bachireddy C, Shyamprasad S, Goldfine AB, Brownstein JS. Association between fine particulate matter and diabetes prevalence in the U.S. *Diabetes Care*. (2010) 33:2196–201. doi: 10.2337/dc10-0698

36. Eze IC, Foraster M, Schaffner E, Vienneau D, Heritier H, Rudzik F, et al. Long-term exposure to transportation noise and air pollution in relation to incident diabetes in the sapaldia study. *Int J Epidemiol*. (2017) 46:1115–25. doi: 10.1093/ije/dyx020

37. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ Health Perspect*. (2003) 111:455–60. doi: 10.1289/ehp.6000

38. Liu C, Yang C, Zhao Y, Ma Z, Bi J, Liu Y, et al. Associations between long-term exposure to ambient particulate air pollution and type 2 diabetes prevalence, blood glucose and glycosylated hemoglobin levels in China. *Environ Int*. (2016) 92-93:416–21. doi: 10.1016/j.envint.2016.03.028

39. Qiu H, Schooling CM, Sun S, Tsang H, Yang Y, Lee RS, et al. Long-term exposure to fine particulate matter air pollution and type 2 diabetes mellitus in elderly: A cohort study in hong kong. *Environ Int*. (2018) 113:350–6. doi: 10.1016/j.envint.2018.01.008

40. Hansen AB, Ravnskjaer L, Loft S, Andersen KK, Brauner EV, Baastrup R, et al. Long-term exposure to fine particulate matter and incidence of diabetes in the danish nurse cohort. *Environ Int*. (2016) 91:243–50. doi: 10.1016/j.envint.2016.02.036

41. Kramer U, Herder C, Sugiri D, Strassburger K, Schikowski T, Ranft U, et al. Traffic-related air pollution and incident type 2 diabetes: results from the salia cohort study. *Environ Health Perspect*. (2010) 118:1273–9. doi: 10.1289/ehp.0901689

42. Puett RC, Hart JE, Schwartz J, Hu FB, Liese AD, Laden F. Are particulate matter exposures associated with risk of type 2 diabetes? *Environ Health Perspect*. (2011) 119:384–9. doi: 10.1289/ehp.1002344

43. Coogan PF, White LF, Yu J, Burnett RT, Marshall JD, Seto E, et al. Long term exposure to no2 and diabetes incidence in the black women's health study. *Environ Res*. (2016) 148:360–6. doi: 10.1016/j.envres.2016.04.021

44. Andersen ZJ, Raaschou-Nielsen O, Ketzel M, Jensen SS, Hvidberg M, Loft S, et al. Diabetes incidence and long-term exposure to air pollution: A cohort study. *Diabetes Care*. (2012) 35:92–8. doi: 10.2337/dc11-1155

45. Eze IC, Schaffner E, Fischer E, Schikowski T, Adam M, Imboden M, et al. Long-term air pollution exposure and diabetes in a population-based swiss cohort. *Environ Int*. (2014) 70:95–105. doi: 10.1016/j.envint.2014.05.014

46. Li S, Guo B, Jiang Y, Wang X, Chen L, Wang X, et al. Long-term exposure to ambient pm2.5 and its components associated with diabetes: evidence from a large population-based cohort from China. *Diabetes Care*. (2023) 46:111–9. doi: 10.2337/dc22-1585

47. Chen H, Burnett RT, Kwong JC, Villeneuve PJ, Goldberg MS, Brook RD, et al. Risk of incident diabetes in relation to long-term exposure to fine particulate matter in ontario, Canada. *Environ Health Perspect*. (2013) 121:804–10. doi: 10.1289/ehp.1205958

48. Brook RD, Jerrett M, Brook JR, Bard RL, Finkelstein MM. The relationship between diabetes mellitus and traffic-related air pollution. *J Occup Environ Med*. (2008) 50:32–8. doi: 10.1097/JOM.0b013e31815dba70

49. Dijkema MB, Mallant SF, Gehring U, van den Hurk K, Alssema M, van Strien RT, et al. Long-term exposure to traffic-related air pollution and type 2 diabetes prevalence in a cross-sectional screening-study in the Netherlands. *Environ Health*. (2011) 10:76. doi: 10.1186/1476-069X-10-76

50. Li X, Tang K, Jin XR, Xiang Y, Xu J, Yang LL, et al. Short-term air pollution exposure is associated with hospital length of stay and hospitalization costs among inpatients with type 2 diabetes: A hospital-based study. *J Toxicol Environ Health A*. (2018) 81:819–29. doi: 10.1080/15287394.2018.1491912

51. Yitshak-Sade M, Kloog I, Liberty IF, Katra I, Novack L, Novack V. Air pollution and serum glucose levels: A population-based study. *Med (Baltimore)*. (2015) 94:e1093. doi: 10.1097/MD.0000000000001093

52. Lucht SA, Hennig F, Matthiessen C, Ohlwein S, Icks A, Moebus S, et al. Air pollution and glucose metabolism: an analysis in non-diabetic participants of the heinz nixdorf recall study. *Environ Health Perspect*. (2018) 126:047001. doi: 10.1289/EHP2561

53. Chen L, Zhou Y, Li S, Williams G, Kan H, Marks GB, et al. Air pollution and fasting blood glucose: A longitudinal study in China. *Sci Total Environ*. (2016) 541:750–5. doi: 10.1016/j.scitotenv.2015.09.132

54. Brook RD, Xu X, Bard RL, Dvonch JT, Morishita M, Kaciroti N, et al. Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. *Sci Total Environ*. (2013) 448:66–71. doi: 10.1016/j.scitotenv.2012.07.034

55. Yitshak Sade M, Kloog I, Liberty IF, Schwartz J, Novack V. The association between air pollution exposure and glucose and lipids levels. *J Clin Endocrinol Metab*. (2016) 101:2460–7. doi: 10.1210/jc.2016-1378

56. Li W, Dorans KS, Wilker EH, Rice MB, Kloog I, Schwartz JD, et al. Ambient air pollution, adipokines, and glucose homeostasis: the framingham heart study. *Environ Int*. (2018) 111:14–22. doi: 10.1016/j.envint.2017.11.010

57. Siddhartha M, Suganthi J, Dimple K, Joel DS, Nikhil T, Viswanathan M, et al. Pm2.5 exposure, glycemic markers and incidence of type 2 diabetes in two large Indian cities. *BMJ Open Diabetes Res & Care*. (2023) 11:e003333. doi: 10.1136/bmjdrc-2023-003333

58. Chen Z, Salam MT, Toledo-Corral C, Watanabe RM, Xiang AH, Buchanan TA, et al. Ambient air pollutants have adverse effects on insulin and glucose homeostasis in mexican americans. *Diabetes Care*. (2016) 39:547–54. doi: 10.2337/dc15-1795

59. Li H, Cai J, Chen R, Zhao Z, Ying Z, Wang L, et al. Particulate matter exposure and stress hormone levels: A randomized, double-blind, crossover trial of air purification. *Circulation*. (2017) 136:618–27. doi: 10.1161/CIRCULATIONAHA.116.026796

60. Chuang KJ, Yan YH, Chiu SY, Cheng TJ. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. *Occup Environ Med*. (2011) 68:64–8. doi: 10.1136/oem.2009.052704

61. Riant M, Meirhaeghe A, Giovannelli J, Occelli F, Havet A, Cuny D, et al. Associations between long-term exposure to air pollution, glycosylated hemoglobin, fasting blood glucose and diabetes mellitus in northern France. *Environ Int*. (2018) 120:121–9. doi: 10.1016/j.envint.2018.07.034

62. Brook RD, Cakmak S, Turner MC, Brook JR, Crouse DL, Peters PA, et al. Long-term fine particulate matter exposure and mortality from diabetes in Canada. *Diabetes Care*. (2013) 36:3313–20. doi: 10.2337/dc12-2189

63. Hwang SE, Kwon H, Yun JM, Min K, Kim HJ, Park JH. Association between long-term air pollution exposure and insulin resistance independent of abdominal adiposity in korean adults. *Sci Rep*. (2022) 12:19147. doi: 10.1038/s41598-022-23324-4

64. Honda T, Pun VC, Manjourides J, Suh H. Associations between long-term exposure to air pollution, glycosylated hemoglobin and diabetes. *Int J Hyg Environ Health*. (2017) 220:1124–32. doi: 10.1016/j.ijheh.2017.06.004

65. Khafaie MA, Salvi SS, Ojha A, Khafaie B, Gore SD. Yajnik CS. Particulate matter and markers of glycemic control and insulin resistance in type 2 diabetic patients: result from wellcome trust genetic study. *J Expo Sci Environ Epidemiol*. (2018) 28:328–36. doi: 10.1038/s41370-017-0001-1

66. Wolf K, Popp A, Schneider A, Breitner S, Hampel R, Rathmann W, et al. Association between long-term exposure to air pollution and biomarkers related to insulin resistance, subclinical inflammation, and adipokines. *Diabetes*. (2016) 65:3314–26. doi: 10.2337/db15-1567

67. Zanobetti A, Schwartz J. Are diabetics more susceptible to the health effects of airborne particles? *Am J Respir Crit Care Med*. (2001) 164:831–3. doi: 10.1164/ajrccm.164.5.2012039

68. O'Neill MS, Veves A, Zanobetti A, Sarnat JA, Gold DR, Economides PA, et al. Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. *Circulation*. (2005) 111:2913–20. doi: 10.1161/CIRCULATIONAHA.104.517110

69. Park Sung K, O'Neill Marie S, Vokonas Pantel S, Sparrow D, Schwartz J. Effects of air pollution on heart rate variability: the va normative aging study. *Environ Health Perspect*. (2005) 113:304–9. doi: 10.1289/ehp.7447

70. Zeka A, Zanobetti A, Schwartz J. Individual-level modifiers of the effects of particulate matter on daily mortality. *Am J Epidemiol*. (2006) 163:849–59. doi: 10.1093/aje/kwj116

71. Pope CA, Muhlestein JB, May HT, Renlund DG, Anderson JL, Horne BD. Ischemic heart disease events triggered by short-term exposure to fine particulate air pollution. *Circulation*. (2006) 114:2443–8. doi: 10.1161/CIRCULATIONAHA.106.636977

72. Peel JL, Metzger KB, Klein M, Flanders WD, Mulholland JA, Tolbert PE. Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. *Am J Epidemiol*. (2006) 165:625–33. doi: 10.1093/aje/kwk051

73. Pope CA, Turner MC, Burnett RT, Jerrett M, Gapstur SM, Diver WR, et al. Relationships between fine particulate air pollution, cardiometabolic disorders, and cardiovascular mortality. *Circ Res*. (2015) 116:108–15. doi: 10.1161/CIRCRESAHA.116.305060

74. Vora R, Zareba W, Utell MJ, Pietropaoli AP, Chalupa D, Little EL, et al. Inhalation of ultrafine carbon particles alters heart rate and heart rate variability in people with type 2 diabetes. *Part Fibre Toxicol*. (2014) 11:31. doi: 10.1186/s12989-014-0031-y

75. Liu J, Yi SS, Russo R, Mayer VL, Wen M, Li Y. Trends and disparities in diabetes and prediabetes among adults in the United States, 1999-2018. *Public Health*. (2022) 214:163–70. doi: 10.1016/j.puhe.2022.10.021

76. Saydah S, Lochner K. Socioeconomic status and risk of diabetes-related mortality in the U. *S. Public Health Rep*. (2010) 125:377–88. doi: 10.1177/003335491012500306

77. Zhu Y, Sidell MA, Arterburn D, Daley MF, Desai J, Fitzpatrick SL, et al. Racial/ethnic disparities in the prevalence of diabetes and prediabetes by bmi: patient outcomes research to advance learning (Portal) multisite cohort of adults in the U.S. *Diabetes Care*. (2019) 42:2211–9. doi: 10.2337/dc19-0532

78. Hajat A, Hsia C, O'Neill MS. Socioeconomic disparities and air pollution exposure: A global review. *Curr Environ Health Rep*. (2015) 2:440–50. doi: 10.1007/s40572-015-0069-5

79. Tessum CW, Apte JS, Goodkind AL, Muller NZ, Mullins KA, Paolella DA, et al. Inequity in consumption of goods and services adds to racial-ethnic disparities in air pollution exposure. *Proc Natl Acad Sci U.S.A*. (2019) 116:6001–6. doi: 10.1073/pnas.1818859116

80. Bowe B, Xie Y, Yan Y, Al-Aly Z. Burden of cause-specific mortality associated with pm2.5 air pollution in the United States. *JAMA Netw Open*. (2019) 2:e1915834. doi: 10.1001/jamanetworkopen.2019.15834

81. Lane HM, Morello-Frosch R, Marshall JD, Apte JS. Historical redlining is associated with present-day air pollution disparities in U.S. Cities. *Environ Sci Technol Lett*. (2022) 9:345–50. doi: 10.1021/acs.estlett.1c01012

82. Munzel T, Sorensen M, Lelieveld J, Hahad O, Al-Kindi S, Nieuwenhuijsen M, et al. Heart healthy cities: genetics loads the gun but the environment pulls the trigger. *Eur Heart J*. (2021) 42:2422–38. doi: 10.1093/eurheartj/ehab235

83. Wild CP. Complementing the genome with an "Exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev*. (2005) 14:1847–50. doi: 10.1158/1055-9965.EPI-05-0456

84. Munzel T, Sorensen M, Hahad O, Nieuwenhuijsen M, Daiber A. The contribution of the exposome to the burden of cardiovascular disease. *Nat Rev Cardiol*. (2023) 20:651–69. doi: 10.1038/s41569-023-00873-3

85. Hamra GB, Buckley JP. Environmental exposure mixtures: questions and methods to address them. *Curr Epidemiol Rep*. (2018) 5:160–5. doi: 10.1007/s40471-018-0145-0

86. Patel CJ. Analytic complexity and challenges in identifying mixtures of exposures associated with phenotypes in the exposome era. *Curr Epidemiol Rep*. (2017) 4:22–30. doi: 10.1007/s40471-017-0100-5

87. Sicard P, Agathokleous E, Anenberg SC, De Marco A, Paoletti E, Calatayud V. Trends in urban air pollution over the last two decades: A global perspective. *Sci Total Environ*. (2023) 858:160064. doi: 10.1016/j.scitotenv.2022.160064

88. Li C, van Donkelaar A, Hammer MS, McDuffie EE, Burnett RT, Spadaro JV, et al. Reversal of trends in global fine particulate matter air pollution. *Nat Commun*. (2023) 14:5349. doi: 10.1038/s41467-023-41086-z

89. Abbasi-Kangevari M, Malekpour M-R, Masinaei M, Moghaddam SS, Ghamari S-H, Abbasi-Kangevari Z, et al. Effect of air pollution on disease burden, mortality, and life expectancy in north africa and the middle east: A systematic analysis for the global burden of disease study 2019. *Lancet Planetary Health*. (2023) 7:e358–e69. doi: 10.1016/S2542-5196(23)00053-0

90. Tian L, Sun S. Comparison of health impact of air pollution between China and other countries. In: Dong G-H, editor. *Ambient air pollution and health impact in China*. Singapore, Singapore: Springer (2017). p. 215–32.

91. Karagulian F, Belis CA, Dora CFC, Prüss-Ustün AM, Bonjour S, Adair-Rohani H, et al. Contributions to cities' Ambient particulate matter (Pm): A systematic review of local source contributions at global level. *Atmospheric Environ*. (2015) 120:475–83. doi: 10.1016/j.atmosenv.2015.08.087

92. Zhang G, Ding C, Jiang X, Pan G, Wei X, Sun Y. Chemical compositions and sources contribution of atmospheric particles at a typical steel industrial urban site. *Sci Rep*. (2020) 10:7654. doi: 10.1038/s41598-020-64519-x

93. Fullerton DG, Bruce N, Gordon SB. Indoor air pollution from biomass fuel smoke is a major health concern in the developing world. *Trans R Soc Trop Med Hyg*. (2008) 102:843–51. doi: 10.1016/j.trstmh.2008.05.028

94. Diabetes Air Pollution Collaborators GBD. Estimates, trends, and drivers of the global burden of type 2 diabetes attributable to pm(2.5) air pollution, 1990-2019: an analysis of data from the global burden of disease study 2019. *Lancet Planet Health*. (2022) 6:e586–600. doi: 10.1016/S2542-5196(22)00122-X

95. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. (2011) 11:98–107. doi: 10.1038/nri2925

96. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol*. (2019) 70:809–24. doi: 10.26402/jpp.2019.6.01

97. Timper K, Bruning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech*. (2017) 10:679–89. doi: 10.1242/dmm.026609

98. Valdearcos M, Robblee MM, Benjamin DI, Nomura DK, Xu AW, Koliwad SK. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. *Cell Rep*. (2014) 9:2124–38. doi: 10.1016/j.celrep.2014.11.018

99. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking tnf-alpha function. *Nature*. (1997) 389:610–4. doi: 10.1038/39335

100. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest*. (2012) 122:153–62. doi: 10.1172/JCI59660

101. Pimentel GD, Ganeshan K, Carvalheira JB. Hypothalamic inflammation and the central nervous system control of energy homeostasis. *Mol Cell Endocrinol*. (2014) 397:15–22. doi: 10.1016/j.mce.2014.06.005

102. Burgos-Ramos E, Gonzalez-Rodriguez A, Canelles S, Baquedano E, Frago LM, Revuelta-Cervantes J, et al. Differential insulin receptor substrate-1 (Irs1)-related modulation of neuropeptide Y and proopiomelanocortin expression in nondiabetic and diabetic irs2-/- mice. *Endocrinology*. (2012) 153:1129–40. doi: 10.1210/en.2011-1278

103. Sun Q, Zhang G, Chen R, Li R, Wang H, Jiang A, et al. Central ikk2 inhibition ameliorates air pollution-mediated hepatic glucose and lipid metabolism dysfunction in mice with type ii diabetes. *Toxicol Sci*. (2018) 164:240–9. doi: 10.1093/toxsci/kfy079

104. Campolim CM, Weissmann L, Ferreira CKO, Zordao OP, Dornellas APS, de Castro G, et al. Short-term exposure to air pollution (Pm(2.5)) induces hypothalamic inflammation, and long-term leads to leptin resistance and obesity *via* tlr4/ikbke in mice. *Sci Rep*. (2020) 10:10160. doi: 10.1038/s41598-020-67040-3

105. Devlin RB, McKinnon KP, Noah T, Becker S, Koren HS. Ozone-induced release of cytokines and fibronectin by alveolar macrophages and airway epithelial cells. *Am J Physiol*. (1994) 266:L612–9. doi: 10.1152/ajplung.1994.266.6.L612

106. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity*. (2022) 55:31–55. doi: 10.1016/j.immuni.2021.12.013

107. Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. *Circulation*. (2009) 119:538–46. doi: 10.1161/CIRCULATIONAHA.108.799015

108. Zhang Y, Ji X, Ku T, Sang N. Inflammatory response and endothelial dysfunction in the hearts of mice co-exposed to so(2) , no(2) , and pm(2.5). *Environ Toxicol*. (2016) 31:1996–2005. doi: 10.1002/tox.22200

109. Ohkawa F, Ikeda U, Kanbe T, Kawasaki K, Shimada K. Effects of inflammatory cytokines on vascular tone. *Cardiovasc Res*. (1995) 30:711–5. doi: 10.1016/S0008-6363(95)00101-8

110. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol*. (2008) 103:398–406. doi: 10.1007/s00395-008-0733-0

111. Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, et al. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *JAMA*. (2005) 294:3003–10. doi: 10.1001/jama.294.23.3003

112. Chen T, Jia G, Wei Y, Li J. Beijing ambient particle exposure accelerates atherosclerosis in apoe knockout mice. *Toxicol Lett*. (2013) 223:146–53. doi: 10.1016/j.toxlet.2013.09.004

113. Du X, Jiang S, Zeng X, Zhang J, Pan K, Zhou J, et al. Air pollution is associated with the development of atherosclerosis *via* the cooperation of cd36 and nlrp3 inflammasome in apoe(-/-) mice. *Toxicol Lett*. (2018) 290:123–32. doi: 10.1016/j.toxlet.2018.03.022

114. Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. *Rev Endocr Metab Disord*. (2013) 14:5–12. doi: 10.1007/s11154-012-9229-1

115. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. *J Toxicol*. (2011) 2011:487074. doi: 10.1155/2011/487074

116. Finlayson-Pitts BJ, Pitts JN Jr. Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles. *Science*. (1997) 276:1045–52. doi: 10.1126/science.276.5315.1045

117. Ma JY, Ma JK. The dual effect of the particulate and organic components of diesel exhaust particles on the alteration of pulmonary immune/inflammatory responses and metabolic enzymes. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. (2002) 20:117–47. doi: 10.1081/GNC-120016202

118. Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, et al. Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (Ros) after exposure of human blood to ozone. *J Biol Regul Homeost Agents*. (1998) 12:67–75.

119. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. (2010) 107:1058–70. doi: 10.1161/CIRCRESAHA.110.223545

120. Nishikawa T, Kukidome D, Sonoda K, Fujisawa K, Matsuhisa T, Motoshima H, et al. Impact of mitochondrial ros production on diabetic vascular complications. *Diabetes Res Clin Pract*. (2007) 77 Suppl 1:S41–5. doi: 10.1016/j.diabres.2007.01.031

121. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. (2000) 404:787–90. doi: 10.1038/35008121

122. Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, et al. Myocardial cell death in human diabetes. *Circ Res*. (2000) 87:1123–32. doi: 10.1161/01.res.87.12.1123

123. Cave AC, Brewer AC, Narayanapanicker A, Ray R, Grieve DJ, Walker S, et al. Nadph oxidases in cardiovascular health and disease. *Antioxid Redox Signal*. (2006) 8:691–728. doi: 10.1089/ars.2006.8.691

124. Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: still a therapeutic target? *Nutrients*. (2019) 11(9):2090. doi: 10.3390/nu11092090

125. Miller MR, Shaw CA, Langrish JP. From particles to patients: oxidative stress and the cardiovascular effects of air pollution. *Future Cardiol*. (2012) 8:577–602. doi: 10.2217/fca.12.43

126. Sun Q, Yue P, Ying Z, Cardounel AJ, Brook RD, Devlin R, et al. Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of rho/rock. *Arterioscler Thromb Vasc Biol*. (2008) 28:1760–6. doi: 10.1161/ATVBAHA.108.166967

127. Gurgueira SA, Lawrence J, Coull B, Murthy GG, Gonzalez-Flecha B. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. *Environ Health Perspect*. (2002) 110:749–55. doi: 10.1289/ehp.02110749

128. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. (2001) 286:327–34. doi: 10.1001/jama.286.3.327

129. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based european prospective investigation into cancer and nutrition (Epic)-potsdam study. *Diabetes*. (2003) 52:812–7. doi: 10.2337/diabetes.52.3.812

130. Marques-Vidal P, Schmid R, Bochud M, Bastardot F, von Kanel R, Paccaud F, et al. Adipocytokines, hepatic and inflammatory biomarkers and incidence of type 2 diabetes. The colaus study. *PloS One*. (2012) 7:e51768. doi: 10.1371/journal.pone.0051768

131. Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, et al. The relation of markers of inflammation to the development of glucose disorders in the elderly: the cardiovascular health study. *Diabetes*. (2001) 50:2384–9. doi: 10.2337/diabetes.50.10.2384

132. Rosenbaum JL, Melhorn SJ, Schoen S, Webb MF, De Leon MRB, Humphreys M, et al. Evidence that hypothalamic gliosis is related to impaired glucose homeostasis in adults with obesity. *Diabetes Care*. (2022) 45:416–24. doi: 10.2337/dc21-1535

133. Berkseth KE, Rubinow KB, Melhorn SJ, Webb MF, Rosalynn BDLM, Marck BT, et al. Hypothalamic gliosis by mri and visceral fat mass negatively correlate with plasma testosterone concentrations in healthy men. *Obes (Silver Spring)*. (2018) 26:1898–904. doi: 10.1002/oby.22324

134. Sewaybricker LE, Schur EA, Melhorn SJ, Campos BM, Askren MK, Nogueira GAS, et al. Initial evidence for hypothalamic gliosis in children with obesity by quantitative T2 mri and implications for blood oxygen-level dependent response to glucose ingestion. *Pediatr Obes*. (2019) 14:e12486. doi: 10.1111/ijpo.12486

135. Kullmann S, Abbas Z, Machann J, Shah NJ, Scheffler K, Birkenfeld AL, et al. Investigating obesity-associated brain inflammation using quantitative water content mapping. *J Neuroendocrinol*. (2020) 32:e12907. doi: 10.1111/jne.12907

136. Wolf D, Ley K. Immunity and inflammation in atherosclerosis. *Circ Res*. (2019) 124:315–27. doi: 10.1161/CIRCRESAHA.118.313591

137. Geovanini GR, Libby P. Atherosclerosis and inflammation: overview and updates. *Clin Sci (Lond)*. (2018) 132:1243–52. doi: 10.1042/CS20180306

138. Xu Z, Wang W, Liu Q, Li Z, Lei L, Ren L, et al. Association between gaseous air pollutants and biomarkers of systemic inflammation: A systematic review and meta-analysis. *Environ pollut*. (2022) 292:118336. doi: 10.1016/j.envpol.2021.118336

139. Arias-Perez RD, Taborda NA, Gomez DM, Narvaez JF, Porras J, Hernandez JC. Inflammatory effects of particulate matter air pollution. *Environ Sci pollut Res Int*. (2020) 27:42390–404. doi: 10.1007/s11356-020-10574-w

140. Pryor JT, Cowley LO, Simonds SE. The physiological effects of air pollution: particulate matter, physiology and disease. *Front Public Health*. (2022) 10:882569. doi: 10.3389/fpubh.2022.882569

141. Hantrakool S, Kumfu S, Chattipakorn SC, Chattipakorn N. Effects of particulate matter on inflammation and thrombosis: past evidence for future prevention. *Int J Environ Res Public Health*. (2022) 19(14):8771. doi: 10.3390/ijerph19148771

142. Rajagopalan S, Al-Kindi SG, Brook RD. Air pollution and cardiovascular disease: jacc state-of-the-art review. *J Am Coll Cardiol*. (2018) 72:2054–70. doi: 10.1016/j.jacc.2018.07.099

143. Tang H, Cheng Z, Li N, Mao S, Ma R, He H, et al. The short- and long-term associations of particulate matter with inflammation and blood coagulation markers: A meta-analysis. *Environ pollut*. (2020) 267:115630. doi: 10.1016/j.envpol.2020.115630

144. Azzouz M, Xu Y, Barregard L, Fagerberg B, Zoller B, Molnar P, et al. Air pollution and biomarkers of cardiovascular disease and inflammation in the malmo diet and cancer cohort. *Environ Health*. (2022) 21:39. doi: 10.1186/s12940-022-00851-1

145. Abohashem S, Osborne MT, Dar T, Naddaf N, Abbasi T, Ghoneem A, et al. A leucopoietic-arterial axis underlying the link between ambient air pollution and cardiovascular disease in humans. *Eur Heart J*. (2021) 42:761–72. doi: 10.1093/eurheartj/ehaa982

146. Zhang L, Fang B, Wang H, Zeng H, Wang N, Wang M, et al. The role of systemic inflammation and oxidative stress in the association of particulate air pollution metal content and early cardiovascular damage: A panel study in healthy college students. *Environ pollut*. (2023) 323:121345. doi: 10.1016/j.envpol.2023.121345

147. Pasqua LA, Damasceno MV, Cruz R, Matsuda M, Martins MAG, Marquezini MV, et al. Exercising in the urban center: inflammatory and cardiovascular effects of prolonged exercise under air pollution. *Chemosphere*. (2020) 254:126817. doi: 10.1016/j.chemosphere.2020.126817

148. Thompson AM, Zanobetti A, Silverman F, Schwartz J, Coull B, Urch B, et al. Baseline repeated measures from controlled human exposure studies: associations between ambient air pollution exposure and the systemic inflammatory biomarkers il-6 and fibrinogen. *Environ Health Perspect*. (2010) 118:120–4. doi: 10.1289/ehp.0900550

149. Forchhammer L, Moller P, Riddervold IS, Bonlokke J, Massling A, Sigsgaard T, et al. Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function. *Part Fibre Toxicol*. (2012) 9:7. doi: 10.1186/1743-8977-9-7

150. Wittkopp S, Staimer N, Tjoa T, Stinchcombe T, Daher N, Schauer JJ, et al. Nrf2-related gene expression and exposure to traffic-related air pollution in elderly subjects with cardiovascular disease: an exploratory panel study. *J Expo Sci Environ Epidemiol*. (2016) 26:141–9. doi: 10.1038/jes.2014.84

151. Puett RC, Yanosky JD, Mittleman MA, Montresor-Lopez J, Bell RA, Crume TL, et al. Inflammation and acute traffic-related air pollution exposures among a cohort of youth with type 1 diabetes. *Environ Int*. (2019) 132:105064. doi: 10.1016/j.envint.2019.105064

152. Fernandez-Friera L, Fuster V, Lopez-Melgar B, Oliva B, Sanchez-Gonzalez J, Macias A, et al. Vascular inflammation in subclinical atherosclerosis detected by hybrid pet/mri. *J Am Coll Cardiol*. (2019) 73:1371–82. doi: 10.1016/j.jacc.2018.12.075

153. Miller MR, Raftis JB, Langrish JP, McLean SG, Samutrtai P, Connell SP, et al. Inhaled nanoparticles accumulate at sites of vascular disease. *ACS Nano*. (2017) 11:4542–52. doi: 10.1021/acsnano.6b08551

154. Halliwell B. Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? *Am J Clin Nutr*. (2000) 72:1082–7. doi: 10.1093/ajcn/72.5.1082

155. Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants (Basel)*. (2019) 8(3):72. doi: 10.3390/antiox8030072

156. Moller P, Loft S. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environ Health Perspect*. (2010) 118:1126–36. doi: 10.1289/ehp.0901725

157. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. (2008) 57:1349–54. doi: 10.2337/db08-0063

158. Ceriello A, Mercuri F, Quagliaro L, Assaloni R, Motz E, Tonutti L, et al. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. *Diabetologia*. (2001) 44:834–8. doi: 10.1007/s001250100529

159. Tannous M, Rabini RA, Vignini A, Moretti N, Fumelli P, Zielinski B, et al. Evidence for inos-dependent peroxynitrite production in diabetic platelets. *Diabetologia*. (1999) 42:539–44. doi: 10.1007/s001250051192

160. Hadi HA, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Risk Manag*. (2005) 1:183–98.

161. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*. (2000) 101:1899–906. doi: 10.1161/01.cir.101.16.1899

162. Clapp BR, Hingorani AD, Kharbanda RK, Mohamed-Ali V, Stephens JW, Vallance P, et al. Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc Res*. (2004) 64:172–8. doi: 10.1016/j.cardiores.2004.06.020

163. Dandona P, Aljada A, Chaudhuri A, Mohanty P. Endothelial dysfunction, inflammation and diabetes. *Rev Endocr Metab Disord*. (2004) 5:189–97. doi: 10.1023/B:REMD.0000032407.88070.0a

164. Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, et al. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. *Hypertension*. (2009) 54:659–67. doi: 10.1161/HYPERTENSIONAHA.109.130237

165. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res*. (2000) 87:840–4. doi: 10.1161/01.res.87.10.840

166. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. *J Intern Med*. (2002) 251:87–101. doi: 10.1046/j.1365-2796.2002.00932.x

167. Hahad O, Wild PS, Prochaska JH, Schulz A, Hermanns I, Lackner KJ, et al. Endothelial function assessed by digital volume plethysmography predicts the development and progression of type 2 diabetes mellitus. *J Am Heart Assoc*. (2019) 8:e012509. doi: 10.1161/JAHA.119.012509

168. Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, et al. Blood flow and muscle metabolism: A focus on insulin action. *Am J Physiol Endocrinol Metab*. (2003) 284:E241–58. doi: 10.1152/ajpendo.00408.2002

169. Hagberg CE, Mehlem A, Falkevall A, Muhl L, Fam BC, Ortsater H, et al. Targeting vegf-B as a novel treatment for insulin resistance and type 2 diabetes. *Nature*. (2012) 490:426–30. doi: 10.1038/nature11464

170. Giroix MH, Irminger JC, Lacraz G, Noll C, Calderari S, Ehses JA, et al. Hypercholesterolaemia, signs of islet microangiopathy and altered angiogenesis precede onset of type 2 diabetes in the goto-kakizaki (Gk) rat. *Diabetologia*. (2011) 54:2451–62. doi: 10.1007/s00125-011-2223-4

171. Bayo Jimenez MT, Hahad O, Kuntic M, Daiber A, Munzel T. Noise, air, and heavy metal pollution as risk factors for endothelial dysfunction. *Eur Cardiol*. (2023) 18:e09. doi: 10.15420/ecr.2022.41

172. Wittkopp S, Walzer D, Thorpe L, Roberts T, Xia Y, Gordon T, et al. Portable air cleaner use and biomarkers of inflammation: A systematic review and meta-analysis. *Am Heart J Plus: Cardiol Res Pract*. (2022) 18:100182. doi: 10.1016/j.ahjo.2022.100182

173. Wittkopp S, Anastasiou E, Hu J, Liu M, Langford AT, Brook RD, et al. Portable air cleaners and home systolic blood pressure in adults with hypertension living in new york city public housing. *J Am Heart Assoc*. (2023) 12:e029697. doi: 10.1161/JAHA.123.029697

174. Walzer D, Gordon T, Thorpe L, Thurston G, Xia Y, Zhong H, et al. Effects of home particulate air filtration on blood pressure: A systematic review. *Hypertension*. (2020) 76:44–50. doi: 10.1161/HYPERTENSIONAHA.119.14456

175. Rajagopalan S, Brauer M, Bhatnagar A, Bhatt DL, Brook JR, Huang W, et al. Personal-level protective actions against particulate matter air pollution exposure: A scientific statement from the american heart association. *Circulation*. (2020) 142:e411–e31. doi: 10.1161/CIR.0000000000000931

176. Villarreal-Calderon R, Torres-Jardon R, Palacios-Moreno J, Osnaya N, Perez-Guille B, Maronpot RR, et al. Urban air pollution targets the dorsal vagal complex and dark chocolate offers neuroprotection. *Int J Toxicol*. (2010) 29:604–15. doi: 10.1177/1091581810383587

177. Villarreal-Calderon R, Reed W, Palacios-Moreno J, Keefe S, Herritt L, Brooks D, et al. Urban air pollution produces up-regulation of myocardial inflammatory genes and dark chocolate provides cardioprotection. *Exp Toxicol Pathol*. (2012) 64:297–306. doi: 10.1016/j.etp.2010.09.002

178. Oliveira B, Falkenhain K, Little JP. Sugar-free dark chocolate consumption results in lower blood glucose in adults with diabetes. *Nutr Metab Insights*. (2022) 15:11786388221076962. doi: 10.1177/11786388221076962

179. Shah SR, Alweis R, Najim NI, Dharani AM, Jangda MA, Shahid M, et al. Use of dark chocolate for diabetic patients: A review of the literature and current evidence. *J Community Hosp Intern Med Perspect*. (2017) 7:218–21. doi: 10.1080/20009666.2017.1361293

180. Calderon-Garciduenas L, Mora-Tiscareno A, Franco-Lira M, Cross JV, Engle R, Aragon-Flores M, et al. Flavonol-rich dark cocoa significantly decreases plasma endothelin-1 and improves cognition in urban children. *Front Pharmacol*. (2013) 4:104. doi: 10.3389/fphar.2013.00104

181. Sesso HD, Manson JE, Aragaki AK, Rist PM, Johnson LG, Friedenberg G, et al. Effect of cocoa flavanol supplementation for the prevention of cardiovascular disease events: the cocoa supplement and multivitamin outcomes study (Cosmos) randomized clinical trial. *Am J Clin Nutr*. (2022) 115:1490–500. doi: 10.1093/ajcn/nqac055

182. Li H, Liu Q, Zou Z, Chen Q, Wang W, Baccarelli AA, et al. L-arginine supplementation to mitigate cardiovascular effects of walking outside in the context of traffic-related air pollution in participants with elevated blood pressure: A randomized, double-blind, placebo-controlled trial. *Environ Int*. (2021) 156:106631. doi: 10.1016/j.envint.2021.106631

183. Piatti PM, Monti LD, Valsecchi G, Magni F, Setola E, Marchesi F, et al. Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. *Diabetes Care*. (2001) 24:875–80. doi: 10.2337/diacare.24.5.875

184. Bo L, Jiang S, Xie Y, Kan H, Song W, Zhao J. Effect of vitamin E and omega-3 fatty acids on protecting ambient pm2.5-induced inflammatory response and oxidative stress in vascular endothelial cells. *PloS One*. (2016) 11:e0152216. doi: 10.1371/journal.pone.0152216

185. Possamai FP, Junior SA, Parisotto EB, Moratelli AM, Inacio DB, Garlet TR, et al. Antioxidant intervention compensates oxidative stress in blood of subjects exposed to emissions from a coal electric-power plant in south Brazil. *Environ Toxicol Pharmacol*. (2010) 30:175–80. doi: 10.1016/j.etap.2010.05.006

186. Wilhelm Filho D, Avila S Jr., Possamai FP, Parisotto EB, Moratelli AM, Garlet TR, et al. Antioxidant therapy attenuates oxidative stress in the blood of subjects exposed to occupational airborne contamination from coal mining extraction and incineration of hospital residues. *Ecotoxicology*. (2010) 19:1193–200. doi: 10.1007/s10646-010-0503-2

187. Huang K, Yu D, Fang H, Ju L, Piao W, Guo Q, et al. Association of fine particulate matter and its constituents with hypertension: the modifying effect of dietary patterns. *Environ Health*. (2023) 22:55. doi: 10.1186/s12940-023-01000-y

188. Janssen NAH, Schwartz J, Zanobetti A, Suh HH. Air conditioning and source-specific particles as modifiers of the effect of pm(10) on hospital admissions for heart and lung disease. *Environ Health Perspect*. (2002) 110:43–9. doi: 10.1289/ehp.0211043

189. Bell ML, Ebisu K, Peng RD, Dominici F. Adverse health effects of particulate air pollution: modification by air conditioning. *Epidemiology*. (2009) 20:682–6. doi: 10.1097/EDE.0b013e3181aba749

190. Franklin M, Zeka A, Schwartz J. Association between pm2.5 and all-cause and specific-cause mortality in 27 us communities. *J Exposure Sci Environ Epidemiol*. (2007) 17:279–87. doi: 10.1038/sj.jes.7500530

191. Lin L-Y, Chuang H-C, Liu IJ, Chen H-W, Chuang K-J. Reducing indoor air pollution by air conditioning is associated with improvements in cardiovascular health among the general population. *Sci Total Environ*. (2013) 463-464:176–81. doi: 10.1016/j.scitotenv.2013.05.093

192. Pui DYH, Qi C, Stanley N, Oberdörster G, Maynard A. Recirculating air filtration significantly reduces exposure to airborne nanoparticles. *Environ Health Perspect*. (2008) 116:863–6. doi: 10.1289/ehp.11169

193. Muala A, Sehlstedt M, Bion A, Osterlund C, Bosson JA, Behndig AF, et al. Assessment of the capacity of vehicle cabin air inlet filters to reduce diesel exhaust-induced symptoms in human volunteers. *Environ Health Global Access Sci Source*. (2014) 13:16. doi: 10.1186/1476-069X-13-16

194. Mallach G, Shutt R, Thomson EM, Valcin F, Kulka R, Weichenthal S. Randomized cross-over study of in-vehicle cabin air filtration, air pollution exposure, and acute changes to heart rate variability, saliva cortisol, and cognitive function. *Environ Sci Technol*. (2023) 57:3238–47. doi: 10.1021/acs.est.2c06556

195. Repace JL, Lowrey AH. Indoor air pollution, tobacco smoke, and public health. *Science*. (1980) 208:464–72. doi: 10.1126/science.7367873

196. Invernizzi G, Ruprecht A, Mazza R, Rossetti E, Sasco A, Nardini S. Boffi R. Particulate matter from tobacco versus diesel car exhaust: an educational perspective. *Tob Control*. (2004) 13:219. doi: 10.1136/tc.2003.005975

197. Northrup TF, Jacob P3rd, Benowitz NL, Hoh E, Quintana PJE, Hovell MF, et al. Thirdhand smoke: state of the science and a call for policy expansion. *Public Health Rep (Washington DC 1974)*. (2016) 131:233–8. doi: 10.1177/003335491613100206

198. Li L, Lin Y, Xia T, Zhu Y. Effects of electronic cigarettes on indoor air quality and health. *Annu Rev Public Health*. (2020) 41:363–80. doi: 10.1146/annurev-publhealth-040119-094043

199. Air Quality Index (Aqi) Basics*AirNowgov*.

200. Amann M, Kiesewetter G, Schöpp W, Klimont Z, Winiwarter W, Cofala J, et al. Reducing global air pollution: the scope for further policy interventions. *Philos Trans R Soc A: Mathematical Phys Eng Sci*. (2020) 378:20190331. doi: 10.1098/rsta.2019.0331

201. Barry E, Roberts S, Oke J, Vijayaraghavan S, Normansell R, Greenhalgh T. Efficacy and effectiveness of screen and treat policies in prevention of type 2 diabetes: systematic review and meta-analysis of screening tests and interventions. *BMJ*. (2017) 356:i6538. doi: 10.1136/bmj.i6538

202. den Braver NR, Lakerveld J, Rutters F, Schoonmade LJ, Brug J, Beulens JWJ. Built environmental characteristics and diabetes: A systematic review and meta-analysis. *BMC Med*. (2018) 16:12. doi: 10.1186/s12916-017-0997-z

203. Mozaffarian D. Dietary and policy priorities for cardiovascular disease, diabetes, and obesity. *Circulation*. (2016) 133:187–225. doi: 10.1161/CIRCULATIONAHA.115.018585

204. Amuda AT, Berkowitz SA. Diabetes and the built environment: evidence and policies. *Curr Diabetes Rep*. (2019) 19:35. doi: 10.1007/s11892-019-1162-1

205. Newman JD, Bhatt DL, Rajagopalan S, Balmes JR, Brauer M, Breysse PN, et al. Cardiopulmonary impact of particulate air pollution in high-risk populations: jacc state-of-the-art review. *J Am Coll Cardiol*. (2020) 76:2878–94. doi: 10.1016/j.jacc.2020.10.020

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Glossary

AQI	air quality index
BC	black carbon
BP	blood pressure
C3c	complement component 3
CVD	cardiovascular disease
DM	diabetes mellitus type 2
FBG	fasting blood glucose
FMD	flow-mediated dilation
GDM	gestational diabetes mellitus
HOMA-IR	homeostasis model assessment of insulin resistance
HRV	heart rate variability
IL-1β	interleukin-1 beta
IL-6	interleukin-6
IL-8	interleukin-8
LMIC	lower- and middle-income countries
LOS	length of stay
Lp-PLA2	lipoprotein-associated phospholipase A2
MACE	major adverse cardiovascular events
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLR	neutrophil-lymphocyte ratio
OM	organic matter
PAC	portable air cleaners
PM	particulate matter
$PM_{2.5}$	fine particulate matter
PM_{10}	coarse particulate matter
$PM_{2.5-10}$	PM between 2.5 and 10 μm in aerodynamic diameter
PN_{AM}	PM between 0.1-1 μm in aerodynamic diameter
ROS	reactive oxygen species
sCD36	soluble CD36
SES	socioeconomic status
TLR4	toll-like receptor 4
WBC	white blood cell count
WHO	World Health Organization

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Sex and age significantly modulate cardiovascular disease presentation in type 2 diabetes: a large population-based cohort study

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Aims: We aimed to describe and compare the incidence of the first cardiovascular event and its major subtypes, coronary heart disease (CHD), cerebrovascular disease, heart failure (HF), or peripheral artery disease (PAD), according to age and sex in a population-based cohort of individuals with type 2 diabetes (T2D) from a Mediterranean region.

Material and methods: We used linked primary care electronic medical reports, pharmacy-invoicing data, and hospital admission disease registry records from the SIDIAP database, which contains linked data for 74% of the Catalonian population. We selected individuals with T2D aged 30 to 89 years free of cardiovascular disease (CVD). The primary outcome was the first presentation of CVD.

Results: The study cohort included 247,751 individuals (48.6% women, 66.8 ± 11.9 years). During a 6.99-year follow-up, the cumulative incidence of the first cardiovascular event was 23.4%. Men were at higher risk for CVD (hazard ratio [HR]: 1.47 95%CI: 1.45-1.50), CHD (HR: 1.52 95%CI: 1.47-1.57), cerebrovascular disease (HR:1.07 95%CI: 1.03-1.10) and PAD (HR: 2.30 95%CI: 2.21-2.39) than women but at a lower risk for HF (HR:0.70 95%CI: 0.68-0.73). CHD and PAD were the most frequent CVD presentations among men (28.1% and 27.5%) and HF (40.1%) in women. CHD predominated among young participants of both sexes, while HF predominated among women older than 65 and men older than 75.

Conclusions: In individuals with T2D, the overall risk and the type of first CVD manifestation largely varied by sex and age. This epidemiological evidence should be considered in clinical practice.

KEYWORDS

first cardiovascular event, primary prevention, heart failure, peripheral artery disease, sex difference, age-related, cumulative incidence

1 Introduction

Patients with type 2 diabetes (T2D) are at a higher risk for a wide range of cardiovascular diseases (1). Although a substantial reduction in incidence rates of cardiovascular complications has been observed over the last two decades, cardiovascular disease (CVD) risk in T2D remains higher than in age and sex-matched controls. Furthermore, despite steadily decreasing rates for atherosclerotic diseases, the decline in heart failure (HF) incidence has plateaued in recent years (2).

Given the varied impact of modifiable cardiovascular risk factors on different manifestations of CVD, acquiring a comprehensive understanding of the epidemiology surrounding initial cardiovascular events in individuals with T2D could foster the creation of more personalized primary prevention approaches (2). This might be especially relevant among young adults, as evidenced by recent data from the Global Burden of Disease (GBD) study, which highlights an increasing trend in cardiovascular morbidity and mortality in this age cohort over the last three decades (3).

In this context, there is a growing acknowledgment that CVD pathophysiology and presentation exhibit variations based on sex and age (4). Of note, within the general population, CVD predominantly presents as coronary heart disease (CHD) in men, whereas women are more prone to experiencing cerebrovascular disease or HF as their primary event, with these manifestations typically occurring more frequently at older ages (5). Whether such age and sex differences in the first CVD event are also present in individuals with T2D has seldom been explored.

Several studies and meta-analyses have demonstrated a stronger association between T2D and HF, CHD, and cerebrovascular disease in women than in men (6–11). Fewer studies have explored sex differences in absolute risk for the various forms of CVD manifestation, and none focused on first events (2, 6, 12). In addition, whether age modulates sex-specific risks for the different CVD subtypes in men and women with T2D is poorly defined as most previous studies applied broad age groups or did not consider the various CVD components separately (2, 6, 12).

In this work, we aimed to describe and compare the incidence rates of the first cardiovascular event and its major subtypes (CHD, PAD, cerebrovascular disease, and HF) according to sex and age in a large cohort of individuals with T2D from a primary care setting.

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; EA, early adulthood; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; MA, middle adulthood; MVO, middle to very old; PAD, peripheral artery disease; UAE, urinary albumin excretion; Y, young; YO, young, old.

2 Materials and methods

2.1 Study design and settings

We performed a longitudinal, retrospective cohort study. Data for this study was obtained from the Sistema d'Informació per al Desenvolupament de l'Investigació en Atenció Primària (SIDIAP) database (www.sidiap.org). This database, created in 2010 for research purposes, contains pseudo-anonymous, routinely collected healthcare information of over 5,000,000 patients registered by 3,414 general practitioners at 274 different primary care practices in Catalonia (a northeast area of Spain). The population in the SIDIAP database corresponds to more than 74% of the Catalonian population, providing a representative sample of the primary care population.

The SIDIAP database contains information on clinical diagnoses, anthropometric measures, and laboratory tests. In addition, the CMBD database (data from hospitalisation discharge statistics and specialised out-patient care from the hospitals of the National Health System) and pharmacological treatments (data from the pharmacy-invoicing system provided by the CatSalut) are automatically collected and linked to the SIDIAP database. The Ethics Committee of the Primary Healthcare University Research Institute (IDIAP), Jordi Gol (Barcelona, Spain), approved the study (code P17/087).

2.2 Study population

For this analysis, we selected all individuals with a registered diagnosis of T2D (n=333,036) on January 1st, 2010 (study index date). T2D diagnosis was based on the presence of the diagnostic codes (International Statistical Classification of Diseases and Related Health Problems 10th Revision-ICD-10): E11, E13, and

E14. Subjects with other types of diabetes, such as type 1, secondary, or gestational diabetes (ICD-10: E10, E12, O24), were excluded from the analysis.

Individuals with previous cardiovascular events (n=68,982, 20.7%) or known atrial fibrillation (n=22,673, 6.8%) were excluded, as well as those aged under 27 or above 89 years (n=8,310, 2.4%) for whom cardiovascular risk evaluation was not systematically recommended or CVD prevention of a first event was not a clinical priority. The remaining participants (n=247,751) were followed until the cardiovascular event, death, or the end of the study (December 2016). Study flow charts are included in the Supplementary material (Supplementary Figure 1).

The study population was divided into five different age groups according to baseline age and sex (young (Y): <35 years; early adulthood (EA): 35-55 for men and 35-60 for women; middle adulthood (MA): 55-65 for men and 60-65 for women; young old (YO): 65-75 years; middle-to-very old (MVO): >75 years). This age grouping was selected considering the age range at which systematic cardiovascular risk evaluation is recommended (Framingham-REGICOR strategy 35-75 years) in our Health Care System, and also according to the age limit to define premature events according to sex, i.e., <55 years for men and <60 years for women (13).

2.3 Study variables

At the study index date, the following data were collected from the SIDIAP database: age, sex, socioeconomic condition, the presence of diagnoses and comorbidities, including microvascular complications (retinopathy, nephropathy, polyneuropathy), and hypertension (based on registers of ICD-9/ICD-10 diagnostic codes recorded in the database) use of concomitant medications (based on the Anatomical Therapeutic Chemical-ATC classification system), blood pressure, anthropometric (body weight, height, and body mass index) and laboratory data [lipids, creatinine, urinary albumin excretion (UAE)].

Specific diagnosis codes for retinopathy, nephropathy, polyneuropathy, and hypertension are displayed in Supplementary Table 1.

Any microvascular complication was defined as the presence of a diagnostic code of retinopathy or/and nephropathy or/and polyneuropathy. Obesity was described as a body mass index ≥30 Kg/m2. The socioeconomic condition was evaluated by the MEDEA index. The MEDEA index is a socioeconomic deprivation index based on indicators recorded by census tract and validated in the Spanish population. Higher index values correspond to greater deprivation (14). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

2.4 Study outcomes

Cardiovascular events involving four major conditions occurring after the index date and before the end of the study period were recorded and reported as CHD, cerebrovascular disease, PAD, and HF. CHD was defined as fatal or non-fatal myocardial infarction, angina or unstable angina, undetermined ischemic heart disease, and coronary revascularisation (coronary artery bypass grafting, percutaneous coronary intervention). Cerebrovascular disease was defined as fatal or non-fatal ischemic or hemorrhagic stroke, transient ischemic attack, and intracerebral revascularisation. PAD was defined as intermittent claudication, extracerebral artery stenosis, and carotid or peripheral revascularisation (endovascular, stenting, or surgical bypass). HF included congestive/acute HF and other HF diagnoses (systolic and diastolic, chronic, or undetermined). Diagnoses were based on ICD-9/ICD-10 codes. Specific diagnosis codes for CVD can be found in the online Supplementary information (Supplementary Table 2).

The overall mortality during the study period was also retrieved from the SIDIAP database. However, specific causes of death were not available in this study.

2.5 Statistical methods

The baseline characteristics were described as frequencies and percentages for categorical variables, while for continuous variables, the mean and standard deviation (SD) or median and quartiles were calculated. In comparing groups (sex), the p-value was calculated using the Fisher exact test for qualitative variables and the independent samples t-test for quantitative variables. Fisher exact test and ANOVA analysis were applied when comparing age categories.

Cumulative incidence and incidence rates of cardiovascular manifestations were computed using the exact method. For each event of interest, the overall incidence rate and age-sex-specific incidence rate were calculated as the number of incident events divided by the persons-years (PY) during the follow-up and were expressed as per 100 PY. Hazard ratios (HR) and 95% confidence intervals (95% CI) were computed for each baseline characteristic. The *compare Groups* R Package (Version 4.6.0) 10 was used to perform group descriptions for several variables and to estimate each HR. Cox proportional hazard models were used to evaluate time to death according to the type of first-ever cardiovascular disease presentation. Hazard ratios (HR) in these models were adjusted by age and sex.

All statistical analyses were performed using the free R statistical software version 3.6.1 (https://www.r-project.org/).

3 Results

The study cohort included 247,751 individuals (48.6% women) with T2D. Table 1 displays the clinical characteristics at baseline for the whole cohort and stratified by age.

The mean age was 66.8 ± 11.9 years, HbA1c was 6.8 ± 1.5%, and body mass index (BMI) was 30.2 ± 5.1 Kg/m^2. The most common treatment modalities were oral hypoglycemic agents in monotherapy or combined. Metformin (59.3%) and sulfonylureas (29.5%) were the most frequently prescribed hypoglycemic medications. In contrast, a mere 0.3% of participants utilized

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TABLE 1 Clinical characteristics at baseline for the whole cohort and stratified by age.

	% with Available data	Whole cohort (n=247,751)	Young (n=1,204)	Early adulthood (n=51,295)	Middle Adulthood (n=52,636)	Young old (n=72,655)	Middle to very old (n=69,961)	p_{trend}
Females (n, (%))	100.0	120,405 (48.6)	540 (44.9)	25,399 (49.5)	15,907 (30.2)	36,113 (49.7)	42,446 (60.7)	<0.01
Age (years)	100.0	66.8 ± 11.9	32.9 ± 1.38	50.4 ± 5.75	61.0 ± 2.71	69.9 ± 2.95	80.7 ± 3.91	<0.01
Deprivation index (Q5) (n, (%))	70.0	29,521 (17.0)	88 (10.2)	5,080 (13.6)	65,45 (17.0)	8,624 (16.4)	9,184 (20.8)	<0.01
Smoking (n, (%)								
No	86.5	137,190 (64.0)	483 (50.9)	21,723 (49.5)	23,778 (49.5)	43,809 (68.4)	47,397 (79.8)	<0.01
Former		39,370 (18.4)	359 (37.8)	15,025 (34.2)	11,932 (25.5)	8,362 (13.0)	3,692 (6.2)	
Current		37,622 (17.6)	107 (11.3)	7,157 (16.3)	10,223 (23.3)	11,847 (17.5)	8,288 (14.0)	
T2D duration (years)	100.0	6.3 ± 5.2	3.6 ± 3.3	4.7 ± 4.0	5.6 ± 4.5	6.7 ± 5.2	7.6 ± 6.1	<0.01
T2D therapy (n, (%))								
No treatment	100.0	66,806 (27.0)	527 (43.8)	15,139 (29.5)	13,827 (26.3)	18,204 (25.1)	19,109 (27.3)	<0.01
OHA monotherapy		84,419 (34.1)	359 (29.8)	18,059 (35.2)	18,496 (35.1)	24,790 (34.1)	22,715 (32.5)	
OHA combined		57,469 (23.2)	139 (11.5)	10,565 (20.6)	12,883 (24.5)	18,106 (24.9)	15,776 (22.5)	
OHA plus insulin		2,6015 (10.5)	100 (8.3)	5,113 (10.0)	5,180 (9.8)	8,212 (11.3)	7,410 (10.6)	
Insulin without OHA		13,042 (5.3)	79 (6.6)	2,419 (4.7)	2,250 (4.3)	3,343 (4.6)	4,951 (7.1)	
Use of non-insulin hypoglycemic medications (n, (%))								
Metformin	100.0	146,793 (59.3)	565 (46.9)	31,380 (61.2)	33,292 (63.2)	44,842 (61.7)	36,714 (52.5)	<0.01
Sulfonylureas		72,955 (29.4)	132 (11.0)	11,677 (22.8)	14,910 (28.3)	22,862 (31.5)	23,374 (33.4)	<0.01
DPP-4 inhibitors		13,080 (5.3)	48 (4.0)	2,950 (5.8%)	3,207 (6.1%)	4,114 (5.7)	2,761 (3.9)	<0.01
SGLT2-inhibitors		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	<0.01
GLP1-analogs		722 (0.3)	9 (0.7)	353 (0.7)	198 (0.4)	142 (0.2)	20 (0.0)	<0.01
Pioglitazone		8,954 (3.6)	33 (2.7)	1,927 (3.8)	2,115 (4.0)	2,930 (4.0)	1,949 (2.8)	<0.01
Glinides		4,406 (1.8)	3 (0.2)	377 (0.7)	677 (1.3)	1,426 (2.0)	1.923 (2.7)	<0.01
FPG (mg/dl)	70.1	146 ± 46.6	149 ± 64.6	155 ± 55.5	151 ± 47.8	144 ± 42.2	137 ± 41.6	<0.01
HbA1c (%)	65.6	6.8 ± 1.5	7.1 ± 2.1	7.1 ± 1.8	6.9 ± 1.6	6.7 ± 1.4	6.7 ± 1.3	
BMI (Kg/m^2)	54.9	30.2 ± 5.1	31.9 ± 7.0	31.6 ± 5.9	30.5 ± 4.9	30.2 ± 4.8	29.1 ± 4.6	<0.01
BMI≥30 Kg/m^2 (n, (%))	54.9	61,632 (46.3)	280 (57.0)	14,137 (56.4)	13,524 (48.3)	19,745 (45.9)	13,910 (38.1)	<0.01
Hypertension (n (%))	100.0	156,967 (63.4)	177 (14.7)	22,108 (43.1)	30,635 (58.2)	50,770 (69.9)	53,277 (76.2)	<0.01
SBP (mmHg)	75.5	136 ± 15.6	127 ± 14.1	132 ± 15.2	136 ± 15.2	137 ± 15.4)	138 ± 16.1	<0.01
DBP (mmHg)	75.5	76.8 ± 9.4	78.6 ± 10.6	80.4 ± 9.4	78.9 ± 9.0	76.3 ± 8.9	73.6 ± 9.2	<0.01
Hypotensive treatment (n (%))	100.0	162,424 (65.6)	181 (15.0)	23,118 (45.1)	32,147 (61.1)	52,729 (72.6)	54,249 (77.5)	<0.01
Total cholesterol (mg/dl)	68.7	197 ± 38.8	196 ± 50.1	206 ± 42.4	199 ± 39.2	195 ± 36.8	193 ± 37.0	<0.01
HDL-cholesterol (mg/dl)	60.1	50.2 ± 13.0	43.9 ± 12.8	47.5 ± 12.6	48.8 ± 12.6	51.0 ± 12.7	52.3 ± 13.5	<0.01
Triglycerides (mg/dl)	64.8	133.0 [97.0-186]	157.0 [103-235]	152.0 [107-224]	139.0 [100-197]	129.0 [96.0-177]	122.0 [92.0-165]	<0.01

(Continued)

TABLE 1 Continued

	% with Available data	Whole cohort (n=247,751)	Young (n=1,204)	Early adulthood (n=51,295)	Middle Adulthood (n=52,636)	Young old (n=72,655)	Middle to very old (n=69,961)	p_{trend}
LDL-cholesterol (mg/dl)	60.1	118 ± 32.8	117 ± 32.9	124 ± 34.7	120 ± 33.4	116 ± 31.7	114 ± 31.4	<0.01
Lipid-lowering treatment (n,(%))	100.0	123,901 (50.0)	194 (16.1)	21,117 (41.2)	27,402 (52.1)	41,141 (56.6)	34,047 (48.7)	<0.01
Statin use (n,(%))	100.0	112,905 (45.6)	134 (11.1)	17,825 (34.8)	24,557 (46.7)	38,361 (52.8)	32,028 (45.8)	<0.01
Fibrates use (n,(%))	100.0	15,647 (6.3)	76 (6.3)	4,563 (8.9)	4,212 (8.0)	4,133 (5.7)	2,663 (3.8)	<0.01
eGFR categories (n, (%))								
>60 ml/min	67.2	136,906 (82.2)	575 (99.5)	31,007 (97.5)	32,796 (93.7)	43,239 (83.9)	29,289 (61.6)	<0.01
30-60 ml/min		27,662 (16.6)	2 (0.3)	728 (2.3)	2,070 (5.9)	7,904 (15.3)	16,958 (35.7)	
<30 ml/min		1,874 (1.1)	1 (0.2)	61 (0.2)	121 (0.3)	393 (0.8)	1,298 (2.7)	
Diabetic retinopathy (n (%))	100.0	11,041 (4.5)	9 (0.7)	1,488 (2.9)	2,361 (4.5)	3,623 (5.0)	3,560 (5.1)	<0.01
Diabetic nephropathy (n (%))	100.0	15,645 (6.3)	24 (2.0)	1,684 (3.3)	2,310 (4.4)	4,402 (6.1)	7225 (10.3)	<0.01
Diabetic neuropathy (n (%))	100.0	4,278 (1.7)	2 (0.2)	594 (1.2)	858 (1.6)	1,358 (1.9)	1,466 (2.1)	<0.01
Any microvascular complication (n (%))	100.0	27,846 (11.2)	31 (2.6)	3,392 (6.6)	4,928 (9.4)	8,373 (11.5)	11,122 (15.9)	<0.01

Data are expressed as mean ± standard deviation, median [25th percentile-75th percentile], or number of participants (n) (%).
OHA, oral hypoglycemic agents; FPG, fasting plasma glucose; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate.

glucagon-like peptide 1 analogs, and none were prescribed sodium glucose transporter inhibitors (SGLT2-i). The prevalence of hypertension, statin use, current smoking, and microvascular complications was 63.4%, 45.6%, 17.6%, and 11.2%, respectively.

Compared to the older age groups, the younger groups had a higher proportion of males, a shorter diabetes duration, were less often treated with insulin, and had a lower prevalence of hypertension and microvascular complications. In contrast, they showed worse glycemic control and lipid profile, had a higher prevalence of obesity, and were less frequently treated with lipid-lowering therapies. The prevalence of active smokers was the lowest among the young, increasing until middle adulthood and decreasing after that (Table 1).

3.1 Incidence of first cardiovascular event stratified by age and sex

The cumulative incidence of overall CVD and its major components in men and women according to age category is displayed in Figure 1.

During 1,435,568 person-years, first-time cardiovascular events occurred in 57,152 individuals, corresponding to a cumulative incidence of 21.2% in women and 24.8% in men and a crude incidence rate of 3.61% per year in women and 4.34% in men (p<0.01).

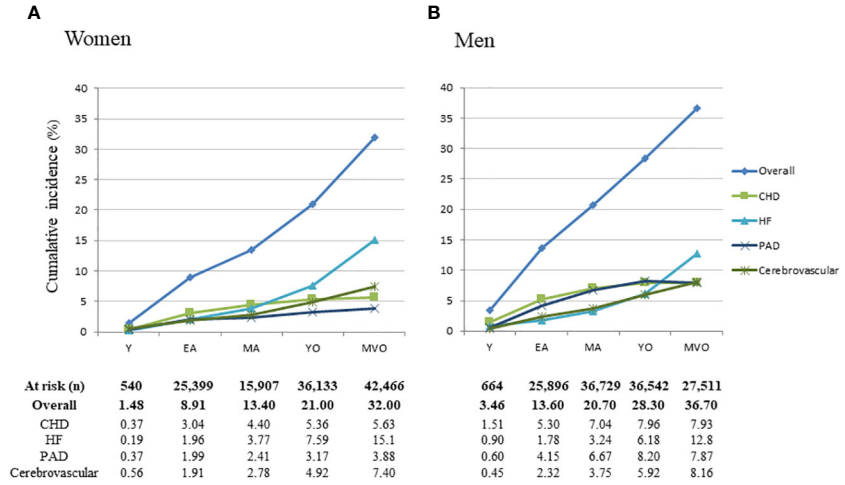
The incidence of cardiovascular events increased across age categories in both men and women (p_{trend}<0.01), with a maximum incidence among middle to very-old adults (HR: 4.03, 95%CI: 3.91-4.16, with young as the reference). Men were at a higher absolute risk than women. The excess risk for men was the highest in young adults (HR: 2.38 (95%CI: 1.06 to 5.32) and the lowest among middle to very-old adults (HR: 1.25; 95%CI: 1.22 to 1.28).

When different CVD subtypes were considered individually, men were at higher risk than women for CHD, PAD, and cerebrovascular disease (p<0.01). In contrast, women showed a higher risk than men for HF (p<0.01) (Figures 1, 2).

3.2 The first cardiovascular event reported

Figure 3; Supplementary Figure 2 display the relative contribution of cardiovascular disease main categories and specific subtypes to the first CVD event in men and women for the entire cohort and stratified by age.

In men, the most frequent CVD presentation was CHD (28.7%), followed by PAD (27.5%), while in women, HF clearly predominated (40.1%).



At risk (n)	540	25,399	15,907	36,133	42,466	664	25,896	36,729	36,542	27,511
Overall	1.48	8.91	13.40	21.00	32.00	3.46	13.60	20.70	28.30	36.70
CHD	0.37	3.04	4.40	5.36	5.63	1.51	5.30	7.04	7.96	7.93
HF	0.19	1.96	3.77	7.59	15.1	0.90	1.78	3.24	6.18	12.8
PAD	0.37	1.99	2.41	3.17	3.88	0.60	4.15	6.67	8.20	7.87
Cerebrovascular	0.56	1.91	2.78	4.92	7.40	0.45	2.32	3.75	5.92	8.16

FIGURE 1
Cumulative incidence rates for first cardiovascular events in women **(A)** and men **(B)** by age categories. CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure; PAD, peripheral artery disease; Y, young; EA, early adulthood; MA, middle-adulthood; YO, young old; MVO, middle to very old.

Furthermore, the first CVD event differed according to age in both men and women. Although more frequent among men, CHD was the most frequent first CVD manifestation in both men and women during early and middle adulthood, decreasing after that and becoming less common in older groups. Despite this reducing incidence with age, CHD remained the most frequent presentation of CVD among men below 75 years and women below 65 years. Similar trends were observed for PAD, which was much more common among men than women and more frequent among younger than older groups.

The opposite was observed for HF, which, although more common in women than men at any age, was relatively infrequent in younger age categories but markedly increased in the older groups. In women, the contribution of HF to overall CVD doubled between EA and MVO (22.0% to 47.1%), while in men, it tripled (13.3% to 34.8%), representing the most common presentation of CVD in the YO and MVO groups among women (i.e., >65 years), and the MVO group among men (i.e., >75 years). The proportion of cerebrovascular disease as the first cardiovascular event varied less with age and between sexes (18.7% to 22.7%) (Figure 3).

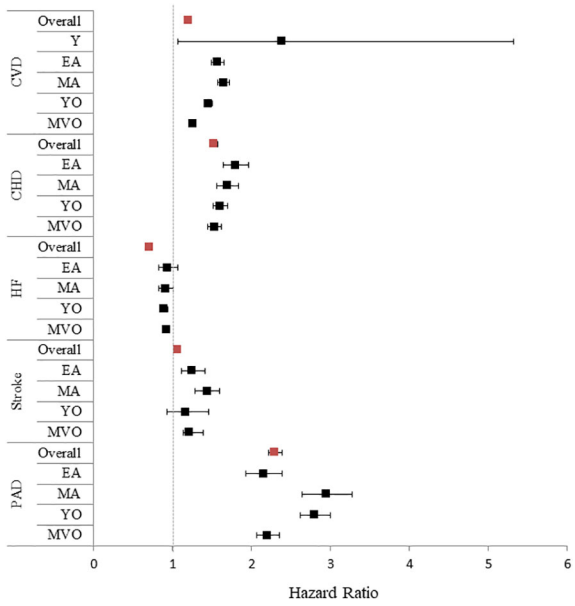


FIGURE 2
Forrest plot for a first cardiovascular event stratified by age in women vs. men Women were considered as the reference. Given the low number of events among the young age category, the hazard ratios for cardiovascular disease subtypes were not included in the figure. Red squares: whole cohort; black squares: pre-defined age subgroups. Y, young; EA, early adulthood; MA, middle-adulthood; YO, young old; MVO, middle to very old. CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure; PAD, peripheral artery disease.

3.3 Factors related to cardiovascular events stratified by age and sex

The baseline characteristics of individuals with or without cardiovascular events during the follow-up are shown in Supplementary Table 3.

In sex-stratified analyses adjusted by age, most CVD risk factors were significantly and similarly associated with CVD in both men and women. However, compared to men, women had a higher negative impact of deprivation [HR: 1.24 (95%CI: 1.17-1.30) vs. HR: 1.12 (95% CI: 1.07-1.17)], obesity [(HR: 1.31 (95%CI: 1.26-1.35) vs. HR: 1.13 (95%CI: 1.10-1.17)], and hypertension (HR: 1.43 (95%CI: 1.38-1.47) vs. HR: 1.27 (95%CI: 1.24-1.30)] (Supplementary Figure 3), Factors more strongly associated with CVD in these analyses were those related to T2D duration, control, and complications.

In age-stratified analyses, we observed a higher relative risk associated with classical cardiovascular risk factors, metabolic

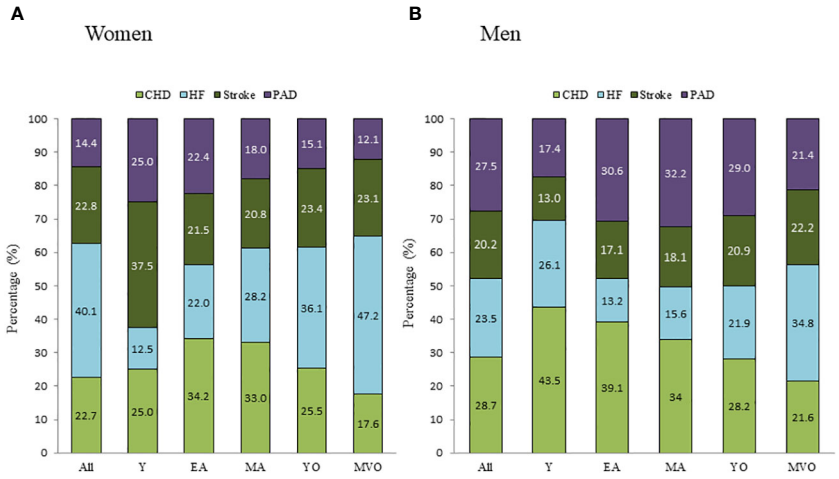


FIGURE 3
Distribution of the first-ever cardiovascular event subtype stratified by sex in the different age categories. **(A)**: women; **(B)**: men. Y, young; EA, early adulthood; MA, middle-adulthood; YO, young old; MVO, middle to very old; CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure; PAD, peripheral artery disease.

control, and end-organ damage in younger vs. older individuals (Supplementary Figure 4).

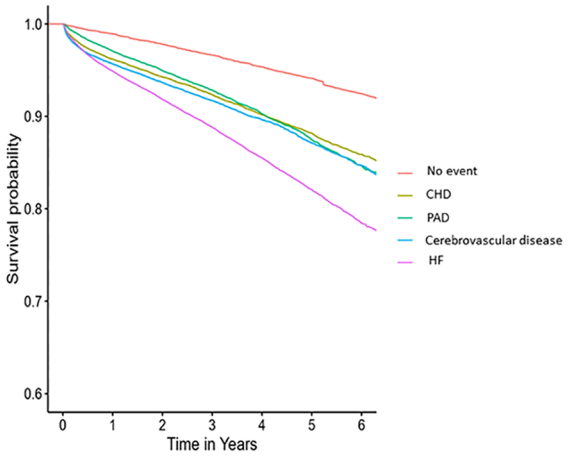
3.4 Mortality during follow-up

During the follow-up, 20,811 men (16.3%) and 18,866 women (15.7%) died. The mean age at the time of death was 73.7 ± 10.2 years in men and 78.4 ± 8.5 years in women. Before the death date, 52.7% of deceased men and 47.9% of dead women had had a previous first cardiovascular event. The specific cause of death was not available.

In Cox regression analysis, experiencing a cardiovascular event, regardless of type, was found to be associated with elevated all-cause mortality. However, the initial occurrence of heart failure (HF) was linked to a greater risk, independently of age and sex, compared to coronary heart disease (CHD), peripheral artery disease (PAD), or cerebrovascular disease (Figure 4).

4 Discussion

In this longitudinal retrospective analysis of a large cohort of individuals with T2D diabetes in primary prevention from a primary care setting in a western Mediterranean region, we found that HF and PAD substantially contribute to CVD. We also observed a considerable influence of sex and age on overall cardiovascular risk and the form of the initial clinical presentation of CVD. Within our cohort, we observed that men exhibited a higher susceptibility to overall CVD, CHD, cerebrovascular disease, and PAD compared to women. However, men demonstrated a lower risk for HF across all age groups. CHD



	HR	95%CI
None (reference)	1	----
CHD	2.15	2.07-2.23
Cerebrovascular disease	2.41	2.32-2.50
PAD	2.10	2.01-2.18
HF	3.30	3.20-3.39

FIGURE 4
Survival plot for all-cause mortality according to first cardiovascular event form of manifestation and the corresponding hazard ratios CHD, coronary heart disease; HF, heart failure; PAD, peripheral artery disease.

emerged as the predominant CVD presentation among men and younger individuals, whereas HF predominated among women and older participants.

We found an annual incidence rate of CVD of around 4%. This rate was similar to that reported by Shah et al. in a recent study that included 34,198 individuals with T2D free of cardiovascular disease from primary care settings in England but higher than the 1 to 2% per year observed in other studies (1, 15–20). Several factors could explain the variability between studies; however, one of the most relevant might be the number and type of outcomes included in the CVD definition. As in the study mentioned above by Shah et al. (1), we applied a broad definition that included HF and PAD. Notably, both conditions combined accounted for more than 50% of the first-ever individual events in our cohort and 1 in 3 in the study of Shah et al. (1). In contrast, in most previous works, CVD was restricted to coronary and cerebrovascular events. Only a few studies included HF (17), and even fewer included PAD (17, 19).

Our findings that HF and PAD are leading contributors to T2D cardiovascular burden reinforce the importance of considering them in routine clinical practice and risk estimation strategies.

That being said, there were considerable variations in the absolute risk for the various manifestations of CVD by sex and age.

First, as in previous studies, we observed that women had lower overall CVD risk and lower risk for atherosclerotic diseases than men, independent of age (6). In contrast, we found higher incidence rates for HF in women than men at any age. Previous studies assessing sex differences in absolute HF risk in individuals with T2D are conflicting. In older studies, a higher absolute risk was observed in men (21, 22), whereas, in more contemporary works, women showed numerically higher rates for HF than men (12, 21–23). Nonetheless, none of these studies focused primarily on first events or considered the full effect of age. Thus, our results further reinforce the importance of HF as a primary driver of CVD in women with T2D. Mechanisms explaining a higher HF risk in women are not yet fully elucidated and might include differences in cardiac structure, function, and metabolism, differential myocardial response to classical cardiovascular risk factors, and exposure in women to unique risks, such as pre-eclampsia (24–26). It should also underscored that, in our cohort, the presentation of cardiovascular disease as HF was associated with a higher risk for all-cause mortality than other CVD subtypes, independently of age and sex. These results, which are in line with previous literature suggesting a profound impact of HF on overall survival, might further reinforce the importance of strategies primarily addressing its prevention (27).

Second, although sex patterns for CVD's first manifestation were consistent across age groups, atherosclerotic diseases, mainly CHD, accounted for most premature events in both men and women. In contrast, HF predominated in the aging population of both sexes. This finding agrees with previous epidemiological studies conducted in the general population, showing that HF is disproportionally distributed among older adults, with a prevalence that doubles for each decade of life (28).

Finally, we observed a sex-differential association between hypertension, obesity, socio-economic status, and incident cardiovascular disease. In our work, as in previous studies, the relative contribution of these factors was higher for women than for men (29, 30). Similarly, as in earlier cohort studies, in our work, the relative risk associated with classical cardiovascular risk factors, metabolic control, and end-organ damage was higher in younger vs. older individuals (31). These data have important clinical implications. First, it should help evaluate and discuss cardiovascular risks and preventive strategies in the clinical setting and facilitate shared decision-making from a more individualised perspective. Second, the evidence of large sex and age differences in CVD epidemiology and the possibility of a sex and age-differential impact of classical and non-classical cardiovascular risk factors among individuals with T2D should prompt us to evaluate the ongoing cardiovascular preventive strategies. Of note, current cardiovascular risk management in T2D is based on evidence from clinical trials mainly focused on coronary and cerebrovascular disease prevention conducted in predominantly middle-aged populations where women were frequently underrepresented (32–36). Although this approach has been proven to be highly effective in diminishing cardiovascular morbidity and mortality, T2D-related cardiovascular burdens might be further reduced by considering sex and age-specific risks and by modulating medical interventions in accordance.

In this regard, hypertension treatment dramatically reduces the incidence of HF and stroke but has a smaller impact on CHD (37, 38). Furthermore, observational data suggested that cardiovascular risk in women rises at lower blood pressure thresholds and that hypertension might confer a greater risk for HF in women than in men (30). In this same line, the recent Systolic Blood Pressure Intervention Trial (SPRINT), which unfortunately excluded participants with T2D, suggested a more significant benefit of lowering blood pressure goals in older vs. younger participants (37). On the other hand, lowering LDL-cholesterol dose-dependently decreases the incidence of atherosclerotic events, especially CHD (39). However, statin treatment is associated with limited improvements in preventing first non-fatal HF hospitalisations (40). Finally, the relative benefits of the newest T2D therapies vary. While sodium-glucose transport type 2 inhibitors have been shown to significantly impact HF, the cardiovascular benefits of glucagon-like peptide 1 agonists mostly rely on preventing atherosclerotic diseases (41). Thus, it could be speculated that the absolute cardiovascular benefits of these newer therapies might also vary by age and sex. Unfortunately, no clinical trials have been designed to primarily explore sex or age-specific targets or therapies for CVD prevention. Our findings further reinforce the need to fill this lack of evidence on these clinically relevant issues.

Our study has several limitations. First, diagnoses were recorded by treating physicians based on ICD-9/ICD-10 codes. Thus, some inter-individual variability in definitions cannot be excluded. Also, over 23% of Catalonians receive health care outside the National Health Care System (*Institut Català de la*

Salut) due to health agreements between the government and the private sector. This population is not included in the SIDIAP database. Nonetheless, the data quality of the SIDIAP database has been previously validated, and more specifically, data on CVD has proven to be of high quality and suitable for epidemiological studies (42, 43). Second, there was a substantial proportion of missing values for some variables (including BMI, blood pressure, or lipid profile). Therefore, the analysis assessing the association between risk factors and cardiovascular disease should be interpreted cautiously. Thirdly, it's important to note that in Spain, sodium-glucose transporters 2 inhibitors and long-acting glucagon-like 1 peptide analogs were not accessible at the baseline study date, and our dataset does not extend beyond 2016. Therefore, we cannot assess whether these newer agents' prescriptions might impact cardiovascular disease incidence and presentation. However, it is worth highlighting that the utilization of these advanced therapies remains minimal within the Catalonian population. As of 2018, their usage had stayed within 5% (41). Further studies comparing cardiovascular disease and its main subtypes incidence before and after full implementation of SGLT2-i and GLP1-analogs in the clinical practice help to delineate their impact in a real-world setting. Finally, the specific cause of death was only available for a limited proportion of study participants.

5 Conclusion

In conclusion, our study demonstrated that HF and PAD are significant contributors to cardiovascular disease burden in subjects with T2D and that, as in the general population, in women and older individuals, CVD more frequently manifests as HF. This epidemiological evidence should be considered in clinical practice and risk estimation strategies and might be relevant in designing future clinical trials.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The Ethics Committee of the Primary Healthcare University Research Institute (IDIAP), Jordi Gol (Barcelona, Spain), approved the study (code P17/087). The study was conducted in accordance with the local legislation and institutional requirements. Minimal risk research permitted the authors to conduct the investigation without obtaining informed consent from patients. Data was anonymized and is untraceable.

Author contributions

AJ: Writing – original draft. BV: Data curation, Methodology, Writing – review & editing. MM: Conceptualization, Investigation, Writing – review & editing. JR: Formal analysis, Methodology, Writing – review & editing. DM: Conceptualization, Funding acquisition, Investigation, Writing – review & editing. JF: Conceptualization, Funding acquisition, Investigation, Writing – review & editing. EO: Conceptualization, Funding acquisition, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1344007/full#supplementary-material

References

1. Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. *Lancet Diabetes Endocrinol.* (2015) 3:105–13. doi: 10.1016/S2213-8587(14)70219-0

2. Sattar N, McMurray J, Boren J, Rawshani A, Omerovic E, Berg N, et al. Years of cardiovascular complications and risk factors in patients with type 2 diabetes: A nationwide Swedish cohort study. *Circulation*. 147(25):1872–86. doi: 10.1161/CIRCULATIONAHA.122.063374

3. Lababidi H, Salerno PR, Wass SY, Shafiabadi Hasani N, Bourges-Sevenier B, Al-Kindi S. The Global Burden of premature cardiovascular disease, 1990-2019. *Int J Cardiol Cardiovasc Risk Prev.* (2023) 19:200212. doi: 10.1016/j.ijcrp.2023.200212

4. Geraghty L, Figtree GA, Schutte AE, Patel S, Woodward M, Arnott C. Cardiovascular disease in women: from pathophysiology to novel and emerging risk factors. *Heart Lung Circ.* (2021) 30:9–17. doi: 10.1016/j.hlc.2020.05.108

5. Leening MJG, Ferket BS, Steyerberg EW, Kavousi M, Deckers JW, Nieboer D, et al. Sex differences in lifetime risk and first manifestation of cardiovascular disease: prospective population based cohort study. *BMJ.* (2014) 349. doi: 10.1136/bmj.g5992

6. Malmborg M, Schmiegelow MDS, Nørgaard CH, Munch A, Gerds T, Schou M, et al. Does type 2 diabetes confer higher relative rates of cardiovascular events in women compared with men? *Eur Heart J.* (2020) 41:1346–53. doi: 10.1093/eurheartj/ehz913

7. Al-Salameh A, El bouzegaoui N, Saraval-Gross M. Diabetes and cardiovascular risk according to sex: An overview of epidemiological data from the early Framingham reports to the cardiovascular outcomes trials. *Ann Endocrinol (Paris).* (2023) 84:57–68. doi: 10.1016/j.ando.2022.09.023

8. Kalyani RR, Lazo M, Ouyang P, Turkbey E, Chevalier K, Brancati F, et al. Sex differences in diabetes and risk of incident coronary artery disease in healthy young and middle-aged adults. *Diabetes Care.* (2014) 37:830–8. doi: 10.2337/dc13-1755

9. Chase-Vilchez AZ, Chan IHY, Peters SAE, Woodward M. Diabetes as a risk factor for incident peripheral arterial disease in women compared to men: a systematic review and meta-analysis. *Cardiovasc Diabetol.* (2020) 19:151. doi: 10.1186/s12933-020-01130-4

10. Dong X, Cai R, Sun J, Huang R, Wang P, Sun H, et al. Diabetes as a risk factor for acute coronary syndrome in women compared with men: a meta-analysis, including 10 856 279 individuals and 106 703 acute coronary syndrome events. *Diabetes Metab Res Rev.* (2017) 33(5). doi: 10.1002/dmrr.2887

11. Peters TM, Holmes MV, Brent Richards J, Palmer T, Forgetta V, Lindgren CM, et al. Sex differences in the risk of coronary heart disease associated with type 2 diabetes: A mendelian randomization analysis. *Diabetes Care.* (2021) 44:556–62. doi: 10.2337/dc20-1137

12. Shen Y, Shi L, Nauman E, Katzmarzyk PT, Price-Haywood EG, Yin P, et al. Race and sex differences in rates of diabetic complications. *J Diabetes.* (2019) 11:449–56. doi: 10.1111/1753-0407.12869

13. Patel J, Al Rifai M, Scheuner MT, Shea S, Blumenthal RS, Nasir K, et al. Basic vs more complex definitions of family history in the prediction of coronary heart disease: the multi-ethnic study of atherosclerosis. *Mayo Clin Proc.* (2018) 93:1213–23. doi: 10.1016/j.mayocp.2018.01.014

14. Domínguez-Berjón MF, Borrell C, Cano-Serral G, Esnaola S, Nolasco A, Pasarín MI, et al. [Constructing a deprivation index based on census data in large Spanish cities (the MEDEA project)]. *Gac Sanit.* (2008) 22:179–87. doi: 10.1157/13123961

15. Basu S, Sussman JB, Berkowitz SA, Hayward RA, Yudkin JS. Development and validation of Risk Equations for Complications Of type 2 Diabetes (RECODe) using individual participant data from randomised trials. *Lancet Diabetes Endocrinol.* (2017) 5:788–98. doi: 10.1016/S2213-8587(17)30221-8

16. Berkelmans GFN, Gudbjörnsdottir S, Visseren FLJ, Wild SH, Franzen S, Chalmers J, et al. Prediction of individual life-years gained without cardiovascular events from lipid, blood pressure, glucose, and aspirin treatment based on data of more than 500 000 patients with Type 2 diabetes mellitus. *Eur Heart J.* (2019) 40:2899–906. doi: 10.1093/eurheartj/ehy839

17. Davis WA, Hellbusch V, Hunter ML, Bruce DG, Davis TME. Contemporary cardiovascular risk assessment for type 2 diabetes including heart failure as an outcome: the fremantle diabetes study phase II. *J Clin Med.* (2020) 9(5):1428. doi: 10.3390/jcm9051428

18. Mata-Cases M, De Prado-Lacueva C, Salido-Valencia V, Fernández-Bertolín E, Casermeiro-Cortés J, García-Durán M, et al. Incidence of complications and mortality in a type 2 diabetes patient cohort study followed up from diagnosis in a primary healthcare centre. *Int J Clin Pract.* (2011) 65:299–307. doi: 10.1111/ijcp.2011.65.issue-3

19. Piniés JA, González-Carril F, Arteagoitia JM, Irigoien I, Altzibar JM, Rodriguez-Murua JL, et al. Development of a prediction model for fatal and non-fatal coronary heart disease and cardiovascular disease in patients with newly diagnosed type 2 diabetes mellitus: the Basque Country Prospective Complications and Mortality Study risk engine (BASCORE). *Diabetologia.* (2014) 57:2324–33. doi: 10.1007/s00125-014-3370-1

20. Pylypchuk R, Wells S, Kerr A, Poppe K, Harwood M, Mehta S, et al. Cardiovascular risk prediction in type 2 diabetes before and after widespread screening: a derivation and validation study. *Lancet.* (2021) 397:2264–74. doi: 10.1016/S0140-6736(21)00572-9

21. Gottdiener JS, Arnold AM, Aurigemma GP, Polak JF, Tracy RP, Kitzman DW, et al. Predictors of congestive heart failure in the elderly: the Cardiovascular Health Study. *J Am Coll Cardiol.* (2000) 35:1628–37. doi: 10.1016/S0735-1097(00)00582-9

22. He J, Ogden LG, Bazzano LA, Vupputuri S, Loria C, Whelton PK. Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Arch Intern Med.* (2001) 161:996–1002. doi: 10.1001/archinte.161.7.996

23. Zinman B, Inzucchi SE, Wanner C, Hehnke U, George JT, Johansen OE, et al. Empagliflozin in women with type 2 diabetes and cardiovascular disease – an analysis of EMPA-REG OUTCOME®. *Diabetologia.* (2018) 61:1522–7. doi: 10.1007/s00125-018-4630-2

24. Beale AL, Meyer PMD, Marwick TH, Lam CSP, Kaye DM. Sex differences in cardiovascular pathophysiology: why women are overrepresented in heart failure with preserved ejection fraction. *Circulation.* (2018) 138:198–205. doi: 10.1161/CIRCULATIONAHA.118.034271

25. Gori M, Lam CSP, Gupta DK, Santos ABS, Cheng S, Shah AM, et al. Sex-specific cardiovascular structure and function in heart failure with preserved ejection fraction. *Eur J Heart Fail.* (2014) 16:535–42. doi: 10.1002/ejhf.67

26. Scantlebury DC, Borlaug BA. Why are women more likely than men to develop heart failure with preserved ejection fraction? *Curr Opin Cardiol.* (2011) 26:562–8. doi: 10.1097/HCO.0b013e32834b7faf

27. Shah KS, Xu H, Matsouaka RA, Bhatt DL, Heidenreich PA, Hernandez AF, et al. Heart failure with preserved, borderline, and reduced ejection fraction: 5-year outcomes. *J Am Coll Cardiol.* (2017) 70:2476–86. doi: 10.1016/j.jacc.2017.08.074

28. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol.* (2016) 13:368–78. doi: 10.1038/nrcardio.2016.25

29. Joseph JJ, Williams A, Azap RA, Zhao S, Brock G, Kline D, et al. Role of sex in the association of socioeconomic status with cardiovascular health in black Americans: the jackson heart study. *J Am Heart Assoc.* (2023) 12(23):e030695. doi: 10.1161/JAHA.123.030695

30. Ji H, Niiranen TJ, Rader F, Henglin M, Kim A, Ebinger JE, et al. Sex differences in blood pressure associations with cardiovascular outcomes. *Circulation.* (2021) 143:761–3. doi: 10.1161/CIRCULATIONAHA.120.049360

31. Antza C, Gallo A, Boutari C, Ershova A, Gurses KM, Lewek J, et al. Prevention of cardiovascular disease in young adults: Focus on gender differences. A collaborative review from the EAS Young Fellows. *Atherosclerosis.* (2023) 384:117272. doi: 10.1016/j.atherosclerosis.2023.117272

32. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med.* (2009) 360:129–39. doi: 10.1056/NEJMoa0808431

33. Patel A, ADVANCE Collaborative Group, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet.* (2007) 370:829–40. doi: 10.1016/S0140-6736(07)61303-8

34. ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med.* (2008) 358:2560–72. doi: 10.1056/NEJMoa0802987

35. Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med.* (2008) 358:2545–59. doi: 10.1056/NEJMoa0802743

36. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HAW, Livingstone SJ, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): Multicentre randomised placebo-controlled trial. *Lancet.* (2004) 364:685–96. doi: 10.1016/S0140-6736(04)16895-5

37. SPRINT Research Group, Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM, et al. A randomized trial of intensive versus standard blood-pressure control. *N Engl J Med.* (2015) 373:2103–16. doi: 10.1056/NEJMoa1511939

38. Mefford MT, Goyal P, Howard G, Durant RW, Dunlap NE, Safford MM, et al. The association of hypertension, hypertension duration, and control with incident heart failure in black and white adults. *J Clin Hypertens.* (2020) 22:857–66. doi: 10.1111/jch.13856

39. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet.* (2010) 376:1670–81. doi: 10.1016/S0140-6736(10)61350-5

40. Preiss D, Campbell RT, Murray HM, Ford I, Packard CJ, Sattar N, et al. The effect of statin therapy on heart failure events: a collaborative meta-analysis of unpublished data from major randomized trials. *Eur Heart J.* (2015) 36:1536–46. doi: 10.1016/j.atherosclerosis.2015.04.086

41. Palmer SC, Tendal B, Mustafa RA, Vandvik PO, Li S, Hao Q, et al. Sodium-glucose cotransporter protein-2 (SGLT-2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists for type 2 diabetes: systematic review and network meta-analysis of randomised controlled trials. *BMJ.* (2021) 372:m4573. doi: 10.1136/bmj.m4573

42. Ramos R, Balló E, Marrugat J, Elosua R, Sala J, Grau M, et al. Validity for use in research on vascular diseases of the SIDIAP (Information System for the Development of Research in Primary Care): the EMMA study. *Rev Esp Cardiol (Engl Ed)*. (2012) 65:29–37. doi: 10.1016/j.rec.2011.07.016

43. Del Mar García-Gil M, Hermosilla E, Prieto-Alhambra D, Fina F, Rosell M, Ramos R, et al. Construction and validation of a scoring system for the selection of high-quality data in a Spanish population primary care database (SIDIAP). *Inform Prim Care*. (2011) 19:135–45. doi: 10.14236/jhi.v19i3.806

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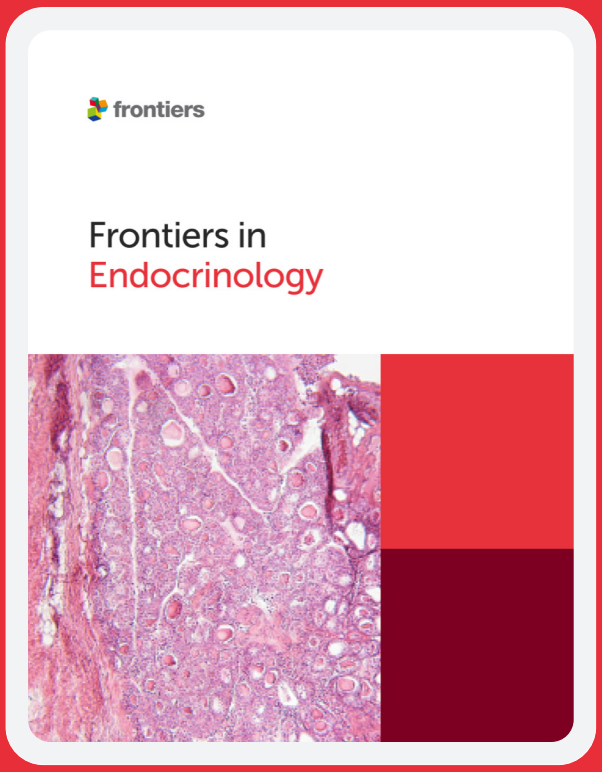

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