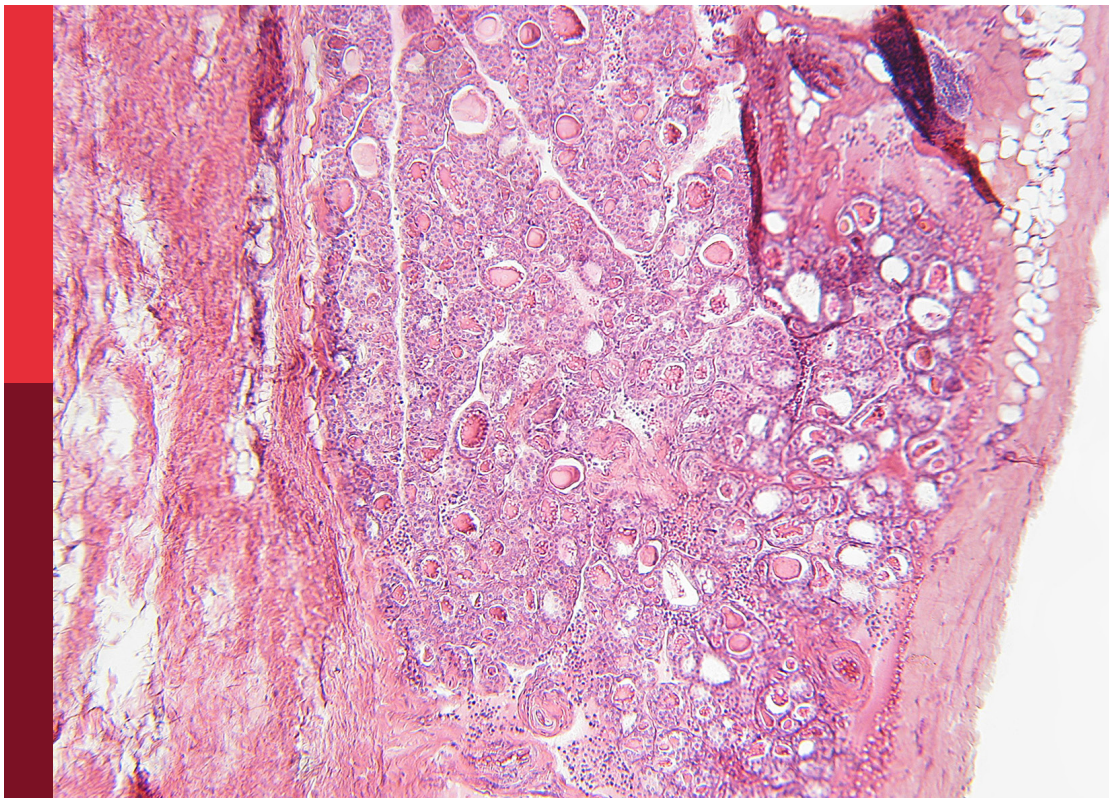
frontiers | Research Topics

Prediabetes and endocrine function

Edited by
Sam Dagogo-Jack and Andile Khathi

Published in
Frontiers in Endocrinology
Frontiers in Public Health



ISSN 1664-8714
ISBN 978-2-8325-3394-9
DOI 10.3389/978-2-8325-3394-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Prediabetes and endocrine function

Topic editors

Sam Dagogo-Jack — University of Tennessee Health Science Center (UTHSC), United States

Andile Khathi — University of KwaZulu-Natal, South Africa

Citation

Dagogo-Jack, S., Khathi, A., eds. (2023). *Prediabetes and endocrine function*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3394-9

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 17 August 2023
DOI 10.3389/fendo.2023.1268552

OPEN ACCESS

EDITED AND REVIEWED BY
Åke Sjöholm,
Gävle Hospital, Sweden

*CORRESPONDENCE
Andile Khathi
khathia@ukzn.ac.za

RECEIVED 28 July 2023
ACCEPTED 03 August 2023
PUBLISHED 17 August 2023

CITATION
Khathi A and Dagogo-Jack S (2023) Editorial: Prediabetes and endocrine function.
Front. Endocrinol. 14:1268552.
doi: 10.3389/fendo.2023.1268552

Editorial: Prediabetes and endocrine function

Andile Khathi[1]* and Sam Dagogo-Jack[2]

[1]School of Laboratory Medicine and Medical Sciences, Discipline of Human Physiology, University of KwaZulu-Natal, Durban, South Africa, [2]Division of Endocrinology, Diabetes & Metabolism, The University of Tennessee Health Science Center, Memphis, TN, United States

KEYWORDS

prediabetes, endocrine function, frequency and distribution of studies, biomarkers, surrogate markers

Editorial on the Research Topic
Prediabetes and endocrine function

Introduction

Type 2 diabetes mellitus (T2DM) is a condition characterized by chronic hyperglycaemia caused by insulin resistance or insulin insufficiency (1). T2DM accounts for approximately 90% of the cases worldwide and leads to a variety of microvascular and macrovascular complications (2). By 2040, it was predicted by the International Diabetes Federation (IDF) that around 642 million people worldwide are expected to be diagnosed with T2DM (2). The onset of T2DM is often preceded by an asymptomatic condition known as prediabetes where the blood glucose levels are above the normal range but below the threshold for the diagnosis of T2DM (3). Pre-diabetes is asymptomatic and has been shown to increasing in prevalence (3). The main risk factors that have been identified for this condition include chronic consumption of high calorie diets, obesity and sedentary lifestyles (4). Studies have shown that the intermediate hyperglycemia associated with prediabetes may result in chronic sub-clinical inflammation and increased generation of reactive oxygen species (ROS) which may in turn have consequences on metabolic function. Indeed, studies have shown that the complications that occur in T2DM begin in pre-diabetes (5–7). In this Research Topic, we compiled papers looking at the effects of prediabetes on endocrine function as well as looked at possible new biomarkers and surrogate markers for prediabetes.

Frequency and distribution of studies on prediabetes

Prediabetes is defined as a condition of intermediate hyperglycaemia characterized by the impaired fasting glucose, impaired glucose tolerance and elevated levels of glycated

haemoglobin (3). Prediabetes is a growing public health concern worldwide and this has prompted studies globally. This is evidenced by the bibliometric analysis done by Zhao and Li, they looked at worldwide trends of prediabetes from 1985 to 2022. The study highlighted research hotspots as well as development patterns in the field of prediabetes with a strong focus on global research outcomes. The study highlighted the increasing frequency of publications in this field over the last few decades. More significantly, the study showed the wide distribution in the journals where the work was published as well as the countries and universities where the work was being done.

Effects on endocrine function

Several studies have previously shown that the complications often associated with type 2 diabetes actually begin prediabetes (8, 9). In this Research Topic, Naidoo et al., showed that there are derangements in calcium homeostasis due to compromised renal function during the prediabetic state. The study further showed that calcium-regulating organs compensate for renal calcium wastage and are aimed at maintaining normocalcaemia. The effects associated with prediabetes on calcium-regulating organs are directed towards promoting increased renal calcium reabsorption, increased renal vitamin D activation, increased intestinal calcium absorption and decreased bone resorption followed by increased bone formation. This was evidenced by increased expression of renal calcium transport markers and intestinal calcium transport markers in addition to increased osteocalcin and decreased deoxypyridinoline levels. This was supported by another research article in this topic by Liu et al.. This study analysed bone mineral density in initially normoglycaemic participants in the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study. This was in relation to the incidence of prediabetes during 5 years of follow-up. The main finding was that study participants who developed incident prediabetes during 5 years of follow-up tended to have higher baseline bone mineral density suggesting compensatory mechanisms in calcium homeostasis during prediabetes. Another study conducted by Krisnamurti et al. in a prediabetic rat model further provided evidence of the involvement of vitamin D in the development of prediabetes. Vitamin D deficiency has been frequently linked to insulin resistance and diabetes. In this study, the authors show that vitamin D supplementation reduces insulin resistance in prediabetic rats and that the reduction might be due to the effects of vitamin D on IRS, PPARg, and NF-kB expression. These studies collectively show both the effects of prediabetes on calcium homeostasis as well as how vitamin D supplementation could be used to reduce insulin resistance in the prediabetic state.

Identification of biomarkers and surrogate markers

Various studies have embarked on identifying novel biomarkers and surrogate markers to assist with early identification and risk markers of prediabetes. Jiang et al. showed that higher visceral adiposity index values are positively associated with insulin resistance while Wang et al. showed that monitoring AST/ALT ratio could be beneficial as a predictor of insulin resistance in both males and females. Han et al. on the other hand demonstrated a positive non-linear relationship between the triglyceride glucose-BMI value and the risk of developing T2DM in patients that already have prediabetes. Yang et al. showed the use of a metabolite-based biomarker for early patient diagnosis and treatment of prediabetes while Al Akl et al. showed that the triglyceride-waist-height ratio may be a good marker in detecting prediabetes.

Conclusion

Recent literature shows an upsurge in the study of prediabetes and the effects that it has on endocrine function. While there is still a lot more work to be done in this field, the latest studies have suggested novel markers that may be used in the early detection of prediabetes.

Author contributions

AK: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. SD-J: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – review & editing.

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. David L. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* (2020) 41(2):255–323.

2. Centers for Disease Control Prevention. *US department of health human services, centers for disease control prevention* Vol. 201. Atlanta, GA (2011) p. 2568–9.

3. Di Pino A SR, Calanna S, Urbano F, Mantegna C, Rabuazzo AM, Purrello F, et al. Cardiovascular risk profile in subjects with prediabetes and new-onset type 2 diabetes identified by HbA(1c) according to American Diabetes Association criteria. *Diabetes Care* (2014) 37(5):1447–53. doi: 10.2337/dc13-2357

4. Stefanaki C, Bacopoulou F, Peppa M. Prediabetes and adolescence—trends, causes, effects, and screening. *US Endocrinol* (2016) 12(2):94–8. doi: 10.17925/USE.2016.12.02.94

5. Gamede M, Mabuza L, Ngubane P, Khathi A. The effects of plant-derived oleanolic acid on selected parameters of glucose homeostasis in a diet-induced pre-diabetic rat model. *Molecules* (2018) 23(4):794. doi: 10.3390/molecules23040794

6. Akinnuga AM, Siboto A, Khumalo B, Sibiya NH, Ngubane P, Khathi A. Ameliorative Effects of Bredemolic Acid on Markers Associated with Renal Dysfunction in a Diet-Induced Prediabetic Rat Model. *Oxid Med Cell Longevity* (2020) 2020. doi: 10.1155/2020/2978340

7. Luvuno M, Mabandla M, Khathi A, Ponte J. (2018) 74.

8. Mkhize B MP, Ngubane P, Sibiya N, Khathi A. Diet-induced prediabetes: Effects on the systemic and renal renin-angiotensin-aldosterone system. *J Diabetes Invest* (2022) 13(5):768–80. doi: 10.1111/jdi.13690

9. Mosili P MB, Ngubane P, Sibiya N, Khathi A. The dysregulation of the hypothalamic-pituitary-adrenal axis in diet-induced prediabetic male Sprague Dawley rats. *Nutr Metab* (2020) 17(1):104. doi: 10.1186/s12986-020-00532-1

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 11 July 2022
doi: 10.3389/fendo.2022.914189

Investigating the Effects of Diet-Induced Pre-Diabetes on the Functioning of Calcium-Regulating Organs in Male Sprague Dawley Rats: Effects on Selected Markers

Karishma Naidoo, Phikelelani S. Ngubane and Andile Khathi*

School of laboratory Medicine and Medical Sciences, Discipline of Human Physiology, University of KwaZulu-Natal, KwaZulu- Natal, South Africa

OPEN ACCESS

Edited by:
Mohd Imtiaz Nawaz, King Saud University, Saudi Arabia

Reviewed by:
Diana Nur Afifah, Diponegoro University, Indonesia
Luciana Da Conceição Antunes, Federal University of Santa Catarina, Brazil
Abulaish Ansari, NYU Langone Health, NYU, United States

***Correspondence:**
Karishma Naidoo
karishma280199@gmail.com

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *06 April 2022*
Accepted: *10 June 2022*
Published: *11 July 2022*

Citation:
Naidoo K, Ngubane PS and Khathi A (2022) Investigating the Effects of Diet-Induced Pre-Diabetes on the Functioning of Calcium-Regulating Organs in Male Sprague Dawley Rats: Effects on Selected Markers. Front. Endocrinol. 13:914189. doi: 10.3389/fendo.2022.914189

Derangements to the functioning of calcium-regulating organs have been associated with type 2 diabetes mellitus (T2DM), a condition preceded by pre-diabetes. Type 2 diabetes has shown to promote renal calcium wastage, intestinal calcium malabsorption and increased bone resorption. However, the changes to the functioning of calcium-regulating organs in pre-diabetes are not known. Subsequently, the effects of diet-induced pre-diabetes on the functioning of calcium-regulating organs in a rat model for pre-diabetes was investigated in this study. Male Sprague Dawley rats were separated into two groups (n=6, each group): non-pre-diabetic (NPD) group and a diet-induced pre-diabetic (DIPD) group for 20 weeks. After the experimental period, postprandial glucose and HOMA-IR were analysed in addition to plasma and urinary calcium concentrations. Gene expressions of intestinal vitamin D (VDR), intestinal calbindin-D9k, renal 1-alpha hydroxylase and renal transient receptor potential vanilloid 5 (TRPV5) expressions in addition to plasma osteocalcin and urinary deoxypyridinoline concentrations were analysed at week 20. The results demonstrated significantly increased concentrations of postprandial glucose, HOMA-IR and urinary calcium in addition to unchanged plasma calcium levels in the DIPD group by comparison to NPD. Renal TRPV5, renal 1-alpha hydroxylase, intestinal VDR and intestinal calbindin-D9k expressions were increased in the DIPD group by comparison to NPD. Furthermore, plasma osteocalcin levels were increased and urine deoxypyridinoline levels were decreased in the DIPD group by comparison to NPD. These observations may suggest that calcium-regulating organs compensate for the changes to calcium homeostasis by inducing increased renal calcium reabsorption, increased intestinal calcium absorption and decreased bone resorption followed by increased bone formation.

Keywords: pre-diabetes, calcium-regulating organs, high-fat high carbohydrate diet, homeostasis, normocalcaemia

INTRODUCTION

Urban lifestyle and the chronic consumption of diets which contain high fat and carbohydrate content has shown to promote the development of type 2 diabetes mellitus (T2DM), a condition which is preceded by pre-diabetes (1). Pre-diabetes is an intermediate state of hyperglycaemia with glycaemic parameters above the homeostatic range yet below the threshold for diagnosis of clinical diabetes (1). Pre-diabetes is associated with the simultaneous presence of insulin resistance and β-cell dysfunction (2). In 2017, the International Diabetes Federation (IDF) reported that 352 million people worldwide were diagnosed with pre-diabetes, while it is further estimated that by 2045 the prevalence of pre-diabetes is expected to increase by 8.3% (2). While T2DM is often associated with macro- and microvascular complications, studies have shown that T2DM impairs calcium homeostasis by disrupting the functioning of calcium-regulating organs, namely the intestine, kidney and bone (3, 4).

Fluxes of calcium between the small intestine, bone and kidney are controlled by parathyroid hormone (PTH), calcitonin and calcitriol (5). Calcium-regulating organs participate in supplying calcium to the blood and removing it from blood when necessary (4). The small intestine is the site where dietary calcium is absorbed, the bone serves as a calcium reservoir and the kidneys regulate urinary calcium excretion (6). Several studies have shown physiological changes to calcium-regulating organs in T2DM individuals (4, 7, 8). Type 2 diabetes mellitus promotes impaired intestinal calcium absorption, renal calcium wasting and bone deterioration (4). Furthermore, it also leads to dysregulation of calciotropic hormones, thereby worsening the already impaired functioning of calcium-regulating organs (6). The maintenance of calcium homeostasis is important because calcium modulates many important functions (4). Calcium is responsible for bone mineralization, hormone communication, regulation of the nervous system and muscle tone (5). By interrupting calcium homeostasis, processes within the body that are dependent on calcium would be impaired as well as conditions such as hypocalcaemia and osteoporosis may develop (4).

Previous studies in our laboratory developed a high fat high carbohydrate (HFHC) diet-induced pre-diabetic animal model which mimics the human condition of pre-diabetes (9, 10). Several studies using this model have revealed that various complications seen in T2DM begin in the pre-diabetic state (9, 11). While the changes to the functioning of calcium-regulating organs have been well documented in the diabetic state, these changes have not yet been investigated during the pre-diabetic state (12, 13). Hence, the aim of this study is to determine the effects of diet-induced pre-diabetes on the functioning of calcium-regulating organs in male Sprague Dawley rats.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats (150-180 g) were bred and housed at the University of KwaZulu-Natal's Biomedical Research Unit (BRU). The protocol for animal experimentation and conditions were followed according to the Animal Research Ethics Committee of the University of KwaZulu-Natal (ETHICS#: AREC/00003627/2021). Procedures involving animals care were conducted in conformity with the institutional guidelines for animal care of the University of KwaZulu-Natal. The animals were maintained under standard conditions of a constant room temperature (22± 2°C), carbon dioxide content (<5000 p.m.), relative humidity (55± 5°C) and illumination (12 h light/dark cycle, with lights on at 7am). The noise levels in the room were maintained below 65 decibels and the animals had access to rat chow and water *ad libitum*. An experimental acclimatization interval of one week was carried out, whereby the rats were fed standard rat chow (Meadows Feeds, South Africa) and tap water, prior to the induction of pre-diabetes (10).

Outcome and Outcome Measures

The primary outcome was evaluation of intestinal calcium transport, renal calcium transport and bone turnover. The secondary outcome was evaluation of body calcium status, glucose tolerance and insulin resistance. Intestinal calcium transport was measured by evaluating intestinal VDR and intestinal calbindinD9k expression. Renal calcium transport was measured by evaluating renal TRPV5 and renal 1-alpha hydroxylase expression. Bone turnover was measured by evaluating plasma osteocalcin and urine deoxypyridinoline levels. Body calcium status was measured by evaluating plasma calcium and urine calcium levels. Glucose tolerance and insulin resistance was measured by evaluating OGTT, plasma insulin and HOMA-IR.

Induction of Pre-Diabetes

The rats were randomly assigned following simple randomisation procedures (computerised random numbers) to 1 of 2 two groups (n=6, per group) and fed their respective diets for an experimental period of 20 weeks. Six rats per group were the minimum amount of animals needed to achieve statistical significance according to the resource method equation (14). Experimental pre-diabetes was induced in the animals using a previously described protocol by 10 (10). Basically, one group was fed a standard rat diet and tap water, whereas the other group was fed a HFHC diet and 15% fructose supplemented water (AVI Products (Pty) Ltd, Waterfall, South Africa), for the purpose of inducing pre-diabetes. After 20 weeks, the animals were tested to confirm pre-diabetes using the criteria from the American Diabetes Association. Animals with a fasting blood glucose (FBG) concentration of 5.6 to 7.1 mmol/L, oral glucose tolerance test (OGTT) 2-h glucose concentration of 7.1–11.1 mmol/L and plasma triglycerides concentration greater than 2 mmol/L were regarded as pre-diabetic. The animals that were fed the standard diet were also tested at week 20 to confirm normoglycaemia.

Experimental Design

This study comprised of two groups, namely a diet-induced pre-diabetic (DIPD) group and non-pre-diabetic (NPD) group (n=6, in each group). The animals that consumed the standard rat chow for 20 weeks and did not have pre-diabetes were regarded as the NPD group, whereas the animals that consumed the

HFHC diet for the same number of weeks and diagnosed with pre-diabetes, were regarded as the DIPD group. Baseline OGTT and HOMA-IR could not be performed due to analysis being limited to changes in glycaemic parameters at week 20 after dietary intervention, in addition blood samples were not obtained at baseline.

Oral Glucose Tolerance Response

At week 20, an OGTT was conducted following glucose loading, to determine the glucose tolerance response of animals subjected to the chronic ingestion of the HFHC diet. The OGT responses were monitored in the animals according to a well-established protocol (9, 10, 15). Briefly, after a 12 hour fast, glucose levels were measured (time, 0 min) in all animals. Thereafter, the animals were loaded with glucose (glucose; 0.86 g/kg, p.o) through an oral gavage (18-gauge gavage needle, 38mm long curved with 21/4 mm ball end). To measure glucose concentration, blood was collected using the tail-prick method (16). Glucose concentrations were measured by a OneTouch select glucometer (Lifescan, Mosta, Malta, United Kingdom). The glucose concentrations were measured at 15, 30, 60, and 120 minutes following glucose loading.

Urine Collection, Blood Collection and Tissue Harvesting

At the end of the experimental period, all animals were housed individually in Makrolon polycarbonate metabolic cages (Techniplats, Labotec, South Africa) for a 24-hour urine collection period. After the 24-hours, urine samples from all animals were collected and stored in a Bio Ultra freezer (Labotec, Umhlanga, South Africa) at -80°C, thereafter the animals were anaesthetized with Isofor (100 mg/kg) (Safeline Pharmaceuticals (Pty) Ltd, Roodeport, South Africa) for 3 minutes *via* a gas anaesthetic chamber (Biomedical Resource Unit, UKZN, South Africa). While the rats are unconscious, blood was collected by cardiac puncture and then injected into individual pre-cooled heparinized containers. The blood was centrifuged (Eppendorf centrifuge 5403, LGBW Germany) at 4°C, 503 g for 15 minutes. Plasma was separated from blood and stored at -70°C in a Bio Ultra freezer (Labotec, Umhlanga, South Africa) until biochemical analysis as previously described by 10. Following blood collection, the kidney and small intestine were removed and placed in pre-cooled Eppendorf containers and snap-frozen in liquid nitrogen before storage in a Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) at − 80°C. Of note, plasma, urine, kidney and intestinal tissue were obtained from a previous study which had ethical approval.

HOMA-IR Index

The homeostatic model assessment (HOMA) was used to measure insulin resistance from fasting blood glucose and insulin levels (17). The HOMA-IR index was calculated using the HOMA2 Calculator v2.2.3 program (18.). Values < 1.0 = insulin sensitive, >1.9 = early insulin resistance, > 2.9= significant insulin resistance.

Biochemical Analysis

Plasma calcium, urinary calcium and creatinine concentrations were measured with an autoanalyzer (IDEXX VetLab station, Hoofddorp, Netherlands). The plasma insulin, plasma osteocalcin and urinary deoxypyridinoline concentrations were measured using separate specific ELISA kits according to the manufacturer's instructions (Elabscience and Biotechnology, Wuhan, China).

Quantitative Real-Time PCR

The harvested kidney and small intestine tissue was subjected to RNA extraction using a ReliaPrep tissue Miniprep system (Promega, USA). The purity and concentration of RNA was determined by Nanodrop 2000 (Thermo Scientific, Roche, South Africa). A purity ratio (A260/A280) of 1.7-2.1 was considered acceptable for conversion to cDNA. Synthesis of cDNAs was performed by reverse transcription reactions with 2 µg of total RNA using GoTaq® 2-Step RT-qPCR System as a cDNA synthesis kit (Promega, USA) as described by the manufacturer.

The ROCHE light cycler SYBR Green I master mix was used for amplication according to the manufacturer's instructions on the ROCHE light cycler system. The primer sequences (Metabion, Germany) used in this study can be found in **Table 1** below. The cycling conditions were: Pre-incubation was carried out at 95°C for 60s, followed by a 3-step amplication of 45 cycles at 95°C for 15s, 60°C for 30s, and 72° C for 30s. Melting was effectuated at 95°C for 10s, 65°C for 60s and 97°C for 1s. Furthermore, cooling was achieved at 37°C for 30s. Glyceraldehyde-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. Gene expression values were represented using the $2^{-\Delta\Delta Ct}$ relative quantication method.

Table 1 List of primers used in this study

Statistical Analysis

All data was expressed as mean ± S.E.M. Statistical comparisons were performed with Graph Pad InStat Software (version 5.00, Graph Pad Software, Inc., San Diego, California, USA) using the student t test. A value of $p < 0.05$ was considered statistically significant.

TABLE 1 | List of primers used in this study.

Gene of interest	Sequence
TRPV5	Forward: 5'-TGTGAGCCATTTGTAGGTCAG-3' Reverse: 5'-GAGGTTGTGGGAACTTCGA-3'
CYP27B1(1-alpha hydroxylase)	Forward: 5'-CACCCATTTGCATCTCTTCC -3' Reverse: 5'-GATGGATGCTCCTCTCAGGT -3'
VDR	Forward: 5′-GTGACTTTGACCGGAACGTG-3′ Reverse: 5'- ATCATCTCCCTCTTACGCTG -3'
S100G(CalbindinD9k)	Forward: 5'CCCGAAGAAATGAAGAGCATTTT-3' Reverse: 5'-TTCTCCATCACCGTTCTTATCCA-3'
GAPDH	Forward: 5'-AGTGCCAGCCTCGTCTCATA-3' Reverse: 5'-GATGGTGATGGGTTTCCCGT-3'

RESULTS

Oral Glucose Tolerance Test

The OGTT and Area under curve (AUC) were analysed in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group after the experimental period (n=6, per group). The results (**Figure 1**) showed that at time 0, the FBG concentration significantly (p= 0.0020) increased in the DIPD group by comparison to the NPD group. At 120 min post-load of glucose, the glucose concentrations of the DIPD group was significantly (p= 0.0386) increased by comparison to the NPD group. Furthermore, the AUC (**Figure 1**) was significantly (p=<0.0001) higher in the DIPD group by comparison to NPD.

Homeostatic Model Assessment for Insulin Resistance

The HOMA-IR values were calculated from the fasting plasma glucose and insulin concentrations after the experimental period (n=6, per group). The results (**Table 2**) showed that the fasting plasma glucose (p=< 0.0001) and insulin (p=< 0.0001) concentrations were significantly higher in the DIPD group by comparison to the NPD group. The HOMA-IR value for NPD was within the insulin-sensitive range (< 1.0) while the DIPD group had a significantly (p=< 0.0001) higher HOMA-IR value compared to the NPD which was in the range of significant insulin resistance (>2.9).

Table 2 Plasma glucose, plasma insulin concentrations and HOMA-IR indices in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic group (DIPD) (n=6, per group). Values are presented as mean ± SEM. ****= p<0.0001 in comparison with NPD

Plasma Calcium and Urinary Calcium From 24-Hour Urine Samples

Plasma and urinary calcium concentrations were analysed in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group after the experimental period (n=6, per group). The results (**Figure 2**) showed that there was no significant (p=<0.0001) change to plasma calcium concentration in the DIPD group by comparison to NPD. The urinary calcium concentration (**Figure 2**) were significantly (p= <0.0001) higher in the DIPD group by comparison to NPD.

Evaluation of Bone Turnover Through Plasma Osteocalcin and Urine Deoxypyridinoline Levels

Plasma osteocalcin and urine deoxypyridinoline concentrations were analysed in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group after the experimental period (n=6, per group). The results (**Figure 3**) showed that plasma osteocalcin concentration was significantly higher (p= 0.0002) in the DIPD group by comparison to NPD. The urinary deoxypyridinoline concentration (**Figure 3**) was significantly (p=<0.0001) lower in the DIPD group by comparison to the NPD group. A Pearson's correlation analysis was performed in both non-pre-diabetic (NPD) and diet-induced pre-diabetic (DIPD) rats between plasma osteocalcin and HOMA-IR. The results (**Supplementary Table 1**) showed that plasma osteocalcin levels were positively correlated (r=0.87, p=0.02) with HOMA-IR in the pre-diabetic state.

Evaluation of Renal Calcium Transport Through Renal TRPV5 and 1-Alpha Hydroxylase Expression

Renal Transient receptor potential cation channel subfamily V5 (TRPV5) gene expression was analysed in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group after the experimental period (n=6, per group). The results (**Figure 4**) showed that the relative expression of renal TRPV5 was significantly (p=<0.0001) increased by 3.89-fold in the DIPD group relative to the NPD group. The relative expression of renal 1-alpha hydroxylase (**Figure 4**) was significantly (p=<0.0001) increased by 10.96-fold in the DIPD group relative to the NPD group.

Evaluation of Intestinal Calcium Transport Through Intestinal VDR and CalbindinD9k Expression

Intestinal vitamin D receptor (VDR) gene expression was analysed in the non-pre-diabetic (NPD) group and diet-

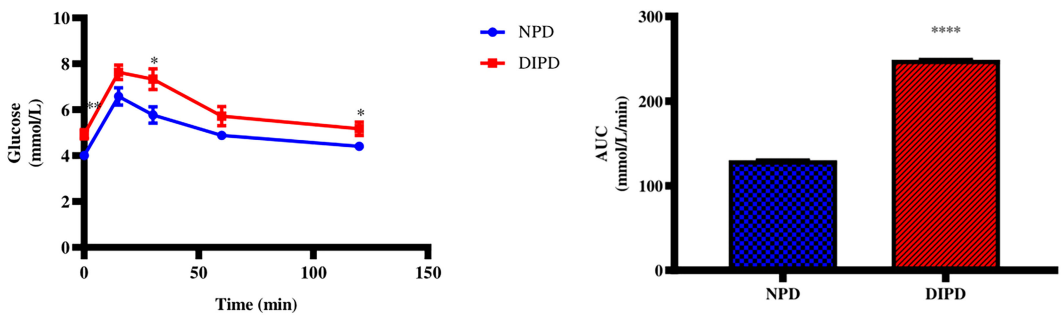


FIGURE 1 | The OGT response and AUC values in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic group (DIPD) (n=6, per group). Values are presented as mean ± SEM. *=p < 0.05, **=p < 0.01, ****= p < 0.0001 by comparison with NPD.

TABLE 2 | Plasma glucose, plasma insulin concentrations and HOMA-IR indices in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic group (DIPD) (n=6, per group).

Groups(n=6)	Plasma glucose (mmol/L)	Plasma insulin(ng/mL)	HOMA-IR values
NPD	4.40 ± 0.20	3.47 ± 0.12	0.68 ± 0.05
DIPD	6.72 ± 0.12****	10.87 ± 0.06****	3.24 ± 0.06****

*Values are presented as mean ± SEM. ****= p < 0.0001 in comparison with NPD.*

induced pre-diabetic (DIPD) group after the experimental period (n=6, per group). The results (**Figure 5**) showed that the relative expression of intestinal VDR was significantly (p=<0.0001) increased by 5.55-fold in the DIPD group relative to the NPD group. The relative expression of intestinal calbindinD9k expression (**Figure 5**) was significantly (p=<0.0001) increased by 9.13-fold in the DIPD group relative to the NPD group.

DISCUSSION

Several studies have shown that the functioning of calcium-regulating organs are disturbed in T2DM (4, 19). However, no studies have been conducted to assess the functioning of calcium-regulating organs during the pre-diabetic state. Hence, this study aimed to investigate the effects of diet-induced pre-diabetes on the functioning of calcium-regulating organs, namely the kidney, intestine and bone. The current study found a significant change in the functioning of calcium-regulating organs induced by the pre-diabetic state. In this study, there was increased concentrations of postprandial glucose, plasma insulin and HOMA-IR index in the DIPD group by comparison to NPD. Furthermore, we have found that the pre-diabetic state induced by HFHC diet increases the levels of urinary calcium as well as the expressions of renal TRPV5, renal 1-alpha hydroxylase, intestinal VDR and intestinal calbindinD9k. The pre-diabetic group have also presented increased plasma osteocalcin, decreased urinary deoxypyridinoline concentrations, but unchanged plasma calcium. The findings highlight the physiological compensatory role of calcium-regulating organ systems in the pathogenesis of pre-diabetes.

Pre-diabetes is characterised as a combination of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) which can be attributed to moderate insulin resistance in insulin-dependent tissues (16). Blood glucose levels must be constantly maintained within a physiological range including a fasting glucose level of less than 5.6 mmol/L and postprandial glucose level of less than 7.8 mmol/L (20). In the postprandial state of normal glucose tolerant (NGT) individuals, blood glucose concentration increases and insulin is secreted to enhance glycogenesis and inhibit glycogenolysis (1). As a result, plasma glucose levels are maintained followed by plasma insulin levels returning towards the homeostatic range (21). However, in the pre-diabetic state endogenous glucose production is excessive before eating and fails to appropriately suppress after eating in pre-diabetic individuals (22). This is due to impaired insulin-induced peripheral glucose uptake in insulin-dependent tissue (10). This accounts for fasting plasma glucose, insulin, postprandial glucose levels and HOMA-IR been higher in pre-diabetic individuals by comparison to NGT individuals (23). In this study, there was a significant increase in the postprandial glucose concentration at 120 min, AUC and HOMA-IR value in DIPD group by comparison to NPD. The results corroborated with previous findings that have shown significantly higher plasma glucose, insulin, 2-hour postprandial glucose levels and HOMA-IR in pre-diabetic patients by comparison to NPD (2, 24). In the DIPD group, the elevated plasma insulin, impaired fasting glucose and HOMA-IR value in the range of insulin resistance may suggest that there is some insulin resistance from peripheral tissue against the uptake of glucose. High dietary fat promotes an increase in circulating triacylglyceride which breakdown to free fatty acids (FFA) (25). The increase in FFAs around insulin-dependent tissue results in insulin resistance which decreases glucose uptake resulting in compensatory hyperinsulinemia, as seen in the DIPD group (10).

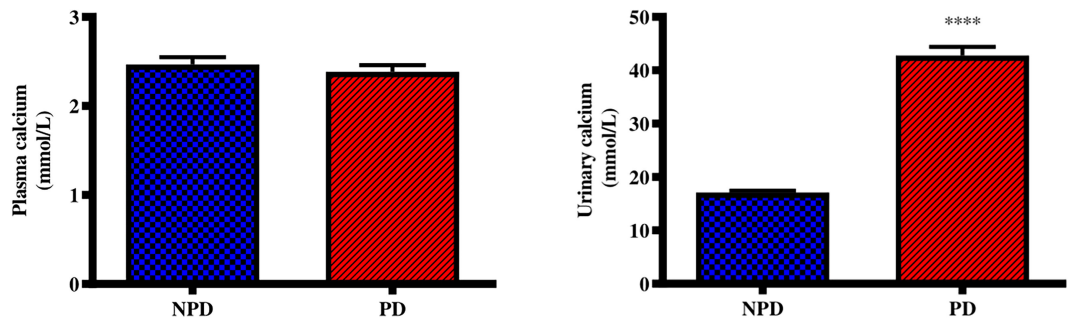


FIGURE 2 | Plasma and urinary calcium concentrations in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic group (DIPD) (n=6, per group). Values are presented as mean ± SEM. ****= p < 0.0001 by comparison with NPD.

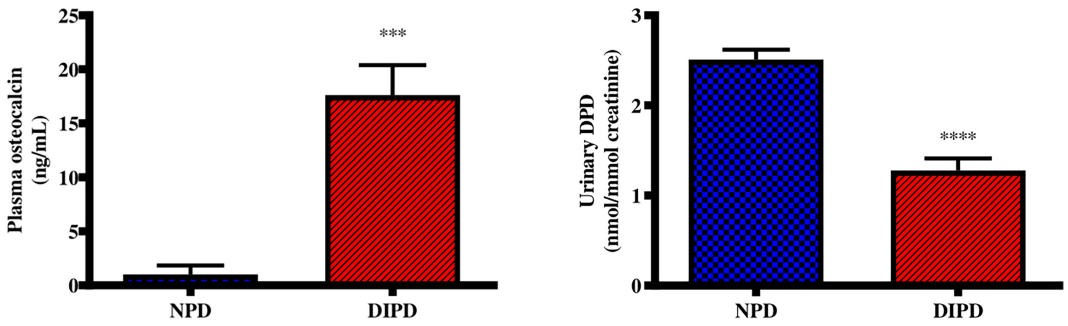


FIGURE 3 | Evaluation of bone turnover through plasma osteocalcin and urine deoxypyridinoline concentrations in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group (n=6, per group).Values are presented as mean ± SEM. ***=p < 0.001, ****=p < 0.0001 by comparison with NPD.

Elevated plasma glucose concentrations and the onset of insulin resistance in T2DM has shown to interfere with the functioning of calcium-regulating organs in the diabetic state.

Calcium plays a crucial role in various physiological processes and plasma calcium levels are kept within a narrow range through the interplay of calcium-regulating organs (26). Calcium-regulating organs maintain plasma calcium levels by regulating renal calcium reabsorption, bone turnover and intestinal calcium absorption (6). Previous studies have shown decreased plasma calcium concentrations in type 2 diabetic patients by comparison to normoglycaemic individuals (19, 27). These observations suggested that renal dysfunction and abnormal vitamin D metabolism were responsible for inducing a state of hypocalcaemia in the diabetic state (26). However, other studies have shown normal plasma calcium levels in diabetic individuals (28, 29). These studies have stated that calcium-regulating organs compensate for the reduced plasma calcium levels by inducing an increase in intestinal calcium absorption, renal calcium reabsorption and bone resorption (30). In this study, the DIPD group had no significant change to plasma calcium levels by comparison to the NPD group. The findings in this study coincided with prior literature that have shown no significant change to plasma calcium levels by comparison to diabetic patients (31, 32). The possible reason for no significant change to plasma calcium levels in the pre-diabetic state may have been due to calcium-regulating organs compensating for the changes to plasma calcium levels. Interestingly, it may be speculated that during the pre-diabetic state there may be counter-regulatory mechanisms remaining that are aimed at the maintenance of calcium homeostasis. The early insulin resistance and intermediate hyperglycaemia in pre-diabetes may activate these counter-regulatory mechanisms, where compensation may be the initial response to disturbances to calcium homeostasis. However, it may be observed that serum alterations of calcium seem to be observed only after renal injury progression culminating in diabetes mellitus.

The kidneys contribute to calcium homeostasis by adjusting the reabsorption and excretion of filtered calcium (6). Disturbances in renal calcium reabsorption can lead to excessive urinary calcium excretion and kidney stone formation (8). TRPV5 is a calcium channel which mediates calcium reabsorption in the kidney and plays an important role in the regulation of urinary calcium (33). Studies have reported elevated urinary calcium levels along with decreased

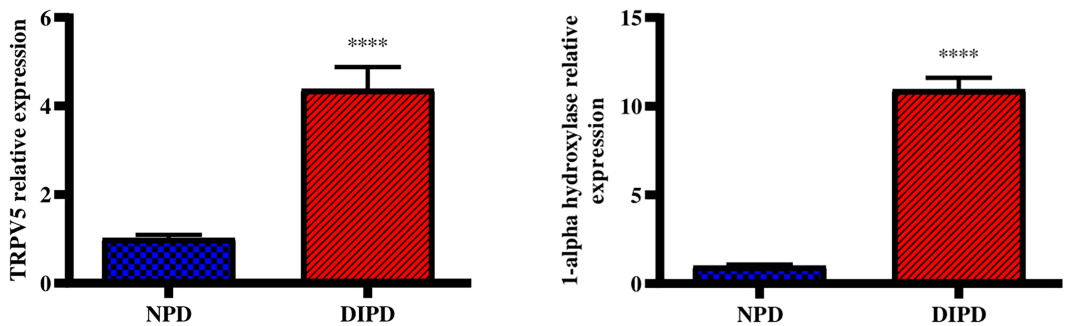


FIGURE 4 | Evaluation of renal calcium transport through renal TRPV5 and 1-alpha hydroxylase gene expression in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group (n=6, per group).Values are presented as mean ± SEM. ****= p < 0.0001 by comparison to NPD.

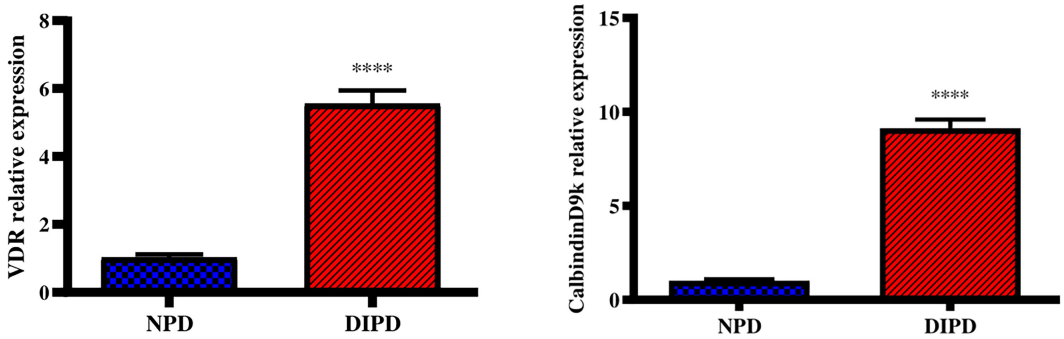


FIGURE 5 | Evaluation of intestinal calcium transport through intestinal VDR and calbindinD9k gene expression in the non-pre-diabetic (NPD) group and diet-induced pre-diabetic (DIPD) group (n=6, per group).Values are presented as mean ± SEM. ****= $p < 0.0001$ by comparison to NPD.

renal TRPV5 expression in diabetes (34, 35). Studies have also shown that decreased renal TRPV5 expression was associated with reduced renal calcium reabsorption (36, 37). These observations suggested that hyperglycaemia-induced renal damage may have downregulated renal TRPV5 expression (37). The downregulation in renal TRPV5 expression promotes renal calcium wastage and hypocalcaemia in diabetics (38). In addition, elevated urinary calcium levels have shown to result from intestinal hyperabsorption of calcium and excessive bone resorption in T2DM (39). In this study, the DIPD group had significantly increased urinary calcium concentrations in the range of hypercalciuria by comparison to NPD. This was accompanied by a significant increase in the expression of renal TRPV5 in the DIPD group by comparison to NPD. This study's results corroborated with previous studies that have shown elevated urine calcium and an upregulation in renal TRPV5 expression in T2DM patients (40, 41). The increased urine calcium may have occurred as a result of kidney damage in the pre-diabetic state, which may have decreased the ability of the kidneys to reabsorb calcium. There may have been other contributors to the increased urine calcium such as increased intestinal calcium absorption and bone resorption (28). Therefore, the kidneys may try to compensate for the increased plasma calcium by excreting it into urine. However, the simultaneous increase in urinary calcium excretion and renal TRPV5 may suggest a compensatory mechanism against renal calcium wastage. The increased renal TRPV5 expression in the DIPD group may have promoted increased renal calcium reabsorption from urinary filtrate. Interestingly, renal TRPV5 expression is regulated by vitamin D, which is known to be catalyzed to its active form in the kidney (5).

Renal-1 alpha hydroxylase is mainly expressed in the proximal convoluted tubules and is the key enzyme involved in the synthesis of calcitriol (42). Disturbances in kidney function and vitamin D metabolism can lead to excessive urinary calcium excretion and hyperparathyroidism (26). Studies have shown that a loss of kidney function in T2DM leads to a decline in circulating plasma calcitriol concentrations (43, 44). Renal injury and the accumulation of metabolites in the diabetic kidney contribute to 1-alpha hydroxylase inhibition and lower circulating calcitriol levels (43). In this study, the DIPD group had significantly increased renal-1 alpha hydroxylase expression by comparison to the NPD group. The findings of this study corroborated with previous studies that have shown an upregulation in renal 1-alpha hydroxylase expression in diabetic patients (42, 45). However, the findings of this study contrasted other studies that have shown decreased renal-1 alpha hydroxylase expression in the diabetic state (43, 44). The upregulation in renal-1 alpha hydroxylase in the DIPD group may suggest that there is an increased demand to synthesis calcitriol in the pre-diabetic state. The kidneys may compensate for the hypercalciuria by upregulating the expression of renal-1 alpha hydroxylase, in order to maintain normal plasma calcium levels. In addition, the regulation of renal-1 alpha hydroxylase is dependent on the calciotropic hormones (46). It is evident that renal cells still appear be responsive to calciotropic hormones in the pre-diabetic state, in attempt to conserve plasma calcium levels.

Intestinal calcium absorption is a crucial physiological process for maintaining calcium homeostasis (30). The small intestine is the site where dietary calcium is absorbed and can physiologically adapt according to the conditions of the body (47, 48). Efficient absorption of calcium in the small intestine is dependent on the expression of calcium-binding proteins and vitamin D receptor (VDR) (47, 49). Vitamin D metabolites regulate calcium absorption in the intestine through activation of the vitamin D receptor (VDR) which results in increased expression of calcium transport proteins including calbindinD9k (47). Type 2 diabetes is associated with profound deterioration of calcium metabolism, partly from impaired intestinal calcium absorption (6, 50). Previous studies in diabetic rats reported that the reduction in intestinal calcium absorption occurred concurrently with decreases in VDR and calcium-binding protein calbindin-D9k in the enterocytes (4, 51). It was noted that intestinal VDR and calcium-binding proteins were downregulated due to impaired production of calciotropic hormones in T2DM (6). Subsequently, the ability of the intestine to adapt to disturbances to low plasma calcium levels

is compromised during the diabetic state (6). However, other studies have shown an upregulation in intestinal calcium transporter expression in diabetic rats (47, 52). The increased intestinal VDR number promoted increased VDR-calcitriol complexes and increased intestinal calcium transport (6). Hence, the present study investigated intestinal VDR and calbindinD9k expression to evaluate intestinal calcium transport. In this study, there was a significant increase in intestinal VDR and calbindinD9k expression in the DIPD group by comparison to the NPD group. The findings of this study contrasted previous results that have shown a downregulation in intestinal VDR and calbindinD9k expression in the diabetic state. The elevated intestinal VDR and calbindinD9k expression in the DIPD group may suggest that there is an increase in intestinal calcium absorption. The upregulation of calcium transport genes in the intestine of the DIPD group may have been a compensatory response to renal calcium wastage.

Bone regulates plasma calcium levels by releasing calcium through a process known as bone resorption and storing calcium through a process known as bone formation (53). Bone formation is coupled to bone resorption, where increased bone resorption is followed by increased bone formation (5). An imbalance between bone resorption and bone formation may result in bone diseases including osteoporosis (54, 55). Bone resorption and formation can be determined indirectly by measurement of plasma concentrations of bone markers (29). These markers include bone matrix components released into circulation during bone formation or resorption (56). Osteocalcin is a marker of bone formation, whereas deoxypyridinoline is a marker of bone resorption (56). Some studies have shown increased bone turnover in type 2 diabetic patients, where bone resorption exceeds formation (20, 57). This was evidenced by decreased plasma osteocalcin levels and increased deoxypyridinoline levels (4, 58). These observations suggested that during the diabetic state there is an increased demand to mobilize calcium from bone to compensate for hypocalcaemia; however the normal bone coupling process becomes compromised (4, 58). Hyperglycaemia has shown to decrease bone formation by inhibiting osteoblast synthesis and differentiation (28). However, other studies have reported increased bone formation in type 2 diabetes (20, 59). It was stated that hyperinsulinemia shifts the balance between bone formation and resorption in favour of bone formation (56). Hence, the present study focused on investigating the levels of plasma osteocalcin and urine deoxypyridinoline in the pre-diabetic state, to evaluate bone turnover. In this study, the DIPD group had increased plasma osteocalcin concentration and decreased urinary deoxypyridinoline concentration in the DIPD group by comparison to NPD. The findings of this study corroborated with previous results that have shown increased plasma osteocalcin levels and decreased urinary deoxypyridinoline levels (59, 60). These observations may suggest that there is increased bone formation and decreased resorption in the pre-diabetic state. The increased bone formation and decreased bone resorption may have been induced by calciotropic hormones to compensate for hypercalcaemia. Interestingly, studies have shown that increased intestinal calcium absorption by calciotropic hormones in the diabetic state may overcompensate for renal calcium wastage inducing a state of hypercalcaemia (61, 62). In this study the intestine may have overcompensated for renal calcium wastage inducing a state of hypercalcaemia. This leads to the speculation that during the pre-diabetic state there may be some resistance in the detection of plasma calcium levels. Subsequently, bone may have suppressed bone resorption and promoted bone formation to compensate for hypercalcaemia. Insulin is an anabolic hormone which has shown to promote bone formation and inhibit bone resorption (59). In the pre-diabetic state, early insulin resistance leads to a compensatory increase in insulin secretion (63). The elevated plasma insulin levels in the DIPD group may have promoted increased bone formation and suppressed bone resorption. Furthermore, previous studies have shown a positive correlation between HOMA-IR and plasma osteocalcin level in diabetic patients (64, 65). It has been demonstrated that osteocalcin can stimulate insulin secretion, acting directly on proliferation and secretion of pancreatic beta-cells (20). Interestingly, there was a positive correlation between plasma osteocalcin and HOMA-IR in the DIPD group. It may be speculated that the elevated plasma osteocalcin concentration in the DIPD group may have been a compensatory response to cope with the early insulin resistance. This may be an early adaptation mechanism for insulin resistance, which fails with the onset of overt T2DM.

The findings elucidated in this study may have the potential to provide an understanding into the physiological processes that occur in calcium-regulating organs during pre-diabetes. From a clinical perspective, pre-diabetes is asymptomatic and many people progress towards the development of T2DM due to being unaware. The findings of this study will not only add to academic knowledge but may serve as a novel marker in the identification of pre-diabetes. This study targets some of the complications and disrupted processes involved in T2DM. Furthermore, these findings may provide an early insight into the pathogenesis involved in the associated complications of T2DM. A future prospective would be to use these findings as insights to understand the possible changes that may occur to pre-diabetic humans.

It is evident that during the pre-diabetic state there are changes to the functioning of calcium-regulating organs which compensate for disturbances to plasma calcium levels. This was made evident by increased urinary calcium levels along with increased expressions of renal TRPV5, renal 1-alpha hydroxylase, intestinal VDR and intestinal calbindinD9k. In addition, there was increased plasma osteocalcin and decreased urinary deoxypyridinoline concentrations along with unchanged plasma calcium in the pre-diabetic state. The normocalcaemia present in the pre-diabetic state may have been conserved due to increased renal calcium reabsorption, increased renal vitamin D activation, increased intestinal calcium absorption and increased bone formation followed by decreased bone resorption.

CONCLUSION

Taken together, calcium-regulating organs compensate for renal calcium wastage and are aimed at maintaining normocalcaemia in HFHC diet-induced pre-diabetes. The effects associated with pre-diabetes on calcium-regulating organs are directed towards promoting increased renal calcium reabsorption, increased renal vitamin D activation, increased intestinal calcium absorption and decreased bone resorption followed by increased bone formation. This was evidenced by increased expression of renal calcium transport markers and intestinal calcium transport markers in addition to increased osteocalcin and decreased deoxypyridinoline levels. Collectively, these observations may suggest that calcium-regulating organs compensate for the changes to calcium homeostasis in the pre-diabetic state.

LIMITATIONS

A limitation of the study was the lack of blinding during the diet intervention stage and the lack of baseline measurement for hepatic and muscle insulin resistance marker (OGTT and HOMA-IR). A second limitation to this study is that it only focuses on mRNA expression and not proteins.

FUTURE RECOMMENDATIONS

In future, a further insight into the mechanisms in which bone turnover by-products participate in glucose homeostasis in the pre-diabetic state should be investigated.

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

This animal study was reviewed and approved by the Animal Research Ethics Committee (AREC) of the University of KwaZulu-Natal, Durban, South Africa (AREC/00003627/2021).

AUTHOR CONTRIBUTIONS

KN contributed to the study design, conducted the experiments, collected, analysed and interpreted data as well as being involved in writing the manuscript. PN and AK was involved in the conceptualization of the study, study design and editing of the manuscript as well as provide funding. All authors have read the manuscript and approved its submission.

FUNDING

This study was funded by the UKZN College of Health Science and the National Research Foundation under the grant number (UID130598).

ACKNOWLEDGMENTS

The authors would like to acknowledge that the content of this manuscript has previously appeared in the corresponding author's KN dissertation and is the only form in which it has appeared (66).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.914189/full#supplementary-material

REFERENCES

1. Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of Prediabetes. *Med Clin North Am* (2011) 95(2):327–39vii–viii. doi: 10.1016/j.mcna.2010.11.005
2. Stefanaki C, Bacopoulou F, Peppa M. Prediabetes and Adolescence—Trends, Causes, Effects, and Screening. *US Endocrinol* (2016) 12(2):94–8. doi: 10.17925/USE.2016.12.02.94
3. Bansal N. Prediabetes Diagnosis and Treatment: A Review. *World J Diabetes* (2015) 6(2):296. doi: 10.4239/wjd.v6.i2.296
4. Wongdee K, Krishnamra N, Charoenphandhu N. Derangement of Calcium Metabolism in Diabetes Mellitus: Negative Outcome From the Synergy Between Impaired Bone Turnover and Intestinal Calcium Absorption. *J Physiol Sci* (2017) 67(1):71–81. doi: 10.1007/s12576-016-0487-7
5. Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH, Christakos S. Vitamin D, Calcium Homeostasis and Aging. *Bone Res* (2016) 4(1):1–7. doi: 10.1038/boneres.2016.41
6. Moe SM. Calcium Homeostasis in Health and in Kidney Disease. *Compr Physiol* (2016) 6(4):1781–800. doi: 10.1002/cphy.c150052
7. Peacock M. Calcium Metabolism in Health and Disease. *Clin J Am Soc Nephrol* (2010) 5(Supplement 1):S23–30. doi: 10.2215/CJN.05910809
8. De Brito Galvao JF, Nagode LA, Schenck PA, Chew DJ. Calcitriol, Calcidiol, Parathyroid Hormone, and Fibroblast Growth Factor-23 Interactions in Chronic Kidney Disease. *J Veterinary Emergency Crit Care* (2013) 23 (2):134–62. doi: 10.1111/vec.12036
9. Gamede M, Mabuza L, Ngubane P, Khathi A. The Effects of Plant-Derived Oleanolic Acid on Selected Parameters of Glucose Homeostasis in a Diet-Induced Pre-Diabetic Rat Model. *Molecules* (2018) 23(4):794. doi: 10.3390/molecules23040794
10. Luvuno M, Mabandla M, Khathi A. Voluntary Ingestion of a High-Fat High-Carbohydrate Diet: A Model for Prediabetes. *J Ponte Int Sci Res* (2018) 74 (5):119–43. doi: 10.21506/j.ponte.2018.5.11
11. Mosili P, Mkhize BC, Ngubane P, Sibiya N, Khathi A. The Dysregulation of the Hypothalamic-Pituitary-Adrenal Axis in Diet-Induced Prediabetic Male Sprague Dawley Rats. *Nutrition Metabolism: Clinical and Experimental* (2020) 17(1):1–12. doi: 10.1186/s12986-020-00532-1
12. Akinnuga AM, Siboto A, Khumalo B, Sibiya NH, Ngubane P, Khathi A. Ameliorative Effects of Bredemolic Acid on Markers Associated With Renal

Dysfunction in a Diet-Induced Prediabetic Rat Model. *Oxid Med Cell Longevity 2020* (2020) 2020:1–12. doi: 10.1155/2020/2978340
13. Mkhize B, Mosili P, Ngubane P, Sibiya N, Khathi A. Diet-Induced Prediabetes: Effects on the Systemic and Renal Renin-Angiotensin-Aldosterone System. *J Diabetes Investig* (2020) 13(5):768–80. doi: 10.1111/jdi.13690
14. Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. *Malaysian J Med Sciences: MJMS* (2017) 24(5):101. doi: 10.21315/mjms2017.24.5.11
15. Mabuza LP, Gamede MW, Maikoo S, Booysen IN, Ngubane PS, Khathi A. Effects of a Ruthenium Schiff Base Complex on Glucose Homeostasis in Diet-Induced Pre-Diabetic Rats. *Molecules* (2018) 23(7):1721. doi: 10.3390/molecules23071721
16. Tabák AG, Herder C, Rathmann W, Brunner EJ , Kivimäki M. Prediabetes: A High-Risk State for Diabetes Development. *Lancet* (2012) 379(9833):2279–90. doi: 10.1016/S0140-6736(12)60283-9
17. Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC. Validation of HOMA-IR in a Model of Insulin-Resistance Induced by a High-Fat Diet in Wistar Rats. *Arch Endocrinol Metabolism: Clin Exp* (2016) 60:138–42. doi: 10.1590/2359-3997000000169
18. Diabetes TOCF. Endocrinology and Metabolism, in: *Homa Calculator*. Available at: http://www.dtu.ox.ac.uk/ (Accessed 2016).
19. Ahn C, Kang J-H, Jeung E-B. Calcium Homeostasis in Diabetes Mellitus. *J veterinary Sci* (2017) 18(3):261–6. doi: 10.4142/jvs.2017.18.3.261
20. Sanches CP, Vianna AGD, Barreto F. The Impact of Type 2 Diabetes on Bone Metabolism. *Diabetol Metab Syndrome* (2017) 9:85–5. doi: 10.1186/s13098-017-0278-1
21. Jeon JY, Ko S-H, Kwon H-S, Kim NH, Kim JH, Kim CS, et al. Prevalence of Diabetes and Prediabetes According to Fasting Plasma Glucose and HbA1c. *Diabetes Metab J* (2013) 37(5):349–57. doi: 10.4093/dmj.2013.37.5.349
22. Abdul-Ghani MA, DeFronzo RA. Pathophysiology of Prediabetes. *Curr Diabetes Rep* (2009) 9(3):193–9. doi: 10.1007/s11892-009-0032-7
23. Kabadi UM. Major Pathophysiology in Prediabetes and Type 2 Diabetes: Decreased Insulin in Lean and Insulin Resistance in Obese. *J Endocrine Soc* (2017) 1(6):742–50. doi: 10.1210/js.2016-1116
24. Tankova T, Chakarova N, Dakovska L, Atanassova I. Assessment of HbA1c as a Diagnostic Tool in Diabetes and Prediabetes. *Acta Diabetologica* (2012) 49 (5):371–8. doi: 10.1007/s00592-011-0334-5
25. Centers for Disease Control Prevention. *National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United State* Vol. 201. Atlanta, GA: US department of health human services, centers for disease control prevention (2011) p. 2568–9. Available at: http://www.cdc.gov/nchs/nhanes.htm.
26. Dusso AS. Kidney Disease and Vitamin D Levels: 25-Hydroxyvitamin D, 1,25-Dihydroxyvitamin D, and VDR Activation. *Kidney Int Suppl* (2011) 1(4):136–41. doi: 10.1038/kisup.2011.30
27. Nwankwor HC, Nwatu CB, Okwara CC, Young EE, Olisaka LC, Ezomike NC, et al. Low Serum Calcium Levels Occur in Nigerian Adults With Type 2 Diabetes and Correlates Negatively With Their Glycosylated Hemoglobin Levels: A Case-Control Study. *Nigerian J Med* (2020) 29(2):229. doi: 10.4103/NJM.NJM_25_20
28. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. Normocalcemia is Maintained in Mice Under Conditions of Calcium Malabsorption by Vitamin D–induced Inhibition of Bone Mineralization. *J Clin Invest* (2012) 122(5):1803–15. doi: 10.1172/JCI45890
29. Blaine J, Chonchol M, Levi M. Renal Control of Calcium, Phosphate, and Magnesium Homeostasis. *Clin J Am Soc Nephrol* (2015) 10(7):1257–72. doi: 10.2215/CJN.09750913
30. Christakos S, Dhawan P, Porta A, Mady LJ, Seth T. Vitamin D and Intestinal Calcium Absorption. *Mol Cell Endocrinol* (2011) 347(1-2):25–9. doi: 10.1016/j.mce.2011.05.038
31. Gosink J. Parathyroid Hormone, Calcitonin and Vitamin D Testing in Calcium and Bone Metabolic Disorders. *Medlab magazine* (2015) 2:26–8. Retrieved from: https://www.euroimmun.com/fileadmin/user_upload/News/Professional articles/HA_6411_L_UK_A.pdf.
32. Hus A, Tahleel B, Hasan AEI, Albagir EH, Mohammad MA, Salah S, et al. Serum Calcium Level in Type 2 Diabetes Mellitus in Khartoum State. *Clin Microbiol* (2019) 8(332):2. doi: 10.4172/2327-5073.1000331
33. Keung L, Perwad F. Vitamin D and Kidney Disease. *Bone Rep* (2018) 9:93–100. doi: 10.1016/j.bonr.2018.07.002
34. Woudenberg-Vrenken TE, Bindels RJ, Hoenderop JG. The Role of Transient Receptor Potential Channels in Kidney Disease. *Nat Rev Nephrol* (2009) 5 (8):441–9. doi: 10.1038/nrneph.2009.100
35. Tajiri M, Nakahashi O, Kagawa T, Masuda M, Ohminami H, Iwano M, et al. Association of Increased Renal Cyp24a1 Gene Expression With Low Plasma 1, 25-Dihydroxyvitamin D Levels in Rats With Streptozotocin-Induced Diabetes. *J Clin Biochem Nutr* (2020) 66(1):49–56. doi: 10.3164/jcbn.19-79
36. Raskin P, Stevenson MR, Barilla DE, Pak CY. The Hypercalciuria of Diabetes Mellitus: Its Amelioration With Insulin. *Clin Endocrinol* (1978) 9(4):329–35. doi: 10.1111/j.1365-2265.1978.tb02218.x
37. Nijenhuis T, Hoenderop JG, Bindels RJ. Downregulation of Ca2+ and Mg2+ Transport Proteins in the Kidney Explains Tacrolimus (FK506)-Induced Hypercalciuria and Hypomagnesemia. *J Am Soc Nephrol* (2004) 15(3):549–57. doi: 10.1097/01.ASN.0000113318.56023.B6
38. Yamada H, Funazaki S, Suzuki D, Saikawa R, Yoshida M, Kakei M, et al. Association Between Urinary Calcium Excretion and Estimated Glomerular Filtration Rate Decline in Patients With Type 2 Diabetes Mellitus: A Retrospective Single-Center Observational Study. *J Clin Med* (2018) 7 (7):171. doi: 10.3390/jcm7070171
39. Christakos S, Lieben L, Masuyama R, Carmeliet G. Vitamin D Endocrine System and the Intestine. *BoneKEy Rep* (2014) 3:1–7. doi: 10.1038/bonekey.2013.230
40. Lee C, Lien YH, Lai L, Chen J, Lin C, Chen H. Increased Renal Calcium and Magnesium Transporter Abundance in Streptozotocin-Induced Diabetes Mellitus. *Kidney Int* (2006) 69(10):1786–91. doi: 10.1038/sj.ki.5000344
41. Wilson PC, Wu H, Kirita Y, Uchimura K, Ledru N, Rennke HG, et al. The Single-Cell Transcriptomic Landscape of Early Human Diabetic Nephropathy. *Proc Natl Acad Sci* (2019) 116(39):19619–25. doi: 10.1073/pnas.1908706116
42. Wang Y, Zhou J, Minto A, Hack B, Alexander J, Haas M, et al. Altered Vitamin D Metabolism in Type II Diabetic Mouse Glomeruli may Provide Protection From Diabetic Nephropathy. *Kidney Int* (2006) 70(5):882–91. doi: 10.1038/sj.ki.5001624
43. Huang C, Ma G, Tao M, Ma X, Feng J, Liu Q. The Relationship Between Renal Injury and Change in Vitamin D Metabolism in Aged Rats With Insulin Resistance or Type 2 Diabetes Mellitus. *J Int Med Res* (2008) 36(2):289–95. doi: 10.1177/147323000803600211
44. Huang C, Ma G, Tao M, Ma X, Liu Q, Feng J. The Relationship Among Renal Injury, Changed Activity of Renal 1-α Hydroxylase and Bone Loss in Elderly Rats With Insulin Resistance or Type 2 Diabetes Mellitus. *J endocrinological Invest* (2009) 32(3):196–201. doi: 10.1007/BF03346452
45. Fowlkes JL, Bunn RC, Cockrell GE, Clark LM, Wahl EC, Lumpkin CK, et al. Dysregulation of the Intrarenal Vitamin D Endocytic Pathway in a Nephropathy-Prone Mouse Model of Type 1 Diabetes. *Exp Diabetes Res* (2011) 2011:1–7. doi: 10.1155/2011/269378
46. Chen W, Roncal-Jimenez C, Lanaspa M, Gerard S, Chonchol M, Johnson RJ, et al. Uric Acid Suppresses 1 Alpha Hydroxylase *In Vitro* and *In Vivo*. *Metabolism: Clin Exp* (2014) 63(1):150–60. doi: 10.1016/j.metabol.2013.09.018
47. de Barboza GD, Guizzardi S, de Talamoni NT. Molecular Aspects of Intestinal Calcium Absorption. *World J Gastroenterology: WJG* (2015) 21(23):7142. doi: 10.3748/wjg.v21.i23.7142
48. Diaz de Barboza G, Guizzardi S, Moine L, Tolosa de Talamoni N. Oxidative Stress, Antioxidants and Intestinal Calcium Absorption. *World J Gastroenterol* (2017) 23(16):2841–53. doi: 10.3748/wjg.v23.i16.2841
49. Keller J, Schinke T. The Role of the Gastrointestinal Tract in Calcium Homeostasis and Bone Remodeling. *Osteoporos Int* (2013) 24(11):2737–48. doi: 10.1007/s00198-013-2335-4
50. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and Diabetes. *Rheum Dis Clin North Am* (2012) 38(1):179–206. doi: 10.1016/j.rdc.2012.03.015
51. Douard V, Asgerally A, Sabbagh Y, Sugiura S, Shapses SA, Casirola D, et al. Dietary Fructose Inhibits Intestinal Calcium Absorption and Induces Vitamin D Insufficiency in CKD. *J Am Soc Nephrol* (2010) 21(2):261–71. doi: 10.1681/ASN.2009080795
52. Stone LA, Weaver VM, Bruns E, Christakos S, Welsh J. Vitamin D Receptors and Compensatory Tissue Growth in Spontaneously Diabetic BB Rats. *Ann Nutr Metab* (1991) 35(4):196–202. doi: 10.1159/000177645
53. Jongwattanapisan P, Suntornsaratoon P, Wongdee K, Dorkkam N, Krishnamra N, Charoenphandhu N. Impaired Body Calcium Metabolism

With Low Bone Density and Compensatory Colonic Calcium Absorption in Cecectomized Rats. *Am J Physiology-Endocrinology Metabolism: Clin Exp* (2012) 302(7):E852–63. doi: 10.1152/ajpendo.00503.2011
54. Anderson PH. Vitamin D Activity and Metabolism in Bone. *Curr Osteoporosis Rep* (2017) 15(5):443–9. doi: 10.1007/s11914-017-0394-8
55. Rathinavelu S, Guidry-Elizondo C, Banu J. Molecular Modulation of Osteoblasts and Osteoclasts in Type 2 Diabetes. *J Diabetes Res* (2018) 2018:6354787. doi: 10.1155/2018/6354787
56. Picke A-K, Campbell G, Napoli N, Hofbauer LC, Rauner M. Update on the Impact of Type 2 Diabetes Mellitus on Bone Metabolism and Material Properties. *Endocrine Connections* (2019) 8(3):R55–70. doi: 10.1530/EC-18-0456
57. Capoglu I, Ozkan A, Ozkan B, Umudum Z. Bone Turnover Markers in Patients With Type 2 Diabetes and Their Correlation With Glycosylated Haemoglobin Levels. *J Int Med Res* (2008) 36(6):1392–8. doi: 10.1177/147323000803600629
58. Cassidy FC, Shortiss C, Murphy CG, Kearns SR, Curtin W, De Buitléir C, et al. Impact of Type 2 Diabetes Mellitus on Human Bone Marrow Stromal Cell Number and Phenotypic Characteristics. *Int J Mol Sci* (2020) 21(7):2476. doi: 10.3390/ijms21072476
59. Ivaska KK, Heliövaara MK, Ebeling P, Bucci M, Huovinen V, Väänänen HK, et al. The Effects of Acute Hyperinsulinemia on Bone Metabolism. *Endocrine connections* (2015) 4(3):155–62. doi: 10.1530/EC-15-0022
60. Sultan E, Taha I, Saber LM. Altered Bone Metabolic Markers In Type 2 Diabetes Mellitus: Impact of Glycemic Control. *J Taibah Univ Med Sci* (2008) 3(2):104–16. doi: 10.1016/S1658-3612(08)70059-3
61. Guerra V, Vieira Neto OM, Laurindo AF, de Paula FJA, Moysés Neto M. Hypercalcemia and Renal Function Impairment Associated With Vitamin D Toxicity: Case Report. *Braz J Nephrol* (2016) 38:466–9. doi: 10.5935/0101-2800.20160074
62. Galuška D, Pácal L, Kaňková K. Pathophysiological Implication of Vitamin D in Diabetic Kidney Disease. *Kidney Blood Pressure Res* (2021) 46(2):152–61. doi: 10.1159/000514286
63. Islam MS, Venkatesan V. Experimentally-Induced Animal Models of Prediabetes and Insulin Resistance: A Review. *Acta Pol Pharm* (2016) 73 (4):827–34. Retrieved from: https://europepmc.org/article/med/29648707
64. Kanazawa I, Yamaguchi T, Tada Y, Yamauchi M, Yano S, Sugimoto T. Serum Osteocalcin Level is Positively Associated With Insulin Sensitivity and Secretion in Patients With Type 2 Diabetes. *Bone* (2011) 48(4):720–5. doi: 10.1016/j.bone.2010.12.020
65. Wang Q, Zhang B, Xu Y, Xu H , Zhang N. The Relationship Between Serum Osteocalcin Concentration and Glucose Metabolism in Patients With Type 2 Diabetes Mellitus. *Int J Endocrinol* (2013) 2013:1–8. doi: 10.1155/2013/842598
66. Naidoo K. Investigating the Effects of Diet-induced Pre-diabetes on Calcium Homeostasis in Male Sprague Dawley Rats. [Master's dissertation]. ResearchSpace Library [Online] University of KwaZulu-Natal (2022).

Conflict of Interest: The co-author AK of this manuscript is the editor of the Frontiers Journal Thieme (Pre-diabetes and endocrine function)

The remaining 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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Endocrinology

ORIGINAL RESEARCH
published: 22 July 2022
doi: 10.3389/fendo.2022.921067

Association Between Visceral Adiposity Index and Insulin Resistance: A Cross-Sectional Study Based on US Adults

Kai Jiang[1†], *Hong Luan*[2†], *Xiaolu Pu*[3], *Mingxiang Wang*[3], *Jiahui Yin*[4*] *and Rongpeng Gong*[3*]

[1] Department of Cardiovascular Medicine, The Bozhou Hospital Affiliated to Anhui Medical University, Bozhou, China, [2] Department of Cardiovascular Medicine,People's Hospital of Ningxia Hui Autonomous Region, YinChuan, China, [3] Medical College of Qinghai University, Xining, China, [4] College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China

OPEN ACCESS

Edited by:
Andile Khathi,
University of KwaZulu-Natal, South Africa

Reviewed by:
Luisa Fernández-Chirino,
Monterrey Institute of Technology and Higher Education (ITESM), Mexico
Ibrahim Solak,
Konya Beyhekim Training and Research Hospital, Turkey
Lishun Liu,
China Agricultural University, China

***Correspondence:**
Rongpeng Gong
gongrongpeng@qhu.edu.cn
4728955@qq.com
Jiahui Yin
1074374992@qq.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: 15 April 2022
Accepted: 22 June 2022
Published: 22 July 2022

Citation:
Jiang K, Luan H, Pu X, Wang M, Yin J and Gong R (2022) Association Between Visceral Adiposity Index and Insulin Resistance: A Cross-Sectional Study Based on US Adults. Front. Endocrinol. 13:921067. doi: 10.3389/fendo.2022.921067

Background: Visceral obesity index (VAI) is an empirical mathematical model used to evaluate the distribution and function of fat. Some studies have shown that VAI may be associated with the development of insulin resistance. In view of the differences in insulin resistance among different ethnic groups, this study attempts to analyze the special relationship between VAI and insulin resistance in American adults.

Methods: We conducted a cross-sectional study through NHANES database. A total of 27309 patients over the age of 18 from the United States took part in the survey. It was divided into two groups: the IR-positive group and the IR-negative group. The association of VAI with IR was evaluated by logistic regression analyses mainly, including univariate analysis, multivariate regression analysis, curve fitting analysis and subgroup analysis.

Results: The results showed that in the full-adjusted model, there is a strong positive association between VAI level and insulin resistance (OR: 1.28 (1.2~1.37), P<0.001) and there is a threshold effect.

Conclusions: This study suggests that higher VAI levels are associated with insulin resistance. VAI index may be used as a predictor of insulin resistance.

Keywords: cross-sectional study, american adult, VAI, IR, NHANES

BACKGROUND

Insulin resistance (IR) is a pathological condition caused by genetic and environmental factors, in which insulin promotes the decrease of glucose uptake and utilization rate, as well as the body's decreased responsiveness and sensitivity to the physiological action of insulin (1, 2). It is the pathological basis of type 2 diabetes (2–6). Recently, the prevalence of diabetes has risen rapidly in all developing and developed countries (7), and type2 diabetes is the most common type of diabetes,

Abbreviations: VAI, visceral obesity index; WC, waist circumference; TG, triglyceride; HDL-C, density lipoprotein cholesterol; ALT, alanine transaminase; GGT, γ-glutamyl transpeptadase.

accounting for approximately 90% of all people with diabetes (8, 9). It is estimated that by 2030, the number of people with type 2 diabetes will reach 439 million (10). Current studies have demonstrated that understanding IR is important for developing prevention measures and determining optimal treatment. Unfortunately, the methods available to determine IR (such as pancreatic suppression tests, high insulin-normal blood sugar tuse and glucose digestion and the minimum model of metabolism) are complex and expensive, so they apply only to small-scale studies (11–13). In view of these characteristics, it is necessary to find alternative parameters that are low-cost and convenient. Visceral obesity index (VAI) is a gender-specific mathematical index that has been proposed to assess fat distribution and function. VAI is estimated with the use of simple anthropometric [body mass index (BMI) and waist circumference (WC) and biochemical triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C)] parameters (14). VAI is generally considered a as a marker of adipose tissue dysfunction. As a simple technique, the index has been widely accepted for epidemiological or clinical research. Some recent studies have indicated that visceral obesity index was also correlated with IR (15–19). Homeostasis model assessment of high insulin-normal glucose (HIEG) forcers and IR (HOMA-IR), which is the gold standard and common method for IR assessment. Given the small sample size of previous studies, the ethnic specificity of IR, and the unexplored relationship between VAI and IR in these studies, it is necessary to conduct new, large-sample studies to understand VAI and IR.

Visceral obesity index (VAI) is an empirical mathematical model that has been proposed to assess fat distribution and function. It is a sex-specific index based on simple anthropometry (BMI and WC) and metabolic parameters (TG and HDL-C). Interesting results have been produced by the application of VAI in populations of patients with endocrine diseases with varying degrees of cardiometabolic risk, such as acromegaly, polycystic ovary syndrome, type 2 diabetes, and prolactinoma (20–24). This has led to the hypothesis that VAI can be regarded as a marker of adipose tissue dysfunction. Some recent studies have indicated that the VAI can be successfully used to detect the distribution and function of visceral fat, IR and increased cardiometabolic risk (25, 26). In a study conducted by Jablonowska-Lietz et al. (18) VAI index was also significantly correlated with glucose, insulin, HOMA-IR, and visceral adipose tissue predicted by bioimpedance analysis. Stepien et al. also suggested a positive correlation between IR and VAI in obese patients (15). In another study, Borruel et al. (19) reported that VAI levels were more strongly correlated with serum insulin levels and HOMA-IR than WC and BMI levels. Because of its simple technique, the index has been widely accepted for epidemiological or clinical research. Given the small sample size of previous studies, the ethnic specificity of IR, and the unexplored relationship between VAI and IR in these studies, it is necessary to conduct new, large-sample studies to understand VAI and IR.

Therefore, we explored the relationship between VAI index and IR in a larger and more representative sample of various ethnic groups in the United States.

METHODS

Data Sources

It was a large cross-sectional study using data from the National Institutes of Health National Health and Nutrition Examination Survey (NHANES) database for 11 cycles (1999-2020). The NHANES program is a multiagency collaboration aimed at improving the health of Americans, with a focus on diet, detailed elsewhere (27). NHANES used a multi-stage stratified probability design in a sample population to obtain a nationally representative sample of non-institutionalized civilians in the United States. Data from these samples consisted of demographic informatics data, dietary data, body measurement data, laboratory data and questionnaire data. In this study, data from 11 cycles were standardized and combined with fasting weights as recommended by the National Center for Health Statistics (NCHS).

Study Design and Participants

This study was designed to be cross-sectional. The target independent variable was the participant's VAI at the time of testing, and the target dependent variable was whether the participant was diagnosed with IR at the time of testing. Simultaneously, the occurrence of IR was divided into two groups, including 11936 patients in the positive group of IR and 15373 patients in the negative group of IR.

A total of 116,876 participants were included in NHANES 1999-2020, and 27,309 were included in the final analysis. Other participants were excluded for the following reasons: 1. Participants younger than 18 years old (n= 47979); 2. Participants who did not undergo insulin test (n= 39465); 3. Participants who were taking insulin drugs or insulin related drugs affecting metabolism (n=855); 4. Participants lack VAI data detection (n= 1268) (**Figure 1**).

Data Collection

All data were collected and recorded by uniformly trained investigators. The data used in this study included demographics (age, sex, race, education level, etc.), anthropometry (WC, BMI, etc.), health-related behaviors (smoking, drinking, etc.), and biochemical indicators (TG, VAI, etc.). Basic information was immediately collated by investigators, and biochemical samples were stored and managed scientifically before being sent to the University of Minnesota laboratory and the University of Missouri-Columbia for testing and analysis.

Measurement of VAI

VAI is a simple clinical index that integrates anthropometric data and metabolic parameters, and can better assess visceral fat. It was calculated as follows: Man = [WC(cm)/39.68 + (1.88 ×BMI)]×(TG (mmol/L)/1.03)×(1.31/HDL(mmol/L)); Women = [WC (cm)/ 36.58 + 1.89 × (BMI)] × (TG (mmol/L)/0.81) × (1.52/HDL (mmol/L)). BMI is calculated based on height and weight. Height was measured using electronic Sports Measurements (Seca Ltd, Medical Scales and Measurement Systems, Birmingham, UK) with an accuracy of millimetres. Weight was

measured by researchers using a digital Scale (Toledo Scale; Mettler-toledo, LLC, Columbus,OH, USA) and convert pounds to kilograms when the measurement is complete. The formula is BMI =weight (kg)/height (m^2). WC was measured using electronic Sports Measurements (Seca Ltd, Medical Scales and Measurement Systems, Birmingham, UK) with an accuracy of millimetres. HDL was measured by the Magnesium sulfate/glucan method and TG was measured based on the Wahlefeld method. Both HDL and TG were measured in the University of Minnesota laboratory. Please refer to the official NHANES website for more detailed information.

Measurement of Insulin Resistance

HOMA-IR is recognized by many experts as a good indicator of IR. The formula was fasting blood glucose (FPG, mmol/L) × fasting insulin (FINS, μU/mL)/22.5 (**Figure 2**). We followed previous studies that defined homA-IR index ≥ 2.73 as positive insulin resistance, and < 2.73 as negative insulin resistance. Fasting blood glucose was measured by hexokinase (HK) method, and fasting insulin was measured by insulin radioimmunoassay. Both blood sugar and insulin measurements were tested at the University of Missouri-Columbia. Enter the official NHANES website for more detailed information.

Definitions of Other Variables

Age: Adults 18 and older in the United States.

Sex: man, woman.

Race: Includes Mexican Americans, non-Hispanic whites, non-Hispanic Blacks, other Hispanics, and other races.

Education: not graduated from high school, high school, graduate and above.

Smoking: current smokers, former smokers and never smokers. Participants were considered current smokers if they had smoked 100 or more cigarettes in the past and reported smoking several days or daily at the time of the interview. Participants who had smoked fewer than 100 cigarettes in the past but did not currently smoke were considered former smokers. Participants who had fewer than 100 cigarettes in their past were considered nonsmokers. Alcohol consumption: Includes both drinkers and non-drinkers.

Alcohol consumption (minus 1 point for alcoholics) is defined as more than one drink per day for women and more than two drinks per day for men, according to the US Department of Health and Human Services/US Department of Agriculture Dietary Guidelines for Americans 2015-2020.

Hypertension: Including those with hypertension and normal blood pressure, the diagnostic criteria are SBP higher than 140mmHg and/or DBP higher than 90mmHg.

Diabetes: Including diabetic patients and people with normal blood glucose.Diabetes is diagnosed if one of the following conditions is met: (1) fasting blood glucose ≥ 7.0mmol/L, (2) OGTT ≥ 11.1mmol/L, (3) doctor's diagnosis, (4) self-report diabetes or taking diabetes drugs.

Laboratory Quality Control

NHANES Quality Control and Quality Assurance Protocols (QA/QC) meet the requirements of the Clinical Laboratory Improvement Act 1988. Detailed QA/QC instructions are discussed in the NHANES LPM.

Statistical Methods

All data were analyzed using version R 4.1.2, with continuous variables represented by a detailed sample description, an average confidence interval of 95%, and categorical variables represented by counting and weighted percentages. The normal distribution is described by median and standard deviation, and the skewness distribution is based on median and quartiles. Continuous variables were compared between groups using mann-Whitney U test or Student T test based on distribution normality. $P < 0.05$ (bilateral) was considered statistically significant. The choice of the covariate was based on the previous literature, international standards and related clinical experience of synthetically considering may influence factors of IR and visceral fat index, including sex, age, race, smoking, drinking, education degree, diabetes, hypertension. In order to maximize statistical efficiency and minimize bias, multiple imputation was used to fill in covariates within the range of missing and extreme values. In addition, sensitivity analysis was performed to observe if the new complete data were significantly varied from the original data. However, these studies revealed that there was no significant difference between the data after multiple interpolation and the original data ($P > 0.05$). Therefore, all the results of our multivariate analysis are based on the data set after multiple interpolation according to Rubin's criterion. In this study, four multivariable logistics regression models were established to analyze the relationship between VAI and IR in U.S. adults. In order to verify whether the results are inapplicable to the current population, we divided the results into groups according to sex, age, race, smoking, alcohol consumption, BMI, education level, diabetes, hypertension, etc., to observe whether the results are stable in each subgroup. Additionally, trend test was carried out to transform the VAI from continuous variable to categorical variable, and a smooth fitting curve and threshold effect model were constructed to ensure the stability of results.

RESULTS

Description of Basic Information About Participants

A total of 27309 participants were included in this study, including 11936 insulin resistance positive and 15373 insulin resistance negatives. The mean age and standard deviation of insulin resistance positive participants (49.0 ± 18.4) were higher than those of insulin resistance negative participants (45.9 ± 19.1), and the difference was significant ($p < 0.001$). There were 6,012 men (50.4%) slightly higher than 5,924 women (49.6%). In the racial distribution of the United States, non-Hispanic whites taken up the highest proportion of 4,555 cases (38.2%), while non-Hispanic blacks accounted for the lowest proportion of 1,145 cases (9.6%). BMI (32.2 vs 25.9), WC (106.9cm vs 90.7cm), TG (1.5mmol/L vs 1.0mmol/L), alanine transaminase (ALT) (23.0mmol/L vs 18.0mmol/L), γ-glutamyl transpeptadase

(GGT) (24.0mmol/L vs 17.0mmol/L), blood urea nitrogen (BUN) (4.6mmol/L vs 4.3mmol/L), VAI (2.0 vs 1.1) were higher than those in the insulin resistance negative group. Compared to insulin resistance negative group, insulin resistance positive group have higher BMI (32.2 vs 25.9), WC (106.9cm vs 90.7cm), TG (1.5mmol/L vs 1.0mmol/L), alanine transaminase (ALT) (23.0mmol/L vs 18.0mmol/L), γ-glutamyl transpeptidase (GGT) (24.0mmol/L vs 17.0mmol/L), blood urea nitrogen (BUN) (4.6mmol/L vs 4.3mmol/L), VAI (2.0 vs 1.1). The difference was significant ($P < 0.001$). In contrast, HDL (1.4mmol/L vs 1.2mmol/L) and totalbilirubin (TBIL) (12.0mmol/L vs 10.3mmol/L) in the insulin resistance negative group were higher than those in the insulin resistance positive group, and the difference was significantly ($P < 0.001$). In addition, there were differences in education level, smoking, alcohol consumption, hypertension and diabetes between the two groups (all $P < 0.001$) (**Table 1**).

Univariate Analysis

We analyzed correlations between age, sex, race, education, BMI, WC, smoking, alcohol consumption, and several biochemical markers and IR in the U.S. population. We found that age was positively correlated with IR, and the effect value OR and 95% confidence interval were 1.01 (1.01, 1.02), respectively. Compared with men, women had a lower risk of IR, with an effect value and 95%CI of 0.76 (0.65, 0.90), respectively. Among ethnic groups, non-Hispanic whites had a lower incidence of IR, with an effect value of 0.57 (95%CI, 0.42, 0.76). The incidence of IR was lower in those with higher education than in those with lower education and secondary education, and the effect value and 95%CI were 0.68 (0.54, 0.85), respectively. Compared with non-smokers, former smokers had a higher risk of IR, with an effect value and 95%CI of 1.24 (1.02, 1.51), respectively. Current smokers had a low incidence of IR, with an effect value and 95% CI of 0.81 (0.65, 1.00), respectively. Compared with non-

TABLE 1 | Basic crowd information description.

Variables	Total (n = 27309)	IR-negative (n = 15373)	IR-positive (n = 11936)	P-value
Age, Mean ± SD	47.2 ± 18.9	45.9 ± 19.1	49.0 ± 18.4	< 0.001
Gender, n (%)				< 0.001
male	13299 (48.7)	7287 (47.4)	6012 (50.4)	
female	14010 (51.3)	8086 (52.6)	5924 (49.6)	
Race, n (%)				< 0.001
Mexican American	4937 (18.1)	2350 (15.3)	2587 (21.7)	
Other Hispanic	5677 (20.8)	3107 (20.2)	2570 (21.5)	
Non-Hispanic white	11562 (42.3)	7007 (45.6)	4555 (38.2)	
Non-Hispanic black	2333 (8.5)	1188 (7.7)	1145 (9.6)	
Other races	2800 (10.3)	1721 (11.2)	1079 (9)	
Education, n (%)				< 0.001
poorly educated	6503 (23.8)	3290 (21.4)	3213 (26.9)	
Moderately educated	5877 (21.5)	3212 (20.9)	2665 (22.3)	
highly educated	13053 (47.8)	7717 (50.2)	5336 (44.7)	
NA	1876 (6.9)	1154 (7.5)	722 (6)	
BMI, Mean ± SD	28.6 ± 6.7	25.9 ± 4.9	32.2 ± 6.9	< 0.001
Waist, Mean ± SD	97.8 ± 16.2	90.7 ± 13.0	106.9 ± 15.5	< 0.001
Smoke, n (%)				< 0.001
never smoking	14303 (52.4)	8034 (52.3)	6269 (52.5)	
former smokers	6337 (23.2)	3256 (21.2)	3081 (25.8)	
Current smoker	5300 (19.4)	3238 (21.1)	2062 (17.3)	
NA	1369 (5.0)	845 (5.5)	524 (4.4)	
Alcohol use, n (%)				< 0.001
no	16185 (59.3)	8775 (57.1)	7410 (62.1)	
yes	11124 (40.7)	6598 (42.9)	4526 (37.9)	
Hypertension, n (%)				< 0.001
no	17302 (63.4)	10953 (71.3)	6349 (53.2)	
yes	10003 (36.6)	4418 (28.7)	5585 (46.8)	
DM, n (%)				< 0.001
no	4343 (15.9)	1053 (6.8)	3290 (27.6)	
yes	22966 (84.1)	14320 (93.2)	8646 (72.4)	
HDL, Median (IQR)	1.3 (1.1, 1.6)	1.4 (1.2, 1.7)	1.2 (1.0, 1.4)	< 0.001
TG, Median (IQR)	1.2 (0.8, 1.7)	1.0 (0.7, 1.4)	1.5 (1.0, 2.1)	< 0.001
ALT, Median (IQR)	20.0 (15.0, 28.0)	18.0 (14.0, 24.0)	23.0 (17.0, 32.0)	< 0.001
AST, Median (IQR)	22.0 (18.0, 27.0)	22.0 (18.0, 26.0)	22.0 (19.0, 28.0)	< 0.001
GGT, Median (IQR)	20.0 (14.0, 30.0)	17.0 (13.0, 25.0)	24.0 (17.0, 36.0)	< 0.001
TBIL, Median (IQR)	10.3 (8.6, 13.7)	12.0 (8.6, 15.4)	10.3 (6.8, 13.7)	< 0.001
BUN, Median (IQR)	4.6 (3.6, 5.7)	4.3 (3.6, 5.4)	4.6 (3.6, 5.7)	< 0.001
VAI, Median (IQR)	1.4 (0.9, 2.4)	1.1 (0.7, 1.8)	2.0 (1.3, 3.3)	< 0.001

BMI, Body Mass Index; DM, diabetes mellitus; HDL, high-density lipoprotein; TG, triglyceride; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, γ-glutamyl transpeptadase; TBIL, total bilirubin; BUN, blood urea nitrogen; VAI, visceral obesity index.

drinkers, drinkers had a lower risk of IR, with an effect value and 95%CI of 0.75 (0.63, 0.89), respectively. Meanwhile, we found that BMI, WC and some biochemical indicators were positively correlated with the occurrence of IR, including TG, ALT, GGT and VAI (**Table 2**).

Multi-Factor Analysis

We established four logistic regression models to analyze the relationship between VAI and IR, the effect value of the model can be interpreted as with the increase of VAI, the probability of IR increases correspondingly. For example, in model 1 (Unadjusted model), the incidence of IR increased by 60% with each increase of variance of VAI, and the effect value OR and 95%CI were 1.60 (1.50, 1.71), respectively. Model 2 was adjusted according to population characteristics (age, gender and race), and its effect value OR and 95%CI were 1.62 (1.51, 1.73), respectively. The effect value OR and 95%CI of model 3 were 1.29 (1.20, 1.38), respectively. The effect value OR and 95%CI of model 4 were 1.28 (1.20, 1.37), respectively. The results of model 3 and model 4 were similar, indicating that the adjustment strategy of model 4 was sufficient. Collectively, VAI is independently positively correlated with the occurrence of IR, which can be used as a predictor of IR. Further, in order to ensure the stability of the results, the trend test was carried out in this study. The VAI was transformed from continuous variable to categorical variable and grouped into four levels according to the quartiles of VAI. Q1 was taken as the reference, the incidence of VAI and IR represented a monotonically increasing trend in all models (All P for trend < 0.001). This suggests that VAI is positively correlated with the occurrence of IR and the results are stable (**Table 3**).

TABLE 2 | Univariate analysis for IR. (weight).

Variable	OR (95%CI)	P-value
Age	1.01 (1.01~1.02)	<0.001
Gender		
male	1	
female	0.76 (0.65~0.9)	0.001
Race		
Mexican American	1	
Other Hispanic	0.75 (0.52~1.07)	0.116
Non-Hispanic white	0.57 (0.42~0.76)	<0.001
Non-Hispanic black	0.82 (0.53~1.25)	0.359
Other races	0.61 (0.4~0.92)	0.017
Education		
poorly educated	1	
Moderately educated	0.86 (0.66~1.1)	0.23
highly educated	0.68 (0.54~0.85)	0.001
BMI	1.25 (1.22~1.27)	<0.001
Waist	1.1 (1.09~1.11)	<0.001
Smoke		
never smoking	1	
former smokers	1.24 (1.02~1.51)	0.029
Current smoker	0.81 (0.65~1)	0.052
Alcohol1 use		
no	1	
yes	0.75 (0.63~0.89)	0.001
HDL	0.13 (0.1~0.17)	<0.001
TG	2.11 (1.89~2.36)	<0.001
ALT	1.03 (1.02~1.04)	<0.001
AST	1.01 (1~1.01)	0.02
GGT	1.01 (1.01~1.02)	<0.001
TBIL	0.96 (0.94~0.98)	<0.001
BUN	1.08 (1.03~1.13)	0.001
VAI	1.6 (1.5~1.71)	<0.001

BMI, Body Mass Index; DM, diabetes mellitus; HDL, high-density lipoprotein; TG, triglyceride; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, γ-glutamyl transpeptadase; TBIL, total bilirubin; BUN, blood urea nitrogen.

Subgroup Analysis

We did a subgroup analysis by age, education, BMI, and so on to observe if the results were not applicable to the current population. As shown in **Figure 3**, the relationship between VAI and insulin resistance remained stable across all subgroups, including age, education, BMI, diabetes, race, and sex.

Curve Fitting and Threshold Effect Analysis

Here, a smooth curve fitting diagram was drawn to visually describe the relationship between VAI and IR, and the linear relationship was tested. As shown in **Figure 3**, the correlation between VAI and insulin resistance is sloping, and P for non-linearity < 0.001, which indicates that the correlation between VAI and IR cannot be assessed by a single logistics regression equation. Therefore, the threshold effect is analyzed. As shown in **Table 4**, there was a threshold effect between VAI and insulin resistance, with an inflection point of 1.92. After adjustment according to model 4, the effect value OR and 95%CI on the left side of inflection point were 2.647 (2.406, 2.913), respectively. The OR and 95%CI on the right side of inflection point were 1.327 (1.245, 1.414), respectively. Moreover, the effect values on the left and right sides of the inflection point are different, and P for Likelihood Ratio test is less than 0.001.

DISCUSSION

This is a large cross-sectional study using 11 cycles of data from the NHANES database to investigate the association between VAI index and IR in America adults. The results revealed that VAI was independently positively correlated with the incidence of IR among all ethnic groups in the United States. and could be used as one of the predictors of IR.

In current clinical practice, heterogeneity in insulin measurement between laboratories in various countries is ubiquitous, which clearly raise the cost and accuracy of determining IR. Therefore, a simple and convenient IR determination system is extremely vital for clinical purposes. VAI determined by WC, BMI, fasting TG, and HDL-C has been established and is considered a more comprehensive IR indicator. VAI exhibit simple, accessible, cheap and at the forefront of glucose and insulin, unlike gold standard HIEG forceps and other IR replacement markers which are complex, time-consuming and costly and dependent on glucose and insulin. The study confirmed a significant association between VAI and HIEG clamps and insulin resistance (16, 17), indicating

TABLE 3 | The association between VAI and IR in a multiple logistics regression model.

Variable	n(%)	Model 1		Model 2		Model 3		Model 4	
		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
VAI	27309	1.6 (1.5~1.71)	<0.001	1.62 (1.51~1.73)	<0.001	1.29 (1.2~1.38)	<0.001	1.28 (1.2~1.37)	<0.001
VAI group									
Q1	6827	1(Ref)		1(Ref)		1(Ref)		1(Ref)	
Q2	6827	2.55 (1.91~3.4)	<0.001	2.69 (2.01~3.61)	<0.001	1.87 (1.34~2.61)	<0.001	1.79 (1.28~2.5)	0.001
Q3	6827	5.52 (4.18~7.29)	<0.001	6.01 (4.51~8.03)	<0.001	3.06 (2.2~4.27)	<0.001	2.79 (2~3.91)	<0.001
Q4	6828	12.27 (9.25~16.28)	<0.001	13.73 (10.22~18.45)	<0.001	5.73 (4.07~8.06)	<0.001	5.03 (3.56~7.12)	<0.001
P for trend	27309	2.27 (2.09~2.47)	<0.001	2.35 (2.15~2.57)	<0.001	1.77 (1.6~1.97)	<0.001	1.69 (1.52~1.88)	<0.001

Model 1: non-adjusted.
Model 2: adjutesd age, gender, race.
Model 3: adjutesd age, gender, race, education, smoke, Alcohol use, diabetes, hypertension.
Model 3: adjutesd age, gender, race, education, smoke, Alcohol use, diabetes, hypertension, ALT, AST, GGT, BUN.

the great potential of VAI as a useful indicator of accurate IR levels.

In a previous study on the relationship between VAI and IR, Randria Arisoa et al. (28)reported that VAI was positively correlated with HOMA-IR in non-diabetic Germans ($\beta = 0.42$, $P < 0.0001$). A prospective cohort study conducted by Ji et al. (29) also found that very high VAI was the main risk factor for the rise of HOMA-IR in Chinese adults. Similarly, Stepien et al. (15) also revealed a positive correlation between IR and VAI levels in obese patients. In another study, Borruel et al. (19) suggested that VAI levels were more strongly correlated with serum insulin levels and HOMA-IR than WC and BMI levels. In the Framingham Heart Study, Preis et al. (30) reported that visceral adipose tissue and abdominal subcutaneous adipose

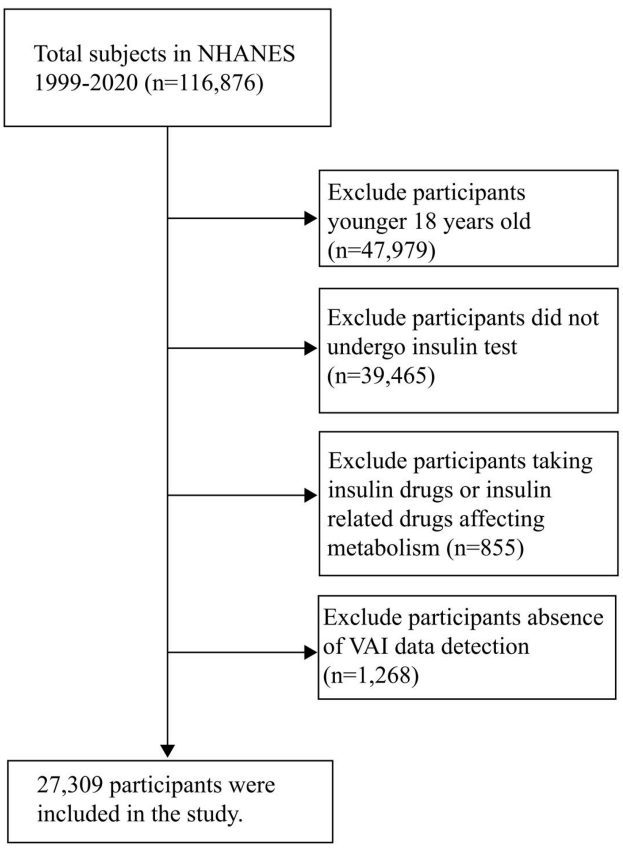


FIGURE 1 | Flowchart of patient selection.

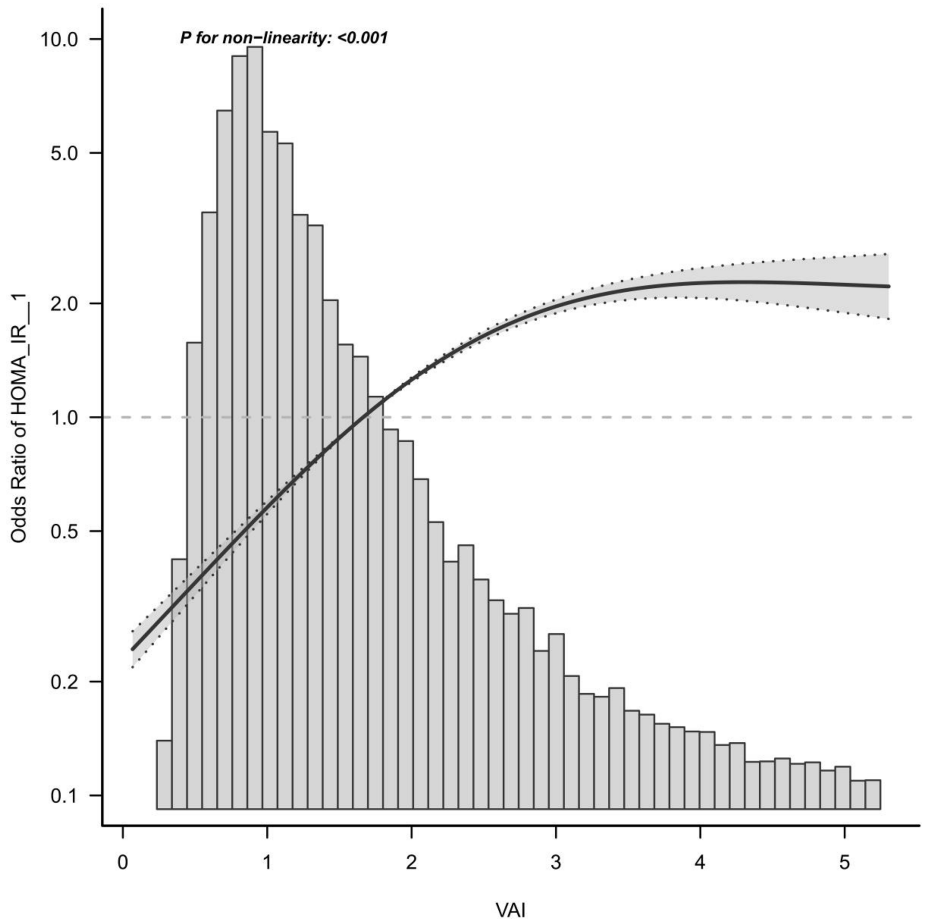


FIGURE 2 | Curve fitting analysis of VIA and IR.

tissue were positively correlated with IR, and that visceral adipose tissue was more strongly correlated with IR than abdominal subcutaneous adipose tissue.

The possible mechanism of the relationship between VAI and insulin resistance is as follows:Visceral adipocytes secrete adipose-specific cytokines, such as leptin and adiponectin, as well as inflammatory cytokines (tumor necrosis factor-α and interleukin 6), which increase IR (31, 32). Macrophages accumulate in visceral adipose tissue and release these inflammatory cytokines, including tumor necrosis factor-α and interleukin-6, which impair insulin sensitivity (33). Excess adipose tissue can promote inflammation by increasing the level of resistin or tumor necrosis factor-α, thus increasing IR (34, 35). Reduced adiponectin levels associated with excess adipose tissue can exacerbate metabolic disorders and IR (36).

Several limitations of this study should be mentioned. (1) The time span is long, and there are differences in the methods used to determine IR. However, more samples are allowed to be included according to our current approach, and the detection method is very clear. Considering that all samples are tested by professional testing institutions, this effect can be ignored. (2) Certain deviations are inevitable in cross-sectional studies. We will conduct cohort studies in the future when conditions permit. (3) This study assessed IR using HOMA-IR index. The "gold standard" method (e.g., hyperinsulin-normal glucose clamp and hyperglycemic clamp tests) is more accurate than the HOMA index in measuring IR. Although HOMA indices are not "gold standard" methods, they may be more suitable in large epidemiological studies (37). Because of the limited sample size, we can't analyze special populations and other ethnic groups. Therefore, whether this result is applicable to special populations and populations in other countries needs further research. We will collect these samples for analysis in future studies to cover the deficiencies of this study.

Despite these limitations, there are some significant advantages. (1) The data used is large and generalized. (2) NHANES is an internationally recognized high-quality database exhibit comprehensive and reliable, which greatly enriches research data. (3) Our study used curve fitting and threshold effect analysis to further analyze the relationship between VAI and IR. (4) A more advanced multiple interpolation method was adopted to deal with missing data,

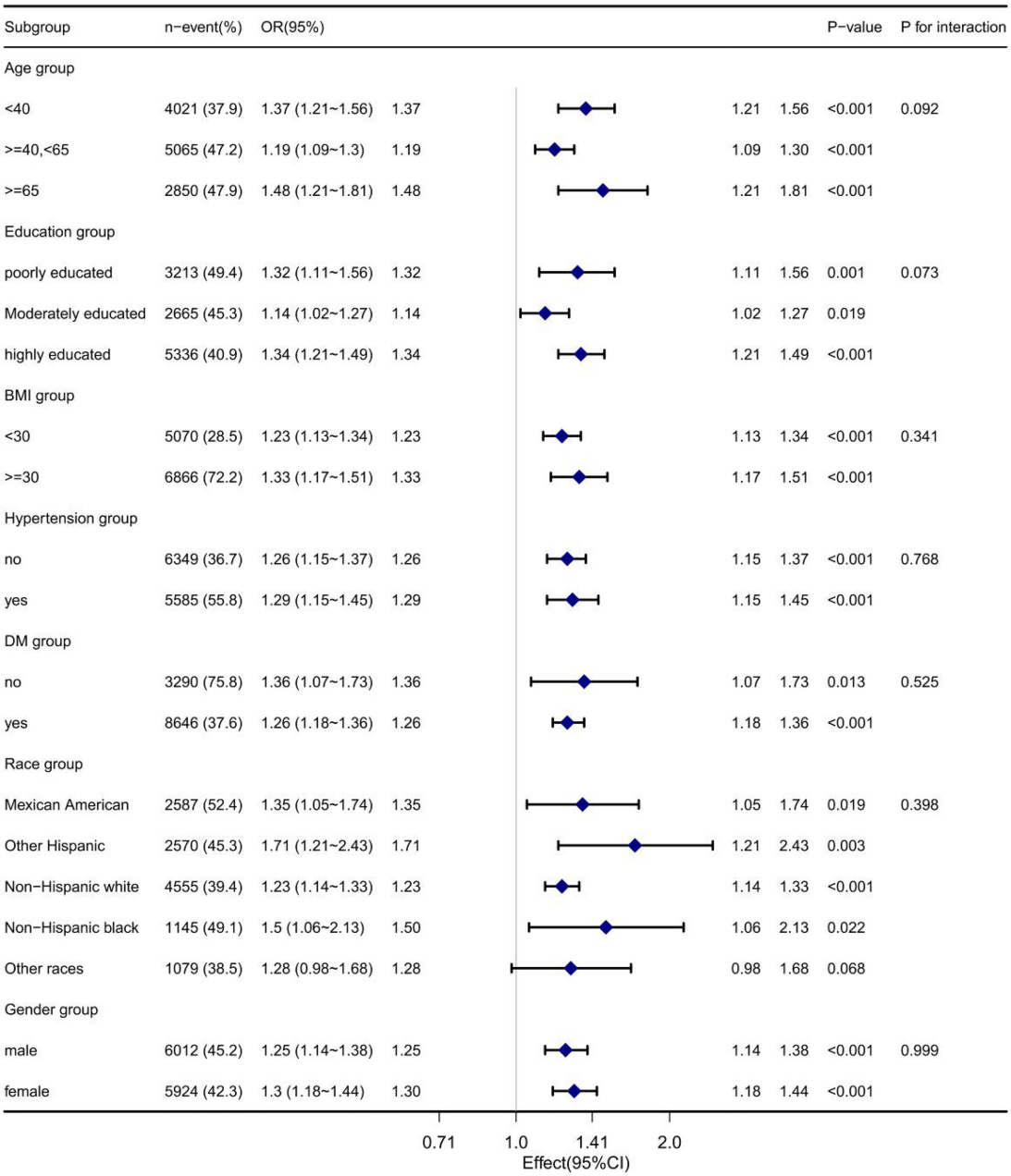


FIGURE 3 | Subgroup analyses of the association between VIA and IR.

TABLE 4 | Threshold effect analysis.

Outcome	OR (95%)	*P*-vale
Break Point	1.92 (1.883,1.956)	NA
slope1	2.647 (2.406~2.913)	<0.001
slope2	1.327 (1.245~1.414)	<0.001
Likelihood Ratio test	–	<0.001
Non-linear Test*1	–	<0.001
Non-linear Test*2	–	<0.001

Covariates are adjusted using variables in Model 4.

and the sensitivity of interpolation data was analyzed. The results indicated that the interpolated data not much differed from the original data, which makes our results more convincing.

CONCLUSION

This study explored the relationship between VAI and IR in depth. The association between VAI and IR was a threshold effect after adjustment for potential confounders. At the right of

the inflection point, the association between VAI and IR was weakened, which has great significance for the further development of predictive models of IR in the U.S. population. However, the causal relationship between VAI and IR cannot be determined owing to the cross-sectional nature of this study, and a large number of prospective studies are still needed to investigate.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/.

AUTHOR CONTRIBUTIONS

RG and KJ conceived the idea. RG, KJ, and MW wrote the manuscript. RG read through and corrected the manuscript. KJ is the first author. RG and JY are the corresponding authors of this paper. All authors contributed to the article and approved the submitted version.

FUNDING

This study was Supported by the Young Teachers Research Fund of Anhui University of Science and Technology (QN2018128).

REFERENCES

1. Xu Z, Gong R, Luo G, Wang M, Li D, Chen Y, et al. Association Between Vitamin D3 Levels and Insulin Resistance: A Large Sample Cross-Sectional Study. *Sci Rep* (2022) 12(1):119.
2. Liu K, Jin X, Zhang X, Lian H, Ye J. The Mechanisms of Nucleotide Actions in Insulin Resistance. *J Genet Genomics* (2022) 49(4):299–307.
3. Xing H, Lu J, Yoong SQ, Tan YQ, Kusuyama J, Wu XV, et al. Effect of Aerobic and Resistant Exercise Intervention on Inflammaging of Type 2 Diabetes Mellitus in Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis. *J Am Med Dir Assoc* (2022) 23(5):823–830.e13.
4. Gong R, Tang X, Jiang Z, Luo G, Dong C, Han X, et al. Serum 25(Oh)D Levels Modify the Association Between Triglyceride and IR: A Cross-Sectional Study. *Int J Endocrinol* (2022) 2022:5457087.
5. Liu Y, Gong R, Luo G, Li J, Li Q, Yang L, et al. Associations of Triglycerides/High-Density Lipoprotein Cholesterol Ratio With Insulin Resistance, Impaired Glucose Tolerance, and Diabetes in American Adults at Different Vitamin D3 Levels. *Front Endocrinol (Lausanne)* (2021) 12:735736.
6. Gong R, Liu Y, Luo G, Yang L. Dietary Magnesium Intake Affects the Vitamin D Effects on HOMA-β and Risk of Pancreatic β-Cell Dysfunction: A Cross-Sectional Study. *Front Nutr* (2022) 9:849747.
7. Gong R, Xu Z, Wei X. The Association Between Vitamin D3 and Diabetes in Both Hyperuricemia and non-Hyperuricemia Populations. *Endocrine* (2021) 74(1):90–9.
8. Gong R, Liu Y, Luo G, Liu W, Jin Z, Xu Z, et al. Associations of TG/HDL Ratio With the Risk of Prediabetes and Diabetes in Chinese Adults: A Chinese Population Cohort Study Based on Open Data. *Int J Endocrinol* (2021) 2021:9949579.
9. Neuenschwander M, Ballon A, Weber KS, Norat T, Aune D, Schwingshackl L, et al. Role of Diet in Type 2 Diabetes Incidence: Umbrella Review of Meta-Analyses of Prospective Observational Studies. *Bmj* (2019) 366:l2368.
10. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention. *Int J Med Sci* (2014) 11(11):1185–200.
11. De Fronzo RA, Tobin JD, Andres R. Glucose Clamp Technique: A Method for Quantifying Insulin Secretion and Resistance. *Am J Physiol* (1979) 237(3):E214–23.
12. Bergman RN, Prager R, Volund A, Olefsky JM. Equivalence of the Insulin Sensitivity Index in Man Derived by the Minimal Model Method and the Euglycemic Glucose Clamp. *J Clin Invest* (1987) 79(3):790–800.
13. Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G. Assessment of Insulin Resistance With the Insulin Suppression Test and the Euglycemic Clamp. *Diabetes* (1981) 30(5):387–92.
14. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, et al. Visceral Adiposity Index: A Reliable Indicator of Visceral Fat Function Associated With Cardiometabolic Risk. *Diabetes Care* (2010) 33(4):920–2.
15. Stepien M, Stepien A, Wlazel RN, Paradowski M, Rizzo M, Banach M, et al. Predictors of Insulin Resistance in Patients With Obesity: A Pilot Study. *Angiology* (2014) 65(1):22–30.
16. Fiorentino TV, Marini MA, Succurro E, Andreozzi F, Sesti G. Relationships of Surrogate Indexes of Insulin Resistance With Insulin Sensitivity Assessed by Euglycemic Hyperinsulinemic Clamp and Subclinical Vascular Damage. *BMJ Open Diabetes Res Care* (2019) 7(1):e000911.
17. Lee J, Kim B, Kim W, Ahn C, Choi HY, Kim JG, et al. Lipid Indices as Simple and Clinically Useful Surrogate Markers for Insulin Resistance in the U.S. Population. *Sci Rep* (2021) 11(1):2366.
18. Jabłonowska-Lietz B, Wrzosek M, Włodarczyk M, Nowicka G. New Indexes of Body Fat Distribution, Visceral Adiposity Index, Body Adiposity Index, Waist-to-Height Ratio, and Metabolic Disturbances in the Obese. *Kardiol Pol* (2017) 75(11):1185–91.
19. Borruel S, Moltó JF, Alpañés M, Fernández-Durán E, Álvarez-Blasco F, Luque-Ramírez M, et al. Surrogate Markers of Visceral Adiposity in Young Adults: Waist Circumference and Body Mass Index are More Accurate Than Waist Hip Ratio, Model of Adipose Distribution and Visceral Adiposity Index. *PloS One* (2014) 9(12):e114112.
20. Ciresi A, Amato MC, Pizzolanti G, Galluzzo Giordano C. Visceral Adiposity Index is Associated With Insulin Sensitivity and Adipocytokine Levels in Newly Diagnosed Acromegalic Patients. *J Clin Endocrinol Metab* (2012) 97 (8):2907–15.
21. Ciresi A, Amato MC, Pivonello R, Nazzari E, Grasso LF, Minuto F, et al. The Metabolic Profile in Active Acromegaly is Gender-Specific. *J Clin Endocrinol Metab* (2013) 98(1):E51–9.
22. Amato MC, Verghi M, Galluzzo A, Giordano C. The Oligomenorrhoic Phenotypes of Polycystic Ovary Syndrome are Characterized by a High Visceral Adiposity Index: A Likely Condition of Cardiometabolic Risk. *Hum Reprod* (2011) 26(6):1486–94.
23. Bozorgmanesh M, Hadaegh F, Azizi F. Predictive Performance of the Visceral Adiposity Index for a Visceral Adiposity-Related Risk: Type 2 Diabetes. *Lipids Health Dis* (2011) 10:88.
24. Ciresi A, Amato MC, Guarnotta V, Lo Castro F, Giordano C. Higher Doses of Cabergoline Further Improve Metabolic Parameters in Patients With Prolactinoma Regardless of the Degree of Reduction in Prolactin Levels. *Clin Endocrinol (Oxf)* (2013) 79(6):845–52.
25. de Koning L, Merchant AT, Pogue J, Anand SS. Waist Circumference and Waist-to-Hip Ratio as Predictors of Cardiovascular Events: Meta-Regression Analysis of Prospective Studies. *Eur Heart J* (2007) 28(7):850–6.
26. Goldani H, Adami FS, Antunes MT, Rosa LH, Fassina P, Quevedo Grave MT, et al. Applicatility Of The Visceral Adiposity Index (Vai) In The Prediction Of The Components Of The Metabolic Syndrome In Elderly. *Nutr Hosp* (2015) 32(4):1609–15.
27. Ahluwalia N, Dwyer J, Terry A, Moshfegh A, Johnson C. Update on NHANES Dietary Data: Focus on Collection, Release, Analytical Considerations, and Uses to Inform Public Policy. *Adv Nutr* (2016) 7(1):121–34.
28. Randrianarisoa E, Lehn-Stefan A, Hieronimus A, Rietig R, Fritsche A, Machann J, et al. Visceral Adiposity Index as an Independent Marker of Subclinical Atherosclerosis in Individuals Prone to Diabetes Mellitus. *J Atheroscler Thromb* (2019) 26(9):821–34.
29. Ji B, Qu H, Wang H, Wei H, Deng H. Association Between the Visceral Adiposity Index and Homeostatic Model Assessment of Insulin Resistance in Participants With Normal Waist Circumference. *Angiology* (2017) 68(8):716–21.

30. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, et al. Abdominal Subcutaneous and Visceral Adipose Tissue and Insulin Resistance in the Framingham Heart Study. *Obes (Silver Spring)* (2010) 18(11):2191–8.
31. Rytka JM, Wueest S, Schoenle EJ, Konrad D. The Portal Theory Supported by Venous Drainage-Selective Fat Transplantation. *Diabetes* (2011) 60(1):56–63.
32. Makki K, Froguel P, Wolowczuk I. Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. *ISRN Inflammation* (2013) 2013:139239.
33. Hardy OT, Czech MP, Corvera S. What Causes the Insulin Resistance Underlying Obesity? *Curr Opin Endocrinol Diabetes Obes* (2012) 19(2):81–7.
34. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The Hormone Resistin Links Obesity to Diabetes. *Nature* (2001) 409 (6818):307–12.
35. Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB Sr., Wilson PW, et al. Associations of Adiponectin, Resistin, and Tumor Necrosis Factor-Alpha With Insulin Resistance. *J Clin Endocrinol Metab* (2008) 93(8):3165–72.
36. Coker RH, Williams RH, Yeo SE, Kortebein PM, Bodenner DL, Kern PA, et al. Visceral Fat and Adiponectin: Associations With Insulin Resistance are Tissue-Specific in Women. *Metab Syndr Relat Disord* (2009) 7(1):61–7.
37. Wallace TM, Levy JC, Matthews DR. Use and Abuse of HOMA Modeling. *Diabetes Care* (2004) 27(6):1487–95.

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 04 January 2023
DOI 10.3389/fpubh.2022.1045141

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal,
South Africa

REVIEWED BY
Chenyu Sun,
AMITA Health Saint Joseph Hospital
Chicago, United States
Wenle Li,
Xiamen University, China
Qilin Yang,
The Second Affiliated Hospital of
Guangzhou Medical University, China

*CORRESPONDENCE
Lin Ji
✉ kkw190721@163.com
Hang Zhao
✉ zhaohang17777@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Public Health

RECEIVED 15 September 2022
ACCEPTED 05 December 2022
PUBLISHED 04 January 2023

CITATION
Wang X, Li H, Ji L, Cang J and Zhao H (2023) Association between aspartate aminotransferase to alanine aminotransferase ratio and the risk of diabetes in Chinese prediabetic population: A retrospective cohort study.
Front. Public Health 10:1045141.
doi: 10.3389/fpubh.2022.1045141

Association between aspartate aminotransferase to alanine aminotransferase ratio and the risk of diabetes in Chinese prediabetic population: A retrospective cohort study

Xiaoqing Wang[1†], He Li[2†], Lin Ji[3*], Jing Cang[1†] and Hang Zhao[3*]

[1]Department of Anaesthesiology, Zhongshan Hospital, Fudan University, Shanghai, China, [2]Department of Anesthesiology, Affiliated Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [3]Department of Anesthesiology, Yancheng Third People's Hospital, The Yancheng School of Clinical Medicine of Nanjing Medical University, Yancheng, Jiangsu, China

Background: Accumulating evidence has revealed that the aspartate aminotransferase to alanine aminotransferase (AST/ALT) ratio is a promising novel biomarker for insulin resistance (IR) and metabolic diseases. However, research on the association between the AST/ALT ratio and the incidence of diabetes progressing from prediabetes remains lacking. Herein, this study aimed to evaluate the relationship between the baseline AST/ALT ratio and risks of diabetes in patients with prediabetes.

Methods: This was a retrospective cohort study involving a total of 82,683 participants across 32 regions and 11 cities in China from 2010 to 2016. Data was obtained based on the DATADRYAD database from the health check screening program. Participants were stratified according to the interquartile range of the AST/ALT ratio (groups Q1 to Q4). The Cox proportional hazard model and smooth curve fitting were used to explore the relationship between the baseline AST/ALT ratio and the risk of diabetes in prediabetic patients. In addition, subgroup analysis was used to further validate the stability of the results.

Results: The mean age of the selected participants was 49.9 ± 14.0 years, with 66.8% of them being male. During the follow-up period 1,273 participants (11.3%) developed diabetes progressing from prediabetes during the follow-up period. Participants who developed diabetes were older and were more likely to be male. The fully-adjusted Cox proportional hazard model revealed that the AST/ALT ratio was negatively associated with the risk of diabetes in prediabetic patients (HR = 0.40, 95% CI: 0.33 to 0.48, $P < 0.001$). Higher AST/ALT ratio groups (Q4) also presented with a lower risk of progressing into diabetes (HR = 0.35, 95% CI: 0.29 to 0.43, $P < 0.001$, respectively) compared with the lowest quintile group (Q1). Through subgroup analysis and interaction tests, it was found that the association stably existed in all subgroup variables, and there

were a stronger interactive effects in people with age <45 years, and TG ≤ 1.7 mmol/L in the association between AST/ALT ratio and diabetes incidences in patients with prediabetes (*P* for interaction <0.05).

Conclusion: According to our study, a higher AST/ALT ratio is associated with a lower risk of progressing into diabetes from prediabetes. Regular monitoring of AST/ALT ratio dynamics and corresponding interventions can help prevent or slow prediabetes progression for diabetes.

KEYWORDS

aspartate aminotransferase, alanine aminotransferase, prediabetes, association, Kaplan-Meier curve, subgroup analysis

Introduction

Diabetes is emerging as one of the most important public health challenges of the 21st century. The World Health Organization (WHO) documents that diabetes caused an estimated 1.6 million deaths in 2016 and was the seventh leading cause of death globally (1). By the end of 2015, the global number of people with diabetes had reached 415 million. And this number is predicted to increase to 642 million by 2040 (2, 3). The prevalence of diabetes among adults in China has been reported to be as high as 12.8%, with the total number of patients in mainland China estimated at 129.8 million, which ranks first in the world. By 2,035, the number of diabetes cases in China is expected to reach 143 million (4, 5). Diabetes is usually accompanied by severe complications, including retinopathy and blindness, renal failure, heart failure, coronary artery disease, stroke, as well as peripheral neuropathy (6, 7). In 2015, the total cost for treating diabetes and its associated complications was USD 673 billion, which is anticipated to increase to USD 802 billion by the year 2040 (8). Diabetes and its complications have a detrimental impact on patients, families, and society, and impose a significant economic burden. Thus, early diagnosis and intervention are important, especially for prediabetes, to reduce the serious harm caused by diabetes and its complications.

Prediabetes is defined as blood glucose concentrations above normal but below the threshold for diabetes. It includes impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Recent studies indicate that more than one-third of individuals have prediabetes in China. It is estimated that up to 70% of people with pre-diabetes will develop diabetes over many years (9, 10). Most people with prediabetes do not have any obvious clinical symptom, which is usually ignored by people. In fact, prediabetes is a high-risk metabolic state that predicts an increased probability of developing diabetes and may itself be associated with health risks and complications (9). Recent studies have shown that prediabetes is associated with an increased risk of all-cause mortality and cardiovascular disease, such as atherosclerotic cardiovascular disease, heart failure, etc (11–13). However, currently, there are no specific and feasible methods for diabetes or prediabetes prediction. Therefore, there is an urgent global need for simple, sensitive, and cost-effective screening strategies to enhance the early identification and prevention of diabetes or prediabetes.

The liver is a key organ of systemic metabolism and contributes significantly to the development of insulin resistance and type 2 diabetes mellitus (T2DM) (14–16). Non-alcoholic fatty liver disease (NAFLD) has a bidirectional association with T2DM. Patients with NAFLD usually have IR. Meanwhile, many T2DM patients develop NAFLD with the inflammatory complication, nonalcoholic steatohepatitis (NASH) (16). The major serum liver enzymes (AST and ALT) are the most sensitive indicators for the clinical evaluation of liver cell damage and death (17). A well-characterized multiethnic cohort trial named the Insulin Resistance Atherosclerosis Study (IRAS) found that liver injury markers, including AST and ALT, were closely associated with T2DM risk (18). The serum AST/ALT ratio concept was first proposed by De Ritis and is known as the De Ritis Ratio. The De Ritis ratio is used to diagnose various chronic liver diseases, including alcoholic and non-alcoholic fatty liver diseases (NAFLD), hepatitis, and autoimmune liver diseases (19–22). Besides, this ratio is also associated with other non-liver diseases, such as metabolic syndromes (MS), T2DM, cardiovascular diseases, acute stroke, and several malignant tumors (23–28). Several studies have investigated the relationship between the indicators of liver function (AST, ALT, and AST/ALT) and the risk of T2DM (29–33). However, studies of the relationship between the AST/ALT ratio and the risk of diabetes in pre-diabetic patients are very limited currently. Therefore, the purpose of this study was to clarify the association between the AST/ALT ratio and the risk of progression to diabetes from prediabetes in Chinese adults.

Methods

Data sources

We obtained the using data from the Dryad Digital Repository (www.Datadryad.org). The Dryad Digital Repository is a Data Platform aiming to make published scientific articles shareable, freely available for secondary use, and citable. We downloaded the raw data free of charge. In the present study, we cited the Dryad data package (Data from: Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. Dryad Digital Repository. https://doi.org/10.1136/bmjopen-2018-021768) (34). Since Chen et.al have authorized the ownership of the original data to the datadryad website. According to Dryad's Terms of Service, we can use this data to perform secondary data analysis on a different hypothesis without infringing on the authors' rights. The initial study conducted by Chen et.al was approved by the Rich Healthcare Group review committee. As this study was retrospective, no ethical approval was required for this secondary analysis. This study followed the principles of the Declaration of Helsinki. All methods were carried out in accordance with the relevant guidelines and regulations, including the statements in the declarations section. All reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (35).

Study participants

In the present study, we retrospectively analyzed data of 6,85,277 adult Chinese participants aged 20 years and over, who underwent a healthy screening at least two visits between 2010 and 2016 across 32 sites and 11 cities in China (Beijing, Shanghai, Guangzhou, Shenzhen, Nanjing, Wuhan, Hefei, Chengdu, Suzhou, Changzhou, Nantong). These clinical records were extracted from a computerized database established by the Rich Healthcare Group in China. This study was approved by the Rich Healthcare Group Review Board before data collection. After initial screening 6,74,031 participants were excluded due to different reasons presented in Figure 1. Consequently 11,246 participants were finally included to assess the relationship between the AST/ALT ratio and incidence of diabetes in the prediabetic population.

Study design and measurement of covariant

As mentioned in the previous research, participants were asked to finish a questionnaire concerning general demographic (age, gender), living habits (smoking status, drinking status), personal health and medication history, and family history of chronic disease (family history of diabetes) in as much detail as possible at each healthy examination. Height, weight, and blood pressure were measured by trained staff. BMI was obtained by dividing weight (kg) by the square of height (m). Blood pressure was measured with a standard mercury sphygmomanometer. Drinking status is divided into current drinker, ever drinker, and never drinker. Smoking status is divided into current smoker, ever smoker, and never smoker. For all participants, fasting venous blood samples were collected after at least 10 h of fasting for each examination. Serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), AST, and ALT were measured using autoanalyzer (Beckman 5800). Determination of serum glucose levels using the glucose oxidase method on an automated analyser (Beckman 5800). The target independent variable is AST/ALT ratio obtained at baseline and recorded as a continuous variable. The dependent variable is the incidence of diabetes progressing from prediabetes (dichotomous variable).

Definitions

The AST/ALT ratio was defined as AST divided by ALT. The diagnostic criteria of diabetes were described as follows: fasting plasma glucose $>$ 7.00 mmol/L and/or self-reported diabetes during the follow-up period (34). According to the American Diabetes Association 2022 criteria, prediabetes is defined as participants who had an FPG level between 5.6 and 6.9 mmol/L (36).

Statistical analysis

In order to investigate whether the AST/ALT ratio of the selected participants in the prediabetic stage is related to the incidence of diabetes, the procedure of statistical analysis includes 6 main steps. First, for missing values, we used multiple imputations to replace based on 5 replications. Second, the baseline characteristics of participants were described according to the quartile of AST/ALT ratio and with/ without diabetes. Continuous variables were presented using the mean and the standard deviation (SD) when normally distributed or otherwise the median and interquartile range (IQR). Categorical variables were presented as proportions and percentages of the total. Comparisons between groups were assessed using the X2 test or Fisher's exact test (for categorical variables) and Student *t*-test (for continuous variables) or Mann-Whitney U-test (for continuous variables). Third, univariate and multivariate cox proportional hazards models were built to evaluate HR with a 95% confidence interval (CI) of diabetes for AST/ALT ratio. Fourth, we used the cox proportional hazards model to calculate

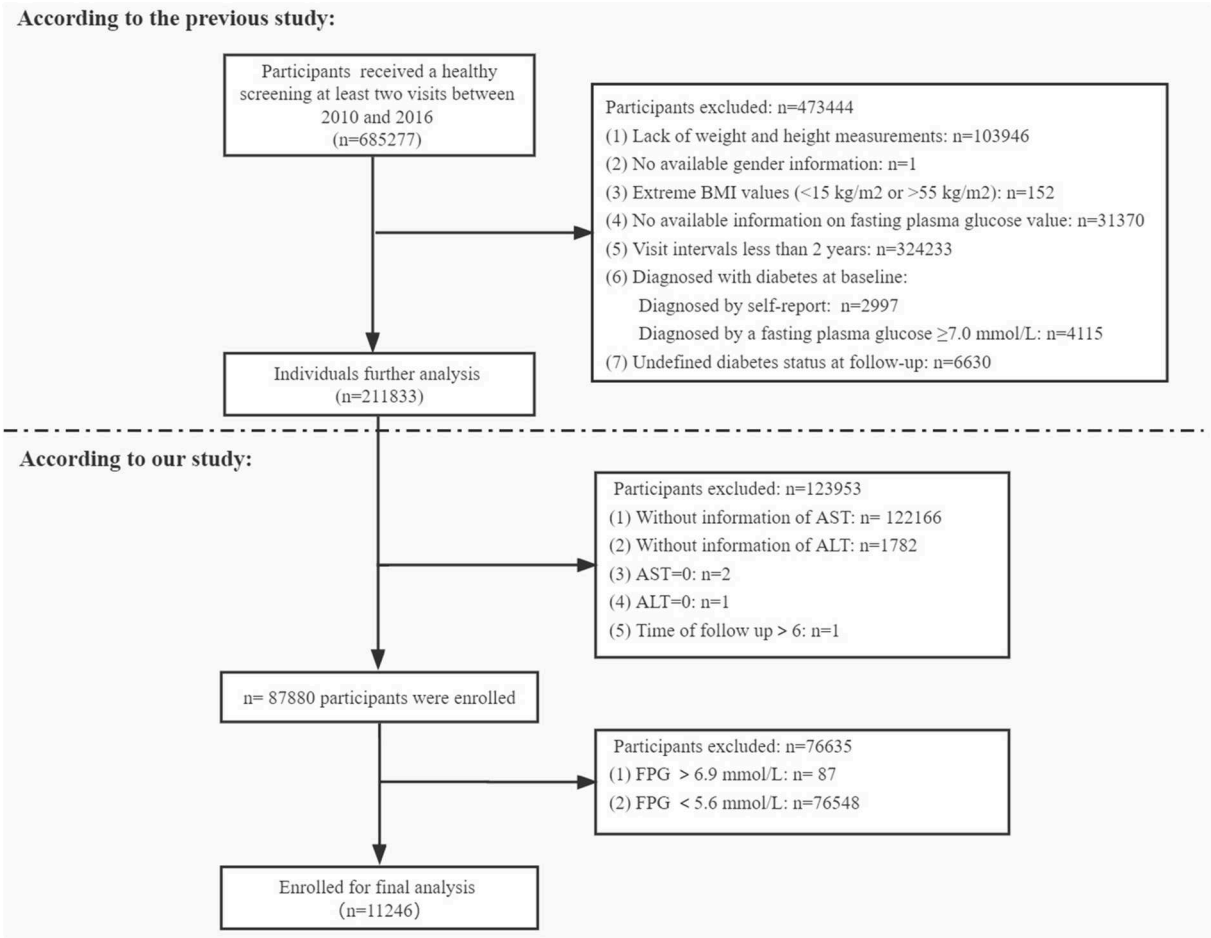


FIGURE 1
Flow chart of the study population.

the association between AST/ALT ratio and the incident diabetes with baseline AST/ALT ratio fitted as continuous (per SD increment) or categorical (tertiles) variables. Model I did not adjust any confounders. Model II adjusted for age and gender. Model III additionally adjusted for age, gender, BMI, SBP, DBP, TG, TC, HDL-C, LDL-C, BUN, Scr, smoking status, drinking status, and family history of diabetes. Fifth, the restricted cubic spline model was used for the dose-response analysis. Sixth, based on stratified cox proportional hazard models, we assessed the consistency of the association between the AST/ALT ratio and the incidence of diabetes by subgroup analyses. We transformed continuous variables into categorical variables regarding clinical cut points or using quartile, and then tested for interaction. Finally, the Kaplan–Meier probabilities of diabetes-free survival were compared using the log-rank test among the quartile of AST/ALT ratio. All the analyses were performed with R statistics software and FreeStatistics software. A two-tailed test was performed and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of selected participants

A total of 11,246 participants were selected for the final data analysis (Figure 1). The mean age of the selected participants was 49.9 ± 14.0 years, of which approximately 66.8% were male. The average follow-up year was 3.0 ± 0.9 years, and 1,273 participants (11.3%) developed diabetes progressing from prediabetes during the follow-up period. The mean value of the AST/ALT ratio was 1.2 ± 0.5, and the mean value of BMI was 24.8 ± 3.3 kg/m2. The baseline characteristics of participants based on quartiles of AST/ALT ratio and the proportion of diabetes occurring were presented in Tables 1, 2, respectively. Among quartiles of the AST/ALT ratio, there were great differences in all baseline characteristics. Participants with the highest quartiles of the AST/ALT ratio had the lowest BMI, SBP, DBP, TC, TG, LDL-C, BUN, and Scr ($P < 0.05$;

10.3389/fpubh.2022.1045141

TABLE 1 Baseline characteristics of participants stratified by quartiles of AST/ALT ratio.

Variables	AST/ALT ratio quartile				*P*-value
	Q1 (<0.8155)	Q2 (0.8155–1.0830)	Q3 (1.0830–1.4149)	Q4 (>1.4149)	
Participants	2,809	2,800	2,825	2,812	
Age (years)	44.3 ± 11.2	49.8 ± 12.9	52.3 ± 14.2	53.4 ± 15.6	< 0.001
Gender, *n (%)*					< 0.001
Male	2,431 (86.5)	2,075 (74.1)	1,719 (60.8)	1,291 (45.9)	
Female	378 (13.5)	725 (25.9)	1,106 (39.2)	1,521 (54.1)	
BMI (Kg/m2)	26.5 ± 3.2	25.3 ± 3.1	24.3 ± 3.1	23.2 ± 3.1	< 0.001
SBP (mmHg)	128.4 ± 16.0	127.6 ± 17.3	127.8 ± 18.5	126.6 ± 18.9	0.002
DBP (mmHg)	80.4 ± 10.7	78.9 ± 10.9	78.1 ± 11.3	76.4 ± 11.4	< 0.001
TG (mmol/L)	1.8 (1.2, 2.7)	1.6 (1.1, 2.3)	1.3 (0.9, 1.9)	1.1 (0.8, 1.5)	< 0.001
TC (mmol/L)	5.1 ± 1.0	5.0 ± 0.9	5.0 ± 0.9	4.9 ± 0.9	< 0.001
HDL-C (mmol/L)	1.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	< 0.001
LDL (mmol/L)	3.0 ± 0.7	2.9 ± 0.7	2.9 ± 0.7	2.8 ± 0.7	< 0.001
AST (mmol/L)	31.3 ± 14.8	25.6 ± 9.9	24.2 ± 8.6	24.2 ± 10.5	< 0.001
ALT (mmol/L)	51.0 ± 31.1	27.3 ± 11.2	19.7 ± 7.3	13.8 ± 5.9	< 0.001
AST/ALT	0.7 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.8 ± 0.5	< 0.001
BUN (mmol/L)	5.1 ± 1.2	5.1 ± 1.2	5.0 ± 1.3	5.0 ± 1.3	0.013
Scr (mmol/L)	77.0 ± 14.0	75.3 ± 15.7	73.1 ± 16.2	70.7 ± 17.5	< 0.001
Smoking status, *n (%)*					< 0.001
Current smoker	806 (28.7)	657 (23.5)	531 (18.8)	386 (13.7)	
Ever smoker	176 (6.3)	131 (4.7)	108 (3.8)	74 (2.6)	
Never smoker	1,827 (65)	2,012 (71.9)	2,186 (77.4)	2,352 (83.6)	
Drinking status, *n (%)*					< 0.001
Current drinker	116 (4.1)	117 (4.2)	129 (4.6)	137 (4.9)	
Ever drinker	701 (25)	572 (20.4)	476 (16.8)	313 (11.1)	
Never drinker	1,992 (70.9)	2,111 (75.4)	2,220 (78.6)	2,362 (84)	
Family history of diabetes, *n (%)*					0.007
No	2,720 (96.8)	2,735 (97.7)	2,775 (98.2)	2,748 (97.7)	
Yes	89 (3.2)	65 (2.3)	50 (1.8)	64 (2.3)	
Follow-up (years)	3.0 ± 0.9	3.0 ± 0.9	3.0 ± 0.9	3.0 ± 0.9	< 0.001
Incident diabetes, *n (%)*					< 0.001
No	2,366 (84.2)	2,434 (86.9)	2,537 (89.8)	2,636 (93.7)	
Yes	443 (15.8)	366 (13.1)	288 (10.2)	176 (6.3)	

Data are shown as mean ± standard deviation or number (%).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; FPG, fasting plasma glucose; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; Scr, serum creatinine; BUN, blood urea nitrogen; TC, total cholesterol; HDL-C, high-density lipid cholesterol; LDL-C, low-density lipid cholesterol.

Table 1). In addition, participants in the group with the lowest AST/ALT ratio had the lowest age and HDL-C values ($P < 0.05$; Table 1). When compared with participants without diabetes during follow-up, participants who developed diabetes were older, more likely to be male, had greater values of BMI, SBP, DBP, TC, TG, had lower levels of HDL-C, AST/ALT ratio, had a higher proportion of current smoker and drinker, and more likely to have the family history of diabetes ($P < 0.05$; Table 2).

TABLE 2 Baseline characteristics of study participants with/without diabetes.

Variables	Total ($n = 11246$)	Subgroups of patients		*P*-value
		Non-diabetes ($n = 9973$)	diabetes ($n = 1273$)	
Age (years)	49.9 ± 14.0	49.2 ± 14.1	55.6 ± 12.3	< 0.001
Gender, *n* (%)				< 0.001
Male	7,516 (66.8)	6,608 (66.3)	908 (71.3)	
Female	3,730 (33.2)	3,365 (33.7)	365 (28.7)	
BMI (Kg/m2)	24.8 ± 3.3	24.7 ± 3.3	26.2 ± 3.3	< 0.001
SBP (mmHg)	127.6 ± 17.7	126.9 ± 17.5	132.7 ± 18.6	< 0.001
DBP (mmHg)	78.4 ± 11.2	78.1 ± 11.1	81.0 ± 11.6	< 0.001
TG (mmol/L)	1.4 (1.0, 2.1)	1.4 (0.9, 2.1)	1.7 (1.2, 2.6)	< 0.001
TC (mmol/L)	5.0 ± 0.9	5.0 ± 0.9	5.1 ± 1.0	< 0.001
HDL-C (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	0.037
LDL (mmol/L)	2.9 ± 0.7	2.9 ± 0.7	2.9 ± 0.7	0.632
ALT (mmol/L)	27.9 ± 22.3	27.0 ± 21.1	35.3 ± 28.6	< 0.001
AST/ALT (mmol/L)	1.2 ± 0.5	1.2 ± 0.5	1.0 ± 0.4	< 0.001
AST (mmol/L)	26.3 ± 11.6	26.0 ± 11.2	29.3 ± 14.0	< 0.001
BUN (mmol/L)	5.0 ± 1.3	5.0 ± 1.3	5.1 ± 1.3	0.328
Scr (umol/L)	74.0 ± 16.1	74.0 ± 16.0	74.1 ± 17.2	0.861
Smoking status, *n* (%)				< 0.001
Current smoker	2,380 (21.2)	2,027 (20.3)	353 (27.7)	
Ever smoker	489 (4.3)	402 (4)	87 (6.8)	
Never smoker	8,377 (74.5)	7,544 (75.6)	833 (65.4)	
Drinking status, *n* (%)				< 0.001
Current drinker	499 (4.4)	412 (4.1)	87 (6.8)	
Ever drinker	2,062 (18.3)	1,811 (18.2)	251 (19.7)	
Never drinker	8,685 (77.2)	7,750 (77.7)	935 (73.4)	
Family history of diabetes, *n* (%)				0.001
No	10,978 (97.6)	9,752 (97.8)	1,226 (96.3)	
Yes	268 (2.4)	221 (2.2)	47 (3.7)	
Follow-up (years)	3.0 ± 0.9	3.0 ± 0.9	3.3 ± 0.9	< 0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FPG, fasting plasma glucose; TC, total cholesterol; HDL-C, high-density lipid cholesterol; Scr, serum creatinine; BUN, blood urea nitrogen; LDL-C, low-density lipid cholesterol.

Risk factors for diabetes in the prediabetic population

As shown in Table 3. By univariate analysis, we found that age, BMI, SBP, DBP, TG, HDL-C, AST/ALT, AST, ALT, family history of diabetes, smoking, and drinking status were correlated with the incidence of diabetes in prediabetic patients (all $P < 0.05$). Furthermore, after adjusting for potential confounding factors according to univariate analysis, the multivariate analysis revealed that age, BMI, DBP, TG, HDL-C, AST/ALT, AST, ALT, and family history of diabetes have a significant association with the incident of diabetes progressing from prediabetes.

Figure 2 shows the Kaplan–Meier curve of the cumulative hazards of incident diabetes risk stratified by AST/ALT ratio categories. The risk of incident diabetes was significantly different between the three AST/ALT groups (Log-rank test, $P < 0.0001$). With an increased AST/ALT ratio, the cumulative risk of incident diabetes gradually decreased, rendering the

10.3389/fpubh.2022.1045141

TABLE 3 Results of univariate and multivariate analysis and risk factors of diabetes.

Covariables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Age (years)	1.03 (1.02,1.03)	< 0.001	1.02 (1.01–1.02)	< 0.001
Gender				
Male	Ref			
Female	0.89 (0.79,1.01)	0.063		
BMI (Kg/m2)	1.11 (1.09,1.12)	< 0.001	1.07 (1.05–1.09)	<0.001
SBP (mmHg)	1.02 (1.01,1.02)	< 0.001	1.00 (1.00–1.01)	0.097
DBP (mmHg)	1.02 (1.01,1.02)	< 0.001	1.01 (1.00–1.01)	0.027
TC (mmol/L)	1.04 (0.98,1.10)	0.215		
TG (mmol/L)	1.12 (1.09,1.15)	< 0.001	1.09 (1.06–1.12)	<0.001
HDL-C (mmol/L)	1.55 (1.39,1.73)	< 0.001	1.64 (1.5–1.79)	<0.001
LDL (mmol/L)	1.00 (0.93,1.08)	0.933		
ALT (mmol/L)	1.01 (1.01,1.01)	< 0.001	1.01 (1.00–1.01)	<0.001
AST/ALT	0.49 (0.42,0.56)	< 0.001	0.42 (0.35–0.5)	<0.001
AST (mmol/L)	1.01 (1.01,1.01)	< 0.001	1.01 (1.00–1.01)	<0.001
BUN (mmol/L)	1.04 (0.99,1.08)	0.106		
Scr (mmol/L)	1.00 (1.00,1.00)	0.294		
Smoking status				
Current smoker	Ref		Ref	
Ever smoker	1.34 (1.06,1.70)	0.014	1.66 (1.31–2.1)	<0.001
Never smoker	0.81 (0.71,0.92)	< 0.001	0.94 (0.83–1.07)	0.327
Drinking status				
Current drinker	Ref		Ref	
Ever drinker	0.65 (0.51,0.82)	< 0.001	0.82 (0.64–1.05)	0.111
Never drinker	0.67 (0.54,0.84)	< 0.001	0.83 (0.66–1.03)	0.092
Family history of diabetes				
No	Ref		Ref	
Yes	1.59 (1.19,2.12)	0.002	1.76 (1.31–2.36)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FPG, fasting plasma glucose; TC, total cholesterol; HDL-C, high-density lipid cholesterol; Scr, serum creatinine; BUN, blood urea nitrogen; LDL-C, low-density lipid cholesterol.

minimum AST/ALT ratio group with the maximum risk of incident diabetes in prediabetic patients.

Effect of AST/ALT ratio on the incident of diabetes progressing from prediabetes

In this study, we constructed three models to analyze the independent effects of the AST/ALT ratio on the incidence of diabetes (univariate and multivariate Cox proportional hazard model). The effect sizes [Hazard ratio (HR)] and 95% confidence intervals (CI) were listed in Table 4. When AST/ALT ratio was a continuous variable, AST/ALT ratio showed a negative correlation with the incidence of diabetes progressing from prediabetes in the unadjusted model (model I), for every 1 unit increase in the AST/ALT ratio, the risk of incident of diabetes in the prediabetic patients decreased by 51% (HR = 0.49, 95% CI: 0.42 to 0.56, $P < 0.001$). In the minimum-adjusted model (model II) adjusted for age and gender, the trend did not have obvious changes, the risk of incident diabetes decreased by 69% as the AST/ALT ratio increased (HR = 0.31, 95% CI: 0.26 to 0.37, $P < 0.001$). In the fully-adjusted model (model III) adjusted for age, gender, BMI, SBP, TG, TC, HDL-C, LDL-C, BUN, Scr, smoking

10.3389/fpubh.2022.1045141

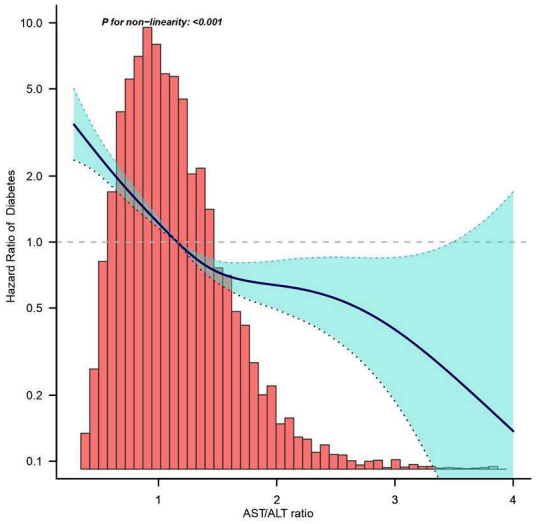


FIGURE 2
Dose-response relationship between AST/ALT ratio and the incidence of diabetes in prediabetic patients.

status, drinking status, family history of diabetes age, for every 1 unit increase in the AST/ALT ratio, the risk of incident of diabetes decreased by 60% (HR = 0.40, 95% CI: 0.33 to 0.48, $P <$ 0.001). For the purpose of sensitivity analysis, we converted the AST/ALT ratio from the continuous variable to the categorical variable (quartiles of AST/ALT ratio). When comparing with the lowest quartile of AST/ALT ratio, the multivariate HRs for the incident of diabetes were 0.71 (0.62–0.82) for Q2, 0.50 (0.42–0.59) for Q3, 0.35 (0.29–0.43) for Q4, The *P* for trend of AST/ALT ratio with categorical variables in the fully-adjusted model was consistent with the result when AST/ALT ratio was a continuous variable.

We divided the total population into two groups, one with normal values of liver function indicators (AST≤40 U/L and ALT≤40 U/L) and the other with abnormal liver function indicators (AST>40 U/L or ALT>40 U/L). Then we constructed three models to analyze the independent effects of the AST/ALT ratio on the incidence of diabetes (univariate and multivariate Cox proportional hazard model). The effect sizes (HR and 95% CI) were listed in Supplementary Table S1.

As is well known, the normal range of AST/ALT ratio is 0.8–1.5. According to this, we divided the total population into three groups. Then we constructed three models to analyze the independent effects of the AST/ALT ratio on the incidence of diabetes (univariate and multivariate Cox proportional hazard model). The effect sizes (HR and 95% CI) were listed in Supplementary Table S2.

In addition, the Kaplan–Meier curve of the cumulative hazards of incident diabetes risk stratified by AST/ALT ratio categories were presented in Supplementary Figure S1.

Figure 3 shows the dose-response relationship between the AST/ALT ratio and the risk of diabetes in prediabetic patients. We found a decreasing trend of incidence of diabetes progressing from prediabetes with a higher AST/ALT ratio.

Subgroup analysis

We further performed subgroup analyses to stratify the association between AST/ALT ratio and incident of diabetes by age, BMI, TG, HDL-C, and family history of diabetes as provided in Table 5. We observed that the negative relationship between the AST/ALT ratio and the risk of diabetes in the prediabetic population remained consistent across all subgroup variables. Meanwhile, we observed that only a small number of interactions including age and TG in the association between the AST/ALT ratio and the risk of diabetes in the prediabetic population (all *P*-values for interaction < 0.05). In this study, a stronger association was detected in the population with age <45 years, and TG ≤ 1.7 mmol/L.

Discussion

In this retrospective cohort study, we established a negative association between the AST/ALT ratio and the risk of diabetes progressing from prediabetes. These results remained stable after adjustment for all potential confounding factors. Furthermore, the negative association between the AST/ALT ratio and the risk of diabetes in prediabetic patients was more evident in participants with age <45 years, and TG ≤ 1.7 mmol/L.

Several previous studies reported the relationship between the AST/ALT ratio and diabetes risk (29, 37, 38). A recent study investigated the relationship between AST/ALT ratio and incident T2DM in populations with or without obesity and demonstrated that non-obese individuals with AST/ALT ≤0.875 have a higher risk of developing T2DM than obese individuals with AST/ALT ≥ 0.875 (38). Chen et al. performed a retrospective cohort study involving 15,291 Japanese individuals from 2004 to 2015 and demonstrated that the AST/ALT ratio was negatively correlated with T2DM (HR = 0.617, 95% CI: 0.405–0.938) (29). However, inconsistent with our findings, their relationship was non-linear and had a saturation effect, and the inflection point was 0.882. We postulated that these differences were due to: i. Key differences between populations in both studies in terms of age range and ethnicity/race; ii. Variations in covariates were included as potential confounders in the studies. Similarly, a cross-sectional study of the fifth Korean National Health and Nutrition Examination Survey (KNHANES V), 2011–2016, found that the AST/ALT ratio was inversely associated with T2DM risk (39). This study found that prediabetic patients with age <45 years old and TG≤1.70

TABLE 4 Relationship between AST/ALT ratio and the risk of diabetes in prediabetic patients in different models.

Exposure	Model I (HR, 95%CI)	*P*-value	Model II (HR, 95%CI)	*P*-value	Model III (HR, 95%CI)	*P*-value
AST/ALT ratio	0.49 (0.42–0.56)	<0.001	0.31 (0.26–0.37)	<0.001	0.40 (0.33–0.48)	<0.001
AST/ALT ratio quartile						
Q1	Reference		Reference		Reference	
Q2	0.80 (0.69–0.92)	0.001	0.63 (0.54–0.72)	<0.001	0.71 (0.62–0.82)	<0.001
Q3	0.59 (0.51–0.68)	<0.001	0.41 (0.35–0.49)	<0.001	0.50 (0.42–0.59)	<0.001
Q4	0.43 (0.36–0.51)	<0.001	0.27 (0.22–0.33)	<0.001	0.35 (0.29–0.43)	<0.001
P for Trend	<0.001		<0.001		<0.001	

Results are shown as hazard ratios (HRs) with 95% confidence intervals (CIs). AST, aspartate aminotransferase; ALT, alanine aminotransferase.
Model I adjust for none.
Model II adjust for age and gender.
Model III adjust for age, gender, BMI, SBP, DBP, TG, TC, HDL-C, LDL-C, BUN, Scr, smoking status, drinking status, family history of diabetes.

mmol/L had a lower risk of progressing into diabetes than others. Currently, we do not have an obvious explanation for this discrepancy. Possible explanations are as follows. Age growth has been shown to be an important risk factor for diabetes. Based on this, youth itself was previously considered a relative protective factor in the development of diabetes. Meanwhile, it is well known that people who tend to maintain better health are at lower risk of diabetes (40, 41). Furthermore, Abnormal lipid metabolism is associated with the pathogenesis of diabetes, which may be associated with impaired insulin reactivity and abnormal blood glucose control (42). Cui et.al found that elevated TG is an independent risk factor for T2DM incidence in the general Chinese population (43). A cross-sectional survey of 15,928 diabetic patients found that high TG patients accounted for 49.7% of the total participants (44). However, since its exact mechanism is unclear, the results of the subgroup and the interaction analysis should be interpreted with caution. And additional large trials are needed for definitive conclusions.

Biological mechanisms involved in the association between AST/ALT ratio and diabetes have not been elucidated, however, there are some potential explanations. As is well known, there have been several studies on the relationship between AST/ALT ratio and non-alcoholic fatty liver disease (NAFLD). The liver plays a crucial role in the control of gluconeogenesis, glycogenolysis, glycolysis, and gluconeogenesis, which are key steps in maintaining glucose homeostasis (45). Any damage to the liver may lead to changes in cell membrane permeability, resulting in leakage of hepatic AST and ALT into the circulatory system (46, 47). The AST/ALT ratio reflects the severity of hepatic steatosis and inflammation. Excess accumulation of fat and inflammation in the liver without heavy alcohol intake leads to NAFLD (48, 49). A recent longitudinal cohort study involving 12,127 Chinese non-obese participants reported that a lower AST/ALT ratio was independently associated with new-onset NAFLD during a 5-year follow-up (50). Moreover, ectopic deposition of lipids in hepatocytes during NAFLD (steatosis) directly or indirectly inhibits key parts of the insulin signaling pathway and significantly increases the risk of T2DM. Accumulating evidence has revealed that NAFLD is closely associated with IR and the AST/ALT ratio is indicative of insulin resistance (IR) (51–53). In a cross-sectional study involving 2,747 adults from the National Health and Nutrition Examination Survey (NHANES) 2011–2016, Visaria et al. discovered that a low AST/ALT ratio is associated with increased IR among

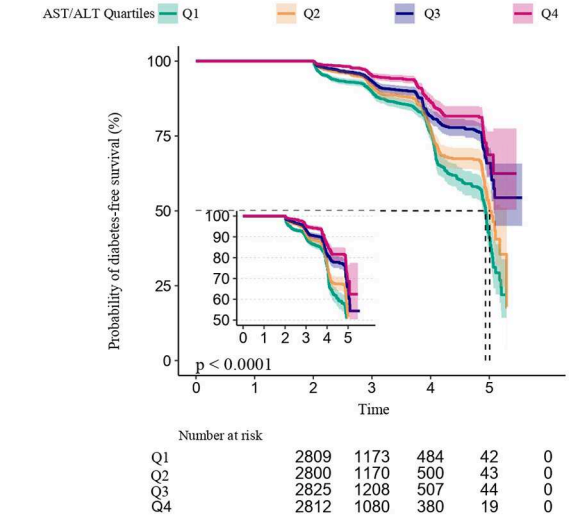


FIGURE 3
Kaplan–Meier event-free survival curve based on AST/ALT ratio quartiles and the incidence of diabetes in prediabetic patients (log-rank, $P < 0.0001$). Each color of lines indicates a quintile group. The color range indicates the 95% confidence interval (CI) range of cumulative incidence of diabetes at a different follow-up time.

TABLE 5 Subgroup analysis between AST/ALT ratio and diabetes in participants with prediabetes.

Characteristic	No of participants	Event (%)	HR (95%CI)	*P*-value	*P* for interaction
Age (years)					
<45	4,359	255 (5.8)	0.37 (0.25–0.56)	<0.001	0.004
45–65	5,032	741 (14.7)	0.43 (0.34–0.54)	<0.001	
>65	1,855	277 (14.9)	0.56 (0.41–0.77)	<0.001	
BMI (Kg/m2)					
≤25	5,982	463 (7.7)	0.31 (0.24–0.41)	<0.001	0.588
>25	5,264	810 (15.4)	0.44 (0.35–0.55)	<0.001	
TG (mmol/L)					
≤1.70	6,973	608 (8.7)	0.30 (0.23–0.38)	<0.001	0.002
>1.70	4,273	665 (15.6)	0.60 (0.47–0.77)	<0.001	
HDL-C (mmol/L)					
≤1.04	1,531	201 (13.1)	0.32 (0.19–0.54)	<0.001	0.181
>1.04	9,715	1,072 (11)	0.42 (0.35–0.50)	<0.001	
Family history of diabetes					
No	10,978	1,226 (11.2)	0.40 (0.34–0.48)	<0.001	0.865
Yes	268	47 (17.5)	0.54 (0.21–1.43)	0.215	

Results are shown as hazard ratios (HRs) with 95% confidence intervals (CIs).
BMI, body mass index; TG, triglyceride; HDL-C, high-density lipid cholesterol.
Models are adjusted for age, gender, BMI, SBP, DBP, TG, TC, HDL-C, LDL-C, BUN, Scr, smoking status, drinking status, and family history of diabetes except for the subgroup variable itself.

those without liver dysfunctions (54). Comparable findings were found in the Chinese population (55). Therefore, the mechanisms involved in the correlation between AST/ALT ratio and diabetes have not been conclusively determined, which warrants further investigation.

This study has substantial strengths. First, our sample size is relatively large and is more representative of the Chinese population. Second, the follow-up duration in this cohort study was up to 6 years, which made the results more convincing. Third, we adjusted for potential confounders to minimize residual confounders in the multivariate analysis, which made the results more reliable. Fourth, sensitivity analysis was performed by handling the AST/ALT ratio as both continuous variables and categorical variables, which reduced contingency in data analysis and enhanced the stability of results. Furthermore, a subgroup analysis was conducted to ensure the robustness of the presented results. Finally, our findings have potential significant clinical implications. AST/ALT ratio is a simple, inexpensive, and routine clinical measurement, which predates traditional predictors of T2DM. It is well known that a high AST/ALT ratio may be a sign of abnormal liver function. However, a low AST/ALT ratio may not necessarily be advantageous. Our study found that a low AST/ALT ratio may increase the risk of developing diabetes. The normal range for AST/ALT ratio is 0.8–1.5. When the AST/ALT ratio is below the normal value, screening for diabetes is suggested. The findings of this study will help to identify patients at high risk of diabetes at an early stage and to make timely lifestyle modifications and interventions related to early diabetes, which may reduce the incidence of diabetes in the long term.

However, this study is associated with a few limitations. First, we only studied Chinese adults. Therefore, our conclusions may not be generalizable to other age- and ethnic groups. Second, due to the nature of the secondary analysis of published data, some important variables were not included, such as physical activities and pre-existing cardiovascular diseases. Studies should assess the relationship between AST/ALT ratio and T2DM. Third, in this study, diabetes was defined by fasting glucose levels ≥7.00 mmol/L and/or self-reported diabetes during follow-up, rather than the test of oral glucose tolerance or measurement of glycosylated hemoglobin, which may underestimate T2DM incidences. However, practically, it is not feasible to perform oral glucose tolerance tests on all participants. Fourth, this study did not classify diabetes as type 1 diabetes and T2DM. However, because T2DM accounts for about 95% of all diabetes cases, our findings are likely more representative of T2DM. Finally. the AST/ALT ratio is dynamic and changes over time. However, we only measured AST/ALT ratio at baseline.

Conclusion

In summary, this present study suggests that a lower AST/ALT ratio is independently associated with a higher risk of diabetes onset in Chinese adults with prediabetes. Regular monitoring of AST/ALT ratio dynamics can help avoid progression to diabetes from prediabetics. The AST/ALT ratio might thus be a useful tool for detecting prediabetic individuals at a high risk of developing diabetes. However, further prospective studies are needed to validate our study findings.

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 human participants were reviewed and approved by the Rich Healthcare Group Review Board. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JC and XW conceived the study idea. HL and LJ analyzed the data. HZ and HL reviewed the literature and wrote the first draft. LJ and HZ critically reviewed, edited the manuscript, and approved the final version. All authors read and approved the final manuscript.

Acknowledgments

We gratefully thank all participants in this cohort study. We gratefully thank the Rich Healthcare Group Review Board for presenting raw data.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1045141/full#supplementary-material

SUPPLEMENTARY TABLE1
Association between AST/ALT ratio and the risk of diabetes progressing from prediabetes by value of liver function.

SUPPLEMENTARY TABLE2
Relationship between AST/ALT ratio and the risk of diabetes in prediabetic patients in different models.

SUPPLEMENTARY FIGURE S1
Kaplan–Meier curve of the cumulative hazards of incident diabetes risk stratified by AST/ALT ratio categories in patients with prediabetes (log-rank, $P < 0.0001$). Each color of lines indicates a quintile group. The color range indicates the 95% confidence interval (CI) range of cumulative incidence of diabetes at a different follow-up time.

References

1. WHO.int. *Diabetes*. Available online at: https://www.who.int/news-room/fact-sheets/detail/diabetes. (accessed December 6, 2019).

2. International Diabetes Federation. *IDF Diabetes Atlas*. Available online at: http://www.diabetesatlas.org/. (accessed August 25, 2016).

3. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* (2001) 414:782–7. doi: 10.1038/414782a

4. Hu C, Jia W. Diabetes in China: epidemiology and genetic risk factors and their clinical utility in personalized medication. *Diabetes.* (2018) 67:3–11. doi: 10.2337/dbi17-0013

5. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. *BMJ.* (2020) 369:m997. doi: 10.1136/bmj.m997

6. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2003) 26(Suppl. 1:S5–20. doi: 10.2337/diacare.26.2007.S5

7. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet.* (2008) 371:1800–9. doi: 10.1016/S0140-6736(08)60768-0

8. Allender S, Foster C, Hutchinson L, Arambepola C. Quantification of urbanization in relation to chronic diseases in developing countries: a systematic review. *J Urban Health.* (2008) 85:938–51. doi: 10.1007/s11524-008-9325-4

9. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet.* (2012) 379:2279–90. doi: 10.1016/S0140-6736(12)60283-9

10. Brannick B, Dagogo-Jack S. Prediabetes and cardiovascular disease: pathophysiology and interventions for prevention and risk reduction. *Endocrinol Metab Clin North Am.* (2018) 47:33–50. doi: 10.1016/j.ecl.2017.10.001

11. Cai X, Zhang Y, Li M, Wu JH, Mai L, Li J, et al. Association between prediabetes and risk of all cause mortality and cardiovascular disease: updated meta-analysis. *BMJ.* (2020) 370:m2297. doi: 10.1136/bmj.m2297

12. Cai X, Liu X, Sun L, He Y, Zheng S, Zhang Y, et al. Prediabetes and the risk of heart failure: a meta-analysis. *Diabetes Obes Metab.* (2021) 23:1746–53. doi: 10.1111/dom.14388

13. Mai L, Wen W, Qiu M, Liu X, Sun L, Zheng H, et al. Association between prediabetes and adverse outcomes in heart failure. *Diabetes Obes Metab.* (2021) 23:2476–83. doi: 10.1111/dom.14490

14. Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: a call to action. *Diabetes Care.* (2017) 40:419–30. doi: 10.2337/dc16-1787

15. Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci.* (2018) 14:1483–96. doi: 10.7150/ijbs.27173

16. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol.* (2017) 14:32–42. doi: 10.1038/nrgastro.2016.147

17. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology.* (2002) 123:1367–84. doi: 10.1053/gast.2002.36061

18. Hanley AJG, Williams K, Festa A, Wagenknecht LE, Jr RBD, Kempf J, et al. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes.* (2004) 53:2623–32. doi: 10.2337/diabetes.53.10.2623

19. De Ritis F, Mallucci L, Coltorti M, Giusti G, Caldera M. Anicteric virus hepatitis in a closed environment as shown by serum transaminase activity. *Bull World Health Organ.* (1959) 20:589–602.

20. Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol Alcohol.* (2004) 39:336–9. doi: 10.1093/alcalc/agh074

21. Nyblom H, Nordlinder H, Olsson R. High aspartate to alanine aminotransferase ratio is an indicator of cirrhosis and poor outcome in patients with primary sclerosing cholangitis. *Liver Int.* (2007) 27:694–9. doi: 10.1111/j.1478-3231.2007.01471.x

22. Hofmeister MG, Xing J, Foster MA, Augustine RJ, Burkholder C, Collins J, et al. Factors associated with hepatitis a mortality during person-to-person outbreaks: a matched case-control study-United States, 2016–2019. *Hepatology.* (2021) 74:28–40. doi: 10.1002/hep.31645

23. Liu H, Zha X, Ding C, Hu L, Li M, Yu Y, et al. AST/ALT ratio and peripheral artery disease in a chinese hypertensive population: a cross-sectional study. *Angiology.* (2021) 72:916–22. doi: 10.1177/00033197211004410

24. Zhou J, He Z, Ma S, Liu R. AST/ALT ratio as a significant predictor of the incidence risk of prostate cancer. *Cancer Med.* (2020) 9:5672–7. doi: 10.1002/cam4.3086

25. Ewid M, Sherif H, Allihimy AS, Alharbi SA, Aldrewesh DA, Alkuraydis SA, et al. AST/ALT ratio predicts the functional severity of chronic heart failure with reduced left ventricular ejection fraction. *BMC Res Notes.* (2020) 13:178. doi: 10.1186/s13104-020-05031-3

26. Li L, Zeng Q, Xue N, Wu M, Liang Y, Xu Q, et al. A Nomogram Based on Aspartate Aminotransferase/Alanine Aminotransferase (AST/ALT) ratio to predict prognosis after surgery in gastric cancer patients. *Cancer Control.* (2020) 27:1073274820954458. doi: 10.21203/rs.3.rs-18794/v1

27. Gao F, Chen C, Lu J, Zheng J, Ma X-C, Yuan X-Y, et al. De Ritis ratio (AST/ALT) as an independent predictor of poor outcome in patients with acute ischemic stroke. *Neuropsychiatr Dis Treat.* (2017) 13:1551–7. doi: 10.2147/NDT.S139316

28. Preuss HG, Kaats GR, Mrvichin N, Swaroop A, Bagchi D, Clouatre D, et al. Examining the relationship between nonalcoholic fatty liver disease and the metabolic syndrome in nondiabetic subjects. *J Am Coll Nutr.* (2018) 37:457–65. doi: 10.1080/07315724.2018.1443292

29. Chen L, Zhang K, Li X, Wu Y, Liu Q, Xu L, et al. Association between aspartate aminotransferase to alanine aminotransferase ratio and incidence of type 2 diabetes mellitus in the Japanese Population: a secondary analysis of a retrospective cohort study. *Diabetes Metab Syndr Obes.* (2021) 14:4483–95. doi: 10.2147/DMSO.S337416

30. Morling JR, Fallowfield JA, Guha IN, Nee LD, Glancy S, Williamson RM, et al. Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: the Edinburgh type 2 diabetes study. *J Hepatol.* (2014) 60:384–91. doi: 10.1016/j.jhep.2013.10.017

31. Wannamethee SG, Shaper AG, Lennon L, Whincup PH. Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. *Diabetes Care.* (2005) 28:2913–8. doi: 10.2337/diacare.28.12.2913

32. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM, et al. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. *Diabetes Care.* (2005) 28:1757–62. doi: 10.2337/diacare.28.7.1757

33. Goessling W, Massaro JM, Vasan RS, D'Agostino RB, Sr., Ellison RC, Fox CS. Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology.* (2008) 135:1935–44. doi: 10.1053/j.gastro.2008.09.018

34. Chen Y, Zhang X-P, Yuan J, Cai B, Wang X-L, Wu X-L, et al. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. *BMJ Open.* (2018) 8:e021768. doi: 10.1136/bmjopen-2018-021768

35. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med.* (2007) 4:e297. doi: 10.1371/journal.pmed.0040297

36. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. *Diabetes Care.* (2022) 45:S17–38. doi: 10.2337/dc22-S002

37. Niu H, Zhou Y. Nonlinear relationship between AST-to-ALT ratio and the incidence of type 2 diabetes mellitus: a follow-up study. *Int J Gen Med.* (2021) 14:8373–82. doi: 10.2147/IJGM.S341790

38. Nakajima H, Okada H, Hamaguchi M, Kurogi K, Murata H, Ito M, et al. Low aspartate aminotransferase/alanine aminotransferase ratio is a predictor of diabetes incidence in Japanese people: Population-based Panasonic cohort study 5. *Diabetes Metab Res Rev.* (2022) 38:e3553. doi: 10.1002/dmrr.3553

39. Ko SH, Baeg MK, Han KD, Ko SH, Ahn YB. Increased liver markers are associated with higher risk of type 2 diabetes. *World J Gastroenterol.* (2015) 21:7478–87. doi: 10.3748/wjg.v21.i24.7478

40. Lascar N, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S. Type 2 diabetes in adolescents and young adults. *Lancet Diabetes Endocrinol.* (2018) 6:69–80. doi: 10.1016/S2213-8587(17)30186-9

41. Lyu YS, Kim SY, Bae HY, Kim JH. Prevalence and risk factors for undiagnosed glucose intolerance status in apparently healthy young adults aged <40 years: the Korean nationa l health and nutrition examination survey 2014-2017. *Int J Environ Res Public Health.* (2019) 16:2393. doi: 10.3390/ijerph16132393

42. Chakrabarti P, Kandror KV. The role of mTOR in lipid homeostasis and diabetes progression. *Curr Opin Endocrinol Diabetes Obes.* (2015) 22:340–6. doi: 10.1097/MED.0000000000000187

43. Cui J, Ma P, Sun JP, Baloch Z, Yin F, Xin HL, et al. The ability of baseline triglycerides and total cholesterol concentrat ions to predict incidence of type 2 diabetes mellitus in chinese men a nd women: a longitudinal study in Qingdao, China. *Biomed Environ Sci.* (2019) 32:905–13. doi: 10.3967/bes2019.113

44. Tang X, Yan X, Zhou H, Huang G, Niu X, Jiang H, et al. Associations of insulin resistance and beta-cell function with abnormal lipid profile in newly diagnosed diabetes. *Chin Med J (Engl).* doi: 10.1097/CM9.0000000000002075. [Epub ahead of print].

45. Han HS, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective. *Exp Mol Med.* (2016) 48:e218. doi: 10.1038/emm.2015.122

46. Zhao H, Lai Q, Zhang J, Huang C, Jia L. Antioxidant and hypoglycemic effects of acidic-extractable polysaccharides from cordyceps militaris on type 2 diabetes mice. *Oxid Med Cell Longev.* (2018) 2018:9150807. doi: 10.1155/2018/9150807

47. Li W, Jin G, Wu H, Wu R, Xu C, Wang B, et al. Interpretable clinical visualization model for prediction of prognosis in osteosarcoma: a large cohort data study. *Front Oncol.* (2022) 12:945362. doi: 10.3389/fonc.2022.945362

48. Long MT, Pedley A, Colantonio LD, Massaro JM, Hoffmann U, Muntner P, et al. Development and validation of the framingham steatosis index to identify persons with hepatic steatosis. *Clin Gastroenterol Hepatol.* (2016) 14:1172–80.e2. doi: 10.1016/j.cgh.2016.03.034

49. Marjot T, Moolla A, Cobbold JF, Hodson L, Tomlinson JW. Nonalcoholic fatty liver disease in adults: current concepts in etiology, outcomes, and management. *Endocr Rev.* (2020) 41:9. doi: 10.1210/endrev/bnz009

50. Zou Y, Zhong L, Hu C, Sheng G. Association between the alanine aminotransferase/aspartate aminotransferase ratio and new-onset non-alcoholic fatty liver disease in a nonobese Chinese population: a population-based longitudinal study. *Lipids Health Dis.* (2020) 19:245. doi: 10.1186/s12944-020-01419-z

51. Lee K, Yang JH. Which liver enzymes are better indicators of metabolic syndrome in adolescents: the Fifth Korea National Health and Nutrition Examination Survey, 2010. *Metab Syndr Relat Disord.* (2013) 11:229–35. doi: 10.1089/met.2012.0153

52. Muzurović E, Mikhailidis DP, Mantzoros C. Non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk. *Metabolism.* (2021) 119:154770. doi: 10.1016/j.metabol.2021.154770

53. Li W, Dong S, Wang B, Wang H, Xu C, Zhang K, et al. The construction and development of a clinical prediction model to assess lymph node metastases in osteosarcoma. *Front Public Health.* (2021) 9:813625. doi: 10.3389/fpubh.2021.813625

54. Visaria A, Pai S, Cheung M, Ahlawat S. Association between aspartate aminotransferase-to-alanine aminotransferase ratio and insulin resistance among US adults. *Eur J Gastroenterol Hepatol.* (2021). doi: 10.1097/MEG.0000000000002215

55. Zhao L, Cheng J, Chen Y, Li Q, Han B, Chen Y, et al. Serum alanine aminotransferase/aspartate aminotransferase ratio is one of the best markers of insulin resistance in the Chinese population. *Nutr Metab (Lond).* (2017) 14:64. doi: 10.1186/s12986-017-0219-x

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 January 2023
DOI 10.3389/fendo.2022.1065527

OPEN ACCESS

EDITED BY
Christian Göbl,
Medical University of Vienna, Austria

REVIEWED BY
Alpesh Goyal,
All India Institute of Medical Sciences, India
Elena Succurro,
University of Magna Graecia, Italy

*CORRESPONDENCE
Sam Dagogo-Jack
sdj@uthsc.edu

SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 09 October 2022
ACCEPTED 19 December 2022
PUBLISHED 05 January 2023

CITATION
Liu Z, Asuzu P, Patel A, Wan J and Dagogo-Jack S (2023) Association of bone mineral density with prediabetes risk among African-American and European-American adult offspring of parents with type 2 diabetes. *Front. Endocrinol.* 13:1065527. doi: 10.3389/fendo.2022.1065527

Association of bone mineral density with prediabetes risk among African-American and European-American adult offspring of parents with type 2 diabetes

Zhao Liu[1], Peace Asuzu[1], Avnisha Patel[1], Jim Wan[2] and Sam Dagogo-Jack[1]*

[1]Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Tennessee Health Science Center, Memphis, TN, United States, [2]Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, United States

Introduction: Type 2 diabetes mellitus (T2DM) is associated with alterations in bone mineral density (BMD), but association between prediabetes and BMD is unclear.

Methods: We analyzed BMD among the initially normoglycemic participants in the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study in relation to incident prediabetes during 5 years of follow-up.

Results and Discussion: A total of 343 participants (193 Black, 150 White) underwent DEXA during Year 1 of POP-ABC and were followed quarterly for 5 years. The mean age was 44.2 ± 10.6 years; BMI was 30.2 ± 7.23 kg/m^2. At baseline, the mean BMD was 1.176 ± 0.135 g/cm^2 (1.230 ± 0.124 g/cm^2 in men vs. 1.154 ± 0.134 g/cm^2 in women, P<0.0001; 1.203 ± 0.114 g/cm^2 in Black vs. 1.146 ± 0.150 g/cm^2 in White participants, P=0.0003). During 5 years of follow-up, 101 participants developed prediabetes and 10 subjects developed T2DM (progressors); 232 were nonprogressors. Progressors to prediabetes had numerically higher baseline BMD and experienced lower 1-year decline in BMD (P<0.0001) compared with nonprogressors. From Kaplan-Meier analysis, the time to 50% prediabetes survival was 2.15 y among participants in the lowest quartile of baseline BMD, longer than those in higher quartiles (1.31 – 1.41 y). Values for BMD correlated inversely with age and adiponectin levels, and positively with BMI. In logistic regression analysis, BMD z score significantly predicted incident prediabetes: more negative BMD z scores were associated with decreased incident prediabetes (odds ratio 0.598 [95% confidence interval 0.407 - 0.877], P=0.0085), after controlling for age, BMI, change in BMI, ethnicity, blood glucose and adiponectin.

Conclusions: Among initially normoglycemic individuals, higher baseline BMD was associated with higher risk of incident prediabetes during 5 years of follow-up.

KEYWORDS

bone mineral density, impaired fasting glucose, impaired glucose tolerance, prospective study, race/ethnicity

1 Introduction

Diabetes mellitus appears to exhibit a complex relationship with bone health. Cross-sectional studies have reported lower bone mineral density (BMD) in people with type 1 diabetes mellitus (T1DM) (1–3) but similar or higher BMD in those with type 2 diabetes (T2DM) (4–6) compared with healthy control subjects. In one study, the mean BMD in patients with T2DM was ~10% higher than that of age-matched individuals without diabetes (4). In a meta-analysis of 15 observational studies with a pooled population of 3,437 T2DM patients and 19,139 controls, BMD was significantly higher by 0.04 g/cm^2 at the femoral neck, 0.06 g/cm^2 at the hip and 0.06 g/cm^2 at the spine in T2DM patients versus controls (5). The mechanisms for the higher BMD in people with T2DM are not known precisely but may be related to adiposity, hyperglycemia, or hyperinsulinemia (6, 7). The Rotterdam study found that patients with inadequately controlled T2DM had higher BMD compared with healthy subjects or patients with adequately controlled T2DM (6).

Paradoxically, the normal or higher BMD observed in people with T2DM is not associated with the expected decrease in the risk of fracture. In fact, increased fracture risk may be higher in people with diabetes versus healthy control (4, 6–8). In the prospective Japanese Nurses' Health Study, among women 34-59 years old the incidence of hip fractures was six-fold higher in patients with T1DM and two-fold higher in those with T2DM compared with healthy subjects, after adjustments for body mass index (BMI), smoking, physical activity, menopausal status, postmenopausal hormone use, and daily intake of calcium, vitamin D and protein (8). Multiple factors than can contribute to increased fracture risk in people with diabetes include alterations in bone microstructure, increased cortical porosity, and reduced cortical density (8–13). Furthermore, insulin deficiency and low levels of IGF-1 in patients with type 1 diabetes impair osteoblast function, leading to low peak bone mass at a young age (10). Additional diabetes-related deleterious factors include formation of advanced glycation end products, inflammatory cytokines, osteocyte production of sclerostin, and bone microvascular disease (8–13). Finally, certain medications used for treating diabetes have been associated with alterations in bone metabolism and fracture risk (14).

We explored the ontogeny of the association between diabetes and increased BMD by studying normoglycemic individuals who developed prediabetes during prospective follow-up. We reasoned that a true biological association between increased bone mass and T2DM might be discernible at the more proximal stage of prediabetes. Among persons at genetic risk for T2DM, the transition from normoglycemia to diabetes often follows a predictable course through an intermediate stage of prediabetes, defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (15–17). The Centers for Disease Control and Prevention estimates that approximately 96 million Americans aged 18 years and older have prediabetes (18). Unlike in patients with established diabetes, the relation between BMD and prediabetes has not been well studied. In one report, based on data from the U.S. National Health and Nutrition Examination Surveys (NHANES) from 2005 to 2014, adults 40 years of age or older with prediabetes had higher BMD but greater hip fracture risk compared with adults with normal glucose tolerance (19). In another cross-sectional study (based on NHANES 2005-2018 data), there was an increasing trend of BMD at the hip, femoral neck, and lumbar spine across the glycemic spectrum from normoglycemia, prediabetes, to diabetes in adults aged 40 years or older (20). However, these cross-sectional observations do not reveal the direction of the association between BMD and diabetes or prediabetes, nor do they permit causal inferences. Prospective studies are needed to demonstrate directionality and enable the identification of possible causal

Abbreviations: AIR, Acute insulin response to intravenous glucose; BMC, Bone mineral content; BMD, Bone mineral density; BMI, Body mass index; T1DM, Type 1 diabetes; T2DM, Type 2 diabetes; FPG, Fasting plasma glucose; GCRC, General Clinical Research Center; hsCRP, high sensitivity C-reactive protein; IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance test; POP-ABC, Pathobiology of Prediabetes in a Biracial Cohort; 2hPG, Two-hour plasma glucose; UTHSC, University of Tennessee Health Science Center.

mechanisms of the association between bone mass and disorders of glucose metabolism.

The Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study enrolled self-reported African American and European American adults with parental T2DM and assessed progression from normoglycemia to T2DM during for 5 years of follow-up (21–26). The primary results of the POP-ABC study, which showed no ethnic disparity in the incidence of prediabetes among people with similar parental history of T2DM, identified baseline weight, insulin sensitivity, insulin secretion and inflammatory markers as significant associations of prediabetes risk (26). In the present *post-hoc* analysis, we examined the association between BMD at enrollment and incident prediabetes risk in the POP-ABC study. We further assessed the relationship between BMD and several demographic, biochemical, and glucoregulatory variables, to explore potential mechanisms for any association between BMD and prediabetes. The prospective design of the POP-ABC study enabled us to track initially normoglycemic individuals until the occurrence of IFG or IGT and determine whether baseline BMD is associated with such an outcome. Furthermore, by studying a normoglycemic population, we avoided the confounding effects of anti-diabetes medications on bone metabolism that plagued cross-sectional studies of people with established diabetes.

2 Materials and methods

2.1 Study subjects

The study subjects were participants in the POP-ABC study (21–23). Eligible for enrolment in the POP-ABC study were healthy, normoglycemic adults aged 18–65 years who self-reported as being of non-Hispanic white (European American) or non-Hispanic black (African American) ancestry and had one or both biological parents with T2DM. The standard 75-gram oral glucose tolerance test (OGTT) was used to screen prospective participants and those with normal fasting plasma glucose (FPG, <100 mg/dL [5.6 mmol/L]) and normal glucose tolerance (NGT, 2-hour plasma glucose [2hPG] <140 mg/dL [7.8 mmol/L]), based on American Diabetes Association criteria, were enrolled (15, 24). Excluded from participation were individuals with a history of diabetes, those taking glucocorticoids or medications known to alter body weight, blood glucose or bone metabolism, or persons enrolled in behavioral weight loss programs or having a history of bariatric surgery. Individuals self-reported their race/ethnicity, based on the 1990 US Census questionnaire (25). The University of Tennessee Institutional Review Board approved the study protocol. All participants gave written informed consent before initiation of the study, which was conducted at the University of Tennessee General Clinical Research Center (GCRC).

2.2 Assessments

Participants made outpatient visits to the GCRC after an overnight fasting at baseline and every 3 months during 5 years of follow-up. Assessments at baseline included anthropometric measurements (weight, height, BMI, waist circumference), body composition (total fat mass, trunk fat mass) and bone densitometry by dual-energy x-ray absorptiometry (DEXA) (Hologic Discovery A80044A, Hologic Inc., Bedford, MA), OGTT, and biochemistries (21–23). Assessments during year one included insulin sensitivity (ISI) measured with hyperinsulinemic euglycemic clamp and insulin secretion using intravenous glucose tolerance test (IVGTT), as previously described (21–23). Other follow-up assessments included quarterly FPG, and annual OGTT, IVGTT and DEXA.

2.3 Definition of outcome measures

The primary outcome was the occurrence of prediabetes (IFG and/or IGT) or diabetes, defined by the 2003 revised American Diabetes Association criteria (15, 24, 26). For participants reaching any of those endpoints, a confirmatory test using 75-g OGTT was performed within six weeks of initial endpoint occurrence, as previously described (26). All endpoints were independently adjudicated by the Institutional Data and Safety Officer (Murray Heimberg, MD, PhD).

2.4 Statistical analysis

This is a *post hoc* analysis of baseline data from the POP-ABC study. Data were reported as means ± standard deviations. Differences in continuous or categorical variables between defined groups were analyzed using unpaired t test or chi square test, as appropriate. Linear regression models were used to analyze the relationship between BMD and demographic, anthropometric, glycemic, and glucoregulatory variables, and predictors of incident prediabetes were modeled using logistic regression. The annual change in BMD was analyzed using paired t test. The incidence of prediabetes across quartiles of baseline BMD was analyzed using Kaplan-Meier plots. Significance level was set as $P< 0.05$ (two-tailed). All analyses were performed using StatView statistical software (SAS Institute Inc., Cary, NC).

3 Results

3.1 Baseline cohort characteristics

A total of 343 participants (193 Black, 150 White; 71% women) underwent DEXA during Year 1 of the POP-ABC study. The mean age was 44.2 ± 10.6 years; BMI was 30.2 ± 7.23 kg/m^2. The mean FPG was 91.8 ± 6.77 mg/dl, 2hPG 124 ± 25.8 mg/dl, and HbA1c was 5.54 ± 0.44% at enrollment. The mean baseline BMD was 1.176 ± 0.135 g/cm^2 for the entire cohort, higher in men than women (1.230 ± 0.124 g/cm^2 vs. 1.154 ± 0.134 g/cm^2, P<0.0001). Table 1 shows the baseline characteristics of study subjects by ethnicity. The BMD was higher in Black vs. White participants (1.203 ± 0.114 g/cm^2 vs. 1.146 ± 0.150 g/cm^2, P=0.0003). Compared with White participants, African American participants had a lower mean age and higher BMI, but similar values for total and trunk fat mass (Table 1). Trunk fat mass and body fat mass were not significantly different by race/ethnicity. Baseline BMD was correlated inversely with age (r^2= -0.063, P<0.0001) and directly with BMI (r^2 = 0.073, P<0.0001) among the Black and White POP-ABC study participants (Figure 1).

3.2 BMD and prediabetes risk

During 5 years of follow-up, 101 participants developed prediabetes and 10 subjects developed T2DM (progressors) and 232 maintained normoglycemia (nonprogressors). Participants who developed T2DM were not included in the present report. Table 2 shows the demographic, clinical and biochemical characteristics of progressors to prediabetes versus nonprogressors. Compared with nonprogressors, participants who progressed to prediabetes were older, more likely to be male, and had significantly higher FPG, HbA1c, baseline BMI, and 1-year increase in BMI. Progressors also had higher insulin sensitivity and trunk fat mass but lower adiponectin levels at baseline, compared with nonprogressors (Table 2). Progressors to prediabetes had numerically but insignificantly higher BMD (1.177± 0.114 g/cm2 vs. 1.175 ± 0.146 g/cm^2, P=0.88) and bone mineral content (BMC) (2.60 kg ± 0.46 vs. 2.49 kg ± 0.50, P=0.07) at baseline compared with nonprogressors. Furthermore, progressors to prediabetes experienced a significantly slower 1-year decrease in BMD compared with nonprogressors (-0.019 ± 0.46 027 vs. -0.038 ± 0.14, P<0.0001) (Figure 2A).

In logistic regression models, BMC and BMD z score significantly predicted incident prediabetes, after adjusting for age, BMI, change in BMI, ethnicity, FPG, 2hPG, total fat mass and trunk fat mass, and adiponectin at enrollment. More negative BMD z scores (indicating lower bone mass referenced to age- and sex-matched control) were associated with decreased risk of incident prediabetes (adjusted odds ratio 0.598 [95% confidence interval 0.407 - 0.877], P=0.0085). In contrast, higher BMC at baseline predicted increased risk of incident prediabetes (adjusted odds ratio 1.001[95% confidence interval 1.000 – 1.002], P=0.0052).

We stratified participants by quartiles of baseline BMD (Figure 2B) and analyzed the development of prediabetes

TABLE 1 Baseline characteristics of POP-ABC study subjects by race/ethnicity.

	African American	European American	P-Value
Number	193	150	
Age (yr)	42.5 ± 10.3	46.5 ± 10.5	0.0003
Weight (kg)	87.8 ± 21.1	81.8 ± 20.9	0.004
BMI (kg/m^2)	31.2 ± 7.40	28.8 ± 6.78	0.0015
FPG (mg/dl)	90.8 ± 6.81	93.1 ± 6.50	0.001
2hPG (mg/dl)	123 ± 27.4	125 ± 23.3	0.45
HbA1c (%)	5.63 ± 0.47	5.44 ± 0.32	<0.0001
BMD, (g/cm^2) Female Male	1.203 ± 0.114 1.184 ± 0.103 1.256 ± 0.127	1.146 ± 0.150 1.118 ± 0.156 1.206 ± 0.116	0.0003
Trunk fat mass(kg)	15.3 ± 7.50	14.8 ± 6.96	0.48
Total body fat mass (kg)	31.8 ± 13.76	29.4 ± 13.21	0.11
Insulin sensitivity (μmol/kg FFM.min^{-1}/pM)	0.12 ± 0.07	0.14 ± 0.06	0.0352
Insulin Secretion (AIR) (μU/ml)	105 ± 88.8	61.3 ± 39.0	<0.0001

AIR, acute insulin response to i.v. glucose; BMD, bone mineral density; BMI, body mass index; AIR, acute insulin response to i.v. glucose; FPG, fasting plasma glucose; 2hPG, two-hour plasma glucose; To convert FPG and 2hPG to mmol/l, multiply by 0.56.

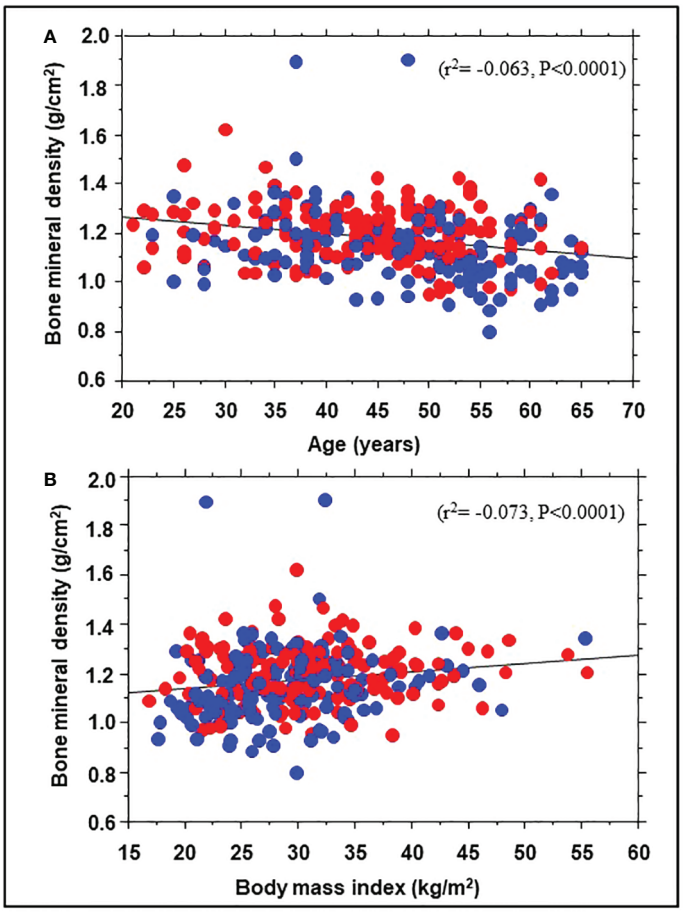


FIGURE 1
Correlation of bone mineral density with age **(A)** and body mass index **(B)** in African American (red symbols) and European American (blue symbols) participants at enrollment in the Pathobiology of Prediabetes in a Biracial Cohort study.

across BMD strata (Figure 2C). From Kaplan-Meier analysis, the time to 50% prediabetes survival was 2.15 years among participants with the lowest BMD at baseline (Quartile 1) versus 1.31 – 1.41 years among subjects in higher BMD quartiles.

3.3 Potential underlying mechanisms

To explore possible mechanisms for the association of BMD with incident prediabetes, we examined the relationship between BMD and several baseline variables. Univariate linear regression showed significant correlations between BMD and body weight (r^2 = 0.10, P<0.0001), BMI (r^2 = 0.029, P=0.0028), total body fat mass (r^2 = 0.044, p=0.0003), trunk fat mass (r^2 = 0.033, P= 0.0021), 2hPG (r^2= -0.017, p=0.027), and adiponectin levels (r^2= -0.036, P=0.0008) but not FPG, HbA1c, insulin sensitivity, insulin secretion, or C-reactive protein (Figure 3). A multivariate regression model was run, with BMD as dependent variable and BMI along with 2hPG, adiponectin levels, C-reactive protein, insulin sensitivity and insulin secretion as independent variables. The significant predictors of BMD were BMI (beta coefficient 0.18, P=0.05), 2hPG (beta coefficient -0.16, P=0.028), and adiponectin (beta coefficient -0.20, P=0.0098).

4 Discussion

In our prospective study of healthy offspring of parents with T2DM, bone density at enrollment had the expected relationships with age, sex, and ethnicity. Study participants who developed incident prediabetes during 5 years of follow-up tended to have higher baseline BMD and BMC and showed a significantly slower 1-year decline in BMD compared with nonprogressors. After controlling for baseline variables (including age, BMI, and blood glucose), higher bone mass predicted increased 5-year risk of progression from

10.3389/fendo.2022.1065527

TABLE 2 Demographic, clinical and biochemical characteristics in progressors to prediabetes versus nonprogressors.

	Progressor	Nonprogressor	P value
Number	111	232	
Black/White	58/53	135/97	0.30
Women/Men	65/46	180/52	0.0003
Premenopausal/ postmenopausal	35/30	115/65	0.15
Age (yr)	47.3 ± 8.94	43.8 ± 10.8	0.0031
Weight (kg)	90.0 ± 20.1	83.1 ± 21.9	0.0051
Baseline BMI (kg/m^2)	31.4 ± 6.88	29.6 ± 7.40	0.034
Delta BMI (1-yr) (kg/m^2)	0.50 ± 1.48	0.16 ± 1.63	0.088
Delta BMI (2-yr) (kg/m^2)	0.61 ± 1.85	0.30 ± 1.98	0.24
FPG (mg/dl)	94.0 ± 6.75	91.0 ± 6.49	<0.0001
HbA1c (%)	5.66 ± 0.47	5.52 ± 0.43	0.0059
BMD (g/cm^2)	1.177± 0.114	1.175 ± 0.146	0.88
BMC (kg)	2.60 ± 0.46	2.49 ± 0.50	0.07
Delta BMD (1-yr) (g/cm^2)	-0.02 ± 0.46	-0.04 ± 0.14	<0.0001
Trunk fat mass (kg)	16.6 ± 6.85	14.3 ± 7.34	0.0079
Total fat mass (kg)	32.0 ± 1.26	29.9 ± 1.40	0.19
Insulin sensitivity (μmol/kg FFM.min^{-1}/pM)	0.12 ± 0.07	0.15 ± 0.06	0.0014
Insulin secretion (AIR) (μu/ml)	81.3 ± 73.7	88.2 ± 74.2	0.44
hsCRP (mg/L)	4.35 ± 6.67	3.55 ± 5.38	0.24
Adiponectin (μg/ml)	8.53 ± 4.35	9.87 ± 5.69	0.031

AIR, acute insulin response to i.v. glucose; BMD, bone mineral density; BMI, body mass index; FFM, fat-free mass; FPG, fasting plasma glucose; hsCRP, high sensitivity C-reactive protein; 2hPG, two-hour plasma glucose; To convert FPG and 2hPG to mmol/l, multiply by 0.56.

normoglycemia to prediabetes. These findings suggest an inverse relationship between baseline bone mass and incident prediabetes risk.

Previous cross-sectional studies had reported higher BMD in people with T2DM compared with individuals without diabetes (4–6). The findings from our prospective POP-ABC study demonstrate a similar association between BMD and prediabetes, consistent with previous findings from cross-sectional surveys (19, 20). Exploring possible mechanisms, we observed significant correlations between baseline BMD and measures of adiposity and glucose tolerance (2hPG) among our study participants. However, our findings associating higher bone mass with incident prediabetes risk persisted after adjusting for adiposity and glycemia. No significant associations were observed between BMD and insulin sensitivity, insulin secretion, or hsCRP levels in our POP-ABC participants, all of whom were normoglycemic at baseline.

The link between higher BMD and increased prediabetes risk requires further mechanistic insights. The higher BMD reported in people with T2DM could be explained at least in part by obesity. The association of BMD with adiposity measures in our present study also supports a role for obesity as a contributing factor for the higher BMD in progressors versus nonprogressors to prediabetes. Besides obesity, hyperglycemia, insulin resistance, or hyperinsulinemia might be possible mediators of increased bone density in people with T2DM and prediabetes (5–7, 27). As a corollary, the lower BMD reported in people with T1DM would be consistent with the underlying beta-cell failure and insulin deficiency (10). Insulin stimulates osteoblast formation and promotes proliferation, differentiation, and survival of osteoblasts, with an overall balance in favor of bone formation (10). Thus, the relative hyperinsulinemia observed in insulin-resistant individuals with obesity, T2DM, and prediabetes would favor accrual of bone mass, although the

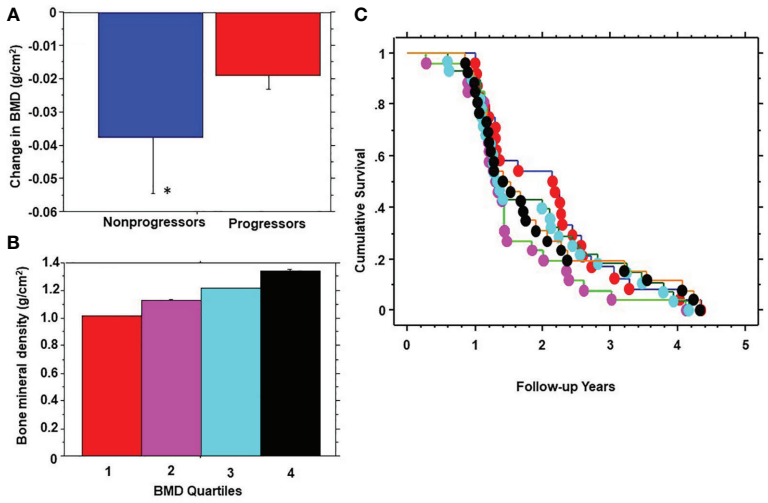


FIGURE 2
One-year change in bone mineral density (BMD) in progressors to prediabetes vs. nonprogressors **(A)**; stratification of participants by quartiles (Q) of baseline BMD **(B)**; and Kaplan-Meier plot of prediabetes survival by baseline BMD quartile **(C)** in the Pathobiology of Prediabetes in a Biracial Cohort study. BMD quartiles: 1 red, 2 purple, 3 blue, 4 black. * P<0.0001.

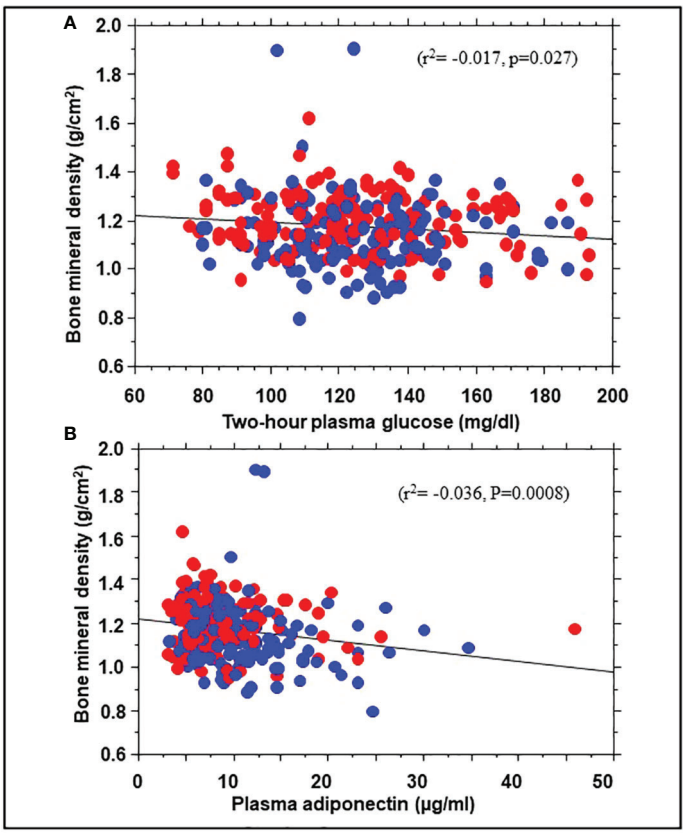


FIGURE 3
Correlation of bone mineral density with 2hPG **(A)** and adiponectin levels **(B)** in African American (red symbols) and European American (blue symbols) participants at enrollment in the Pathobiology of Prediabetes in a Biracial Cohort study.

effect may be modified by the severity of insulin resistance and ambient adipocytokines (4–7, 27, 28).

Plasma adiponectin levels were lower in progressors to prediabetes compared with nonprogressors, and inversely correlated with BMD in our study cohort. Adiponectin, the most abundant secreted product of adipocytes, is a beneficial marker of cardiometabolic health that has been associated with decreased risks of development of diabetes and progression from prediabetes T2DM (29, 30). In a previous report from the POP-ABC study, lower baseline adiponectin levels predicted higher risk of progression from normoglycemia to prediabetes (31). Taken together, our findings of lower baseline adiponectin levels in progressors to prediabetes versus nonprogressors, an inverse correlation between adiponectin and BMD, and a positive association between BMD and incident prediabetes, implicate adiponectin as a possible mediator of the link between BMD and prediabetes risk. Previous reports have also shown a negative correlation between adiponectin and BMD (32, 33). The mechanisms underlying the negative association between adiponectin and BMD are unclear, but increased bone marrow adipogenesis with associated increase in adiponectin production has been proposed to mediate decreased BMD (34, 35).

In addition to the mechanisms involving insulinemia and adiponectin on BMD, there might be a possible mechanism linking bone metabolism to dysglycemia *via* osteocyte production of sclerostin, an inhibitor of wnt signaling pathway. The possible metabolic effects of inhibiting wnt signaling pathway include downstream consequences on adipogenesis, TCF7L2 gene expression, incretin processing and glucose dysregulation (36–39). Another putative mechanism might involve osteocalcin. In a recent study of 240 women with prior gestational diabetes mellitus, participants with prediabetes or diabetes tended to have higher BMD and significantly lower serum osteocalcin levels compared with normoglycemic control (40). Osteocalcin levels declined serially as glycemic status shifted from normoglycemia to prediabetes to diabetes, and showed significant associations with BMD, plasma glucose, insulin sensitivity and insulin secretion in the study population (40).

The strengths of study include the prospective design, enrolment of a diverse study cohort, and the use of robust methodologies for assessment of prediabetes endpoints, insulin sensitivity and insulin secretion. Despite these strengths, our study has some limitations. First, the associations between BMD and prediabetes risk, and the related mechanisms that we observed, do not indicate causality. Second, we studied a special population (offspring of T2DM parents), which may limit the extrapolation of our findings to the general population of individuals without a family history of T2DM. Third, we used fasting plasma glucose and 2-hour OGTT plasma glucose values for definition of prediabetes and did not include HbA1c as one of the criteria. Thus, we may have underdiagnosed individuals with normal fasting and 2-hour plasma glucose values but prediabetes-range HbA1c levels. Fourth, we did not assess vitamin D level, bone micro-architecture, or bone turnover markers.in our participants. Vitamin D deficiency has been associated with increased risks of T2DM and prediabetes (41, 42). However, vitamin D deficiency leads to osteomalacia and decreased bone density (43). Thus, our present finding of an association between higher bone density and increased risk of prediabetes is not likely explained by mechanisms involving vitamin D status (44). Furthermore, our conclusions based on baseline assessments do not account for possible temporal changes in those parameters that might have occurred during the follow-up period. In conclusion, our prospective study demonstrates that the previously reported association between higher bone density and T2DM is discernible in people with prediabetes risk. Thus, putative mechanisms linking bone metabolism with dysglycemia could be operational long before the occurrence of clinical diabetes. Thus, our findings suggest that BMD might be a biomarker for incident glycemic deterioration among normoglycemic individuals.

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 human participants were reviewed and approved by University of Tennessee Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SD-J, as the principal investigator, developed the study concept and design, analyzed data, and wrote the manuscript; ZL collected data, reviewed and revised the manuscript; PA collected data, reviewed and revised the manuscript; AP collected data, reviewed and revised the manuscript, JW analyzed data, reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The POP-ABC study was sponsored by National Institutes of Health Grant R01 DK067269 (to SD-J), American Diabetes Association Grant 7-07-MN-13, and Grant E070166010 from the State of Tennessee Clinical Research Center fund (University of Tennessee Health Science Center Clinical Research Center). The funding sources had no role in the production of the manuscript or in the decision to submit it for publication.

Acknowledgments

The authors gratefully acknowledge the kind assistance of Mary Peterson, RN, and staff at GCRC, and the generosity of the participants who volunteered for the POP-ABC study.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Campos Pastor MM, López-Ibarra PJ, Escobar-Jiménez F, Serrano Pardo MD, García-Cervigón AG. Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: a prospective study. *Osteoporos Int* (2000) 11(5):455–9. doi: 10.1007/s001980070114

2. Eller-Vainicher C, Zhukouskaya VV, Tolkachev YV, Koritko SS, Cairoli E, Grossi E, et al. Low bone mineral density and its predictors in type 1 diabetic patients evaluated by the classic statistics and artificial neural network analysis. *Diabetes Care* (2011) 34(10):2186–91. doi: 10.2337/dc11-0764

3. Joshi A, Varthakavi P, Chadha M, Bhagwat N. A study of bone mineral density and its determinants in type 1 diabetes mellitus. *J Osteoporos.* (2013) 2013:397814. doi: 10.1155/2013/397814

4. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, et al. Risk of fracture in women with type 2 diabetes: The women's health initiative observational study. *J Clin Endocrinol Metab* (2006) 91(9):3404–10. doi: 10.1210/jc.2006-0614

5. Ma L, Oei L, Jiang L, Estrada K, Chen H, Wang Z, et al. Association between bone mineral density and type 2 diabetes mellitus: A meta-analysis of observational studies. *Eur J Epidemiol.* (2012) 27(5):319–32. doi: 10.1007/s10654-012-9674-x

6. Oei L, Zillikens MC, Dehghan A, Buitendijk GH, Castaño-Betancourt MC, Estrada K, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: The Rotterdam study. *Diabetes Care* (2013) 36(6):1619–28. doi: 10.2337/dc12-1188

7. Napoli N, Conte C, Pedone C, Strotmeyer ES, Barbour KE, Black DM, et al. Effect of insulin resistance on BMD and fracture risk in older adults. *J Clin Endocrinol Metab* (2019) 104(8):3303–10. doi: 10.1210/jc.2018-02539

8. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: the nurses' health study. *Diabetes Care* (2006) 29(7):1573–8. doi: 10.2337/dc06-0440

9. Pritchard JM, Giangregorio LM, Atkinson SA, Beattie KA, Inglis D, Ioannidis G, et al. Changes in trabecular bone microarchitecture in postmenopausal women with and without type 2 diabetes: a two year longitudinal study. *BMC Musculoskelet Disord* (2013) 14:114. doi: 10.1186/1471-2474-14-114

10. Hough FS, Pierroz DD, Cooper C, Ferrari SL. IOF CSA bone and diabetes working group. MECHANISMS IN ENDOCRINOLOGY: Mechanisms and evaluation of bone fragility in type 1 diabetes mellitus. *Eur J Endocrinol* (2016) 174(4):R127–38. doi: 10.1530/EJE-15-0820

11. Napoli N, Strollo R, Paladini A, Briganti SI, Pozzilli P, Epstein S. The alliance of mesenchymal stem cells, bone, and diabetes [published correction appears in int J endocrinol. *2017;2017:5924671]. Int J Endocrinol* (2014) 2014:690783. doi: 10.1155/2014/690783

12. Gennari L, Merlotti D, Valenti R, Ceccarelli E, Ruvio M, Pietrini MG, et al. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. *J Clin Endocrinol Metab* (2012) 97(5):1737–44. doi: 10.1210/jc.2011-2958

13. Yamamoto M, Yamauchi M, Sugimoto T. Elevated sclerostin levels are associated with vertebral fractures in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* (2013) 98(10):4030–7. doi: 10.1210/jc.2013-2143

14. Palermo A, D'Onofrio L, Eastell R, Schwartz AV, Pozzilli P, Napoli N. Oral anti-diabetic drugs and fracture risk, cut to the bone: Safe or dangerous? a narrative review. *Osteoporos Int* (2015) 26(8):2073–89. doi: 10.1007/s00198-015-3123-0

15. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* (2022) 45(Suppl. 1):S17–38. doi: 10.2337/dc22-S002

16. Meigs JB, Muller DC, Nathan DM, Blake DR, Andres R. Baltimore Longitudinal study of aging. the natural history of progression from normal glucose tolerance to type 2 diabetes in the Baltimore longitudinal study of aging. *Diabetes.* (2003) 52(6):1475–84. doi: 10.2337/diabetes.52.6.1475

17. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: Implications for care. *Diabetes Care* (2007) 30(3):753–9. doi: 10.2337/dc07-9920

18. Centers for Disease Control and Prevention. *National diabetes statistics report, 2020.* Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services (2020). Available at: https://www.cdc.gov/diabetes/data/statistics-report/index.html.

19. Chen C, Chen Q, Nie B, Zhang H, Zhai H, Zhao L, et al. Trends in bone mineral density, osteoporosis, and osteopenia among U.S. adults with prediabetes, 2005-2014. *Diabetes Care* (2020) 43(5):1008–15. doi: 10.2337/dc19-1807

20. Yuan J, Jia P, Zhou JB. Comparison of bone mineral density in US adults with diabetes, prediabetes and normoglycemia from 2005 to 2018. *Front Endocrinol (Lausanne).* (2022) 13:890053. doi: 10.3389/fendo.2022.890053

21. Dagogo-Jack S, Edeoga C, Nyenwe E, Chapp-Jumbo E, Wan J. Pathobiology of prediabetes in a biracial cohort (POP-ABC): Design and methods. *Ethn Dis* (2011) 21:33–9.

22. Ebenibo S, Edeoga E, Ammons A, Egbuonu N, Dagogo-Jack S. Recruitment strategies and yields for the pathobiology of prediabetes in a biracial cohort: A prospective natural history study of incident dysglycemia. *BMC Med Res Method* (2013) 13(1):64. doi: 10.1186/1471-2288-13-64

23. Dagogo-Jack S, Edeoga C, Ebenibo S, Chapp-Jumbo E. Pathobiology of prediabetes in a biracial cohort (POP-ABC) study: Baseline characteristics of enrolled subjects. *J Clin Endocrinol Metab* (2013) 98:120–8. doi: 10.1210/jc.2012-2902

24. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Expert committee on the diagnosis and classification of diabetes mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* (2003) 26:3160–7. doi: 10.2337/diacare.26.11.3160

25. Bureau of the Census. 1990 census of the population. Washington, DC: U.S. Government Printing Office (1990).

26. Dagogo-Jack S, Edeoga C, Ebenibo S, Nyenwe E, Wan J. Pathobiology of prediabetes in a biracial cohort (POP-ABC) research group. lack of racial disparity in incident prediabetes and glycemic progression among black and white offspring of parents with type 2 diabetes: The pathobiology of prediabetes in a biracial cohort (POP-ABC) study. *J Clin Endocrinol Metab* (2014) 99:E1078–87. doi: 10.1210/jc.2014-1077

27. Kindler JM, Lobene AJ, Vogel KA, Martin BR, McCabe LD, Peacock M, et al. Adiposity, insulin resistance, and bone mass in children and adolescents. *J Clin Endocrinol Metab* (2019) 104:892–899. doi: 10.1210/jc.2018-00353

28. Miazgowski T, Noworyta-Ziętara M, Safranow K, Ziemak J, Widecka K. Serum adiponectin, bone mineral density and bone turnover markers in post-menopausal women with newly diagnosed type 2 diabetes: A 12-month follow-up. *Diabetes Med* (2012) 29(1):62–9. doi: 10.1111/j.1464-5491.2011.03381.x

29. Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, et al. Adiponectin and the development of type 2 diabetes: The atherosclerosis risk in communities study. *Diabetes* (2004) 53:2473–8. doi: 10.2337/diabetes.53.9.2473

30. Mather KJ, Funahashi T, Matsuzawa Y, Edelstein S, Bray GA, Kahn SE, et al. Adiponectin, change in adiponectin, and progression to diabetes in the diabetes prevention program. *Diabetes* (2008) 57:980–6. doi: 10.2337/db07-1419

31. Jiang Y, Owei I, Wan J, Ebenibo S, Dagogo-Jack S. Adiponectin levels predict prediabetes risk: The pathobiology of prediabetes in a biracial cohort (POP-ABC) study. *BMJ Open Diabetes Res Care* (2016) 4(1):e000194. doi: 10.1136/bmjdrc-2016-000194

32. Tai TY, Chen CL, Tsai KS, Tu ST, Wu JS, Yang WS. A longitudinal analysis of serum adiponectin levels and bone mineral density in postmenopausal women in Taiwan. *Sci Rep* (2022) 12(1):8090. doi: 10.1038/s41598-022-12273-7

33. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Yano S, Sugimoto T. Relationships between serum adiponectin levels versus bone mineral density, bone metabolic markers, and vertebral fractures in type 2 diabetes mellitus. *Eur J Endocrinol* (2009) 160(2):265–73. doi: 10.1530/EJE-08-0642

34. Wang F, Wang PX, Wu XL, Dang SY, Chen Y, Ni YY, et al. Defciency of adiponectin protects against ovariectomy-induced osteoporosis in mice. *PLoS One* (2013) 8(7):e68497. doi: 10.1371/journal.pone.0068497

35. Herrmann M. Marrow fat-secreted factors as biomarkers for osteoporosis. *Curr Osteoporos. Rep* (2019) 17(6):429–37. doi: 10.1007/s11914-019-00550-w

36. Umekwe N, Owei I, Stentz F, Dagogo-Jack S. Plasma FGF-21 and sclerostin levels, glycemia, adiposity, and insulin sensitivity in normoglycemic black and white adults. *J Endocr Soc* (2021) 6(1):bvab183. doi: 10.1210/jendso/bvab183

37. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: Components, mechanisms, and diseases. *Dev Cell* (2009) 17(1):9–26. doi: 10.1016/j.devcel.2009.06.016

38. Christodoulides C, Lagathu C, Sethi JK, Vidal-Puig A. Adipogenesis and WNT signalling. *Trends Endocrinol Metab* (2009) 20(1):16–24. doi: 10.1016/j.tem.2008.09.002

39. García-Martín A, Rozas-Moreno P, Reyes-García R, Morales-Santana S, García-Fontana B, García-Salcedo JA, et al. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* (2012) 97(1):234–41. doi: 10.1210/jc.2011-2186

40. Kubihal S, Gupta Y, Goyal A, Kalaivani M, Tandon N. Bone microarchitecture, bone mineral density and bone turnover in association with glycemia and insulin action in women with prior gestational diabetes. *Clin Endocrinol (Oxf).* (2022) 96(4):531–8. doi: 10.1111/cen.14641

41. Mitri J, Pittas AG. Vitamin d and diabetes. *Endocrinol Metab Clin North Am* (2014) 43(1):205–32. doi: 10.1016/j.ecl.2013.09.010

42. Yu L, Zhai Y, Shen S. Association between vitamin d and prediabetes: A PRISMA-compliant meta-analysis. *Med (Baltimore).* (2020) 99(8):e19034. doi: 10.1097/MD.0000000000019034

43. Reid IR. Vitamin d effect on bone mineral density and fractures. *Endocrinol Metab Clin North Am* (2017) 46(4):935–45. doi: 10.1016/j.ecl.2017.07.005

44. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari S. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol* (2017) 13(4):208–19. doi: 10.1038/nrendo.2016.153

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 20 January 2023
DOI 10.3389/fendo.2022.1065665

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal, South Africa

REVIEWED BY
Ruth Gutierrez-Aguilar,
Research Division, Faculty of Medicine,
National Autonomous University of Mexico,
Mexico
E-Hu Liu,
China Pharmaceutical University, China

*CORRESPONDENCE
Zhigang Wang
wangzhigang0513@vip.163.com
Dan Yan
danyan@ccmu.edu.cn
Na Guo
guona5246@126.com

SPECIALTY SECTION
This article was submitted to
Clinical Diabetes,
a section of the journal
Frontiers in Endocrinology

RECEIVED 10 October 2022
ACCEPTED 30 December 2022
PUBLISHED 20 January 2023

CITATION
Yang J, Wang M, Yang D, Yan H, Wang Z, Yan D and Guo N (2023) Integrated lipids biomarker of the prediabetes and type 2 diabetes mellitus Chinese patients. *Front. Endocrinol.* 13:1065665. doi: 10.3389/fendo.2022.1065665

Integrated lipids biomarker of the prediabetes and type 2 diabetes mellitus Chinese patients

Jiaying Yang[1,2], Mei Wang[3], Dawei Yang[4], Han Yan[1], Zhigang Wang[2*], Dan Yan[5*] and Na Guo[1*]

[1]Experimental Research Center, China Academy of Chinese Medical Sciences, Beijing, China, [2]College of Pharmacy, Heilongjiang University of Traditional Chinese Medicine, Heilongjiang, China, [3]LU-European Center for Chinese Medicine and Natural Compounds, Institute of Biology, Leiden University, Leiden, Netherlands, [4]Zhong Yuan Academy of Biological Medicine, Liaocheng People's Hospital, Liaocheng, Shandong, China, [5]Beijing Institute of Clinical Pharmacy, Beijing Friendship Hospital, Capital Medical University, Beijing, China

Introduction: Dyslipidemia is a hallmark of T2DM, and as such, analyses of lipid metabolic profiles in affected patients have the potential to permit the development of an integrated lipid metabolite-based biomarker model that can facilitate early patient diagnosis and treatment.

Methods: Untargeted and targeted lipidomics approaches were used to analyze serum samples from newly diagnosed 93 Chinese participants in discovery cohort and 440 in validation cohort *via* UHPLC-MS and UHPLC-MS/MS first. The acid sphingomyelinase protein expression was analyzed by Western blot.

Results and Discussion: Through these analyses, we developed a novel integrated biomarker signature composed of LPC 22:6, PC(16:0/20:4), PE(22:6/16:0), Cer (d18:1/24:0)/SM(d18:1/19:0), Cer(d18:1/24:0)/SM(d18:0/16:0), TG(18:1/18:2/18:2), TG(16:0/16:0/20:3), and TG(18:0/16:0/18:2). The area under the curve (AUC) values for this integrated biomarker signature for prediabetes and T2DM patients were 0.841 (cutoff: 0.565) and 0.894 (cutoff: 0.633), respectively. Furthermore, theresults of western blot analysis of frozen adipose tissue from 3 week (prediabetes) and 12 week (T2DM) Goto–Kakizaki (GK) rats also confirmed that acid sphingomyelinase is responsible for significant disruptions in ceramide and sphingomyelin homeostasis. Network analyses of the biomarkers associated with this biosignature suggested that the most profoundly affected lipid metabolism pathways in the context of diabetes include *de novo* ceramide synthesis, sphingomyelin metabolism, and additional pathways associated with phosphatidylcholine synthesis. Together, these results offer new biological insights regarding the role of serum lipids in the context of insidious T2DM development, and may offer new avenues for future diagnostic and/or therapeutic research.

KEYWORDS

prediabetes, type 2 diabetes mellitus, lipidomics, ceramide, UHPLC-MS

1 Introduction

T2DM makes up over 90% of human diabetes cases (1), and is among the most rapidly growing threats to human health throughout the globe (2). T2DM develops over several years in prediabetic individuals (3, 4), early diagnosis and treatment can effectively prevent the development of diabetes. Therefore, the detection of reliable biomarkers associated with prediabetes and T2DM is an area of active research, and multiple biomarkers including fasting blood glucose (FBG) and glycated hemoglobin A1c(HbA1c) (5–8) have been proposed as tools to assess the risk of diabetes (3–8). While valuable, however, these biomarkers fail to fully capture the complexity of T2DM development, and may also fail to detect at-risk individuals prior to disease onset (4, 9–11).

Dyslipidemia, and lipoprotein metabolism abnormalities are commonly detected in those with diabetes (12–14). Detecting these shifts in lipid profiles thus represents a promising approach to identifying high-risk patients at earlier time points. Lipidomic analyses of overall lipid profiles can also offer additional insight into the pathophysiology of diseases (15–17), including diabetes (15, 18–22). Several lipidomics studies have provided evidence that comprehensive lipid profiles have the potential to improve diabetes risk assessment relative to the use of conventional clinic indices alone Certain subclasses of lipids including ceramides, sphingolipids, phospholipids, triglycerides (TGs) having been linked to human prediabetes and T2DM (23–34)s T2DM is highly prevalent in European nations (35–37), and the human serum lipidome is highly complex (38, 39), a majority of recent studies have employed lipidomics approaches to analyze the serum lipid profiles of European individuals with prediabetes and T2DM (40–42). However, diabetes rates are rising rapidly in China such that it is now home to the highest global diabetes incidence (43), with prediabetes affecting a remarkable 35.7% of the population (44). Chinese dietary composition and obesity rates are very distinct from those in Western nations, and relative to European T2DM patients, those from China are often diagnosed at younger ages and with lower body mass index (BMI) values (45). As such, more in-depth analyses of the roles of endogenous lipids in the pathophysiology of prediabetes and T2DM in Chinese patients is essential to guide the development of novel preventative measures or treatment strategies. Furthermore, in recent years, an increasing number of studies have shown that synthesis of ceramide by sphingomyelinase hydrolysis sphingomyelin is considered to be one of the major causes for insulin resistance (29).

Sphingomyelinase-regulated balance of ceramides and sphingolipids plays an important role in many diseases (30, 46, 47). Sphingomyelinase especially acid sphingomyelinase has a central function for the re-organization of molecules within the cell upon stimulation and thereby for the response of cells to stress and the induction of cell death but also proliferation and differentiation (31). The role and mechanism of ASM research in many diseases has made great progress, which fully confirmed the important role of ASM/ceramides pathway in T2DM, However, there are few studies on prediabetes. It is important to further study the exact regulation mechanism of ASM pathway in pathophysiology of prediabetes. Previous studies have suggested that patients with long-standing T2DM and had worse metabolic profiles when compared with the newly diagnosed (48), and multiple complications such as chronic kidney disease (CKD) and diabetic kidney disease (DKD) remain common in diabetics in the decade after diagnosis (49). In addition, long-term use of hypoglycemic drugs such as metformin and acarbose also could alter the lipid profile of human (50, 51), revealing metabolic changes of diseases. Thus, it is very key for study of lipid metabolic profiles of participants with prediabetes and T2DM with the newly diagnosed. Untargeted lipidomics analyses are limited by their narrow linear range, poor reproducibility, and low sensitivity (52, 53), whereas targeted approaches exhibit reduced metabolomics coverage such that they have the potential to miss metabolites of interest. As such, combining targeted and untargeted lipidomics strategies can overcome potential misannotation owing to the structural diversity and complexity of lipid molecules, thereby enabling the better confirmation of results to offer insight into lipid metabolism in the pathophysiology of metabolic diseases.

Herein, we employed untargeted and targeted UHPLC-MS and UHPLC-MS/MS approaches to analyze the serum lipid profiles of Chinese individuals with newly diagnosed patients or without prediabetes or T2DM. Subsequently, western blot analysis of ASM in different ages of GK rats was performed in order to explore and confirm whether ASM is responsible for significant disruptions in ceramide and sphingomyelin homeostasis and the important role of ASM/ceramides pathway in prediabetes and T2DM patients. The resultant data were analyzed with both commercial and in-house software applications. The overall goals of this study were to systematically screen for potential lipid biomarkers associated with prediabetes and T2DM incidence in Chinese patients in order to both better understand lipid pathway dysregulation and to develop a new integrated biosignature that may aid in diagnosing these conditions.

2 Materials and methods

2.1 Participant recruitment and grouping

All study participants were recruited from Beijing Shijitan hospital at the Capital Medical University (Beijing, China), Beijing Jiao Tong University Community Health Center (Beijing, China), The First Affiliated Hospital of Zhengzhou University (Henan, China), The First Affiliated Hospital of Henan University of Chinese Medicine (Henan, China), and Kaifeng Hospital of Traditional Chinese Medicine (Henan, China). All subjects underwent a physical examination during which their height, weight, and BMI were recorded. They then completed a face-to-face interview during which they detailed their demographics, medical history, family medical history, and other lifestyle factors. Blood samples were additionally collected to measure participant plasma total cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), triglyceride (TG), FBG, alanine transaminase (ALT), and aspartate transaminase (AST) levels. Individuals were eligible for final study enrollment if they met the following criteria: (1)

patients exhibited an FBG > 7.0 mmol/L (54) or met the diagnostic criteria for prediabetes (FBG: 5.6-6.9 mmol/L) (55, 56); (2) patients were 20-70 years of age. Patients were excluded if they: (1) exhibited a history of cardiovascular or cerebrovascular events; (2) had impaired liver/kidney function; (3) had a fasting triglyceride level ≥ 10mmol/L; (4) suffered from other endocrine, autoimmune, renal, cancerous, or otherwise serious diseases; (5) were undergoing treatment with antibiotics, glucocorticoids, or traditional Chinese herbal medicines; (6) were pregnant or expecting to become pregnant; (7) were currently breastfeeding; (8) suffered from mental health conditions; (9) declined or were unable to comply with study dietary guidelines; or (10) suffered from severe infectious diseases. Based upon this criteria, participants were grouped into control (n=35), prediabetes (n=31), and T2DM (n=27) discovery cohorts as well as control (n=150), prediabetes (n=170), and T2DM (n=120) validation cohorts. The Ethics Committee of Scientific Research, Beijing Shijitan Hospital, Capital Medical University approved this study, and all participants provided written informed consent to participate.

2.2 Chemicals and materials

Liquid chromatography/mass spectrometry (LC/MS)-grade methanol, acetonitrile, 2-propanol, ammonium formate, and HPLC-grade methyl tert-butyl ether (MTBE) were obtained from Fisher Scientific (PA, USA). LC/MS-grade ammonium formate was from Sigma-Aldrich (MO, USA). A Milli-Q system (MA, USA) was used to prepare ultra-pure water (18.2 MΩ).

Lysophosphatidylcholine (LPC 19:0), Phosphatidylethanolamine (PE 12:0/13:0), Ceramide (Cer d18:1/17:0), Sphingomyelin (SM (d18:1/12:0), TG (15:0/15:0/15:0), and Phosphatidylcholine (PC 12:0/13:0) were purchased for use as internal standard (IS) compounds from Avanti Polar Lipids (AL, USA). Antibodies used in this study were rabbit anti-acid sphingomyelinase polyclonal antibody (Absin, Shanghai, China).

2.3 Sample preparation

For untargeted lipidomics analyses, serum (10 μL) and cold methanol containing IS compounds (125 μL) were mixed for 30 s, followed by the addition of MTBE (500 μL). Lipids were then extracted by constantly agitating these samples for 20 min at room temperature, followed by the addition of water (125 μL), shaking for 30 s, and centrifugation at 16,826×g for 10 min at 4°C. For untargeted analyses, 200 μL of the resultant supernatant was dried with a concentrator prior to resuspension in a 100 μL volume of water: isopropanol: acetonitrile (5:30:65 (v/v/v). These samples were then agitated for an additional 30 s, followed by centrifugation at 16,826×g for 5 min at 4°C. The isolated supernatants were then evaluated *via* ultra-performance liquid chromatography/time of flight-mass spectrometry (UHPLC/TOF-MS) as soon as they had been collected. For targeted lipidomic analyses, a 100 μL volume of the supernatant prepared above was dried, resuspended in a 200 μL volume, and analyzed *via* UHPLC/MS-MS. A quality control (QC) serum sample was also generated for further analyses by mixing together 5 μL of each serum sample. These QC samples were processed for analysis in the sample manner as individual samples throughout the duration of our analyses. The QC samples were injected every 10 injections, and analyzed 10 times (discovery cohort) and 44 times (validation cohort) between samples to verify the stability of the LC-MS system respectively.

2.4 Untargeted and targeted UHPLC-MS lipidomics analyses

An Acquity UPLC BEH C_8 column (2.1 × 100 mm, 1.7 μm) was used for lipid separation using a mobile phase composed of 5 mM ammonium formate with acetonitrile/water (A, 6:4; v/v) and 5 mM ammonium formate with isopropanol/acetonitrile (B, 9:1; v/v). Linear elution gradient settings for separation were: 0–1.0 min, 100% A; 1.0–2.0 min, 100–70% A; 2.0–12.0 min, 70–30% A; 12.0–12.5 min, 30–5% A; 12.5–13.0 min, 5–0% A; 13.0–14.0 min, 0% A; 14.0–14.1 min, 0–100% A; and 14.1–16.0 min, 100% A. The column was maintained at 55°C. An ACQUITY UPLC connected to a XEVO-G2XS quadrupole time-of-flight (QTOF) mass spectrometer (Waters, Manchester, NH, USA) in ESI+ mode was used for untargeted lipidomics analyses with the following settings: desolvation gas at 800 L/h and 400°C; cone gas at 50 L/h; source temperature at 100°C; capillary and sampling voltages of 2,000 V and 40 V, respectively. Mass data were acquired in MS^E mode at a ramping collision energy of 10–60 V. Data accuracy was ensured using a LockSpray™ source, with the (M+H)+ ions of leucine-enkephalin being set at m/z 556.2771 for the lock mass in ESI + mode. Sample profiling data were acquired from 50 - 1,200 Da. A UHPLC system (Waters Acquity) with a Xevo TQ-S mass spectrometer and an ESI ionization source was used for targeted lipidomics analyses conducted using multiple reaction monitoring (MRM) in positive ion modes.

2.5 Experimental animals and adipose tissue collection

Goto–Kakizaki (GK) rat is one of the best characterized animal models of spontaneous T2DM. This model was established by selectively breeding of normal Wistar rats with signs of impaired glucose tolerance (57). It displays hyperglycemia, impaired glucose tolerance, insulin resistance and also defects in insulin secretion. In most of the GK studies, Wistar rats of outbreed origin are used as control animals (58). In addition, GK pups become overtly hyperglycemic for the first time after 3–4weeks of age only (i.e., during the weaning period). The occurrence of basal hyperglycemia and diabetes in the GK rat is therefore preceded by a period of prediabetes (22-28 days) (59). This study involved 10 T2DM GK male rats (12 week); 10 prediabetic GK male rats (3 week) and 10 control Wistar male rats obtained from Nanjing Junke Biotechnology Co., Ltd. (Jiangsu, China). A GLU Assay Kit (KOFA, China) and an

automatic biochemistry analyzer (Hitachi 7020, Tokyo, Japan) were used to measure glucose concentration of GK rats. Rats were anesthetized using pentobarbital sodium (3%, 0.2 ml/100 g) and sacrificed by abdominal aortic exsanguination. After the adipose tissues of the rats was collected, snap-frozen in liquid nitrogen, and transferred to a -80 freezer until analysis. The experiments were approved by the China Pharmaceutical University Animal Care and Use Committee.

2.6 Western blotting

The ASM protein expression was analyzed by Western blot. The adipose tissue were washed twice by phosphate-Buffered Saline (PBS) and lysed in radio immunoprecipitation assay (RIPA) lysis buffer. The protein concentrations were determined by the bicinchoninic acid (BCA) protein assay kit. 30ul proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes, and blocked by immersing the membrane completely in 5% bovine serum albumin-tris buffered saline tween (BSA-TBST) and incubating on a horizontal shaker for 1 h. The membranes were probed with the primary antibodies of ASM (1:1000), overnight at 4°C followed by incubation with the secondary antibody goat anti rabbit IgG (H+L) at room temperature for 1 h. glyceraldehyde-3-phosphate dehydrogenase (GADPH) were used as control protein. The resulting complexes were visualized using chemoluminescence Western blotting detection reagents enhanced chemiluminescence (ECL). The blot was detected by chemiluminescent detection systems with LumiGlo and Peroxide (1:1, BU). Densitometric analysis of the images was performed with Image Pro Plus software (v.6.0) (Media Cybernetics, Inc, MD, USA).

2.7 Statistical analyses

The Waters MarkerLynx software (Waters; Micromass MS Technologies, Manchester, UK) was utilized to analyze data from untargeted lipidomics analyses in an effort to identify serum biomarkers specifically associated with prediabetes and T2DM patients. Waters Progenesis QI Applications Manager (v2.3) was utilized for peak finding, filtering, and alignment with the following data collection parameters: retention time = 0.5-15.5 min; mass = 50-1,200 Da. SIMCA-P (v13.0) (Umetrics, Umea, Sweden) was used to conduct multivariate statistical analyses of the resultant data. Partial least squares discriminant analysis (PLS-DA) was conducted in order to visualize the global metabolic difference of individuals between the control, prediabetes and T2DM groups. To validate the PLS-DA model, permutation tests were performed (n = 200). The Skyline software (v21.1) (MacCoss Lab; WA, USA) was used for data acquisition and peak processing for targeted lipidomics analyses. MetaboAnalyst 5.0 Web service (www.MetaboAnalyst.ca) was used to normalize raw data for next statistical analyses. Data have a normal distributed by Kolmogorov-Smirnov test and Quantile-Quantile plots (Q-Q plots). Independent samples t-tests and ROC curve analyses were performed using SPSS (v26.0) (IBM, NY, USA) P-value < 0.05 corrected by FDR was used as the cutoff for significance of differential metabolites. Column diagrams and forest plots were drawn by GraphPad Prism 9.0 (GraphPad Software Inc., USA). Python was used to generate heat maps highlighting correlations between putative biomarkers and specific clinical parameters calculated based upon Pearson correlation coefficients.

3 Results

3.1 Patient characteristics

In total, 533 participants ultimately met the criteria for enrollment of this study, of whom 93 were included in a discovery cohort (control = 35, prediabetes = 31, and T2DM = 27) and 440 were included in a validation cohort (control = 150, prediabetes = 170, and T2DM = 120). Patient clinical characteristics are summarized in Table 1. As expected, patients in the prediabetes and T2DM groups in both cohorts exhibited higher FBG and TG concentrations relative to controls. T2DM patients also exhibited a significant reduction in HDL content relative to control participants (P = 0.01), with a similar downward trend being observed for prediabetes patients in the validation cohort (P < 0.001) together with an increase in their TC levels (P < 0.001). There were no differences among groups with respect to age, gender, BMI, ALT, or AST, nor were there any differences in TC or LDL levels among the discovery cohorts.

3.2 Reproducibility of the lipidomic analysis

Base peak chromatograms generated in positive ion mode in an untargeted lipidomics analysis are shown in Figures 1A–C. To validate the method being used herein for biomarker detection, system stability and result reproducibility were assessed by analyzing pooled QC samples and determining relative standard deviation (RSD%) values corresponding to the peak area for IS compounds (Table S1). RSD% values corresponding to the peak area for IS compounds are 6.86%-27.61%. This approach confirmed the high reproducibility and stability of these analyses.

3.3 Exploration of distinct lipidomic profiles associated with prediabetes and T2DM

Next, we sought to explore differences in the serum lipidomic profiles of control, prediabetes, and T2DM study subjects by using a PLS-DA model to evaluate the global lipid profiles of these groups as detected through the untargeted lipidomics approach validated above. The resultant 2D and 3D score plots achieved satisfactory classification, revealing that the lipid metabolic state in the serum of prediabetes and T2DM patients was distinct from that in healthy control serum (Figures 1D, E). These results suggested that T2DM is

10.3389/fendo.2022.1065665

TABLE 1 Baseline patient characteristics in the discovery and validation cohorts.

	Discovery						Validation					
	Control n=35	prediabetes n=31	T2DM n=27	P_1 value	P_2 value	P_3 value	Control n=150	prediabetes n=170	T2DM n=120	P_1 value	P_2 value	P_3 value
Females (%)	25.71	35.48	25.92	**0.985**	**0.793**	**0.490**	43.33%	35.88%	30.83%	**0.1938**	**0.0505**	**0.6206**
Age (years)	49.26 ± 12.21	46.29 ± 13.38	47.71 ± 12.74	**0.829**	**0.874**	**0.576**	45.96 ± 9.79	47.91 ± 10.96	49.98 ± 9.95	**0.1195**	**0.2071**	**0.2044**
FBG (mmol/L)	5.31 ± 0.45	6.37 ± 0.19	8.20 ± 2.27	**0.00001**	**0.00001**	**0.001**	5.05 ± 0.51	6.43 ± 0.24	9.54 ± 2.57	**0.00001**	**0.00001**	**0.00001**
BMI (kg/m2)	24.42 ± 2.42	24.65 ± 2.40	25.60 ± 3.30	**0.226**	**0.877**	**0.478**	24.80 ± 2.84	25.44 ± 3.36	25.67 ± 4.32	**0.0980**	**0.0619**	**0.7773**
Total cholesterol (mmol/L)	4.53 ± 0.28	4.62 ± 0.34	4.85 ± 0.87	**0.103**	**0.943**	**0.383**	4.70 ± 0.46	4.98 ± 0.73	4.77 ± 0.96	**0.0003**	**0.4920**	**0.1267**
Triglyceride (mmol/L)	1.16 ± 0.25	1.14 ± 0.27	1.59 ± 0.77	**0.013**	**0.821**	**0.017**	1.10 ± 0.28	2.04 ± 1.17	2.07 ± 1.31	**0.00001**	**0.00001**	**0.9590**
HDL-C (mmol/L)	1.45 ± 0.24	1.47 ± 0.25	1.20 ± 0.48	**0.045**	**0.859**	**0.043**	1.43 ± 0.27	1.37 ± 0.27	1.12 ± 0.26	**0.0902**	**0.00001**	**0.00001**
LDL-C (mmol/L)	2.54 ± 0.26	2.66 ± 0.31	2.78 ± 0.72	**0.093**	**0.480**	**0.434**	2.86 ± 0.40	3.01 ± 0.65	3.16 ± 0.78	**0.0400**	**0.0001**	**0.1867**
ALT (U/L)	18.89 ± 5.07	20.00 ± 6.11	24.71 ± 18.86	**0.193**	**0.702**	**0.493**	20.68 ± 6.97	22.87 ± 11.16	23.84 ± 16.56	**0.0780**	**0.0589**	**0.7886**
AST (U/L)	19.54 ± 3.09	18.90 ± 3.87	20.86 ± 11.63	**0.637**	**0.655**	**0.466**	20.54 ± 3.63	20.4 ± 5.40	20.34 ± 9.45	**0.7883**	**0.8071**	**0.9409**

Values are given as mean ± SD or number of individuals (%), unless otherwise indicated. P-value; independent t-test and adjusted by FDR, "P_1" Control VS Prediabetes, "P_2" Control VS T2DM, "P_3" Prediabetes VS T2DM. BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C,low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase; NGT, normal glucose tolerance; IFG, impaired fasting glucose; T2DM, type 2 diabetes mellitus.
Bold values mean P value.

associated with the disruption of endogenous metabolic processes such that patients exhibit a distinct metabolic fingerprint. Notably, we also observed substantial separation between prediabetes and T2DM patient samples in these PLS-DA plots, suggesting that prediabetic and diabetic individuals also exhibit distinct lipid metabolic profiled. R^2 Y represents the goodness of fit of the PLS-DA model on the Y-axis, while Q^2 estimates predictive capability (60). The R^2 Yand Q^2 of the established PLS-D model were 0.925 and 0.609. A permutation test (n=200) was additionally used to validate this model, confirming the goodness of fit and predictive reliability (Figure S1).

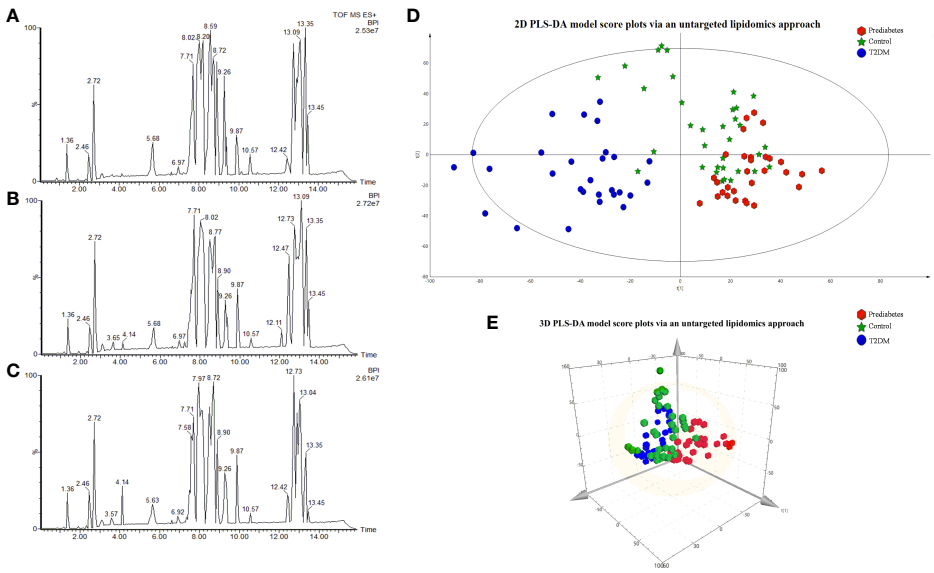


FIGURE 1
The BPI chromatograms of samples via the untargeted lipidomics approach in the Control group **(A)**; prediabetes group **(B)**; T2DM group **(C)**. 2D PLS-DA model score plots for individual serum samples in the control (green), prediabetes (red), and T2DM (blue) groups analyzed via an untargeted lipidomics approach 3D PCS-DA model score plots **(E)**.

3.4 Identification of putative prediabetes- and T2DM- related biomarkers *via* untargeted and targeted lipidomics analyses

For untargeted lipidomics analyses, the Progenesis QI software was used to detect tens of thousands of features in the LC-MS data. Based on ion fragmentation patterns, accurate compound masses, published data, and chemical standards, 166 lipids were identified in these serum samples (Table S2). To screen for metabolites that were differentially abundant in the serum of prediabetes and T2DM patients, we next conducted independent sample t-tests with P-value < 0.05 corrected by FDR. 49 candidate lipids show similar significant trends in prediabetes and T2DM relative to controls in untargrted lipidomics analyse (discovery cohort). These differences were additionally emphasized through heatmaps and clustering analyses (Figure 2). Based on these results from the discovery cohort, subsequently, a high selectivity, reproducibility and sensitivity targeted lipidomics approach including more than 200 lipids of interest was used to assess the serum lipid profiles of patients in the validation cohort (Table S3). In this analysis, 37 lipids including LPCs, LPEs, PCs, PEs, SMs, Cers, and TGs were significantly differentially abundant in samples from the control group and the prediabetes/T2DM groups (Figure 3). Levels of all of these lipids were significantly elevated in those with prediabetes/T2DM, suggesting the dysregulation of the ceramide synthesis, SM metabolism, PC biosynthesis pathways (Figure 3). By venn diagram (Figure S2), 9 potential biomarkers including LPC 22:6, PC(16:0/20:4), PE(22:6/16:0), Cer(d18:1/24:0), Cer(d18:1/23:0), Cer(d18:1/22:0), TG(18:1/18:2/18:2), TG(16:0/16:0/20:3), and TG(18:0/16:0/18:2) (FDR < 0.05 and P < 0.05) were overlapping between 49 candidate lipids metabolites screened from non-targeted lipidomic data (discovery cohort) and 37 differential lipids from targeted lipidomic data (validation cohort), and they show similar significant trends in prediabetes and T2DM relative to controls (Table 2 and Figure 4). A one standard deviation change in the levels of these 9 putative biomarkers was associated with prediabetes and T2DM effect sizes ranging from odds ratios (ORs) of 1.235 - 8.306 and 1.189 - 11.479, respectively (Figure 4).

3.5 Integrated biomarker development and validation

While no significant differences in SMs levels were observed among groups in the discovery cohort, levels of SM (d18:2/24:1), SM (d18:1/24:1), SM (d18:2/23:0), SM (d18:1/19:1), SM (d18:1/19:0), SM (d16:0/19:0) and SM (d18:0/16:0) trended downwards in prediabetes and T2DM samples from the validation cohort (Figure 4). Ceramides and SMs are closely linked through the sphingomyelinase pathway, and several ceramide levels trended upward in the prediabetes and T2DM groups in both cohorts. Sphingomyelinase-regulated Cer/SM balance plays a variety of roles in cancer, coronary heart disease and neurodegenerative disorders progression and prevention (16, 61, 62), To investigate whether Cer/SM can predict prediabetes and T2DM, we have carried out binary logistic regression and ROC curve analyses for Cer(d18:1/24:0), Cer (d18:1/23:0), Cer(d18:1/22:0) first. The results show that Cer(d18:1/24:0) have higher predictive power in prediabetes and T2DM compared with Cer(d18:1/23:0) and Cer(d18:1/22:0) (Figures S3A, B). Then we performed binary logistic regression and ROC curve analyses for the ratio of Cer(d18:1/24:0) to 7 different SM such as Cer(d18:1/24:0)/SM(d18:2/24:1), Cer(d18:1/24:0)/SM(d18:1/24:1), Cer(d18:1/24:0)/SM(d18:2/23:0), Cer(d18:1/24:0)/SM(d18:1/19:1), Cer(d18:1/24:0)/SM(d18:1/19:0), Cer(d18:1/24:0)/SM(d16:0/19:0) and Cer(d18:1/24:0)/SM(d18:0/16:0). The results show that Cer(d18:1/24:0)/SM(d18:1/19:0) and Cer(d18:1/24:0)/SM(d18:0/16:0) have higher predictive power in prediabetes and T2DM compared with others candidate features (Figures S3C, D). As such, we selected Cer(d18:1/24:0)/SM(d18:1/19:0) and Cer(d18:1/24:0)/SM (d18:0/16:0) as candidate features for the development of an integrated diagnostic biosignature for prediabetes and T2DM. The resultant integrated potential biomarker model consisted of LPC 22:6, PC(16:0/20:4), PE(22:6/16:0), Cer(d18:1/24:0)/SM(d18:1/19:0), Cer (d18:1/24:0)/SM(d18:0/16:0), TG(18:1/18:2/18:2), TG(16:0/16:0/20:3), and TG(18:0/16:0/18:2), and was assessed through binary logistic regression and ROC curve analyses. As shown in Figures 5A, B, the AUC values for this integrated biomarker in prediabetes and T2DM patients were 0.841 (cutoff: 0.565) and 0.894

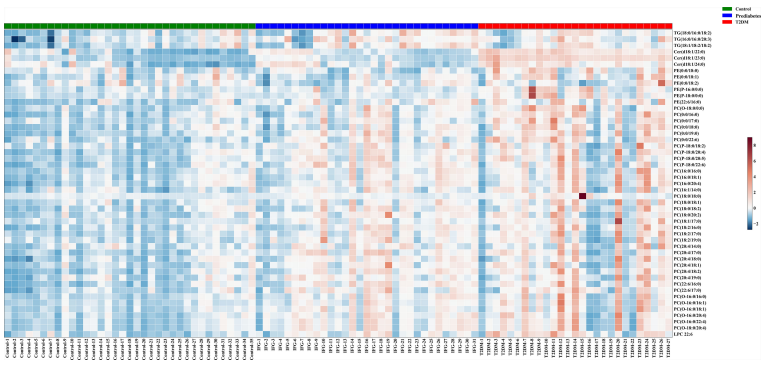


FIGURE 2
Metabolites that were significantly differentially abundant among groups in the discovery cohort were arranged in a heatmap, with increased and decreased metabolites being shown in red and blue, respectively.

10.3389/fendo.2022.1065665

TABLE 2 Potential serum biomarkers.

	Discovery			Validation		
	FDR-adjusted P-value and trend			FDR-adjusted P-value		
Lipid	Control VS Prediabetes	Control VS T2DM	Prediabetes VS T2DM	Control VS Prediabetes	Control VS T2DM	Prediabetes VS T2DM
LPC 22:6	**0.0078 (↑)**	**0.0004 (↑)**	**0.4031 (-)**	**0.0007 (↑)**	**0.00001 (↑)**	**0.7424 (-)**
PC(16:0/20:4)	**0.0081 (↑)**	**0.0181 (↑)**	**0.8881 (-)**	**0.0058 (↑)**	**0.0076 (↑)**	**0.9945 (-)**
PE(22:6/16:0)	**0.0069 (↑)**	**0.0367 (↑)**	**0.7589 (-)**	**0.0008 (↑)**	**0.00001 (↑)**	**0.2894 (-)**
Cer(d18:1/24:0)	**0.0130 (↑)**	**0.0005 (↑)**	**0.0033 (↑)**	**0.0004 (↑)**	**0.00001 (↑)**	**0.2065 (-)**
Cer(d18:1/23:0)	**0.0059 (↑)**	**0.0012 (↑)**	**0.000001 (↑)**	**0.0007 (↑)**	**0.00001 (↑)**	**0.0422 (↑)**
Cer(d18:1/22:0)	**0.0296 (↑)**	**0.0030 (↑)**	**0.000001 (↑)**	**0.0006 (↑)**	**0.00001 (↑)**	**0.0453 (↑)**
TG(18:1/18:2/18:2)	**0.0140 (↑)**	**0.0314 (↑)**	**0.6526 (-)**	**0.00001 (↑)**	**0.0001 (↑)**	**0.8134 (-)**
TG(16:0/16:0/20:3)	**0.0042 (↑)**	**0.0087 (↑)**	**0.6971 (-)**	**0.00001 (↑)**	**0.0003 (↑)**	**0.9209 (-)**
TG(18:0/16:0/18:2)	**0.0122 (↑)**	**0.0054 (↑)**	**0.5711 (-)**	**0.00001 (↑)**	**0.0003 (↑)**	**0.1559 (-)**

P-value corrected by FDR; "↑" means a higher level of metabolites; "↓" means a lower level of metabolites; "–" represents no statistically significant difference Control represents control group; prediabetes represents prediabetes group; T2DM represents T2DM group.
Bold values mean P value.

(cutoff: 0.633), respectively. As all of these values were > 0.5, this indicated that this model is reliable and able to effectively diagnose prediabetes and T2DM. Pearson correlation analyses were then performed to assess relationships between these biomarkers and clinical parameters, revealing the levels of all of these biomarkers to be positively correlated with patient FBG (Figure 5C). We additionally found that Cer(d18:1/24:0)/SM(d18:1/19:0) and Cer (d18:1/24:0)/SM(d18:0/16:0) were significantly negatively correlated with sex (Figure 5C). PE(22:6/16:0) and TG (18:0/16:0/18:2) levels were positively correlated with TG. In addition, TG (18:0/16:0/18:2) level was significantly negatively correlated with LDL level, and PE (22:6/16:0) level were significantly negatively correlated with HDL.

3.6 Increased ASM protein expression in prediabetes and T2DM rats

As shown in Figure 5D, Wistar rats and prediabetic rats had comparable non-fasting blood glucose, and the non-fasting blood glucose values of T2DM rats were about >2 times higher compared to wistar rats. The intensity of individual ASM bands were obtained by western blot analysis of GK rat adipose tissue. Compared with wistar rats, the levels of ASM in prediabetic rats (3-week GK rat) and T2DM rats (12-week GK rat) were significantly increased ($p < 0.05$) (Figures 5E, F), which demonstrated the process of diabetes could affect the changes of ASM content in the patient.

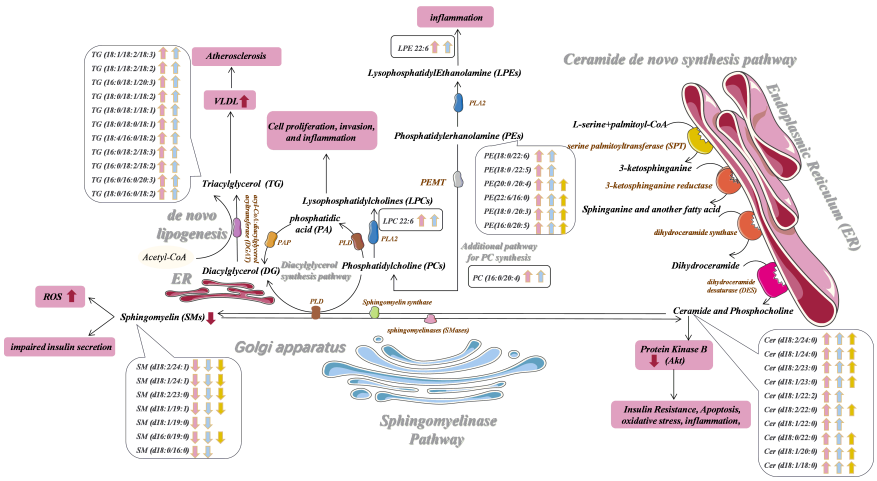


FIGURE 3
Potential prediabetes and T2DM-related serum biomarker networks. Arrows ("↑↓") indicated metabolites that were significantly up- and down-regulated in prediabetes (pink) and T2DM (blue) patients relative to healthy controls. Metabolites that were significantly altered in the prediabetes group relative to the T2DM group are also shown in yellow. PLD, phospholipase; PAP, phosphatidic acid phosphatase; phospholipase AZ, PLA2; PEMT, phosphatidylethanolamine N-methyltransferase.

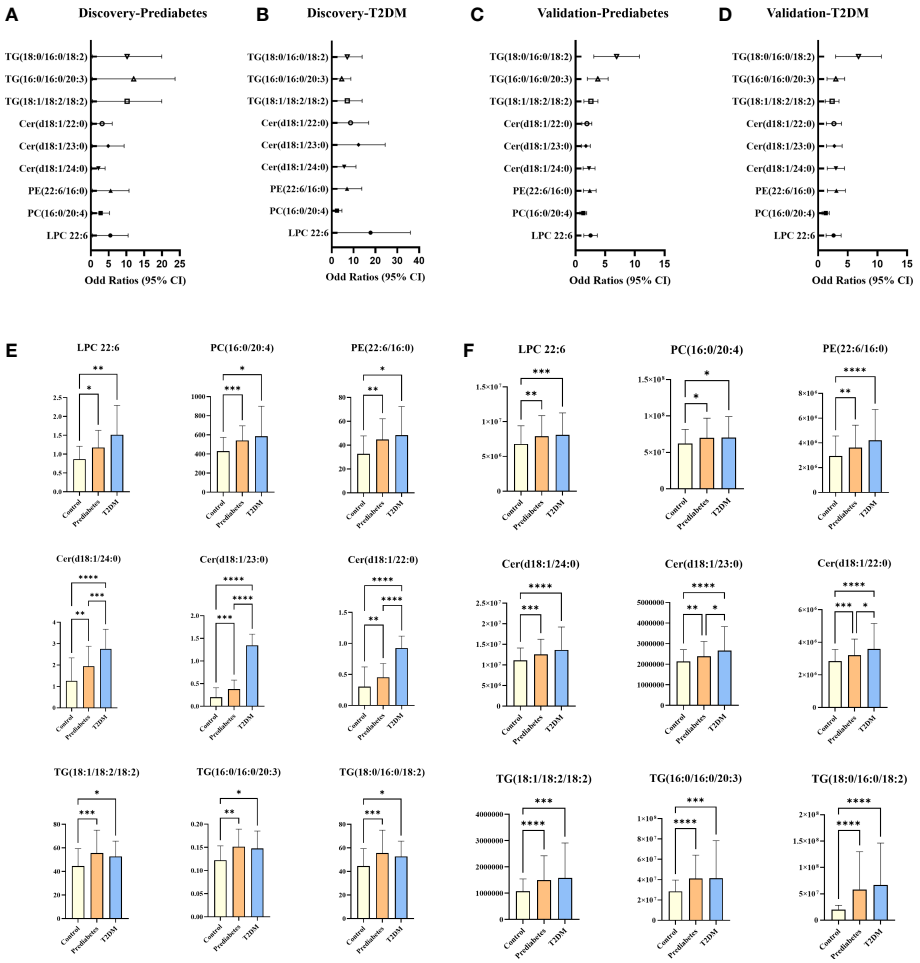


FIGURE 4
Plot of ORS per one SD increment and 95% CIs of lipids that emerged significant (FDR < 0.05 and P < 0.05) in the discovery and validation cohorts **(A–D)**; potential serum biomarkers in discovery cohort **(E)** and validation cohort **(F)**. *P < 0.05, **P < 0.01, ***P < 0.001, ***P < 0.0001.

4 Discussion

In this study, we employed targeted and untargeted approaches to identify serum lipid profiles in control, prediabetes, and T2DM patients *via* UHPLC-MS and UHPLC-MS/MS. This approach led to the identification of LPC, PC, PE, Cer, SM, and TG lipids that were differentially abundant in those with prediabetes/T2DM relative to control individuals.

Ceramides are the simplest sphingolipid family molecules and are central to sphingolipid metabolism such that they can impact important T2DM-related processes such as insulin resistance, oxidative stress, inflammation, and apoptosis (63, 64). There are three primary ceramide synthesis pathways (65, 66). The first of these involved *de novo* ceramide synthesis within the endoplasmic reticulum (ER) from L-serine and palmitoyl-CoA *via* a multi-stage process (Figure 3) (67, 68). Enhanced *de novo* ceramide synthesis can promote protein phosphatase 2A (PPA2) activation, thereby inhibiting insulin sensitivity and β-cell function through the inactivation of protein kinase B (Akt) in the insulin-signaling pathway (69–71). Sphingosine can be used to generate ceramide by many enzymes through a recycling pathway, such as lysosomal ceramidase and ceramide synthetase in the ER (72, 73). Ceramides can also be synthesized through the hydrolysis of SM and glycosphingolipids by sphingomyelinase (SMase) within the Golgi. Through the activity of sphingomyelin synthase (SMS) and phospholipase (PLD), the phosphocholine portion of PC can be transferred to the primary hydroxyl group of ceramide to yield diacylglycerol (DG) and SM, the latter of which is an important bioactive lipid associated with cellular proliferation, migration, and survival (74, 75). We did not detect significant differences in SM levels among groups for serum samples in the discovery cohort. Whereas in the validation cohort, compared with controls, we observed significantly lower levels of SM (d18:2/24:1), SM (d18:1/24:1), SM (d18:2/23:0), SM (d18:1/19:1), SM (d18:1/19:0), SM (d16:0/19:0) and SM (d18:0/16:0) in prediabetes and T2DM patient serum samples. This may suggest that the limited number of samples in the discovery cohort may have yielded false-negative results. We also found that ceramides including Cer(d18:1/24:0), Cer(d18:1/23:0), and Cer (d18:1/22:0) were significantly more abundant in prediabetes and T2DM patients relative to controls in both cohorts. Multiple prior analyses (76, 77), including the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study (78), have found

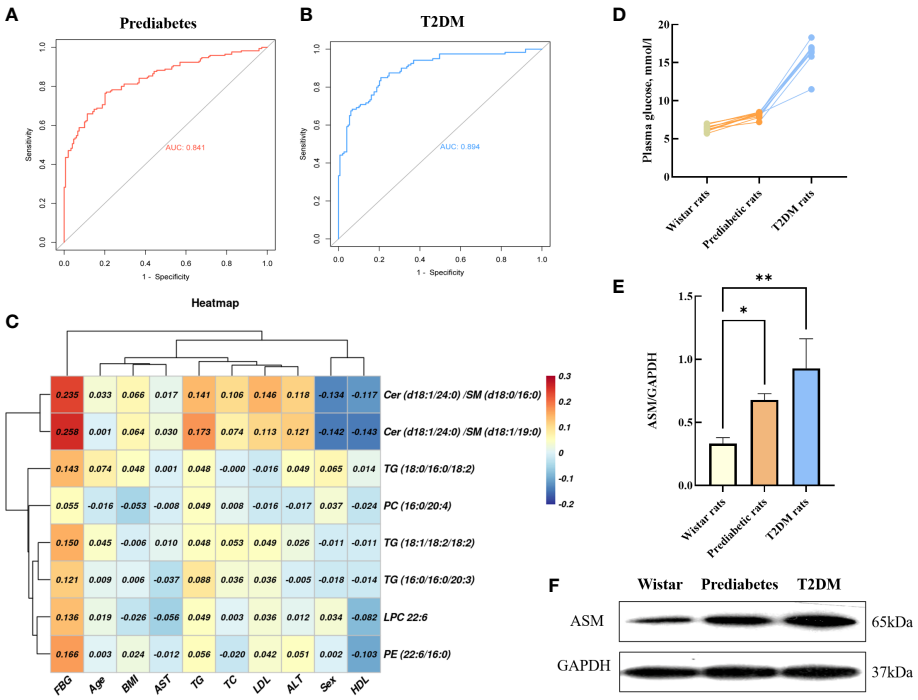


FIGURE 5
ROC curves of the integrated biomarker in prediabetes group **(A)**, T2DM group **(B)**. Heat map of the Pearson correlation coefficients between potential biomarkers and clinical parameters **(C)**. Basal plasma glucose in GK and control Wistar rats **(D)**. Representative Western blot gel documents and summarized data showed the expression of ASM in adipose tissue **(E, F)**. *P < 0.05, **P < 0.01.

SM levels to be negatively correlated with T2DM incidence. Similarly, one large cohort analysis of prediabetic and diabetic individuals found that odd-chain SMs were negatively correlated with T2DM risk (27), in line with our findings. We detected significant disruptions in ceramide and SM homeostasis in prediabetes and T2DM patients. This may be the result of the increased expression of enzymes responsible for regulating the conversion between Cer and SM, such as Smases like acid sphingomyelinase (79),. The results of western blot analysis of frozen adipose tissue from 3- and 12-week GK rats also confirmed that ASM is responsible for significant disruptions in ceramide and sphingomyelin homeostasis in prediabetes and T2DM patients. Mice in which SM synthase has been knocked out exhibited reduced SM levels, ceramide accumulation, and impaired mitochondrial activity resulting in impaired ATP production, increased reactive oxygen species (ROS) levels, and decreased glucose-induced insulin secretion, consistent with our hypothesis (80). This ceramide/SM homeostasis has been suggested to be a promising target for therapeutic intervention in multiple pathological contents (81), though whether glucose supplementation can effectively modulate sphingolipid metabolism within β cells by enhancing ceramide to SM conversion remains to be confirmed (82). We ultimately selected Cer(d18:1/24:0)/SM(d18:1/19:0) [(OR: 2.980; 95% CI:1.874-4.737 in prediabetes) and [(OR: 5.507; 95% CI: 3.233-9.379 in T2DM)] and Cer(d18:1/24:0)/SM (d18:0/16:0) [(OR: 2.883; 95% CI:1.801-4.614 in prediabetes) and (OR: 8.308; 95% CI: 4.778-14.445 in T2DM)] as one of components of an integrated biomarker model capable of predicting prediabetes and T2DM risk.

PE synthesis is important in the metabolic processing of lipids in the muscle tissue, and muscle PE levels may be linked to insulin resistance (83). Plasma PE levels have been shown to rise in individuals affected by insulin resistance in population studies (84). In line with such findings, we observed significant increases in PE (22:6/16:0) levels in the serum of prediabetes and T2DM patients in the discovery and verification cohorts. PC is the most common phospholipid in the body, wherein it is produced both by the Kennedy pathway and by additional synthetic pathways in the liver catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) (85). Samad et al. (86) reported that individuals with diabetes exhibit plasma PC levels distinct from those in healthy individuals. Consistently, we found that PC (16:0/20:4) levels were significantly altered in prediabetes and T2DM patients in both cohorts. Phospholipase A2 (PLA2) can catalyze the formation of LPC from PC (87). LPC is a lipid that serves as an important signaling molecule in the context of cellular proliferation and invasion, and increase levels of LPC 22:6 have previously been reported in obese individuals and those with prediabetes or diabetes (88, 89). The proinflammatory properties of LPC have also been previously documented, as it can both drive inflammatory molecule upregulation (90) and increase vascular endothelial permeability (91). Following PC synthesis through the additional pathway in the liver, PC and ceramide can processed by PLD to yield DG and SM. DG in turn gives rise to TG under the action of acyl-CoA: diacylglycerol acyltransferase (DGAT). Aberrant PC metabolism may increase levels of TG through the activation of SREBP-1 and the induction of *de novo* lipogenesis (92–94). Levels of TG, in turn, are well-studied as a risk factor linked to

dysregulated glucose metabolism in the general population. Our Pearson correlation analyses revealed a positive correlation between plasma TG levels and FBG, with plasma TG (18:1/18:2/18:2), TG (16:0/16:0/20:3), and TG (18:0/16:0/18:2) levels being significantly elevated in prediabetes and T2DM patients relative to controls. T2DM patients inevitably exhibit hyperlipidemia, while individuals with prediabetes frequently present with higher circulating TG and free fatty acid (FFA) levels (95), in part owing to impaired lipid processing within adipose tissue (96). Diabetes-related dyslipidemia is also linked with a marked increase in cardiovascular risk (97).

5 Conclusions

In this study, we first herein conducted an untargeted lipidomics analysis of newly diagnosed Chinese prediabetic and T2DM patients in a discovery cohort, leading to the identification of changing phospholipid and sphingolipid profiles associated with prediabetes and T2DM that were confirmed for the first time in a separate validation cohort *via* targeted lipidomics analyses. Furthermore, potential biomarkers were confirmed for the first time in separate validation cohort *via* targeted lipidomics analyses. Moreover, the results confirmed ASM is responsible for significant disruptions in ceramide and sphingomyelin homeostasis in prediabetic and T2DM. Finally, this study developing a new integrated biomarker signature that may better aid in the diagnosis of Chinese prediabetes and T2DM, and provides a better biological understanding of the insidious progression to diabetes from a lipid perspective.

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 human participants were reviewed and approved by The Ethics Committee of Scientific Research, Beijing Shijitan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by The China Pharmaceutical University Animal Care and Use Committee.

Author contributions

NG and DanY conceived the project. NG contributed to the concept development, study design, and edited the manuscript. DanY collected the clinical information, interpreted the data. JY performed the experiments, performed data analysis and wrote the manuscript. DawY, HY and ZW were contributed to the supervision of the experimental process and helped write the manuscript. MW revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Fundamental Research Funds for the central public welfare research institutes (Grant JBGS2021002); China Academy of Chinese Medical Sciences (CACMS) innovation fund (Grant CI2021A05031); National Science and Technology Major Project (Grant 2017ZX09301040); Open Research Funding of Beijing Key Laboratory of Bio-characteristic Profiling for Evaluation of Rational Drug Use (Grant 2019-KF14); National Natural Science Foundation of China (grant number 82130112); Youth Beijing Scholar (No. 2022–051) and the Beijing Excellent Talent Project (grant numbers DFL20190702).

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.2022.1065665/full#supplementary-material

References

1. Leung PS. Current research of the ras in diabetes mellitus. *Adv Exp Med Biol* (2010) 690:131–53. doi: 10.1007/978-90-481-9060-7_8

2. Khursheed R, Singh SK, Wadhwa S, Kapoor B, Gulati M, Kumar R, et al. Treatment strategies against diabetes: Success so far and challenges ahead. *Eur J Pharmacol* (2019) 862:172625. doi: 10.1016/j.ejphar.2019.172625

3. Khan RMM, Chua ZJY, Tan JC, Yang Y, Liao Z, Zhao Y. From pre-diabetes to diabetes: Diagnosis, treatments and translational research. *Medicina (Kaunas)* (2019) 55 (9):546. doi: 10.3390/medicina55090546

4. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: A high-risk state for diabetes development. *Lancet* (2012) 379(9833):2279–90. doi: 10.1016/s0140-6736(12)60283-9

5. Peters AL, Davidson MB, Schriger DL, Hasselblad V. A clinical approach for the diagnosis of diabetes mellitus: An analysis using glycosylated hemoglobin levels. meta-analysis research group on the diagnosis of diabetes using glycated hemoglobin levels. *JAMA* (1996) 276(15):1246–52. doi: 10.1001/jama.1996.03540150048030

6. Who Guidelines Approved by the Guidelines Review Committee. *Use of glycated haemoglobin (Hba1c) in the diagnosis of diabetes mellitus: Abbreviated report of a who consultation* Vol. 2011. . Geneva: World Health Organization (2011).

7. Noble D, Mathur R, Dent T, Meads C, Greenhalgh T. Risk models and scores for type 2 diabetes: Systematic review. *BMJ* (2011) 343:d7163. doi: 10.1136/bmj.d7163

8. Ding L, Xu Y, Liu S, Bi Y, Xu Y. Hemoglobin A1c and diagnosis of diabetes. *J Diabetes* (2018) 10(5):365–72. doi: 10.1111/1753-0407.12640

9. Chen T, Ni Y, Ma X, Bao Y, Liu J, Huang F, et al. Branched-chain and aromatic amino acid profiles and diabetes risk in Chinese populations. *Sci Rep* (2016) 6:20594. doi: 10.1038/srep20594

10. Yu H, Di J, Bao Y, Zhang P, Zhang L, Tu Y, et al. Visceral fat area as a new predictor of short-term diabetes remission after roux-En-Y gastric bypass surgery in Chinese patients with a body mass index less than 35 Kg/M2. *Surg Obes Relat Dis* (2015) 11(1):6–11. doi: 10.1016/j.soard.2014.06.019

11. Herder C, Baumert J, Zierer A, Roden M, Meisinger C, Karakas M, et al. Immunological and cardiometabolic risk factors in the prediction of type 2 diabetes and coronary events: Monica/Kora augsburg case-cohort study. *PloS One* (2011) 6(6): e19852. doi: 10.1371/journal.pone.0019852

12. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: Evidence and guidance for management. *Eur Heart J* (2011) 32(11):1345–61. doi: 10.1093/eurheartj/ehr112

13. Savelieff MG, Callaghan BC, Feldman EL. The emerging role of dyslipidemia in diabetic microvascular complications. *Curr Opin Endocrinol Diabetes Obes* (2020) 27 (2):115–23. doi: 10.1097/med.0000000000000533

14. Sunil B, Ashraf AP. Dyslipidemia in pediatric type 2 diabetes mellitus. *Curr Diabetes Rep* (2020) 20(10):53. doi: 10.1007/s11892-020-01336-6

15. Markgraf DF, Al-Hasani H, Lehr S. Lipidomics-reshaping the analysis and perception of type 2 diabetes. *Int J Mol Sci* (2016) 17(11):1841. doi: 10.3390/ijms17111841

16. Ding M, Rexrode KM. A review of lipidomics of cardiovascular disease highlights the importance of isolating lipoproteins. *Metabolites* (2020) 10(4):163. doi: 10.3390/metabo10040163

17. Kalli E. Nutritional lipidomics in alzheimer's disease. *Adv Exp Med Biol* (2020) 1195:95–104. doi: 10.1007/978-3-030-32633-3_14

18. Nie Q, Xing M, Chen H, Hu J, Nie S. Metabolomics and lipidomics profiling reveals hypocholesterolemic and hypolipidemic effects of arabinoxylan on type 2 diabetic rats. *J Agric Food Chem* (2019) 67(38):10614–23. doi: 10.1021/acs.jafc.9b03430

19. O'Brien PD, Guo K, Eid SA, Rumora AE, Hinder LM, Hayes JM, et al. Integrated lipidomic and transcriptomic analyses identify altered nerve triglycerides in mouse models of prediabetes and type 2 diabetes. *Dis Model Mech* (2020) 13(2):dmm042101. doi: 10.1242/dmm.042101

20. Bagheri M, Tiwari HK, Murillo AL, Al-Tobasei R, Arnett DK, Kind T, et al. A lipidome-wide association study of the lipoprotein insulin resistance index. *Lipids Health Dis* (2020) 19(1):153. doi: 10.1186/s12944-020-01321-8

21. Kulkarni H, Mamtani M, Wong G, Weir JM, Barlow CK, Dyer TD, et al. Genetic correlation of the plasma lipidome with type 2 diabetes, prediabetes and insulin resistance in Mexican American families. *BMC Genet* (2017) 18(1):48. doi: 10.1186/s12863-017-0515-5

22. Yan L, Han P, Man J, Tian Y, Wang F, Wang J. Discovery of lipid profiles of type 2 diabetes associated with hyperlipidemia using untargeted uplc q-Tof/Ms-Based lipidomics approach. *Clin Chim Acta* (2021) 520:53–62. doi: 10.1016/j.cca.2021.05.031

23. Imamura F, Fretts A, Marklund M, Ardisson Korat AV, Yang WS, Lankinen M, et al. Fatty acid biomarkers of dairy fat consumption and incidence of type 2 diabetes: A pooled analysis of prospective cohort studies. *PloS Med* (2018) 15(10):e1002670. doi: 10.1371/journal.pmed.1002670

24. Telle-Hansen VH, Gaundal L, Myhrstad MCW. Polyunsaturated fatty acids and glycemic control in type 2 diabetes. *Nutrients* (2019) 11(5):1067. doi: 10.3390/nu11051067

25. Jackson KH, Harris WS. Blood fatty acid profiles: New biomarkers for cardiometabolic disease risk. *Curr Atheroscl Rep* (2018) 20(5):22. doi: 10.1007/s11883-018-0722-1

26. Zhong H, Fang C, Fan Y, Lu Y, Wen B, Ren H, et al. Lipidomic profiling reveals distinct differences in plasma lipid composition in healthy, prediabetic, and type 2 diabetic individuals. *GigaScience* (2017) 6(7):1–12. doi: 10.1093/gigascience/gix036

27. Meikle PJ, Wong G, Barlow CK, Weir JM, Greeve MA, MacIntosh GL, et al. Plasma lipid profiling shows similar associations with prediabetes and type 2 diabetes. *PloS One* (2013) 8(9):e74341. doi: 10.1371/journal.pone.0074341

28. Burns JL, Nakamura MT, Ma DWL. Differentiating the biological effects of linoleic acid from arachidonic acid in health and disease. *Prostaglandins Leukot Essent Fatty Acids* (2018) 135:1–4. doi: 10.1016/j.plefa.2018.05.004

29. Verma MK, Yateesh AN, Neelima K, Pawar N, Sandhya K, Poornima J, et al. Inhibition of neutral sphingomyelinases in skeletal muscle attenuates fatty-acid induced defects in metabolism and stress. *SpringerPlus* (2014) 3:255. doi: 10.1186/2193-1801-3-255

30. Choi RH, Tatum SM, Symons JD, Summers SA, Holland WL. Ceramides and other sphingolipids as drivers of cardiovascular disease. *Nat Rev Cardiol* (2021) 18(10):701–11. doi: 10.1038/s41569-021-00536-1

31. Henry B, Ziobro R, Becker KA, Kolesnick R, Gulbins E. Acid sphingomyelinase. *Handb Exp Pharmacol* (2013) 215):77–88. doi: 10.1007/978-3-7091-1368-4_4

32. Chew WS, Torta F, Ji S, Choi H, Begum H, Sim X, et al. Large-Scale lipidomics identifies associations between plasma sphingolipids and T2dm incidence. *JCI Insight* (2019) 5(13):e126925. doi: 10.1172/jci.insight.126925

33. Jiang M, Huang S, Duan W, Liu Q, Lei M. Inhibition of acid sphingomyelinase activity ameliorates endothelial dysfunction in Db/Db mice. *Biosci Rep* (2019) 39(4): BSR20182144. doi: 10.1042/bsr20182144

34. Li X, Jin SJ, Su J, Li XX, Xu M. Acid sphingomyelinase down-regulation alleviates vascular endothelial insulin resistance in diabetic rats. *Basic Clin Pharmacol Toxicol* (2018) 123(6):645–59. doi: 10.1111/bcpt.13073

35. Goff LM. Ethnicity and type 2 diabetes in the uk. *Diabetes Med* (2019) 36(8):927–38. doi: 10.1111/dme.13895

36. Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. the third national health and nutrition examination survey, 1988-1994. *Diabetes Care* (1998) 21(4):518–24. doi: 10.2337/diacare.21.4.518

37. Fang M. Trends in the prevalence of diabetes among U.S. adults: 1999-2016. *Am J Prev Med* (2018) 55(4):497–505. doi: 10.1016/j.amepre.2018.05.018

38. Burdge GC, Calder PC. Introduction to fatty acids and lipids. *World Rev Nutr Diet* (2015) 112:1–16. doi: 10.1159/000365423

39. Vanni S, Riccardi L, Palermo G, De Vivo M. Structure and dynamics of the acyl chains in the membrane trafficking and enzymatic processing of lipids. *Acc Chem Res* (2019) 52(11):3087–96. doi: 10.1021/acs.accounts.9b00134

40. Razquin C, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, et al. Plasma lipidomic profiling and risk of type 2 diabetes in the predimed trial. *Diabetes Care* (2018) 41(12):2617–24. doi: 10.2337/dc18-0840

41. Aristizabal-Henao JJ, Jones CM, Lippa KA, Bowden JA. Nontargeted lipidomics of novel human plasma reference materials: Hypertriglyceridemic, diabetic, and African-American. *Anal Bioanal Chem* (2020) 412(27):7373–80. doi: 10.1007/s00216-020-02910-3

42. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kröger J, Schulze MB, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: The epic-interact case-cohort study. *Lancet Diabetes Endocrinol* (2014) 2(10):810–8. doi: 10.1016/s2213-8587(14)70146-9

43. IDF. *Idf diabetes atlas*. Brussels: The International Diabetes Federation (2019).

44. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. *JAMA* (2017) 317(24):2515–23. doi: 10.1001/jama.2017.7596

45. Hu C, Jia W. Diabetes in China: Epidemiology and genetic risk factors and their clinical utility in personalized medication. *Diabetes* (2018) 67(1):3–11. doi: 10.2337/dbi17-0013

46. Insausti-Urkia N, Solsona-Vilarrasa E, Garcia-Ruiz C, Fernandez-Checa JC. Sphingomyelinases and liver diseases. *Biomolecules* (2020) 10(11):1497. doi: 10.3390/biom10111497

47. Kady N, Yan Y, Salazar T, Wang Q, Chakravarthy H, Huang C, et al. Increase in acid sphingomyelinase level in human retinal endothelial cells and Cd34(+) circulating angiogenic cells isolated from diabetic individuals is associated with dysfunctional retinal vasculature and vascular repair process in diabetes. *J Clin Lipidol* (2017) 11(3):694–703. doi: 10.1016/j.jacl.2017.03.007

48. Ramazani M, Qujeq D, Moazezi Z. Assessing the levels of l-carnitine and total antioxidant capacity in adults with newly diagnosed and long-standing type 2 diabetes. *Can J Diabetes* (2019) 43(1):46–50.e1. doi: 10.1016/j.jcjd.2018.03.009

49. González-Pérez A, Saéz ME, Vizcaya D, Lind M, García Rodríguez LA. Impact of chronic kidney disease definition on assessment of its incidence and risk factors in patients with newly diagnosed type 1 and type 2 diabetes in the uk: A cohort study using primary care data from the united kingdom. *Prim Care Diabetes* (2020) 14(4):381–7. doi: 10.1016/j.pcd.2019.11.002

50. Horska K, Kotolova H, Karpisek M, Babinska Z, Hammer T, Prochazka J, et al. Metabolic profile of methylazoxymethanol model of schizophrenia in rats and effects of three antipsychotics in long-acting formulation. *Toxicol Appl Pharmacol* (2020) 406:115214. doi: 10.1016/j.taap.2020.115214

51. Salemi Z, Rafie E, Goodarzi MT, Ghaffari MA. Effect of metformin, acarbose and their combination on the serum visfatin level in Nicotinamide/Streptozocin-induced type 2 diabetic rats. *Iranian Red Crescent Med J* (2016) 18(3):e23814. doi: 10.5812/ircmj.23814

52. Cui L, Lu H, Lee YH. Challenges and emergent solutions for lc-Ms/Ms based untargeted metabolomics in diseases. *Mass Spectrom Rev* (2018) 37(6):772–92. doi: 10.1002/mas.21562

53. Lee HC, Yokomizo T. Applications of mass spectrometry-based targeted and non-targeted lipidomics. *Biochem Biophys Res Commun* (2018) 504(3):576–81. doi: 10.1016/j.bbrc.2018.03.081

54. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2021. *Diabetes Care* (2021) 44(Suppl 1):S15–33. doi: 10.2337/dc21-S002

55. Commission CP. Pharmacopoeia of the people's republic of China. *China Med Sci Technol* (2020).

56. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2013) 36 Suppl 1(Suppl 1):S67–74. doi: 10.2337/dc13-S067

57. Portha B. Programmed disorders of beta-cell development and function as one cause for type 2 diabetes? the gk rat paradigm. *Diabetes Metab Res Rev* (2005) 21(6):495–504. doi: 10.1002/dmrr.566

58. Hao J, Shen W, Tian C, Liu Z, Ren J, Luo C, et al. Mitochondrial nutrients improve immune dysfunction in the type 2 diabetic goto-kakizaki rats. *J Cell Mol Med* (2009) 13 (4):701–11. doi: 10.1111/j.1582-4934.2008.00342.x

59. Portha B, Giroix MH, Tourrel-Cuzin C, Le-Stunff H, Movassat J. The gk rat: A prototype for the study of non-overweight type 2 diabetes. *Methods Mol Biol* (2012) 933:125–59. doi: 10.1007/978-1-62703-068-7_9

60. Zhang Y, Shi J, Fei Q, Huang Y, Zhu F, Wang J, et al. Plasma and urine metabonomics coupled with uplc-Q-Tof/Ms and multivariate data analysis on potential biomarkers in anemia and hematinic effects of steamed notoginseng. *Phytochem Lett* (2020) 38:55–64. doi: 10.1016/j.phytol.2020.05.003

61. Taniguchi M, Okazaki T. Role of Ceramide/Sphingomyelin (Sm) balance regulated through "Sm cycle" in cancer. *Cell Signal* (2021) 87:110119. doi: 10.1016/j.cellsig.2021.110119

62. Signorelli P, Conte C, Albi E. The multiple roles of sphingomyelin in parkinson's disease. *Biomolecules* (2021) 11(9):1311. doi: 10.3390/biom11091311

63. Ilan Y. Compounds of the sphingomyelin-Ceramide-Glycosphingolipid pathways as secondary messenger molecules: New targets for novel therapies for fatty liver disease and insulin resistance. *Am J Physiol Gastrointest Liver Physiol* (2016) 310(11):G1102–17. doi: 10.1152/ajpgi.00095.2016

64. Boden G. Ceramide: A contributor to insulin resistance or an innocent bystander? *Diabetologia* (2008) 51(7):1095–6. doi: 10.1007/s00125-008-1015-y

65. Bandet CL, Tan-Chen S, Bourron O, Le Stunff H, Hajduch E. Sphingolipid metabolism: New insight into ceramide-induced lipotoxicity in muscle cells. *Int J Mol Sci* (2019) 20(3):479. doi: 10.3390/ijms20030479

66. Kojta I, Chacińska M, Błachnio-Zabielska A. Obesity, bioactive lipids, and adipose tissue inflammation in insulin resistance. *Nutrients* (2020) 12(5):1305. doi: 10.3390/nu12051305

67. Kurek K, Piotrowska DM, Wiesiołek P, Chabowski A, Zendzian-Piotrowska M. [Role of sphingolipids in digestive system]. *Postepy higieny i medycyny doswiadczalnej (Online)* (2012) 66:868–75. doi: 10.5604/17322693.1019650

68. Pewzner-Jung Y, Ben-Dor S, Futerman AH. When do lasses (Longevity assurance genes) become cers (Ceramide synthases)?: insights into the regulation of ceramide synthesis. *J Biol Chem* (2006) 281(35):25001–5. doi: 10.1074/jbc.R600010200

69. Rico JE, Myers WA, Laub DJ, Davis AN, Zeng Q, McFadden JW. Hot topic: Ceramide inhibits insulin sensitivity in primary bovine adipocytes. *J Dairy Sci* (2018) 101 (4):3428–32. doi: 10.3168/jds.2017-13983

70. Wu Y, Song P, Xu J, Zhang M, Zou MH. Activation of protein phosphatase 2a by palmitate inhibits amp-activated protein kinase. *J Biol Chem* (2007) 282(13):9777–88. doi: 10.1074/jbc.M608310200

71. Campana M, Bellini L, Rouch C, Rachdi L, Coant N, Butin N, et al. Inhibition of central *De novo* ceramide synthesis restores insulin signaling in hypothalamus and enhances B-cell function of obese zucker rats. *Mol Metab* (2018) 8:23–36. doi: 10.1016/j.molmet.2017.10.013

72. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: From synthesis to breakdown. In: Chalfant C, Poeta MD, editors. *Sphingolipids as signaling and regulatory molecules*. New York, NY: Springer New York (2010). p. 1–23.

73. Kitatani K, Idkowiak-Baldys J, Hannun YA. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal* (2008) 20(6):1010–8. doi: 10.1016/j.cellsig.2007.12.006

74. Taniguchi M, Okazaki T. The role of sphingomyelin and sphingomyelin synthases in cell death, proliferation and migration-from cell and animal models to human disorders. *Biochim Biophys Acta* (2014) 1841(5):692–703. doi: 10.1016/j.bbalip.2013.12.003

75. Tafesse FG, Ternes P, Holthuis JC. The multigenic sphingomyelin synthase family. *J Biol Chem* (2006) 281(40):29421–5. doi: 10.1074/jbc.R600021200

76. Yaney GC, Korchak HM, Corkey BE. Long-chain acyl coa regulation of protein kinase c and fatty acid potentiation of glucose-stimulated insulin secretion in clonalβ -cells*. *Endocrinology* (2000) 141(6):1989–98. doi: 10.1210/endo.141.6.7493

77. Chaurasia B, Kaddai VA, Lancaster GI, Henstridge DC, Sriram S, Galam DL, et al. Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism. *Cell Metab* (2016) 24(6):820–34. doi: 10.1016/j.cmet.2016.10.002

78. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* (2013) 62(2):639–48. doi: 10.2337/db12-0495

79. Błachnio-Zabielska AU, Pułka M, Baranowski M, Nikołajuk A, Zabielski P, Górska M, et al. Ceramide metabolism is affected by obesity and diabetes in human adipose tissue. *J Cell Physiol* (2012) 227(2):550–7. doi: 10.1002/jcp.22745

80. Yano M, Watanabe K, Yamamoto T, Ikeda K, Senokuchi T, Lu M, et al. Mitochondrial dysfunction and increased reactive oxygen species impair insulin secretion in sphingomyelin synthase 1-null mice. *J Biol Chem* (2011) 286(5):3992–4002. doi: 10.1074/jbc.M110.179176

81. Taniguchi M, Okazaki T. Ceramide/Sphingomyelin rheostat regulated by sphingomyelin synthases and chronic diseases in murine models. *J Lipid Atheroscl* (2020) 9(3):380–405. doi: 10.12997/jla.2020.9.3.380

82. Pearson GL, Mellett N, Chu KY, Boslem E, Meikle PJ, Biden TJ. A comprehensive lipidomic screen of pancreatic B-cells using mass spectroscopy defines novel features of glucose-stimulated turnover of neutral lipids, sphingolipids and plasmalogens. *Mol Metab* (2016) 5(6):404–14. doi: 10.1016/j.molmet.2016.04.003

83. Selathurai A, Kowalski GM, Burch ML, Sepulveda P, Risis S, Lee-Young RS, et al. The cdp-ethanolamine pathway regulates skeletal muscle diacylglycerol content and mitochondrial biogenesis without altering insulin sensitivity. *Cell Metab* (2015) 21 (5):718–30. doi: 10.1016/j.cmet.2015.04.001

84. Wentworth JM, Naselli G, Ngui K, Smyth GK, Liu R, O'Brien PE, et al. Gm3 ganglioside and phosphatidylethanolamine-containing lipids are adipose tissue markers of insulin resistance in obese women. *Int J Obes (Lond)* (2016) 40(4):706–13. doi: 10.1038/ijo.2015.223

85. Vance DE. Phospholipid methylation in mammals: From biochemistry to physiological function. *Biochim Biophys Acta (BBA) - Biomembr* (2014) 1838(6):1477–87. doi: 10.1016/j.bbamem.2013.10.018

86. Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: A potential mechanism for cardiovascular and metabolic risk. *Diabetes* (2006) 55(9):2579–87. doi: 10.2337/db06-0330

87. Law SH, Chan ML, Marathe GK, Parveen F, Chen CH, Ke LY. An updated review of lysophosphatidylcholine metabolism in human diseases. *Int J Mol Sci* (2019) 20 (5):1149. doi: 10.3390/ijms20051149

88. Ahmad MI, Umair Ijaz M, Hussain M, Ali Khan I, Mehmood N, Siddiqi SM, et al. High fat diet incorporated with meat proteins changes biomarkers of lipid metabolism, antioxidant activities, and the serum metabolomic profile in Glrx1(-/-) mice. *Food Funct* (2020) 11(1):236–52. doi: 10.1039/c9fo02207d

89. Pietiläinen KH, Sysi-Aho M, Rissanen A, Seppänen-Laakso T, Yki-Järvinen H, Kaprio J, et al. Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects–a monozygotic twin study. *PloS One* (2007) 2(2):e218 p. doi: 10.1371/journal.pone.0000218

90. Lin P, Welch EJ, Gao XP, Malik AB, Ye RD. Lysophosphatidylcholine modulates neutrophil oxidant production through elevation of cyclic amp. *J Immunol* (2005) 174 (5):2981–9. doi: 10.4049/jimmunol.174.5.2981

91. Qiao J, Huang F, Naikawadi RP, Kim KS, Said T, Lum H. Lysophosphatidylcholine impairs endothelial barrier function through the G protein-coupled receptor Gpr4. *Am J Physiol Lung Cell Mol Physiol* (2006) 291(1):L91–L101. doi: 10.1152/ajplung.00508.2005

92. Jacobs RL, van der Veen JN, Vance DE. Finding the balance: The role of s-adenosylmethionine and phosphatidylcholine metabolism in development of nonalcoholic fatty liver disease. *Hepatology* (2013) 58(4):1207–9. doi: 10.1002/hep.26499

93. Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, et al. A conserved srebp-1/Phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* (2011) 147(4):840–52. doi: 10.1016/j.cell.2011.09.045

94. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, et al. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear srebp-1c in adipose tissue: Model for congenital generalized lipodystrophy. *Genes Dev* (1998) 12 (20):3182–94. doi: 10.1101/gad.12.20.3182

95. Corpeleijn E, Mensink M, Kooi ME, Roekaerts PMHJ, Saris WHM, Blaak EE. Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. *Obesity* (2008) 16(5):1025–32. doi: 10.1038/oby.2008.24

96. Frayn K. Adipose tissue as a buffer for daily lipid flux. *Diabetologia* (2002) 45 (9):1201–10. doi: 10.1007/s00125-002-0873-y

97. Ye X, Kong W, Zafar MI, Chen LL. Serum triglycerides as a risk factor for cardiovascular diseases in type 2 diabetes mellitus: A systematic review and meta-analysis of prospective studies. *Cardiovasc Diabetol* (2019) 18(1):48. doi: 10.1186/s12933-019-0851-z

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 03 February 2023
DOI 10.3389/fpubh.2023.1028461

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal, South Africa

REVIEWED BY
Soham Al Snih,
University of Texas Medical Branch at Galveston, United States
Peter Fasching,
Vienna Health Association, Austria

*CORRESPONDENCE
Zhe Deng
✉ zdeng_emergency@126.com
Dehong Liu
✉ dhliu_emergency@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Public Health

RECEIVED 26 August 2022
ACCEPTED 12 January 2023
PUBLISHED 03 February 2023

CITATION
Han Y, Hu H, Li Q, Deng Z and Liu D (2023) Triglyceride glucose-body mass index and the risk of progression to diabetes from prediabetes: A 5-year cohort study in Chinese adults. *Front. Public Health* 11:1028461. doi: 10.3389/fpubh.2023.1028461

Triglyceride glucose-body mass index and the risk of progression to diabetes from prediabetes: A 5-year cohort study in Chinese adults

Yong Han[1†], Haofei Hu[2†], Qiming Li[1†], Zhe Deng[1*] and Dehong Liu[1*]

[1]Department of Emergency, Shenzhen Second People's Hospital, Shenzhen, Guangdong, China, [2]Department of Nephrology, Shenzhen Second People's Hospital, Shenzhen, Guangdong, China

Objective: Evidence regarding the relationship between the triglyceride glucose-body mass index (TyG-BMI) and the risk of progression from prediabetes to diabetes remains limited. Our study aimed to investigate the relationship between them in patients with prediabetes.

Methods: In this retrospective cohort study, data were collected from 25,279 patients with prediabetes who received health checks between 2010 and 2016. We used a Cox proportional-hazards regression model to examine the relationship between TyG-BMI and diabetes risk. We used Cox proportional hazards regression with cubic spline functions and smooth curve fitting to identify the nonlinear relationship between them. In addition, A series of sensitivity and subgroup analyses were also conducted.

Results: The mean age of the included participants was 49.29 ± 13.82 years old, and 1,6734 (66.2%) were male. The mean TyG-BMI was 219.47. The median follow-up time was 2.89 years, and 2,687 (10.63%) individuals had a final diagnosis of diabetes. After adjusting for covariates, TyG-BMI was positively linked with incident diabetes in patients with prediabetes (HR = 1.011, 95%CI 1.010–1.012). TyG-BMI had a non-linear connection with diabetes risk, and its inflection point was 231.66. Right and left effects sizes (HR) at the inflection point were 1.017 (95%CI:1.014–1.019) and 1.007 (95%CI:1.005–1.009), respectively.The sensitivity analysis demonstrated the robustness of these results.

Conclusion: This study demonstrated a positive, non-linear relationship between the TyG-BMI and diabetes risk in Chinese patients with prediabetes. When the TyG-BMI was <231.66, there was a significant positive association between TyG-BMI and the risk of progression from prediabetes to diabetes. This study serves as a reference to promote clinical consultation and optimize diabetes prevention decisions for patients with prediabetes.

KEYWORDS

triglyceride-glucose index, prediabetes, diabetes, smooth curve fitting, non-linear relationship

Introduction

Diabetes mellitus (DM) is a very common complex of endocrine and metabolic disorders that afflicts hundreds of millions of people worldwide (1). According to the International Diabetes Federation Diabetes Atlas, diabetes affects 425 million people worldwide in 2017. It is estimated that the number of people with diabetes will increase to 629 million by 2045 (2). It is well known that diabetes can have long-term complications affecting the kidneys, nerves, eyes, and cardiovascular system (3–5). Besides, diabetes is a leading cause of disability and mortality (6). Therefore, diabetes is a serious health concern that imposes a heavy economic burden on societies worldwide.

Prediabetes is an intermediate stage between normoglycemia and diabetes, characterized by impaired glucose metabolism. It generally reflects the presence of either or both impaired glucose tolerance and fasting glucose. According to the International Diabetes Federation (IDF), around 374 million adults in the world had prediabetes in 2017, with a global prevalence of 7.7%. In 2045, there will be 548 million adults with prediabetes, equivalent to 8.4% of the world's population (2). In the US alone, 86 million adults aged ≥18 had prediabetes (7). The prevalence of prediabetes among adults has reached 35.7% in a nationwide cross-sectional survey in China (8). Seventy percent of those with prediabetes will eventually develop diabetes, according to an American Diabetes Association (ADA) expert panel (7). Thus, prediabetes is often viewed as a warning sign. However, most patients with prediabetes often ignore this metabolic abnormality and neglect its significance. Therefore, knowing the risk factors for progression from prediabetes to diabetes is particularly important in preventing or delaying diabetes and its complications.

It has been recognized that insulin resistance (IR) plays an essential role in many metabolic disorders, such as metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), diabetes, and obesity (9–12). IR is one of the major factors in the development of diabetes, so identifying individuals with IR before diabetes develops is crucial. The hyperinsulinemic euglycemia clamp remains the gold standard for IR measurement, but it is not widely applicable in clinical practice by its labor intensity, cost, and ethical issues (13). Thus, a simple, reproducible, and reliable index for detecting IR is urgently needed. Researchers have demonstrated that the triglyceride-glucose index (TyG) index consists of the product of fasting plasma glucose (FPG) levels and triglyceride (TG) and is highly sensitive and specific for recognizing IR compared to the euglycemic hyperinsulinemic clamp test (14, 15). The triglyceride glucose-body mass index (TyG-BMI) has been developed as an obesity-related parameter in recent years. It is the product of the body mass index (BMI) and the TyG index. According to a recent study, TyG-BMI can simultaneously capture several clinical variables, such as BMI, blood glucose, and lipid profile, and more closely reflect IR than index alone (16). Since IR plays an important role in diabetes pathogenesis, we hypothesized that TyG-BMI might be a useful predictor of diabetes. Unfortunately, the current research on the relationship between diabetes and TyG-BMI is limited, with only two studies addressing the topic (17, 18). In addition, previous studies investigating the association between TyG-BMI and diabetes were for the general population. The relationship between them has not been reported in patients with prediabetes, a population at high risk of developing diabetes. Therefore, in order to determine the relationship between TyG-BMI and the risk of progression from prediabetes to diabetes, we conducted a retrospective cohort study using published data.

Abbreviations: BMI, body mass index; TyG, the triglyceride-glucose index; TyG-BMI, triglyceride glucose-body mass index; TC, total cholesterol; TG, triglyceride; BUN, blood urea nitrogen; HDL-c, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; LDL-c, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; Scr, serum creatinine; FPG, fasting plasma glucose; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; DBP, diastolic blood pressure; NAFLD, Non-alcoholic fatty liver disease; IDF, International Diabetes Federation; GAM, generalized additive model; HR, hazard ratio; Ref, reference; CI, confidence interval.

Methods

Study design

A retrospective cohort study design was used in this study, and data were collected from a Chinese computerized database by Chinese researchers (Chen et al. 19). TyG-BMI was the target-independent variable. Diabetes (DM) (dichotomous: 0 = non-DM, 1 = DM) was the outcome variable.

Data source

The raw data were obtained free of charge from DATADRYAD (www.datadryad.org), which was provided by Chen et al. (19). Dataset was derived from a published article -association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study (https://doi.org/10.5061/dryad.ft8750v). This is an open-access article given in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which enables people to share, remix, modify, and create a derivative work from this work for non-commercial purposes as long as the author and source are credited (19).

Study population

The original researchers extracted data from a computerized database created by the Rich Healthcare Group in China, which contains all medical records for participants who received health checks in 32 regions and 11 cities between 2010 and 2016 (19). The original study was initially approved by the Rich Healthcare Group Review Board, and the data was retrieved retroactively. The institutional ethics committee did not require informed consent or approval for the retrospective study (19). Therefore, ethical approval was not required for the current secondary analysis. Furthermore, the original study was carried out in compliance with the Helsinki Declaration. So did this secondary analysis.

685,277 participants who were at least 20 years old and had passed at least two health examinations were initially enrolled in the original study. 473,744 participants were excluded after that. Finally, the original study included 211,833 individuals in its analysis (19). Following are the exclusion criteria for the original study: (i) participants diagnosed with diabetes at enrolment; (ii) no information about FPG value, sex, height, and weight at baseline; (iii) extreme BMI values (<15 or >55 kg/m^2); (iv) Participants with <2 years between visits; (v) participants with unknown diabetes status at follow-up (19). We first included 26,018 participants with baseline FPG of 5.6–6.9 mmol/l in the current study. Prediabetes is defined as an FPG level of 5.6–6.9mmol/L according to the American Diabetes Association 2021 criteria (20). After that, we excluded participants who lacked TG data (n = 618) and those who had abnormal or extreme TyG-BMI (greater or <3 standard deviations from the mean) (n = 317). Ultimately, 25,279 participants were included in the current secondary analysis. Figure 1 shows how participants were selected.

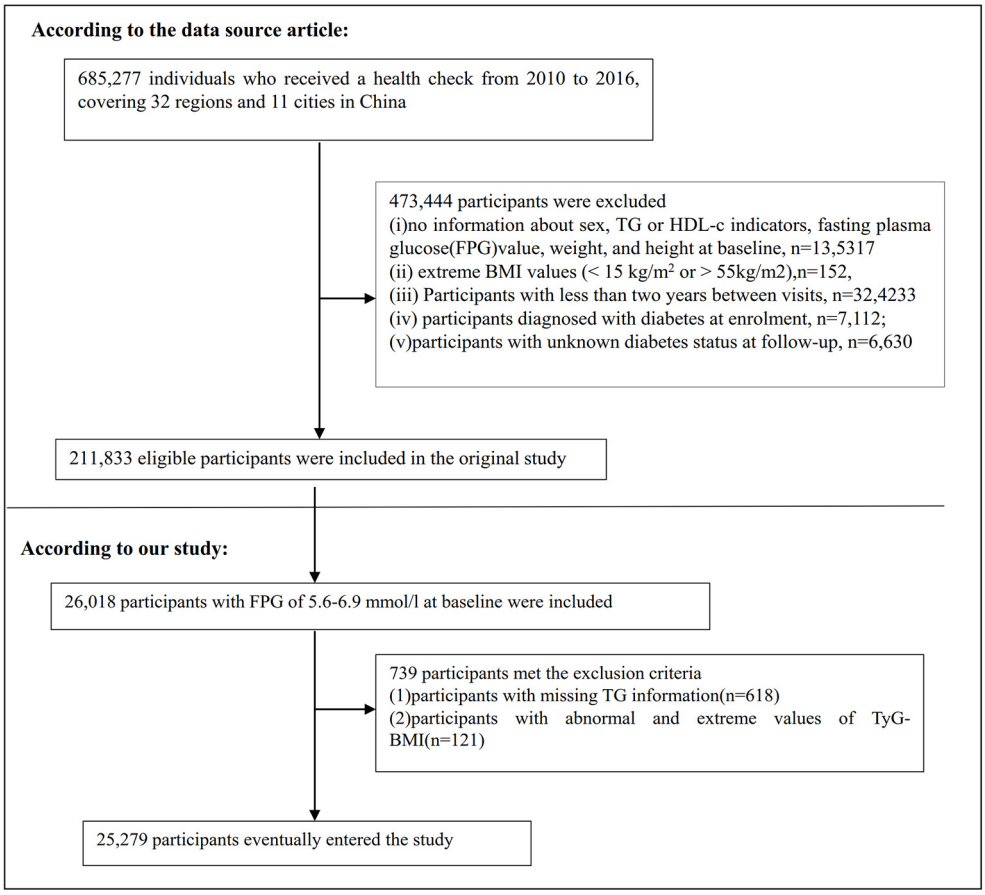


FIGURE 1
Flowchart of study participants.

Variables

Independent variable

The detailed procedure for defining TyG-BMI was as follows: TyG-BMI = BMI × TyG index, where TyG index = ln [FPG (mg/dL) × TG (mg/dL)/2] and BMI = weight / height^2 (16). It was important to note that relevant information for TG, BMI, and FPG was obtained at baseline.

Outcome measures

The outcome variable of interest in our investigation was incident diabetes (dichotomous variable: 1 = DM and 0 = non-DM). FPG≥7.0 mmol/L or self-report at follow-up evaluation was used to define incident diabetes (19).

Covariates

The covariates in our study were selected according to the previous literature and our medical experience. Covariates included the following: (i) continuous variables: weight, height, age, aspartate aminotransferase (AST), blood urea nitrogen (BUN), alanine aminotransferase (ALT), serum creatinine (Scr), BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDL-c), total cholesterol (TC), low-density lipid cholesterol (LDL-c); (ii) categorical variables: drinking status, family history of diabetes, sex, and smoking status.

Data collection

In the initial study, qualified researchers used standardized questionnaires to gather baseline data on lifestyle (drinking and smoking status), demographics (age and sex), and family history of diabetes. Standard mercury sphygmomanometers measured blood pressure. During each visit, fasting venous blood samples were taken at least 10 h after a fast. A Beckman 5,800 autoanalyzer was used to measure plasma glucose, TC, TG, HDL-c, and LDL-c (19).

Missing data processing

In this second analysis, the number of participants whose data are missing of SBP, DBP, ALT, Scr, BUN, LDL-c, HDL-c, AST, drinking status, and smoking status was 7 (0.03%), 7 (0.03%), 211 (0.84%), 1,119 (4.43%), 2,439 (9.65%), 9,291 (36.75%), 9,921 (39.25%), 14,118 (55.85%), 16,727 (66.17%), and 16,727 (66.17%), respectively. This

study used multiple imputations for missing data to reduce the variation brought on by missing variables (21). The imputation model (type was Linear regression, iterations were 10) included sex, age, DBP, HDL-c, TC, AST, BUN, Scr, SBP, ALT, LDL-c, drinking status, family history of diabetes, and smoking status. Missing-at-random (MAR) assumptions are used in missing data analysis procedures (21, 22).

Statistical analysis

We stratified the participants by quartiles of TyG-BMI. The means and standard deviations were presented for continuous variables with Gaussian distributions, medians were reported for skewed distributions, and percentages and frequencies were presented for categorical variables. We used the Kruskal-Wallis H-test (skewed distribution), the One-Way ANOVA test (normal distribution), or $\chi 2$ (categorical variables) to test for differences among different TyG-BMI groups.

We examined the link between TyG-BMI and diabetes risk in patients with prediabetes using univariate and multivariate Cox proportional-hazards regression models after collinearity screening. These included a non-adjusted model (no covariates adjusted), a minimally adjusted model (Model I: adjusted age and sex), and a fully adjusted model were used (Model II: adjusted age, sex, BUN, DBP, SBP, AST, ALT, HDL-c, LDL-c, drinking status, smoking status and family history of diabetes). HR and 95% confidence intervals (CI) were recorded in this study. The collinearity screening also excluded TC from the final multivariate Cox proportional hazards regression equation since it was collinear with other variables (Supplementary Table S1).

Besides, we used Cox proportional hazards regression with cubic spline functions and smooth curve fitting to explore the non-linear relationship between the TyG-BMI and diabetes risk in participants with prediabetes. Recursive algorithms were used to calculate the inflection point if non-linearity was found. Then a two-piecewise linear regression model was fitted to calculate the threshold effect of the TyG-BMI on incident diabetes according to the smoothed graph.

A stratified Cox proportional hazard regression model was used to conduct subgroup analysis across several subgroups (age, sex, SBP, smoking status, and drinking status). Firstly, the interaction test between these variables and TyG-BMI was performed before the subgroup analysis. Secondly, continuous variables, including SBP, and age, were converted into categorical variables based on clinical cut-off points (age: <30, ≥ 30 to <40, ≥ 40 to <50, ≥ 50 to <60, ≥ 60 to <70, ≥ 70 years old; SBP: <140, ≥ 140 mmHg) (23). Thirdly, we adjusted each stratification for all other factors (sex, age, AST, SBP, ALT, DBP, HDL-c, BUN, LDL-c, smoking status, family history of diabetes, and drinking status) besides the stratification factor itself. Ultimately, the likelihood ratio test was used to determine whether interaction terms existed in models with and without interaction terms. The likelihood ratio test compares models with and without the multiplicative interaction term(s); the log-likelihood of models with main effects was compared with the log-likelihood of models that contained main effects and the interaction terms to determine the statistical significance of interactions (24).

To test the robustness of the results, we performed a series of sensitivity analyses. We converted the TyG-BMI into a categorical variable according to the quartile and calculated the *P*-value for the trend to test the results of the TyG-BMI as a continuous variable and to explore the possibility of non-linearity. Previous studies have suggested that drinking status, family history of diabetes, and BMI are significantly related to diabetes (25–27). Therefore, we performed sensitivity analyses after excluding alcohol drinkers and smokers and participants with a family history of diabetes. Additionally, we further explored the association between the TyG-BMI and diabetes risk in participants with BMI<25 kg/m^2. Further, to further confirm our findings' reliability, we used a generalized additive model (GAM), which included the continuity covariate as a curve in the equation.

Finally, we construct a receiver operating characteristic (ROC) curve to estimate the ability of TyG-BMI, BMI, TyG, TG, and TG/HDL-c ratio to predict the risk of diabetes in patients with prediabetes.

All results were written according to the STROBE statement (28). Both Empower Stats (X&Y Solutions, Inc. Boston, MA, http://www.empowerstats.com) and the R statistical software packages (http://www.r-project.org, The R Foundation) were used to conduct all analyses. Statistical significance was defined as *P*-values under 0.05 (two-sided).

Results

Characteristics of participants

The study participants' demographic and clinical characteristics are presented in Table 1. The mean age was 49.29 ± 13.82 years old, and 16,734 (66.2%) were male. The mean TyG-BMI was 219.47. Over a median follow-up period of 2.89 years, 2,687 (10.63%) participants developed diabetes. We divided adults into subgroups based on TyG-BMI quartiles (Q1: <193.08, Q2: 193.08–218.21, Q3: 218.21–244.04, Q4: ≥ 244.04). The highest quartile (Q4: ≥ 244.04) showed significant increases in age, height, weight, BMI, DBP, SBP, TG, LDL-c, TC, AST, ALT, TyG-BMI, TyG, Scr, and BUN in comparison with the lowest quartile (Q1:<193.08); however, HDL-c showed the opposite trend. Moreover, the highest quartile had a higher proportion of men, current drinkers, and current smokers. The TyG-BMI presents a normal distribution, ranging from 116. 94 to 334.08, with a mean of 219.47 (Figure 2). The presence or absence of diabetes at the last follow-up visit was used to split the participants into two groups. Supplementary Figure S1 shows the distribution of TyG-BMI for the two groups. The TyG-BMI distribution level was lower in the non-diabetes group. In contrast, the group with diabetes had a higher distribution level of the TyG-BMI.

The incidence rate of diabetes in patients with prediabetes

Among participants with prediabetes, 2,687 (10.63%) individuals developed diabetes. Specifically, the incidence rate of diabetes among participants with prediabetes in the TyG-BMI quartiles was 13.16, 27.09, 41.11, and 55.22 per 1,000 person-years, respectively. During a median follow-up period of 2.89 years, the overall cumulative incidence of diabetes was 11.46%, and the cumulative incidences of diabetes in each TyG-BMI quartile were Q1: 3.83%, Q2: 7.97%, Q3: 12.23%, and Q4: 16.47% (Figure 3). There was a higher incidence of

TABLE 1 The baseline characteristics of participants.

TyG-BMI quartile	Q1 (<193.08)	Q2 (193.08–218.21)	Q3 (218.21–244.04)	Q4 (≥244.04)	*P*-value
Participants	6,319	6,320	6,320	6,320	
Age (years)	45.25 ± 14.30	50.29 ± 13.79	51.63 ± 13.34	50.00 ± 12.96	<0.001
Height (cm)	165.18 ± 8.29	166.32 ± 8.40	167.17 ± 8.25	168.06 ± 8.16	<0.001
Weight (kg)	57.30 ± 7.40	65.93 ± 7.50	71.97 ± 7.97	81.05 ± 9.93	<0.001
BMI (kg/m^2)	20.94 ± 1.66	23.77 ± 1.28	25.69 ± 1.39	28.63 ± 2.21	<0.001
SBP (mmHg)	120.70 ± 16.57	126.39 ± 17.05	129.17 ± 17.32	132.41 ± 17.26	<0.001
DBP (mmHg)	73.91 ± 10.09	77.40 ± 10.60	79.76 ± 10.86	82.44 ± 11.17	<0.001
TG (mmol/L)	0.84 (0.63–1.11)	1.24 (0.96–1.65)	1.67 (1.25–2.24)	2.33 (1.70–3.34)	<0.001
TYG	8.28 ± 0.43	8.69 ± 0.43	8.99 ± 0.46	9.38 ± 0.55	<0.001
TyG-BMI	173.25 ± 14.59	206.08 ± 7.26	230.53 ± 7.42	267.99 ± 19.45	<0.001
TC (mmol/L)	4.65 ± 0.88	4.93 ± 0.93	5.07 ± 0.92	5.25 ± 0.99	<0.001
FPG (mmol/L)	5.86 ± 0.27	5.92 ± 0.30	5.97 ± 0.33	6.03 ± 0.34	<0.001
HDL-c (mmol/L)	1.42 ± 0.31	1.34 ± 0.31	1.29 ± 0.28	1.27 ± 0.30	<0.001
LDL-c (mmol/L)	2.68 ± 0.68	2.90 ± 0.71	2.96 ± 0.71	3.00 ± 0.75	<0.001
ALT (U/L)	15.50 (12.00–21.30)	20.00 (15.00–28.00)	24.50 (18.00–35.60)	32.00 (22.30–48.30)	<0.001
AST (U/L)	22.47 ± 8.95	24.65 ± 11.10	27.02 ± 10.51	31.18 ± 14.62	<0.001
BUN (mmol/L)	4.82 ± 1.25	5.00 ± 1.23	5.07 ± 1.26	5.06 ± 1.25	<0.001
Scr (μmol/L)	68.32 ± 15.32	72.37 ± 15.49	74.45 ± 16.42	75.80 ± 15.69	<0.001
Sex					<0.001
Male	3,092 (48.93%)	4,067 (64.35%)	4,586 (72.56%)	4,989 (78.94%)	
Female	3,227 (51.07%)	2,253 (35.65%)	1,734 (27.44%)	1,331 (21.06%)	
Smoking status					<0.001
Current smoker	880 (13.93%)	1,324 (20.95%)	1,600 (25.32%)	1,911 (30.24%)	
Ever smoker	208 (3.29%)	252 (3.99%)	271 (4.29%)	303 (4.79%)	
Never smoker	5,231 (82.78%)	4,744 (75.06%)	4,449 (70.40%)	4,106 (64.97%)	
Drinking status					<0.001
Current drinker	145 (2.29%)	195 (3.09%)	222 (3.51%)	362 (5.73%)	
Ever drinker	714 (11.30%)	968 (15.32%)	1,028 (16.27%)	1,161 (18.37%)	
Never drinker	5,460 (86.41%)	5,157 (81.60%)	5,070 (80.22%)	4,797 (75.90%)	
Family history of diabetes					0.220
No	6,177 (97.75%)	6,148 (97.28%)	6,175 (97.71%)	6,154 (97.37%)	
Yes	142 (2.25%)	172 (2.72%)	145 (2.29%)	166 (2.63%)	

Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as percentage (%).
FPG, fasting plasma glucose; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglyceride; SBP, systolic blood pressure; TyG, the triglyceride-glucose index; TyG-BMI, triglyceride glucose-body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL-C, low-density lipid cholesterol; BUN, blood urea nitrogen; Scr, serum creatinine. HDL-c, high-density lipoprotein cholesterol.

diabetes among those with the highest TyG-BMI (Q4) compared to those with the lowest TyG-BMI (Q1) ($p < 0.001$ for trend) (Table 2, Figure 3).

Regardless of their age groups, men with prediabetes were more likely to develop diabetes than women in the age stratification by ten intervals (Figure 4). In addition, both males and females showed an increase in diabetes incidence with age.

Factors influencing the risk of diabetes in patients with prediabetes analyzed by univariate Cox proportional hazards regression

Based on univariate analyses, the risk of progression to diabetes from prediabetes was not related to Scr ($P > 0.05$), but was positively correlated with age, DBP, BMI, SBP, AST, TG, ALT, TyG-BMI, TC,

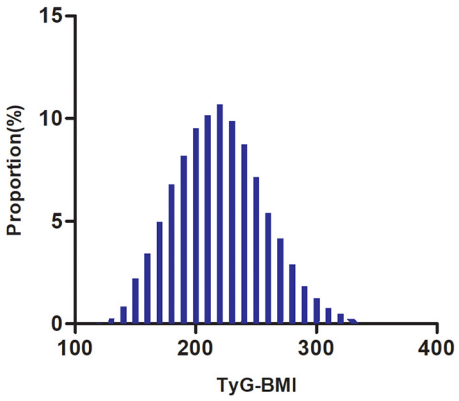


FIGURE 2
Distribution of TyG-BMI. It presented a normal distribution, ranging from 116.94 to 334.08, with a mean of 219.47.

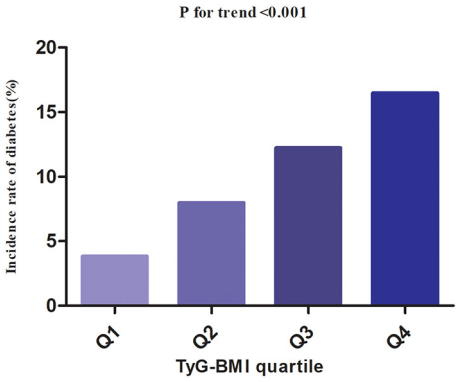


FIGURE 3
The incidence rate for diabetes according to the quartiles of TyG-BMI. Participants with the highest TyG-BMI(Q4) had higher diabetes incidence rates than those with the lowest TyG-BMI(Q1) ($p < 0.001$ for trend).

FPG, LDL-c, BUN, family history of diabetes, and current drinking (all $P < 0.05$; Table 3).

Using the TyG-BMI quartile as stratification, Figure 5 presented Kaplan-Meier survival curves for diabetes-free survival probability. Among the quartiles of TyG-BMI, there were statistically significant differences in the probability of diabetes-free survival (log-rank test, $p < 0.001$). Prediabetic patients with the greatest TyG-BMI had the highest risk of progression to diabetes.

The results of multivariable analyses using Cox proportional-hazards regression models

Three models were constructed using the Cox proportional-hazards regression model to investigate the association between the TyG-BMI and incident diabetes in patients with prediabetes (Table 4). An increase of 1 unit of TyG-BMI was associated with a 1.2% increase in diabetes risk in the crude model (HR = 1.012, 95%CI:1.011–1.013, $p < 0.001$). For participants with prediabetes, each additional unit of TyG-BMI increased their diabetes risk by 1.2%

TABLE 2 Incidence rate of diabetes in participants with prediabetes (% or Per 1,000 person-year).

TyG-BMI	Participants (*n*)	Diabetes events (*n*)	Incidence rate (95% CI) (%)	Per 1,000 person-year
Total	25,279	2,560	10.13(9.76–10.50)	34.27
Q1(<193.08)	6,319	242	3.83(3.36–4.30)	13.16
Q2 (193.08–218.21)	6,320	504	7.97(7.70–8.34)	27.09
Q3 (218.21–244.04)	6,320	773	12.23(11.42–13.04)	41.11
Q4 (≥244.04)	6,320	1,041	16.47(15.56–17.39)	55.22
P for trend			<0.001	

TyG-BMI, triglyceride glucose-body mass index; CI, confidence interval.

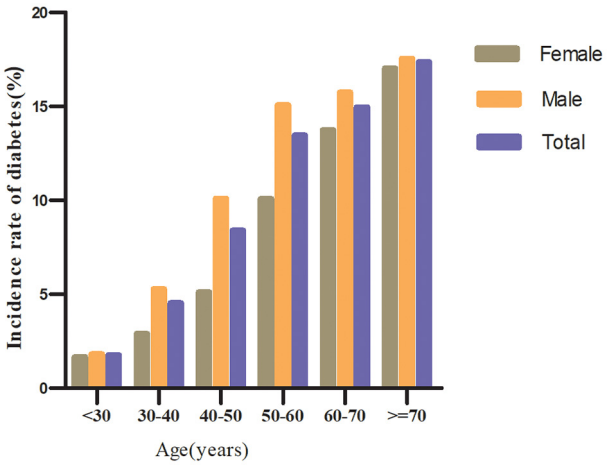


FIGURE 4
The incidence of diabetes in prediabetic patients of age stratification by 10 intervals. It shows that the incidence of diabetes in participants with prediabetes was higher in men than in women, regardless of age group. It was also found that the incidence of diabetes increased with age in both men and women.

(HR = 1.012, 95% CI: 1.011–1.013) in the minimally adjusted model (Model I). As a result of the fully adjusted model (Model II), every 1 unit increase in TyG-BMI was associated with an increase in diabetes risk of 1.1% in participants with prediabetes (HR = 1.011, 95% CI 1.010–1.012). As evidenced by the confidence interval distribution, the model indicated a reliable relationship between TyG-BMI and diabetes risk in subjects with prediabetes.

Sensitivity analysis

A series of sensitivity analyses were carried out to confirm the robustness of our conclusions. The TyG-BMI was first converted into quartile-based categorical variables, and the categorically modified TyG-BMI was then added back to the regression equation.

10.3389/fpubh.2023.1028461

TABLE 3 Factors influencing the risk of diabetes in patients with prediabetes analyzed by univariate Cox proportional hazards regression.

Exposure	Characteristics	HR (95%CI)	*P*-value
Age (years)	49.292 ± 13.819	1.031 (1.028, 1.034)	<0.001
Sex			
Male	16,734 (66.197%)	Ref	
Female	8,545 (33.803%)	0.835 (0.766, 0.910)	<0.001
BMI (kg/m^2)	24.758 ± 3.265	1.119 (1.106, 1.132)	<0.001
SBP (mmHg)	127.169 ± 17.586	1.015 (1.013, 1.017)	<0.001
DBP (mmHg)	78.377 ± 11.135	1.017 (1.014, 1.020)	<0.001
FPG (mmol/L)	5.945 ± 0.317	9.796 (8.889, 10.795)	<0.001
TG (mmol/L)	1.410 (0.960–2.110)	1.116 (1.096, 1.137)	<0.001
TyG	8.835 ± 0.619	1.800 (1.700, 1.907)	<0.001
TyG-BMI	219.465 ± 37.051	1.012 (1.011, 1.013)	<0.001
TC (mmol/L)	4.975 ± 0.957	1.082 (1.040, 1.125)	<0.001
HDL-c(mmol/L)	1.330 ± 0.303	1.246 (1.097, 1.416)	<0.001
LDL-c (mmol/L)	2.886 ± 0.724	1.064 (1.010, 1.122)	0.021
ALT (U/L)	22.000 (15.400–33.000)	1.005 (1.004, 1.006)	<0.001
AST (U/L)	26.328 ± 11.927	1.011 (1.009, 1.012)	<0.001
BUN (mmol/L)	4.991 ± 1.251	1.051 (1.019, 1.083)	0.002
Scr (μmol/L)	72.735 ± 15.988	1.001 (0.999, 1.004)	0.392
Smoking status			
Current smoker	5,715 (22.608%)	Ref	
Ever smoker	1,034 (4.090%)	1.059 (0.878, 1.276)	0.550
Never smoker	18,530 (73.302%)	0.823 (0.754, 0.899)	<0.001
Drinking status			
Current drinker	924 (3.655%)	Ref	
Ever drinker	3,871 (15.313%)	0.797 (0.650, 0.977)	0.029
Never drinker	20,484 (81.032%)	0.793 (0.659, 0.953)	0.013
Family history of diabetes			
No	24,654 (97.528%)	Ref	
Yes	625 (2.472%)	1.446 (1.191, 1.754)	<0.001

Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as percentage (%).

FPG, fasting plasma glucose; BMI, body mass index; DBP, diastolic blood pressure; TC, total cholesterol, SBP, systolic blood pressure; TG triglyceride; ALT, alanine aminotransferase; TyG, the triglyceride-glucose index; TyG-BMI, triglyceride glucose-body mass index; BUN, blood urea nitrogen; LDL-c, low-density lipid cholesterol; Scr, serum creatinine, HDL-c, high-density lipoprotein cholesterol; AST aspartate aminotransferase.

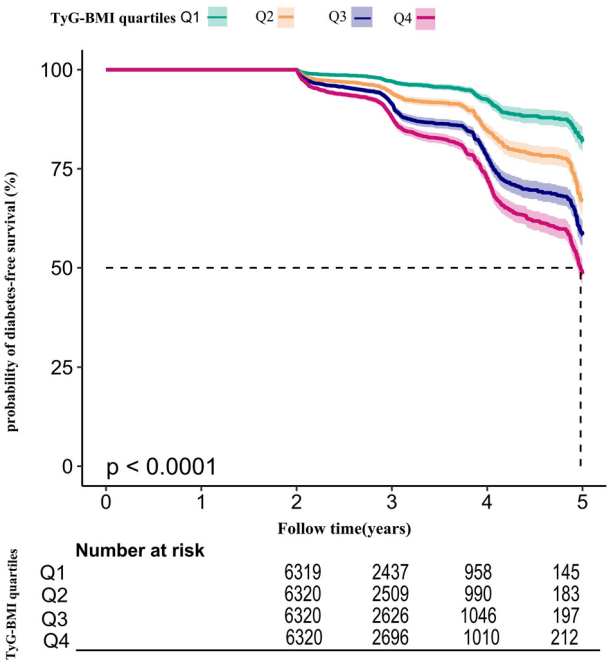


FIGURE 5
Kaplan–Meier event-free survival curve. Kaplan–Meier event-free survival curve. The probability of diabetes-free survival differed significantly between the TyG-BMI quartiles (log-rank test, $p < 0.001$). The probability of diabetes-free survival gradually decreased with increasing TyG-BMI, suggesting that the group with the highest TyG-BMI had the highest risk of diabetes.

The findings revealed that the effect sizes between groups were equidistant, and the trend of effect sizes was consistent with the result when the TyG-BMI was a continuous variable (Table 4).

Additionally, we introduced the continuity covariate as a curve into the equation using a GAM. As shown in Table 4, the outcome of Model III was reasonably consistent with the fully adjusted model (HR = 1.010, 95%CI: 1.009–1.011, $p < 0.001$).

Furthermore, we conducted a sensitivity analysis on participants who had never consumed alcohol ($n = 20,484$). After adjusting for confounding variables, the findings indicated that the TyG-BMI was also positively associated with the risk of diabetes (HR = 1.011, 95% CI:1.010–1.012, $p < 0.001$). We also excluded patients with a family history of diabetes for the sensitivity analyses. After adjusting for confounding variables, the results suggested that the TyG-BMI was still positively associated with diabetes risk in individuals with prediabetes (HR = 1.011, 95% CI:1.010–1.012, $p < 0.001$). In addition, restricting the analysis to participants with BMI<25 kg/m^2, the results suggested that the HR between the TyG-BMI and diabetes risk was 1.015 (95% CI:1.011–1.018, $P < 0.001$) (Table 5). Based on all the sensitivity analyses, it is evident that our findings were robust.

Cox proportional hazards regression model with cubic spline functions to address non-linearity

We observed that the relationship between TyG-BMI and diabetes risk in prediabetic patients was non-linear using the Cox proportional hazards regression model with cubic spline functions (Figure 6). The *P*-value for the log-likelihood ratio test was <0.001. We first determined that the inflection point of the TyG-BMI was 231.66 by the recursive algorithm and then used a two-piecewise Cox proportional hazards regression model to calculate the HR and CI for each side of the inflection point. Right before the inflection point, the HR was 1.007 (95% CI:1.005–1.009), while left after it was 1.017(95%CI:1.014–1.019) (Table 6).

TABLE 4 Relationship between TyG-BMI and the risk of diabetes in prediabetic patients in different models.

Exposure	Crude model (HR, 95%CI)	Model I (HR, 95%CI) *P*	Model II (HR, 95%CI) *P*	Model III (HR, 95% CI) *P*
TyG-BMI	1.012 (1.011, 1.013) <0.001	1.012 (1.011, 1.013) <0.001	1.011 (1.010, 1.012) <0.001	1.010 (1.009, 1.011) <0.001
TyG-BMI quartile				
Q1	Ref	Ref	Ref	Ref
Q2	1.994 (1.710, 2.324) <0.001	1.743 (1.494, 2.034) <0.001	1.756 (1.502, 2.052) <0.001	1.645 (1.405, 1.927) <0.001
Q3	2.950 (2.553, 3.408) <0.001	2.496 (2.156, 2.889) <0.001	2.525 (2.174, 2.933) <0.001	2.268 (1.943, 2.647) <0.001
Q4	3.966 (3.449, 4.562) <0.001	3.502 (3.038, 4.038) <0.001	3.371 (2.906, 3.910) <0.001	2.920 (2.497, 3.414) <0.001
P for trend	1.514 (1.458, 1.572) <0.001	1.477 (1.420, 1.536) <0.001	1.451 (1.393, 1.511) <0.001	1.387 (1.328, 1.449) <0.001

Crude model: we did not adjust other covariates.
Model I: we adjusted age, sex.
Model II: we adjusted age, sex, SBP, DBP ALT, AST, BUN, LDL-C, HDL-c, family history of diabetes, drinking status, and smoking status.
Model III: we adjusted age (smooth), sex, SBP (smooth), DBP (smooth), ALT (smooth), AST (smooth), LDL-c (smooth), HDL-c (smooth), smoking status, drinking status, family history of diabetes.
HR, Hazard ratios; CI: confidence, Ref: reference.

TABLE 5 Relationship between TyG-BMI and the risk of diabetes in participants with prediabetes in different sensitivity analyses.

Exposure	Model I (HR, 95%CI) *P*	Model II (HR, 95%CI) *P*	Model III (HR, 95%CI) *P*
TyG-BMI	1.011 (1.010, 1.012) <0.001	1.011 (1.010, 1.012) <0.001	1.015 (1.011, 1.018) <0.001
TyG-BMI quartile			
Q1	Ref	Ref	Ref
Q2	1.738 (1.466, 2.061) <0.001	1.775 (1.513, 2.084) <0.001	1.687 (1.431, 1.989) <0.001
Q3	2.541 (2.158, 2.992) <0.001	2.570 (2.204, 2.996) <0.001	2.510 (2.076, 3.035) <0.001
Q4	3.272 (2.776, 3.856) <0.001	3.458 (2.970, 4.026) <0.001	1.869 (1.187, 2.944) 0.007
P-for trend	<0.001	<0.001	<0.001

Model I was a sensitivity analysis performed on participants who had never consumed alcohol ($N = 20,484$). We adjusted age, sex, SBP, DBP ALT, AST, BUN, LDL-C, HDL-c, family history of diabetes, and smoking status.
Model II was a sensitivity analysis performed on participants without a family history of diabetes ($N = 24,654$). We adjusted age, sex, SBP, DBP ALT, AST, BUN, LDL-C, HDL-c, drinking status, and smoking status.
Model III was a sensitivity analysis performed after excluding participants with BMI≥25 kg/m^2 ($N = 13,571$). We adjusted age, sex, SBP, DBP ALT, AST, BUN, LDL-C, HDL-c, drinking status, smoking status, and family history of diabetes.
HR, Hazard ratios; CI, confidence; Ref, reference.

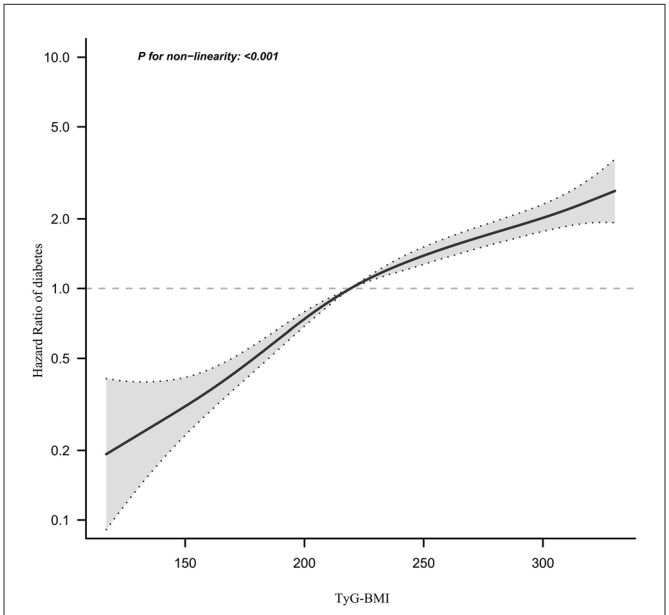


FIGURE 6
The non-linear relationship between TyG-BMI and the risk of diabetes in prediabetic patients. We used a Cox proportional hazards regression model with cubic spline functions to evaluate the relationship between the TyG-BMI and diabetes risk. The result showed that the relationship between the TyG-BMI and diabetes risk in prediabetic patients was non-linear, with the inflection point of the TyG-BMI ratio being 231.66.

The results of subgroup analyses

Interaction tests performed before **subgroup analyses** showed that age and SBP interacted with TyG-BMI ($P < 0.001$). In contrast, sex, smoking, and alcohol consumption did not interact with TyG-BMI ($P > 0.05$) (Supplementary Table S2). Therefore, further subgroup analyses with prespecified or exploratory age and SBP were performed (Table 7). However, there was no significant interaction of SBP as a categorical variable with TyG-BMI (*P* for interaction >0.05). The results showed that in the age subgroup, the interaction between TyG-BMI and age was significant (*P* for interaction <0.001). Specifically, a stronger relationship between TyG-BMI and diabetes risk was observed in participants aged <50 years. Among participants aged <30 years, 30–40 years, and 40–50 years, the HRs for the association between TyG-BMI and the risk of diabetes in prediabetic patients were 1.020, 1.019, and 1.016, respectively (all $P < 0.001$). In contrast, a weaker relationship between TyG-BMI and diabetes risk was observed in prediabetic participants aged >50 years. Among participants aged 50–60, 60–70, and ≥70, the HRs for the relationship between TyG-BMI and the risk of diabetes was 1.009, 1.006, and 1.008, respectively (all $P < 0.001$).

The results of the ROC curve analysis

In addition, we drew a ROC curve to measure the ability of TyG-BMI, BMI, TyG, TG, and TG/HDL-c ratio to predict the risk of

TABLE 6 The result of two-piecewise linear regression model.

Incident diabetes:	HR, 95%CI	*P*
Fitting model by standard Cox regression	1.011 (1.010, 1.012)	<0.001
Fitting model by two-piecewise Cox regression		
Inflection points of TyG-BMI	231.66	
<231.66	1.017 (1.014, 1.019)	<0.001
≥231.66	1.007 (1.005, 1.009)	<0.001
P-for log-likelihood ratio test		<0.001

We adjusted age, sex, SBP, DBP ALT, AST, BUN, LDL-C, HDL-c, family history of diabetes, drinking status, and smoking status.

TABLE 7 Stratified associations between TyG-BMI and diabetes in participants with prediabetes by age and SBP.

Characteristic	No of participants	HR (95%CI)	*P*-value	*P*-for interaction
Age (years)				<0.001
<30	1,464	1.020 (1.011, 1.029)	<0.001	
30 to <40	6,055	1.019 (1.017, 1.022)	<0.001	
40 to <50	5,514	1.016 (1.014, 1.019)	<0.001	
50 to <60	5,907	1.009 (1.007, 1.011)	<0.001	
60 to <70	4,278	1.006 (1.003, 1.008)	<0.001	
≥70	2,061	1.008 (1.005, 1.011)	<0.001	
SBP (mmHg)				0.2484
<140	19,902	1.012 (1.010, 1.013)	<0.001	
≥140	5,377	1.010 (1.008, 1.012)	<0.001	

Above model adjusted for age, sex, SBP, DBP ALT, AST, BUN, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status.
In each case, the model is not adjusted for the stratification variable.
HR, Hazard ratios; CI, confidence; Ref, reference.

diabetes (Figure 7). The areas under the curve of each variable were as follows: TG: 0.615 < TG/HDL-c ratio:0.621<BMI: 0.628 < TyG: 0.640 <TyG-BMI:0.656. The highest Youden index of TG, TG/HDL-c ratio, BMI, TyG, and TyG-BMI was 0.1670, 0.1700,0.1954, 0.2030, 0.2513, and the corresponding optimal cut-off value was 1.595, 1.1463, 24.760, 8.808, 220.238, respectively. The Youden index and AUC of TyG-BMI were the biggest, so the predictive ability of TyG-BMI to incident diabetes was better than that of other variables (Supplementary Table S3).

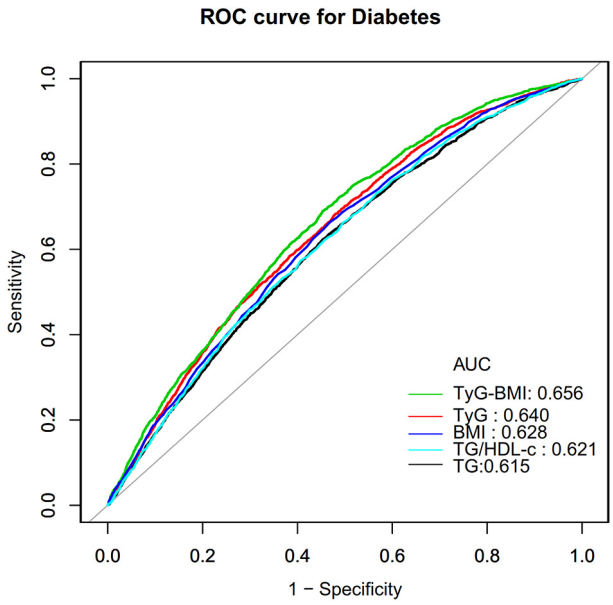


FIGURE 7
The results of ROC curve analysis for measuring the ability of TyG-BMI, BMI, TG, TyG, and TG/HDL-c ratio to predict the risk of diabetes.

Discussion

In this retrospective cohort study, we examined the link between the TyG-BMI and diabetes risk in patients with prediabetes. We found that a higher TyG-BMI was linked to a significantly higher risk of diabetes among patients with prediabetes. Additionally, an inflection point was identified, and different relationships between the TyG-BMI and diabetes risk were detected on both sides.

A Japanese study found an 8.5% progression rate from prediabetes to diabetes within five years (29). Americans with prediabetes aged 70–79 have a 10.6% chance of progressing to diabetes over the next seven years (30). Participants with prediabetes in the present study had a cumulative incidence of diabetes of 10.63% over a median follow-up of 2.89 years. Participants' age, follow-up time, and ethnicity may be responsible for these differences in diabetes incidence among these patients. It is worth noting that all studies have shown that patients with prediabetes are at high risk for developing diabetes. Therefore, it is crucial to actively identify various other risk factors for progressing to diabetes from prediabetes.

The TyG index is a combined marker containing FPG and TG and is considered an alternative marker of IR (14, 31). In recent years, TyG-BMI, a metric that combines BMI with TyG, has emerged as a new obesity-related parameter. Studies have shown that TyG-BMI is a better predictor of IR than traditional lipids, BMI, and TyG index (16). Furthermore, several recent studies have found that TyG-BMI is strongly associated with non-alcoholic fatty liver, stroke, prehypertension, and diabetes (32–34). However, the connection between the TyG-BMI index and the prevalence of diabetes has received little attention with only two research covering the subject. 116,661 participants in a cohort study had their physicals examined. Multivariate Cox regression analysis revealed an independent relationship between the TyG-BMI index and new-onset diabetes in the general population (HR 1.50/standard deviation

increase, 95% CI: 1.40 to 1.60, $P < 0.0001$) (18). Another cross-sectional study conducted in Spain supported this conclusion. According to results from multivariate-adjusted models, participants in the fourth quartile of the TyG-BMI index had a 3.63-fold higher risk of getting diabetes than those in the first quartile (17). Our study complemented the existing literature, which supported the hypothesis that an elevated TyG-BMI index is positively associated with the risk of new-onset diabetes. In contrast to earlier studies, the independent variables in our study used both the TyG-BMI index as a categorical variable and a continuous variable to explore their relationship with diabetes risk, thus reducing information loss and quantifying the relationship between them. Second, to the best of our knowledge, this is the first study to examine the association between the TyG-BMI and diabetes risk in individuals with prediabetes, a population with a high propensity to develop diabetes. Identifying TyG-BMI as a risk factor for progression from prediabetes to diabetes and clarifying the association between them would be beneficial for diabetes prevention in patients with prediabetes.

In addition, based on the population with prediabetes, our previous study identified an important lipid index, TG/HDL-c ratio, as an important risk predictor for diabetes (35). Therefore, we constructed ROC curves to estimate the ability of TyG-BMI, BMI, TyG, TG, and TG/HDL-c ratio to predict the risk of progression to diabetes in patients with prediabetes. We found that the AUC and highest Youden index of the TyG-BMI index fared better than any other component of the TyG-BMI and TG/HDL-c ratio. This finding shows that TyG-BMI may be a valuable marker for predicting the onset of diabetes in patients with prediabetes. These indicators provide significant risk predictors for future risk prediction models for the progression of prediabetes to diabetes. Furthermore, sensitivity analyses found that their association persisted in prediabetic patients with a BMI<25 kg/m^2, no family history of diabetes, and no alcohol consumption. This study encourages clinical consultation and provides a reference for enhancing diabetes prevention in individuals with prediabetes.

The underlying mechanisms relating TyG-BMI to diabetes risk remain unclear, but it may be associated with IR. Research has confirmed that IR plays a crucial role in diabetes occurrence and progression (36). TyG-BMI represents a combination of FPG, TG, and BMI. FPG levels are a reflection of insulin sensitivity in the liver and insulin secretion by the pancreas (37). A higher level of FPG is associated with an increased risk of diabetes among people with normal FPG levels (38). In addition, the role of BMI and TG in identifying IR has been well established in previous studies (39–41). Therefore, the underlying mechanism of the relationship between TyG-BMI and the risk of developing diabetes may be related to the association of three factors, FPG, TG, and BMI, with IR.

Furthermore, our study observed a non-linear relationship between the TyG-BMI and diabetes risk in individuals with prediabetes for the first time. After controlling for confounders, the TyG-BMI inflection point was 231.66. When the TyG-BMI was <231.66, each unit increase was associated with a 1.7% increase in the risk of diabetes. A 1-unit increase in TyG-BMI was associated with a 0.7% increase in the risk of diabetes when the TyG-BMI was >231.66. It could be found that compared to participants with a TyG-BMI>231.66, those with TyG-BMI ≤ 231.66 generally are younger and have lower DBP, LDL-c, AST, SBP, and ALT. In addition, those with a TyG-BMI≤ 231.66 had a lower proportion of currently drinking and smoking (Supplementary Table S4). However, these indicators are strongly linked to incident diabetes (42–46). Because of the presence of these risk factors, when the TyG-BMI was >231.66, the TyG-BMI had a relatively weak effect on diabetes. On the contrary, among those with a TyG-BMI of <231.66, these diabetes risk factors were lower, the impact on diabetes was lower, and the effect of TyG-BMI was relatively enhanced. Furthermore, non-linear relationships are those in which the change in one variable does not correspond to the same constant change in the other variable. In other words, it could imply that the relationship between two variables is either non-existent or unpredictable. Non-linear entities, on the other hand, can be related to each other in predictable but more complex ways than linear ones. Because of the complexities of the relationship between TyG-BMI and diabetes risk, the non-linear relationship may be closer to the true relationship. The discovery of a curvilinear relationship between the TyG-BMI and incident diabetes in prediabetic patients has significant clinical implications. It serves as a resource for promoting clinical consultation and optimizing diabetes prevention decision-making in patients with prediabetes.

Subgroup analysis revealed some interesting findings in this study. Young adults have a higher risk of diabetes associated with their TyG-BMI than other age groups. After further analysis of the baseline information of the study population grouped according to age, young-aged people (<50 years old) were found had lower ALT, DBP, LDL-c, SBP, and a lower proportion of currently drinking (Supplementary Table S5). Therefore, the level of these risk factors for diabetes was lower in young adults, the impact on diabetes was reduced, and the effect of the TyG-BMI was relatively enhanced.

Several strengths are worthy of attention in this investigation. (i)This is the first time Chinese individuals with prediabetes have been employed as a research population to explore the association between TyG-BMI and diabetes risk. (ii) We elucidated the non-linear association between TyG-BMI and diabetes risk and identified the inflection point. This is a great improvement compared to other previous studies. (iii) Multiple imputations were used to account for missing data. This strategy maximizes statistical power while minimizing the bias caused by missing covariate data. (iv) To ensure the robustness of the conclusions, a series of sensitivity analyses were conducted, including converting TyG-BMI into a categorical variable, using a GAM to insert the continuity covariate into the equation as a curve, and reanalyzing the association between TyG-BMI and incident diabetes after excluding alcohol and cigarette consumers as well as participants with a family history of diabetes.

Nonetheless, the following restrictions should be noted: (i) Because all of the participants were Chinese, additional studies are needed to assess the association between this new risk marker TyG-BMI and the risk of progression from prediabetes to diabetes. In the future, we will collaborate with investigators outside of China to validate their association in other populations of different genetic backgrounds. (ii) Diabetes was defined as a fasting plasma glucose (FPG) level of 7.00 mmol/L and/or self-reported diabetes during the follow-up period, but not a measurement of glycosylated hemoglobin or a 2-h oral glucose tolerance test. Consequently, the incidence of diabetes may be understated. (iii) Certain indications related to diabetes and IR were absent from the raw data, including waist-to-hip ratio, waist circumference, and insulin concentration. In addition, the present investigation only evaluated TG, FPG, BMI, and other parameters at baseline;

TyG-BMI variations over time were not included. In the future, we may seek to construct our studies or partner with other researchers to collect as many factors as feasible, including TyG-BMI change information over time. (iv) The type of diabetes cannot be determined. Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes in China, accounting for more than 90% of all diabetes cases (47). Consequently, our results are representative of T2DM. Finally, this retrospective observational study did not demonstrate a causal relationship between the TyG-BMI and the risk of diabetes in patients with prediabetes; rather, it established an association.

Conclusion

This study demonstrates a positive and non-linear relationship between the TyG-BMI and the risk of incident diabetes in Chinese adults with prediabetes. When the TyG-BMI was <231.66, there was a significant positive association with the risk of progression from prediabetes to diabetes. The present study offers more references to promote clinical consultation and to optimize diabetes prevention decisions for patients with prediabetes.

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/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Rich Healthcare Group Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. No identifiable images data statement are presented in this manuscript.

Author contributions

YH, HH, and QL conceived of the study, conducted statistical analysis, and drafted the manuscript. ZD and DL revised the manuscript and designed the study. All authors have reviewed and approved the final version of the text.

Acknowledgments

As a secondary analysis, the majority of the information and methodology were obtained from the following studies: Chen et al. (19). Data from: Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study, Dryad, Dataset, https://doi.org/10.5061/dryad.ft8750v. The authors of the original study deserve our appreciation.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1028461/full#supplementary-material

References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. *Nat Rev Endocrinol.* (2011) 8:228–36. doi: 10.1038/nrendo.2011.183

2. Cho NH, Shaw JE, Karuranga S, Huang Y, Da RFJ, Ohlrogge AW, et al. Idf diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* (2018) 138:271–81. doi: 10.1016/j.diabres.2018.02.023

3. Fradkin JE, Cowie CC, Hanlon MC, Rodgers GP. Celebrating 30 years of research accomplishments of the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes.* (2013) 62:3963–7. doi: 10.2337/db13-1108

4. Preis SR, Hwang SJ, Coady S, Pencina MJ, D'Agostino RS, Savage PJ, et al. Trends in all-cause and cardiovascular disease mortality among women and men with and without diabetes mellitus in the framingham heart study, 1950 to 2005. *Circulation.* (2009) 119:1728–35. doi: 10.1161/CIRCULATIONAHA.108.829176

5. Tandon N, Ali MK, Narayan KM. Pharmacologic prevention of microvascular and macrovascular complications in diabetes mellitus: implications of the results of recent clinical trials in type 2 diabetes. *Am J Cardiovasc Drugs.* (2012) 12:7–22. doi: 10.2165/11594650-000000000-00000

6. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet.* (2018) 392:1923–94. doi: 10.1016/S0140-6736(18)32225-6

7. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet.* (2012) 379:2279–90. doi: 10.1016/S0140-6736(12)60283-9

8. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. Prevalence and ethnic pattern of diabetes and prediabetes in china in 2013. *Jama.* (2017) 317:2515–23. doi: 10.1001/jama.2017.7596

9. McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. *Clin Dermatol.* (2018) 36:14–20. doi: 10.1016/j.clindermatol.2017.09.004

10. Barazzoni R, Gortan CG, Ragni M, Nisoli E. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord.* (2018) 23:149–57. doi: 10.1007/s40519-018-0481-6

11. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (nafld). *Metabolism.* (2016) 65:1038–48. doi: 10.1016/j.metabol.2015.12.012

12. Chen C, Cohrs CM, Stertmann J, Bozsak R, Speier S. Human beta cell mass and function in diabetes: recent advances in knowledge and technologies to understand disease pathogenesis. *Mol Metab.* (2017) 6:943–57. doi: 10.1016/j.molmet.2017.06.019

13. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* (1979) 237:E214–23. doi: 10.1152/ajpendo.1979.237.3.E214

14. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab.* (2010) 95:3347–51. doi: 10.1210/jc.2010-0288

15. Lee EY, Yang HK, Lee J, Kang B, Yang Y, Lee SH, et al. Triglyceride glucose index, a marker of insulin resistance, is associated with coronary artery stenosis in asymptomatic subjects with type 2 diabetes. *Lipids Health Dis.* (2016) 15:155. doi: 10.1186/s12944-016-0324-2

16. Er LK, Wu S, Chou HH, Hsu LA, Teng MS, Sun YC, et al. Triglyceride glucose-body mass index is a simple and clinically useful surrogate marker for insulin resistance in nondiabetic individuals. *PLoS ONE.* (2016) 11:e149731. doi: 10.1371/journal.pone.0149731

17. Ramírez-Vélez R, Pérez-Sousa MÁ, González-Ruíz K, Cano-Gutierrez CA, Schmidt-RioValle J, Correa-Rodríguez M, et al. Obesity- and lipid-related parameters in the identification of older adults with a high risk of prediabetes according to the american diabetes association: an analysis of the 2015 health, well-being, and aging study. *Nutrients.* (2019) 11:2654. doi: 10.3390/nu11112654

18. Wang X, Liu J, Cheng Z, Zhong Y, Chen X, Song W. Triglyceride glucose-body mass index and the risk of diabetes: a general population-based cohort study. *Lipids Health Dis.* (2021) 20:99. doi: 10.1186/s12944-021-01532-7

19. Chen Y, Zhang XP, Yuan J, Cai B, Wang XL, Wu XL, et al. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. *BMJ Open.* (2018) 8:e21768. doi: 10.1136/bmjopen-2018-021768

20. Ramírez-Vélez R, Pérez-Sousa MÁ, González-Ruíz K, Cano-Gutierrez CA, Schmidt-RioValle J, Correa-Rodríguez M, et al. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. *Diabetes Care.* (2021) 44:S15–33. doi: 10.2337/dc21-S002

21. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med.* (2011) 30:377–99. doi: 10.1002/sim.4067

22. Groenwold RH, White IR, Donders AR, Carpenter JR, Altman DG, Moons KG. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *CMAJ.* (2012) 184:1265–9. doi: 10.1503/cmaj.110977

23. Consultation WE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* (2004) 363:157–63. doi: 10.1016/S0140-6736(03)15268-3

24. Pond SL, Frost SD, Muse SV. Hyphy: hypothesis testing using phylogenies. *Bioinformatics.* (2005) 21:676–9. doi: 10.1093/bioinformatics/bti079

25. Guo Z, Liu L, Yu F, Cai Y, Wang J, Gao Y, et al. The causal association between body mass index and type 2 diabetes mellitus-evidence based on regression discontinuity design. *Diabetes Metab Res Rev.* (2021) 37:e3455. doi: 10.1002/dmrr.3455

26. Wagner R, Thorand B, Osterhoff MA, Müller G, Böhm A, Meisinger C, et al. Family history of diabetes is associated with higher risk for prediabetes: a multicentre analysis from the german center for diabetes research. *Diabetologia.* (2013) 56:2176–80. doi: 10.1007/s00125-013-3002-1

27. Knott C, Bell S. Britton A. Alcohol consumption and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of more than 19 million individuals from 38 observational studies. *Diabetes Care.* (2015) 38:1804–12. doi: 10.2337/dc15-0710

28. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies. *Int J Surg.* (2014) 12:1495–9. doi: 10.1016/j.ijsu.2014.07.013

29. Heianza Y, Hara S, Arase Y, Saito K, Fujiwara K, Tsuji H, et al. Hba1c 5·7-6·4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in japan (topics 3): a longitudinal cohort study. *Lancet.* (2011) 378:147–55. doi: 10.1016/S0140-6736(11)60472-8

30. Lipska KJ, Inzucchi SE, Van Ness PH, Gill TM, Kanaya A, Strotmeyer ES, et al. Elevated hba1c and fasting plasma glucose in predicting diabetes incidence among older adults: are two better than one? *Diabetes Care.* (2013) 36:3923–9. doi: 10.2337/dc12-2631

31. Lanfranchi AL, Braicovich PE, Cantatore D, Irigoitia MM, Farber MD, Taglioretti V, et al. Influence of confluent marine currents in an ecotonal region of the south-west Atlantic on the distribution of larval anisakids (nematoda: anisakidae). *Parasit Vectors.* (2018) 11:583. doi: 10.1186/s13071-018-3119-7

32. Wang R, Dai L, Zhong Y, Xie G. Usefulness of the triglyceride glucose-body mass index in evaluating nonalcoholic fatty liver disease: insights from a general population. *Lipids Health Dis.* (2021) 20:77. doi: 10.1186/s12944-021-01506-9

33. Zeng ZY, Liu SX, Xu H, Xu X, Liu XZ, Zhao XX. Association of triglyceride glucose index and its combination of obesity indices with prehypertension in lean individuals: a cross-sectional study of Chinese adults. *J Clin Hypertens (Greenwich).* (2020) 22:1025–32. doi: 10.1111/jch.13878

34. Du Z, Xing L, Lin M, Sun Y. Estimate of prevalent ischemic stroke from triglyceride glucose-body mass index in the general population. *Bmc Cardiovasc Disord.* (2020) 20:483. doi: 10.1186/s12872-020-01768-8

35. Sun Y, Wang Z, Huang Z, Hu H, Han Y. The association between the triglyceride-to-high-density lipoprotein cholesterol ratio and the risk of progression to diabetes from prediabetes: a 5-year cohort study in Chinese adults. *Front Endocrinol.* (2022) 13:947157. doi: 10.3389/fendo.2022.947157

36. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet.* (2005) 365:1333–46. doi: 10.1016/S0140-6736(05)61032-X

37. Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes.* (2010) 59:2697–707. doi: 10.2337/db10-1032

38. O'Malley G, Santoro N, Northrup V, D'Adamo E, Shaw M, Eldrich S, et al. High normal fasting glucose level in obese youth: a marker for insulin resistance and beta cell dysregulation. *Diabetologia.* (2010) 53:1199–209. doi: 10.1007/s00125-010-1693-0

39. Stancáková A, Javorský M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M. Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 finnish men. *Diabetes.* (2009) 58:1212–21. doi: 10.2337/db08-1607

40. Watts GF, O'Brien SF, Silvester W, Millar JA. Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. *Clin Sci.* (1996) 91:567–73. doi: 10.1042/cs0910567

41. Tirosh A, Shai I, Bitzur R, Kochba I, Tekes-Manova D, Israeli E, et al. Changes in triglyceride levels over time and risk of type 2 diabetes in young men. *Diabetes Care.* (2008) 31:2032–7. doi: 10.2337/dc08-0825

42. Niu H, Zhou Y. Nonlinear relationship between ast-to-alt ratio and the incidence of type 2 diabetes mellitus: a follow-up study. *Int J Gen Med.* (2021) 14:8373–82. doi: 10.2147/IJGM.S341790

43. Siegel LC, Sesso HD, Bowman TS, Lee IM, Manson JE, Gaziano JM. Physical activity, body mass index, and diabetes risk in men: a prospective study. *Am J Med.* (2009) 122:1115–21. doi: 10.1016/j.amjmed.2009.02.008

44. Climie RE, van Sloten TT, Bruno RM, Taddei S, Empana JP, Stehouwer C, et al. Macrovasculature and microvasculature at the crossroads between type 2 diabetes mellitus and hypertension. *Hypertension.* (2019) 73:1138–49. doi: 10.1161/HYPERTENSIONAHA.118.11769

45. Wakabayashi I. Light-to-moderate alcohol drinking reduces the impact of obesity on the risk of diabetes mellitus. *J Stud Alcohol Drugs.* (2014) 75:1032–8. doi: 10.15288/jsad.2014.75.1032

46. Jaiswal M, Schinske A, Pop-Busui R. Lipids and lipid management in diabetes. *Best Pract Res Clin Endocrinol Metab.* (2014) 28:325–38. doi: 10.1016/j.beem.2013.12.001

47. Jia W, Weng J, Zhu D, Ji L, Lu J, Zhou Z, et al. Standards of medical care for type 2 diabetes in china 2019. *Diabetes Metab Res Rev.* (2019) 35:e3158. doi: 10.1002/dmrr.3158

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 13 February 2023
DOI 10.3389/fpubh.2023.1072521

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal, South Africa

REVIEWED BY
Sathish Thirunavukkarasu,
Emory University, United States
Dongbo Shi,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Min Li
✉ limin-72114@163.com

SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Public Health

RECEIVED 17 October 2022
ACCEPTED 23 January 2023
PUBLISHED 13 February 2023

CITATION
Zhao J and Li M (2023) Worldwide trends in prediabetes from 1985 to 2022: A bibliometric analysis using bibliometrix R-tool. *Front. Public Health* 11:1072521. doi: 10.3389/fpubh.2023.1072521

Worldwide trends in prediabetes from 1985 to 2022: A bibliometric analysis using bibliometrix R-tool

JingYi Zhao and Min Li*

Guang'anmen Hospital of China Academy of Chinese Medical Sciences, Institute of Metabolic Diseases, Beijing, China

Background: Prediabetes is a widespread condition that represents the state between normal serum glucose and diabetes. Older individuals and individuals with obesity experience a higher rate of prediabetes. Prediabetes is not only a risk factor for type 2 diabetes mellitus (t2dm) but is also closely related to microvascular and macrovascular complications. Despite its importance, a bibliometric analysis of prediabetes is missing. The purpose of this study is to provide a comprehensive and visually appealing overview of prediabetes research.

Methods: First, the Web of Science (WOS) database was searched to collect all articles related to prediabetes that were published from 1985 to 2022. Second, R language was used to analyze the year of publication, author, country/region, institution, keywords, and citations. Finally, network analysis was conducted using the R package bibliometrix to evaluate the hotspots and development trends of prediabetes.

Results: A total of 9,714 research articles published from 1985 to 2022 were retrieved from WOS. The number of articles showed sustained growth. Rathmann W was the most prolific author with 71 articles. *Diabetes Care* was the journal that published the highest number of articles on prediabetes (234 articles), and Harvard University (290 articles) was the most active institution in this field. The United States contributed the most articles (2,962 articles), followed by China (893 articles). The top five clusters of the keyword co-appearance network were "prediabetes", "diabetes mellitus", "glucose", "insulin exercise", and "oxidative stress". The top three clusters of the reference co-citation network were "Knowler. WC 2002", "Tabak AG 2012", and "Matthews DR1985".

Conclusions: The combined use of WOS and the R package bibliometrix enabled a robust bibliometric analysis of prediabetes papers, including evaluation of emerging trends, hotspots, and collaboration. This study also allowed us to validate our methodology, which can be used to better understand the field of prediabetes and promote international collaboration.

KEYWORDS

prediabetes, diabetes, bibliometrics, R language, bibliometrix

1. Introduction

Prediabetes is a major worldwide public health issue. Individuals with prediabetes have a high risk of progression to diabetes and elevated risks of kidney disease, cardiovascular disease, and death (1). The concept of prediabetes emerged in the late 1970s to better understand the process of diabetes (2, 3). However, it is unclear whether prediabetes should be classified as a unique pathogenic state because it is a status that lies between healthy glucose homeostasis and the pathological condition of diabetes (4). Prediabetes is a degree of impairment between euglycemia

and the hyperglycemia of type 2 diabetes (5). Professional societies such as the American Diabetes Association (ADA), the World Health Organization (WHO), and the International Expert Committee (IEC) have issued definitions of prediabetes. These definitions are based on a variety of hyperglycemia-related parameters such as FBG, 2hBG, and HbA1C (6, 7).

Nevertheless, there is still no consistent definition of prediabetes, and different definitions correspond to different groups of individuals in epidemiologic studies (8). For example, large surveys of Chinese adults using all three glycemic tests (HbA1C, FBG, or 2hBG) revealed the prevalence of prediabetes, ranging from 36% in one study to as high as 50.1% in another (9). Previous literature also suggested that, for individuals over 40 years of age or with a higher risk of diabetes, FBG and/or HbA1C were more effective (10). For individuals with prediabetes, pharmacological and lifestyle changes could reduce cardiovascular risk and cost-effectively prevent diabetes (11), and restoring normoglycemia can produce long-lasting remission (10). Hence, the National Institute for Health and Care Excellence (NICE) suggested that individuals with prediabetes should initially undergo lifestyle intervention in the form of intensive group education programs (12). However, the effectiveness of these interventions relies on a consistent and accurate definition of prediabetes.

Insulin resistance, B-cell dysfunction, increased lipolysis, inflammation, poor incretin response, and hepatic glucose overproduction are all pathophysiologic abnormalities that underlie prediabetes (13). Obesity-related metabolic abnormalities increase the risk of macrovascular and microvascular problems by impairing endothelial vasodilators and fibrinolytic activity. Additionally, prediabetes has been linked to an increased risk of cancer and dementia (14, 15).

Bibliometric analysis has evolved into the most effective tool for investigating detailed research trends in a research field over time. It objectively presents research contributions related to particular scientific fields from different countries, institutions, journals, and authors through statistical analysis and forecasts future directions or hotspots (16). It is important to note that hotspots flag emerging problems in a specific field that have not been resolved and are of great concern to global academics, and future research directions forecast research that must be undertaken urgently and that will have a significant impact in the future. Furthermore, bibliometric analysis has played a significant role in the development of policy and clinical guidelines for a variety of diseases. However, to date, no bibliometric analysis of prediabetes has been conducted, and even less attention has been given to the prediction of research hotspots.

In this research, we retrieved prediabetes-related articles from the Web of Science (WOS) database and used bibliometric analysis tools to examine the literature characteristics and research hotspots. The Web of Science (WOS) is the most comprehensive and authoritative citation database in which peer review is a requirement in the journal evaluation process for inclusion. Therefore, we chose WOS in this study. The goal of this study is to provide a comprehensive and visually appealing overview of prediabetes studies and to lay a robust foundation for future research.

2. Methods

The Core Collection of WOS was searched to obtain relevant literature. The search strategy was as follows: TS = Prediabet* AND PY = (1985–2022). The search was performed on 17 August 2022. Only articles and reviews were included in the analysis. Two researchers independently retrieved and downloaded the literature. After data confirmation and standardization, the online literature was exported to plain text format, including full documents and cited references. The data were then imported into R for analysis.

We used the R package bibliometrix to clean, analyze, and visualize the literature data. Bibliometrix was created by Massimo Aria and Corrado Cuccurullo and built in R, a programming language for statistical computing and graphics (17). It contains all the necessary instruments to pursue a complete bibliometric analysis, following the Science Mapping Workflow. It is a powerful tool because it makes bibliometric analysis more sophisticated and replicable.

3. Results

We used the R package bibliometrix to analyze the quantity of prediabetes literature, and publications of different journals, authors, countries, and institutions. We used keyword analysis, themes, and theme evolution to understand the main research areas of prediabetes articles. We also used citation analysis to explore the logical relationships between the literature and a collaboration network to show the collaboration between countries, institutions, and authors in this field.

3.1. Annual literature quantity and growth forecast

A total of 9,714 papers were collected from the WOS (see the workflow in Figure 1). We excluded 161 non-English papers and other 1,917 papers, including early access publications, book chapters, retracted publications, proceedings papers, and editorial materials. Thereafter, 7,636 publications remained for analysis. The first article in the field of prediabetes was written by A R Dian and published in the journal "Diabetologia" (18). As shown in Figure 2, the number of articles each year has exhibited a sustained growth trend since 2005 and reached 876 in 2021. Furthermore, we ran a polynomial regression model to predict how many articles will be published in 2022. The predicted number of articles in 2022 was 906 with a 95% confidence interval of 876 to 935.

The above pattern suggests that prediabetes is an emerging field. As shown in Figure 3, the number of articles by country also demonstrated an increasing growth trend. The United States published the most articles, followed by China, Germany, Canada, and South Korea. However, while studies on prediabetes have increased significantly over the past few decades, it is still a relatively new and promising area of research. China, India, Pakistan, and the United States (US) are the countries with the largest numbers of patients with diabetes aged 20–79 years in 2021. The US and China have the highest interest in the area of prediabetes because of the high prevalence of diabetes and the high economic level in these countries. India and Pakistan ranked only 10th and 44th in the prediabetes field in terms of the number of publications, which may be related to the investment in research and the emphasis on diabetes prevention.

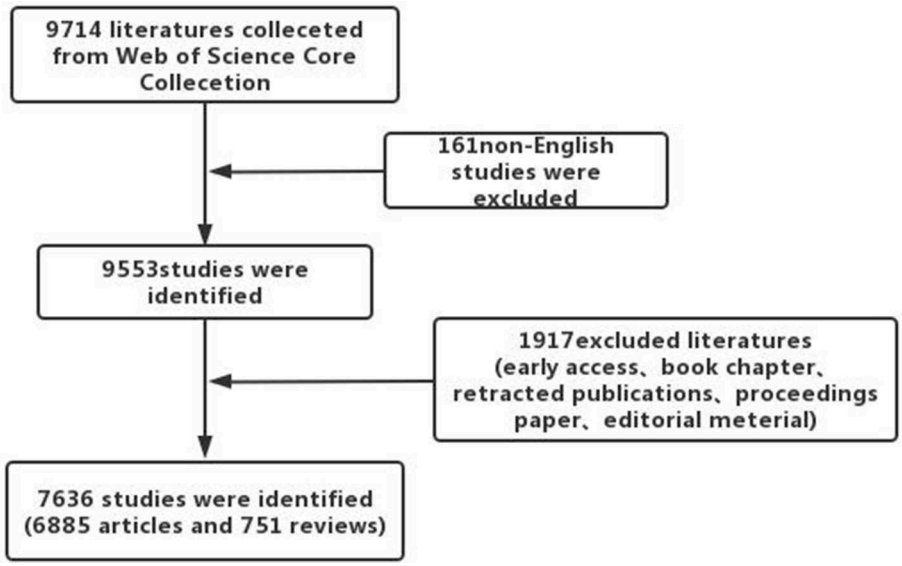


FIGURE 1
Bibliometric analysis of prediabetes presented in the workflow.

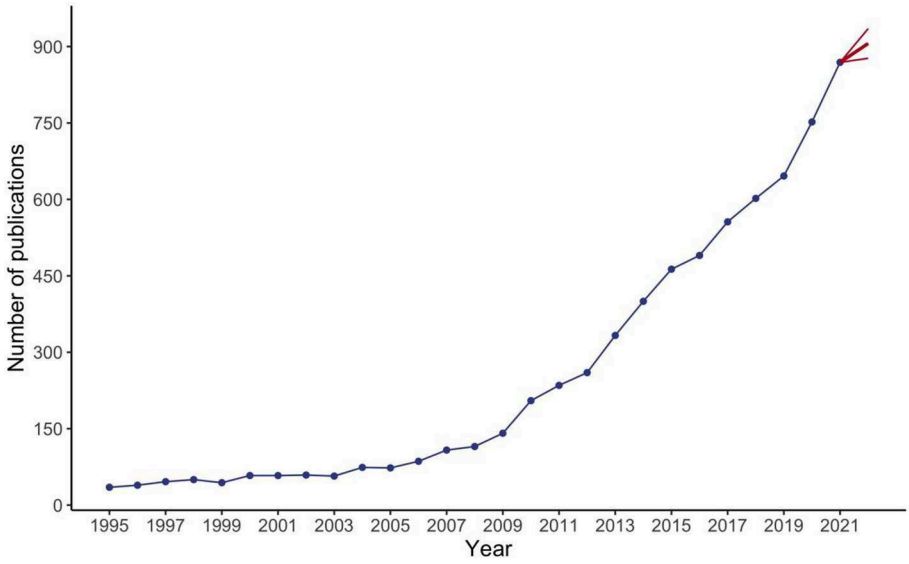


FIGURE 2
Growth trend and prediction of prediabetes.

3.2. Distribution of literature

We then analyzed the distribution of authors, journals, and institutions of the literature. More than 34,914 authors contributed to the 7,636 prediabetes-related studies published in the WOS. Among the 20 most-productive authors, Rathmann W had the most publications (71 articles), followed by Peters A (58 articles). Haring HU and Meisinger C were tied for third place (47 articles each) (Supplementary material 1).

The articles on prediabetes were published in more than 1,549 journals. *Diabetes Care* published 234 articles, which accounted for 3.02% of all articles, followed by "PLoS ONE" (193 articles), "Diabetes Research and Clinical Practice" (187 articles), "Diabetologia" (186 articles), and "Diabetes" (167 articles) (Supplementary material 2). The impact factor (IF) is a widely used indicator measuring the academic impact of a journal and the quality of its publications. Among the top five journals, *Diabetes Care* had the highest IF, reaching 17.152 in 2022; the IF of the other four journals, i.e., *Diabetologia, Diabetes, Diabetes Research, Clinical Practice,* and *PLoS ONE* were 10.12, 9.46, 5.60, and 3.75, respectively. A majority of the prediabetes-related articles published in these journals were of high quality and worth further analysis.

According to the retrieval results of the WOS database, the authors were affiliated with 139 countries/regions. The United States was the country with the highest number of publications (2,962 articles), followed by China (893 articles), Germany (471 articles), England (446 articles), and Canada (398 articles) (Supplementary material 3). Notably, the literature on prediabetes in China has shown rapid growth in the last decade.

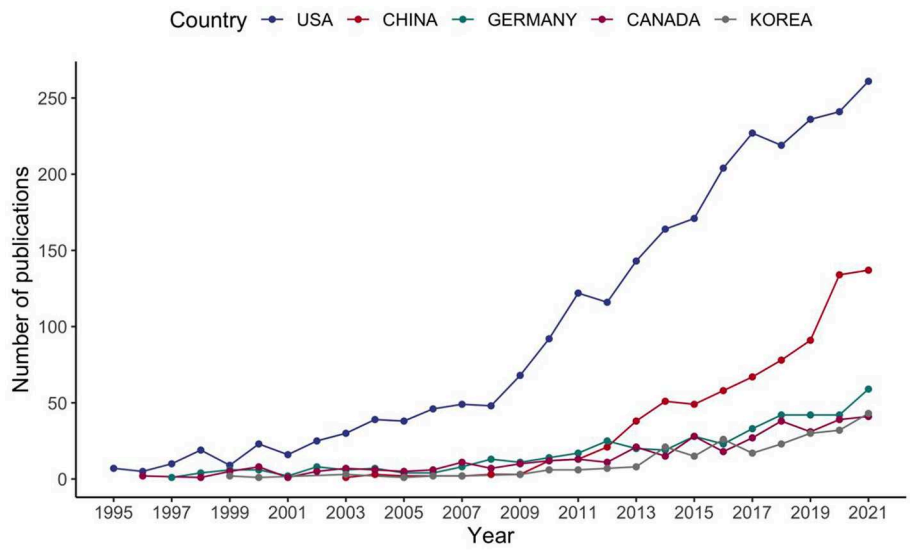


FIGURE 3
Number of publications in different countries and their growth trends.

In terms of affiliations, there were 7,834 institutes involved in the field of prediabetes. Harvard University ranked first with 290 articles on prediabetes, followed by the University of California (269 articles), the U.S. Department of Veterans Affairs (194 articles), Veterans Health Administration (192 articles), and the University of Texas system (191 articles). Eight of the top 10 institutions were located in the US (Supplementary material 4). There is no doubt that the US has maintained its lead in the field of prediabetes. Shanghai Jiao Tong University was the top Chinese institution in terms of the number of articles on prediabetes and ranked 22nd overall.

3.3. Keywords analysis

Keywords are brief phrases used in indexing or classifying to describe the topic of an article accurately and concisely. Through keyword analysis, we can gain a general understanding of the themes and features of publications (19). Co-occurrence analysis assumes that keywords in the same documents are strongly related to the conceptual space of the research area. Clustering the keyword co-occurrence network provides a method to identify the subfields of a research area (20). We used author keywords in the following analysis and built a network with 8,960 nodes and 51,101 links.

Research frontiers can be identified by examining the frequency and centrality of keywords (21). The top 20 most common keywords are shown in Table 1. "Prediabetes" was the most commonly used keyword in the literature, followed by "diabetes", "type 2 diabetes", "diabetes mellitus", "insulin resistance", "obesity", "metabolic syndrome", "HBA1C", "IGT", "IFG", and "Insulin".

The top 100 keywords can be classified into five groups: prediabetes-related diseases, diagnostic criteria, risk factors, intervention modalities, and pathological mechanisms (Supplementary material 5). Diseases frequently addressed in the literature on prediabetes are "obesity", "metabolic syndrome", and "cardiovascular disease". Among them, "type 2 diabetes" appeared more frequently as a keyword than "type 1 diabetes". "Insulin resistance", "inflammation", and "sensitivity to insulin" were popular pathological mechanisms in prediabetes. The discussions of the diagnostic criteria in order of frequency were "HBA1C", "OGTT", and "FPG". Physiological indicators such as "BMI", "blood pressure", and "waist circumference" caused relatively high concern; "metformin", "exercise", and "physical activity" were the most frequently studied interventions in the field of prediabetes.

TABLE 1 Top 20 keywords of prediabetes.

Ranking	Counts	Centrality	Keywords
1	2,284	0.387	Prediabetes
2	1,497	0.247	Diabetes
3	903	0.118	Type 2 diabetes
4	526	0.048	Insulin resistance
5	478	0.043	Obesity
6	256	0.013	Metabolic syndrome
7	246	0.011	HBA1C
8	226	0.012	IGT
9	179	0.007	IFG
10	151	0.015	Insulin
11	150	0.018	Type 1 diabetes
12	143	0.008	Inflammation
13	137	0.005	Cardiovascular disease
14	135	0.007	Prevention
15	134	0.004	Risk factors
16	127	0.005	Hyperglycemia
17	106	0.004	Hypertension
18	106	0.005	Secretion of insulin
19	106	0.007	Metformin
20	104	0.005	Epidemiology

3.4. Cluster analysis of keywords: Cooccurrence

In Figure 4, we demonstrate the co-occurrence network of the top 400 most frequent keywords. They are clustered into five categories: "prediabetes", "type 2 diabetes", "insulin resistance", "exercise", and "insulin".

Keywords in the same cluster were presented by the same color, and they were clustered together because they often appeared together in the same article. The purple cluster contained four major keywords: type 1 diabetes, nod, glucose, and insulin. "Nod" is commonly used for modeling "type 1 diabetes", and loss of "insulin" secretion is a key mechanism for the progression of prediabetes to "type 1 diabetes". The red cluster gathered the most articles. It represented some basic questions about prediabetes such as prevalence, risk factors, and screening. The green cluster was related to the study of prediabetes mechanisms, such as insulin resistance, inflammation, and oxidative stress, and the clustering of prediabetes-related diseases, such as hypertension, metabolic syndrome, and atherosclerosis. The brown cluster contained some common measures of prediabetes, such as IFG, IGT, OGTT, insulin secretion, and insulin sensitivity. The blue cluster contained common types of prediabetes, such as IFG and IGT, and combined the most directly related diseases together, such as type 2 diabetes and cardiovascular disease. The orange cluster mainly reflected lifestyle interventions for prediabetes such as diet, exercise, and weight loss.

When taking the time dimension into the analysis, Figures 5, 6 show that *prediabetes, diabetes,* and *type 2 diabetes* were the top three keywords in almost all periods, demonstrating the dominance of these three keywords. Between 2005 and 2007, *obesity* ranked in the top three one time, and then, it was surpassed by other keywords. Another interesting pattern is that the use of *insulin resistance* as a keyword increased very quickly since 2010 and ranked fourth in 2020, which may suggest that it is an emerging research direction.

3.5. Themes and thematic evolution

The themes included the title, abstract and keywords, and features by conceptualization and normalization. To investigate the dynamic pattern of the research theme over time, we mapped all clusters into a strategic diagram using two metrics: centrality and density; the degree of interaction between clusters is referred to as centrality, and the degree of internal cohesion is referred to as density (22). The strategic diagram has four quadrants (Figure 7) and the themes can be categorized into four groups: (a) motor themes in the upper-right quadrant which are well-developed and relevant for the research field; (b) basic and transversal themes in the lower-right quadrant which are considered relevant for the research field, but not fully developed; (c) emerging or declining themes in the lower left quadrant which are poorly or marginally developed, and (d) highly developed and isolated themes in the upper-left quadrant which are well-developed but not relevant for the research area. The size of a given cluster is dependent on the number of keywords it contains, and the label cluster conforms to the cluster's most frequently used word. The Walktrap algorithm was used to cluster the data in this study (23).

As shown in Figure 7, the total period was split into four sub-periods: 1990–2005, 2006–2013, 2014–2018, and 2019–2022. The reason for keeping the last period so short, at only 4 years, was to gain a better understanding of current trends.

In the first period (1990–2005), the fully developed themes were related to "type 1 diabetes", "prediabetes", "hyperglycemia", and "nitric oxide". At that time, scientists believed that the early prediabetic process may be a suitable target for immunomodulation aimed at delaying or preventing progression to type 1 diabetes. The niche themes included Chinese hamster and glucose tolerance, which were not developed into moto themes in the following period. Diabetes was among the basic themes.

In the second period (2006–2013), "type 1 diabetes" remained a fully developed theme. Taken together, the period from 1990 to 2013 had much research focused on type 1 diabetes. However, after 2014, research on "type 2 diabetes" emerged and finally became the motor theme in the last 4 years (2019-2022). Notably, "obesity" and "diabetes" were the other two developed themes in the second period. "Prediabetes" was still a basic theme, despite its larger density. There were also several new niche themes, such as gene expression, palatability, and tissue Doppler imaging.

From 2014 to 2018, the theme of "type 1 diabetes" decreased while the density and centrality of "type 2 diabetes" increased. "Prediabetes" became a new motor theme, together with "insulin resistance". Moreover, "meta-analysis" emerged as a new theme with moderate centrality and density. After a period of development, scholars reviewed and examined the existing findings of prediabetes.

In the most recent period from 2019 to 2022, "prediabetes" remained a motor theme, and "type 2 diabetes" finally joined the motor quadrant. The research on "insulin" merged into a single cluster in this period. The basic theme quadrant included two new clusters: "metformin" and "hyperglycemia". Moreover, "hyperglycemia" was a motor theme during the first period.

In summary, the most solid theme identified in the thematic evolution was "prediabetes", which is also the most frequent keyword over time. We also found that the research interest shifted from "type 1 diabetes" to "type 2 diabetes". "Obesity" and "insulin" topics were also relatively solid. However, the identified niche themes were basically different for different periods. This may suggest that the research interests changed rapidly over time.

3.6. References analysis

Table 2 presents the top 20 most highly cited references. Eleven of these articles were written in the United States, followed by China (three articles). The epidemiology of prediabetes was the subject of one-fourth of the 20 most frequently cited articles. The most frequently cited article "Prevalence of diabetes among men and women in China" (24), was published by Yang WY in the *New England Journal of Medicine* in 2010.

The literature type was assessed by reading the title and abstract of the top 100 articles. Table 3 shows the literature types of the 100 most frequently cited articles in the last 3 years. Cardio-cerebrovascular complications and gut microbiota-related studies are the two research directions that have been highly cited in the past 3 years, accounting for 20% of the 100 most frequently cited articles. Clinical trials and randomized controlled trials are the most common types of literature in the field

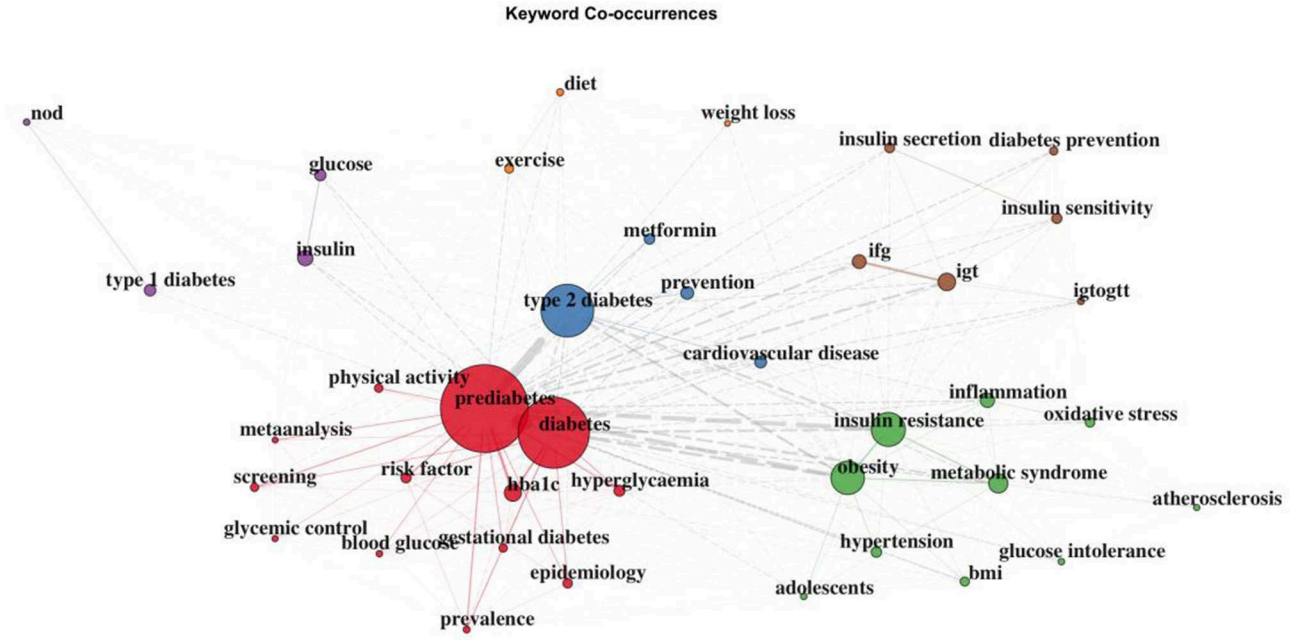


FIGURE 4
Co-occurrence network of the top 400 keywords.

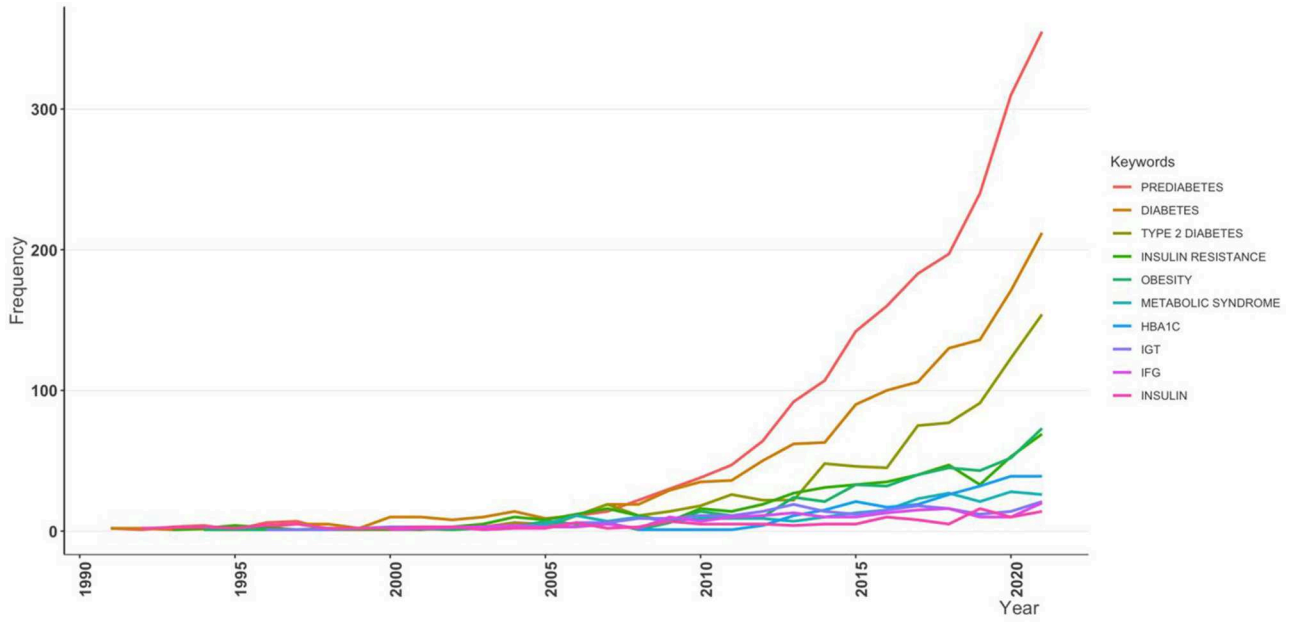


FIGURE 5
Growth trend of the top 10 keywords.

of prediabetes, accounting for 20% of the 100 most frequently cited articles.

3.7. Cluster analysis of references: Co-citations

To better understand the relationship among the references, we clustered them based on the co-citation network using bibliometrix. As shown in Figure 8, three groups were obtained: "Knowler. Wc 2002", "Tabak ag 2012", and "Matthews Dr1985-1". The most highly cited article in the red cluster, "Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin" (25), was published in 2002 in the *New England Journal of Medicine*. The most highly cited article in the green cluster, "Prediabetes: a high-risk state for diabetes development" (26), was published in 2012 in *Lancet*. The most highly cited article in the blue cluster, "Homeostasis model assessment: insulin resistance and beta-cell function from

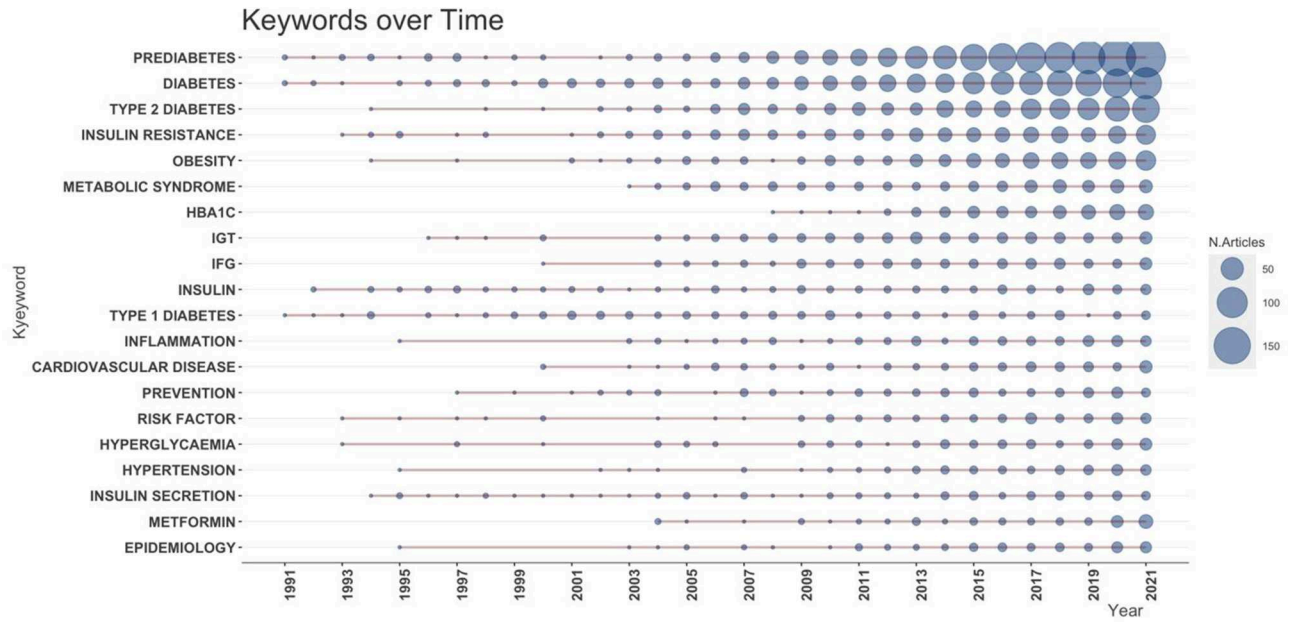


FIGURE 6
Production of the top 20 keywords over time.

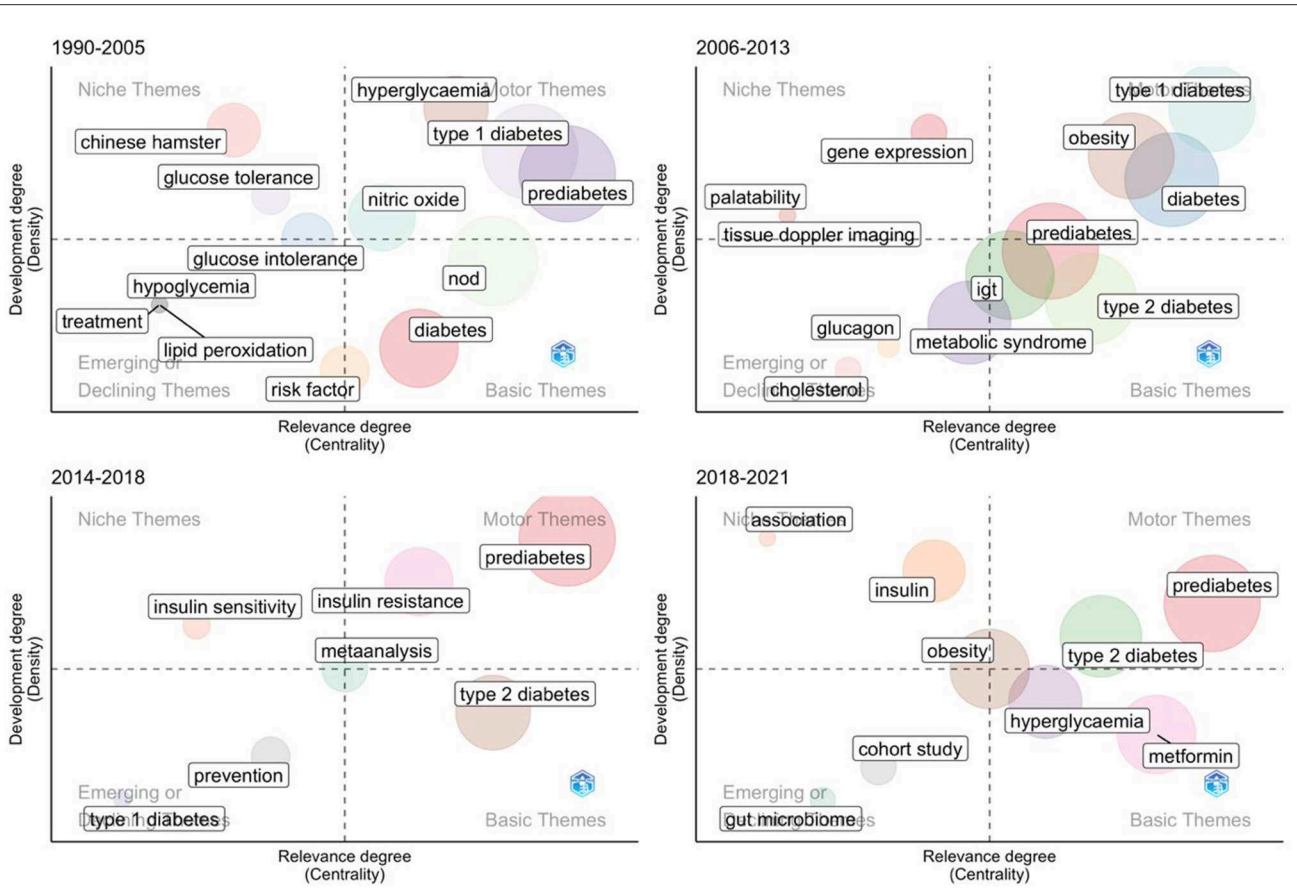


FIGURE 7
Strategic diagram for four periods.

TABLE 2 Top 20 most highly cited articles of prediabetes.

Rank	Citations	Citations/year	Centrality	Year	First author	Journal
1	2,315	178.1	5.01E-03	2010	Yang WY	New England Journal of Medicine
2	2,042	204.2	3.18E-03	2013	Xu Y	JAMA
3	1,653	71.9	8.06E-04	2000	Salomon B	Immunity
4	1,329	120.8	5.10E-02	2012	Tabak AG	Lancet
5	1,288	92	1.14E-04	2009	Scheer FAJL	Proceedings of the National Academy of Sciences of United States of America
6	1,276	116	1.01E-02	2012	Chen L	Nature Reviews Endocrinology
7	1,151	143.9	4.38E-03	2015	Menke A	JAMA
8	1,127	140.9	3.02E-04	2015	Zeevi D	Cell
9	1,097	99.7	3.74E-06	2012	Booth FW	Comprehensive Physiology
10	1,037	207.4	2.72E-04	2018	Saklayen MG	Current Hypertension Reports
11	981	163.5	3.08E-03	2017	Wang LM	JAMA
12	947	30.5	2.01E-03	1992	Martin BC	Lancet
13	944	37.8	3.11E-04	1998	Shimabukuro M	Proceedings of the National Academy of Sciences of United States of America
14	917	34	1.48E-05	1996	Yamagata K	Nature
15	868	31	8.33E-04	1995	Unger RH	Diabetes
16	863	107.9	6.79E-04	2015	Pi-Sunyer X	New England Journal of Medicine
17	856	25.9	2.14E-03	1990	Haffner SM	JAMA
18	854	47.4	5.46E-06	2005	Krentz AJ	Drugs
19	689	28.7	2.86E-05	1999	Perseghin G	Diabetes
20	687	49.1	4.75E-04	2009	Eizirik DL	Nature Reviews Endocrinology

fasting plasma glucose and insulin concentrations in man" (27), was published in 1985 in *Diabetologia*.

Citations featured in the red cluster had the highest number of total citations, and their prediabetes related articles were especially significant in the first period. Most of them were published in high-impact journals such as the *Lancet* and *the New England Journal of Medicine*. The majority of the studies were long-term follow-up studies to investigate the prevalence of diabetes and related diseases and the impact of lifestyle interventions. Citations featured in the blue cluster were also less consistent in terms of their topics. They covered the longest time span (1972–2009). Most of the cited literature focuses on the detection, evaluation, and treatment of blood glucose, blood pressure, and blood lipids. Most were published in *Diabetes Care*. The green cluster cited many important prediabetes guidelines and expert consensus. These studies were relatively new, concentrated in the third period, and were mostly published in *Diabetes Care*.

3.8. Collaboration network

We clustered the countries and authors based on their collaboration network using bibliometrix. The nodes in the collaboration network were authors or countries, and the links represented co-authorship.

The collaboration network between countries can be seen in Figure 9. The US was the country with the most international collaboration in the field, followed by the United Kingdom, Germany, Denmark, and Australia. It is worth noting that, while China was the second most active country in terms of the number of articles, it ranked sixth in international collaboration. In terms of the frequency of collaboration, the top five country pairs were all between the United States, China, the UK, Italy, Canada, and Germany.

The authors' collaboration network (Figure 10) was mapped into four clusters. Each color in this network represents a single cluster or a group of collaborating authors. Figure 10 shows that the collaborators were mostly from the same country or region. Most of the collaborative studies were large clinical trials, cohort studies, or randomized controlled studies of diabetes, prediabetes, and related diseases. These studies require collaboration between research institutions. Authors clustered in the red and orange groups were from Germany. However, the authors of the orange cluster were all from the University of Tübingen. The authors clustered in the blue and purple clusters were from China; the authors of the blue cluster were all from Shanghai Jiao Tong University.

There were four clusters in the institution collaboration network (Figure 11). In the purple cluster, all but Imperial College London were Finnish universities. In the green cluster, all institutions were Chinese universities and hospitals, except for Tulane University and Johns Hopkins University. The US and Canadian universities comprised the red cluster. Mahidol University in Thailand also belonged to the red cluster. Finally, two institutions from Spain formed the blue cluster.

4. Discussion

The general term "prediabetes" refers to the stage between normal glucose tolerance and T2DM. It is generally recognized that individuals with prediabetes are at a high risk of developing T2DM (28). The bibliometric analysis is a useful tool for mining information about a research field. Through a bibliometric analysis, researchers can quickly capture the characteristics and hot spots of the literature in a specific field (19). Therefore, a comprehensive understanding of prediabetes could be obtained by using the bibliometric analysis method, which contributes to subsequent research and clinical treatment.

TABLE 3 The research types of the 100 most highly cited articles (2020–2022).

Type of research	Number	Percentage
Cardio-cerebrovascular complications	13	13%
Clinical Trial	12	12%
Randomized controlled trial	11	11%
Epidemiology	10	10%
Review	10	10%
Experimental Research	8	8%
Gut Microbiota-related studies	7	7%
Meta-analysis	6	6%
Pathophysiology	5	5%
Diagnostic techniques	4	4%
Cohort study	4	4%
Medical care	4	4%
Guidelines	3	3%
Lifestyle intervention	2	2%

However, there are differing viewpoints regarding the necessity of and criteria for the diagnosis and intervention of prediabetes. Institutions such as the WHO, the National Institute for Health and Care Excellence (NICE), the European Association of the Study of Diabetes (EASD), and the International Diabetes Federation (IDF) do not use or emphasize the term prediabetes, and they normally advise treatment only when blood sugar levels approach those of diabetes. The ADA and the Centers for Disease Control and Prevention (CDC) fund much of the nation's research and programs on diabetes prevention. The ADA criteria and the WHO criteria are currently the two most commonly used criteria for prediabetes. Different from the WHO standard, in the ADA standard, the lower FPG cutoff point value of IFG was reduced to 5.6 mmol/L and included glycosylated hemoglobin (HbA1C) from 5.7 to 6.4% as one of the diagnostic criteria for prediabetes. The lower cutoff value defined by the ADA guidelines led to much higher prevalence rates compared with those defined by the WHO guidelines. In a cohort of 1,547 American adults without diabetes, changing the lower IFG threshold from 110 to 100 mg/dL resulted in an increase in prediabetes prevalence from 19.8 to 34.6% (29).

Currently, the frequency and rate of prediabetes progression to diabetes are unclear. Whether prediabetes itself causes harm is not clear, particularly when a person's average glucose levels are at the low

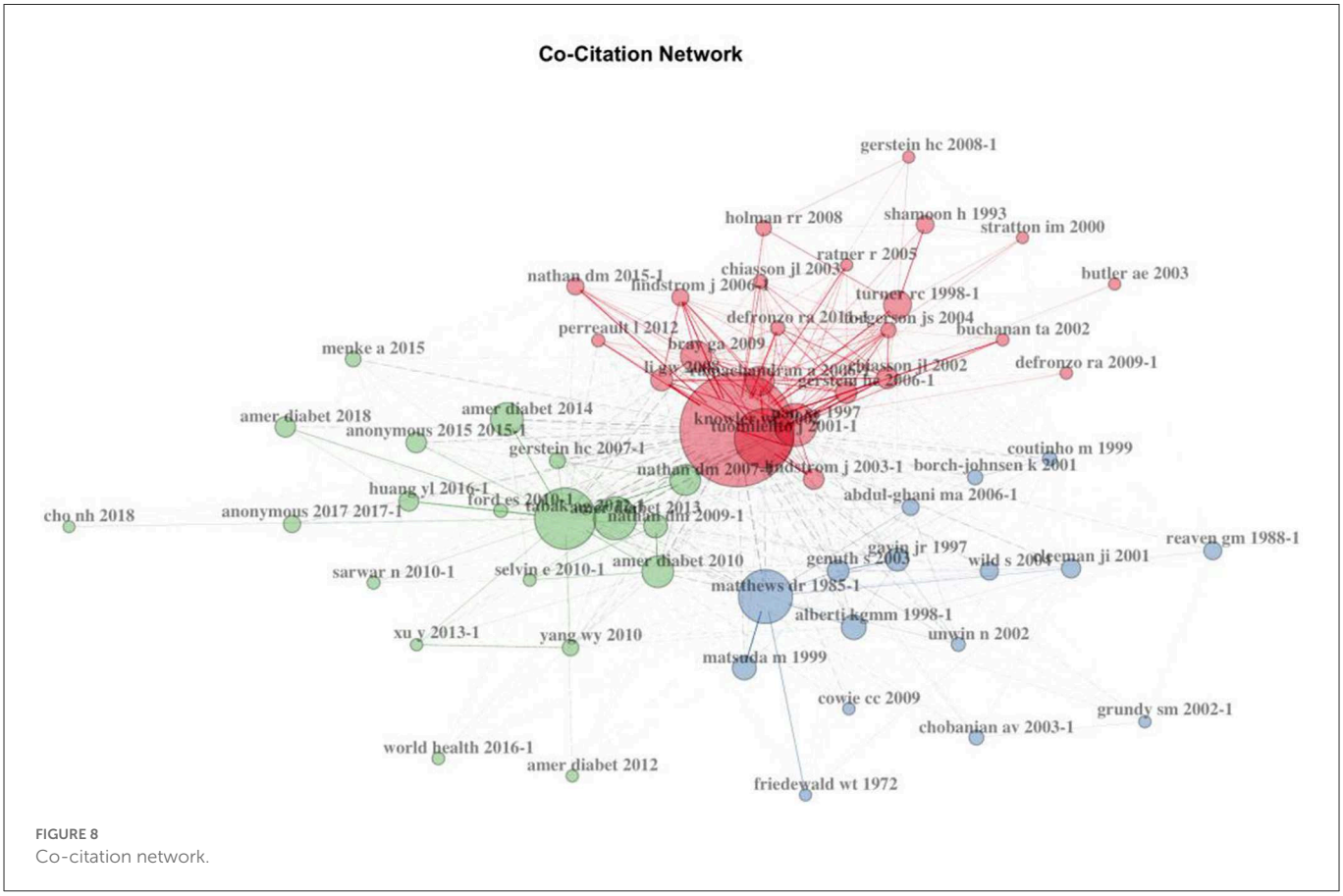


FIGURE 8
Co-citation network.

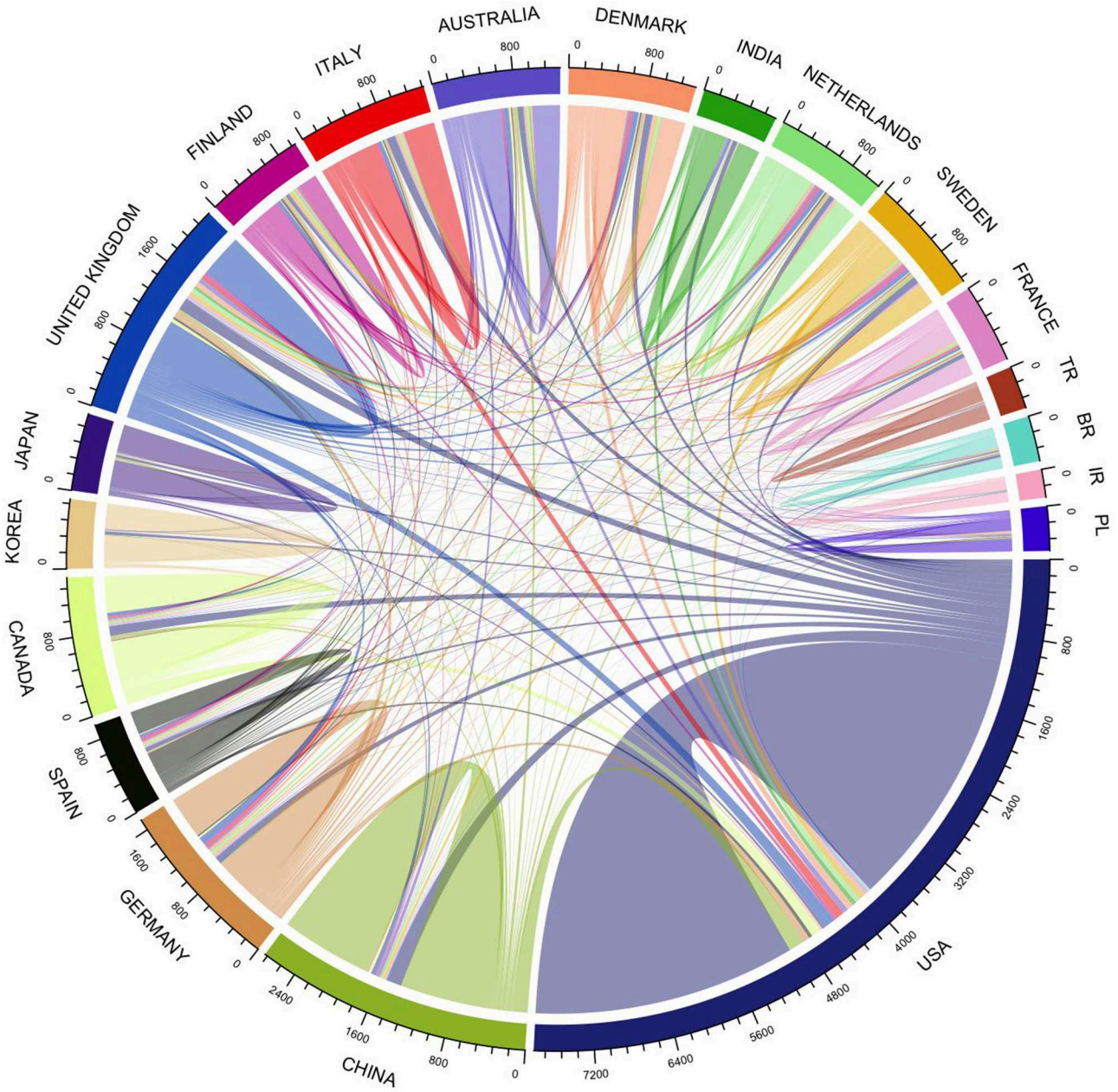


FIGURE 9
Country collaboration network.

end of the test result spectrum. The CDC data show a progression from prediabetes to diabetes at a rate of <2% per year or <10% in 5 years. The Cochrane Library in London showed that up to 59% of prediabetes patients returned to normal glycemic values over 1–11 years with no treatment whatsoever. Therefore, the diagnosis and treatment of prediabetes is not only a medical problem but also a social and economic problem. More research is still needed to determine a suitable definition and other tipping points in identifying the risk of progression to diabetes and other complications[1]

This study examined the progression of prediabetes-related research during the last 37 years. Since 2005, the number of articles on prediabetes has been increasing steadily. With the improvement of living standards and unhealthy behaviors such as physical inactivity, the incidence of prediabetes has increased significantly (30). The booming literature on prediabetes reflects the growing awareness of the importance of detecting and treating prediabetes. Over 98% of the articles were written in English. The majority of the articles were published by corresponding authors from the United States, China, Germany, Canada, and South Korea. These countries face a high incidence of diabetes and emphasize disease prevention (31). The majority of prediabetes relationships are similarly based in the United States, which is consistent with the country's substantial contribution to this academic subject, indicating that collaborations with other countries/territories should be strengthened. As a country with the second-highest number of prediabetes articles, China only

1 https://www.science.org/content/article/war-prediabetes-could-be-boon-pharma-it-good-medicine

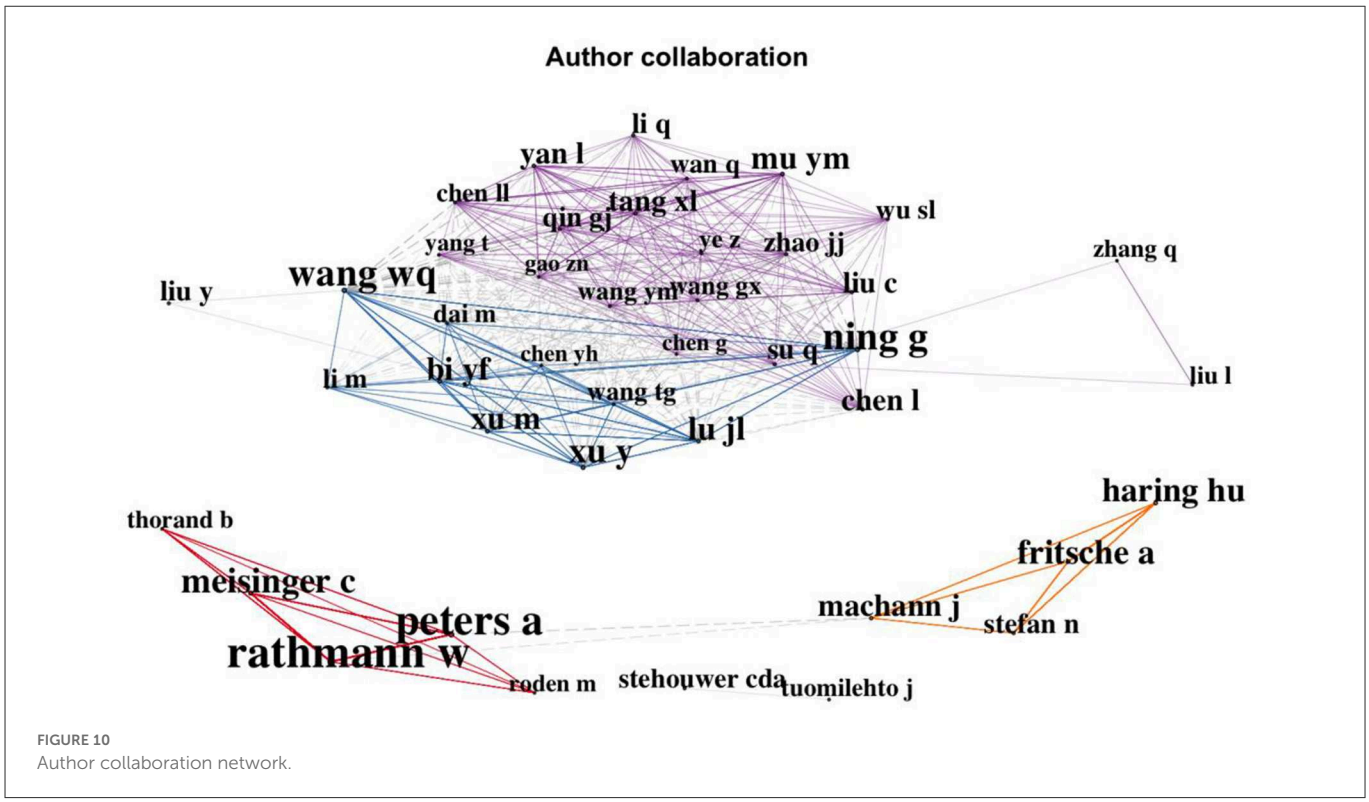


FIGURE 10
Author collaboration network.

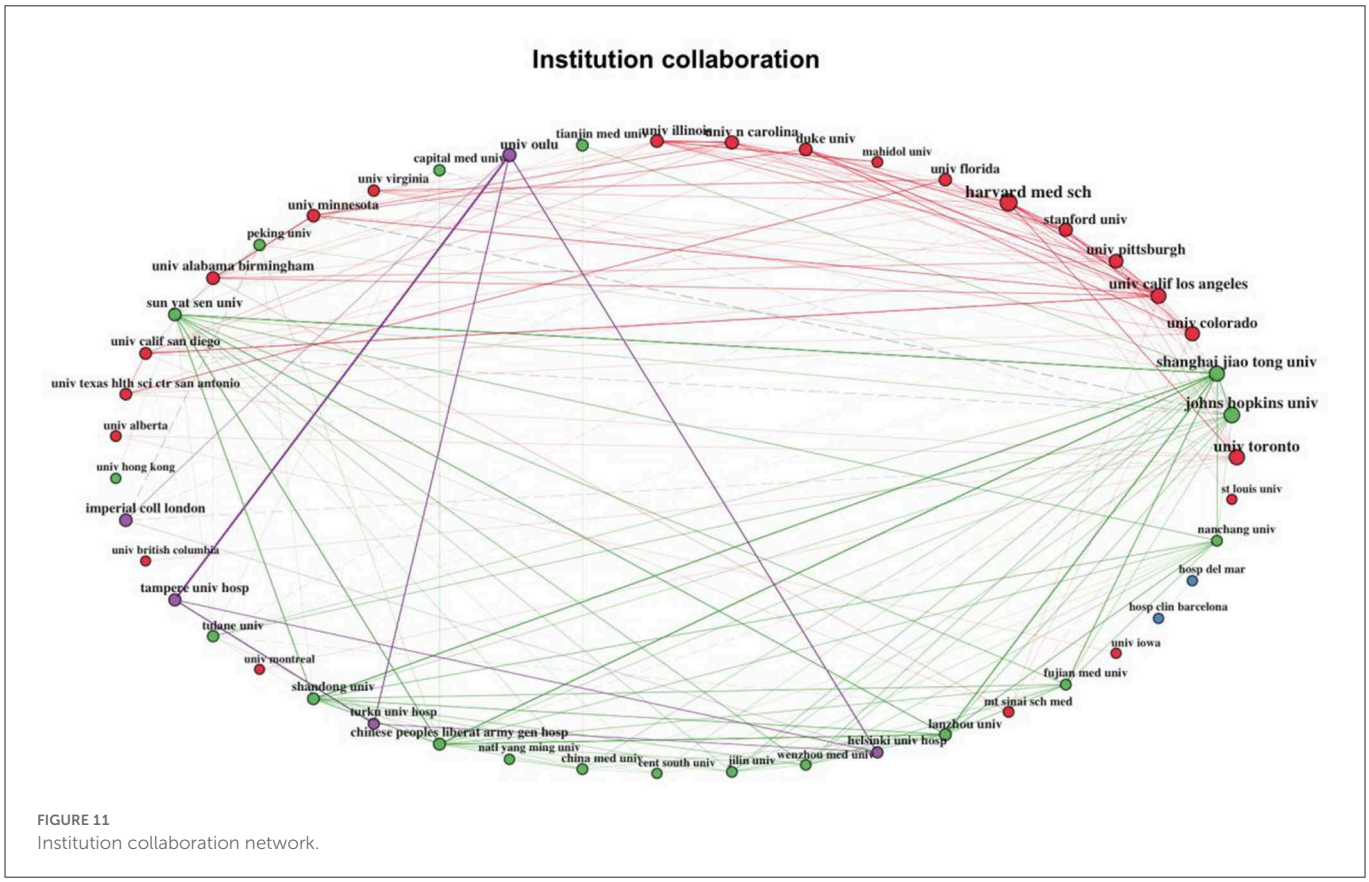


FIGURE 11
Institution collaboration network.

ranks sixth in international collaboration. Therefore, as the country with the highest incidence of diabetes, China should strengthen international collaboration in the future to improve the ability to diagnose, prevent, and treat prediabetes.

In terms of authorship, the 20 most prolific authors have written 786 articles, accounting for 10.3% of all papers. They have made significant contributions to the development and progression of prediabetes research. Rathmann W was the most prolific author

(71 articles) followed by Peters A (58 articles). The journal *Diabetes Care* published most of the literature relevant to prediabetes among the top 20 medical journals. It also had the highest IF, which reached 17.152 in 2021, demonstrating its superiority in quantity and quality. Furthermore, eight of the top 20 journals were American journals, reflecting the US's considerable interest and leadership in this field. Eight of the top 10 institutions were from the US, and Harvard University ranked first. The collaborators tended to come from the same country or region. China and Germany were the two countries with the highest concentration of collaboration networks, especially Shanghai Jiao Tong University in China and the University of Tubingen in Germany.

According to the 2021 worldwide diabetes atlas issued by the International Diabetes Federation (IDF), China, India, and Pakistan had the highest number of people with diabetes among the 20- to 79-year-old population in 2021(31). The highest diabetes-related health expenditure was observed in the United States (USD $379.5 billion), followed by China and Brazil (USD $165.3 billion and USD $42.9 billion, respectively). Both China and the United States attach great importance to diabetes prevention. The idea of "preventive treatment of disease" has existed in China since ancient times and a series of guidelines, such as the "Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition)"(32), which has been published. The Diabetes Prevention Program (MDPP) had already been launched in 25 centers in the United States (33). As shown in this study, the United States and China have published the most articles in the prediabetes field. Low- and middle-income countries have higher numbers of people with diabetes and higher growth rates of diabetes prevalence. However, we found that their attention to prediabetes is low. In terms of economic development, although large-scale screening and education for prediabetes also require high financial investment, it may reduce the incidence of diabetes and the economic burden of diabetes and diabetes complications in the long run. India and Pakistan rank third and fourth in the number of patients with diabetes, respectively, but rank 10th and 44th in the area of prediabetes publications. India's research and development (R&D) intensity was only 0.66% in 2018 (34), much lower than that of 2.14% in China[2]. The disparity is a result of less research investment, lower diabetes-related health expenditures, and insufficient attention to diabetes prevention (35). On the one hand, the national annual cost associated with the diagnosis of diabetes is USD $327.2 billion and that for prediabetes is $43.4 billion. The economic burden of diagnosed diabetes may be reduced by intervening in prediabetes (36). On the other hand, screening and education for prediabetes may also pose a financial burden. The ADA, CDC, and other organizations have already spent billions on research, education, and health improvement programs. To date, no studies have been undertaken to calculate whether the investment in diagnosing and treating prediabetes can reduce the cost of diabetes treatment due to failure to intervene early.

A detailed reading of the literature in the field of prediabetes over the past 3 years revealed that 13% of the articles were related to cardiovascular risk (37). "Insulin resistance", "inflammation", and "sensitivity to insulin" are common mechanisms in the field of prediabetes (38–40). Research related to gut microbes is an emerging hot topic in the field of prediabetes over the past 3 years (41). Epidemiological studies accounted for 10% of prediabetes studies. Much attention has been given to the prevalence of prediabetes. However, there is no consensus on the definition of prediabetes. The complexity of defining prediabetes makes it challenging to obtain profiles of relative prediabetes prevalence from the literature (42). At least five different definitions have been endorsed by different clinical organizations and guidelines. Comparisons of incidence rates between countries will be meaningful only if diagnostic criteria are standardized.

The classification of prediabetes is mainly based on plasma glucose, which is divided into impaired fasting glucose (IFG) with elevated FPG and normal OGTT and into impaired glucose tolerance (IGT) with elevated OGTT and normal FPG. In addition, there are classification of IFG + IGT as well as classification with elevated glycated hemoglobin (HbA1C) (43). In the ranking of keywords in articles related to prediabetes, IGT appeared as a keyword in 205 prediabetes articles and ranked 9th. IFG appeared as a keyword in 165 articles and ranked 11th. HBA1c appeared as a keyword in 135 articles and ranked 14th. It can be seen that the type of prediabetes has received much attention in the field of prediabetes. We are not sure about the effectiveness of different types of prediabetes for the assessment and prevention of diabetes conversion. Further research is needed to explore blood glucose (FPG, OGTT) and HbA1C in identifying the risk of progression to diabetes and whether there are other tipping points. Further research is needed to determine which of the current definitions of prediabetes has the highest ability to discriminate between individuals who transition to diabetes and those that do not and to see how their performance varies with age, sex, and geographic location.

Clinical trials and randomized controlled trials accounted for 23% of the prediabetes literature in the last 3 years. This shows the strong need to develop an appropriate prediabetes intervention. To date, no drugs have been approved specifically for prediabetes, meaning that doctors are limited to prescribing diabetes drugs or other medications "off label" to treat the condition. Metformin is the most commonly used drug (44). However, metformin is not always prescribed for prediabetes, even if a patient meets the prediabetes criteria. Only people who are at a higher risk for developing type 2 diabetes or who have more risk factors may benefit from metformin therapy. Risk factors include having a higher body mass index (BMI) and prior gestational diabetes (45). Exercise, physical activity, and diet are common lifestyle interventions (46–48). With early detection and simple lifestyle changes (such as diet and exercise), prediabetes is often reversible (49–51). However, 38% of the lifestyle treatment group failed to maintain the strict regimen after only 6 months. More studies are needed to determine the best method and timing for intervention in prediabetes.

This study explores research trends and hotspots of prediabetes, which is useful to many researchers. On the one hand, researchers can use the research trend to prevent certain obsolete research on specific themes, reduce the repetitive effort in research initiatives, and reduce project funding waste. On the other hand, depending on research hotspots, researchers can optimize and improve their study design, making prediabetes research more novel and realistic. This study also presents a timeline of the changes in prediabetes research. It lays the groundwork for precise prediabetes prevention and treatment and provides a necessary reference value for the formulation of prediabetes guidelines and the adjustment of medical

2 https://www.indexmundi.com/facts/china/research-and-development-expenditure

insurance policies. Ultimately, more individuals will benefit from lessening the medical load as well as the economic costs associated with prediabetes prevention and treatment around the world.

However, the limitations of this study must be mentioned. First, this study only examined publications in English, which might have led to bias in the study outcomes. Second, we only retrieved data from the WOS database and did not search additional databases or preprint articles for information, resulting in inadequate literature collection. Finally, while bibliometric analysis is a strong tool for revealing precise study trends, it provides little information about research content, such as methods or results. More review studies are needed to go deeper into the research content to enhance prediabetes research.

5. Conclusion

The current study examined the research hotspots, frontiers, and development patterns in the field of prediabetes, with a focus on global research outcomes. The number of articles on prediabetes has increased over the last few decades, indicating that this new topic is gaining traction. Our findings provide an overview of the current status of diabetes research and have significant implications for future research directions.

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

JZ wrote the first draft of the editorial. ML reviewed and provided feedback on the manuscript. Both authors contributed to the article and approved the submitted version.

Funding

This work was supported by Scientific and technological innovation project of China Academy of Chinese Medical Sciences (CI2021A01606); Beijing Natural Science Foundation and Beijing-Tianjin-Hebei Project (J200019); The National Natural Science Foundation of China (81973837).

Acknowledgments

The authors would like to thank the group for their deliberation and assistance.

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/fpubh.2023.1072521/full#supplementary-material

References

1. Echouffo-Tcheugui JB, Selvin E. Prediabetes and what it means: the epidemiological evidence. *Annu Rev Public Health.* (2021) 42:59–77. doi: 10.1146/annurev-publhealth-090419-102644

2. Fajans SSI. Identification of chemical diabetes. The definition of chemical diabetes. *Metabolism.* (1973) 22:211–7. doi: 10.1016/0026-0495(73)90163-7

3. Forouhi NG, Luan J, Hennings S. Incidence of Type 2 diabetes in England and its association with baseline impaired fasting glucose: the Ely study 1990-2000. *Diabet Med.* (2007) 24:200–7. doi: 10.1111/j.1464-5491.2007.02068.x

4. Rett K, Gottwald-Hostalek U. Understanding prediabetes: definition, prevalence, burden and treatment options for an emerging disease. *Curr Med Res Opin.* (2019) 35:1529–34. doi: 10.1080/03007995.2019.1601455

5. Braga T, Kraemer-Aguiar LG, Docherty NG. Treating prediabetes: why and how should we do it? *Minerva Med.* (2019) 110:52–61. doi: 10.23736/S0026-4806.18.05897-4

6. American Diabetes Association. Standards of medical care in diabetes—2010. *Diabetes Care.* (2010) 33:S11–61. doi: 10.2337/dc10-S011

7. Gillett MJ. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: diabetes care (2009) 32:1327–34. *Clin Biochem Rev.* (2009) 30:197–200. doi: 10.2337/dc09-9033

8. Barbu E, Popescu MR, Popescu AC, Balanescu SM. Phenotyping the prediabetic population-a closer look at intermediate glucose status and cardiovascular disease. *Int J Mol Sci.* (2021) 22:6864. doi: 10.3390/ijms22136864

9. Wang L, Gao P, Zhang M. Prevalence and ethnic pattern of diabetes and prediabetes in China in (2013). *JAMA.* (2017) 317:2515–23. doi: 10.1001/jama.2017.7596

10. Khetan AK, Rajagopalan S. Prediabetes. *Can J Cardiol.* (2018) 34:615–23. doi: 10.1016/j.cjca.2017.12.030

11. Fritsche A, Wagner R, Heni M. Different effects of lifestyle intervention in high- and low-risk prediabetes: results of the randomized controlled prediabetes lifestyle intervention study (PLIS). *Diabetes.* (2021) 70:2785–95. doi: 10.2337/db21-0526

12. Mahat RK, Singh N, Arora M, Rathore V. Health risks and interventions in prediabetes: a review. *Diabetes Metab Syndr.* (2019) 13:2803–11. doi: 10.1016/j.dsx.2019.07.041

13. Lawal Y, Bello F, Kaoje YS. Prediabetes deserves more attention: a review. *Clin Diabetes.* (2020) 38:328–38. doi: 10.2337/cd19-0101

14. Cefalu WT. "Prediabetes": are there problems with this label? No, we need heightened awareness of this condition! *Diabetes Care.* (2016) 39:1472–7. doi: 10.2337/dc16-1143

15. Brannick B, Wynn A, Dagogo-Jack S. Prediabetes as a toxic environment for the initiation of microvascular and macrovascular complications. *Exp Biol Med (Maywood).* (2016) 241:1323–31. doi: 10.1177/1535370216654227

16. Rodríguez-Soler R, Uribe-Toril J, Valenciano J D P. Worldwide trends in the scientific production on rural depopulation, a bibliometric analysis using bibliometrix R-tool. *Land Use Policy.* (2020) 97:104787. doi: 10.1016/j.landusepol.2020.104787

17. Aria M, Cuccurullo C. bibliometrix: an R-tool for comprehensive science mapping analysis. *J Informetr.* (2017) 11:959–75. doi: 10.1016/j.joi.2017.08.007

18. Diani AR, Peterson T, Sawada GA. Elevated levels of vasoactive intestinal peptide in the eye and urinary bladder of diabetic and prediabetic Chinese hamsters. *Diabetologia.* (1985) 28:302–7. doi: 10.1007/BF00271690

19. Di Cosmo A, Pinelli C, Scandurra A. Research trends in octopus biological studies. *Animals (Basel).* (2021) 11:1808. doi: 10.3390/ani11061808

20. Zhou X, Zhou M, Huang D, Cui L. A probabilistic model for co-occurrence analysis in bibliometrics. *J Biomed Inform.* (2022) 128:104047. doi: 10.1016/j.jbi.2022.104047

21. Cobo MJ, López-Herrera AG, Herrera-Viedma E, Herrera F. An approach for detecting, quantifying, and visualizing the evolution of a research field: a practical application to the Fuzzy Sets Theory field. *J Informetr.* (2011) 5:146–66. doi: 10.1016/j.joi.2010.10.002

22. Callon M, Courtial J P, Laville F. Co-word analysis as a tool for describing the network of interactions between basic and technological research: the case of polymer chemsitry. *Scientometrics*. (1991) 22:155–205. doi: 10.1007/BF02019280

23. Pons P, Latapy M. Computing communities in large networks using random walks. In: *International Symposium on Computer and Information Sciences*. Berlin, Heidelberg: Springer. (2005) p. 284–293. doi: 10.1007/11569596_31

24. Yang W, Lu J, Weng J. China national diabetes and metabolic disorders study group. Prevalence of diabetes among men and women in China. *N Engl J Med.* (2010) 362:1090–101. doi: 10.1056/NEJMc1004671

25. Knowler WC, Barrett-Connor E, Fowler SE. Diabetes prevention program research group. reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* (2002) 346:393–403. doi: 10.1056/NEJMoa012512

26. Tabák AG, Herder C, Rathmann. Prediabetes: a high-risk state for diabetes development. *Lancet.* (2012) 379:2279–90. doi: 10.1016/S0140-6736(12)60283-9

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

28. Beulens J, Rutters F, Rydén L. Risk and management of pre-diabetes. *Eur J Prev Cardiol.* (2019) 26:47–54. doi: 10.1177/2047487319880041

29. Karve A, Hayward RA. Prevalence, diagnosis, and treatment of impaired fasting glucose and impaired glucose tolerance in nondiabetic US adults. *Diabetes Care.* (2010) 33:2355–9. doi: 10.2337/dc09-1957

30. Weisman A, Fazli GS, Johns A. Evolving trends in the epidemiology, risk factors, and prevention of type 2 diabetes: a review. *Can J Cardiol.* (2018) 34:552–64. doi: 10.1016/j.cjca.2018.03.002

31. IDF. *Diabetes Atlas. 10th ed.* Brussels: International Diabetes Federation. (2021).

32. Chinese Elderly Type 2 Diabetes Prevention and Treatment of Clinical Guidelines Writing Group, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Society, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Health Care Society, Geriatric Professional Committee of Beijing Medical Award Foundation, National Clinical Medical Research Center for Geriatric Diseases (PLA General Hospital). Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition). *Zhonghua Nei Ke Za Zhi.* (2022) 61:12–50. doi: 10.3760/cma.j.cn112138-20211027-00751

33. O'Connell JM, Manson SM. Understanding the economic costs of diabetes and prediabetes and what we may learn about reducing the health and economic burden of these conditions. *Diabetes Care.* (2019) 42:1609–11. doi: 10.2337/dci19-0017

34. Li Y, Teng D, Shi X, Qin G. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. *BMJ.* (2020) 369:m997. doi: 10.1136/bmj.m997

35. Burt T, Sharma P, Dhillon S, Manchanda M, Mittal S, Trehan N. Clinical research environment in india: challenges and proposed solutions. *J Clin Res Bioeth.* (2014) 5:1–8. doi: 10.4172/2155-9627.1000201

36. Joan M, O'Connell, Spero M. Manson; understanding the economic costs of diabetes and prediabetes and what we may learn about reducing the health and economic burden of these conditions diabetes care 1 September. *Diabetes Care.* (2019) (9):1609–11.

37. Cai X, Zhang Y, Li M. Association between prediabetes and risk of all-cause mortality and cardiovascular disease: updated meta-analysis. *BMJ.* (2020) 370:m2297. doi: 10.1136/bmj.m2297

38. Wen J, Cai X, Zhang J. Relation of adipose tissue insulin resistance to prediabetes. *Endocrine.* (2020) 68:93–102. doi: 10.1007/s12020-020-02186-8

39. Luc K, Schramm-Luc A, Guzik TJ. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol.* (2019) 70:6. doi: 10.26402/jpp.2019.6.01

40. Ramírez-Rodríguez AM, González-Ortiz M, Martínez-Abundis E. Effect of dapagliflozin on insulin secretion and insulin sensitivity in patients with prediabetes. *Exp Clin Endocrinol Diabetes.* (2020) 128:506–11. doi: 10.1055/a-0664-7583

41. Allin KH, Tremaroli V, Caesar R, Hansen T, Bäckhed F, Pedersen O. IMI-DIRECT consortium aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia.* (2018) 61:810–20. doi: 10.1007/s00125-018-4550-1

42. Hostalek U. Global epidemiology of prediabetes–present and future perspectives. *Clin Diabetes Endocrinol.* (2019) 5:5. doi: 10.1186/s40842-019-0080-0

43. Davidson MB. Historical review of the diagnosis of prediabetes/intermediate hyperglycemia: case for the international criteria. *Diabetes Res Clin Pract.* (2022) 185:109219. doi: 10.1016/j.diabres.2022.109219

44. Zhuo C, Xu Y, Wang H. Clozapine induces metformin-resistant prediabetes/diabetes that is associated with poor clinical efficacy in patients with early treatment-resistant schizophrenia. *J Affect Disord.* (2021) 295:163–72. doi: 10.1016/j.jad.2021.08.023

45. Davidson MB. Metformin should not be used to treat prediabetes. *Diabetes Care.* (2020) 43:1983–7. doi: 10.2337/dc19-2221

46. Wang S, Li J, Zhang C. Effects of aerobic exercise on the expressions and activities of nitric oxide synthases in the blood vessel endothelium in prediabetes mellitus. *Exp Ther Med.* (2019) 17:4205–12. doi: 10.3892/etm.2019.7437

47. Wang J, Wu Y, Ning F. The association between leisure-time physical activity and risk of undetected prediabetes. *J Diabetes Res.* (2017) 2017:4845108. doi: 10.1155/2017/4845108

48. Costello E, Goodrich J, Patterson WB. Diet quality is associated with glucose regulation in a cohort of young adults. *Nutrients.* (2022) 14:3734. doi: 10.3390/nu14183734

49. Davidson KW, Barry MJ, Mangione CM. Screening for prediabetes and type 2 diabetes: US preventive services task force recommendation statement. *JAMA.* (2021) 326:736–43. doi: 10.1001/jama.2021.12531

50. Basit A, Fawwad A, Abdul Basit K. Glycated hemoglobin (HbA1c) as diagnostic criteria for diabetes: the optimal cut-off points values for the Pakistani population; a study from second National Diabetes Survey of Pakistan (NDSP) 2016-2017. *BMJ Open Diabetes Res Care.* (2020) 8:e001058. doi: 10.1136/bmjdrc-2019-001058

51. Zhang P, Zhu X, Du Y. Screening and functional studies of long noncoding RNA in subjects with prediabetes. *Endocrine.* (2020) 68:296–305. doi: 10.1007/s12020-020-02226-3

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 06 April 2023
DOI 10.3389/fpubh.2023.1086771

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal,
South Africa

REVIEWED BY
Giovanni Messina,
University of Foggia,
Italy
Ntethelelo Sibiya,
Rhodes University,
South Africa

*CORRESPONDENCE
Abdelilah Arredouani
✉ aarredouani@hbku.edu.qa

SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Public Health

RECEIVED 01 November 2022
ACCEPTED 17 March 2023
PUBLISHED 06 April 2023

CITATION
Al Akl NS, Haoudi EN, Bensmail H and Arredouani A (2023) The triglyceride glucose-waist-to-height ratio outperforms obesity and other triglyceride-related parameters in detecting prediabetes in normal-weight Qatari adults: A cross-sectional study. *Front. Public Health* 11:1086771. doi: 10.3389/fpubh.2023.1086771

The triglyceride glucose-waist-to-height ratio outperforms obesity and other triglyceride-related parameters in detecting prediabetes in normal-weight Qatari adults: A cross-sectional study

Neyla S. Al Akl[1], Elias N. Haoudi[2], Halima Bensmail[3] and Abdelilah Arredouani[1,4]*

[1]Qatar Foundation, Diabetes Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar, [2]Qatar Foundation, Qatar Academy Doha, Doha, Qatar, [3]Qatar Computing Research Institute, Hamad Bin Khalifa University, Doha, Qatar, [4]Qatar Foundation, College of Health and Life Sciences, Hamad Bin Khalifa University (HBKU), Doha, Qatar

Introduction: The triglyceride-glucose (TyG)-driven indices, incorporating obesity indices, have been proposed as reliable markers of insulin resistance and related comorbidities such as diabetes. This study evaluated the effectiveness of these indices in detecting prediabetes in normal-weight individuals from a Middle Eastern population.

Methods: Using the data of 5,996 adult Qatari participants from the Qatar Biobank cohort, we employed adjusted logistic regression to assess the ability of various obesity and triglyceride-related indices to detect prediabetes in normal-weight ($18.5 \leq$BMI <25kg/m^2) adults ($\geq$18years).

Results: Of the normal-weight adults, 13.62% had prediabetes. TyG-waist-to-height ratio (TyG-WHTR) was significantly associated with prediabetes among normal-weight men [OR per 1-SD 2.68; 95% CI (1.67–4.32)] and women [OR per 1-SD 2.82; 95% CI (1.61–4.94)]. Compared with other indices, TyG-WHTR had the highest area under the curve (AUC) value for prediabetes in men [AUC: 0.76, 95% CI (0.70–0.81)] and women [AUC: 0.73, 95% CI (0.66–0.80)], and performed significantly higher than other indices ($p<0.05$) in detecting prediabetes in men. Tyg-WHTR shared similar diagnostic values as fasting plasma glucose (FPG).

Discussion: Our findings suggest that the TyG-WHTR index could be a better indicator of prediabetes for general clinical usage in normal weight Qatari adult men than other obesity and TyG-related indices. TyG-WHTR can help identify a person's risk for developing prediabetes in both men and women when combined with FPG results.

KEYWORDS

prediabetes, diabetes, triglyceride-glucose-related waist-to-height ratio, obesity, normal-weight, Qatar

Introduction

Although most normal-weight adults with BMI between 18.5 and 25 kg/m^2 are seemingly healthy, a significant number of them may be affected by undiagnosed metabolic disorders such as insulin resistance, prediabetes, type 2 diabetes (T2D), and nonalcoholic fatty liver disease (NAFLD) (1). These individuals are classified as Normal-Weight Obese (NWO) because they usually have a high body fat mass but a normal BMI (2). Because of their increased risk of cardiometabolic morbidity and mortality, there is a growing interest in this group of subjects (2–4). The global prevalence of NWO ranges from 4.5 to 22% due to the wide variation in body fat percent cut-offs used to diagnose excess body fat in different populations (5). The exact etiology of NWO is unclear, but genetics, diet, and physical activity have all been associated with the condition. Compared to normal-weight lean (NWL) subjects with a normal BMI and body fat amount, the NOW subjects present changes in body composition, inflammation, and oxidative stress (2).

Screening for prediabetes and diabetes is recommended in overweight or obese adults (6). However, given the prevalence of NWO, these disorders may go undiagnosed in individuals with a seemingly normal weight. Prediabetes is a subclinical high-risk state that could lead to diabetes and conventional diabetes complications (7). Prediabetes is associated with the concomitant presence of insulin resistance and β-cell dysfunction, instigated before detectable glucose modifications (8). Prediabetes is defined as having a Hb1A$_c$ level between 5.7 and 6.4% (39 and 47 mmol/mol), a fasting glucose concentration between 100 and 125 mg/dL (5.6 and 6.9 mmol/L), or a 2 h oral glucose tolerance test between 140 and 200 mg/dL (7.8–11.0 mmol/L) (6). Prediabetes affected 7.5% of the global population in 2019, and this figure is expected to rise to 8.6% by 2045 if no prompt actions are taken (Saeedi, 2019 #5).

According to previous prospective studies, the annualized conversion rate from prediabetes to diabetes is between 5 and 10% (8, 9). Additionally, persons with prediabetes have a 6-year risk of T2D at a rate of 33–65%, compared to 5% of those with normoglycemia (10).

Reports from the Middle East region show that, like T2D, prediabetes is highly prevalent in the region's nations, with rates ranging from 20 to 40% (11, 12). Fortunately, many people with prediabetes can revert to normoglycemia and prevent the progression to T2D in response to sustained lifestyle changes and/or medication (13–16). Hence, identifying convenient clinical markers that can efficiently diagnose prediabetes would benefit from closer monitoring and early intervention to prevent T2D onset.

To date, the gold standard test for prediabetes diagnosis is the oral glucose tolerance test (OGTT), fasting plasma glucose (FPG), and HbA_{1c} levels (11, 12). However, several studies have shown discordance between HbA_{1c} and glycemia (17–21). This discordance could have a significant impact on clinical practice. Consequently, there is a need for improved and more reliable diagnostic tools for prediabetes and diabetes. Numerous obesity indices (22)such as waist circumference (WC) (23), waist-to-height ratio (WHR) (24), Visceral Adiposity Index (VAI) (12) and lipid accumulation product (LAP) (12, 22) have had their potential in predicting diabetes. However, many promising surrogate indices are being studied for predicting diabetes and performed better than the traditional markers identified hereinabove. These markers include the triglyceride glucose (TyG)-related parameters (TyG), TyG–Body mass index (TyG-BMI), TyG-WC, and TyG-WHTR (25–30). Some epidemiological studies also targeted a few surrogate indices in predicting prediabetes. Wen et al. found that TyG performed better as an indicator for prediabetes than the conventional markers in the Chinese Elderly (31). TyG also scored higher as a predictive index for prediabetes among reproductive-age women (32). Furthermore, the TyG index was useful as a surrogate tool to estimate dysglycemia in obese adolescents (33, 34).

To the best of our knowledge, no previous study has investigated the association of triglyceride glucose (TyG)-related indices with prediabetes in a population from the Middle East. Consequently, this cross-sectional study sought to investigate the association of TyG-glucose-related parameters with prediabetes, especially in normal-weight individuals, and evaluate their superiority over conventional dysglycemia risk factors.

Methods

Study population

We obtained cross-sectional clinical, anthropometric, and demographic data of 5,996 Qatari individuals aged between 18 and 86 years and collected between 2012 and 2020 by the Qatar Biobank (QBB), a national institute running a well-phenotyped cohort of individuals from the general population (35). Based on the American Diabetes Association guidelines, 1996 (33.2%) of the 5,996 subjects had prediabetes, defined as having HbA1c levels between 39 mmol/mol (5.7%) and 47 mmol/mol (6.4%).

Anthropometric and clinical measures

Plasma samples of patients fasting for at least 6 h were handled according to a standard protocol within 2 h of blood collection. FPG, HbA1c, triglyceride (TG), total cholesterol (TC), low-density lipid cholesterol (LDL-C), and high-density lipid cholesterol (HDL-C) were analyzed with an automated biochemical analyzer at the central laboratories at the Hamad Medical Corporation in Doha. Bodyweight (kg) and height (cm) were measured in a standing position. BMI (kg/m^2) was calculated as weight (kg) divided by the square of height (m). We used Caucasian BMI cut-off values to categorize BMI into two groups: normal-weight (BMI 18.5–24.9 kg/m^2) and overweight/obese (BMI ≥25 kg/m^2). Prediabetes cases were defined as those individuals with $HbA1_c$ between 39 mmol/mol (5.7%) and 47 mmol/mol (6.4%), whereas controls were those with $HbA1_c$ < 39 mmol/mol (5.7%). An informed written consent to use collected data for research was obtained by the QBB for all the participants. The present project was approved by the IRB of the Qatar biobank (protocol Ex-2018-Res-ACC-0123-0067).

Definitions of obesity and triglyceride indices

We used the formulas in the table below to define the obesity or TyG-related indices.

10.3389/fpubh.2023.1086771

TABLE 1 Obesity and TyG-related indices.

Obesity indices			
Index name	Abbreviation	Formula	References
Waist-to-height ratio	WHTR	WHTR = Waist $_{(cm)}$/Height $_{(cm)}$	(36)
Visceral Adiposity Index	VAI (Men)	$VAI_{(men)} = [(WC_{(in\ cm)}/39.68) + (1.88 \times BMI_{(in\ Kg/m2)})] \times [(TG_{(in\ mmol/L)}/1.03) \times (1.31/HDL\text{-}C_{(in\ mmol/L)})]$	(37)
Visceral Adiposity Index	VAI (Women)	$VAI_{(women)} = [(WC_{(in\ cm)}/36.58) + (1.89 \times BMI_{(in\ Kg/m2)})] \times [(TG_{(in\ mmol/L)}/0.81) \times (1.52/HDL\text{-}C_{(in\ mmol/L)})]$	(37)
Lipid accumulation product	LAP (Men)	$LAP_{(men)} = [WC_{(in\ cm)}\text{-}65] \times TG_{(in\ mmol/L)}$	(38)
Lipid accumulation product	LAP (Women)	$LAP_{(women)} = [WC_{(in\ cm)}\text{-}58] \times TG_{(in\ mmol/L)}$	(38)
TyG related parameters			
Triglyceride-glucose	TyG	$TyG = Ln\ [(TG_{(in\ mg/dL)} \times FBG_{(in\ mg/dL)}/2)]$	(39)
Triglyceride-glucose-BMI	TyG-BMI	TyG-BMI = TyG × BMI	(27)
Triglyceride-glucose-waist circumference	TyG-WC	TyG-WC = TyG × WC	(27)
Triglyceride-glucose-waist-to-height ratio	TyG-WHTR	TyG-WHTR = TyG × WHTR	(27)

Statistical analysis

The subjects were divided into groups for statistical analysis based on gender, prediabetes presence or absence, BMI, and age as needed.

Data analyses were performed using Stata/IC 16.1 software.[1] Descriptive statistics were used to compare the baseline characteristics of the participants. Variables with outliers were winsorized using winsor2 command in Stata. Continuous variables were expressed as means ± standard deviation (SD) and compared using the independent sample T-test between the two groups. Categorical variables were expressed as percentages, and the Chi-squared test was employed to compare two groups. The odds of prediabetes were determined by binary logistic regression, using the continuous variables for both obesity and triglyceride indices as independent variables. Odd ratios (ORs) were standardized by using transformed observations [(observation − mean)/SD] in the models. Results are presented as Odds Ratios (OR) with associated 95% confidence intervals (CI) for 1-SD increase of the independent variables. The predictive value for prediabetes of each index was determined by the area under the curve (AUC) in the Receiver operating characteristic curve (ROC) analyses. The cut-off point was selected according to the Youden index (sensitivity + specificity −1). Statistical significance was set at $p < 0.05$.

1 http://www.stata.com

Results

Demographic and clinical characteristics of participants

Table 1 displays the baseline characteristics of the participants. Of the 5,996 individuals, 1996 had prediabetes (HbA_{1c} between 5.7 and 6.5%). The mean age of normoglycemic subjects and those with prediabetes was 36.37 and 48.17 years old, respectively ($p < 0.001$). Women represented 53.9% of the normoglycemic subjects and 53.3% of the subjects with prediabetes. Subjects with prediabetes showed significantly higher obesity and TyG-related indices than those with normoglycemia ($p < 0.05$).

Associations of indicators with prediabetes risk

Gender-specific multivariate logistic regression models were fitted for each indicator variable to calculate the age-adjusted OR (aOR) per 1-SD with 95% CI for prediabetes. The aORs per 1-SD for the obesity and TyG indices were significant in both men and women (Table 2). Among the tested indices, TyG-WHTR and TyG-WC showed the strongest association with prediabetes [TyG-WHTR: aOR 2.19; 95%CI (1.96–2.46) in men and aOR 2.76; 95%CI (2.30–2.86) in women; TyG-WC: aOR 2.08; 95% (1.87–2.32) in men and aOR 2.68; 95% (2.38–3.02) in women].

Further stratification of subjects by BMI resulted in higher aORs per 1-SD for prediabetes in the normal-weight (NW) individuals compared to overweight/obese (Ow/Ob) for most indices, mainly Tyg-BMI, TyG-WC, and TyG-WHTR (Figure 1). In NW men and women, TyG-BMI had the highest aOR for prediabetes [aOR 3.37; 95%CI (1.71–6.65) and aOR 4.19; 95%CI (1.82–9.61) in NW men and

10.3389/fpubh.2023.1086771

TABLE 2 Baseline demographic and clinical characteristics of the participants.

	NG (n=4,000)	Prediabetes (n=1996)	p-value
Men/Women	1842/2158	928/1068	
Age	36.37 ± 10.42	48.17 ± 11.39	<0.0001
BMI (Kg/m^2)	28.45 ± 5.58	32.02 ± 5.58	<0.0001
NW/(Ow + Ob)	1090/2910	172/1824	<0.0001
SBP (mm Hg)	111.11 ± 12.93	121.67 ± 15.00	<0.0001
DBP (mm Hg)	66.94 ± 9.89	72.22 ± 10.55	<0.0001
TC (mmol/L)	4.94 ± 0.89	5.15 ± 0.94	<0.0001
TG (mmol/L)	1.17 ± 0.64	1.49 ± 0.72	<0.0001
HDL-C (mmol/L)	1.41 ± 0.37	1.31 ± 0.34	<0.0001
LDL-C (mmol/L)	2.99 ± 0.82	3.16 ± 0.88	<0.0001
FPG (mmol/L)	4.88 ± 0.56	5.60 ± 0.89	<0.0001
HbA_{1c} (%)	5.17 ± 0.3	5.9 ± 0.21	<0.0001
TyG-related parameters			
TyG	8.29 ± 0.51	8.68 ± 0.49	<0.0001
TyG-BMI	236.7 ± 51.79	278.35 ± 51.26	<0.0001
TyG- WC	714.85 ± 131.48	834.09 ± 122.77	<0.0001
TyG-WHTR	4.33 ± 0.75	5.11 ± 0.72	<0.0001
Obesity indices			
WC (cm)	85.86 ± 12.92	95 ± 12.11	<0.0001
WHTR	0.52 ± 0.07	0.58 ± 0.07	<0.0001
VAI	1.38 ± 1.08	1.94 ± 1.30	<0.0001
LAP	31.17 ± 26.88	52.46 ± 32.22	<0.0001

Values are presented as range, mean ± SD, or frequencies (%). NW, normal-weight; Ow + Ob, overweight + obese; NG, Normoglycemic; BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoproteins; LDL-C, low-density lipoproteins; TC, total cholesterol; TG, total triglycerides; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; WC, Waist Circumference; WHTR Waist Height-Ratio; VAI, Visceral Adiposity Index; LAP Lipid Accumulation Product; TyG, Triglyceride Glucose; TyG-BMI, TyG related to BMI; TyG-WC TyG related to WC; TyG-WHTR, TyG related to WHTR. Statistical significance is considered at $p < 0.05$.

NW women, respectively]. TyG-WHTR and TyG-WC indices were also strongly associated with prediabetes [TyG-WHTR: aOR, 2.68; 95% CI (1.67–4.32) in NW men and aOR, 2.82; 95%CI (1.61–4.94) in NW women; TyG-WC: aOR, 2.33; 95% CI (1.49–3.66) in NW men and aOR, 2.97; 95% (1.63–5.38) in NW women]. In Ow/Ob individuals, TyG-WC and TyG-WHTR had the highest aORs in men and women compared to all other indices [TyG-WC: aOR 2.16; 95% CI (1.90–2.47) in Ow/Ob men and aOR, 2.76; 95%CI (2.41–3.16) in Ow/Ob women; TyG-WHTR: aOR, 2.30; 95%CI (2.00–2.64) in Ow/Ob men and aOR, 2.65; 95% CI (2.34–3.00) in Ow/Ob women].

The predictive value of each index for prediabetes in normal-weight individuals

We performed ROC analysis to assess the predictive value of each index for prediabetes in NW individuals. The results of the ROC curve analysis for each index are shown in Table 3 and Figure 2. The largest AUC observed in NW men corresponded to TyG-WHTR index [AUC: 0.76, 95% CI (0.70–0.81)] followed by TyG-WC [AUC: 0.74, 95% CI (0.69–0.79)]. The indices with the highest predictive value for prediabetes in NW women were TyG-WHTR [AUC: 0.73, 95% CI (0.66–0.80)] and TyG-WC [AUC: 0.73, 95% CI (0.66–0.79)]. TyG BMI and TyG showed approximately similar predictive ability when predicting prediabetes in normal-weight men and women [AUC ranging between (0.69 and 0.70)]. When predicting prediabetes, TyG-WC and TyG-WHTR had the highest sensitivity (76% for TyG-WC and 74% for TyG-WHTR) and Youden index (0.421 for TyG-WC and 0.432 for TyG-WHTR) in NW men. In NW women, LAP and TyG-WHTR had the highest sensitivity (75% for LAP and 76% for TyG-WHTR), and the WHTR and TyG-WHTR had the highest Youden index (0.413 for WHTR and 0.427 for TyG-WHTR).

Tyg-WHTR index had the highest ability to predict prediabetes

The index with the highest AUC value was contrasted with the other indices in the ROC analysis to determine the superior indicator for prediabetes (Table 4). The AUC of the TyG-WHTR index for prediabetes was significantly higher than all other indices in NW men ($p < 0.05$). In women, the predictive value of TyG-WHTR was significantly higher than the AUC of WC ($p < 0.004$), WHTR ($p < 0.049$), VAI ($p < 0.008$). However, TyG-WHTR was not significantly different from the other TyG-related indices and LAP ($p > 0.05$).

Since FPG levels are one of the determinants for prediabetes diagnosis, subgroup analyses were conducted to assess whether Tyg-WHTR and FPG indices might differentially predict prediabetes. The OR and the AUC of TyG -WHTR for prediabetes were compared to that of FPG in different subgroups, as shown in Table 5. TyG WHTR performed similarly to FPG in NW men [TyG-WHTR: AUC 0.76, 95% CI (0.70–0.81) versus FPG: AUC 0.76, 95% CI (0.72–0.81), $p = 0.76$] and women [TyG-WHTR AUC 0.73, 95% CI (0.66–0.80) versus FPG AUC 0.68, 95% CI (0.61–0.76), $p = 0.13$]. Similarly, no significant difference was found in Ow/Ob men [TyG-WHTR: AUC 0.71, 95% CI (0.69–0.74) versus FPG: AUC 0.74, 95% (0.72–0.76), $p = 0.4$]. In contrast, the predictive value of the TyG-WHTR index was significantly higher than FPG in obese women [TyG-WHTR: AUC 0.77, 95% CI (0.75–0.78) versus FPG: AUC 0.75, 95% CI (0.73–0.77) $p = 0.0034$].

We then estimated the prediabetes risk probabilities of TyG-WHTR based on four age subgroups: less than 30 years old (Q1), between 30 and less than 45 years old (Q2), between 45 and less than 60 years old (Q3), and > 60 years (Q4) (Figure 3). The results indicate that the probability of having prediabetes increases gradually and significantly with age in NW men and OW/Ob men and women but not in NW women.

Discussion

The current study aimed to compare how well obesity and TyG-related indices might detect prediabetes in Qatari people who were normal-weight (NW). In NW men, TyG-WHTR had the highest

10.3389/fpubh.2023.1086771

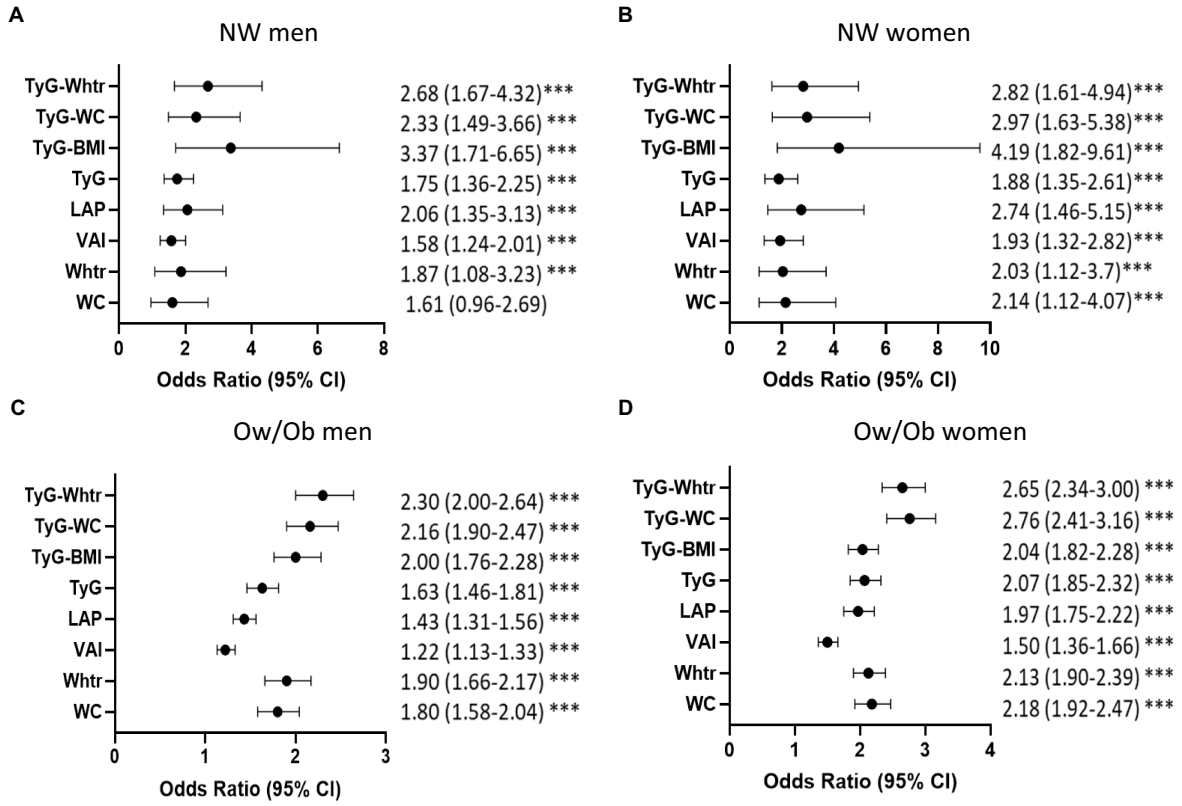


FIGURE 1
Strength of association of obesity and triglyceride indices with prediabetes in normal-weight and overweight/obese individuals. Age-adjusted Odds Ratios and 95%CI for prediabetes in each index by per 1-SD in NW men **(A)**, NW women **(B)**, Ow/Ob men **(C)**, Ow/Ob women **(D)**. ***$p<0.001$. Statistical significance is considered at $p<0.05$. *** indicates $p<0.001$.

predictive value compared to all other and most obesity indices in women. Furthermore, TyG-WHTR performed similarly to the conventionally adapted FPG index in normal-weight men and women, as well as in obese men, but was superior to FPG in obese women. These results suggest using the TyG-WHTR index as a potential predictor of prediabetes for general clinical usage in normal-weight men. When coupled with FPG results, TyG-WHTR can further determine prediabetes predisposition in both men and women. In addition, our results enabled us to determine optimal cut-off points of WC and WHTR for identifying prediabetes in normal-weight Qatari adults; WC ≥ 79.5 cm and WHTR ≥ 0.47 for men, and WC ≥ 71.5 cm and WHTR ≥ 0.45 for women.

According to the World Health Organization, the WC cut-off point for various metabolic disorders varies by ethnicity (40). Although, the present data facilitated the identification of the WC and WHTR cut-off points needed for predicting prediabetes risk in a Middle Eastern population, a person's height can affect the predictive ability of WC (36). As a result, adopting the WHTR marker outweighed BMI and WC in predicting certain metabolic diseases, including diabetes (26, 41, 42). In line with these findings, our results revealed that the association of WHTR with prediabetes was stronger than WC among the normal-weight adult population.

Furthermore, WHTR had higher specificity in normal-weight men and women than WC and higher sensitivity in women. This result suggests that WHTR may have a better predictive ability than WC and may be used to screen for prediabetes. Although these anthropometric parameters performed poorly than the TyG-WHTR index, as indicated by the ROC curve analyses, their predictive value was equivalent to LAP, VAI, and the other TyG-related indices. These findings point to the inclusion of these predictors collectively to improve the accuracy of prediabetes diagnosis over anthropometric indices alone.

Obesity increases the risk of numerous chronic disease, such as T2D, metabolic syndrome, hypertension, dyslipidemia, hyperinsulinemia, coronary artery disease, cardiovascular disease, osteoarthritis, chronic kidney disease, and numerous cancers. It is also linked to non-alcoholic steatohepatitis, sleep apnea, depression and other psychiatric disorders. Studies have also shown that obesity may have an impact on cognitive function and that a higher BMI may increase the chance of dementia or other cognitive impairments in later life (43).

Obesity and being overweight are known diabetes and prediabetes risk factors. However, these disorders can affect people with lower BMIs as well. The fasting triglyceride and glucose parameters have been proposed as alternative surrogate markers for identifying insulin resistance and diabetes (22, 27–30). Our study supports this finding, and all TyG-related parameters predict individuals with prediabetes (AUC > 0.5). On a large scale, when the AUC is equal to 1, it indicates faultless predictive power, and an AUC ≤ 0.55 means that the predictive power of a parameter is not better than chance (44). TyG-BMI showed the highest OR for prediabetes occurrence in normal-weight men. However, as determined by the AUC, its predictive value was not the highest compared to the other indices. The TyG-WHTR had the highest AUC value in men and women and

10.3389/fpubh.2023.1086771

TABLE 3 ROC curve analyses for each index in predicting prediabetes in NW participants stratified by gender.

	AUC (95%CI)	*p*-value	Cut-off	Sens (%)	Spec (%)	Youden index
Men (620)						
WC	0.69 (0.64–0.75)	<0.0001	≥79.5	72%	59%	0.310
WHTR	0.71 (0.65–0.76)	<0.0001	≥0.47	58%	75%	0.329
VAI	0.66 (0.61–0.72)	<0.0001	≥1.15	60%	64%	0.275
LAP	0.71 (0.66–0.77)	<0.0001	≥18.8	68%	73%	0.411
TyG	0.70 (0.65–0.76)	<0.0001	≥ 8.49	61%	73%	0.349
TyG-BMI	0.69 (0.64–0.75)	<0.0001	≥194	67%	67%	0.343
TyG-WC	0.74 (0.69–0.79)	<0.0001	≥669	76%	66%	0.421
TyG-WHTR	0.76 (0.70–0.81)	<0.0001	≥3.92	74%	69%	0.432
Women (642)						
WC	0.69 (0.63–0.76)	<0.0001	≥71.5	67%	65%	0.316
WHTR	0.70 (0.63–0.77)	<0.0001	≥0.45	70%	72%	0.413
VAI	0.65 (0.58–0.73)	<0.0001	≥0.87	65%	61%	0.259
LAP	0.71 (0.64–0.78)	<0.0001	≥10.57	75%	63%	0.378
TyG	0.69 (0.61–0.76)	<0.0001	≥8.19	60%	72%	0.322
TyG-BMI	0.70 (0.63–0.77)	<0.0001	≥191	57%	74%	0.309
TyG-WC	0.73 (0.66–0.79)	<0.0001	≥593	65%	75%	0.404
TyG-WHTR	0.73 (0.66–0.80)	<0.0001	≥3.62	76%	66%	0.427

WC, waist circumference; WHTR, waist height-ratio; VAI, Visceral Adiposity Index; LAP, lipid accumulation product; TyG, triglyceride glucose; TyG-BMI, TyG related to BMI; TyG-WC, TyG related to WC; TyG-WHTR, TyG related to WHTR. Statistical significance is considered at $p < 0.05$.

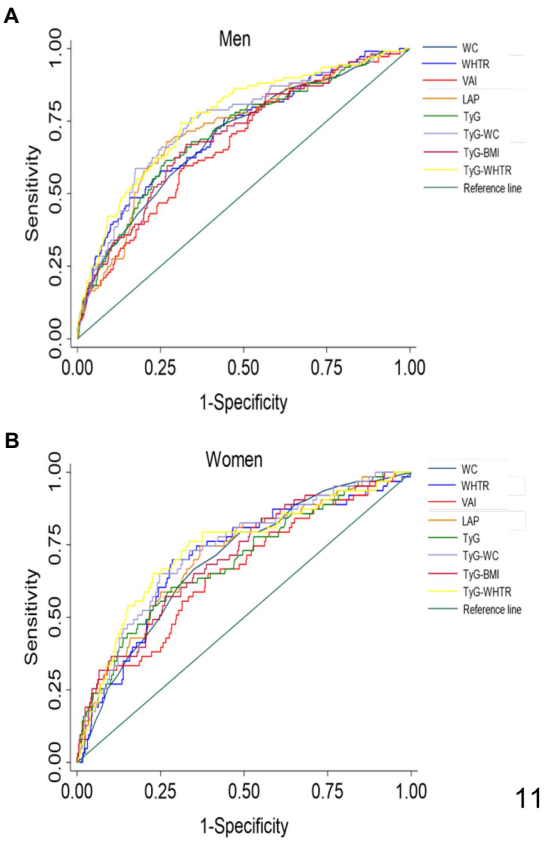


FIGURE 2
ROC curves for each index as predictors of prediabetes in normal-weight men **(A)** and women **(B)**. WC, Waist Circumference; WHTR, Waist Height-Ratio; VAI, Visceral Adiposity Index; LAP, lipid accumulation product; TyG, Triglyceride Glucose; TyG-BMI, TyG related to BMI; TyG-WC, TyG related to WC; TyG-WHTR, TyG related to WHTR.

was selected as the primary index for predicting prediabetes. The cut-off points indicated sensitivity and specificity values between 66 and 76% for both sexes, thus reducing false-positive and false-negative cases. TyG-WHTR proved significantly different from all obesity-related parameters in men and was significantly higher than the remaining TyG-related indices. It also had the highest AUC and was significantly different from all other indices in the obese population (Supplementary Tables S1, S2). Therefore, TyG-WHTR could potentially be adopted for identifying prediabetes in normal-weight and Ow/Ob men. Many TyG-related parameters were assessed for the predictive value of prediabetes and diabetes. However, no evidence has been published regarding the relationship between TyG-WHTR and prediabetes in normal-weight individuals. However, in recently published studies, TyG-WHTR exceeded the commonly used anthropometric markers in predicting diabetes (45, 46).

The TyG-WHTR index's capacity to detect prediabetes was contrasted with FPG's to determine whether it could be a useful screening tool. The results demonstrated that the predictive abilities were all significantly improved. These results indicate that the TyG-WHTR index can act similarly to FPG. Hence, TyG-WHTR can, alongside FPG, function in screening individuals for prediabetes. Knowing that advanced age poses an additional risk for prediabetes (11), further age stratification of the participants demonstrated that TyG-WHTR proved higher predictive probability with advanced age in normal-weight men and obese men and women, highlighting the efficiency of TyG-WHTR in predicting prediabetes.

TyG-WC was previously reported as the best predictor of prediabetes or diabetes (47). TyG-BMI was also suggested as the best index for detecting prediabetes in adults of either sex (25). Further, TyG was suggested as a good index for predicting insulin resistance and prediabetes (29, 31). We have found the TyG-WHTR to be the most effective index for prediabetes prediction. These disparities could be attributed to the ethnic diversity of the populations studied. Nonetheless, the overall conclusion from our findings and previously

10.3389/fpubh.2023.1086771

TABLE 4 Pairwise comparison of AUC of TyG-WHTR in NW participants.

	Men (620)			Women (642)		
	Differences between AUC	95% CI	*p*-value	Differences between AUCs	95% CI	*p*-value
WC	0.08	(0.04–0.11)	<0.0001	0.06	(0.01–0.10)	0.004
WHTR	0.04	(0.015–0.07)	0.0029	0.02	(0.0007–0.058)	0.049
VAI	0.09	(0.05–0.13)	<0.0001	0.07	0.02–0.13	0.008
LAP	0.04	(0.02–0.06)	<0.0001	0.02	(−0.004–0.04)	0.1
TyG	0.05	(0.01–0.09)	0.0031	0.04	(−0.005–0.09)	0.78
TyG-BMI	0.06	(0.03–0.09)	<0.0001	0.03	(−0.012–0.07)	0.15
TyG-WC	0.016	(0.0003–0.32)	0.045	0.003	(−0.016–0.024)	0.72

NW, normal-weight; AUC, area under the curve; WC, waist circumference; WHTR, waist height-ratio; VAI, Visceral Adiposity Index; LAP, lipid accumulation product; TyG, triglyceride glucose; TyG-BMI, TyG related to BMI; TyG-WC, TyG related to WC; TyG-WHTR, TyG related to WHTR. Statistical significance is considered at $p < 0.05$.

TABLE 5 Performance of the TyG-WHTR index versus FPG using adjusted logistic regression and ROC analysis in predicting prediabetes in subgroups with the different characteristics.

	OR (95%)	AUC (95%CI)	Cut-off point	*p*-value
NW men (*n* = 620)				
Tyg-WHTR	2.68 (1.67–4.32)***	0.76 (0.70–0.81)	3.92	Ref
FPG	2.32 (1.73–3.10)***	0.76 (0.72–0.81)	5.11	0.76
NW women (*n* = 642)				
Tyg-WHTR	2.82 (1.61–4.94)***	0.73 (0.66–0.80)	3.62	Ref
FPG	1.90 (1.32–2.72)***	0.68 (0.61–0.76)	4.89	0.13
Ow/Ob men (*n* = 2,150)				
Tyg-WHTR	2.30 (2.00–2.64)***	0.71 (0.69–0.74)	4.94	Ref
FPG	2.35 (2.08–2.66)***	0.74 (0.72–0.76)	5.33	0.4
Ow/Ob women (*n* = 2,584)				
Tyg-WHTR	2.65 (2.34–3.00)***	0.77 (0.75–0.78)	4.73	Ref
FPG	2.61 (2.31–2.95)***	0.75 (0.73–0.77)	5.29	**0.0034**

NW, normal-weight; Ow/Ob, overweight/obese; OR, odds ratio; AUC, area under the curve; TyG-WHTR, TyG related to waist height-ratio; FPG, fasting plasma glucose. Statistical significance is considered at $p < 0.05$. ***$p < 0.001$.

published data is that triglyceride-glucose (TyG)-related parameters outperform obesity parameters alone. The clinical significance of TyG-WHTR rests in its ability to identify persons at risk of developing prediabetes before symptoms appear. By recognizing these patients early, healthcare providers can take steps to prevent or delay illness onset through lifestyle changes like diet and exercise, or medication interventions if necessary. Furthermore, TyG-WHTR can be used to assess the efficacy of programs targeted at lowering the risk of prediabetes in people of normal weight. A patient, for example, may begin an exercise and diet regimen in order to reduce their waist circumference and improve their glucose and lipid levels. Their TyG-WHTR score can be tracked over time to evaluate the intervention's effectiveness and make any necessary modifications.

In some cases, the TyG-WHTR may be considered superior to fasting glucose or HbA1c because: (1) fasting glucose and HbA1c may only show abnormalities after significant metabolic damage has occurred, whereas TyG-WHTR can detect early metabolic changes when interventions are more likely to be effective. (2) TyG-WHTR ca be a better predictor of prediabetes than fasting glucose or HbA1c. This is due to the fact that TyG-WHTR considers both triglyceride and glucose levels, which are both independent risk factors for dysglycemia. (3) TyG-WHTR may be more responsive to metabolic state changes than fasting glucose or HbA1c. For example, if a patient improves their diet and exercise habits, their TyG-WHTR score may improve even if their fasting glucose or HbA1c levels stay unchanged. (4) TyG-WHTR is a straightforward computation that involves only basic laboratory tests and measures found in most healthcare facilities. HbA1c testing, on the other hand, might be more expensive, and not all healthcare settings have the necessary equipment or competence. One of the strengths of our study is the large sample size. Indeed, according to the Qatar Planning and Statistics Authority, the population of Qatar at the end of April 2022 was 2,773,598 people (accessed on 22nd of May, 2022),[2] with Qataris accounting for approximately 12% of the total (i.e., 333,000 individuals). Moreover, in 2015, individuals under 19 made up 47% of all Qatari nationals,[3] and if this percentage did not change in 2022, approximately 176,500 individuals would be adults and would be eligible for our study. As a result, our study (6,000/176,500 = 0.03) has statistically significant power. Additionally, the data we used was obtained from a well-phenotyped cohort from the general population. Furthermore, our study is the first to compare the ability of obesity indices and TyG-related parameters to identify prediabetes in normal-weight individuals in a Middle Eastern population. It is worth noting that T2D is a major public health burden in the Middle East, and early detection of prediabetes in obese and normal-weight individuals is thus critical for implementing strategies to prevent its progression to T2D.

2 https://www.psa.gov.qa/en/Pages/default.aspx

3 https://gulfmigration.org/qatar-population-nationality-qatari-non-qatari-five-year-age-group-2015/

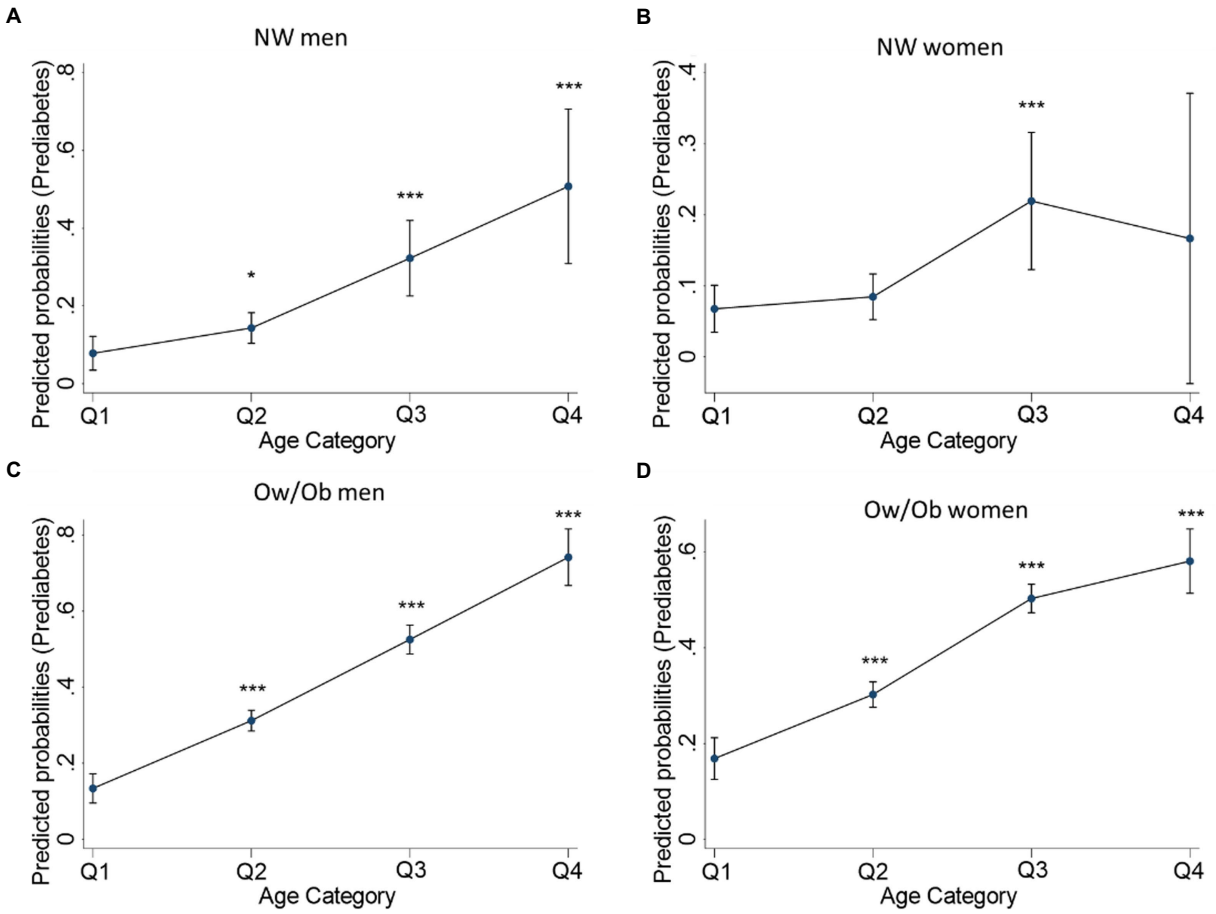


FIGURE 3
TyG-WHTR predicted probabilities for having prediabetes across age quartiles in NW and Ow/Ob participants. Predicted probabilities for Prediabetes in NW men **(A)**, NW women **(B)** Ow/Ob men **(C)**, Ow/Ob women **(D)**. Statistical significance is considered at $p<0.05$. *$p<0.05$, **$p<0.01$ ***$p<0.001$.

Given the shared environmental factors and lifestyle habits, as well as genetic background and ethnicity among many Middle Eastern countries, particularly the Gulf Cooperation Council nations (Qatar, Bahrain, Saudi Arabia, United Arab Emirates, Kuwait, and Oman), our findings may perform similarly in many of these countries.

The main limitation of our study is the cross-sectional design, which does not allow the use of the findings to predict future prediabetes. However, the QBB has recently started to call back the participants for a 5-year follow-up, which will open new avenues for assessing the predictive ability of the different indices longitudinally. We also did not adjust for parameters such as smoking status, medication, or physical activity. Finally, the findings of the present study may not be generalizable to all populations due to the ethnic and geographic characteristics of the study population.

Conclusion

Based on our results, factoring in waist-to-height ratio with simple biochemical measurements of triglyceride and glucose proved to be the best indicator of prediabetes in normal-weight, overweight and obese men, in addition to outperforming most obesity indices in women and having similar predictive effects to FPG in both normal-weight and overweight/obese men and women. We suggest that TyG-WHTR be used in clinical practice as part of routine check-ups as an asserting indicator to FPG for predicting prediabetes in men. Further studies are warranted to confirm the predictive value of these parameters across varying ethnicities.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Clinical, anthropometric, demographic and genetic data can be obtained from the Qatar biobank according to the applied rules.

Ethics statement

The institutional review board approved the current project at the Qatar Biomedical Research Institute (IRB number: 2017–001) and QBB (IRB number: Ex-2018-Res-ACC-0123-0067). All participants gave written informed consent for their data and biospecimens to be used in medical research. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NA, EH, and AA performed the statistical analysis. NA and AA interpreted the results and wrote the manuscript. HB revised the statistical analysis. AA conceived and designed the study, was the guarantor of this work, had full access to all the data in the study, and took responsibility for the data's integrity and the data's accuracy. All authors reviewed the results, edited the manuscript, and approved the final version.

Funding

This project was funded by intermural grants from Qatar the Biomedical Research Institute to AA and from the Qatar Computing Research Institute to HB.

Acknowledgments

We want to thank Qatar Biobank for facilitating access to the data and providing us with expert advice. We are also grateful to all the participants of the study.

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/fpubh.2023.1086771/full#supplementary-material

References

1. Suliga E, Kozieł D, Głuszek S. Prevalence of metabolic syndrome in normal weight individuals. *Ann Agric Environ Med*. (2016) 23:631–5. doi: 10.5604/12321966.1226858.

2. Wijayatunga NN, Dhurandhar EJ. Normal weight obesity and unaddressed cardiometabolic health risk-a narrative review. *Int J Obes*. (2021) 45:2141–55. doi: 10.1038/s41366-021-00858-7

3. Romero-Corral A, Somers VK, Sierra-Johnson J, Korenfeld Y, Boarin S, Korinek J, et al. Normal weight obesity: A risk factor for cardiometabolic dysregulation and cardiovascular mortality. *Eur Heart J*. (2010) 31:737–46. doi: 10.1093/eurheartj/ehp487

4. Correa-Rodríguez M, González-Ruíz K, Rincón-Pabón D, Izquierdo M, García-Hermoso A, Agostinis-Sobrinho C, et al. Normal-weight obesity is associated with increased cardiometabolic risk in young adults. *Nutrients*. (2020) 12:1106. doi: 10.3390/nu12041106

5. Franco LP, Morais CC, Cominetti C. Normal-weight obesity syndrome: Diagnosis, prevalence, and clinical implications. *Nutr Rev*. (2016) 74:558–70. doi: 10.1093/nutrit/nuw019

6. Association AD. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2018. *Diabetes Care*. (2018) 41:S13–s27. doi: 10.2337/dc18-S002

7. Jiang C, Yang R, Kuang M, Yu M, Zhong M, Zou Y. Triglyceride glucose-body mass index in identifying high-risk groups of pre-diabetes. *Lipids Health Dis*. (2021) 20:161. doi: 10.1186/s12944-021-01594-7

8. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: A high-risk state for diabetes development. *Lancet*. (2012) 379:2279–90. doi: 10.1016/S0140-6736(12)60283-9

9. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: Implications for care. *Diabetes Care*. (2007) 30:753–9. doi: 10.2337/dc07-9920

10. de Vegt F, Dekker JM, Jager A, Hienkens E, Kostense PJ, Stehouwer CD, et al. Relation of impaired fasting and postload glucose with incident type 2 diabetes in a Dutch population: The Hoorn study. *JAMA*. (2001) 285:2109–13. doi: 10.1001/jama.285.16.2109

11. Abbas M, Mall R, Errafii K, Lattab A, Ullah E, Bensmail H, et al. Simple risk score to screen for prediabetes: A cross-sectional study from the Qatar biobank cohort. *J Diabetes Investig*. (2021) 12:988–97. doi: 10.1111/jdi.13445

12. Ahn N, Baumeister SE, Amann U, Rathmann W, Peters A, Huth C, et al. Visceral adiposity index (VAI), lipid accumulation product (LAP), and product of triglycerides and glucose (TyG) to discriminate prediabetes and diabetes. *Sci Rep*. (2019) 9:9693. doi: 10.1038/s41598-019-46187-8

13. Jiang L, Johnson A, Pratte K, Beals J, Bullock A, Manson SM. Long-term outcomes of lifestyle intervention to prevent diabetes in American Indian and Alaska native communities: The special diabetes program for Indians diabetes prevention program. *Diabetes Care*. (2018) 41:1462–70. doi: 10.2337/dc17-2685

14. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. (2002) 346:393–403. doi: 10.1056/NEJMoa012512

15. Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, et al. 10-year follow-up of diabetes incidence and weight loss in the diabetes prevention program outcomes study. *Lancet*. (2009) 374:1677–86. doi: 10.1016/S0140-6736(09)61457-4

16. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and diabetes study. *Diabetes Care*. (1997) 20:537–44. doi: 10.2337/diacare.20.4.537

17. Ho-Pham LT, Nguyen UDT, Tran TX, Nguyen TV. Discordance in the diagnosis of diabetes: Comparison between HbA1c and fasting plasma glucose. *PLoS One*. (2017) 12:e0182192. doi: 10.1371/journal.pone.0182192

18. Rathmann W, Bongaerts B, Kostev K. Association of characteristics of people with type 2 diabetes mellitus with discordant values of fasting glucose and HbA1c. *J Diabetes*. (2018) 10:934–41. doi: 10.1111/1753-0407.12823

19. Abdul Murad NA, Abdullah N, Kamaruddin MA, Abd Jalal N, Ismail N, Yusof NAM, et al. Discordance between fasting plasma glucose (FPG) and HbA1c in diagnosing diabetes and pre-diabetes in the Malaysian cohort. *J ASEAN Fed Endocr Soc*. (2021) 36:127–32. doi: 10.15605/jafes.036.02.02

20. Kim JH, Shin JH, Lee HJ, Kim SY, Bae HY. Discordance between HbA1c and fasting plasma glucose criteria for diabetes screening is associated with obesity and old age in Korean individuals. *Diabetes Res Clin Pract*. (2011) 94:e27–9. doi: 10.1016/j.diabres.2011.07.013

21. Wade AN, Crowther NJ, Abrahams-Gessel S, Berkman L, George JA, Gómez-Olivé FX, et al. Concordance between fasting plasma glucose and HbA(1c) in the diagnosis of diabetes in black south African adults: A cross-sectional study. *BMJ Open*. (2021) 11:e046060. doi: 10.1136/bmjopen-2020-046060

22. Ke P, Wu X, Xu M, Feng J, Xu H, Gan Y, et al. Comparison of obesity indices and triglyceride glucose-related parameters to predict type 2 diabetes mellitus among normal-weight elderly in China. *Eat Weight Disord*. (2022) 27:1181–91. doi: 10.1007/s40519-021-01238-w

23. Siren R, Eriksson JG, Vanhanen H. Waist circumference a good indicator of future risk for type 2 diabetes and cardiovascular disease. *BMC Public Health*. (2012) 12:631. doi: 10.1186/1471-2458-12-631

24. Elizalde-Barrera CI, Rubio-Guerra AF, Lozano-Nuevo JJ, Olvera-Gomez JL. Triglycerides and waist to height ratio are more accurate than visceral adiposity and body adiposity index to predict impaired fasting glucose. *Diabetes Res Clin Pract*. (2019) 153:49–54. doi: 10.1016/j.diabres.2019.05.019

25. Er LK, Wu S, Chou HH, Hsu LA, Teng MS, Sun YC, et al. Triglyceride glucose-body mass index is a simple and clinically useful surrogate marker for insulin resistance in nondiabetic individuals. *PLoS One*. (2016) 11:e0149731. doi: 10.1371/journal.pone.0149731

26. Ke JF, Wang JW, Lu JX, Zhang ZH, Liu Y, Li LX. Waist-to-height ratio has a stronger association with cardiovascular risks than waist circumference, waist-hip ratio and body mass index in type 2 diabetes. *Diabetes Res Clin Pract*. (2022) 183:109151. doi: 10.1016/j.diabres.2021.109151

27. Lim J, Kim J, Koo SH, Kwon GC. Comparison of triglyceride glucose index, and related parameters to predict insulin resistance in Korean adults: An analysis of the 2007-2010 Korean National Health and nutrition examination survey. *PLoS One*. (2019) 14:e0212963. doi: 10.1371/journal.pone.0212963

28. Lin J, Li H, Qin Y, Xiang X, Wan Q. Triglyceride glucose-waist circumference to height ratio exceeds commonly used anthropometric markers in predicting 10-year diabetes risk: The REACTION research. *Res Squ*. (2022).

29. Selvi NMK, Nandhini S, Sakthivadivel V, Lokesh S, Srinivasan AR, Sumathi S. Association of triglyceride-glucose index (TyG index) with HbA1c and insulin resistance in type 2 diabetes mellitus. *Maedica*. (2021) 16:375–81. doi: 10.26574/maedica.2021.16.3.375

30. Song K, Park G, Lee HS, Choi Y, Oh JS, Choi HS, et al. Prediction of insulin resistance by modified triglyceride glucose indices in youth. *Life*. (2021) 11:286. doi: 10.3390/life11040286

31. Wen J, Wang A, Liu G, Wang M, Zuo Y, Li W, et al. Elevated triglyceride-glucose (TyG) index predicts incidence of prediabetes: A prospective cohort study in China. *Lipids Health Dis*. (2020) 19:226. doi: 10.1186/s12944-020-01401-9

32. Liberty IA, Kodim N, Sartika RAD, Trihandini I, Tjekyan RMS, Zulkarnain PM, et al. Triglyceride/glucose index (TyG index) as a marker of glucose status conversion among reproductive-aged women in Jakarta, Indonesia: The Bogor cohort study (2011-2016). *Diabetes Metab Syndr*. (2021) 15:102280. doi: 10.1016/j.dsx.2021.102280

33. Dikaiakou E, Vlachopapadopoulou EA, Paschou SA, Athanasouli F, Panagiotopoulos I, Kafetzi M, et al. Triglycerides-glucose (TyG) index is a sensitive marker of insulin resistance in Greek children and adolescents. *Endocrine*. (2020) 70:58–64. doi: 10.1007/s12020-020-02374-6

34. Mohd Nor NS, Lee S, Bacha F, Tfayli H, Arslanian S. Triglyceride glucose index as a surrogate measure of insulin sensitivity in obese adolescents with normoglycemia, prediabetes, and type 2 diabetes mellitus: Comparison with the hyperinsulinemic-euglycemic clamp. *Pediatr Diabetes*. (2016) 17:458–65. doi: 10.1111/pedi.12303

35. al Kuwari H, al Thani A, al Marri A, al Kaabi A, Abderrahim H, Afifi N, et al. The Qatar biobank: Background and methods. *BMC Public Health*. (2015) 15:1208. doi: 10.1186/s12889-015-2522-7

36. Hsieh SD, Yoshinaga H, Muto T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *Int J Obes Relat Metab Disord*. (2003) 27:610–6. doi: 10.1038/sj.ijo.0802259

37. Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, et al. Visceral adiposity index: A reliable indicator of visceral fat function associated with cardiometabolic risk. *Diabetes Care*. (2010) 33:920–2. doi: 10.2337/dc09-1825

38. Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: A population-based comparison. *BMC Cardiovasc Disord*. (2005) 5:26. doi: 10.1186/1471-2261-5-26

39. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord*. (2008) 6:299–304. doi: 10.1089/met.2008.0034

40. World Health Organization. *Waist circumference and waist-hip ratio report of WHO expert consultation*. Geneva: World Health Organization (2008).

41. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. *Obes Rev*. (2012) 13:275–86. doi: 10.1111/j.1467-789X.2011.00952.x

42. Xu Z, Qi X, Dahl AK, Xu W. Waist-to-height ratio is the best indicator for undiagnosed type 2 diabetes. *Diabet Med*. (2013) 30:e201–7. doi: 10.1111/dme.12168

43. Monda V, la Marra M, Perrella R, Caviglia G, Iavarone A, Chieffi S, et al. Obesity and brain illness: From cognitive and psychological evidences to obesity paradox. *Diabetes Metab Syndr Obes*. (2017) 10:473–9. doi: 10.2147/DMSO.S148392

44. Ramírez-Vélez R, Pérez-Sousa M, González-Ruíz K, Cano-Gutierrez CA, Schmidt-Riovalle J, Correa-Rodríguez M, et al. Obesity- and lipid-related parameters in the identification of older adults with a high risk of Prediabetes according to the American Diabetes Association: An analysis of the 2015 health, well-being, and aging study. *Nutrients*. (2019) 11:2654. doi: 10.3390/nu11112654

45. Li W, Wang Y, He F, Liu Z, Dong J, Zhang Y, et al. Association between triglyceride-glucose index and nonalcoholic fatty liver disease in type 2 diabetes mellitus. *BMC Endocr Disord*. (2022) 22:261. doi: 10.1186/s12902-022-01172-7

46. Xuan W, Liu D, Zhong J, Luo H, Zhang X. Impacts of triglyceride glucose-waist to height ratio on diabetes incidence: A secondary analysis of a population-based longitudinal data. *Front Endocrinol*. (2022) 13:949831. doi: 10.3389/fendo.2022.949831

47. Zheng S, Shi S, Ren X, Han T, Li Y, Chen Y, et al. Triglyceride glucose-waist circumference, a novel and effective predictor of diabetes in first-degree relatives of type 2 diabetes patients: Cross-sectional and prospective cohort study. *J Transl Med*. (2016) 14:260. doi: 10.1186/s12967-016-1020-8

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 18 April 2023
DOI 10.3389/fendo.2023.1111791

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal, South Africa

REVIEWED BY
Prem Mishra,
Institute for Social and Economic Change, India
Karim Kohansal,
Shahid Beheshti University of Medical Sciences, Iran
Ibiye Owei,
Texas Tech University Health Sciences Center El Paso, United States

*CORRESPONDENCE
Zhiqiang Huang
huangzhiqiang444@163.com
Dehong Liu
dhliu_emergency@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Obesity, a section of the journal Frontiers in Endocrinology

RECEIVED 30 November 2022
ACCEPTED 28 March 2023
PUBLISHED 18 April 2023

CITATION
Han Y, Hu H, Huang Z and Liu D (2023) Association between body mass index and reversion to normoglycemia from impaired fasting glucose among Chinese adults: a 5-year cohort study. *Front. Endocrinol.* 14:1111791. doi: 10.3389/fendo.2023.1111791

Association between body mass index and reversion to normoglycemia from impaired fasting glucose among Chinese adults: a 5-year cohort study

Yong Han[1†], Haofei Hu[2†], Zhiqiang Huang[1*] and Dehong Liu[1*]

[1]Department of Emergency, Shenzhen Second People's Hospital, Shenzhen, Guangdong, China, [2]Department of Nephrology, Shenzhen Second People's Hospital, Shenzhen, Guangdong, China

Objective: Evidence regarding the relationship between body mass index (BMI) and reversion to normoglycemia from prediabetes is still limited. The purpose of our study is to survey the link of BMI on reversion to normoglycemia among patients with impaired fasting glucose (IFG).

Methods: This study, a retrospective cohort, covered 32 regions and 11 cities in China and collected 258,74 IFG patients who underwent a health check from 2010 to 2016. We investigated the association between baseline BMI and reversion to normoglycemia in patients with IFG using the Cox proportional-hazards regression model. The nonlinear relationship between BMI and reversion to normoglycemia was determined using a Cox proportional hazards regression with cubic spline functions and smooth curve fitting. In addition, we also performed a series of sensitivity analyses and subgroup analyses. A competing risk multivariate Cox regression was performed using progression to diabetes as a competing risk for reversal of normoglycemic events.

Results: After adjusting covariates, the results showed that BMI was negatively related to the probability of reversion to normoglycemia (HR=0.977, 95% CI:0.971-0.984). Compared with participants with normal BMI(<24kg/m^2), overweight (BMI:24-28kg/m^2) participants with IFG had a 9.9% lower probability of returning to normoglycemia (HR=0.901,95%CI:0.863-0.939), while obese patients (BMI ≥ 28kg/m^2) had a 16.9% decreased probability of reverting from IFG to normoglycemia (HR=0.831,95%CI:0.780-0.886). There was also a nonlinear relationship between them, and the inflection point of BMI was 21.7kg/m^2. The effect sizes (HR) on the left sides of the inflection point were 0.972(95%CI:0.964-0.980). The competing risks multivariate Cox's regression and sensitivity analysis demonstrated the robustness of our results.

Conclusion: This study demonstrates a negative and nonlinear relationship between BMI and reversion to normoglycemia in Chinese patients with IFG. Minimizing BMI to 21.7 kg/m^2 in patients with IFG through aggressive intervention may significantly increase the probability of returning to normoglycemia.

KEYWORDS

pre-diabetes, regression to normoglycemia, nonlinear, competitive risk model, smooth curve fitting

Introduction

Diabetes is a major public health concern because of its high prevalence, mortality, and rising costs (1). Prediabetes is an intermediate stage between normal glucose levels and type 2 diabetes mellitus (T2DM). It generally reflects the presence of either or both impaired fasting glucose (IFG) and glucose tolerance (IGT). In 2017, the International Diabetes Federation (IDF) estimated that 374 million adults worldwide had prediabetes, and the number of adults with prediabetes will reach 548 million by 2045, equaling 8.4% of the adult population (2). Approximately 86 million US adults (37%) have prediabetes (3). Among adults in China, the prevalence of prediabetes was about 35.7% (4). Adolescent boys and girls in India have a 12.3% prediabetes prevalence rate (5). Moreover, there is evidence that the prevalence of prediabetes in Korea is as high as 38.3% (6). People with prediabetes have an increased risk of T2DM, with approximately 5-10% of people developing T2DM each year, and up to 70% of them will eventually develop T2DM according to the American Diabetes Association (ADA) expert panel (3, 7). Nevertheless, it is worth noting that some patients with prediabetes do not progress to diabetes but remain in the prediabetic stage, and 20%-50% of individuals with prediabetes may even regress to normoglycemia (8–10). In addition, prediabetes increases the risk of not only T2DM but also cardiovascular disease and microvascular complications (11–13). Previous research has suggested that reversion to normoglycemia, even briefly, is related to a significantly decreased risk of development of T2DM in patients with prediabetes (14). Thus, the clinical benefits of reversion from prediabetes to normoglycemia cannot be overemphasized. The goal of prediabetes screening and treatment should be to revert normoglycemia.

Given that most of the attention on the clinical side seems to be focused on disease progression, finding contributing factors for prediabetes regression to normoglycemia is equally or more important to indicate pathways for prevention and actionable targets for sustaining public health efforts. Unfortunately, few studies have been conducted to determine the rate of reversion to normoglycemia in people with prediabetes and which contributing factors are associated with this. Preliminary evidence from previous epidemiological studies suggests that regression to normoglycemia is associated with factors such as age, baseline fasting glucose, insulin secretion, obesity, beta-cell function, fasting triglycerides, etc (3, 15–18). Studies have shown that an increase in body mass index (BMI) is positively associated with the risk of progression from prediabetes to diabetes (19, 20). However, there is limited research into the relationship between BMI and regression to normoglycemia from prediabetes. A cohort study revealed that an increase in delta-BMI (baseline BMI minus BMI at follow-up) was negatively associated with the likelihood of returning to normoglycemia in participants with prediabetes (21). Another study from Korea showed that in older adults, even modest weight loss helped to return from prediabetes to normoglycemia (22).

Regrettably, neither study performed subgroup analyses nor explored the non-linear relationship between BMI and regression to normoglycemia from prediabetes. Besides, the current study is limited by the small sample size. The link between BMI and reversion to normoglycemia has not yet been widely explored among Chinese adults. Furthermore, given that patients with diabetes at follow-up are no longer likely to regress from prediabetes to euglycemia, observation of the possibility of reversal of prediabetes to euglycemic events or the occurrence of altered events may be hampered. However, no research has attempted to investigate the relationship between them using the competing risk model. Therefore, based on the fact that obesity is a high-risk factor for diabetes, we propose the hypothesis that there may be a negative association between BMI and the likelihood of reversal of prediabetes to normoglycemia in the Chinese population, and that a non-linear relationship between them cannot be excluded. We conducted a retrospective cohort study using published Chinese population-based data to test this hypothesis.

Abbreviations: IFG, impaired fasting glucose; IGT, impaired glucose tolerance; BMI, body mass index; TC, total cholesterol; TG, triglyceride; BUN, blood urea nitrogen; HDL-c, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; LDL-c, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; Scr, serum creatinine; FPG, fasting plasma glucose; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; DBP, diastolic blood pressure; IDF, International Diabetes Federation; GAM, generalized additive model; HR, hazard ratio; Ref, reference; CI, confidence interval.

Methods

Study design

This study used a retrospective cohort study design, and the data were obtained from a retrospective cohort study previously undertaken by Chinese researchers (Chen et al.) from a computerized database in China (23). The target-independent variable was BMI at baseline. The outcome variable was reversion to normoglycemia from prediabetes at follow-up.

Data source

The raw data were obtained free of charge from DATADRYAD (www.datadryad.org) and provided by Ying Chen et al. With reference to the terms of service of the Dryad database, the dataset can be used by researchers to be able to share, remix, modify and create derivative works for non-commercial purposes, as long as the author and source are credited. Data information was obtained from an open access article published in 2018 – "Association of body mass index and age with diabetes onset in Chinese adults: a population-based cohort study" (http://dx.doi.org/10.1136/bmjopen-2018-021768). The data can be downloaded at: https://doi.org/10.5061/dryad.ft8750v (23).

Study population

The initial researchers took information from a computerized database created by the Rich Healthcare Group in China. This

database contains all medical records of participants who underwent a health check from 2010 to 2016, spanning 32 regions and 11 cities in China. The Rich Healthcare Group Review Board initially approved the original study, and the information was retrieved retrospectively. For the retrospective study, no informed consent or approval was required by the institutional ethics committee (23). Therefore, the current secondary analysis did not require ethical approval. Additionally, the initial study was conducted in accordance with the Helsinki Declaration (23). So did this secondary analysis.

The original study enrolled 685,277 participants older than 20 who passed at least two health examinations. 473,444 participants meeting the exclusion criteria were excluded. The following were the original study's exclusion criteria: The original study's exclusion criteria were as follows: (i) participants with a visit interval of less than two years; (ii) participants with extreme BMI values (15 kg/m^2 or > 55 kg/m^2); (iii) participants with no information about weight, height, sex, and fasting plasma glucose(FPG) value at baseline; (iv) participants with diabetes at enrollment; and (v) participants whose diabetes status at follow-up was unknown. Finally, the analysis of the initial study comprised a total of 211,833 people (23). In the current study, we first further included 26,018 participants with baseline FPG of 5.6-6. 9 mmol/L. We then excluded participants with missing FPG information at follow-up (n = 12) as well as those with abnormal and extreme BMI (three standard deviations greater or less than three standard deviations from the mean) (n = 132). Finally, the current study included 25,874 people in total. The procedure for choosing participants is shown in Figure 1. It is important to highlight that according to the American Diabetes Association 2022 criteria, prediabetes is defined as the presence of IFG (FPG level of 5.6–6.9 mmol) and/or IGT and/or hemoglobin A1c(HA1c) (24). Our definition of prediabetes is therefore based on FPG. To make the study more accurate, our study population is reported as patients with IFG.

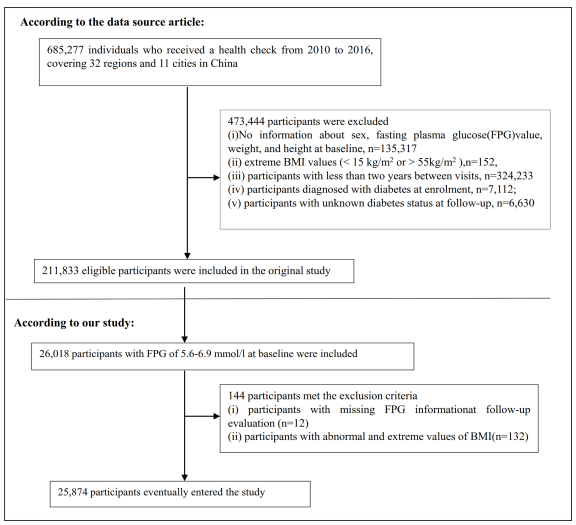


FIGURE 1
Flowchart of study participants.

Variables

Body mass index

BMI was recorded as a continuous variable. The detailed procedure for defining BMI was as follows: BMI = weight/height^2 (kg/m^2). It was important to note that relevant information for height and weight was obtained at baseline. The categories of obesity (BMI≥ 28 kg/m^2), overweight (24≤ BMI < 28 kg/m^2), and normal weight (BMI< 24 kg/m^2) were established according to the definition put forth by the Working Group on Obesity in China (25).

Outcome measures

The occurrences of reversion to normoglycemia were our intriguing outcome variable. Reversion to normoglycemia was based on FPG<5.6mmol/l at follow-up evaluation and the absence of self-reported incident diabetes (26, 27).

Covariates

The covariates in our study were selected based on the original study, previous studies having a correlation to diabetes or prediabetes, and our clinical expertise (18, 22, 23, 28, 29). Covariates included the following variables: (i)categorical variables: sex, smoking status, family history of diabetes, and drinking status. (ii) continuous variables: weight, height, age, serum creatinine (Scr), systolic blood pressure (SBP), aspartate aminotransferase (AST), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), diastolic blood pressure (DBP), alanine aminotransferase (ALT), total cholesterol (TC), blood urea nitrogen (BUN), low-density lipid cholesterol (LDL-c).

Data collection

In the original study, professional researchers used standard questionnaires to gather baseline data on drinking, smoking, and family history of diabetes. Standard mercury sphygmomanometers measured blood pressure. During each visit, fasting venous blood samples were taken at least 10 hours after a fast. A Beckman 5800 autoanalyzer was used to measure plasma glucose, HDL-c, TC, LDL-c, BUN, TG, AST, ALT, and Scr. The time to regression to normoglycemia or progression to diabetes was based on when participants returned for one or more physical examinations.

Missing data processing

In current study, the number of participants whose data are missing of DBP, SBP, TC, ALT, TG, BUN, Scr, LDL-c, HDL-c, AST, drinking status, and smoking status was 7(0.03%), 7(0.03%), 605 (2.34%),232(0.90%), 607(2.35%),2840(10.98%), 1334(5.16%), 9897 (38.25%), 10527(40.69%), 14629(56.54%), 17139(66.24%), and 17139(66.24%), respectively. This study used multiple imputations for missing data to reduce the volatility brought on by missing variables. SBP, age, ALT, sex, LDL-c, DBP, AST, TG, Scr, HDL-c, BUN, TC, drinking status, family history of diabetes, and smoking status were all included in the imputation model (iterations were 10; the type of regression was linear). Missing-at-random (MAR) assumptions are used in missing data analysis processes (30, 31).

Statistical analysis

We divided the individuals into three categories based on the World Health Organization's BMI values for Chinese patients: "normal", "overweight", and "obesity". The means and standard deviations were presented for continuous variables with Gaussian distributions, medians were reported for skewed distributions, and frequencies and percentages were presented for categorical variables. We used the Kruskal-Wallis H test (skewed distribution), the One-Way ANOVA test (normal distribution), or $\chi 2$ (categorical variables) to test for differences among different BMI groups.

Following collinearity screening, we used univariate and multivariate Cox proportional-hazards regression models to examine the relationship between BMI and the reversion rate to normoglycemia in individuals with IFG, including a crude model with no covariates adjusted, a model with just minimal covariates adjusted (Model I with adjusted sex and age), and a model with full covariate adjustments (Model II: adjusted DBP, age, sex, SBP, AST, BUN, ALT, LDL-c, Scr, TG, HDL-c, family history of diabetes, drinking status, and smoking status). Effect sizes (HR) with 95% confidence intervals (CI) were recorded. We adjusted for confounding factors based on clinical experience, literature reports, and the results of univariate analysis. Additionally, the final multivariate Cox proportional hazards regression equation did not include TC since it was collinear with other variables (Supplementary Table S1).

Besides, the Cox proportional hazards regression model with cubic spline functions and smooth curve fitting were performed to account for the nonlinear relationship between BMI and reversion to normoglycemia in participants with IFG. Furthermore, a two-piecewise Cox proportional hazards regression model was used to clarify the nonlinear association between BMI and reversion from IFG to normoglycemia. Finally, a log-likelihood ratio test was performed to choose the best model to explain the association between them in patients with IFG. Considering that patients who experience diabetes at follow-up are no longer likely to recover from IFG to normoglycemia, this may hinder the observation of prediabetes reversal to normoglycemia events or alter the likelihood of events occurring (32, 33). Therefore, competing risks multivariate Cox proportional-hazards regression was performed, as described by Fine and Gray, with progression to diabetes as the competing risk for the reversal to normoglycemia events (33, 34).

Using a stratified Cox proportional hazard regression model, subgroup analyses were performed across various groupings (age, sex, SBP, DBP, smoking status, and drinking status). First, based on clinical cut-off points, continuous data, such as SBP and age, were transformed into categorical variables (age: 30, 30 to 40, 40 to 50, 50 to 60, 60 to 70, 70 years old; SBP: 140, 140 mmHg) (35). In addition to the stratification factor itself, we adjusted each stratification for DBP, age, SBP, sex, Scr, ALT, HDL-c, AST, BUN, TG, LDL-c, drinking status, family history of diabetes, and smoking status. Ultimately, in models with and without interaction terms, the likelihood ratio test was employed to identify whether there were interaction terms or not.

To check the reliability of the findings, we ran a series of sensitivity analyses. Previous studies have suggested that drinking status, TG, and family history of diabetes are significantly related to glucose metabolism (36–38). We also conducted further sensitivity analyses to examine the connection between BMI and reversion to normoglycemia in prediabetic patients. Firstly, we performed a sensitivity analysis on participants who had never consumed alcohol (n=21,010). We also performed a sensitivity analysis after excluding patients with a family history of diabetes (n=25,244). In addition, we further explored the relationship between BMI and reversion to normoglycemia in participants with TG<1.7mmol/L (N= 15,858). The continuity covariate was also incorporated into the equation as a curve using a generalized additive model (GAM) to confirm the reliability of the findings. We also calculated E-values to examine the possibility of unmeasured confounding between BMI and reversion from IFG to normoglycemia (39).

All results were written in accordance with the STROBE statement (40). Empower Stats (X&Y Solutions, Inc., Boston, MA, http://www.empowerstats.com) and R statistical software packages (http://www.r-project.org, The R Foundation) were used for all analyses. Statistical significance was set at P values lower than 0.05 (two-sided). Supplementary Figure S1 showed the analytical framework for this study.

Results

Characteristics of participants

The study participants' demographic and clinical characteristics are presented in Table 1. The mean age was 49.07 ± 13.82 years old, and 17,168 (66.35%) were male. The median follow-up time was 3.05 years, and 11,856 (45.82%) participants had a final reversion to normoglycemia. BMI presents a normal distribution, ranging from 15.2 to 34.9kg/m^2, with a mean of 24.74kg/m^2 (Figure 2). We assigned adults into subgroups based on Chinese criteria for BMI categories (normal: < 24, overweight: 24-28, obesity: ≥28kg/m^2). Compared with the normal group, age, height, weight, DBP, SBP, TG, LDL-c, TC, AST, ALT, Scr, and BUN increased significantly in the obesity group, whereas the opposite results were found in the HDL-c covariates. In addition, the proportion of men, current smokers, and current drinkers was higher in the obesity group.

Baseline characteristics according to regression and progression status of patients with IFG are shown in Supplementary Table S2. Participants who progressed to diabetes had significantly higher levels of age, height, weight, BMI, DBP, SBP, TG, LDL-c, TC, AST, ALT, Scr, and BUN than participants with persistent IFG but significantly lower levels of HDL-c. Besides, compared with participants with persistent IFG, age, height, weight, BMI, DBP, SBP, TG, LDL-c, TC, AST, ALT, Scr, and BUN decreased significantly in participants who reverted to normoglycemia, whereas the opposite results were found in the HDL-c covariates.

The reversal rate to normoglycemia from IFG

In participants with IFG, 11,856 individuals developed diabetes. The overall rate of reversion to normoglycemia was 155.33 per 1000 person-years. In particular, the reversal rate to normoglycemia among

TABLE 1 The baseline characteristics of participants.

BMI groups (kg/m^2)	normal (<24)	overweight (24-28)	obesity (≥28)	P-value
participants	10688	11014	4172	
Sex				<0.001
Male	5892 (55.13%)	8107 (73.61%)	3169 (75.96%)	
Female	4796 (44.87%)	2907 (26.39%)	1003 (24.04%)	
SBP (mmHg)	123.15 ± 17.18	128.62 ± 17.05	133.33 ± 17.34	<0.001
DBP (mmHg)	75.42 ± 10.47	79.58 ± 10.87	82.75 ± 11.40	<0.001
Age(years)	47.40 ± 14.37	50.68 ± 13.19	49.12 ± 13.50	<0.001
Height(cm)	165.73 ± 8.39	167.22 ± 8.17	167.80 ± 8.36	<0.001
Weight(kg)	59.79 ± 7.84	72.24 ± 7.78	84.26 ± 9.60	<0.001
BMI (kg/m^2)	21.69 ± 1.67	25.77 ± 1.12	29.84 ± 1.59	<0.001
AST(U/L)	23.78 ± 9.56	27.14 ± 11.72	31.25 ± 15.72	<0.001
HDL-c(mmol/L)	1.40 ± 0.31	1.30 ± 0.28	1.24 ± 0.29	<0.001
TC (mmol/L)	4.85 ± 0.95	5.04 ± 0.95	5.12 ± 0.97	<0.001
TG (mmol/L)	1.39 ± 1.11	1.98 ± 1.58	2.28 ± 1.60	<0.001
LDL-c(mmol/L)	2.81 ± 0.72	2.92 ± 0.71	2.97 ± 0.74	<0.001
ALT(U/L)	17.20 (13.00-24.30)	24.40 (17.70-35.90	32.00 (22.00-50.00)	<0.001
Scr (μmol/L)	70.05 ± 16.13	74.56 ± 15.67	75.10 ± 15.75	<0.001
BUN (mmol/L)	4.86 ± 1.24	5.08 ± 1.24	5.07 ± 1.25	<0.001
Drinking status				<0.001
Current drinker	257 (2.40%)	490 (4.45%)	218 (5.23%)	
Ever drinker	1200 (11.23%)	1879 (17.06%)	820 (19.65%)	
Never	9231 (86.37%)	8645 (78.49%)	3134 (75.12%)	
Smoking status				<0.001
Current smoker	1796 (16.80%)	2896 (26.29%)	1210 (29.00%)	
Ever smoker	379 (3.55%)	537 (4.88%)	214 (5.13%)	
Never	8513 (79.65%)	7581 (68.83%)	2748 (65.87%)	
Family history of diabetes				0.342
No	10419 (97.48%)	10763 (97.72%)	4062 (97.36%)	
Yes	269 (2.52%)	251 (2.28%)	110 (2.64%)	
Follow up-times(years)	2.90 (2.09-3.77)	2.78 (2.10-3.15)	3.03 (2.26-3.98)	<0.001

Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as a percentage (%).
DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol, SBP, systolic blood pressure; TG triglyceride, BMI, body mass index; AST aspartate aminotransferase; LDL-c, low-density lipid cholesterol; ALT, alanine aminotransferase; BUN, blood urea nitrogen; HDL-c, high-density lipoprotein cholesterol; Scr, serum creatinine.

participants with IFG of BMI groups was normal group:186.84, overweight group:139.14, and obesity group:119.25 per 1000 person-years, respectively. The overall cumulative reversal rate of IFG to normoglycemia was 45.82% over a median follow-up period of 3.05 years. The cumulative reversal rate in each BMI group was normal group:54.55%, overweight group: 41.38%, and obesity group: 35.19% (Figure 3). Participants with higher BMI had a significantly lower reversal rate than those with a lower BMI (p<0.001 for trend) (Table 2, Figure 3).

In the age stratification by ten intervals, the rate of reversion to normoglycemia among participants with IFG was higher in women than in men, regardless of their age group (Figure 4). It was also found that the reversal rate decreased with age in both men and women.

Factors influencing reversion to normoglycemia among participants with IFG analyzed by univariate Cox proportional hazards regression

Univariate analyses showed that reversion to normoglycemia in patients with IFG was negatively correlated with age, BMI, DBP,

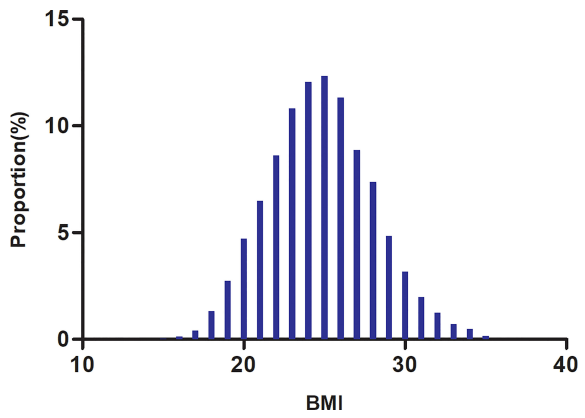


FIGURE 2
Distribution of BMI. It presented a normal distribution, ranging from 15.2 to 34.9kg/m^2, with a mean of 24.74kg/m^2.

SBP, ALT, AST, TG, TC, LDL-c, BUN, and family history of diabetes but was positively related to HDL-c, never smoking and never drinking (all P<0.05; Table 3).

Figure 5 showed the Kaplan-Meier curves for the probability of reversion to normoglycemia from IFG stratified by BMI category. The probability of reversal to normoglycemia from IFG varied significantly between BMI groups (log-rank test, p<0.001). The probability of reversion to normoglycemia decreased progressively with rising BMI, meaning that patients with the highest BMI had the lowest probability of reverting from IFG to normoglycemia. Supplementary Figure S2 presented Kaplan-Meier survival curves for diabetes-free survival probability. Among BMI groups, there were statistically significant differences in the probability of diabetes-free survival (log-rank test, p<0.001). IFG patients with the greatest BMI had the highest risk of progression to diabetes.

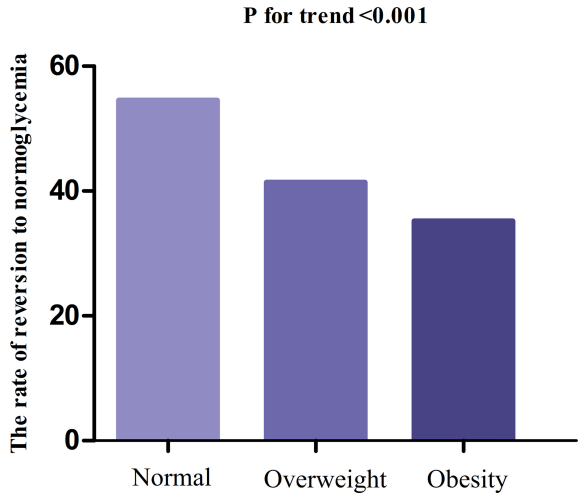


FIGURE 3
The rate of reversion to normoglycemia in people with IFG stratified by BMI. Participants with higher BMI had a significantly lower reversal rate than those with a lower BMI (p<0.001 for trend).

The relationship between BMI and reversion to normoglycemia from IFG analyzed by multivariate Cox proportional-hazards regression model

We constructed three models using the Cox proportional-hazards regression model to investigate the association between BMI and reversion to normoglycemia in patients with IFG. In the crude model, a 1kg/m^2 increase in BMI was associated with a 6.5% decrease in the probability of reversion to normoglycemia (HR=0.935,95%CI 0.930-0.940, p<0.001). In the minimally-adjusted model, when we adjusted for population variables only, each 1kg/m^2 increase in BMI was associated with a 4.6% lower probability of reversion to normoglycemia (HR=0.954, 95%CI 0.949-0.960, p<0.001). The HR between BMI and reversion to normoglycemia from IFG was 0.977 (95% CI: 0.971-0.984, p<0.001) in the fully adjusted model. The distribution of confidence intervals suggested that the link between BMI and reversion to normoglycemia among patients with IFG obtained by the model was reliable (Table 4).

Besides, we transformed BMI from a continuous variable to a categorical variable and then reintroduced the categorically transformed BMI into the model. The results of the multivariate-adjusted model showed that with reference to participants with normal BMI, the HR was 0.901(95%CI:0.863-0.939) for overweight participants and 0.831(95%CI:0.780-0.886) for obese participants. That is, compared with participants with normal BMI(<24kg/m^2), overweight participants (BMI:24-28kg/m^2) with prediabetes had a 9.9% lower probability of returning to normoglycemia, while obese patients (BMI ≥ 28kg/m^2) had a 16.9% decreased probability of reverting from IFG to normoglycemia (Table 4 Model II).

The results of competing risks multivariate Cox proportional-hazards regression

When progression to incident diabetes from IFG was treated as a competing event, the competing analysis results were shown in Table 5. In the crude model, BMI was negatively related to the probability of reversion to normoglycemia (SHR=0.93, 95% CI:0.93-0.94). In the minimally adjusted model (model I: adjusted age, sex), the result did not have a noticeable change (SHR:0.95, 95% CI: 0.95-0.96, p<0.001). In the fully adjusted model (model II) (adjusted age, sex, SBP, DBP ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status), we could also detect a negative association between BMI and reversion to normoglycemia (SHR=0.92, 95%CI: 0.89-0.96). In addition, when BMI was used as a categorical variable, multivariate-adjusted model (fully adjusted model) results showed that overweight participants with IFG had a 10% lower probability of returning to normoglycemia compared with participants with normal BMI (SHR=0.90, 95%CI: 0.86-0.94), while obese patients had a 17.0% decreased probability of reverting from IFG to normoglycemia compared with patients with normal BMI (SHR=0.83, 95%CI: 0.78-0.89).

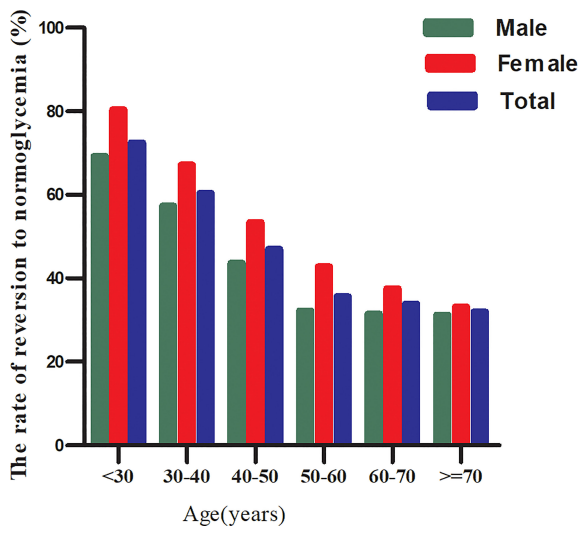


FIGURE 4
The rate of reversion to normoglycemia in IFG patients of age stratification by 10 intervals. Figure showed that the rate of reversion to normoglycemia among participants with IFG was higher in women than in men, regardless of their age group. It was also found that the reversal rate decreased with age in both men and women.

Sensitivity analysis

A series of sensitivity analyses were performed to ensure that our findings were robust. We first introduced the continuity covariate as a curve into the equation using a GAM. As shown in Table 4, the outcome of Model III was consistent with the fully adjusted model. Referring to patients with IFG with normal BMI, obese patients had a 14.5% lower probability of reverting to normoglycemia (HR = 0.855, 95% CI: 0.802-0.912).

Furthermore, we conducted a sensitivity analysis on participants who had never consumed alcohol (n = 21,010). After adjusting for confounding variables (including DBP, age, SBP, sex, TG, ALT, AST, LDL-c, BUN, Scr, HDL-c, family history of diabetes, and smoking status), the findings indicated that BMI was also negatively linked with reversion to normoglycemia from IFG (HR=0.979, 95%CI:0.972-0.986, p<0.001). We also excluded patients with a family history of diabetes for the sensitivity analyses (n=25,244). After adjusting for confounding variables (including BUN, sex, AST, SBP, age, DBP, HDL-c, ALT, Scr, LDL-c, TG, drinking status, and smoking status), the results suggested that BMI was still negatively associated with reversion to normoglycemia from IFG (HR=0.977, 95% CI:0.971-0.984, p<0.001). Besides restricting the analysis to participants with TG<1.7mmol/L (adjusted for age, sex, SBP, DBP, ALT, AST, BUN, Scr, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status), the results suggested that the HR between BMI and probability of reverting to normoglycemia was 0.981 (95% CI:0.973-0.989, P<0.001). Similarly, when BMI was used as a categorical variable, sensitivity analyses of multivariate-adjusted models showed a significantly lower probability of recovery from IFG to normoglycemia in overweight and obese patients compared with participants with normal BMI (Table 6). Based on all the sensitivity analyses, it is evident that our findings were robust. We also calculated an E-value to evaluate the sensitivity to unmeasured confounding. Unknown or unmeasured variables likely had little impact on the association between BMI and recovery from IFG to normoglycemia, as the E-value (1.53) was greater than the relative risk of BMI and unmeasured confounders (1.36).

Cox proportional hazards regression model with cubic spline functions to address nonlinearity

Using a Cox proportional hazards regression model with cubic spline functions, we found that the correlation between BMI and the probability of reversal to normoglycemia in patients with IFG was nonlinear (Figure 6). Additionally, using a standard binary two-piecewise Cox proportional-hazards regression model to fit the data, we chose the model that best fit the data using the log-likelihood ratio test (Table 7). Less than 0.05 was the P-value for the log-likelihood ratio test. By using the recursive technique, we first established the 21.7 kg/m^2 as the BMI inflection point. Next, we utilized a two-piecewise Cox proportional hazards regression model to get the HR and CI for either side of the inflection point. Before the inflection point, the HR was 1.000 (95% CI: 0.978, 1.022, P=0.979), which was not statistically significant, and after the inflection point, the HR was 0.972 (95% CI: 0.964-0.980).

Results of subgroup analysis

In all prespecified or exploratory subgroups assessed (Table 8), sex, age, smoking status, SBP, and alcohol consumption did not

TABLE 2 The rate of reversion to normoglycemia in people with IFG (% or Per 1000 person-year).

BMI Group	Participants(n)	Reversion events(n)	Reversal rate (95% CI) (%)	Per 1000 person-year
Total	25874	11856	45.82(45.21-46.23)	155.33
Normal	10688	5830	54.55(53.60-55.49)	186.84
Overweight	11041	4558	41.38(40.46-42.30)	139.14
Obesity	4172	1468	35.19(33.74-36.64)	119.25
P for trend			<0.001	

BMI, body mass index; CI, confidence interval,

TABLE 3 Factors influencing reversion to normoglycemia among participants with IFG analyzed by univariate Cox proportional hazards regression.

Variable	Characteristics	HR (95% CI)	P-value
Age (years)	49.071 ± 13.818	0.976 (0.975, 0.978)	<0.001
Sex			
Male	17168 (66.352%)	Ref	
Female	8706 (33.648%)	1.267 (1.221, 1.315)	<0.001
BMI (kg/m^2)	24.742 ± 3.251	0.935 (0.930, 0.940)	<0.001
SBP (mmHg)	127.120 ± 17.546	0.990 (0.988, 0.991)	<0.001
DBP (mmHg)	78.371 ± 11.129	0.985 (0.983, 0.987)	<0.001
TC (mmol/L)	4.972 ± 0.957	0.880 (0.863, 0.898)	<0.001
TG (mmol/L)	1.787 ± 1.450	0.891 (0.877, 0.905)	<0.001
HDL-c(mmol/L)	1.331 ± 0.304	1.612 (1.523, 1.706)	<0.001
LDL-c(mmol/L)	2.882 ± 0.722	0.919 (0.896, 0.943)	<0.001
ALT (U/L)	28.363 ± 23.334	0.993 (0.992, 0.994)	<0.001
AST (U/L)	26.413 ± 11.954	0.987 (0.985, 0.989)	<0.001
BUN (mmol/L)	4.986 ± 1.248	0.955 (0.941, 0.969)	<0.001
Scr (μmol/L)	72.784 ± 16.039	0.997 (0.996, 0.998)	<0.001
Smoking status			
Current smoker	5902 (22.811%)	Ref	<0.001
Ever smoker	1130 (4.367%)	1.062 (0.963, 1.171) 0.227	<0.001
Never	18842 (72.822%)	1.255 (1.200, 1.313)	<0.001
Drinking status			
Current drinker	965 (3.730%)	Ref	<0.001
Ever drinker	3899 (15.069%)	1.260 (1.123, 1.413)	<0.001
Never	21010 (81.201%)	1.394 (1.253, 1.551)	<0.001
Family history of diabetes			
No	25244 (97.565%)	Ref	
Yes	630 (2.435%)	0.753 (0.666, 0.852)	< 0.001

Continuous variables were summarized as mean (SD) or medians (quartile interval); categorical variables were displayed as percentage (%).
DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol, SBP, systolic blood pressure; TG triglyceride, BMI, body mass index; AST aspartate aminotransferase; LDL-c, low-density lipid cholesterol; ALT, alanine aminotransferase; BUN, blood urea nitrogen; HDL-c, high-density lipoprotein cholesterol; Scr, serum creatinine.

modify the relationship between BMI and reversion to normoglycemia from IFG. That is, there was no significant interaction between these factors and BMI ($P > 0.05$ for interaction).

Discussion

This retrospective cohort study was designed to examine the link between BMI and reversion to normoglycemia in patients with IFG. We found that the increase in BMI was related to a significantly decreased probability of regression to normoglycemia. A significantly lower probability of reversal from IFG to normoglycemia in overweight and obese patients compared with participants with normal BMI. In addition, a threshold effect curve was discovered, and on both sides of the inflection point, different associations between BMI and reversion to normoglycemia can be identified.

A prospective cohort study of 491 participants showed that during a median follow-up of 2.5 years, 22.6% of participants with prediabetes returned to normoglycemia (41). Results from another study found that one year after the start of follow-up, 54% of participants with prediabetes had returned to normoglycemia, and 6% had developed diabetes (17). Besides, in another cohort study from China, including 14,231 Chinese adults, 44.9% of patients with prediabetes reverted to normoglycemia within 2 years (42). Our study showed that 45.82% of IFG patients returned to normoglycaemia during the 5-year follow-up period. Variations in the rate of reversion to normoglycemia from prediabetes between studies may be attributable to changes in participant age, follow-up

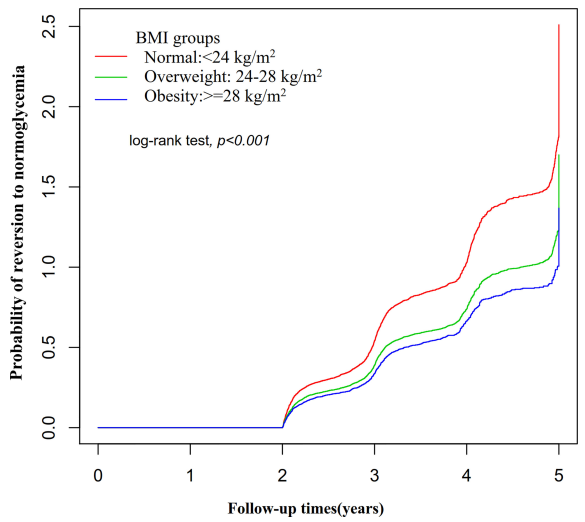


FIGURE 5
Kaplan-Meier curves for the probability of reversion to normoglycemia from IFG. Figure showed the Kaplan-Meier curves for the probability of reversion to normoglycemia from IFG stratified by BMI category. The probability of reversion to normoglycemia decreased progressively with rising BMI, meaning that Patients with the highest BMI had the lowest probability of reverting from IFG to normoglycemia.

length, and ethnicity. It is important to note that all studies have confirmed that a sizable fraction of persons with prediabetes reverts to normoglycemia. Therefore, finding the contributing factors for the reversion to normoglycemia from prediabetes is particularly important for the prevention of diabetes and its complications.

There have been many findings in the past suggesting that elevated BMI is associated with a higher risk of developing diabetes (43–46). In people with prediabetes, increased BMI was also positively associated with the risk of developing diabetes (19). In addition, weight gain is also a high-risk risk factor for prediabetes (21). Several studies have demonstrated that BMI follows a positive dose-response relationship with the risk of prediabetes (47–49). Therefore, we hypothesized that a reduction in BMI may be associated with an increased probability of regression to normoglycemia from prediabetes. Unfortunately, there are few reports on the relationship between them. A study found that a 1 kg/m^2 increase in delta-BMI (BMI follow-up baseline) was related to a 28% decrease in the odds ratio (OR) for regression to normoglycemia in subjects with prediabetes. Results from another study suggested that Each 5.3 kg/m^2 increase in BMI was associated with a 6% reduction in the probability of returning to normoglycemia in patients with prediabetes during a median follow-up of 2.5 years (HR=0.94, 95% CI: 0.91–0.98) (41). Our study complemented the existing literature, which supported the hypothesis that elevated BMI was associated with a reduced probability of reversal to normoglycemia in patients with prediabetes. Compared with other studies, the independent variables in our study used both BMI as a categorical variable and a continuous variable of BMI to explore its relationship with reversion to normoglycemia from prediabetes, which reduced the loss of information and quantified their relationship. In addition, the covariates adjusted for our study were different from those of the previous studies. We adjusted more parameters, including drinking status, smoking status, ALT, AST, and LDL-c. Evidence showed that those parameters were associated with the development of diabetes (50–53). Meanwhile, the sensitivity analysis found that this relationship still exists among participants with TG < 1.7 mmol/l, no family history of diabetes, and never alcohol consumption. Furthermore, we applied a competing risk multivariate Cox regression analysis model, and the results were consistent with those of a multivariate Cox proportional hazards regression model. The results mentioned above have confirmed the relationship stability between BMI and reversion to normoglycemia in patients with IFG. This finding provides a reference for the clinical intervention of BMI levels to increase the probability of reversal to normoglycemia in patients with IFG. It is worth noting that this study addressing nonlinearity is a great improvement compared to previous studies.

The mechanism underlying the inverse relationship between BMI and reversion to normoglycemia in patients with IFG remains unclear, but it may be associated with insulin resistance(IR). Research has confirmed that IR plays a crucial role in the regression and progression of prediabetes (27). In addition, evidence shows that BMI is independently positively related to indices of IR and negatively related to β-cell function adjusted for IR (54).

TABLE 4 Relationship between BMI and reversion to normoglycemia in patients with IFG in different models.

Exposure	Crude model (HR,95%CI)	Model I(HR,95%CI) P	Model II(HR,95%CI) P	Model III(HR,95%CI) P
BMI (kg/m^2)	0.935 (0.930, 0.940) <0.001	0.954 (0.949, 0.960) <0.001	0.977 (0.971, 0.984) <0.001	0.982 (0.976, 0.989) <0.001
BMI Group				
Normal	Ref	Ref	Ref	Ref
Overweight	0.715 (0.688, 0.743) <0.001	0.809 (0.777, 0.842) <0.001	0.901 (0.863, 0.939) <0.001	0.928 (0.888, 0.969) <0.001
Obesity	0.618 (0.584, 0.654) <0.001	0.678 (0.640, 0.719) <0.001	0.831 (0.780, 0.886) <0.001	0.855 (0.802, 0.912) <0.001
P for trend	<0.001	<0.001	<0.001	<0.001

Crude model: we did not adjust other covariates.
Model I: we adjusted age, sex.
Model II: we adjusted age, sex, SBP, DBP ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status.
Model III: we adjusted age(smooth), sex, SBP (smooth), DBP (smooth), Scr(smooth), TG (smooth), ALT(smooth), AST(smooth), LDL-c(smooth), HDL-c(smooth), smoking status, drinking status, family history of diabetes.
HR, Hazard ratios; CI, confidence, Ref, reference.

TABLE 5 Relationship between BMI and reversion to normoglycemia in patients with IFG in different models with competing risk of progression to diabetes.

Exposure	Crude model (SHR,95%CI, P)	Model I(SHR,95%CI, P)	Model II (SHR,95%CI, P)
BMI (kg/m^2)	0.93 (0.93, 0.94) <0.001	0.95 (0.95, 0.96) <0.001	0.98 (0.97, 0.98) <0.001
BMI Group			
Normal	Ref.	Ref.	Ref.
Overweight	0.77 (0.74, 0.81) <0.001	0.81 (0.78, 0.84) <0.001	0.90 (0.86, 0.94) <0.001
Obesity	0.66 (0.63, 0.69) <0.001	0.68 (0.64, 0.72) <0.001	0.83 (0.78, 0.89) <0.001
P for trend	<0.001	<0.001	<0.001

Crude model: we did not adjust other covariates.
Model I: we adjust age, sex,
Model II: we adjust age, sex, SBP, DBP ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status.
SHR, subdistribution hazard ratios; CI, confidence, Ref, reference

Furthermore, this study utilized a model based on a two-piecewise Cox proportional hazards regression to shed light on the nonlinear connections. The findings demonstrated a nonlinear link and threshold effect between BMI and reversion to normoglycemia from IFG. The inflection point of BMI was 21.7kg/m^2 after adjusting for confounders. There was no significant association between elevated BMI and reversal of normoglycemia in IFG patients when BMI was below 21.7 kg/m^2. However, when BMI was greater than 21.7kg/m^2, the probability of reversal to normoglycemia decreased by 2.8% for every 1kg/m^2 increase in BMI. That is to say, as the BMI of patients with IFG decreases, the probability of reversal to normoglycemia will gradually increase, but when it drops to about 21.7kg/m^2, the probability of reversal to normoglycemia will not continue to increase and remain stable. The possible reason for the non-linear association between BMI and reversion to normoglycemia in patients with prediabetes is that the risk of IR decreases as BMI decreases, but the risk of IR does not continue to decrease when BMI decreases to a certain extent (54). In addition, studies have confirmed that skeletal muscle plays an important role in glucose metabolism. It is one of the major components of insulin-mediated glucose metabolism. Maintaining and increasing skeletal muscle mass can improve IR (55). An excessively low BMI is often accompanied by reduced skeletal muscle mass, reduced insulin sensitivity and abnormalities in glucose and fatty acid metabolism (56, 57). Therefore, the reason that the probability of reversal to normoglycemia does not continue to increase with a decrease in BMI to 21.7 kg/m^2 may be that a decrease in skeletal muscle mass offsets the benefits of a continued decrease in BMI. Excellent clinical value can be derived from the finding that BMI and reversion to normoglycemia in patients with prediabetes have a curvilinear relationship. It promotes clinical consultation and offers a reference for decision-making that is optimized for diabetes prevention. The population with prediabetes is at much higher risk not only for T2DM but also for cardiovascular disease and all-cause mortality (58, 59). Previous research has demonstrated that even a brief recovery to normoglycemia is associated with a significantly decreased risk of developing T2DM in patients with prediabetes (14). As a result, prediabetes should be treated, and the goal should be regression to normoglycemia rather than only preventing the potential impacts of prediabetes and lowering the likelihood of advancement to T2DM. Lifestyle interventions including diet and exercise have been demonstrated to be useful in the prevention and treatment of prediabetes and T2DM (60). Our study establishes a BMI threshold for the reversion to normoglycemia in Chinese persons with IFG. That is, controlling BMI around 21.7 kg/m^2 through dietary interventions

TABLE 6 Relationship between BMI and the probability of reverting from IFG to normoglycemia in different sensitivity analyses.

Exposure	Model I(HR,95%CI) P	Model II(HR,95%CI) P	Model III(HR,95%CI) P
BMI (kg/m^2)	0.981 (0.973, 0.989) <0.00001	0.977 (0.971, 0.984) <0.00001	0.979 (0.972, 0.986) <0.00001
BMI Group			
Normal	Ref	Ref	Ref
Overweight	0.900 (0.855, 0.947) 0.00005	0.901 (0.863, 0.940) <0.00001	0.911 (0.869, 0.954) 0.00008
Obesity	0.875 (0.803, 0.955) 0.00267	0.832 (0.780, 0.887) <0.00001	0.849 (0.791, 0.912) <0.00001
P for trend	0.921 (0.887, 0.957) 0.00002	0.909 (0.882, 0.937) <0.00001	0.918 (0.889, 0.949) <0.00001

Model I was a sensitivity analysis performed after excluding participants with TG≥1.7 mmol/L (N= 15858). We adjusted age, sex, SBP, DBP, ALT, AST, BUN, Scr, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status.
Model II was a sensitivity analysis performed on participants without a family history of diabetes (N= 25244). We adjusted age, sex, SBP, DBP, ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, drinking status, and smoking status.
Model III was a sensitivity analysis performed on participants who had never consumed alcohol (N= 21010). We adjusted age, sex, SBP, DBP, ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, family history of diabetes, and smoking status.
HR, Hazard ratios; CI, confidence, Ref, reference.

10.3389/fendo.2023.1111791

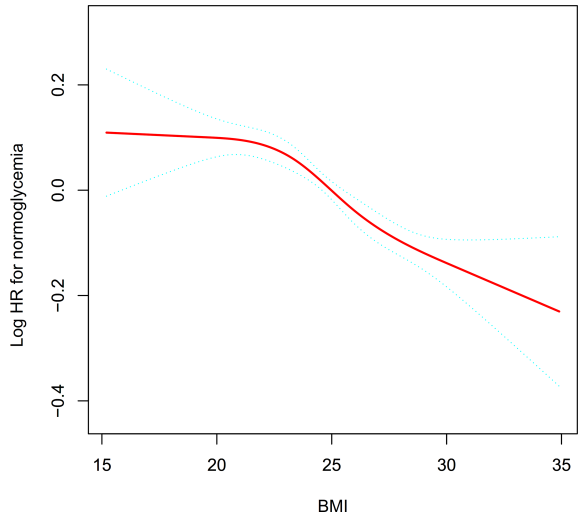


FIGURE 6
The nonlinear relationship between BMI and reversion to normoglycemia in patients with IFG. The result showed that the relationship between BMI and reversion to normoglycemia from IFG was nonlinear, with the inflection point of BMI being 21.7kg/m^2.

TABLE 7 The result of two-piecewise linear regression model.

Outcome: reversion to normoglycemia	HR, 95%CI	P-value
Fitting model by standard Cox regression	0.977 (0.971, 0.984)	<0.001
Fitting model by two-piecewise Cox regression		
Inflection points of BMI(Kg/m^2)	21.7	
< **21.7** kg/m^2	1.000 (0.978, 1.022)	0.979
≥**21.7** kg/m^2	0.972 (0.964, 0.980)	<0.001
P for log-likelihood ratio test	0.035	

and lifestyle changes may significantly increase the probability of reversion to normoglycemia.

This study has several strengths worth mentioning. (i) The non-linear association between BMI and recovery from prediabetes to normoglycemia, and the identification of inflection points, are important findings of this study. (iii) To deal with the missing data,

TABLE 8 Stratified associations between BMI and reversion to normoglycemia in patients with IFG by age, sex, SBP, DBP, smoking status, and drinking status.

Characteristic	No of participants	HR (95%CI)	P value	P for interaction
Age(years)				0.2341
<30	1584	0.996 (0.980, 1.012)	0.6072	
30 to <40	6246	0.993 (0.983, 1.003)	0.1636	
40 to <50	5679	0.967 (0.954, 0.979)	<0.0001	
50 to <60	6015	0.967 (0.953, 0.982)	<0.0001	
60 to <70	4293	0.960 (0.943, 0.977)	<0.0001	
≥70	2057	0.972 (0.949, 0.996)	0.0234	
Sex				0.3776
Male	17168	0.974 (0.966, 0.982)	<0.0001	
Female	8706	0.983 (0.973, 0.992)	0.0004	
SBP (mmHg)				0.1509
<140	20404	0.976 (0.969, 0.983)	<0.0001	
≥140	5470	0.984 (0.970, 0.999)	0.0316	
Drinking status				0.732
Current drinker	965	0.961 (0.927, 0.996)	0.0309	
Ever drinker	3899	0.972 (0.957, 0.987)	0.0004	
Never	21010	0.979 (0.972, 0.986)	<0.0001	
Smoking status				0.1727
Current smoker	5902	0.980 (0.967, 0.993)	0.0034	
Ever smoker	1130	0.950 (0.923, 0.977)	0.0004	
Never	18842	0.978 (0.971, 0.985)	<0.0001	

Above model adjusted for age, sex, SBP, DBP ALT, AST, BUN, Scr, TG, LDL-c, HDL-c, family history of diabetes, drinking status, and smoking status.
In each case, the model is not adjusted for the stratification variable.
HR, Hazard ratios; CI, confidence, Ref, reference.

we used a multiple imputation approach. This approach allows for maximum statistical power while minimizing bias due to missing covariate information. (iv) A series of sensitivity analyses were conducted to ensure the reliability of the findings. In addition, we performed a multivariate Cox proportional hazards regression model of competing risks, taking into account prediabetes development to diabetes as the competing risk for reversion to normoglycemia event.

The following are some possible limitations of this study. First, as the participants in the study were all Chinese, more investigation is needed to determine the association between BMI and return to normoglycemia in people with prediabetes with different genetic backgrounds. Second, IFG does not fully define prediabetes, however, measuring 2-hour oral glucose tolerance tests and HbA1C is difficult for such a large study cohort. In the future we will conduct our study or collaborate with others as we try to collect information on 2-hour oral glucose tolerance tests and HbA1C levels. Third, this study is based on a secondary analysis of published data; therefore, it is impossible to adjust variables not included in the original dataset, such as insulin concentration and waist circumference. However, we calculated the E-value to quantify the potential impact of unmeasured confounders and found that unmeasured confounders were unlikely to explain the results. In addition, this *post hoc* observational investigation established an association inference between BMI and regression of normoglycemia in patients with IFG rather than a causal one. Finally, the BMI and other parameters were only evaluated at baseline in the current study, and their variations over time were not considered. In the future, we can also think about structuring our studies or working with other researchers to get as many data points as we can, such as details on how BMI changes over the course of patient follow-up.

Conclusion

This study showed that BMI was independently associated with regression to normoglycemia in Chinese adults with IFG and that there was a specific non-linear relationship and threshold effect between them. There was a significant negative correlation between BMI and the likelihood of returning to normoglycemia from IFG when BMI was greater than 21.7 kg/m^2. Minimizing BMI to 21.7 kg/m^2 in patients with IFG may significantly increase the probability of returning to normoglycemia.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by The rich healthcare group review board. The ethics committee waived the requirement of written informed consent for participation.

Author contributions

YH, and ZH contributed to the study design and drafted the manuscript. HH and YH are responsible for statistical analysis, research, and interpretation of the data. They are responsible for the data's integrity and the data analysis's accuracy. HH and DL contributed to the discussion and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The following research provides the majority of the data and methodology for this secondary analysis: Chen, Ying et al. (2018), Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study (23). The study's authors deserve our gratitude.

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.2023.1111791/full#supplementary-material

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9(th) edition. *Diabetes Res Clin Pract* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843

2. Cho NH, Shaw JE, Karuranga S, Huang Y, Da RFJ, Ohlrogge AW, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* (2018) 138:271–81. doi: 10.1016/j.diabres.2018.02.023

3. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet* (2012) 379:2279–90. doi: 10.1016/S0140-6736(12)60283-9

4. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. *Jama* (2017) 317:2515–23. doi: 10.1001/jama.2017.7596

5. Kumar P, Srivastava S, Mishra PS, Mooss E. Prevalence of prediabetes/type 2 diabetes among adolescents (10-19 years) and its association with different measures of overweight/obesity in India: a gendered perspective. *BMC Endocr Disord* (2021) 21:146. doi: 10.1186/s12902-021-00802-w

6. Jeon JY, Ko SH, Kwon HS, Kim NH, Kim JH, Kim CS, et al. Prevalence of diabetes and prediabetes according to fasting plasma glucose and HbA1c. *Diabetes Metab J* (2013) 37:349–57. doi: 10.4093/dmj.2013.37.5.349

7. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes Care* (2007) 30:753–59. doi: 10.2337/dc07-9920

8. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* (2002) 346:393–403. doi: 10.1056/NEJMoa012512

9. Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, et al. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* (2006) 368:1096–105. doi: 10.1016/S0140-6736(06)69420-8

10. Busquets-Cortés C, Bennasar-Veny M, López-González ÁA, Fresneda S, Abbate M, Yáñez AM. Utility of fatty liver index to predict reversion to normoglycemia in people with prediabetes. *PloS One* (2021) 16:e249221. doi: 10.1371/journal.pone.0249221

11. Perreault L, Pan Q, Schroeder EB, Kalyani RR, Bray GA, Dagogo-Jack S, et al. Regression from prediabetes to normal glucose regulation and prevalence of microvascular disease in the diabetes prevention program outcomes study (DPPOS). *Diabetes Care* (2019) 42:1809–15. doi: 10.2337/dc19-0244

12. Huang Y, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. *Bmj* (2016) 355:i5953. doi: 10.1136/bmj.i5953

13. Tandon N, Ali MK, Narayan KM. Pharmacologic prevention of microvascular and macrovascular complications in diabetes mellitus: implications of the results of recent clinical trials in type 2 diabetes. *Am J Cardiovasc Drugs* (2012) 12:7–22. doi: 10.2165/11594650-000000000-00000

14. Perreault L, Pan Q, Mather KJ, Watson KE, Hamman RF, Kahn SE. Effect of regression from prediabetes to normal glucose regulation on long-term reduction in diabetes risk: results from the diabetes prevention program outcomes study. *Lancet* (2012) 379:2243–51. doi: 10.1016/S0140-6736(12)60525-X

15. Perreault L, Kahn SE, Christophi CA, Knowler WC, Hamman RF. Regression from prediabetes to normal glucose regulation in the diabetes prevention program. *Diabetes Care* (2009) 32:1583–88. doi: 10.2337/dc09-0523

16. Herman WH, Pan Q, Edelstein SL, Mather KJ, Perreault L, Barrett-Connor E, et al. Impact of lifestyle and metformin interventions on the risk of progression to diabetes and regression to normal glucose regulation in overweight or obese people with impaired glucose regulation. *Diabetes Care* (2017) 40:1668–77. doi: 10.2337/dc17-1116

17. Bodicoat DH, Khunti K, Srinivasan BT, Mostafa S, Gray LJ, Davies MJ, et al. Incident type 2 diabetes and the effect of early regression to normoglycaemia in a population with impaired glucose regulation. *Diabetes Med* (2017) 34:396–404. doi: 10.1111/dme.13091

18. Hwang YC, Cho IJ, Jeong IK, Ahn KJ, Chung HY. Factors associated with regression from prediabetes to normal glucose tolerance in a Korean general population: A community-based 10-year prospective cohort study. *Diabetes Med* (2018) 35:1544–51. doi: 10.1111/dme.13789

19. Hu H, Kawasaki Y, Kuwahara K, Nakagawa T, Honda T, Yamamoto S, et al. Trajectories of body mass index and waist circumference before the onset of diabetes among people with prediabetes. *Clin Nutr* (2020) 39:2881–88. doi: 10.1016/j.clnu.2019.12.023

20. Haghighatdoost F, Amini M, Feizi A, Iraj B. Are body mass index and waist circumference significant predictors of diabetes and prediabetes risk: Results from a population based cohort study. *World J Diabetes* (2017) 8:365–73. doi: 10.4239/wjd.v8.i7.365

21. Nakasone Y, Miyakoshi T, Sato Y, Yamauchi K, Hashikura R, Takayama M, et al. Impact of weight gain on the evolution and regression of prediabetes: a quantitative analysis. *Eur J Clin Nutr* (2017) 71:206–11. doi: 10.1038/ejcn.2016.118

22. Kowall B, Rathmann W, Kuss O, Herder C, Roden M, Stang A, et al. Reversion from prediabetes to normoglycaemia after weight change in older persons: The KORA F4/FF4 study. *Nutr Metab Cardiovasc Dis* (2021) 31:429–38. doi: 10.1016/j.numecd.2020.09.008

23. Chen Y, Zhang XP, Yuan J, Cai B, Wang XL, Wu XL, et al. Association of body mass index and age with incident diabetes in Chinese adults: a population-based cohort study. *BMJ Open* (2018) 8:e21768. doi: 10.1136/bmjopen-2018-021768

24. 2. classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* (2022) 45:S17–38. doi: 10.2337/dc22-S002

25. Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults–study on optimal cut-off points of body mass index and waist circumference in Chinese adults. *BioMed Environ Sci* (2002) 15:83–96. doi: 10.1046/j.1440-6047.11.s8.9.x

26. 2. classification and diagnosis of diabetes: Standards of medical care in diabetes-2021. *Diabetes Care* (2021) 44:S15–33. doi: 10.2337/dc21-S002

27. Lazo-Porras M, Bernabe-Ortiz A, Ruiz-Alejos A, Smeeth L, Gilman RH, Checkley W, et al. Regression from prediabetes to normal glucose levels is more frequent than progression towards diabetes: The CRONICAS cohort study. *Diabetes Res Clin Pract* (2020) 163:107829. doi: 10.1016/j.diabres.2019.107829

28. Sun Y, Wang Z, Huang Z, Hu H, Han Y. The association between the triglyceride-to-High-Density lipoprotein cholesterol ratio and the risk of progression to diabetes from prediabetes: A 5-year cohort study in Chinese adults. *Front Endocrinol (Lausanne)* (2022) 13:947157. doi: 10.3389/fendo.2022.947157

29. An R, Ma S, Zhang N, Lin H, Xiang T, Chen M, et al. AST-to-ALT ratio in the first trimester and the risk of gestational diabetes mellitus. *Front Endocrinol (Lausanne)* (2022) 13:1017448. doi: 10.3389/fendo.2022.1017448

30. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med* (2011) 30:377–99. doi: 10.1002/sim.4067

31. Groenwold RH, White IR, Donders AR, Carpenter JR, Altman DG, Moons KG. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *Cmaj* (2012) 184:1265–69. doi: 10.1503/cmaj.110977

32. Noordzij M, Leffondré K, van Stralen KJ, Zoccali C, Dekker FW, Jager KJ. When do we need competing risks methods for survival analysis in nephrology? *Nephrol Dial Transplant* (2013) 28:2670–77. doi: 10.1093/ndt/gft355

33. Basak R, Mistry H, Chen RC. Understanding competing risks. *Int J Radiat Oncol Biol Phys* (2021) 110:636–40. doi: 10.1016/j.ijrobp.2021.01.008

34. Solbak NM, Al RA, Akawung AK, Lo SG, Kirkpatrick SI, Robson PJ. Strategies to address misestimation of energy intake based on self-report dietary consumption in examining associations between dietary patterns and cancer risk. *Nutrients* (2019) 11:2614. doi: 10.3390/nu11112614

35. Song X, Zhang W, Hallensleben C, Versluis A, van der Kleij R, Jiang Z, et al. Associations between obesity and multidimensional frailty in older Chinese people with hypertension. *Clin Interv Aging* (2020) 15:811–20. doi: 10.2147/CIA.S234815

36. Beshara A, Cohen E, Goldberg E, Lilos P, Garty M, Krause I. Triglyceride levels and risk of type 2 diabetes mellitus: a longitudinal large study. *J Investig Med* (2016) 64:383–87. doi: 10.1136/jim-2015-000025

37. Knott C, Bell S, Britton A. Alcohol consumption and the risk of type 2 diabetes: A systematic review and dose-response meta-analysis of more than 1.9 million individuals from 38 observational studies. *Diabetes Care* (2015) 38:1804–12. doi: 10.2337/dc15-0710

38. Wagner R, Thorand B, Osterhoff MA, Müller G, Böhm A, Meisinger C, et al. Family history of diabetes is associated with higher risk for prediabetes: a multicentre analysis from the German center for diabetes research. *Diabetologia* (2013) 56:2176–80. doi: 10.1007/s00125-013-3002-1

39. Burne RM, Abrahamowicz M. Adjustment for time-dependent unmeasured confounders in marginal structural cox models using validation sample data. *Stat Methods Med Res* (2019) 28:357–71. doi: 10.1177/0962280217726800

40. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg* (2014) 12:1495–99. doi: 10.1016/j.ijsu.2014.07.013

41. Sevilla-González M, Merino J, Moreno-Macias H, Rojas-Martínez R, Gómez-Velasco DV, Manning AK. Clinical and metabolomic predictors of regression to normoglycemia in a population at intermediate cardiometabolic risk. *Cardiovasc Diabetol* (2021) 20:56. doi: 10.1186/s12933-021-01246-1

42. Liu X, Wu S, Song Q, Wang X. Reversion from prediabetes mellitus to normoglycemia and risk of cardiovascular disease and all-cause mortality in a Chinese population: A prospective cohort study. *J Am Heart Assoc* (2021) 10:e19045. doi: 10.1161/JAHA.120.019045

43. Tang ML, Zhou YQ, Song AQ, Wang JL, Wan YP, Xu RY. The relationship between body mass index and incident diabetes mellitus in Chinese aged population: A cohort study. *J Diabetes Res* (2021) 2021:5581349. doi: 10.1155/2021/5581349

44. Xu H, Kuja-Halkola R, Chen X, Magnusson P, Svensson P, Carrero JJ. Higher body mass index is associated with incident diabetes and chronic kidney disease independent of genetic confounding. *Kidney Int* (2019) 95:1225–33. doi: 10.1016/j.kint.2018.12.019

45. Guo Z, Liu L, Yu F, Cai Y, Wang J, Gao Y, et al. The causal association between body mass index and type 2 diabetes mellitus-evidence based on regression discontinuity design. *Diabetes Metab Res Rev* (2021) 37:e3455. doi: 10.1002/dmrr.3455

46. Zhao Q, Laukkanen JA, Li Q, Li G. Body mass index is associated with type 2 diabetes mellitus in Chinese elderly. *Clin Interv Aging* (2017) 12:745–52. doi: 10.2147/CIA.S130014

47. Li CL, Chen SY, Lan C, Pan WH, Chou HC, Bai YB, et al. The effects of physical activity, body mass index (BMI) and waist circumference (WC) on glucose intolerance in older people: a nationwide study from Taiwan. *Arch Gerontol Geriatr* (2011) 52:54–9. doi: 10.1016/j.archger.2010.01.020

48. Li S, Xiao J, Ji L, Weng J, Jia W, Lu J, et al. BMI and waist circumference are associated with impaired glucose metabolism and type 2 diabetes in normal weight Chinese adults. *J Diabetes Complications* (2014) 28:470–76. doi: 10.1016/j.jdiacomp.2014.03.015

49. Chai Y, Liu Y, Yang R, Kuang M, Qiu J, Zou Y. Association of body mass index with risk of prediabetes in Chinese adults: A population-based cohort study. *J Diabetes Investig* (2022) 13:1235–44. doi: 10.1111/jdi.13783

50. Niu H, Zhou Y. Nonlinear relationship between AST-to-ALT ratio and the incidence of type 2 diabetes mellitus: A follow-up study. *Int J Gen Med* (2021) 14:8373–82. doi: 10.2147/IJGM.S341790

51. van de Wiel A. Diabetes mellitus and alcohol. *Diabetes Metab Res Rev* (2004) 20:263–67. doi: 10.1002/dmrr.492

52. Maddatu J, Anderson-Baucum E, Evans-Molina C. Smoking and the risk of type 2 diabetes. *Transl Res* (2017) 184:101–07. doi: 10.1016/j.trsl.2017.02.004

53. Bonilha I, Hajduch E, Luchiari B, Nadruz W, Le Goff W, Sposito AC. The reciprocal relationship between LDL metabolism and type 2 diabetes mellitus. *Metabolites* (2021) 11:807. doi: 10.3390/metabo11120807

54. Okura T, Nakamura R, Fujioka Y, Kawamoto-Kitao S, Ito Y, Matsumoto K, et al. Body mass index ≥23 is a risk factor for insulin resistance and diabetes in Japanese people: A brief report. *PloS One* (2018) 13:e201052. doi: 10.1371/journal.pone.0201052

55. Sylow L, Tokarz VL, Richter EA, Klip A. The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. *Cell Metab* (2021) 33:758–80. doi: 10.1016/j.cmet.2021.03.020

56. Kim JH, Park YS. Low muscle mass is associated with metabolic syndrome in Korean adolescents: the Korea national health and nutrition examination survey 2009-2011. *Nutr Res* (2016) 36:1423–28. doi: 10.1016/j.nutres.2016.09.013

57. Lee SW, Youm Y, Lee WJ, Choi W, Chu SH, Park YR, et al. Appendicular skeletal muscle mass and insulin resistance in an elderly korean population: the korean social life, health and aging project-health examination cohort. *Diabetes Metab J* (2015) 39:37–45. doi: 10.4093/dmj.2015.39.1.37

58. Wang H, Ba Y, Cai RC, Xing Q. Association between diabetes mellitus and the risk for major cardiovascular outcomes and all-cause mortality in women compared with men: a meta-analysis of prospective cohort studies. *BMJ Open* (2019) 9:e24935. doi: 10.1136/bmjopen-2018-024935

59. Beulens J, Rutters F, Rydén L, Schnell O, Mellbin L, Hart HE, et al. Risk and management of pre-diabetes. *Eur J Prev Cardiol* (2019) 26:47–54. doi: 10.1177/2047487319880041

60. Braga T, Kraemer-Aguiar LG, Docherty NG, Le Roux CW. Treating prediabetes: why and how should we do it? *Minerva Med* (2019) 110:52–61. doi: 10.23736/S0026-4806.18.05897-4

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 31 May 2023
DOI 10.3389/fendo.2023.1089298

OPEN ACCESS

EDITED BY
Andile Khathi,
University of KwaZulu-Natal, South Africa

REVIEWED BY
Antonio Brunetti,
Magna Græcia University, Italy
Subhadip Choudhuri,
University of Texas Medical Branch at Galveston, United States

*CORRESPONDENCE
Melva Louisa
melva.louisa@gmail.com

RECEIVED 04 November 2022
ACCEPTED 08 May 2023
PUBLISHED 31 May 2023

CITATION
Krisnamurti DGB, Louisa M, Poerwaningsih EH, Tarigan TJE, Soetikno V, Wibowo H and Nugroho CMH (2023) Vitamin D supplementation alleviates insulin resistance in prediabetic rats by modifying IRS-1 and PPARγ/NF-κB expressions.
Front. Endocrinol. 14:1089298.
doi: 10.3389/fendo.2023.1089298

Vitamin D supplementation alleviates insulin resistance in prediabetic rats by modifying IRS-1 and PPARγ/NF-κB expressions

Desak Gede Budi Krisnamurti[1], Melva Louisa[2]*, Erni H. Poerwaningsih[1], Tri Juli Edi Tarigan[3], Vivian Soetikno[2], Heri Wibowo[4] and Christian Marco Hadi Nugroho[5]

[1]Department of Medical Pharmacy, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [3]Division of Endocrinology and Metabolism, Department of Internal Medicine, Dr. Cipto Mangunkusumo National Referral Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [4]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [5]Research and Development Unit, Medika Satwa Laboratories, Bogor, Indonesia

Background: Prediabetes is a condition of intermediate hyperglycemia that may progress to type 2 diabetes. Vitamin D deficiency has been frequently linked to insulin resistance and diabetes. The study aimed to investigate the role of D supplementation and its possible mechanism of action on insulin resistance in prediabetic rats.

Method: The study was conducted on 24 male Wistar rats that were randomly divided into 6 rats as healthy controls and 18 prediabetic rats. Prediabetic rats were induced with a high-fat and high-glucose diet (HFD-G) combined with a low dose of streptozotocin. Rats with the prediabetic condition were then randomized into three groups of 12-week treatment: one group that received no treatment, one that received vitamin D3 at 100 IU/kg BW, and one group that received vitamin D3 at 1000 IU/kg BW. The high-fat and high-glucose diets were continuously given throughout the twelve weeks of treatment. At the end of the supplementation period, glucose control parameters, inflammatory markers, and the expressions of IRS1, PPARγ, NF-κB, and IRS1 were measured.

Results: Vitamin D3 dose-dependently improves glucose control parameters, as shown by the reduction of fasting blood glucose (FBG), oral glucose tolerance test (OGTT), glycated albumin, insulin levels, and markers of insulin resistance (HOMA-IR). Upon histological analysis, vitamin D supplementation resulted in a reduction of the islet of Langerhans degeneration. Vitamin D also enhanced the ratio of IL-6/IL-

10, reduced IRS1 phosphorylation at Ser307, increased expression of PPAR gamma, and reduced phosphorylation of NF-KB p65 at Ser536.

Conclusion: Vitamin D supplementation reduces insulin resistance in prediabetic rats. The reduction might be due to the effects of vitamin D on IRS, PPARγ, and NF-κB expression.

KEYWORDS

diabetes mellitus, high-fat diet, 25-hydroxyergocalciferol, inflammation, insulin resistance

Introduction

Prediabetes is when an individual has above-average blood sugar levels but does not yet match the diagnostic criteria for diabetes. Prediabetes is not a disease in and of itself but rather an indicator of future health problems, including diabetes and cardiovascular disease (1). The World Health Organization instead called it "Intermediate Hyperglycemia." At the same time, the American Diabetes Association referred to it as a "High-Risk State of Developing Diabetes" (2). Prediabetes is characterized by hyperinsulinemia which leads to insulin resistance. Eventually, chronic hyperinsulinemia will lead to beta cell dysfunction and favor the development of type 2 diabetes mellitus (2–4). Several strategies have been suggested for preventing diabetes in the prediabetic population. However, many few have been proven effective. No pharmacological intervention has been used explicitly to treat insulin resistance (4–6).

Recently, studies have linked vitamin D deficiency to diabetes pathogenesis (7–9). Several studies have shown that vitamin D deficiency may have a role in insulin resistance, yet the findings are still controversial. In some *in-vivo* and clinical studies, the lack of vitamin D levels has been associated with increased insulin resistance and impaired insulin production (7, 10–12). Vitamin D is suggested to promote insulin sensitivity and optimizes the activity of beta cells through several pathways. Vitamin D directly affects pancreatic beta cells by activating beta-cell calcium-dependent endopeptidases to release insulin (10, 12, 13).

In addition to vitamin D deficiency, a high-fat diet and sedentary lifestyle may produce adipocyte hypertrophy and hyperplasia, which aggravates hyperglycemia and hyperinsulinemia (14, 15). In a previous study in a mouse model with a high-fat diet, inflammatory insulin signaling markers were dysregulated. Chronic high fat intake will then be attributed to the development of insulin resistance (16).

Multiple studies have consistently shown reduced serum 25OHD concentrations in diabetic individuals. An analysis of the collective findings coming from multiple studies conducted to investigate the effectiveness of vitamin D supplementation in preventing type 2 diabetes revealed that compared to placebo, vitamin D supplementation reduced the risk of developing type 2 diabetes in people with prediabetes (17, 18). Despite the encouraging benefits of vitamin D, vitamin D supplementation in prediabetic and diabetic individuals has shown inconclusive outcomes in several studies (10, 19–21). Vitamin D supplementation's mechanism of inhibiting insulin resistance in prediabetes has yet to be well understood. Thus, in the present study, we aimed to investigate the effect and mechanism of vitamin D supplementation in prediabetic rats on a high-fat, high-glucose diet.

Methods

Animals and treatments

The Health Research Ethics Committee of the Faculty of Medicine at Universitas Indonesia authorized this study (KET.701/UN2.F1/ETIK/PPM.00.02/2020). The experiments were carried out on male Wistar rats weighing 150–200 grams. The rats were acclimatized for 1 week in the Animal Research Facilities before the experiment. Six of the 24 rats were fed a standard diet (TestDiet™ 5012, Richmond, USA). 18 of the 24 rats were given a high-fat diet (TestDiet™ 58V8 rat chow, Richmond, USA) along with 20% glucose (HFD-G) in their drinking water to induce prediabetes. After three weeks, the rats in the high-fat, high-glucose groups were injected with 30 mg/kg BW streptozotocin. Seventy-two hours after streptozotocin injection, the rats were tested for oral glucose tolerance test (OGTT), fasting blood glucose (FBG), and 2-hour postprandial glucose (2H-PPG) concentrations. To confirm prediabetes conditions, all the rats had to meet 2 out of the 3 criteria: FBG of 100–125 mg/dL, OGTT of 140–199 mg/dL, and 2H-PPG prior to treatment randomization. The prediabetic rats were then randomly assigned to one of three groups of six: HFD-G + vehicle; HFD-G+ vitamin D3 100 IU/kg BW/day; or HFD-G+ vitamin D3 1000 IU/kg BW/day. The treatments were given for 12 weeks. The rat group given a standard diet continued to receive the same diet for an additional 12 weeks. At the end of the experiment, rats were sacrificed, blood samples were taken for biochemical testing, liver samples were used for western blot analysis, and pancreatic tissues were removed and fixed in 10% formal saline for histopathological analysis.

Serum biochemical analysis

The current study measured blood glucose using serum rather than plasma. Even though serum produced lower values than plasma, the difference was not physiologically significant (22). The blood

glucose concentrations were tested shortly after the blood was drawn. Blood glucose concentrations were quantified from serum samples on a Randox Glucose GOD-PAP GL 364 (Randox, UK) colorimetric kit. Blood glucose levels were calculated using the glucose oxidase technique described by Randox Laboratories Ltd (Ardmore, UK).

Histological analysis

Pancreatic tissue samples were collected, dissected, and immediately fixed in 10% formalin for 24 hours, dehydrated using a graded alcohol series, cleaned in xylene, and finally embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) for histopathological analysis (23). All areas were viewed using an OLYMPUS CX43 light microscope using a 400x magnification and shot with an OLYMPUS SC52 camera. The area of the Islet of Langerhans was counted using ImageJ, and two blind histopathologists examined all histological anomalies. The histological state of the pancreas was evaluated and then compared across the various treatment groups for damage and regeneration of pancreatic islet cells.

Enzyme-linked immunoassay

The levels of insulin, glycated albumin, TNF-α, 25-hydroxycholecalciferol, IL-6, and IL-10 were quantified using enzyme-linked immunoassay kits according to the manufacturer's instructions. Rat INS (Insulin) (Cat# ERINS), IL-6 (Cat# BMS625), and IL-10 (Cat# BMS629) ELISA kits were purchased from Thermo Scientific; rat glycated Albumin (Cat# No MBS1600353) and IRS1 (Cat No MBS9501484) ELISA kit from MyBioSource and 25-hydroxycholecalciferol (Cat No CSB-EL006431HV) ELISA kit from Cusabio.

Western blot analysis

Proteins were isolated from liver tissue homogenates using 1x RIPA buffer. Moreover, the protein concentration was determined using a Coomassie Plus (Bradford) assay kit on a microplate reader spectrophotometer at 590 nm. The isolate was used for western blot analysis of protein expressions of NF-κB p65, PPARγ, and p-IRS1.

Primary antibodies used in the present study were obtained from Cell Signaling Technology (Beverly, MA): GAPDH (CST#2118), NF-κB p65 (CST#8242), phospho-NF-κB p65 (CST#3033), PPARγ (CST#2430), phospho-PPARγ (CST#2430), IRS1 (CST#2382), and p-IRS1 (Ser307) (CST#2381). Subsequently, 70 μg proteins were separated using 10% SDS-PAGE and transferred to a PVDF membrane. The quantity of protein used in the study corresponds with the study by Soetikno V. et al. (24). Blocking the membrane was done for 1.5 hours with 5% skimmed milk in phosphate buffer saline with Tween-20. After blocking, the membrane was incubated overnight at 4°C with a 1:1,000 dilution for all primary antibodies. Afterward, the membranes were washed in Tris-buffered saline with Tween-20 and incubated for 1 hour with secondary antibodies against Anti-rabbit IgG, HRP-linked Antibody (CST#7074) at a 1:5,000 dilution rate. Enhanced chemiluminescence (ECL) detection system reagents, Clarity Western (BioRad), were used to examine the targeted protein bands. ImageJ was used to evaluate the densitometry data (version 1.53a; National Institutes of Health). The bands presented were taken from the best acquisition and time in the ChemiDoc Imaging instrument (Biorad™).

Statistical analysis

GraphPad Prism 9.4.1 software was used for the statistical analysis (GraphPad Software, Inc). The data were presented in the mean and standard error of the mean (SEM). Comparison between groups was analyzed using one-way ANOVA followed by Tukey's *post hoc* test. A statistically significant difference was one with a p-value of less than 0.05.

Results

The effects of vitamin D supplementation in prediabetic rats

The baseline serum 25-hydroxyvitamin-D3 (25-OH-D3) concentrations were measured before 12-week vitamin D3 supplementation. The results showed that the 25-OH-D3 average baseline levels in all four groups were below 30 μg/L, which indicates insufficient levels (Figure 1A). In prediabetic rats with no treatment, the 25-OH-D3 concentrations tend to decrease after twelve weeks. However, vitamin D3 supplementation may prevent the decrease of serum 25-OH-D3 levels in prediabetic rats given 100 IU/kg BW. Moreover, in prediabetic rats given 1000 IU/kg BW, there was a slight increase in serum 25-OH-D3 levels (Figure 1B).

Hyperglycemia and hyperinsulinemia were shown in prediabetic rats compared to the control group. The status of insulin resistance was shown in HOMA-IR, and there was a substantial increase in HOMA-IR compared to the control group. Supplementation of vitamin D3 to prediabetic rats resulted in a considerable reduction in glucose control parameters and glycated albumin. As shown in HOMA-IR, insulin resistance was significantly decreased compared to the prediabetic group (Figure 2).

Histopathology examinations of healthy control rat pancreas confirmed the islets of Langerhans' regular shape. The prediabetic rat group induced with a high-fat diet showed pathological changes and cellular damage in the islets of Langerhans. Fat accumulation in pancreatic acinar cells is associated with pancreatic fibrosis and acinar cell damage. The pancreas of prediabetic rats also showed shrinkage of the islets of Langerhans, necrosis, and degeneration of the cells' components (Figure 3A).

The supplementation of vitamin D3 at 100 IU/kg BW and 1000 IU/kg BW may minimize the damage in Langerhans's islet and fatty pancreatic acinar cell atrophy. The pancreas of rats receiving vitamin D treatment has a virtually regular shape. The size of the islets of Langerhans is virtually restored to normal, while the

10.3389/fendo.2023.1089298

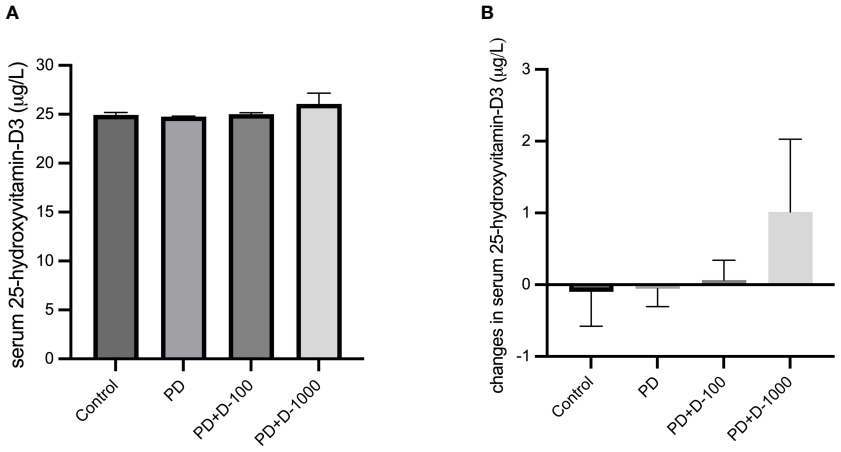


FIGURE 1
(A) Serum 25-hydroxy-vitamin D3 levels at the start of the treatment period; **(B)** changes in serum 25-hydroxy-vitamin D3 levels in healthy control or prediabetic rats after 12 weeks with no treatment or vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day.

number of fatty acinar cells is reduced (Figure 3A). As demonstrated in Figure 3B, there was a lower area of Langerhans islets in prediabetic rats compared to the control group. However, vitamin D3 supplementation tended to increase the area of Langerhans islets.

Reduction of IRS-1 phosphorylation after vitamin D supplementation in prediabetic rats

There was a significant reduction in IRS1 concentrations in prediabetic groups compared to healthy control. However, vitamin D3 supplementation in prediabetic rats did not change the IRS1 concentrations (Figure 4A). Nevertheless, we observed a slight decrease in the phosphorylation of IRS1 after vitamin D3 supplementation at 100 IU/kg BW and 1000 IU/kg BW in the prediabetic group (Figure 4B).

Modulation of serum inflammatory markers after vitamin D supplementation in prediabetic rats

The modulation of serum inflammatory markers in control, prediabetic, and prediabetic groups treated with vitamin D3 100 IU/

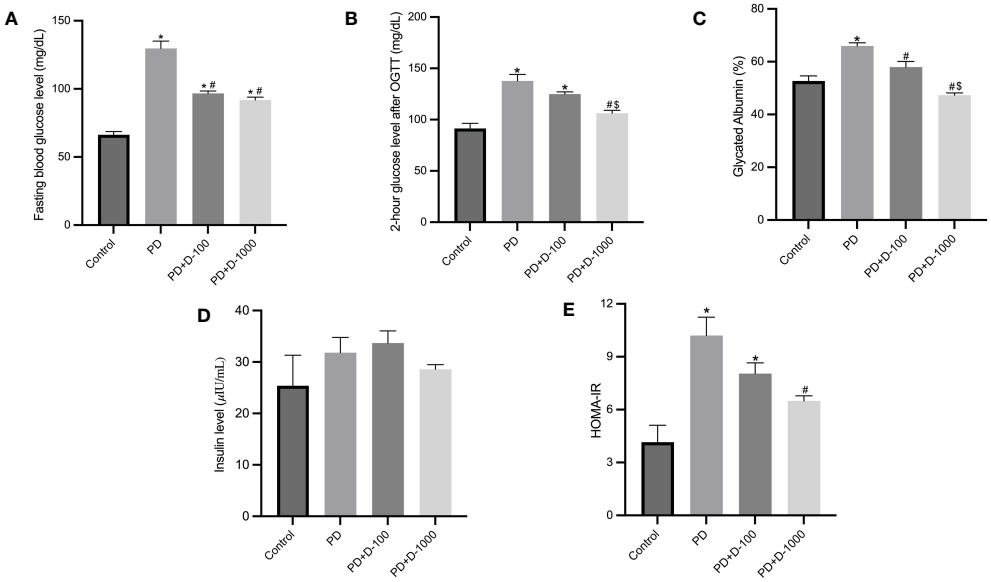


FIGURE 2
Markers of glucose control and insulin resistance in healthy control or prediabetic rats after 12 weeks with no treatment of vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day. **(A)** Fasting blood glucose; **(B)** 2-hour glucose level after oral glucose tolerance test (OGTT); **(C)** glycated albumin; **(D)** insulin level; **(E)** HOMA-IR. *: p<0.05 vs control; #: p<0.05 vs PD group; $: p<0.05 vs vitamin D-100 IU/kg BW/day group.

10.3389/fendo.2023.1089298

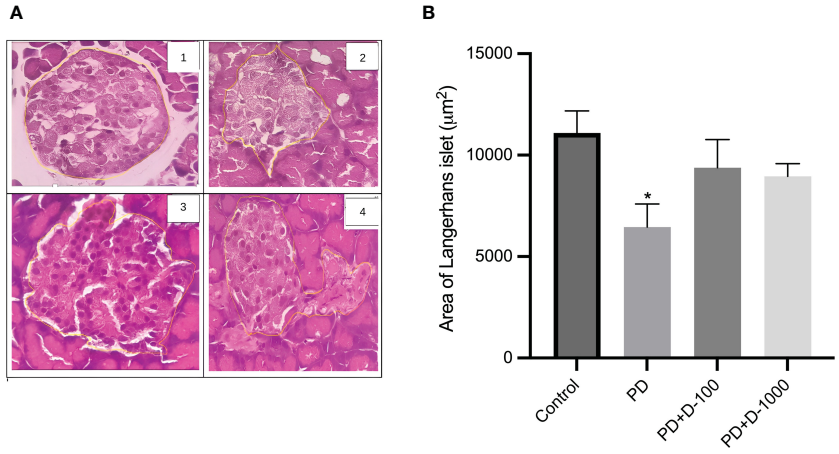


FIGURE 3
(A) Histology of the islet of Langerhans in the rat pancreas; **(B)** islet of Langerhans area in control or prediabetic rats after 12 weeks with no treatment or vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day. Magnification at 400x. The Islet of the Langerhans area was counted using the ImageJ analyzer. *: p<0.05 vs control.

kg BW or vitamin D3 1000 IU/kg BW was observed. There were no differences in TNF-α, IL-6, or IL-10 after 12 weeks of treatment. However, vitamin D3 supplementation tends to decrease the ratio of IL-6/IL-10 compared with the prediabetes group (Figure 5).

Altered expressions of PPARγ and NF-κB phosphorylation after vitamin D supplementation in prediabetic rats

To illustrate the possible mechanism of vitamin D supplementation in prediabetic rats, we investigated PPARγ and NF-κB signaling by analyzing the expression of PPARγ and NF-κB p65 phosphorylation at Serine 536. We observed that supplementation of vitamin D3 at 100 IU/kg BW did little change in PPARγ expressions and NF-κB p65 phosphorylation. However, compared to the prediabetic group, vitamin D3 supplementation at 1000 IU/kg BW tends to increase PPARγ expression and NF-κB p65 phosphorylation (Figure 6).

Discussion

In the present study, we examined the modulating effects of vitamin D supplementation on the molecular mechanism of insulin resistance in prediabetic rats. Our study showed that vitamin D3 administration improved glucose control and ameliorated insulin resistance in prediabetic rats. The modulation of the insulin signaling pathway and improved balance between proinflammatory, and anti-inflammatory cytokines contribute to reducing insulin resistance in prediabetic rats.

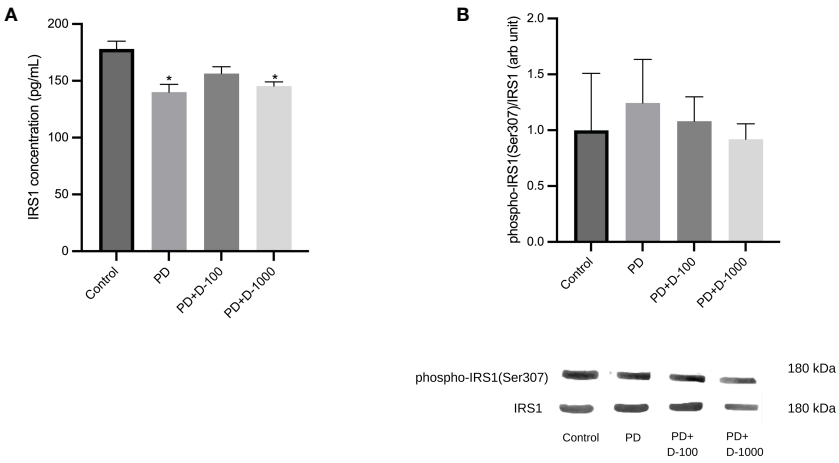


FIGURE 4
(A) Muscle IRS1 concentrations; **(B)** hepatic phospho-IRS1(Ser307)/IRS1 in healthy control or prediabetic rats after 12 weeks with no treatment or vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day. *: p<0.05 vs control.

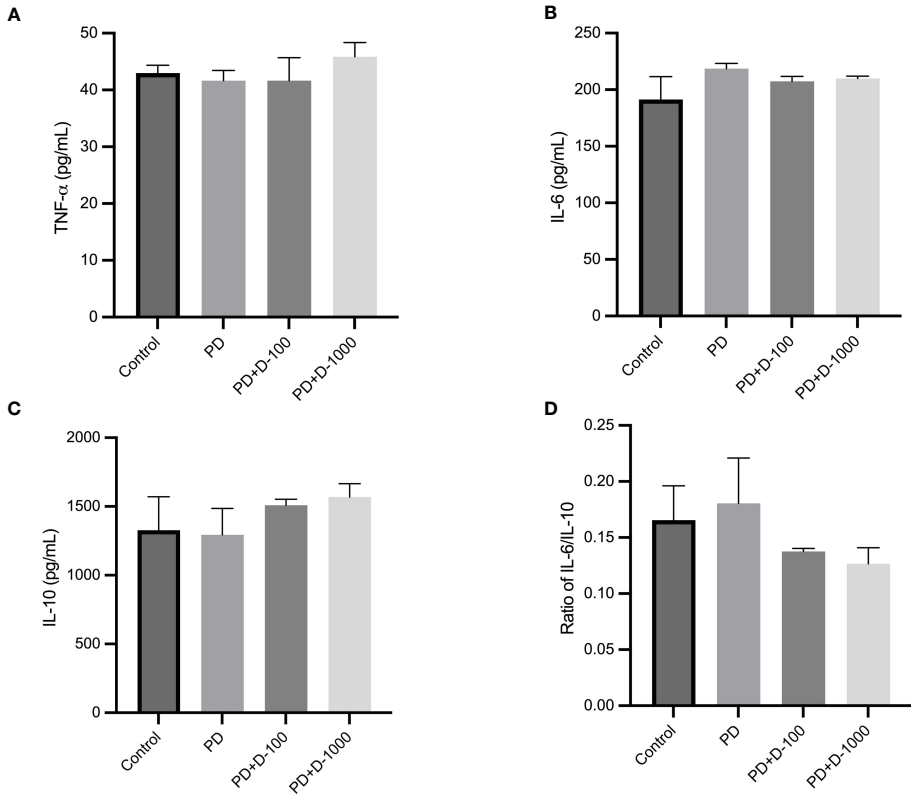


FIGURE 5
(A) Serum TNF-α concentration; **(B)** serum IL-6 concentration; **(C)** serum IL-10 concentration; **(D)** ratio of IL-6/IL-10 in healthy control or prediabetic rats after 12 weeks with no treatment or vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day.

The prediabetic conditions in our study were induced by the chronic administration of a high-fat and high-glucose diet (HFD-G) in combination with a small dose of streptozotocin (30 mg/kg BW). The present model of prediabetes demonstrates impaired glucose homeostasis, as indicated by variations in glucose tolerance such as fasting blood glucose, OGTT, and insulin levels. No specific criteria for prediabetic conditions are currently available for rodents. However, the criteria used in the study paradigm are consistent with other studies (25, 26). HFD-G is widely used in the animal model of diabetes induction by inducing hyperglycemia and insulin resistance. Studies have shown that prolonged administration of a high-fat diet may inhibit the insulin receptor signaling pathway and

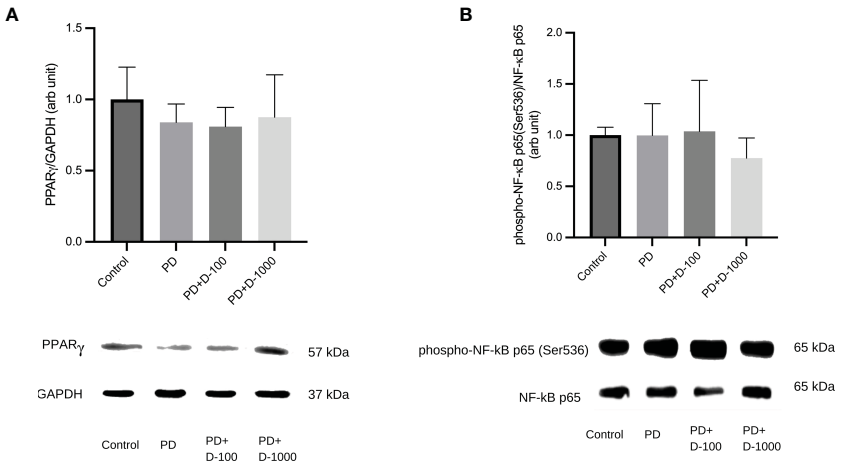


FIGURE 6
(A) Hepatic PPARγ/GAPDH expression; **(B)** Hepatic phospho-NF-κB p65(Ser536)/NF-κB in healthy control or prediabetic rats after 12 weeks with no treatment or vitamin D 100 IU/kg BW/day or vitamin D 1000 IU/kg/BW/day.

trigger insulin resistance (27, 28). A high-fat diet will increase the formation of diacylglycerol (DAG) in the liver and muscles over time. Increased DAG in the liver activates protein kinase C (PKC), which phosphorylates serine residues on IRS1, blocking the phosphorylation of tyrosine residues. Insulin resistance develops because of a reduction in insulin-PI3K-Akt signaling. Reduced insulin-PI3K-Akt in muscle promotes reduced glucose absorption and a decrease in GLUT-4, both of which contribute to insulin resistance (28). In adipocytes, studies revealed a distinct connection between decreased insulin receptor expression and impairment of insulin signaling in adipocytes. One of the most critical roles is the activation of a miRNA (miR-128) in adipocytes, which causes mRNA instability of the insulin receptor (29, 30).

Studies showed impaired insulin signaling and secretion are linked to reduced 25-hydroxy-vitamin D3 concentrations in the blood in prediabetic individuals (7, 31). The current study showed that vitamin D3 supplementation might prevent the decrease of 25-hydroxy-vitamin D3 levels in the prediabetic group. Previous studies showed that a high-fat diet might increase vitamin D3 storage in the liver and adipose tissue, contributing to low serum 25 (OH) D3 levels (32).

Based on findings linked to the role of vitamin D in insulin generation and glucose homeostasis, studies have demonstrated a causal relationship between vitamin D deficiency and diabetes mellitus (8, 9, 20, 21, 33, 34). Compared to the prediabetes group, vitamin D supplementation at 1000 IU/kg BW successfully lowered fasting blood glucose, plasma insulin, and insulin resistance, as shown by HOMA IR. In diabetic individuals, vitamin D levels were negatively associated with insulin resistance (HOMA-IR). Vitamin D deficiency is hypothesized to cause insulin resistance *via* several pathways, including increased proinflammatory cytokines, reduced insulin production by pancreatic beta cells, and decreased glucose absorption in peripheral tissues (20). Another study of diabetic rats given vitamin D supplementation (1000 IU and 2000 IU) for 45 days showed better glucose control and insulin resistance (35). Vitamin D indirectly impacts insulin secretion and interacts *via* β-cells to modulate extracellular calcium or calcium flow (21). Vitamin D may also activate calcium-dependent endopeptidase, which aids in the conversion of proinsulin to insulin (36).

Our findings were supported by histological examination using hematoxylin and eosin staining, which revealed a decrease in Langerhans islets as well as fatty pancreatic acinar cell atrophy while increasing the number of fatty acinar cells. For the histopathology analysis, we utilized the same strategy as earlier research that effectively reported pancreatic histology (37). The morphological differences between the negative and positive control groups, as well as the treatment group, were clearly visible using hematoxylin and eosin (H&E) staining. However, it will be beneficial for future research to add scan and image-based phenotypic analysis utilizing the cell painting approach.

Regarding to the vitamin D doses utilized in the study, were well below the hazardous quantity. Vitamin D toxicity is highly uncommon. The most common way to get vitamin D intoxication is by continuing to take very high dosages of vitamin D over an extended period. More than 150 μg/L may cause vitamin D intoxication and hypercalcemia in humans (38). In rats, the toxicity of vitamin D3 has been documented at extremely high dosages. Ali et al. reported vitamin D toxicity at a dose of 6,750 IU/rat/day, or equivalent to 27,000 IU/kg BW/day, while Chavhan et al. demonstrated toxicity at 2 mg/kg BW/day, or equal to 80,000 IU/kg BW/day (39, 40). In our study, the highest dose used was 1,000 IU/kg BW/daily, lower than those demonstrated in the studies of Ali et al. (39) and Chavhan et al. (40). Additionally, the highest plasma concentrations of vitamin D3 after treatment with vitamin D3 1,000 IU/kg BW were 31 μg/L.

In a rat model of type 2 diabetes mellitus with no vitamin D deficiency, vitamin D therapy was shown to reduce blood glucose levels by 40% (41). In addition to modulating calcium regulation in pancreatic beta cells, vitamin D3 directly impacts pancreatic beta cells *via* the binding to the vitamin D receptor (VDR) in the active form 1.25-hydroxy-vitamin D3. After binding to 1.25-hydroxyvitamin D3, VDR will interact with the vitamin D response element (VDRE), consequently leading to the insulin gene's induced activation (11).

Our findings were consistent with those of Wahba et al., who discovered that vitamin D might improve oral glucose tolerance in prediabetic rats (42). The present study also showed that in both dosages studied, and vitamin D reduced glycated albumin levels. The link between vitamin D supplementation and glycated albumin levels in prediabetics has received little attention. Glycated albumin is a novel biomarker for monitoring short-term glycemic control due to its shorter half-life (2 to 3 weeks) compared to HbA1c (43).

Improvement of insulin resistance by vitamin D supplementation can be partially explained by reducing IRS1 phosphorylation at Serine 307. Insulin signaling is mediated by insulin receptor substrates 1 and 2 (IRS1 and IRS2), which regulate glucose homeostasis and energy metabolism. To date, the increased phosphorylation of IRS1 at serine 307 was considered the best available mechanism to understand the desensitization of insulin signaling (44). Phosphorylation of Ser307 in IRS1 limits insulin action by blocking connections with the insulin receptor (45). Our result was in line with a previous study in a diabetic rat model, which demonstrated that vitamin D supplementation for eight weeks and a high-fat diet reduced Ser307 phosphorylation of IRS1. Increased degradation of IRS1 causes impaired GLUT4 mobilization and decreased glucose uptake in the diabetic rat (46).

Multiple inflammatory responses are closely connected and play critical roles in developing insulin resistance and type 2 diabetes (47). Insulin resistance associated with obesity is characterized by chronic low-grade inflammation. There were increased proinflammatory cytokines and other bioactive compounds such as TNF-α, IL-1β, IL-6, or monocyte attractant protein-1 (MCP-1) (48). VDR, the receptor for 1.25-hydroxy-vitamin D3, is present in more than 38 different tissues and is known to regulate essential genes involved in bone metabolism, oxidative damage, chronic illnesses, and inflammation. Macrophages and dendritic cells express VDR constitutively, indicating that vitamin D likely plays a significant role in regulating the inflammatory response (49). Our study showed that vitamin D3 supplementation did not alter individual concentrations of proinflammatory cytokines (TNF-α, IL-6) and anti-inflammatory cytokines IL-10. Vitamin D3 may restore the balance between proinflammatory and anti-inflammatory

cytokines, as shown by increasing the IL-10 levels and thus reducing the ratio of IL-6/IL-10.

The interaction between PPARγ and NF-κB is a signaling pathway that connects insulin resistance, metabolic syndrome, and inflammation (50, 51). PPARγ is a ligand-activated transcription factor that plays a crucial role in glucose homeostasis and adipocyte formation (52, 53). Several investigations have demonstrated that PPARγ may decrease inflammation by reducing NF-κB transcriptional activity by competing with p65 (51). The transcription factor NF-κB is an essential regulator of inflammation. It is necessary to produce proinflammatory cytokines such as IL-1β and IL-6 (54, 55). According to Ke et al., the inactivation of NF-κB p65 may modulate hepatic insulin sensitivity by elevating cAMP through PDE3B gene transcription suppression (55). Our data showed that 1000 IU/kg BW vitamin D3 supplementation enhanced PPARγ expression while decreasing NF-κB p65 phosphorylation at Ser536. NF-κB p65 phosphorylation at Ser536 is essential in inhibiting NF-κB transcription responses in toll-like receptor-activated macrophages, contributing to inflammation resolution (56).

Conclusions

Our study indicated that vitamin D supplementation improves insulin resistance in prediabetic rats. Additionally, the decreased phosphorylation of IRS1 increased expression of PPARγ and reduced phosphorylation of NF-κB could be attributed to the attenuation of insulin resistance of vitamin D3. Therefore, vitamin D supplementation in a prediabetic state may prevent the progression of insulin resistance to diabetes.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by the ethics committee of The Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

Author contributions

DK, ML, TT, VS, and EP: study design; DK, ML, and VS: data analysis; DK, CN, and ML: funding; HW: acquisition. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by Grants from the Ministry of Education and Culture, Republic of Indonesia No. NKB-804/UN2.RST/HKP.05.00/2022.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Association AD. Classification and diagnosis of diabetes: standards of medical care in diabetes–2021. *Diabetes Care* (2020) 44(Supplement_1):S15–33. doi: 10.2337/dc21-S002

2. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet* (2012) 379(9833):2279–90. doi: 10.1016/S0140-6736(12)60283-9

3. Zhang Y, Shen T, Wang S. Progression from prediabetes to type 2 diabetes mellitus induced by overnutrition. *Hormones (Athens).* (2022) 21(4):591-597. doi: 10.1007/s42000-022-00399-2

4. DIG G, Ciccarelli G, Cefalo CM, Cinti F, Moffa S, Impronta F, et al. Prediabetes: how pathophysiology drives potential intervention on a subclinical disease with feared clinical consequences. *Minerva Endocrinol (Torino)* (2021) 46(3):272–92. doi: 10.23736/S2724-6507.21.03405-9

5. Derosa G, D'Angelo A, Maffioli P. The role of selected nutraceuticals in management of prediabetes and diabetes: an updated review of the literature. *Phytother Res* (2022) 36(10):3709–65. doi: 10.1002/ptr.7564

6. Jonas DE, Vander Schaaf EB, Riley S, Allison B, Middleton JC, Baker C, et al. U.S. preventive services task force evidence syntheses, formerly systematic evidence reviews. In: *Screening for prediabetes and type 2 diabetes mellitus in children and adolescents: an evidence review for the US preventive services task force.* Rockville (MD: Agency for Healthcare Research and Quality (US (2022).

7. Alkhatatbeh MJ, Abdul-Razzak KK. Association between serum 25-hydroxyvitamin d, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* (2018) 9(6):523–30. doi: 10.3892/br.2018.1159

8. Lips P, Eekhoff M, van Schoor N, Oosterwerff M, de Jongh R, Krul-Poel Y, et al. Vitamin d and type 2 diabetes. *J Steroid Biochem Mol Biol* (2017) 173:280–5. doi: 10.1016/j.jsbmb.2016.11.021

9. Pilz S, Kienreich K, Rutters F, de Jongh R, van Ballegooijen AJ, Grübler M, et al. Role of vitamin d in the development of insulin resistance and type 2 diabetes. *Curr Diabetes Rep* (2013) 13(2):261–70. doi: 10.1007/s11892-012-0358-4

10. Rashidi H, Ghaderian SB, Shirinpour Z, Yazdanpanah L, Kaykhaei MA, Aleali A, et al. The effect of vitamin d supplementation on insulin resistance and glycemic

control in patients with type 2 diabetes. *Int J Pharm Technol* (2016) 8:11665–74. doi: 10.3390/nu10030375

11. Sadek KM, Shaheen H. Biochemical efficacy of vitamin d in ameliorating endocrine and metabolic disorders in diabetic rats. *Pharm Biol* (2014) 52(5):591–6. doi: 10.3109/13880209.2013.854812

12. Park S, Kim DS, Kang S. Vitamin d deficiency impairs glucose-stimulated insulin secretion and increases insulin resistance by reducing PPAR-γ expression in nonobese type 2 diabetic rats. *J Nutr Biochem* (2016) 27:257–65. doi: 10.1016/j.jnutbio.2015.09.013

13. Benetti E, Mastrocola R, Chiazza F, Nigro D, D'Antona G, Bordano V, et al. Effects of vitamin d on insulin resistance and myosteatosis in diet-induced obese mice. *PloS One* (2018) 13(1):e0189707. doi: 10.1371/journal.pone.0189707

14. von Frankenberg AD, Marina A, Song X, Callahan HS, Kratz M, Utzschneider KM. A high-fat, high-saturated fat diet decreases insulin sensitivity without changing intra-abdominal fat in weight-stable overweight and obese adults. *Eur J Nutr* (2017) 56 (1):431–43. doi: 10.1007/s00394-015-1108-6

15. Liu Z, Patil IY, Jiang T, Sancheti H, Walsh JP, Stiles BL, et al. High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. *PloS One* (2015) 10(5):e0128274. doi: 10.1371/journal.pone.0128274

16. Denver P, Gault VA, McClean PL. Sustained high-fat diet modulates inflammation, insulin signalling and cognition in mice and a modified xenin peptide ameliorates neuropathology in a chronic high-fat model. *Diabetes Obes Metab* (2018) 20(5):1166–75. doi: 10.1111/dom.13210

17. Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, et al. Skeletal and extraskeletal actions of vitamin d: current evidence and outstanding questions. *Endocr Rev* (2019) 40(4):1109–51. doi: 10.1210/er.2018-00126

18. Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin d supplementation: evidence from human studies. *Nat Rev Endocrinol* (2022) 18(2):96–110. doi: 10.1038/s41574-021-00593-z

19. Xie S, Huang L, Cao W, Hu Y, Sun H, Cao L, et al. Association between serum 25-hydroxyvitamin d and diabetic kidney disease in Chinese patients with type 2 diabetes. *PloS One* (2019) 14(4):e0214728–e. doi: 10.1371/journal.pone.0214728

20. Zhang J, Ye J, Guo G, Lan Z, Li X, Pan Z, et al. Vitamin d status is negatively correlated with insulin resistance in Chinese type 2 diabetes. *Int J Endocrinol* (2016) 2016:1794894. doi: 10.1155/2016/1794894

21. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin d and calcium in type 2 diabetes. a systematic review and meta-analysis. *J Clin Endocrinol Metab* (2007) 92(6):2017–29. doi: 10.1210/jc.2007-0298

22. Frank EA, Shubha MC, D'Souza CJ. Blood glucose determination: plasma or serum? *J Clin Lab Anal* (2012) 26(5):317–20. doi: 10.1002/jcla.21524

23. Bancroft JD, Layton C. 10 - the hematoxylins and eosin. In: Suvarna SK, Layton C, Bancroft JD, editors. *Bancroft's theory and practice of histological techniques (Eighth edition)*. (UK: Elsevier) (2019). p. 126–38.

24. Soetikno V, Sari FR, Veeraveedu PT, Thandavarayan RA, Harima M, Sukumaran V, et al. Curcumin ameliorates macrophage infiltration by inhibiting NF-κB activation and proinflammatory cytokines in streptozotocin induced-diabetic nephropathy. *Nutr Metab (Lond)* (2011) 8(1):35. doi: 10.1186/1743-7075-8-35

25. Burgeiro A, Cerqueira MG, Varela-Rodríguez BM, Nunes S, Neto P, Pereira FC, et al. Glucose and lipid dysmetabolism in a rat model of prediabetes induced by a high-sucrose diet. *Nutrients* (2017) 9(6):638. doi: 10.3390/nu9060638

26. Islam MS, Venkatesan V. Experimentally-induced animal models of prediabetes and insulin resistance: a review. *Acta Pol Pharm* (2016) 73(4):827–34.

27. Liu C, Shao M, Lu L, Zhao C, Qiu L, Liu Z. Obesity, insulin resistance and their interaction on liver enzymes. *PloS One* (2021) 16(4):e0249299. doi: 10.1371/journal.pone.0249299

28. Maulana H, Ridwan A. High-fat diets-induced metabolic disorders to study molecular mechanism of hyperlipidemia in rats. *3BIO: J Biol Science Technol Manage* (2021) 3:38–50. doi: 10.5614/3bio.2021.3.2.5

29. Tomás E, Lin YS, Dagher Z, Saha A, Luo Z, Ido Y, et al. Hyperglycemia and insulin resistance: possible mechanisms. *Ann N Y Acad Sci* (2002) 967:43–51. doi: 10.1111/j.1749-6632.2002.tb04262.x

30. Arcidiacono B, Chiefari E, Foryst-Ludwig A, Currò G, Navarra G, Brunetti FS, et al. Obesity-related hypoxia *via* miR-128 decreases insulin-receptor expression in human and mouse adipose tissue promoting systemic insulin resistance. *EBioMedicine* (2020) 59:102912. doi: 10.1016/j.ebiom.2020.102912

31. Abbasi F, Blasey C, Feldman D, Caulfield MP, Hantash FM, Reaven GM. Low circulating 25-hydroxyvitamin d concentrations are associated with defects in insulin action and insulin secretion in persons with prediabetes. *J Nutr* (2015) 145(4):714–9. doi: 10.3945/jn.114.209171

32. Park CY, Shin Y, Kim J-H, Zhu S, Jung YS, Han SN. Effects of high fat diet-induced obesity on vitamin d metabolism and tissue distribution in vitamin d deficient or supplemented mice. *Nutr Metab* (2020) 17(1):44. doi: 10.1186/s12986-020-00463-x

33. Hidayat R, Setiati S, Soewondo P. The association between vitamin d deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* (2010) 42(3):123–9.

34. Harinarayan CV. Vitamin D. And diabetes mellitus. *Hormones (Athens)* (2014) 13(2):163–81. doi: 10.1007/BF03401332

35. Guareschi ZM, Valcanaia AC, Ceglarek VM, Hotz P, Amaral BK, de Souza DW, et al. The effect of chronic oral vitamin d supplementation on adiposity and insulin secretion in hypothalamic obese rats. *Br J Nutr* (2019) 121(12):1334–44. doi: 10.1017/S0007114519000667

36. Lee S, Clark SA, Gill RK, Christakos S. 1,25-dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin d receptors, gene expression, and insulin secretion. *Endocrinology* (1994) 134(4):1602–10. doi: 10.1210/endo.134.4.8137721

37. Oyouni AAA, Al-Amer OM, Ali FAZ, Altayar MA, Jalal MM, Albalawi RSM, et al. Melatonin ameliorates the adrenal and pancreatic alterations in streptozotocin-induced diabetic rats: clinical, biochemical, and descriptive histopathological studies. *Front Vet Sci* (2022) 9. doi: 10.3389/fvets.2022.1016312

38. Marcinowska-Suchowierska E, Kupisz-Urbańska M, Łukaszkiewicz J, Płudowski P, Jones G. Vitamin d toxicity-a clinical perspective. *Front Endocrinol (Lausanne)* (2018) 9:550. doi: 10.3389/fendo.2018.00550

39. Soad Shaker Ali SHM, Ramziyyah Mohammed A. The effects of hypervitaminosis d in rats on histology and weights of some immune system organs and organs prone to calcification. *Int J Pharm Phytopharmacological Res* (2018) 8 (6):59–71.

40. Chavhan S, Brar R, Banga Hs, Singh N. Cholecalciferol (Vitamin D3) toxicity in animals. *Indian Pet J - Online J Canine Feline Exotic Pets* (2011) 3:54–8.

41. de Souza Santos R, Vianna LM. Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and wistar rats. *Clin Chim Acta* (2005) 358(1-2):146–50. doi: 10.1016/j.cccn.2005.02.020

42. Wahba NS, Ghareib SA, Abdel-Ghany RH, Abdel-Aal M, Alsemeh AE. Renoprotective effects of vitamin D3 supplementation in a rat model of metabolic syndrome. *Eur J Nutr* (2021) 60(1):299–316. doi: 10.1007/s00394-020-02249-6

43. Arasteh A, Farahi S, Habibi-Rezaei M, Moosavi-Movahedi AA. Glycated albumin: an overview of the *In vitro* models of an *In vivo* potential disease marker. *J Diabetes Metab Disord* (2014) 13:49. doi: 10.1186/2251-6581-13-49

44. Copps K, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* (2012) 55:2565–82. doi: 10.1007/s00125-012-2644-8

45. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* (2002) 277(2):1531–7. doi: 10.1074/jbc.M101521200

46. Wang Y, Nishina PM, Naggert JK. Degradation of IRS1 leads to impaired glucose uptake in adipose tissue of the type 2 diabetes mouse model TALLYHO/Jng. *J Endocrinol* (2009) 203(1):65–74. doi: 10.1677/JOE-09-0026

47. Rehman K, Akash MSH. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* (2016) 23 (1):87. doi: 10.1186/s12929-016-0303-y

48. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* (2006) 116(7):1793–801. doi: 10.1172/JCI29069

49. Chagas CEA, Borges MC, Martini LA, Rogero MM. Focus on vitamin d, inflammation and type 2 diabetes. *Nutrients* (2012) 4(1):52–67. doi: 10.3390/nu4010052

50. Bandera Merchan B, Tinahones FJ, Macías-González M. Commonalities in the association between PPARG and vitamin d related with obesity and carcinogenesis. *PPAR Res* (2016) 2016:2308249. doi: 10.1155/2016/2308249

51. Remels AHV, Langen RCJ, Gosker HR, Russell AP, Spaapen F, Voncken JW, et al. PPARγ inhibits NF-κB-dependent transcriptional activation in skeletal muscle. *Am J Physiology-Endocrinology Metab* (2009) 297(1):E174–E83. doi: 10.1152/ajpendo.90632.2008

52. Jay MA, Ren J. Peroxisome proliferator-activated receptor (PPAR) in metabolic syndrome and type 2 diabetes mellitus. *Curr Diabetes Rev* (2007) 3(1):33–9. doi: 10.2174/157339907779802067

53. Ferré P. The biology of peroxisome proliferator-activated receptors. relationship with lipid metabolism and insulin sensitivity. *Diabetes* (2004) 53(suppl 1):S43–50. doi: 10.2337/diabetes.53.2007.S43

54. Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation, and metabolic disease. *Cell Metab* (2011) 13(1):11–22. doi: 10.1016/j.cmet.2010.12.008

55. Ke B, Zhao Z, Ye X, Gao Z, Manganiello V, Wu B, et al. Inactivation of NF-κB p65 (RelA) in liver improves insulin sensitivity and inhibits cAMP/PKA pathway. *Diabetes* (2015) 64(10):3355–62. doi: 10.2337/db15-0242

56. Christian F, Smith EL, Carmody RJ. The regulation of NF-κB subunits by phosphorylation. *Cells* (2016) 5(1):12. doi: 10.3390/cells501001

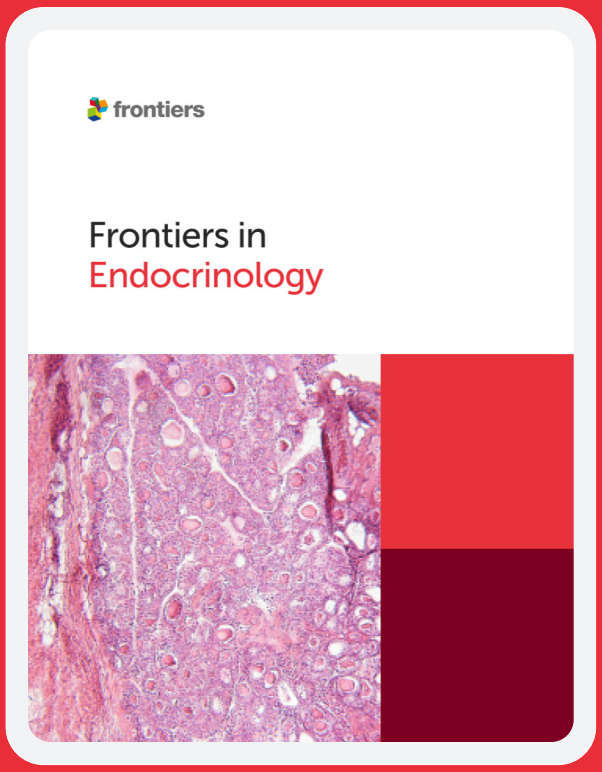
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact