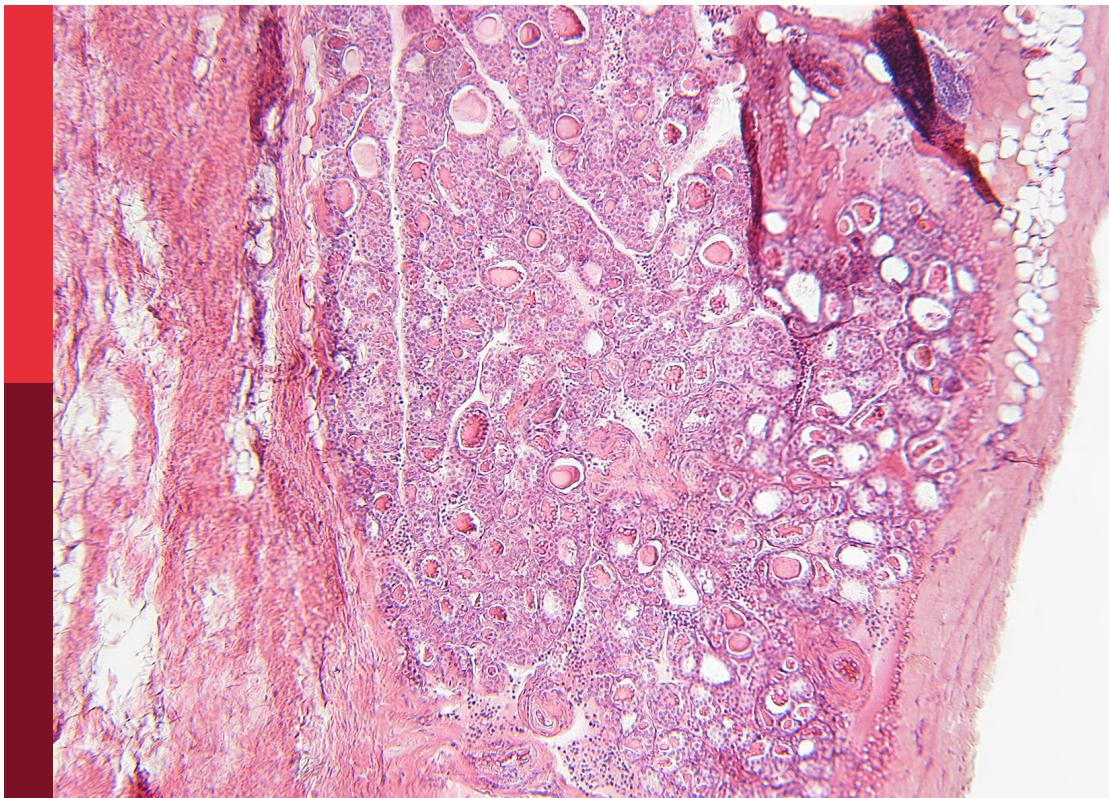
frontiers | Research Topics

Metabolic miscommunication among organs: The missing links

Edited by
Maria Paula Macedo, Maria João Meneses,
Sonia Michael Najjar and Ruben Nogueiras

Published in
Frontiers in Endocrinology
Frontiers in Public Health



ISSN 1664-8714
ISBN 978-2-83251-480-1
DOI 10.3389/978-2-83251-480-1

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

Metabolic miscommunication among organs: The missing links

Topic editors

Maria Paula Macedo — New University of Lisbon, Portugal
Maria João Meneses — Universidade Nova de Lisboa, Portugal
Sonia Michael Najjar — Ohio University, United States
Ruben Nogueiras — University of Santiago de Compostela, Spain

Citation

Macedo, M. P., Meneses, M. J., Najjar, S. M., Nogueiras, R., eds. (2023). *Metabolic miscommunication among organs: The missing links*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-480-1

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.

Table of contents

05 Editorial: Metabolic miscommunication among organs: The missing links
Maria João Meneses and Maria Paula Macedo

08 Mapping Knowledge Landscapes and Emerging Trends of the Links Between Bone Metabolism and Diabetes Mellitus: A Bibliometric Analysis From 2000 to 2021
Kunming Cheng, Qiang Guo, Weiguang Yang, Yulin Wang, Zaijie Sun and Haiyang Wu

26 Distinct impacts of fat and fructose on the liver, muscle, and adipose tissue metabolome: An integrated view
Maria João Meneses, Inês Sousa-Lima, Ivana Jarak, João F. Raposo, Marco G. Alves and Maria Paula Macedo

42 Serum iron is closely associated with metabolic dysfunction-associated fatty liver disease in type 2 diabetes: A real-world study
Jun-Wei Wang, Chun-Hua Jin, Jiang-Feng Ke, Yi-Lin Ma, Yu-Jie Wang, Jun-Xi Lu, Mei-Fang Li and Lian-Xi Li

53 Monocyte-to-high-density lipoprotein ratio and systemic inflammation response index are associated with the risk of metabolic disorders and cardiovascular diseases in general rural population
Pengbo Wang, Xiaofan Guo, Ying Zhou, Zhao Li, Shasha Yu, Yingxian Sun and Yu Hua

68 The association between diabetes and nocturia: A systematic review and meta-analysis
Zhiwei Fu, Fang Wang, Xing Dang and Tao Zhou

79 Impact of admission and early persistent stress hyperglycaemia on clinical outcomes in acute pancreatitis
Xinmin Yang, Na Shi, Linbo Yao, Wenhua He, Ping Zhu, Sheyu Li, Lan Li, Yuying Li, Shiyu Liu, Lihui Deng, Tao Jin, Tingting Liu, Nonghua Lu, John A. Windsor, Robert Sutton, Yin Zhu, Qing Xia and Wei Huang

90 The association of liver enzymes with diabetes mellitus risk in different obesity subgroups: A population-based study
Dinghao Zheng, Xiaoyun Zhang, Lili You, Feng Li, Diaozhu Lin, Kan Sun, Meng Ren, Li Yan and Wei Wang

99 Integrated analysis of potential gene crosstalk between non-alcoholic fatty liver disease and diabetic nephropathy
Qianqian Yan, Zihao Zhao, Dongwei Liu, Jia Li, Shaokang Pan, Jiayu Duan, Jiancheng Dong and Zhangsuo Liu

113 Does aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? A systematic literature review and meta-analysis
Veera Houttu, Julia Bouts, Yasaman Vali, Joost Daams, Aldo Grefhorst, Max Nieuwdorp and Adriaan G. Holleboom

134 Understanding gut-liver axis nitrogen metabolism in Fatty Liver Disease
Teresa C. Delgado, Javier de las Heras and María L. Martínez-Chantar

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 13 January 2023
DOI 10.3389/fendo.2023.1136283

OPEN ACCESS

EDITED AND REVIEWED BY
Åke Sjöholm,
Gävle Hospital, Sweden

*CORRESPONDENCE
Maria João Meneses
maria.meneses@nms.unl.pt
Maria Paula Macedo
paula.macedo@nms.unl.pt

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

RECEIVED 02 January 2023
ACCEPTED 03 January 2023
PUBLISHED 13 January 2023

CITATION
Meneses MJ and Macedo MP (2023) Editorial: Metabolic miscommunication among organs: The missing links. *Front. Endocrinol.* 14:1136283. doi: 10.3389/fendo.2023.1136283

Editorial: Metabolic miscommunication among organs: The missing links

Maria João Meneses[1,2]* and Maria Paula Macedo[1]*

[1]iNOVA4Health, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade Nova de Lisboa, Lisboa, Portugal, [2]DECSIS II Iberia, Évora, Portugal

KEYWORDS

diabetes, metabolism, NAFLD, crosstalk, exercise, inflammation

Editorial on the Research Topic
Metabolic miscommunication among organs: The missing links

Type 2 diabetes (T2D) is one of the modern metabolic diseases that involve impaired or faulty communication between tissues and/or organs. Extracellular vesicles, endocrine hormones, and cytokines are just some of the communication/signaling molecules that may be affected, involving both commensal microbiota and constitutive tissues. For these processes to be identified and characterized in a clinical setting, various disciplines and methods of analysis and diagnosis must be integrated. As an example, the imaging of liver fat and fibrosis could be analyzed along with the characterization of intestinal microbiome activity to understand the role of intestinal dysbiosis in fatty liver disease development.

The goal of this Research Topic was to present new progress in the integration of epidemiology and pathogenic mechanisms that improve the understanding of biological crosstalk within diabetes and related disorders. Indeed, the authors replied to our call and the manuscripts within this Research Topic include the link between diabetes and intestinal disorders, kidney disease, bone metabolism, nocturia, iron and nitrogen metabolism, exercise as well as inflammation and liver disease.

Meneses et al. studied the impact of high-fat and high-fructose feeding on the metabolome of the liver, muscle, white and brown adipose tissue. By feeding male mice with these diets for 12 weeks, it was possible to disclose that although both diets have deleterious effects on the liver, causing non-alcoholic fatty liver disease (NAFLD), high-fat resulted in a higher impact on hepatic glucose and lipid metabolism than high-fructose, leading namely to glucose intolerance. Moreover, while high-fat had alterations in brown adipose tissue metabolites that indicate increased thermogenesis, high-fructose led to increased levels of betaine, known to be a shielding metabolite against fructose-induced inflammation. Overall, this study indicates that high-fat and high-fructose feeding have a negative but distinct effect on the metabolome of the abovementioned organs.

Besides high circulating lipid and fructose levels, iron levels also impact metabolic homeostasis. Wang et al. aimed at exploring the association of serum iron levels with metabolic dysfunction-associated fatty liver disease (MAFLD) in Chinese patients with T2D in a cross-sectional study with 1,467 individuals. Indeed, the association between iron metabolism and insulin resistance has been explored in the last few years, and the present study found a positive correlation between the presence of MAFLD and serum iron levels in

patients with T2D. As such, serum iron levels may act as one indicator for MAFLD risk assessment in those individuals.

The clinical evaluation of circulating and phenotypic characteristics changes with disease progression and aging as observed by Zheng et al. who evaluated the association of liver enzymes with diabetes mellitus risk in different obesity subgroups based on a middle-aged Chinese population. Although serum GGT levels were correlated with diabetes mellitus risk in the middle-aged population, the correlation disappeared when waist circumference was over 98.99 cm.

How organs crosstalk impacts dysmetabolism is a step forward in understanding dysmetabolism. Yan et al. shed the light on the liver-kidney axis by exploring, through machine learning techniques, crosstalk genes in NAFLD and diabetic nephropathy. The authors pinpoint that there seems to be a common pathogenesis between these metabolic disorders and that lipoprotein lipase (LPL) and secreted phosphoprotein 1 (SPP1) are the most relevant crosstalk genes. The contribution of nephropathy for diabetes and nocturia is covered by Fu et al. through a systematic review and meta-analysis. Nocturia is known to be connected to age, but it may be also influenced by hypertension. As there are many confounding factors (e.g., age, gender), the associations need to be carefully performed. Diabetes had a 1.49-fold higher risk of nocturia. The association was stronger for Asian and male subjects or those at a lower nocturia cut-off.

Delgado et al. describe the importance of gut-liver axis nitrogen metabolism for the onset and development of NAFLD. Indeed, the authors depict how disrupted nitrogen metabolism and metabolic miscommunication between the gut and the liver may lead to NAFLD and shed the light on the re-establishment of altered gut-liver axis nitrogenous balance as a possible therapeutic approach to tackle NAFLD.

Yang et al. aimed at determining the impact of glucose levels at admission and during the first week (early phase) on clinical outcomes in patients with acute pancreatitis and to investigate the relationship between stress hyperglycemia and hypertriglyceridemia. Indeed, stress hyperglycemia, both during admission and during the first week of admission worsens the clinical outcomes of patients with acute pancreatitis. These effects were even more noticeable when hypertriglyceridemia co-existed at admission.

Cheng et al. performed a bibliometric analysis from 2000 to 2021 about the link between bone metabolism and diabetes *mellitus* where osteoporosis and associated fractures are the greatest concern in the bone metabolism field. With this publication, it becomes evident that cross-discipline research fields are attracting increased attention. Specifically, these are valuable insights for clinicians to recognize diabetic osteopenia and provide more attention and support to patients at risk of developing osteopenia.

Wang et al. conducted a cross-sectional study to clarify the association between inflammatory indicators and metabolic diseases, and cardiovascular disease risk. Contrary to the neutrophil-to-lymphocyte ratio and systematic immune-inflammation index, the monocyte-to-high-density lipoprotein ratio and systemic inflammation response index had a significant positive association with metabolic diseases and their components. Furthermore, they also correlated with cardiovascular disease, and the increment of these indicators caused a gradually evaluated risk of 10-year cardiovascular disease risk assessed by the Framingham score.

The prevention and disease control was tackled by Houttu et al. who systematically reviewed the effect of aerobic exercise on NAFLD, specifically in non-alcoholic steatohepatitis (NASH) and liver fibrosis. The 24 analyzed studies illustrate that liver fat is decreased by aerobic exercise (moderate-intensity continuous training or high-intensity interval training) with a concomitant decrease of alanine transaminase and aspartate aminotransferase. However, further studies are needed to elucidate the impact of moderate-intensity continuous training versus high-intensity interval training on hepatic inflammation and fibrosis.

Wang et al. provided a commentary on the previously published manuscript by Riedel et al. (1) calling attention to the need to look to T2D beyond hyperglycemia. Indeed, the authors support that T2D is more likely to be a syndrome leading to hyperglycemia, systematic inflammation, insulin resistance, and intestinal bowel disease, indeed a systematic disease as previously supported by Pina et al. (2, 3). As such, hyperglycemia could be treated as a coexistent symptom rather than the central one. We have also shared that opinion in a recent review where we aim to call attention to insulin and C-peptide as central molecules to help in the phenotypic diagnosis of T2D along with glucose, rather than glucose *per se* (4).

In summary, papers on this Research Topic cover a wide spectrum of metabolic miscommunication among organs and underline once more how important is to have a wider view of these disorders and how organs communicate, both trying to compensate or spreading the "disease" message.

Author contributions

MJM drafted the manuscript. MPM reviewed, provided input, and approved the content.

Funding

The authors were funded by Fundação para a Ciência e Tecnologia (FCT), FEDER, Portugal2020, and co-financed by Lisboa2020 and Alentejo2020 (ALT20-03-0247-FEDER-113469 and LISBOA-01-0247-FEDER-113469), iNOVA4Health (UIDB/Multi/04462/2020), and by the Sociedade Portuguesa de Diabetologia,

Acknowledgments

The authors thank all the authors who submitted their study for consideration for the present Research Topic and the reviewers who evaluated the papers.

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. Riedel S, Pheiffer C, Johnson R, Louw J, Muller CJF. Intestinal barrier function and immune homeostasis are missing links in obesity and type 2 diabetes development. *Front Endocrinol* (2022) 12. doi: 10.3389/fendo.2021.833544

2. Pina AF, Meneses MJ, Sousa-Lima I, Henriques R, Raposo JF, Macedo MP. Big data and machine learning to tackle diabetes management. *Eur J Clin Invest* (2023) 53(1): e13890. doi: 10.1111/eci.13890

3. Pina AF, Patarrão RS, Ribeiro RT, Penha-Gonçalves C, Raposo JF, Gardete-Correia L, et al. Metabolic footprint, towards understanding type 2 diabetes beyond glycemia. *J Clin Med* (2020) 9(8):2588. doi: 10.3390/jcm9082588

4. Meneses MJ, Patarrão RS, Pinheiro T, Coelho I, Carriço N, Marques AC, et al. Leveraging the future of diagnosis and management of diabetes: From old indexes to new technologies. *Eur J Clin Invest* (2022):e13934. doi: 10.1111/eci.13934

frontiers | Frontiers in Public Health

ORIGINAL RESEARCH
published: 03 June 2022
doi: 10.3389/fpubh.2022.918483

Mapping Knowledge Landscapes and Emerging Trends of the Links Between Bone Metabolism and Diabetes Mellitus: A Bibliometric Analysis From 2000 to 2021

Kunming Cheng[1*†], *Qiang Guo*[2†], *Weiguang Yang*[3,4], *Yulin Wang*[3,4], *Zaijie Sun*[5*] and *Haiyang Wu*[3,4*]

[1] Department of Intensive Care Unit, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [2] Department of Orthopaedic Surgery, Baodi Clinical College of Tianjin Medical University, Tianjin, China, [3] Graduate School of Tianjin Medical University, Tianjin, China, [4] Department of Orthopaedic Surgery, Clinical College of Neurology, Neurosurgery and Neurorehabilitation, Tianjin Medical University, Tianjin, China, [5] Department of Orthopaedic Surgery, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang, China

Background: Diabetes mellitus (DM) have become seriously threatens to human health and life quality worldwide. As a systemic metabolic disease, multiple studies have revealed that DM is related to metabolic bone diseases and always induces higher risk of fracture. In view of this, the links between bone metabolism (BM) and DM (BMDM) have gained much attention and numerous related papers have been published. Nevertheless, no prior studies have yet been performed to analyze the field of BMDM research through bibliometric approach. To fill this knowledge gap, we performed a comprehensive bibliometric analysis of the global scientific publications in this field.

Methods: Articles and reviews regarding BMDM published between 2000 and 2021 were obtained from the Web of Science after manually screening. VOSviewer 1.6.16, CiteSpace V 5.8.R3, Bibliometrix, and two online analysis platforms were used to conduct the bibliometric and visualization analyses.

Results: A total of 2,525 documents including 2,255 articles and 270 reviews were retrieved. Our analysis demonstrated a steady increasing trend in the number of publications over the past 22 years ($R^2 = 0.989$). The United States has occupied the leading position with the largest outputs and highest H-index. University of California San Francisco contributed the most publications, and Schwartz AV was the most influential author. Collaboration among institutions from different countries was relatively few. The journals that published the most BMDM-related papers were *Bone* and *Osteoporosis International*. Osteoporosis and related fractures are the main bone metabolic diseases of greatest concern in this field. According to co-cited references result, "high glucose environment," "glycation end-product" and "sodium-glucose co-transporter" have been recognized as the current research focus in this domain. The keywords co-occurrence analysis indicated that "diabetic osteoporosis," "osteoarthritis," "fracture risk," "meta-analysis," "osteogenic differentiation," "bone regeneration," "osteogenesis,"

OPEN ACCESS

Edited by:
Ruben Nogueiras, University of Santiago de Compostela, Spain

Reviewed by:
Barbara Ruaro, University of Trieste, Italy
Chengliang Yin, Macau University of Science and Technology, Macao SAR, China
Fei-Long Wei, Fourth Military Medical University, China
Andy Wai Kan Yeung, University of Hong Kong, China

***Correspondence:**
Zaijie Sun
gukeszj@163.com
Kunming Cheng
chengkm2013@163.com
Haiyang Wu
wuhaiyang2021@tmu.edu.cn

†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: *12 April 2022*
Accepted: *16 May 2022*
Published: *03 June 2022*

Citation:
Cheng K, Guo Q, Yang W, Wang Y, Sun Z and Wu H (2022) Mapping Knowledge Landscapes and Emerging Trends of the Links Between Bone Metabolism and Diabetes Mellitus: A Bibliometric Analysis From 2000 to 2021. Front. Public Health 10:918483. doi: 10.3389/fpubh.2022.918483

and "trabecular bone score" might remain the research hotspots and frontiers in the near future.

Conclusion: As a cross-discipline research field, the links between bone metabolism and diabetes mellitus are attracting increased attention. Osteoporosis and related fractures are the main bone metabolic diseases of greatest concern in this field. These insights may be helpful for clinicians to recognize diabetic osteopenia and provide more attention and support to such patients.

Keywords: diabetes mellitus, bone metabolism, bibliometrics, CiteSpace, VOSviewer, Bibliometrix

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease, characterized by disturbances in insulin and glucose metabolism. According to the estimates from World Health Organization (WHO), DM, especially type 2 DM (T2DM), affects 8.5% of world's total population aged 18 years or older, and it will become the 7th leading cause of death worldwide by 2030 (1). DM is able to cause damages to a variety of organs, in particular, nervous system, kidneys, and blood vessels. Substantial evidence suggests that a longer exposure to hyperglycemia also make an impact on bone metabolism (BM) (2, 3). Initially, many clinicians observed that diabetic patients had a higher risk of fractures as well as impaired fracture healing. Until 2007, a meta-analysis conducted by Vestergaard (4) reported for the first time that hip fracture risk was significantly increased in both type 1 DM (T1DM) and T2DM. And compared to subjects without DM, the risk of hip fracture was 6.94 and 1.38 times higher in T1DM and T2DM individuals, respectively. Apart from hip fracture, similar conclusions were reached from several other large sample meta-analyses on the association between DM and fracture risk at other sites including vertebra, upper arm, distal forearm, and ankle (5–7). Since then, osteoporosis and related fractures are the main bone metabolic diseases of greatest concern in this field (8, 9). Many experts have been calling attention to the osteoporosis/bone loss management and fracture risk assessment for DM patients. In 2019, several scholars from the Chinese Medical Association (CMA) even developed a joint expert consensus for the fractures risk management in DM patients (10).

In the light of this, the links between BM and DM (BMDM) have gained widespread attention from researchers recently. A large aggregation of studies devoted to deciphering the complex mechanisms about how diabetes works on bone remodeling (2). To date, despite more than 20 years of intensive research and several hypotheses, such as oxidant injury, accumulation of advanced glycation end products, increased collagen glycation, bone-fat axis hypothesis, and some other intermediate mediators including hormones, cytokines and nutrients, have been proposed, the specific mechanism of diabetes-related reduction of bone mass remains unclear (11–13). In addition, with the development of novel antidiabetic agents, it has been reported that various glucose-lowering medications including thiazolidinedione, insulin, metformin, sodium glucose co-transporter 2 inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors have different influences on bone formation and resorption (14–18).

Motivated by the above concerns, numerous papers related to BMDM have been published. However, in the face of massive information in this topic, researchers need to dedicate a great deal of time to reading and understanding appropriate work in interrelated disciplines. Hence, it is imperative to classify substantial, classical, and evocative evidence to assist the scientific investigation (19). Although several literature reviews and meta-analyses are able to provide useful information and reliable evidence-based medical findings, these methods are often unable to make a summary from a holistic and integrated perspective for a specific field of research (20, 21).

Bibliometric analysis applies statistical and mathematical approaches to quantitatively and qualitatively analyze all carriers of knowledge such as journal articles. It is an important and well-established method to spot active researchers and potential collaborators, sort out hot topics, describe the dynamic trends, and identify future research frontiers (22, 23). Recently, bibliometric analysis and data visualization have received a considerable amount of attention in biomedical fields owing to the explosion in basic and clinical data, and the growing number of freely available bibliometric tools over the past few years (24, 25). Taking DM and metabolic bone disorders as examples, multiple investigators have studied the scientific outputs and publication trends of diabetes-related research across different countries via bibliometric approaches (26–28). DM-associated complications such as diabetic foot ulcers (29), diabetic nephropathy (19, 30), and diabetic ophthalmopathy (31), etc. have also been studied with this approach. Our and other research teams have also investigated the publication status and research hotspots in the field of metabolic bone disorders including osteoporosis (32), hip fracture (33), osteonecrosis

Abbreviations: DM, diabetes mellitus; WHO, World Health Organization; T2DM, type 2 DM; BM, bone metabolism; T1DM, type 1 DM; CMA, Chinese Medical Association; BMDM, bone metabolism and diabetes mellitus; DPP-4, peptidase-4; WoSCC, Web of Science Core Collection; SCIE, Science Citation Index Expanded; TI, titles (TI); AK, author keywords; JIF, journal impact factor; JCR, Journal Citation Report; AAY, average appearing year; BC, betweenness centrality; TLS: total link strength; BMD, bone mineral density; FRAX, WHO Fracture Risk Algorithm; BMSCs, bone marrow stroma cells; ROS, reactive oxygen species; AGEs, advanced glycation end products; RAGE, receptors for AGEs; ASCs, adipose-derived stem cells; SGLT2, Sodium glucose cotransporter-2; CANVAS, Canagliflozin Cardiovascular Assessment Study; PTH, parathyroid hormone.

(34), and osteoarthritis (35) by using bibliometric methods, and mapped the overall knowledge structures and co-citation networks in these areas. Nevertheless, to the best of our knowledge, there are still no previous bibliometric studies reporting the links between BM and DM.

As such, we sought to fill this knowledge gap. In this study, multiple software programs and online platforms were used to analyze BMDM-related literature, as well as draw the scientific knowledge maps. The primary aims of the study were to (i) identify the main contributors including authors, institutions, and countries in the BMDM field from 2000 to 2021; (ii) explore the development and evolution trend of research focus; (iii) predict future research frontiers of this area; (iv) offer some new perspective and ideas for the subsequent studies between diabetes and bone metabolism; (v) call for more attention, especially clinician and researcher on this subject.

MATERIALS AND METHODS

Data Source

The Web of Science Core Collection (WoSCC, Clarivate Analytics, Philadelphia, PA, USA) is one of the most professional and authoritative citation-based databases with powerful indexing functions, which not only contains the basic information including titles, authors, institutions, countries/regions, and author keywords, but particularly includes the references information (22, 23). Therefore, it is considered as the optimal database and widely used in previous bibliometric studies (36). In this study, we selected to retrieve publications related to BMDM in the WoSCC of Science Citation Index Expanded (SCIE).

Data Search Strategy

Considering that the database is still functioning and may update daily, a comprehensive online search was performed on a single day by two authors (CK and WH), to avoid the bias. All potentially relevant publications were collected based on the titles (TI) and author keywords (AK) with the following search formula: #1: [TI = (diabetic* OR diabetes* OR antidiabetic*) OR AK = (diabetic* OR diabetes* OR antidiabetic*); #2: TI = (osteo* OR bone* OR fracture* OR "skelet*) OR AK = (osteo* OR bone* OR fracture* OR "skelet*); Final dataset: #1 AND #2]. In order to achieve as many relevant sources as possible, a wildcard character (*), representing one or more other characters and allowing variable endings of keywords was used. For example, osteo* would also return the terms of osteoporosis, osteopenia, osteoporotic, osteoarthritis, and so on. Then, a timespan of 22 years was set and only studies published from 2000 to 2021 were included. The literature language was restricted to English. The literature types were limited to articles and reviews, with the specific exclusion criteria shown in **Figure 1**. A total of 3,571 documents were retrieved as potential candidates for inclusion from the initial literature search. Then the literature titles, abstracts, and the full text were manually examined by two investigators (CK and GQ) to exclude literature that was irrelevant to study topic (including disease type, research purposes, interventions, outcome indexes, etc.). A total of 2,525 articles and reviews were included for data analysis after the final selection.

Data Extraction and Collection

All the 2,525 retrieved literature was downloaded with "full record and cited references" and exported in plain text or tab-delimited (win, UTF-8) format for the analysis of bibliometric tools. Subsequently, Microsoft Excel 2019 was used for statistical analysis of the bibliometric indicators including annual number of publications and citations, countries/regions, institutions, authors, funding agencies, journals, keywords, as well as research areas. Some inherent deficiencies from the WOS database were checked and merged, and information from various regions was incorporated into their affiliated countries. For example, publications from England, Northern Ireland, Scotland, and Wales were assigned to the UK. The journal impact factor (JIF) and subject category quartile ranks were obtained from the 2020 Journal Citation Report (JCR, http://clarivate.com/products/web-of-science). Based on the value of JIF, JCR splits all the journals within the same discipline into four categories, of the top 25% belonging to Q1 and the top 25–50% being Q2, and so on. Some other bibliometric information including sum of time cited, average number of citations, and H-index was acquired from the "citation report" function of WoSCC.

Bibliometric Analysis

In order to obtain more comprehensive data analysis, three bibliometric software including VOSviewer 1.6.16 (Leiden University, the Netherlands), CiteSpace V 5.8.R3 (Drexel University, the USA), Bibliometrix (University of Naples Federico II, Italy), and two online analysis platforms were used for bibliometric and visualization analyses.

VOSviewer, a free java-based software for bibliometric mapping and clustering analysis, was developed by van Eck and Waltman (24). In this study, country/institution/author co-authorship analysis, author/journal co-citation analysis and keyword co-occurrence analysis were conducted in this study. Generally speaking, in these visual maps, different nodes indicated different items such as authors, countries, institutions, journals and keywords, with the nodes size reflecting the corresponding number of publications citations or occurrences. The links between nodes represented the co-authorship, co-citation or co-occurrence associations between nodes. The color of the nodes and lines indicated different clusters or corresponding average appearing year (AAY) (23, 37).

CiteSpace, developed by Professor Chen Chaomei, is another software tool for visualizing and constructing bibliometric networks (25). In this study, CiteSpace was used to visualize international collaboration among institutions, the co-citation of references and its cluster analysis. We also identified several references that experienced the greatest increase in the citation frequencies over a certain period, which was considered a period of popularity for the study. The parameters of CiteSpace were set as follows: time span (2000–2021), years per slice (1), selection criteria (Top 30), and pruning (minimum spanning tree, pruning sliced networks).

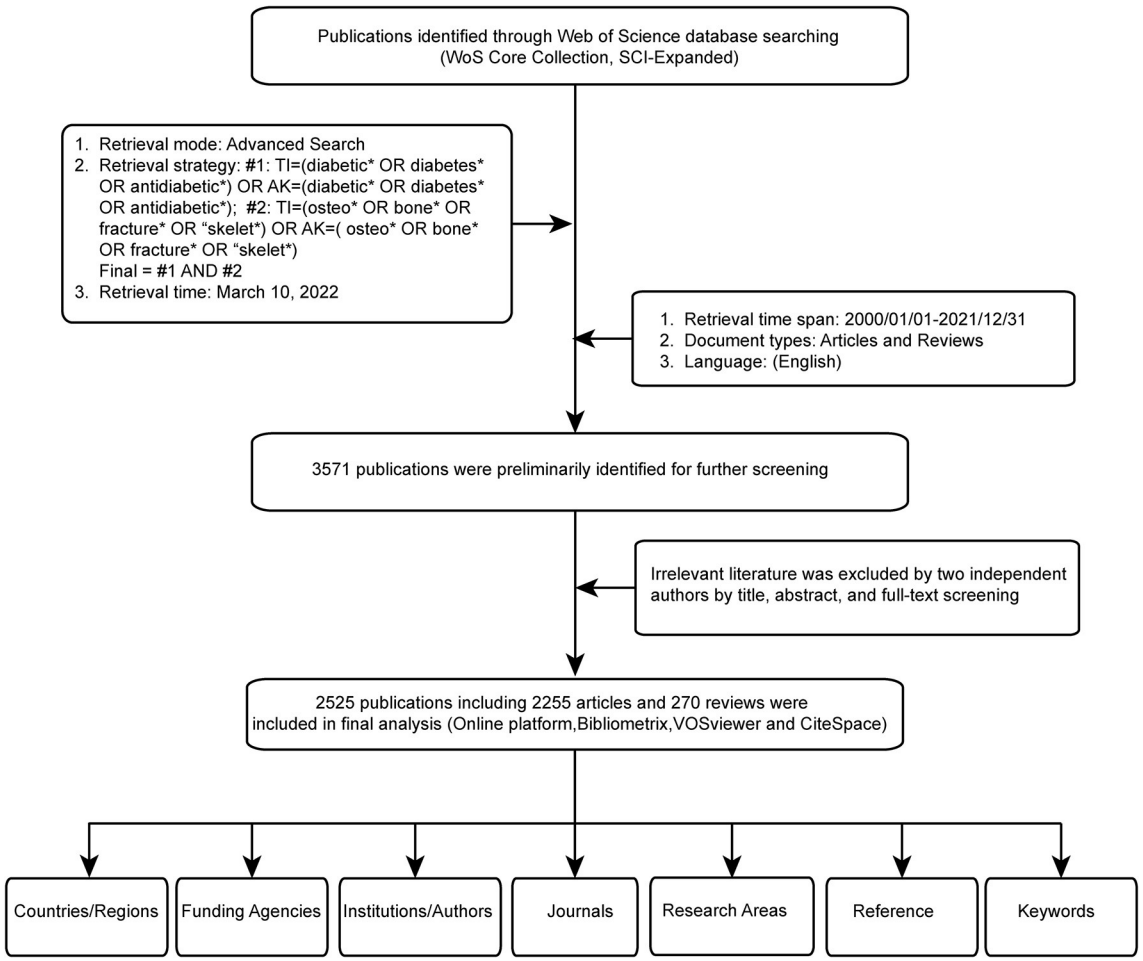


FIGURE 1 | Flowchart of literature search and selection process.

In addition, the online bibliometric analysis platform, available at: https://bibliometric.com/, was used to conduct the collaboration networks between countries, and annual publication trend analysis of the top three most productive countries (38). We also used Bibliometrix to perform descriptive analysis of the authors' production over time and thematic evolution of keywords. Furthermore, gene-based analysis of osteoporosis or osteoarthritis in diabetes mellitus was analyzed by the online website (https://www.citexs.com/Summary). This website allowed us to summarize the gene data of all the studies in a certain field, and the publications were acquired based on Pubmed database.

Statistical Analysis

Descriptive data analysis, graph plotting and curve fitting were performed with Microsoft Excel 2019, R software (v 4.1.0) and GraphPad Prism software (v 8.0). The annual number of publications and citations were calculated with Microsoft Excel, and the following types of functions including exponential, linear, logarithmic, and polynomial were used to fit curves. The best-fit model was selected according to the magnitude of the correlation coefficient (R^2). The growth rate of publications over time was calculated based on the specific calculation formula as follows (23): Growth rate = [(number of publications in the last year ÷ number of publications in the first year)$^{1/(\text{lastyear}-\text{firstyear})}$−1] × 100. Pearson's correlation coefficient test was used to evaluate the correlation between publications and citations, and Pearson correlations corresponding to a p-value < 0.05 were considered significant.

RESULTS

Publication Outputs and Trends

Based on the literature search and screening strategy in **Figure 1**, a total of 2,525 literature studies including 2,255 (89.3%) articles and 270 (10.7%) reviews were finally identified. **Figure 2** depicts the specific amount of annual publications regarding BMDM. From 2000 to 2021, the average growth rate of publications was 13.1%. Moreover, in order to further evaluate the change trend of BMDM studies, the index function $Y = 0.5953X^2 - 0.8034X + 21.325$ ($R^2 = 0.989$, X is the year, Y is the annual publications) of the annual publication trend was created.

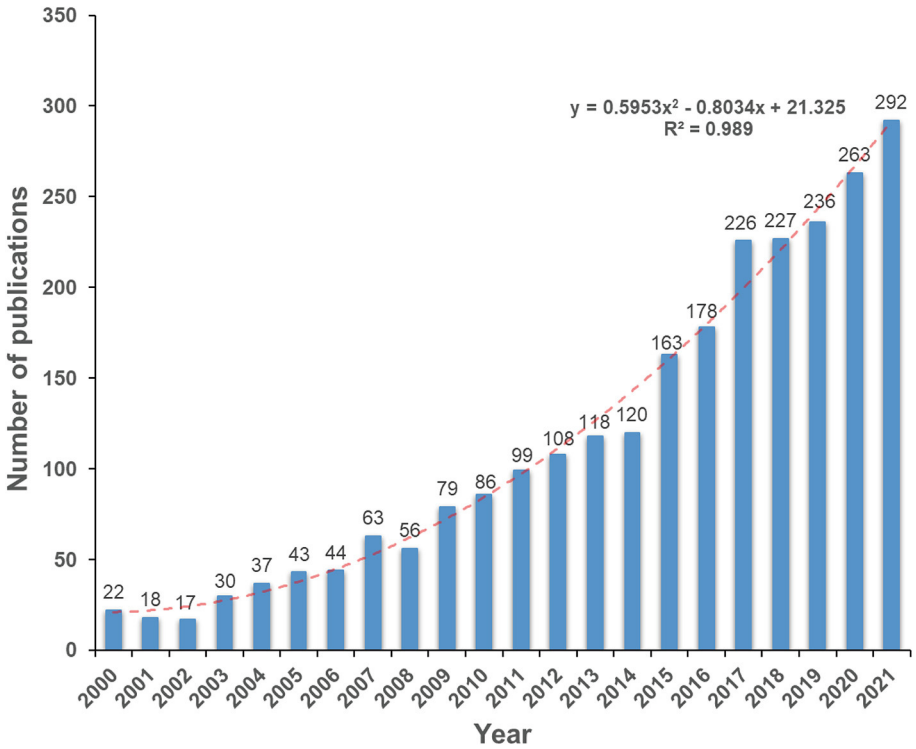


FIGURE 2 | The specific amount of annual publications regarding BMDM from 2000 to 2021.

When it comes to the annual number of citations, it also showed a similar increasing trend ($R^2 = 0.9974$) as the annual publication number (**Supplementary Figure 1**). Upon plotting the correlation between publications and citations, a statistically significant linear correlation was observed with a satisfactory Pearson's correlation coefficient ($r = 0.996$) and explicability ($R^2 = 0.992$). Thus, the variations in the citation rate can mostly be explained by the publication rate.

Most Productive Countries/Regions and Funding Agencies

A total of 78 countries/regions contributed all publications on BMDM research. As can be seen from **Table 1**, the most prolific country is the United States ($n = 656$, accounting for 25.98% of the total), followed by China and Japan. **Figure 3A** summaries the annual publications of these top 3 countries from 2000 to 2021. Among the top 20 most productive countries, the United States has the highest H-index of 97, and far higher than other countries/regions. Nevertheless, based on the average citations per document, the ranking of countries is: Netherlands (60.2), the United States (50.2), and Canada (49.37). **Figure 3B** displays the international cooperation analysis among different countries. As shown in **Figure 3C**, the overlay visualization map of country co-authorship analysis was conducted by VOSviewer. Literature originating from 47 countries/regions was selected, with the minimum number of 5 documents for each country. The United States was situated in a central position of this network map. **Figure 3D** summarizes the data of the top 10 most frequent funding sources in this field, with seven funding agencies based in the United States.

Analysis of Institutional Output

A network visualization map of institutional collaboration was generated by CiteSpace and presented in **Figure 4A**. The top 5 institutions addressed in the largest number of publications were University of California San Francisco, Shanghai Jiao Tong University, Shimane University, Washington University, and Aarhus University Hospital. However, there were relatively few collaborations among institutions from different countries. And only three institutions including University of California San Francisco, Columbia University, and University of Toronto had a betweenness centrality (BC) value more than 0.1. Additionally, institution co-authorship analysis was conducted by VOSviewer (**Figure 4B**). According to the color gradient in the lower right corner, these institutions such as Aarhus University Hospital, Sichuan University, University of Southern Denmark, University of Copenhagen, etc. were given a red color with the larger AAY values. And corresponding to this, University of Pittsburgh, Michigan State University, University of Minnesota were given a blue color with the smaller AAY values.

Analysis of Influential Authors

In terms of author analysis, the top 5 most prolific authors were laid out in **Figure 5A**. Schwartz AV contributed the highest number of publications, followed by Sugimoto T and Kanazawa

TABLE 1 | Top 20 most productive countries in the research field of BMDM.

Ranking	Countries	Publications, n	% of 2,525	H-index	Average citations per document
1	USA	656	25.98	97	50.2
2	China	596	23.60	39	12.6
3	Japan	240	9.50	48	30.9
4	UK	151	5.98	45	39.13
5	Italy	146	5.78	38	33.58
6	Brazil	107	4.24	26	18.24
7	Germany	103	4.08	35	39.31
8	Denmark	99	3.92	30	46.79
9	Canada	86	3.41	30	49.37
10	South Korea	71	2.81	17	20.27
11	Australia	69	2.73	20	20.25
12	Spain	67	2.65	22	21.97
13	Turkey	67	2.65	22	17.97
14	France	66	2.61	28	38.8
15	Netherlands	49	1.94	24	60.2
16	India	43	1.70	14	14.93
17	Sweden	41	1.62	17	38.12
18	Saudi Arabia	39	1.54	14	17.21
19	Belgium	38	1.50	19	23.97
20	Switzerland	37	1.47	21	45.89

Ranking: based on the number of total publications.

I. Schwartz AV was also the author with the highest H-index and average citations per document. **Figure 5B** depicts the annual outputs and citations of the top 5 authors between 2000 and 2021. A cluster density map of author co-authorship analysis is shown in **Figure 5C**. Only authors with more than 5 documents were included. Of these, a total of 11 author clusters were formed. As for author co-citation analysis, 73 authors with at least 100 citations were included. As displayed in **Figure 5D**, the top 3 authors with the greatest total link strength (TLS) were Schwartz AV, Vestergaard P, and Janghorbani M.

Most Active Journals and Research Areas

Table 2 shows the basic information of the top 20 most productive journals in BMDM field. Among them, *Bone* has published the greatest number of 122 papers, followed by *Osteoporosis International*, *Journal of Clinical Endocrinology Metabolism*, *Calcified Tissue International*, and *Journal of Bone and Mineral Research*. More than four fifths of the top 20 journals were categorized in Q1 or Q2 JCR region. As indicated in **Figure 6A**, the network visualization map of journal co-citation analysis was further performed by VOSviewer. Only journals with a minimum of 300 citations were visualized. Of the 58 journals satisfying the criteria, the top 5 co-cited journals were *Journal of Bone and Mineral Research*, *Osteoporosis International*, *Bone*, *Journal of Clinical Endocrinology Metabolism*, *Diabetes Care*. Additionally, according to WoS subject categories, all these literatures are assigned to different research areas. Top 10 research areas ranked by publication counts are exhibited in **Figure 6B**.

Co-cited References and Reference Burst

Table 3 summarizes the characteristics of the top 10 highly cited literature in the research scope of BMDM. The majority of the studies were published before 2010. Of these, three papers were cited over 500 times with all the top 10 cited 370 times or more. Besides that, reference co-citation analysis was also conducted by CiteSpace. As shown in **Figure 7** and **Supplementary Table 1**, all the nodes representing references in the co-citation network map could be clustered into 17 specific clusters. The modularity was 0.89, and the mean silhouette value was 0.9618, reflecting the rationality of this clustering method. All these clusters were generalized and ordered by the number of co-cited references. The first cluster was "#0 glycation end-product," followed by "#1 oxidative stress" and "#2 energy metabolism." Apart from that, the references with strong citation bursts were explored via CiteSpace, and the top 50 references with the strongest citation bursts were identified. As indicated in **Figure 8**, the strongest burst starting from 2008 was from the paper published by Vestergaard P and colleagues in 2007, followed by Schwartz et al. (47), Janghorbani et al. (39).

Analysis of Co-occurring Keywords and Related Genes

After manual merging the keywords with the same meaning, a total of 58 author keywords with a minimum of 20 occurrences were extracted from the 2,525 publications, and an overlay visualization map was created (**Figure 9A**). All these keywords were marked with different colors based on AAY, which could reflect the research hotspots in different periods. **Figure 9B** presents the frequency distribution of the top 20 most frequent occurrences keywords. Among them, the top 5 keywords were as follows: diabetes mellitus (964), type 2 diabetes mellitus (509), osteoporosis (366), bone mineral density (353), and fracture (225). In addition, thematic evolution analysis was also performed by Bibliometrix. Sankey diagram was used to

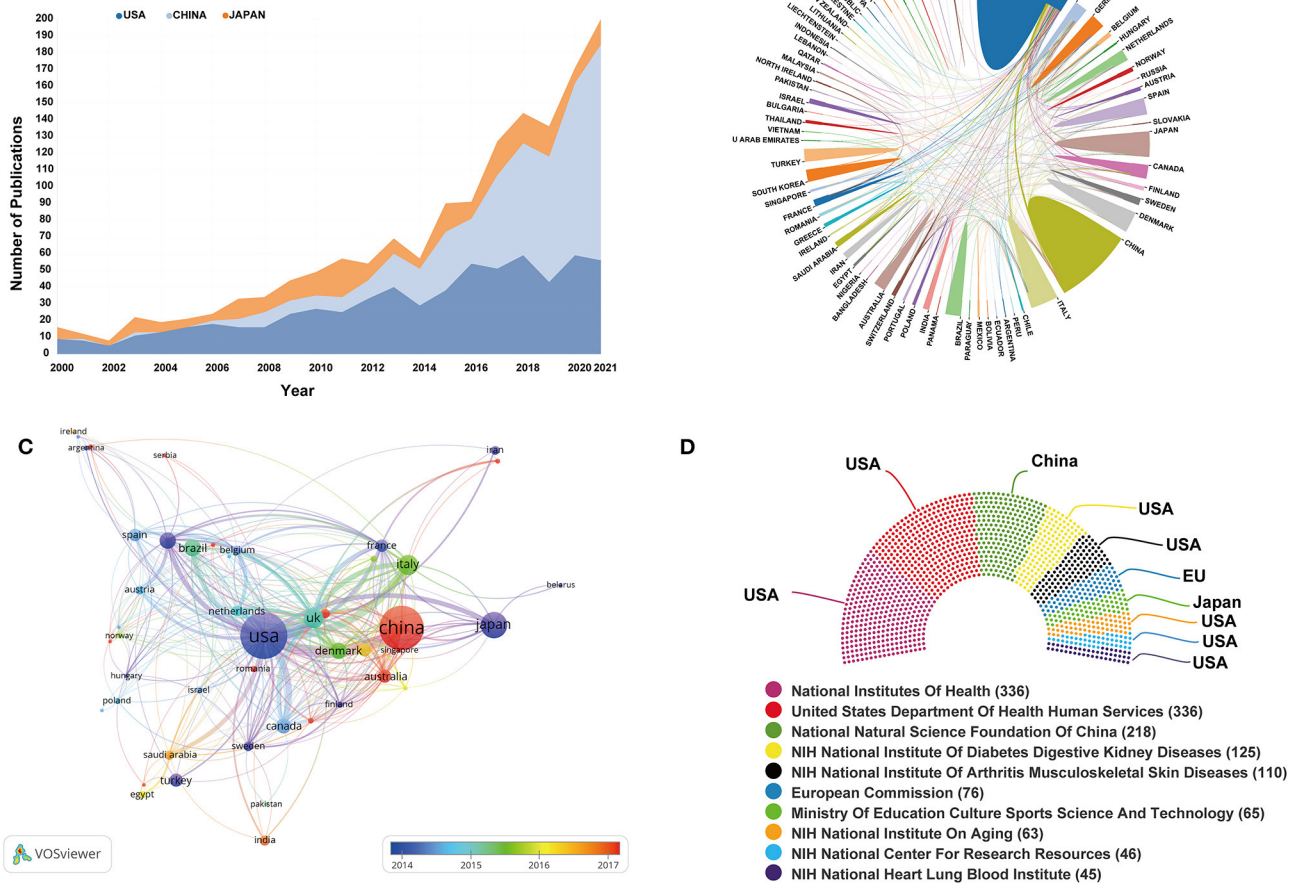


FIGURE 3 | (A) The annual publications of the top 3 countries from 2000 to 2021. **(B)** The international cooperation analysis among different countries. Line thickness between countries reflects the intensity of the closeness. **(C)** The overlay visualization map of country co-authorship analysis conducted by VOSviewer. Each country is represented as a node, and the node size is proportional to the sum of publications. A link between two nodes indicates a co-authorship relationship. Different nodes were given different colors (based on AAY) according to the color gradient in the lower right corner. **(D)** The top 10 most frequent funding sources in this field.

interpret the thematic evolution of three stages in the BMDM research (**Figure 10A**). The change pattern of annual occurrences frequency of author keywords related to diseases such as diabetes, osteoporosis, osteoarthritis, etc. from 2000 to 2021 is illustrated in **Figure 10B**. Moreover, **Figure 10C** illustrates the top 15 most studied genes between osteoporosis and DM, and **Figure 10D** presents the top 15 genes between osteoarthritis and DM.

DISCUSSION

Global Publication Trends in BMDM Research

The change in the amount of academic publications is a vital indicator of the development trend in a field (48). As can be seen from the curve fitting results, the number of publications exhibited an overall rapid growth trend. From the point of view of various stages of development, from 2000 to 2006, the number of scientific publications investigating the links between BM and DM was still very low (no more than 50 publications) and unstable. The study on BMDM was still at an early stage of development, suggesting that the role of bone metabolic aberration occurred in DM did not attract too much attention of medical community at that time. Interestingly, the published studies on BMDM had a steady rise in 2007–2014, and the number has exceeded 100 since 2012. Between 2015 and 2021, the annual publication has drastically increased, and almost 62.7% of them (1,585 papers) were published over the last 7 years. This result may suggest that BMDM has generated increasing attention and interest over recent years. And on this basis, one can predict that the number of publications in this field will further grow with in-depth study of the molecular mechanism and the conduct of clinical experiments on anti-diabetic medicines (44, 49).

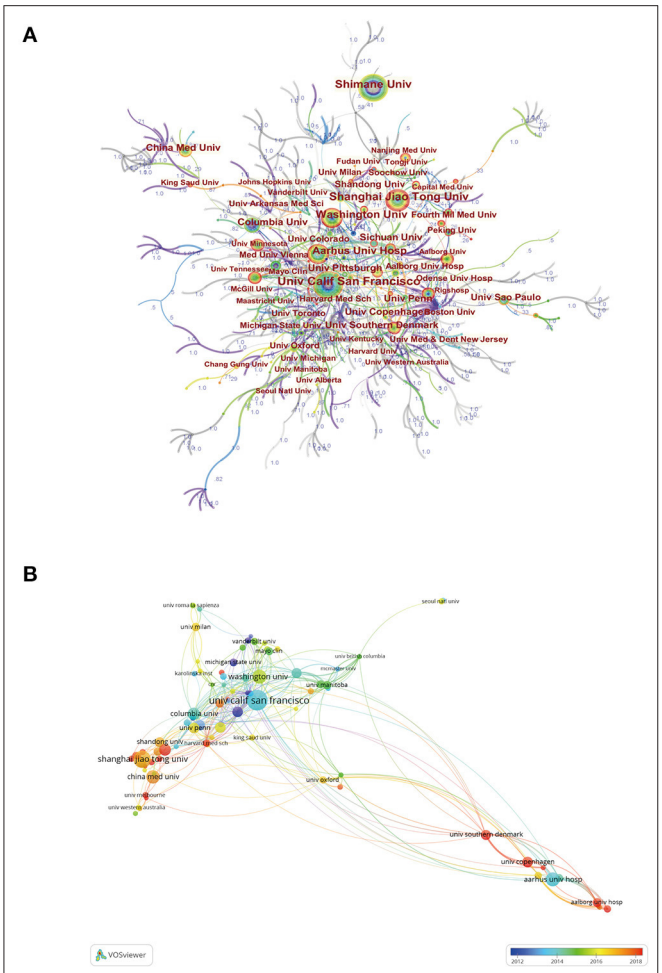


FIGURE 4 | (A) Network visualization map of institutional collaborations generated by CiteSpace. In this map, a node represents an institution, and the size of each node represents its relative quantity of research output. Each line represents the strength of the cooperation relationship between two institutions, and strength value is displayed between lines. **(B)** The overlay visualization map of Institution co-authorship analysis conducted by VOSviewer.

General Knowledge Structures and Major Contributors

Countries and Institutions

The total number of literature by a country serves as an important indicator to reflect a country's output and productivity. The results depicted in **Table 1** show that countries from North America, Europe, and Asia almost accounted for the top 20 contributing nations on BMDM. Among them, we can see that the United States, China, and Japan are the leading countries where BMDM research is occurring. In the meantime, the United States has the highest H-index. The above results reflect that the United States has made tremendous contribution and established its leading position in the domain of BMDM research. In addition to the BMDM research subject, earlier bibliometric studies revealed that the United States also had the highest number of publications in other areas of BM or DM researches (31, 32, 34). As for countries or institutions collaboration analysis, it can be seen from **Figure 3B** that the United States collaborated most closely with China, Italy, and Canada. However, when it comes to an institutional level, there were only three institutions with a BC value more than 0.1. BC is an indicator to assess the importance of nodes in a collaboration network. Generally, BC value >0.1 is regarded as vital node (22). From **Figures 4A,B**, there were relatively few collaboration and exchange of findings among institutions from different countries, and most collaborating institutions were limited to the domestic level. Considering the constant increase in the incidence of DM worldwide, this situation greatly impedes the development of the research field. It is worth noting that, consistent with other bibliometric studies, a lack of cross-institutional cooperation seems to be a general phenomenon in the field of diabetes (50, 51). Therefore, we strongly recommend that institutions from different countries should remove academic barriers and strengthen the cooperation to boost the development of BMDM research.

Authors

Among the top 5 most productive authors, Schwartz AV from University of California San Francisco contributed the most articles, followed by Sugimoto T and Kanazawa I from Shimane University. In addition, as can be seen from **Figures 5A,B**, Schwartz AV was also the author with the highest H-index and average citations per document, and remained high scientific influence in this field from 2000 to 2021. Schwartz et al. mainly focused on the increased fracture risk in diabetic elderly men and women. In 2001, by analyzing the data from 9,654 women aged 65 or older, they have determined diabetes was a risk factor for proximal humerus, hip, and foot fractures in older women. And this study has gained enormous attention and was cited more than 550 times so far (41). Moreover, in another prospective observational study published in *JAMA*, they found that bone mineral density (BMD) T-score of the femoral neck and WHO Fracture Risk Algorithm (FRAX) score were both associated with fracture risk in older adults with T2DM. Nevertheless, compared with these patients without DM, a given T-score or FRAX score was associated with higher fracture risk in older adults with DM (47). This finding has confirmed the useful of BMD T-score and FRAX score for clinical evaluation of fracture risk, and prompted a series of subsequent studies (52, 53).

As for author co-citation analysis, 73 authors with at least 100 citations were included. As displayed in **Figure 5D**, the top 3 authors with the greatest TLS were Schwartz AV, Vestergaard P, and Janghorbani M. The TLS indicates the impact of documents from an author on other authors involved in these studies. This result further confirms that Schwartz AV was the most influential author. At the same time, it is worth noting that albeit with not large number of publications, Vestergaard P from Aarhus University and Janghorbani M from Isfahan University Medical Science still occupied core locations in the co-citation map. This could have been relevant to several highly-cited publications by them. Unsurprisingly, three studies of them were the top 10 most highly-cited articles in this domain (4, 39, 46). And the study by

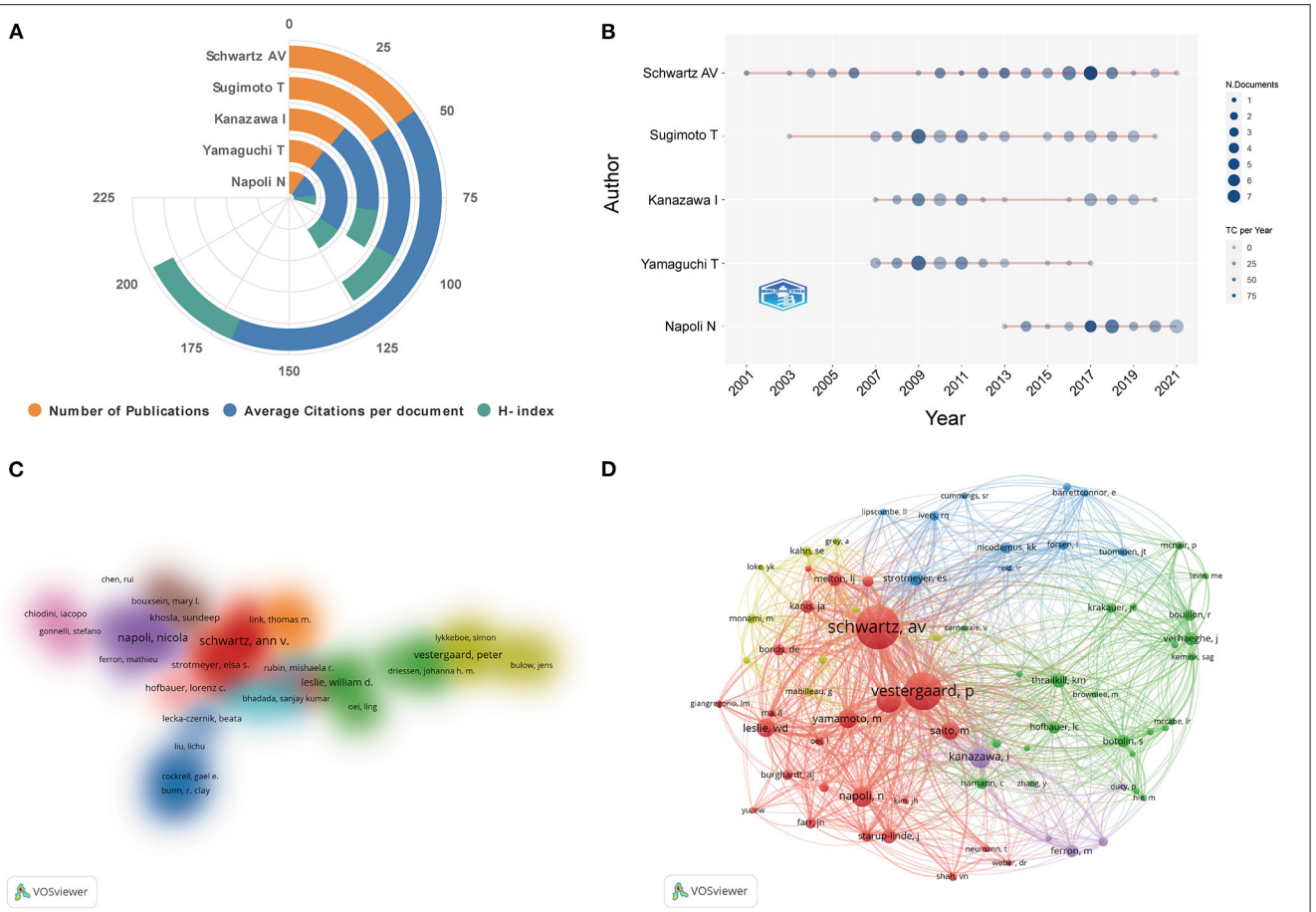


FIGURE 5 | (A) Total number of publications, average citations per document, and H-index of top 5 authors in this area. **(B)** Top 5 authors' production over time. The circle size represents the number of documents (N. Documents), and the shade of the color signifies the total number of citations (TC). **(C)** The cluster density map of author co-authorship analysis generated by VOSviewer. Authors with close cooperation relationship are assigned to one cluster with the same color. **(D)** The network visualization map of author co-citation analysis generated by VOSviewer. Each author is represented as a node, and the node size is proportional to the sum of citations. A link between two nodes indicates a co-citation relationship. The distance between nodes indicates the relatedness, and a smaller distance implies a higher relatedness and will be assigned to one cluster with the same colors.

Vestergaard (4) titled "Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis" was the only one study that received more than 1,200 citations in this field. This result suggests that the number of publications may not definitely reflect the academic influence of an author, since there are many factors that influence the citation frequencies of an article (27).

Journals

Journals are an important vehicle for presenting the results of academic information and knowledge dissemination. Few researchers are able to fully understand all relevant journals in their field, and many of them struggle to select the most proper outlet and target journals for their studies. By summarizing the basic information drawing the co-citation visual network of the most productive and high-impact journals, the most relevant journals in the research field of BMDM could be identified so that researchers can choose the most suitable journals for submitting their manuscripts. Among the top 20 most productive journals, most relevant studies were published in Q1 or Q2 journals, and *Bone*, *Osteoporosis International*, *Journal of Clinical Endocrinology Metabolism*, *Calcified Tissue International*, and *Journal of Bone and Mineral Research* occupy the first five positions, making them zone core and popular journals in BMDM field. In term of co-citation analysis, the top 5 co-cited journals were *Journal of Bone and Mineral Research*, *Osteoporosis International*, *Bone*, *Journal of Clinical Endocrinology Metabolism*, *Diabetes Care*. Research findings related to BMDM that published in these journals may have great potential to be cited and receive more attention. Therefore, scholars may consider these priority journals in the future, and scientific outputs by these journals also deserve special attention to obtain recent advances in this domain.

Research Clusters and Research Focus Transition

Reference and keyword analysis is one of the key methodologies and most significant indicators in bibliometrics. Based on the reference co-citation analysis and keyword co-occurrence

TABLE 2 | Top 20 most productive journals in BMDM field.

Ranking	Sources title	Output	% of 2,525	JIF 2020	JCR quartile 2020
1	Bone	122	4.83	4.398	Q2
2	Osteoporosis International	102	4.04	4.507	Q2
3	Journal of Clinical Endocrinology Metabolism	73	2.89	5.958	Q1
4	Calcified Tissue International	56	2.22	4.333	Q2
5	Journal of Bone and Mineral Research	55	2.18	6.741	Q1
6	Diabetes Care	53	2.10	19.112	Q1
7	PLoS One	43	1.70	3.24	Q2
8	Diabetes Research and Clinical Practice	41	1.62	5.602	Q1
9	Journal of Bone and Mineral Metabolism	33	1.31	2.626	Q3
10	Frontiers in Endocrinology	28	1.11	5.555	Q1
11	Journal of Diabetes and Its Complications	28	1.11	2.852	Q3
12	Current Osteoporosis Reports	26	1.03	5.096	Q2
13	Diabetic Medicine	26	1.03	4.359	Q2
14	Scientific Reports	26	1.03	4.38	Q1
15	Acta Diabetologica	23	0.91	4.28	Q2
16	Diabetes Metabolism Research and Reviews	21	0.83	4.876	Q2
17	Endocrine	21	0.83	3.633	Q3
18	Journal of Periodontology	21	0.83	6.993	Q1
19	Diabetologia	20	0.79	10.122	Q1
20	European Journal of Endocrinology	19	0.75	6.664	Q1

Ranking: based on the number of total publications.

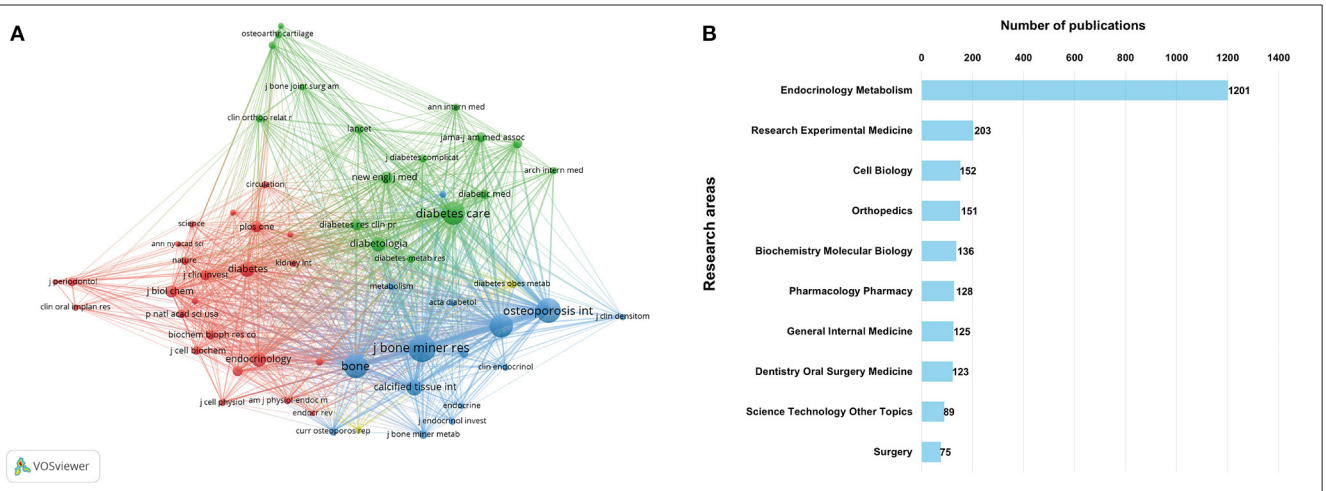


FIGURE 6 | (A) Network visualization map of journal co-citation analysis generated by VOSviewer. **(B)** Top 10 research areas ranked by publication counts.

analysis, the main research directions, hotspots, and evolution process in the field can be uncovered (54, 55).

References Co-citation Analysis

Using the CiteSpace software, a visualization network map of co-cited references was generated in **Figure 7**. All these 17 BMDM research clusters in the past 22 years were ordered from the largest to smallest based on the number of co-cited references. As can be seen from **Supplementary Table 1**, "glycation end-product" was the largest cluster (#0), followed by "oxidative stress" (#1), and "energy metabolism" (#2). In addition, the timeline chart could cluster references and take time into account, which is convenient for us to understand the period of a particular topic and explore the evolution track of this field (34). According to mean year of these clusters, we can see that the research hotspots have shifted to "high glucose environment" (#8), "glycation end-product" (#0), and "sodium-glucose co-transporter" (#7).

High Glucose Environment

Although glucose represent key molecules in cellular energy metabolism, previous studies revealed that hyperglycemia had

TABLE 3 | Characteristics of top 10 highly cited literatures on BMDM.

Ranking	Title	Total citations	Average citation frequency per year	Journal	References
1	Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis	1,248	78	Osteoporosis International	(4)
2	Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture	796	49.75	American Journal of Epidemiology	(39)
3	Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice	674	44.93	Proceedings of the National Academy of Sciences of the United States of America	(40)
4	Older women with diabetes have an increased risk of fracture: a prospective study	557	25.32	Journal of Clinical Endocrinology & Metabolism	(41)
5	Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus	539	41.46	Osteoporosis International	(42)
6	Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women	410	18.64	Diabetes Care	(43)
7	Association of BMD and FRAX Score with Risk of Fracture in Older Adults with Type 2 Diabetes	405	33.75	Jama-Journal of the American Medical Association	(41)
8	Mechanisms of diabetes mellitus-induced bone fragility	388	64.67	Nature Reviews Endocrinology	(44)
9	Rosiglitazone-associated fractures in type 2 diabetes—An analysis from a diabetes outcome progression trial (ADOPT)	388	25.87	Diabetes Care	(45)
10	Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk	370	20.56	Diabetologia	(46)

Ranking: based on the number of total citations.

an adverse effect on bone quality. *In vitro* experiments have proven that high glucose environment exerts an inhibitory action on the osteogenic differentiation and growth of osteoblasts and bone marrow stroma cells (BMSCs) (18, 56). Glycolysis is a fundamental metabolic pathway for glucose catabolism. However, under a high-glucose microenvironment, excess glucose may activate other metabolic pathways such as the polyol pathway, the protein kinase C signaling pathway, the formation of glycation end-products, and the hexosamine pathway, all of which induce the accumulation of oxidative stress and the production of inflammatory cytokines (57, 58). Furthermore, there is also study showing that sustained hyperglycemia may trigger cellular innate immune responses, activate reactive oxygen species (ROS)-producing enzymes localized on the outer mitochondrion, resulting in overproduction of ROS and cell dysfunction (59). Therefore, in recent years, a large number of studies have focused on how to rescue the impaired osteogenesis differentiation ability of BMSCs and osteoblasts (56, 57, 60, 61). That is very critical and meaningful way to find novel therapeutic targets for diabetic osteoporosis.

Glycation End-Product

Advanced glycation end products (AGEs) are irreversibly produced from non-enzymatic glycation and oxidation of nucleic acids, lipids and proteins. The receptors for AGEs (RAGE) are the main receptors involved in the cellular uptake and degradation of AGEs and are also known as a pattern recognition receptor. Previous studies revealed that RAGE and its ligands play important roles in bone homeostasis (62, 63). RAGE knockout mice exhibit decreased bone resorptive activity and increased bone mineral density (62). AGEs were reported to suppress osteogenic differentiation of adipose-derived stem cells (ASCs) via interfering with the Wnt/β-catenin signaling pathway (63). In addition, accumulation of AGEs could also promote expression of transforming growth factor (TGF)-β and IL-6 to induce apoptosis of osteoblasts and elevate osteoclasts activity (64, 65). As previously stated, protracted exposure to excessive blood glucose results in excessive formation and accumulation of AGEs. Increasing evidence indicates that the accumulation of AGEs are important contributors of diabetic bone loss (13). Accordingly, small-molecular inhibitors such as TTP488, FPS-ZM1, etc. targeting RAGE and its intracellular signaling pathway have been developed (66, 67). These small molecule inhibitors hold great promise for the treatment of multiple metabolic bone diseases and merit further investigation.

Sodium-Glucose Co-transporter

Sodium glucose cotransporter-2 (SGLT2) is a sodium-dependent glucose transporter involved in glucose reabsorption in the kidney. The inhibitors of SGLT2 have been established as a novel

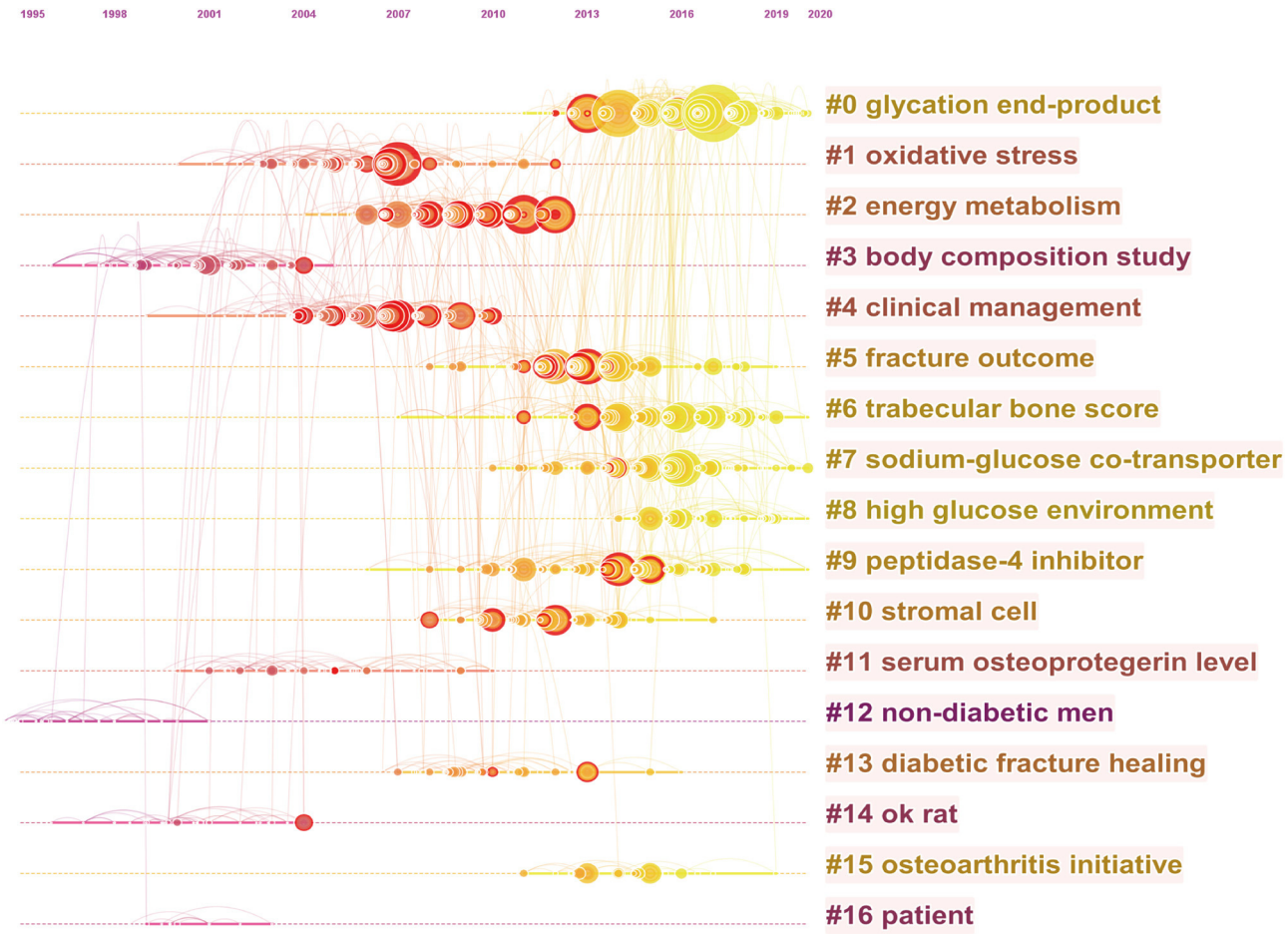


FIGURE 7 | The timeline view map of reference co-citation analysis generated by CiteSpace. Years from 2000 to 2021 are arranged horizontally at the top, and this view map clearly implies the differences in the appearance time point of 17 clusters. The bigger the circle size, the more research on the topic. The label of each cluster (LLR algorithm) is shown at the end of the timeline.

therapeutic approach for diabetic control, working by decrease the reabsorption of glucose and increase the glucose excretion. Since the first SGLT-2 inhibitor, canagliflozin, approved by USA Food and Drug Administration in 2013, more than seven SGLT-2 inhibitors such as empagliflozin, ertugliflozin and dapagliflozin, etc. have been introduced into the clinic nowadays (68). However, there remains controversy about the effects of different SGLT-2 inhibitors on bone metabolism. Among them, canagliflozin is the most studied. In the Canagliflozin Cardiovascular Assessment Study (CANVAS) trial, an increased risk of fractures was observed in patients treated with canagliflozin, which triggered concerns about bone health (69). The exact mechanisms of canagliflozin increasing fracture risk are unclear, and existing evidence suggests that canagliflozin might have an impact on the homeostasis of calcium and phosphate, and the secretion of parathyroid hormone (PTH) (70). Despite all of this, multiple subsequent studies have found that canagliflozin was not associated with an increased risk of fractures (71, 72). Therefore, available evidence on SGLT-2 inhibitors is somehow conflicting and cannot fully support a direct responsibility for bone fractures. In addition, the effects of other kinds of SGLT-2 inhibitors on bone health need more investigations to confirm (73).

References With Citation Burst

Apart from cluster analysis, burst references are considered another important indicator to track and capture the research hotspots and emerging trends over time. Burst references refers to references heavily cited by other studies over a period of time, which implies that they have received particular attention at a certain time period (34). References with outbreak durations ≥5 years were shown in **Figure 8**. Top 50 references with the strongest citation bursts were listed. Among these references, the strongest burst starting from 2008 was from the paper published by Vestergaard P in 2007 (4), followed by Schwartz et al. (47), Janghorbani et al. (39). Additionally, references with citation bursts were first appeared in 2005 due to 5 literatures published in 2004 (74–78). Of these, a multiethnic study conducted by Strotmeyer et al. found that T2DM was associated with higher BMD in all race-gender elderly adults, and independent of central adiposity, increased obesity, or fasting insulin levels. Heap et al.

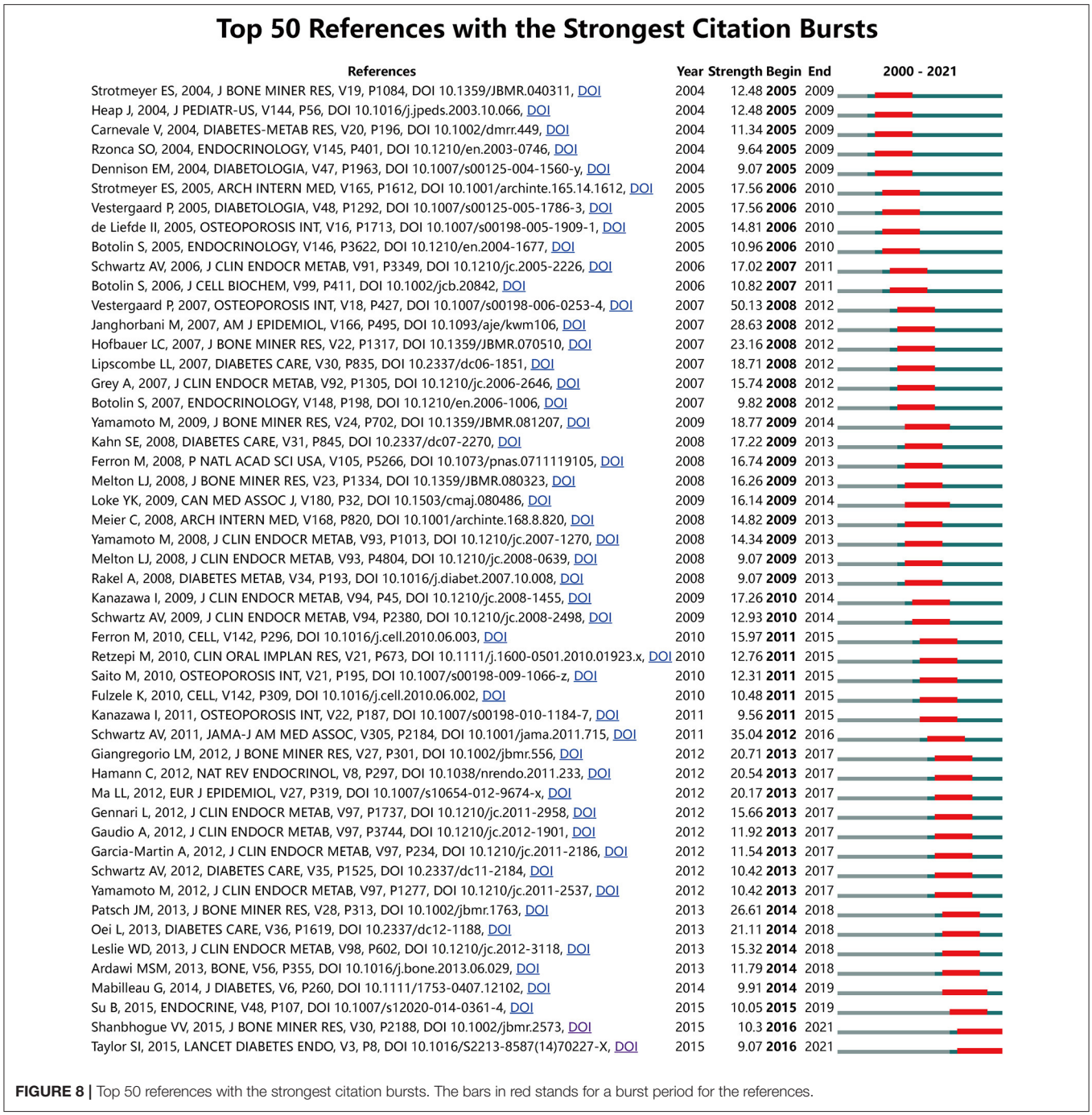


FIGURE 8 | Top 50 references with the strongest citation bursts. The bars in red stands for a burst period for the references.

(75) investigated whether blood glucose regulation and disease duration could influence the bone characteristics in adolescents with T1DM. Their findings suggest that altered BMD acquisition in these adolescents might limit the acquisition of peak bone mass and increase the risk of developing osteoporosis in later life. An elegant review by Carnevale et al. (76) summarized the evidence that confirmed the diabetic patients with higher fracture risk and the possible mechanisms. While Rzonca et al. (77), found that rosiglitazone therapy posed potential risks of adverse skeletal effects based on *in vitro* experiments. Regarding the fifth research, their study confirmed the positive association between BMD and T2DM, and partly mediated by adiposity (78). Of note, there are still two references published in 2015 with ongoing burst, which means they have received substantial attention recently (70, 79). Of them, one cross-sectional *in vivo* study has assessed the bone characteristics in adult T1DM patients with and without diabetic microvascular disease. Their findings suggested that the presence of diabetic microvascular disease was associated with

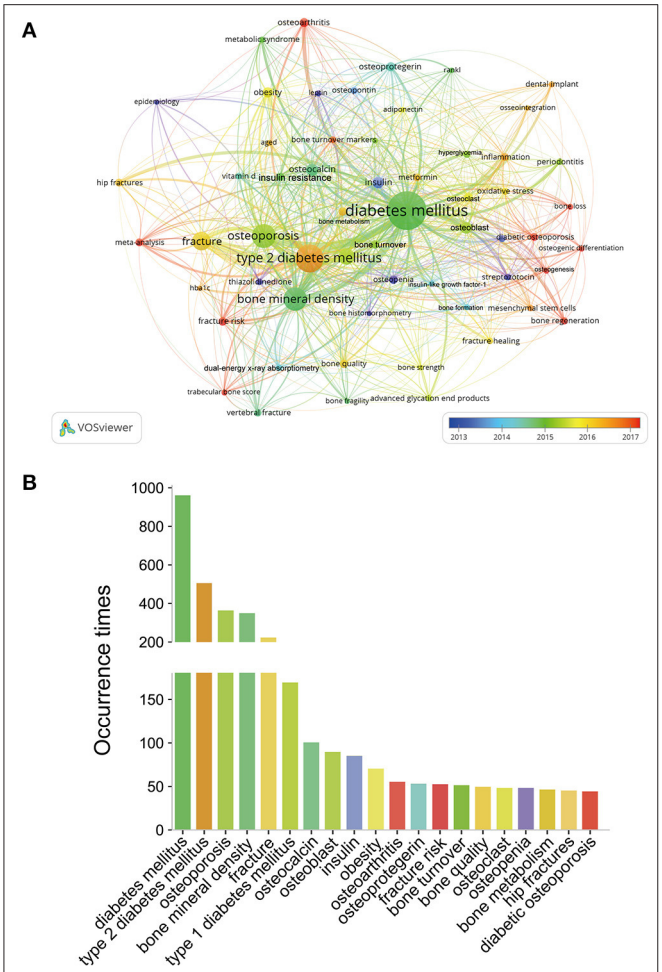


FIGURE 9 | (A) Overlay visualization map of keywords analysis in BMDM field based on the VOSviewer. The node size is proportional to the sum of occurrence times. The color of each node implies the average appearing year according to the color gradient in the lower right corner. The blue color represents the keywords appeared relatively earlier, and the dark red color reflects the recent occurrence. **(B)** Frequency distribution of the top 20 most frequent occurrences keywords.

the deficits in bone microarchitecture. Another study by Taylor et al. (70) explored the possible downstream mechanisms of SGLT2-inhibitors on bone. This finding illustrates again that SGLT2 is a hot topic in BMDM research.

Keywords Analysis and Related Genes

Author keywords are usually the most highly represented terms selected to explain the subject matter of research (34). In this study, we constructed the keyword co-occurrence network map with VOSviewer software. As several author keywords had various forms, but the same meaning. After manual merging the keywords with the same meaning, a total of 58 author keywords were extracted from the 2,525 publications, and an overlay visualization map was created in **Figure 9A**. Besides the keywords related to DM, osteoporosis and related fractures and osteoarthritis are the main bone metabolic diseases of greatest concern in this field. In the meantime, it can be seen that although patients with T1DM have a much higher risk of fracture compared to those with T1DM, researchers' concern about T2DM is relatively higher, which may have relevance to the higher incidence of T2DM. Moreover, Sankey diagram of authors' keywords was conducted to interpret the thematic change and evolution in the BMDM research field. Several popular research topics in a certain period may slow down with the appearance of other novel directions (80). The period of 22 years considered for our included publications was split into 3 periods: 2000–2007, 2008–2014, and 2015–2021. In the first period, thematic evolution was observed in 12 research areas: streptozotocin, bone density, type 1 diabetes mellitus, alveolar bone loss, osteoblast, collagen, bone histomorphometry, hyperglycemia, insulin, osteopontin, diabetes mellitus, epidemiology. As for the second period, seven new thematic domains including osteocalcin, osteoporosis, bone formation, bone quality, charcot foot, bone metabolism, and osteoprotegerin emerged. When it comes to the third period, the research topics were more centrally and diabetes mellitus and osteoporosis remain as the primary themes.

In the overlay visualization map of keyword co-occurrence analysis, different keywords were marked with various colors based on AAY. The blue color represents the keywords appeared relatively earlier, and the dark red color reflects the recent occurrence. Thus, these keywords with blue color such as "insulin," "leptin," "epidemiology," and "streptozotocin" were the major topics during the early stage. And these keywords such as "diabetic osteoporosis," "osteoarthritis," "fracture risk," "meta-analysis," "osteogenic differentiation," "bone regeneration," "osteogenesis," and "trabecular bone score" (81) were colored in red, which suggests that these research topics are attracting attention recently and may remain the research hotspots and frontiers in the near future. Take meta-analysis as an example, it is an important tool for evidence-based medicine. In previous studies, scholars have conducted extensive meta-analyses to assess the relationship between BMD/bone loss/fracture and DM/antidiabetic medication (4, 82, 83). Of these, the most well-known one was conducted by Vestergaard (4). However, due to the lack of large-sample randomized controlled studies in BMDM field, most of meta-analysis were based on retrospective studies. Further randomized controlled trials of high quality are warranted to be performed for update of the results of these meta-analyses.

Moreover, as osteoporosis and osteoarthritis are the most representative metabolic bone diseases associated with DM, we also summarized the most studied genes among them by online data analysis website. As shown in **Figures 10C,D**, *INS*, *BGLAP*, *PTH*, *IGF1*, and *TNF* were the top 5 most studied genes between osteoporosis and DM (84, 85). As for osteoarthritis and DM, the top 5 most related genes were *INS*, *TNF*, *IL-6*, *CRP*, and *IL-1B* (86–88). It can be seen that, unlike diabetic osteoporosis, multiple inflammatory factors are involved in the progress of diabetic osteoarthritis. Recently, increasing studies have uncovered that in diabetic patients with osteoarthritis, diabetic treatment such as DPP-4 could partially improve osteoarthritis symptoms by decreasing the production of inflammatory cytokines such as

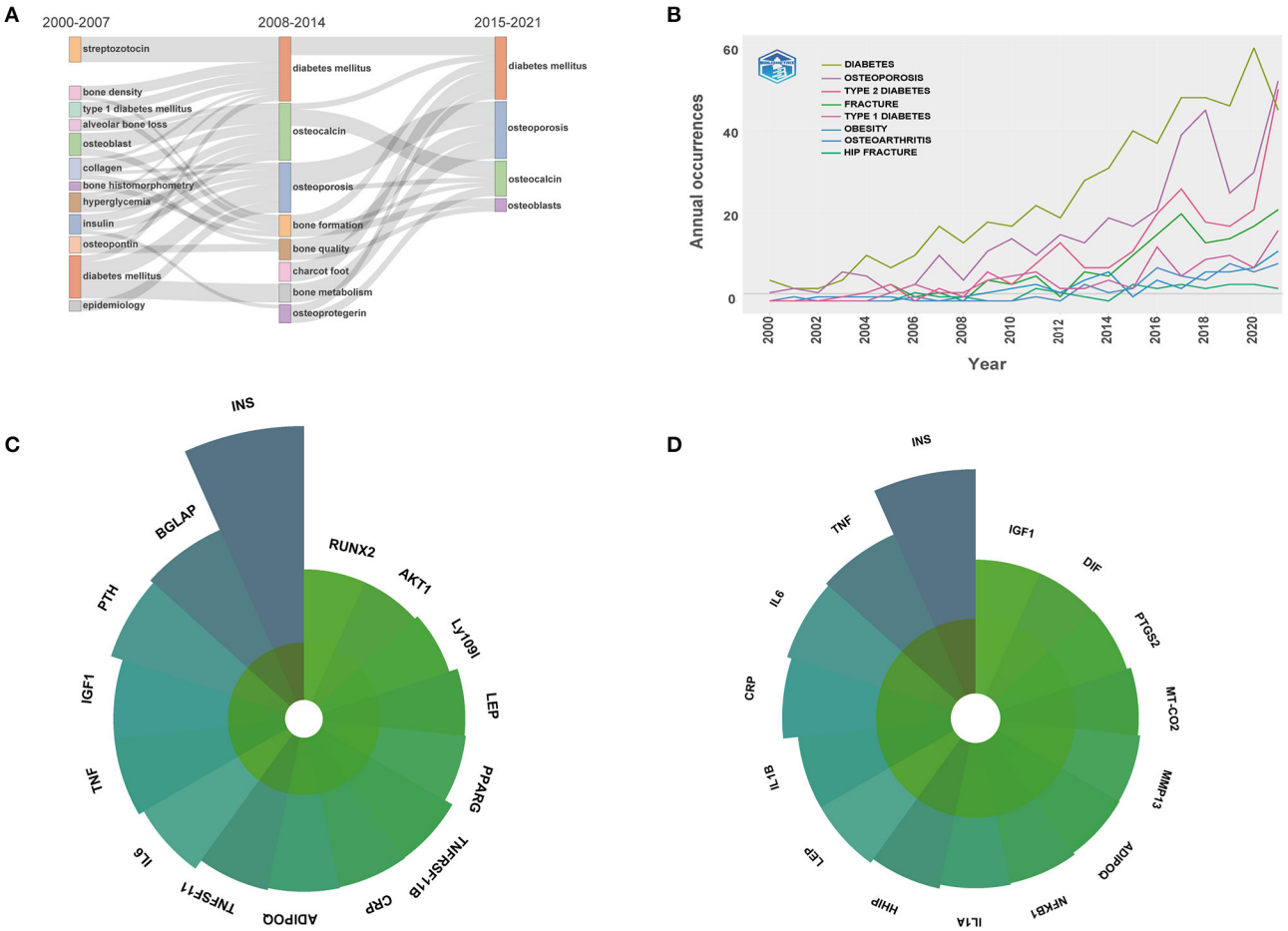


FIGURE 10 | (A) Thematic evolution of the three stages in the BMDM research field. The line between nodes reflects the research topic's evolving focus. The width indicates the amount of shared keywords. The thicker the line, the greater the significance of the two subjects. **(B)** The change pattern of annual occurrences frequency of author keywords related to disease. **(C)** Top 15 most studied genes between osteoporosis and diabetes mellitus. **(D)** Top 15 most studied genes between osteoarthritis and diabetes mellitus.

IL-6, IL-8, and TNF-α (86, 87). These results could serve as a reference for investigators in the field.

STRENGTHS AND LIMITATION

The present study has certain strong points in contrast to previous studies that adopted only meta-analysis or narrative reviews. Most importantly, it is the first bibliometric study to map and characterize the knowledge landscapes on BMDM from 2000 to 2021. Meanwhile, we used multiple types of bibliometric software and tools for analysis and visualization, which would add richness to the results. Another strength is checking the quality of literature included in the final analysis. However, there are also several additional limitations to our work. First of all, data on BMDM were primarily retrieved and collected from the WoSCC database, which would miss several related publications not included in these databases. However, it should be noted that the WoSCC is the most commonly used database for bibliometric analysis, and the data from WoSCC can represent the condition of most publications in a certain field to some extent. There have been few studies used more than two electronic databases in the previous studies owing to the limitation of file formats (22, 23, 36). Secondly, despite our manual screening and normalization procedures, selection bias may have still existed due to the merge of some keywords and continuous updates of the database. Nevertheless, we believe that our findings are still to be an effective representation of the global outputs of bone metabolism in diabetes mellitus. Finally, we only included literature published in English, meaning that some relevant publications may have been missed.

CONCLUSIONS

This study provides a missing analysis of global research progress on diabetes mellitus and bone metabolism. Analysis of all literature published in English showed an overall increasing trend. Major contributions were from North American, European, and Asian countries, institutions, and authors, led

by the United States. The journals that published the most BMDM-related papers were *Bone* and *Osteoporosis International*. Osteoporosis and related fractures are the main bone metabolic diseases of greatest concern in this field. The main funding agencies and collaborations were also found to be from developed regions, showing that increased collaboration is needed to boost the development of BMDM research. Several research topics including high glucose environment, glycation end-product and sodium-glucose co-transporter have been recognized as the current research focus in this domain. The following research directions such as diabetic osteoporosis, osteoarthritis, fracture risk, meta-analysis, osteogenic differentiation, bone regeneration, osteogenesis, and trabecular bone score may remain the research hotspots and frontiers in the near future. All in all, we believe that this bibliometric study can help researchers explore potential cooperation opportunities, and also understand BMDM field's knowledge landscapes, evolution process, and research hotspots in this field. This study represents a call to researchers and clinicians that like other diabetic complications, diabetic osteopenia deserves more attention.

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.

AUTHOR CONTRIBUTIONS

KC, ZS, and HW designed the study. KC, QG, ZS, and WY collected the data. KC, WY, YW, and HW analyzed the data and drafted the manuscript. KC, ZS, WY, and QG revised and approved the final version of the manuscript. All authors read and approved the submitted version.

FUNDING

This work was supported by the Tianjin Municipal Health Bureau (grant number 14KG115) and Key Program of the Natural Science Foundation of Tianjin (grant number 20JCZDJC00730).

ACKNOWLEDGMENTS

The authors thank Dr Yan Zhou and home-for-researchers (www.home-for-researchers.com) for their effort in polishing the English content of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.918483/full#supplementary-material

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* (2006) 3:e442. doi: 10.1371/journal.pmed.0030442
2. Hofbauer LC, Busse B, Eastell R, Ferrari S, Frost M, Müller R, et al. Bone fragility in diabetes: novel concepts and clinical implications. *Lancet Diabetes Endocrinol.* (2022) 10:207–20. doi: 10.1016/S2213-8587(21)00347-8
3. Schousboe JT, Morin SN, Kline GA, Lix LM, Leslie WD. Differential risk of fracture attributable to type 2 diabetes mellitus according to skeletal site. *Bone.* (2022) 154:116220. doi: 10.1016/j.bone.2021.116220
4. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis. *Osteoporos Int.* (2007) 18:427–44. doi: 10.1007/s00198-006-0253-4
5. Koromani F, Oei L, Shevroja E, Trajanoska K, Schoufour J, Muka T, et al. Vertebral fractures in individuals with type 2 diabetes: more than skeletal complications alone. *Diabetes Care.* (2020) 43:137–44. doi: 10.2337/dc19-0925
6. Vilaca T, Schini M, Harnan S, Sutton A, Poku E, Allen IE, et al. The risk of hip and non-vertebral fractures in type 1 and type 2 diabetes: a systematic review and meta-analysis update. *Bone.* (2020) 137:115457. doi: 10.1016/j.bone.2020.115457
7. Gao Y, Chai F. Risk of non-vertebral fractures in men with type 2 diabetes: a systematic review and meta-analysis of cohort studies. *Exp Gerontol.* (2021) 150:111378. doi: 10.1016/j.exger.2021.111378
8. Ala M, Jafari RM, Dehpour AR. Diabetes mellitus and osteoporosis correlation: challenges and hopes. *Curr Diabetes Rev.* (2020) 16:984–1001. doi: 10.2174/1573399816666200324152517
9. Ruaro B, Guiducci S, da Silva J, Wade B, Baratella E, Confalonieri M. Editorial: osteoporosis in rheumatic diseases, what's new?. *Front Med.* (2021) 3:8:808345. doi: 10.3389/fmed.2021.808345
10. Liu JM, Zhu DL, Mu YM, Xia WB, Chinese Society of Osteoporosis Bone Mineral Research, the Chinese Society of Endocrinology, et al. Management of fracture risk in patients with diabetes-Chinese expert consensus. *J Diabetes.* (2019) 11:906–19. doi: 10.1111/1753-0407.12962
11. Ghodsi M, Larijani B, Keshtkar AA, Nasli-Esfahani E, Alatab S, Mohajeri-Tehrani MR. Mechanisms involved in altered bone metabolism in diabetes: a narrative review. *J Diabetes Metab Disord.* (2016) 15:52. doi: 10.1186/s40200-016-0275-1
12. Cheng YZ, Yang SL, Wang JY, Ye M, Zhuo XY, Wang LT, et al. Irbesartan attenuates advanced glycation end products-mediated damage in diabetes-associated osteoporosis through the AGEs/RAGE pathway. *Life Sci.* (2018) 205:184–92. doi: 10.1016/j.lfs.2018.04.042
13. Asadipooya K, Uy EM. Advanced glycation end products (AGEs), receptor for AGEs, diabetes, and bone: review of the literature. *J Endocr Soc.* (2019) 3:1799–818. doi: 10.1210/js.2019-00160
14. Loke YK, Singh S, Furberg CD. Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. *CMA J.* (2009) 180:32–9. doi: 10.1503/cmaj.080486
15. Mamza J, Marlin C, Wang C, Chokkalingam K, Idris I. DPP-4 inhibitor therapy and bone fractures in people with type 2 diabetes - a systematic review and meta-analysis. *Diabetes Res Clin Pract.* (2016) 116:288–98. doi: 10.1016/j.diabres.2016.04.029
16. Tang HL, Li DD, Zhang JJ, Hsu YH, Wang TS, Zhai SD, et al. Lack of evidence for a harmful effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors on fracture risk among type 2 diabetes patients: a network and cumulative meta-analysis of randomized controlled trials. *Diabetes Obes Metab.* (2016) 18:1199–206. doi: 10.1111/dom.12742
17. Salari-Moghaddam A, Sadeghi O, Keshteli AH, Larijani B, Esmaillzadeh A. Metformin use and risk of fracture: a systematic review and meta-analysis of observational studies. *Osteoporos Int.* (2019) 30:1167–73. doi: 10.1007/s00198-019-04948-1
18. Zhang M, Yang B, Peng S, Xiao J. Metformin rescues the impaired osteogenesis differentiation ability of rat adipose-derived stem cells in high glucose by activating autophagy. *Stem Cells Dev.* (2021) 30:1017–27. doi: 10.1089/scd.2021.0181

19. Zhang Y, Jin D, Duan Y, Zhang Y, Duan L, Lian F, et al. Bibliometric analysis of renal fibrosis in diabetic kidney disease from 1985 to 2020. *Front Public Health.* (2022) 10:767591. doi: 10.3389/fpubh.2022.767591
20. Yeung AWK, Heinrich M, Kijjoa A, Tzvetko,v NT. Atanasov AG. The ethnopharmacological literature: an analysis of the scientific landscape. *J Ethnopharmacol.* (2020) 250:112414. doi: 10.1016/j.jep.2019.112414
21. Kokol P. Meta approaches in knowledge synthesis in nursing: a bibliometric analysis. *Nurs Outlook.* (2021) 69:815–25. doi: 10.1016/j.outlook.2021.02.006
22. Shen Z, Wu H, Chen Z, Hu J, Pan J, Kong J, et al. The global research of artificial intelligence on prostate cancer: a 22-year bibliometric analysis. *Front Oncol.* (2022) 12:843735. doi: 10.3389/fonc.2022.843735
23. Wu H, Cheng K, Guo Q, Yang W, Tong L, Wang Y, et al. Mapping knowledge structure and themes trends of osteoporosis in rheumatoid arthritis: a bibliometric analysis. *Front Med.* (2021) 8:787228. doi: 10.3389/fmed.2021.787228
24. van-Eck NJ, Waltman L. Software survey: VOS viewer, a computer program for bibliometric mapping. *Scientometrics.* (2010) 84:523–38. doi: 10.1007/s11192-009-0146-3
25. Synnestvedt MB, Chen C, Holmes JH. CiteSpace II: visualization and knowledge discovery in bibliographic databases. *AMIA Annu Symp Proc.* (2005) 2005:724–8.
26. Gazzaz ZJ, Butt NS, Zubairi NA, Malik AA. Scientometric evaluation of research productivity on diabetes from the Kingdom of Saudi Arabia over the last two decades (2000-2019). *J Diabetes Res.* (2020) 2020:1514282. doi: 10.1155/2020/1514282
27. Okaiyeto K, Oguntibeju OO. Trends in diabetes research outputs in South Africa over 30 years from 2010 to 2019: a bibliometric analysis. *Saudi J Biol Sci.* (2021) 28:2914–24. doi: 10.1016/j.sjbs.2021.02.025
28. Ganasegeran K, Hor CP, Jamil MFA, Suppiah PD, Noor JM, Hamid NA, et al. Mapping the scientific landscape of diabetes research in Malaysia (2000-2018): a systematic scientometrics study. *Int J Environ Res Public Health.* (2021) 18:318. doi: 10.3390/ijerph18010318
29. Deng P, Shi H, Pan X, Liang H, Wang S, Wu J, et al. Worldwide research trends on diabetic foot ulcers (2004-2020): suggestions for researchers. *J Diabetes Res.* (2022) 2022:7991031. doi: 10.1155/2022/7991031
30. Wei Y, Jiang Z. The evolution and future of diabetic kidney disease research: a bibliometric analysis. *BMC Nephrol.* (2021) 22:158. doi: 10.1186/s12882-021-02369-z
31. Koh BMQR, Banu R, Nusinovici S, Sabanayagam C. 100 most-cited articles on diabetic retinopathy. *Br J Ophthalmol.* (2021) 105:1329–36. doi: 10.1136/bjophthalmol-2020-316609
32. Wu H, Sun Z, Tong L, Wang Y, Yan H, Sun Z. Bibliometric analysis of global research trends on male osteoporosis: a neglected field deserves more attention. *Arch Osteoporos.* (2021) 16:154. doi: 10.1007/s11657-021-01016-2
33. Wu H, Li Y, Tong L, Wang Y, Sun Z. Worldwide research tendency and hotspots on hip fracture: a 20-year bibliometric analysis. *Arch Osteoporos.* (2021) 16:73. doi: 10.1007/s11657-021-00929-2
34. Wu H, Cheng K, Tong L, Wang Y, Yang W, Sun Z. Knowledge structure and emerging trends on osteonecrosis of the femoral head: a bibliometric and visualized study. *J Orthop Surg Res.* (2022) 17:194. doi: 10.1186/s13018-022-03068-7
35. Wang K, Xing D, Dong S, Lin J. The global state of research in nonsurgical treatment of knee osteoarthritis: a bibliometric and visualized study. *BMC Muscul Disord.* (2019) 20:407. doi: 10.1186/s12891-019-2804-9
36. Yeung AWK, Georgieva MG, Atanasov AG, Tzvetkov NT. Monoamine oxidases (MAOs) as privileged molecular targets in neuroscience: research literature analysis. *Front Mol Neurosci.* (2019) 12:143. doi: 10.3389/fnmol.2019.00143
37. Yeung AWK, Atanasov AG, Sheridan H, Klager E, Eibensteiner F, Völkl-Kernsock S, et al. Open innovation in medical and pharmaceutical research: a literature landscape analysis. *Front Pharmacol.* (2021) 11:587526. doi: 10.3389/fphar.2020.587526
38. Meng Y, Tao Z, Zhou S, Da W, Tao L. Research hot spots and trends on melatonin from 2000 to 2019. *Front Endocrinol.* (2021) 12:753923. doi: 10.3389/fendo.2021.753923
39. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol.* (2007) 166:495–505. doi: 10.1093/aje/kwm106
40. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci U S A.* (2008) 105:5266–70. doi: 10.1073/pnas.0711119105
41. Schwartz AV, Sellmeyer DE, Ensrud KE, Cauley JA, Tabor HK, Schreiner PJ, et al. Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab.* (2001) 86:32–8. doi: 10.1210/jcem.86.1.7139
42. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int.* (2010) 21:195–214. doi: 10.1007/s00198-009-1066-z
43. Nicodemus KK, Folsom AR; Iowa Women's Health Study. Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. *Diabetes Care.* (2001) 24:1192–7. doi: 10.2337/diacare.24.7.1192
44. Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL, et al. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol.* (2017) 13:208–19. doi: 10.1038/nrendo.2016.153
45. Kahn SE, Zinman B, Lachin JM, Haffner SM, Herman WH, Holman RR, et al. Rosiglitazone-associated fractures in type 2 diabetes: an Analysis from A Diabetes Outcome Progression Trial (ADOPT). *Diabetes Care.* (2008) 31:845–51. doi: 10.2337/dc07-2270
46. Vestergaard P, Rejnmark L, Mosekilde L. Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. *Diabetologia.* (2005) 48:1292–9. doi: 10.1007/s00125-005-1786-3
47. Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA.* (2011) 305:2184–92. doi: 10.1001/jama.2011.715
48. Peng C, He M, Cutrona SL, Kiefe CI, Liu F, Wang Z. Theme trends and knowledge structure on mobile health apps: bibliometric analysis. *JMIR Mhealth Uhealth.* (2020) 8:e18212. doi: 10.2196/18212
49. Meneses MJ, Silva BM, Sousa M, Sá R, Oliveira PF, Alves MG. Antidiabetic drugs: mechanisms of action and potential outcomes on cellular metabolism. *Curr Pharm Des.* (2015) 21:3606–20. doi: 10.2174/1381612821666150710145753
50. Vu GT, Tran BX, McIntyre RS, Pham HQ, Phan HT, Ha GH, et al. Modeling the research landscapes of artificial intelligence applications in diabetes (GAPRESEARCH). *Int J Environ Res Public Health.* (2020) 17:1982. doi: 10.3390/ijerph17061982
51. Liu Y, Wang Y, Qin S, Jin X, Jin L, Gu W, et al. Insights into genome-wide association study for diabetes: a bibliometric and visual analysis from 2001 to 2021. *Front Endocrinol.* (2022) 13:817620. doi: 10.3389/fendo.2022.817620
52. Wang C, Liu J, Xiao L, Liu D, Yan W, Hu T, et al. Comparison of FRAX in postmenopausal Asian women with and without type 2 diabetes mellitus: a retrospective observational study. *J Int Med Res.* (2020) 48:300060519879591. doi: 10.1177/0300060519879591
53. Van Hulten V, Sarodnik C, Driessen JHM, Schaper NC, Geusens PMM, Webers CAB, et al. Prevalent morphometrically assessed vertebral fractures in individuals with type 2 diabetes, prediabetes and normal glucose metabolism: the maastricht study. *Front Endocrinol.* (2022) 13:832977. doi: 10.3389/fendo.2022.832977
54. Lai Y, Suo S, Wang R, Kong X, Hu Y, Tang D, et al. Trends involving monoclonal antibody (mAb) research and commercialization: a scientometric analysis of IMS lifecycle R&D focus database (1980-2016). *Hum Vaccin Immunother.* (2018) 14:847–55. doi: 10.1080/21645515.2017.1420445
55. Deng Z, Wang H, Chen Z, Wang T. Bibliometric analysis of dendritic epidermal T Cell (DETC) research from 1983 to 2019. *Front Immunol.* (2020) 11:259. doi: 10.3389/fimmu.2020.00259
56. Li Y, Wang X. Chrysin attenuates high glucose-induced BMSC dysfunction via the activation of the PI3K/AKT/Nrf2 signaling pathway. *Drug Des Devel Ther.* (2022) 16:165–82. doi: 10.2147/DDDT.S335024
57. Dong K, Hao P, Xu S, Liu S, Zhou W, Yue X, et al. Alpha-lipoic acid alleviates high-glucose suppressed osteogenic differentiation of MC3T3-E1 cells via antioxidant effect and PI3K/Akt signaling pathway. *Cell Physiol Biochem.* (2017) 42:1897–906. doi: 10.1159/000479605
58. Morresi C, Cianfruglia L, Sartini D, Cecati M, Fumarola S, Emanuelli M, et al. Effect of high glucose-induced oxidative stress on paraoxonase 2 expression and activity in caco-2 cells. *Cells.* (2019) 8:1616. doi: 10.3390/cells8121616

59. Lee H, Jose PA. Coordinated contribution of NADPH oxidase- and mitochondria-derived reactive oxygen species in metabolic syndrome and its implication in renal dysfunction. *Front Pharmacol.* (2021) 12:670076. doi: 10.3389/fphar.2021.670076
60. Dong X, Wang X, Xing M, Zhao C, Guo B, Cao J, et al. Inhibition of the negative effect of high glucose on osteogenic differentiation of bone marrow stromal cells by silicon ions from calcium silicate bioceramics. *Regen Biomater.* (2020) 7:9–17. doi: 10.1093/rb/rbz030
61. Zhang YS, Zheng YD, Yuan Y, Chen SC, Xie BC. Effects of anti-diabetic drugs on fracture risk: a systematic review and network meta-analysis. *Front Endocrinol.* (2021) 12:735824. doi: 10.3389/fendo.2021.735824
62. Zhou Z, Immel D, Xi CX, Bierhaus A, Feng X, Mei L, et al. Regulation of osteoclast function and bone mass by RAGE. *J Exp Med.* (2006) 203:1067–80. doi: 10.1084/jem.20051947
63. Li Y, Wang L, Zhang M, Huang K, Yao Z, Rao P, et al. Advanced glycation end products inhibit the osteogenic differentiation potential of adipose-derived stem cells by modulating Wnt/β-catenin signalling pathway via DNA methylation. *Cell Prolif.* (2020) 53:e12834. doi: 10.1111/cpr.12834
64. Notsu M, Kanazawa I, Takeno A, Yokomoto-Umakoshi M, Tanaka KI, Yamaguchi T, et al. Advanced glycation end product 3 (AGE3) increases apoptosis and the expression of sclerostin by stimulating TGF-β expression and secretion in osteocyte-like MLO-Y4-A2 cells. *Calcif Tissue Int.* (2017) 100:402–411. doi: 10.1007/s00223-017-0243-x
65. Takagi M, Kasayama S, Yamamoto T, Motomura T, Hashimoto K, Yamamoto H, et al. Advanced glycation endproducts stimulate interleukin-6 production by human bone-derived cells. *J Bone Miner Res.* (1997) 12:439–46. doi: 10.1359/jbmr.1997.12.3.439
66. Jiang M, Wang X, Wang P, Peng W, Zhang B, Guo L. Inhibitor of RAGE and glucose-induced inflammation in bone marrow mesenchymal stem cells: effect and mechanism of action. *Mol Med Rep.* (2020) 22:3255–62. doi: 10.3892/mmr.2020.11422
67. Kim HJ, Jeong MS, Jang SB. Molecular characteristics of RAGE and advances in small-molecule inhibitors. *Int J Mol Sci.* (2021) 22:6904. doi: 10.3390/ijms22136904
68. Patorno E, Goldfine AB, Schneeweiss S, Everett BM, Glynn RJ, Liu J, et al. Cardiovascular outcomes associated with canagliflozin versus other non-gliflozin antidiabetic drugs: population based cohort study. *BMJ.* (2018) 360:k119. doi: 10.1136/bmj.k119
69. Neal B, Perkovic V, Mahaffey KW, de, Zeeuw. D., Fulcher G, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925
70. Taylor SI, Blau JE, Rother KI. Possible adverse effects of SGLT2 inhibitors on bone. *Lancet Diabetes Endocrinol.* (2015) 3:8–10. doi: 10.1016/S2213-8587(14)70227-X
71. Ruanpeng D, Ungprasert P, Sangtian J, Harindhanavudhi T. Sodium-glucose cotransporter 2 (SGLT2) inhibitors and fracture risk in patients with type 2 diabetes mellitus: a meta-analysis. *Diabetes Metab Res Rev.* (2017) 33:10. doi: 10.1002/dmrr.2903
72. Toulis KA, Bilezikian JP, Thomas GN, Hanif W, Kotsa K, Thayakaran R, et al. Initiation of dapagliflozin and treatment-emergent fractures. *Diabetes Obes Metab.* (2018) 20:1070–4. doi: 10.1111/dom.13176
73. Wang JY, Cheng YZ, Yang SL, An M, Zhang H, Chen H, et al. Dapagliflozin attenuates hyperglycemia related osteoporosis in ZDF rats by alleviating hypercalciuria. *Front Endocrinol.* (2019) 10:700. doi: 10.3389/fendo.2019.00700
74. Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Zmuda JM, et al. Diabetes is associated independently of body composition with BMD and bone volume in older white and black men and women: the health, aging, and body composition study. *J Bone Miner Res.* (2004) 19:1084–91. doi: 10.1359/JBMR.040311
75. Heap J, Murray MA, Miller SC, Jalili T, Moyer-Mileur LJ. Alterations in bone characteristics associated with glycemic control in adolescents with type 1 diabetes mellitus. *J Pediatr.* (2004) 144:56–62. doi: 10.1016/j.jpeds.2003.10.066
76. Carnevale V, Romagnoli E, D'Erasmo E. Skeletal involvement in patients with diabetes mellitus. *Diabetes Metab Res Rev.* (2004) 20:196–204. doi: 10.1002/dmrr.449
77. Rzonca SO, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology.* (2004) 145:401–6. doi: 10.1210/en.2003-0746
78. Dennison EM, Syddall HE, Aihie Sayer A, Craighead S, Phillips DI, et al. Type 2 diabetes mellitus is associated with increased axial bone density in men and women from the hertfordshire cohort study: evidence for an indirect effect of insulin resistance? *Diabetologia.* (2004) 47:1963–8. doi: 10.1007/s00125-004-1560-y
79. Shanbhogue VV, Hansen S, Frost M, Jørgensen NR, Hermann AP, Henriksen JE, et al. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in adult patients with type 1 diabetes mellitus. *J Bone Miner Res.* (2015) 30:2188–99. doi: 10.1002/jbmr.2573
80. Ogunsakin RE, Ebenezer O, Ginindza TG. A bibliometric analysis of the literature on norovirus disease from 1991-2021. *Int J Environ Res Public Health.* (2022) 19:2508. doi: 10.3390/ijerph19052508
81. Thangavelu T, Silverman E, Akhter MP, Lyden E, Recker RR, Graeff-Armas LA. Trabecular bone score and transilial bone trabecular histomorphometry in type 1 diabetes and healthy controls. *Bone.* (2020) 137:115451. doi: 10.1016/j.bone.2020.115451
82. Hidayat K, Fang QL, Shi BM, Qin LQ. Influence of glycemic control and hypoglycemia on the risk of fracture in patients with diabetes mellitus: a systematic review and meta-analysis of observational studies. *Osteoporos Int.* (2021) 32:1693–704. doi: 10.1007/s00198-021-05934-2
83. Zhang Z, Cao Y, Tao Y, E M, Tang J, Liu Y, et al. Sulfonylurea and fracture risk in patients with type 2 diabetes mellitus: a meta-analysis. *Diabetes Res Clin Pract.* (2020) 159:107990. doi: 10.1016/j.diabres.2019.107990
84. Chen Z, Zhao GH, Zhang YK, Shen GS, Xu YJ, Xu NW. Research on the correlation of diabetes mellitus complicated with osteoporosis with lipid metabolism, adipokines and inflammatory factors and its regression analysis. *Eur Rev Med Pharmacol Sci.* (2017) 21:3900–5.
85. Liu GY, Cao GL, Tian FM, Song HP, Yuan LL, Geng LD, et al. Parathyroid hormone (1-34) promotes fracture healing in ovariectomized rats with type 2 diabetes mellitus. *Osteoporos Int.* (2017) 28:3043–53. doi: 10.1007/s00198-017-4148-3
86. Gao F, Wang Y, Wu M. Teneligliptin inhibits IL-1β-induced degradation of extracellular matrix in human chondrocytes. *J Cell Biochem.* (2020) 121:4450–7. doi: 10.1002/jcb.29662
87. Liu J, Zuo Q, Li Z, Chen J, Liu F. Trelagliptin ameliorates IL-1β-impaired chondrocyte function via the AMPK/SOX-9 pathway. *Mol Immunol.* (2021) 140:70–6. doi: 10.1016/j.molimm.2021.09.009
88. Li M, Xiao YB, Wang XT, Zhuang JP, Zhou CL. Proline-Serine-threonine phosphatase-interacting protein 2 alleviates diabetes mellitus-osteoarthritis in rats through attenuating synovial inflammation and cartilage injury. *Orthop Surg.* (2021) 13:1398–407. doi: 10.1111/os.13000

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.

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 17 August 2022
DOI 10.3389/fendo.2022.898471

OPEN ACCESS

EDITED BY
Åke Sjöholm,
Gävle Hospital, Sweden

REVIEWED BY
Ivan Torre-Villalvazo,
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico
Raquel Soares,
University of Porto, Portugal
Ariane Zamoner,
Federal University of Santa Catarina, Brazil

*CORRESPONDENCE
Maria Paula Macedo
paula.macedo@nms.unl.pt

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

RECEIVED 17 March 2022
ACCEPTED 12 July 2022
PUBLISHED 17 August 2022

CITATION
Meneses MJ, Sousa-Lima I, Jarak I, Raposo JF, Alves MG and Macedo MP (2022) Distinct impacts of fat and fructose on the liver, muscle, and adipose tissue metabolome: An integrated view.
Front. Endocrinol. 13:898471.
doi: 10.3389/fendo.2022.898471

Distinct impacts of fat and fructose on the liver, muscle, and adipose tissue metabolome: An integrated view

Maria João Meneses[1,2], Inês Sousa-Lima[1], Ivana Jarak[3,4], João F. Raposo[1,2], Marco G. Alves[5] and Maria Paula Macedo[1,2,5]*

[1]iNOVA4Health, NOVA Medical School/Faculdade de Ciências Médicas (NMS/FCM), Universidade Nova de Lisboa, Lisbon, Portugal, [2]Portuguese Diabetes Association - Education and Research Center (APDP-ERC), Lisbon, Portugal, [3]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal, [4]Department of Anatomy and Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal, [5]Medical Sciences Department, University of Aveiro, Aveiro, Portugal

Objective: In the last years, changes in dietary habits have contributed to the increasing prevalence of metabolic disorders, such as non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM). The differential burden of lipids and fructose on distinct organs needs to be unveiled. Herein, we hypothesized that high-fat and high-fructose diets differentially affect the metabolome of insulin-sensitive organs such as the liver, muscle, and different adipose tissue depots.

Methods: We have studied the impact of 12 weeks of a control (11.50% calories from fat, 26.93% from protein, and 61.57% from carbohydrates), high-fat/sucrose (HFat), or high-fructose (HFruct) feeding on C57Bl/6J male mice. Besides glucose homeostasis, we analyzed the hepatic levels of glucose and lipid-metabolism-related genes and the metabolome of the liver, the muscle, and white (WAT) and brown adipose tissue (BAT) depots.

Results: HFat diet led to a more profound impact on hepatic glucose and lipid metabolism than HFruct, with mice presenting glucose intolerance, increased saturated fatty acids, and no glycogen pool, yet both HFat and HFruct presented hepatic insulin resistance. HFat diet promoted a decrease in glucose and lactate pools in the muscle and an increase in glutamate levels. While HFat had alterations in BAT metabolites that indicate increased thermogenesis, HFruct led to an increase in betaine, a protective metabolite against fructose-induced inflammation.

Conclusions: Our data illustrate that HFat and HFruct have a negative but distinct impact on the metabolome of the liver, muscle, WAT, and BAT.

KEYWORDS

prediabetes, non-alcoholic fatty liver disease, diet, metabolomics, muscle, adipose tissue

Introduction

Dysmetabolism drives obesity and/or diabetes as a result of excessive and abnormal accumulation of body fat impinging on several adverse health effects (1). Although awareness for these health and social problems has increased over the years, its prevalence continues to increase dramatically (2). Genetic background influences dysmetabolism development (3), yet the vast majority of the cases are due to factors that arose in industrialized countries, namely, excessive consumption of highly processed and energy-dense foods, frequently combined with a sedentary lifestyle. Indeed, these diets are usually rich in fructose and fat. While fructose is known to promote hepatic *de novo* lipogenesis, lipid accumulation, and insulin resistance (4), high-fat diets lead not only to hepatic lipid accumulation but also to higher adipose tissue lipolysis (5). However, fats and specifically triglycerides are differently metabolized depending on the fatty acid chain length. While medium-chain triglycerides are absorbed into the portal circulation and transported to the liver, long-chain fatty acids are transported through the lymphatic system *via* chylomicrons (6).

The metabolic and homeostatic dysfunctions (7) caused by the poor lifestyle habits may end up in the onset of hypertension, type 2 diabetes mellitus (T2DM) (8), cardiovascular disease, dyslipidemia (9), and non-alcoholic fatty liver disease (NAFLD) (10). The latter is characterized by the presence of macrovesicular steatosis in ≥5% of hepatocytes, in individuals with no causes for secondary hepatic accumulation, as is the case of abusive alcohol consumption and hereditary disorders (11). NAFLD is the most common cause of chronic liver disease in Western countries, having a reported prevalence of 6%–35% worldwide (12). Although the molecular mechanisms leading to NAFLD are still under discussion, it is known that it is a consequence of several factors, namely, (i) an increased incursion of free fatty acids from insulin-resistant adipose tissue, (ii) impaired metabolism of dietary lipids in the liver or impaired lipid export from the hepatocytes, and (iii) increased *de novo* lipogenesis in the liver (13). The overload of lipids affects not only the liver but also other metabolic organs such as the muscle or adipose tissue (both white and brown). Considering organ crosstalk, dysfunctions in one of these organs will have an impact on others, namely, in cases of insulin resistance and inflammation, and dysmetabolism overall (14, 15).

We hypothesized that dietary content in lipids or fructose differentially affect the metabolome of insulin-sensitive organs. Our aim was to evaluate the metabolome of insulin-sensitive organs, as is the case of the liver, muscle, and white and brown adipose tissue in animals fed with high-fat or high-fructose diet.

Materials and methods

Chemicals

Bicinchoninic acid (BCA) Protein Assay Kit was purchased from Thermo Scientific (Waltham, MA, USA). Dried milk was purchased from Nestlé (Vevey, Switzerland). Amersham ECL was purchased from GE Healthcare (Weßling, Germany). NZYColour Protein Marker II, agarose, NZYDNA Ladder V, and Supreme NZYTaq II 2x Green Master Mix were purchased from NZYTech (Lisbon, Portugal). Fructose was purchased from Enzymatic (Loures, Portugal). Immobilon-P polyvinylidene difluoride (PVDF) membrane was purchased from Merck Millipore (MA, USA). Kits for triglycerides and cholesterol determination were purchased from Spinreact (Girona, Spain). Quantification of free fatty acids was done using a kit from Wako Diagnostics (CA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless stated otherwise.

Animals

Experiments were performed using male C57Bl6/J mice kept on a 12-h light/dark cycle with *ad libitum* access to food and water. Animals were randomly divided in three groups (n=7/group) and fed different diets from 6 to 18 weeks of age: normal chow diet with 11.50% calories from fat, 26.93% from protein, and 61.57% from carbohydrates [Chow group; RM3A(P), Special Diets Services, Witham, Essex, UK]; high-fat diet with 58% of calories from fat, 16.4% from protein, and 25.5% from carbohydrates (HFat; D12331, Research Diets, New Brunswick, NJ) or high fructose diet [HFruct; FRUC-00T-500, Enzymatic, PT; 35% w/v in drinking water and fed with RM3A(P) diet]. Mice were monitored weekly for body weight, blood glucose levels, and for distress signals. The experimental procedures

were approved by the Ethics Committee of the NOVA Medical School and by the Directorate-General for Food and Veterinary that regulates the animal care and use in research (registration number 82/2019/CEFCM and 0421/000/000/2016, respectively). All procedures followed Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines and the European laws (Directive 2010/63/EU) that rule the use of animals in research.

Metabolic measurements

Mice were weighed at 6 weeks and weekly thereafter. Blood glucose levels were measured using a Contour Next glucose meter (Bayer, Leverkusen, Germany). Food and caloric intake were also assessed. For glucose tolerance tests, mice were fasted overnight at 17 weeks of age, and blood glucose levels were measured before and 15, 30, 60, 90, and 120 min after intraperitoneal injection of glucose (2.0 g/kg). The area under the curve (AUC) was calculated using the trapezoidal rule for glucose data (16). For insulin tolerance tests, at 17 weeks of age, mice were fasted for 5 h, and blood glucose levels were measured before and 15, 30, 60, 90, and 120 min after intraperitoneal injection of human insulin (0.75 UI/Kg; Actrapid, Novo Nordisk).

Insulin signaling studies

Mice were fasted overnight and intraperitoneally injected with human insulin (10 UI/kg of body weight; Actrapid, Novo Nordisk) or saline and sacrificed 10 min later. Organs [liver, epididymal white adipose tissue (WAT), interscapular brown adipose tissue (BAT), and gastrocnemius muscle] were harvested, snap frozen, and stored at −80°C until analysis. The liver was homogenized in lysis buffer (in mM: 20 Tris pH 7.5, 5 EDTA, 10 $Na4P_2O_7$, 100 NaF, 2 Na_3VO_4) with 1% NP-40 and protease inhibitors (Roche, Switzerland). Tissue lysates (20 µg protein) were mixed with Laemmli sample buffer (in %: 1.5 Tris, 20 glycerol, 4.1 SDS, 2 β-mercaptoethanol, 0.02 bromophenol blue, pH 6.8) and denatured for 10 min at 95°C. Proteins were fractionated in 10% polyacrylamide gels. The proteins were transferred from gels to previously activated PVDF membranes in TransBlot Turbo (Bio-Rad Laboratories, Hemel Hempstead, UK) and then blocked for 1 h in a 5% non-fat milk solution at room temperature. The membranes were incubated overnight at 4°C with the primary antibodies listed in Supplementary Table S1 and incubated with secondary antibodies for 1 h. Either glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin were used as loading controls. Membranes were reacted with Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare), read in Chemidoc, and quantified using ImageLab (Bio-Rad Laboratories).

Liver histology and lipid assay

Liver was fixed with 10% formalin solution, embedded in paraffin, and cut in 4-µm sections. These sections were then stained with hematoxylin and eosin for the characterization of liver morphology and lipid content. Hepatic lipids were extracted as previously described (17). Briefly, approximately 250 mg of frozen tissue was rapidly mixed with high-performance liquid chromatography (HPLC)-grade methanol (4.6 ml/g) followed by methyl-tert-butyl ether (MTBE) (15.4 ml/g). The mixture was placed in a shaker for 4 h and then centrifuged at 13,000*g* for 10 min. The liquid fraction was collected, and phase separation was induced by adding 1 ml of distilled water and letting it rest at room temperature for 10 min. The liquid was then centrifuged for 10 min at 1,000*g*. The organic phase, containing the lipids, was separated and dried under nitrogen gas in a glass vial protected from light. It was then dissolved in butanol:(Triton X-100:methanol). Hepatic total cholesterol and triglyceride contents were determined by enzymatic method (SpinReact).

Quantitative real-time polymerase chain reaction

The extraction of liver total ribonucleic acid (RNA) was performed using TRIzol (Invitrogen), and the concentrations were determined by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Total RNA was reverse transcribed to complementary DNA (cDNA) using high-capacity cDNA reverse transcription kit (Applied Biosystems, CA, USA). Quantitative real-time polymerase chain reaction (qPCR) was performed to evaluate the abundance of messenger RNA (mRNA) coding for GK, G6Pase, PEPCK, ChREBP, SREBP2, ELOVL2, CD36, SCD1, β-actin, and β-2-microglobulin using SYBR™ Green PCR Master Mix (Applied Biosystems) in an ABI 7500 (Applied Biosystems). Specific exon–exon spanning primers were designed for the amplification of the target and housekeeping transcripts (Supplementary Table S2). β-Actin and β-2-microglobulin transcript levels were used to normalize gene expression levels. Fold variation in gene expression levels was calculated with the $2^{-\Delta\Delta Ct}$ method (18).

Metabolite extraction

To extract the liver, gastrocnemius muscle, epididymal WAT, and interscapular BAT metabolites, the different tissues were homogenized in glass vials using a mixture of methanol and chloroform (2:1). After sonication on ice for 15 min, chloroform and water (1:1) were added, and samples were centrifuged at 10,000*g* for 15 min at 4°C. Polar and apolar fractions were

isolated and evaporated using a flow of nitrogen. The polar fraction was dissolved in D_2O phosphate buffer (0.2 M, pH 7) for proton nuclear magnetic resonance (^{1}H-NMR) analysis.

Proton nuclear magnetic resonance spectroscopy

Proton nuclear magnetic resonance (^{1}H-NMR) spectra of the polar extracts were acquired using a Varian Inova 600 MHz (14.1 T) spectrometer equipped with a 3-mm QXI probe with a z-gradient. ^{1}H-1D NOESY experiments with water presaturation were acquired at 298 K (7.2 kHz spectral width, 0.1 s mixing time, four dummy scans, 4 s relaxation delay with 3 s of water presaturation, 90°C pulse angle, 3 s acquisition time, and a minimum of 128 scans). For further spectral assignment, two-dimensional spectra (TOCSY) were acquired using sweep width of 5.4 kHz in both dimensions, 48 transients, and 400 and 1,024 points in t1 and t2 dimensions, respectively. Spectra were treated by multiplying FIDs with exponential window function (line broadening of 0.3 Hz) and were zero filled to 64 k points prior to Fourier transformation using TopSpin (Bruker Biospin, Karlsruhe, Germany). 2D spectra were processed by applying qsine window function and zero filled to 2,048 points in both dimensions. The comparison of 1D and 2D spectra with reference spectra and public databases such as HMDB allowed for peak assignment and metabolite identification (19). Metabolites were identified according to metabolomics standards initiative guidelines for metabolite identification (20).

Multivariate analysis of NMR data

Processed 1D NOESY spectra were bucketed using one-point bucket (0.6–9.0 ppm, with signal-free, water, and fumarate regions excluded). Data matrix was built in Amix Viewer (BrukerBiospin, Rheinstetten). Icoshift algorithm (21) was used to align bucketed spectra, and total area integral normalization was applied to account for the variations in the overall sample concentrations. Multivariate statistical analysis was applied on unit variance scaled matrix (SIMCA 14, Umetrics, Sweden). Principal component analysis (PCA) was used to provide information on global data structure, and partial least squares discriminant analysis (PLS-DA) was used to assess group separation and to identify the main metabolites that contribute to the group discrimination. PLS-DA models were validated by sevenfold cross-validation and permutation test (n=100) to provide the qualitative measure of predictive power (Q2) and to assess the degree of fit to the data (R2). The corresponding PLS-DA loadings were obtained by multiplying the loading weight factors (w) by the standard deviation of the respective variable and were color-coded according to variable importance in the projection (VIP). All differentially expressed metabolites for each of the diets in each tissue were used to identify the most relevant metabolic pathways affected by diet using MetaboAnalyst (22).

GC-MS analysis

Fatty acid methyl esters were obtained by base-catalyzed transmethylation (2 M KOH in methanol). The resultant fatty acid methyl ester solution was analyzed by gas chromatography using a Shimadzu GC-MS QP2010 UltraGas Chromatograph Mass Spectrometer (Shimadzu, Kyoto, Japan), with a capillary column BPX70 (0.25 mm internal diameter, 0.25 µm film thickness, 30 m long, SGE, Austin, TX, USA). Nonadecanoic fatty acid (C19:0) was used as the internal standard. The injector temperature was set at 250°CC, and 1 µl of each sample was injected with a split ratio of 1:80. Helium was used as the carrier gas. The initial column temperature was 155°CC, followed by a heating rate of 1°CC/min up to 170°CC, 4°CC/min up to 220°CC and 40°CC/min until reaching 250°CC, which was kept for 5 min. The linear velocity was 35 cm/s, with interface temperature of 250°CC, ion source temperature of 225°CC, mass range of 45–500, and event time of 0.3 s. All the measurements were repeated three times, and the average values were reported. Fatty acids were identified by retention time and fragmentation profile and quantified by the internal standard procedure. Results are expressed as a percentage of total fatty acids.

Statistical analysis

Experimental data are shown as mean ± standard error of mean (SEM). Unless stated otherwise, statistical analysis was performed using one-way ANOVA in GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). $p<0.05$ was considered significant.

Results

HFat diet-fed mice develop glucose intolerance, insulin resistance, and non-alcoholic fatty liver disease

Mice were subjected to a Chow, HFat, or HFruct diet. After a 12-week period of diet intervention, mice subjected to an HFat diet had increased body weight gain (37.90 ± 1.06 g) compared to Chow-diet-fed mice (28.14 ± 0.57 g; Figure 1A). Conversely, HFruct-diet-fed mice had decreased body weight (24.86 ± 0.76 g). In fact, after 12 weeks of diet, HFat-diet-fed

10.3389/fendo.2022.898471

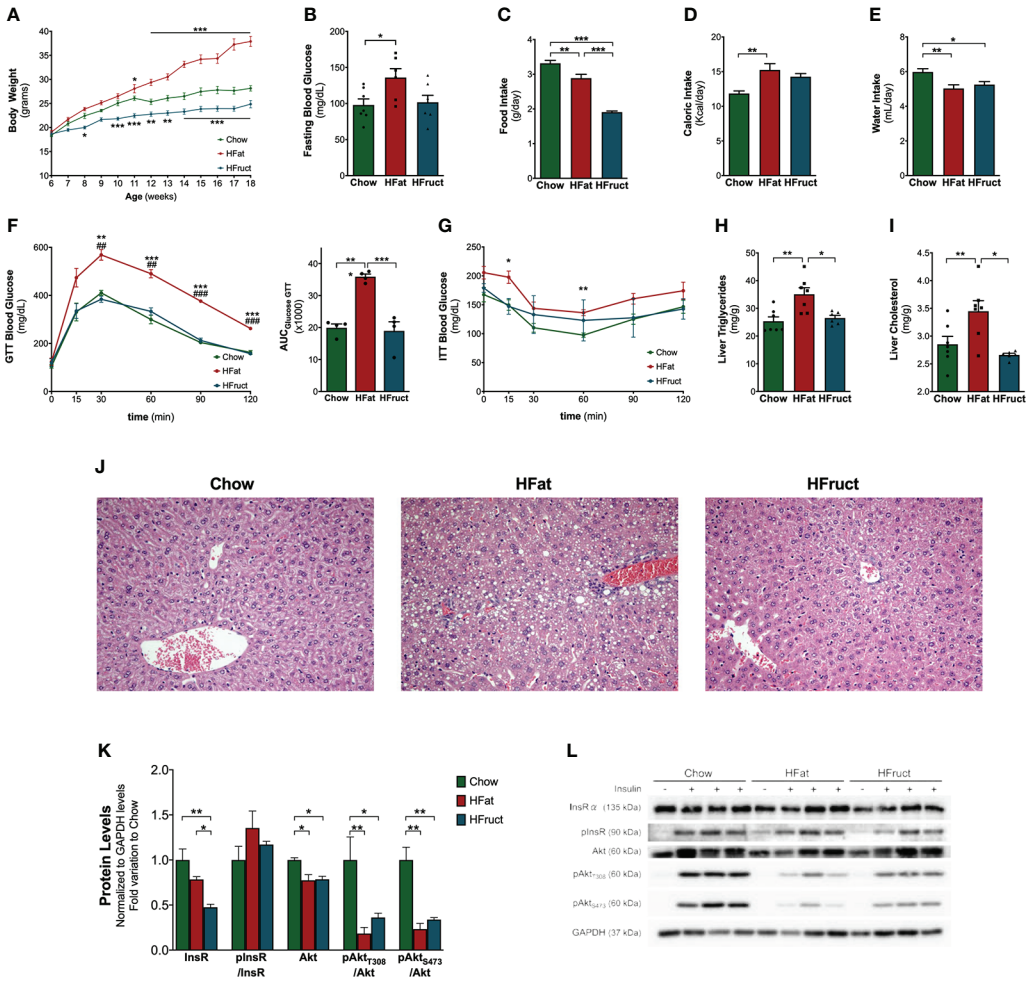


FIGURE 1
Effects of HFat and HFruct diets on metabolic parameters, glucose homeostasis, hepatic lipid deposition, and insulin signaling pathway in C57Bl/6J male mice. **(A)** Body weight, **(B)** intraperitoneal glucose tolerance test (ipGTT) and the respective area under the curve (AUC), **(C)** insulin tolerance test (ITT); **(D)** fasting blood glucose; **(E)** food intake; **(F)** water intake; **(G)** caloric intake; **(H)** hepatic triglycerides; **(I)** hepatic cholesterol levels; **(J)** representative images of hematoxylin and eosin-stained liver sections; **(K)** Chow, high-fat (HFat) and high-fructose (HFruct) hepatic protein levels of insulin receptor α (InsR), phosphorylated-insulin receptor (pInsR; normalized to InsR levels); protein kinase B (PKB/Akt) and its phosphorylation sites ($pAkt_{T308}$ and $pAkt_{S473}$; normalized to Akt levels) normalized to GAPDH levels and represented as fold variation to Chow group; **(L)** representative images of Western blot for the described antibodies. Data are presented as mean ± SEM for three to seven mice. *p<0.05, **p<0.01, ***p<0.001; **(F)** $^{\#\#}$p<0.01 vs. HFruct, $^{\#\#\#}$p<0.001 vs. HFruct.

mice had marked hyperglycemia (136 ± 12 mg/dl), compared to Chow-diet-fed mice (98 ± 8 mg/dl, Figure 1B), while HFruct-diet-fed mice were normoglycemic (102 ± 10 mg/dl). HFruct-diet-fed mice had decreased water intake, concomitant with decreased food intake, compared to Chow-diet-fed mice. However, this was not reflected in a decrease in caloric intake (Figures 1C–E). Caloric intake was increased by 25% in HFat-diet-fed mice, compared to Chow-diet-fed mice (Figure 1D). HFat-diet-fed mice were glucose intolerant, as observed by failure to decrease blood glucose levels after administration of a glucose bolus (Figure 1F). HFruct-diet-fed mice displayed normal glucose tolerance, but the ITT curve was flatter than Chow, revealing insulin resistance, as the capacity to decrease blood glucose levels after an insulin bolus is compromised (Figures 1F, G).

Dysmetabolism is associated with ectopic fat deposition in parenchymatous organs such as the liver. To evaluate this, hepatic triglycerides and cholesterol were measured. Liver content in both triglycerides and cholesterol was increased in HFat-diet-fed mice (35.1 ± 2.3 mg/g and 3.5 ± 0.2 mg/g, respectively) but unchanged in HFruct-diet-fed mice (26.5 ± 0.9 mg/g and 2.7 ± 0.03 mg/g, respectively; Figures 1H, I) when compared with Chow-diet-fed mice (25.4 ± 1.6 mg/g and 2.9 ± 0.1 mg/g, respectively). The

analysis of histological sections demonstrated that HFat-diet-fed mice developed NAFL, i.e., presented lipid droplets in >5% of the sections, whereas HFruct-diet-fed mice had mild hepatic degeneration (Figure 1J). Insulin-stimulated glucose utilization relies on an effective insulin signaling and is known to impact the transcription of several lipid and carbohydrate metabolism-related genes (23). Therefore, the protein levels of the insulin pathway-related components, insulin receptor (InsR) and Akt, in the liver of the mice were determined. After 12 weeks of diet exposure, HFruct-diet-fed mice presented a decrease in InsRα levels. However, the activation of the pathway, observed by the ratio between phosphorylated-insulin receptor (pInsR) and InsR, did not present any differences between the groups, suggesting that InsR are functional (Figures 1K, L). When analyzing Akt phosphorylation levels, there was a marked reduction in both T308 and S473 in both HFat- and HFruct-diet-fed groups, revealing that both diets negatively impact on the activation of the insulin signaling pathway (Figures 1K, L).

Hepatic carbohydrate and lipid and amino acid metabolisms are impaired by HFat and HFruct diets

The liver is a key organ for the maintenance of normal glucose homeostasis. However, this homeostasis is impaired in insulin-resistant states; thus, we evaluated the intracellular levels of key metabolites in the fasting state, and the mRNA expression of some glucose metabolism-related genes. Intra-hepatic glucose levels were decreased in HFat-diet-fed mice compared to Chow-diet-fed mice (Figure 2A). The mRNA levels of glucokinase, the enzyme responsible for the conversion of glucose into glucose-6-phosphate, thus enabling the trapping of glucose inside the cells, are increased in HFat-diet-fed mice (4.32 ± 1.02-fold variation to Chow), when compared to both Chow- and HFruct-diet-fed mice (1.00 ± 0.24- and 1.68 ± 0.45-fold variation to Chow, respectively; Figure 2B). The mRNA expression of glucose-6-phosphatase, the enzyme that converts glucose-6-phosphate to glucose, does not present any significant difference between the groups, although there is a decrease in the HFat group, when compared to Chow (0.45 ± 0.09- and 1.00 ± 0.26-fold variation to Chow, respectively; p=0.06; Figure 2B). The expression of *Pepck*, responsible for glucose production from pyruvate, was also analyzed, although no statistical differences were found. In the fasting state, the liver produces glucose from several substrates, namely, glycogen. However, we observed that both HFat- and HFruct-diet-fed mice present lower levels of glycogen in the liver when compared to Chow-diet-fed mice. Glucose may also enter glycolysis to yield pyruvate; the latter has three major outcomes, namely, acetyl-CoA, acetate, or lactate. However, we observed that hepatic lactate and acetate levels do not vary significantly with the diet (data not shown).

Like for carbohydrates, the liver is essential for fatty acid metabolism. Dysregulations in cholesterol biosynthesis or *de novo* lipogenesis, in a key lipogenic tissue as the liver, may disrupt the overall lipid homeostasis. Thus, we evaluated intracellular levels of key metabolites for lipid metabolism and the mRNA expression of some lipid-metabolism-related genes. No statistical differences were found regarding *CD36* mRNA levels nor *Elovl2*, responsible for the elongation. However, *Srebp2* and *Scd1* mRNA levels were increased in HFat-diet-fed mice (3.06 ± 0.51- and 2.59 ± 0.74-fold variation to Chow; Figure 2C), when compared to both Chow (1.00 ± 0.29- and 1.00 ± 0.19-fold variation to Chow) and HFruct groups (1.23 ± 0.26- and 0.76 ± 0.14-fold variation to Chow; Figure 2C). Glycerol, a product of adipose tissue lipolysis, is an important substrate for both gluconeogenesis and lipogenesis (24). Glycerol levels were increased in the liver of HFat-diet-fed mice, when compared to Chow-diet-fed mice. Choline, which is the precursor for the essential component of the very low density lipoprotein (VLDL) phosphatidylcholine, was increased in HFat-diet-fed mice, compared to Chow-diet-fed mice (Figure 2A). Choline is oxidized to betaine, an osmoregulator and a methyl-group donor (25). Betaine was increased in HFruct-fed-mice compared to both Chow- and HFat-fed mice (Figure 2A). When glycogen is not available to produce glucose as energy substrate, ketogenesis takes place. One of the metabolites produced during this process is 3-hydroxybutyrate, which was increased in HFat-diet-fed mice, when compared to Chow- and HFruct-diet-fed animals (Figure 2A).

Besides being the basic units for protein synthesis, amino acids serve as intermediate metabolites for TCA cycle and lipid and nucleotide biosynthesis and sustain cell proliferation. Amino acid levels were significantly altered in the liver tissue by both high-fat and high-fructose diets. Serine, besides being a precursor of proteins and lipids, is involved in glycogen storage in the liver (26). Its levels were decreased in HFat-diet-fed mice, when compared to both Chow- and HFruct-diet-fed animals (Figure 2A). Serine is inter-convertible with glycine (27). The levels of this amino acid were increased in ~50% in the HFat group, when compared with both Chow- and HFruct-diet-fed mice (Figure 2A). During fasting, alanine participates in gluconeogenesis. Alanine levels were increased in almost 40% in HFruct mice when compared to Chow-diet-fed mice (Figure 2A). α-Ketoglutarate (α-KG), one of the metabolites of the Krebs cycle, may be interconverted into glutamate, which may be, in turn, interconverted in glutamine, an important amino acid and a precursor for gluconeogenesis and glutathione synthesis (28). Both glutamate and glutamine were increased in HFat-diet-fed mice when compared to Chow-diet-fed animals (Figure 2A). A graphical diagram with the most affected metabolites is depicted in Figure 2A, along with the graphs for each metabolite and a heat map (Figure 2D); the most affected pathways in the liver by HFat or HFruct feeding is are shown in Supplementary Figures 1A, B, respectively.

10.3389/fendo.2022.898471

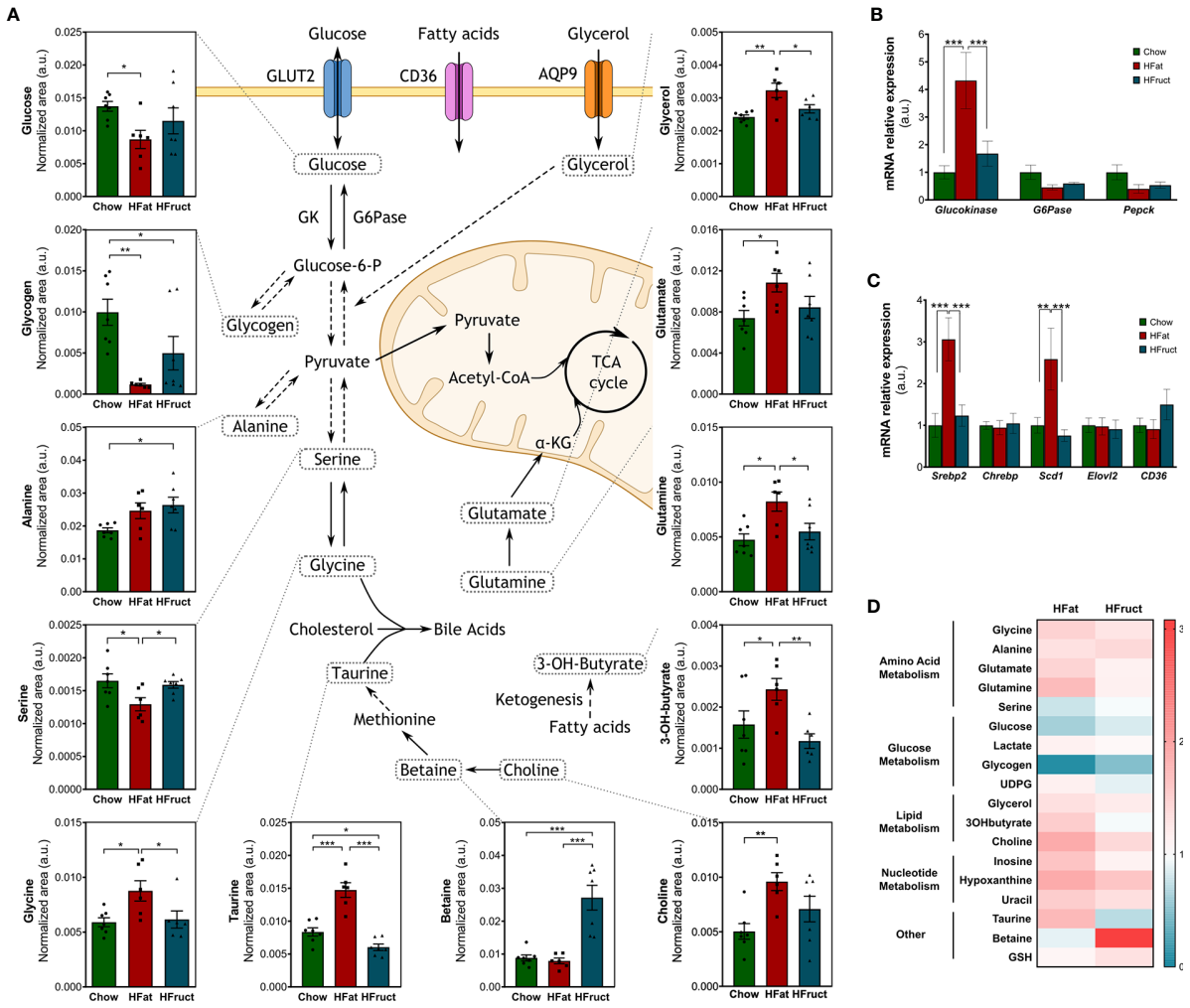


FIGURE 2
Effects of the HFat and HFruct diets on the hepatic metabolism of C57Bl/6J male mice. Chow, high-fat (HFat), and high-fructose (HFruct) hepatic levels of metabolites involved in glycolysis/gluconeogenesis, amino acid metabolism, ketogenesis, bile acid synthesis, and methionine cycle **(A)**, mRNA levels of glucose **(B)**, and lipid-related metabolism genes **(C)**, and heat map of hepatic polar metabolites in comparison to Chow metabolites, which are represented by 1/white **(D)**. α-KG, α-ketoglutarate; AQP9, aquaporin 9; CD36, cluster of differentiation 36; G6Pase, glucose-6-phosphatase; GK, glucokinase; GSH, glutathione; TCA, tricarboxylic acid; UDPG, uridine diphosphate glucose. Data are presented as mean ± SEM for five to seven mice. *p<0.05, **p<0.01; ***p<0.001.

HFat and HFruct diets increase hepatic monounsaturated fatty acids and decrease n-6 polyunsaturated fatty acids

As previously mentioned, the liver is a key lipogenic tissue; thus, fatty acid methyl esters levels were analyzed upon HFat and HFruct feeding for 12 weeks. After grouping fatty acids by saturation degrees, we observed that HFat diet leads to an increase in relative abundance of monounsaturated fatty acids (MUFA; 45.60 ± 1.61) when compared to Chow-fed mice (27.91 ± 1.13; Table 1). Moreover, HFat-fed mice present decreased relative abundance of n-3 and n-6 polyunsaturated fatty acids (PUFAs), when compared to Chow-fed mice and HFruct-fed mice (Table 1). The decrease in n-3 PUFA is mainly attributed to a decrease in docosahexaenoic acid (DHA; C22:6 n3) and α-linolenic acid (C18:3n3), while the decrease in n-6 PUFA is mainly due to a decrease in linoleic acid (C18:2n6).

High-fat and high-fructose diets differentially affect muscle metabolome

Skeletal muscle is a major organ of glucose uptake, storage, and usage. The muscle can store glucose in the form of

10.3389/fendo.2022.898471

TABLE 1 Fatty acid composition of liver tissue obtained from normal chow diet (Chow), high-fat diet (HFat), and high-fructose (HFruct)-fed male C57Bl6/J mice after 12 weeks of diet.

Fatty Acid	Chow	HFat	HFruct
C10:0	0.000 ± 0.000	0.017 ± 0.003	0.000 ± 0.000
C12:0	0.141 ± 0.027	1.370 ± 0.225	0.159 ± 0.028
C14:0	0.517 ± 0.051	3.182 ± 0.272[a]	0.494 ± 0.047[b]
C15:0	0.090 ± 0.005	0.082 ± 0.005	0.081 ± 0.005
C16:0	24.097 ± 0.309	25.915 ± 0.225[a]	20.880 ± 0.318[a,b]
C16:1n7	2.876 ± 0.256	8.025 ± 0.280[a]	3.004 ± 0.197[b]
C18:0	6.801 ± 0.538	4.027 ± 0.272[a]	7.793 ± 0.562[b]
C18:1n9	22.877 ± 0.889	33.708 ± 1.234[a]	29.316 ± 0.961[a,b]
C18:1n7	1.917 ± 0.045	5.440 ± 0.541[a]	2.570 ± 0.047[b]
C18:2n6	25.679 ± 1.135	8.312 ± 0.613[a]	19.856 ± 0.312[a,b]
C18:3n3	0.623 ± 0.063	0.208 ± 0.021	0.414 ± 0.017
C20:1n9	0.236 ± 0.033	0.430 ± 0.036	0.379 ± 0.023
C20:3n6	0.497 ± 0.053	0.503 ± 0.020	0.843 ± 0.069
C20:4n6	8.167 ± 0.764	4.845 ± 0.335[a]	8.260 ± 0.474[b]
C22:4n6	0.197 ± 0.012	0.152 ± 0.009	0.167 ± 0.010
C22:5n6	0.121 ± 0.014	0.263 ± 0.015	0.124 ± 0.009
C22:5n3	0.299 ± 0.012	0.227 ± 0.025	0.256 ± 0.006
C22:6n3	4.863 ± 0.425	3.312 ± 0.244[a]	5.407 ± 0.298[b]
Σ MUFA	27.906 ± 1.134	47.597 ± 1.609[a]	35.270 ± 1.118 [a,b]
Σ n3 PUFA	5.783 ± 1.134	3.745 ± 0.279[a]	6.074 ± 0.289[b]
Σ n6 PUFA	34.661 ± 0.958	14.074 ± 0.895[a]	29.274 ± 0.435[a,b]
Σ SFA	31.650 ± 0.622	34.585 ± 0.625[a]	29.407 ± 0.560[a,b]

Values represent mean ± SEM for five to seven mice per experimental group. [a]$p<0.05$ vs. Chow; [b]$p<0.05$ vs. HFat. Monounsaturated fatty acids (MUFAs) correspond to C16:1n7, C18:1n9, C18:1n7, and C20:1n9. n-3 polyunsaturated fatty acids (PUFAs) correspond to C18:3n3, C22:5n3, and C22:6n3; n-6 PUFA correspond to C18:2n6, C20:3n6, C20:4n6, C22:4n6, and C22:5n6. Saturated fatty acids (SFAs) correspond to C10:0, C12:0, C14:0, C15:0, C16:0, and C18:0.

glycogen, which is crucial for the rapid initiation of energy production even when glucose is not readily available (29). Therefore, we evaluated the muscle intracellular polar metabolites after 12 weeks of HFat or HFruct feeding. Muscular glucose levels were decreased in HFat-diet fed mice, compared with both Chow and HFruct-diet-fed mice (Figure 2A). Lactate, which may be converted into pyruvate, was also decreased in HFat-diet-fed mice compared with both Chow- and HFruct-diet-fed mice. In the need of substrate, fatty acids stored as triglycerides or amino acids may be metabolized (30). In fact, dietary changes had the major impact in amino acids levels. Glycine and glutamine were increased in HFat-diet-fed mice compared to the other diets, which may promote and contribute for protein synthesis (31). Both dietary interventions caused a decrease in valine, whose metabolism produces ammonia by-products that may participate in the conversion of glutamate to glutamine (32), but only HFruct led to decreased levels of glutamate, when compared to Chow-diet-fed mice. A graphical diagram with the most affected metabolites is depicted in Figure 3, along with the graphs for each metabolite, and a heat map is depicted in Supplementary Figure 2A, and the most affected pathways in the muscle by HFat (Supplementary Figure 2B) or HFruct feeding (Supplementary Figure 2C).

Epididymal white adipose tissue metabolome is less affected by high-fat and high-fructose feeding than the liver and muscle

As a source of energy substrates, WAT responds to variations in the body's nutritional status and energy demand (33). Herein, we observed that HFat feeding for 12 weeks led to increased acetate levels (Figure 4E), which might indicate suppressed lipolysis (34), and decreased glycerophosphocholine (Figure 4B) when compared to Chow. Moreover, HFat-fed mice presented decreased creatine, a metabolite known for stimulating energy expenditure (35) (Figure 4D), and increased taurine (Figure 4A), when compared to HFruct-fed mice, all pointing towards an obesogenic mechanism (36). On the other hand, HFruct animals had decreased succinate, when compared to Chow-fed mice (Figure 4C). No significant differences were observed in formate levels (Figure 4F). A heat map with analyzed metabolites is depicted in Supplementary Figure 3A

10.3389/fendo.2022.898471

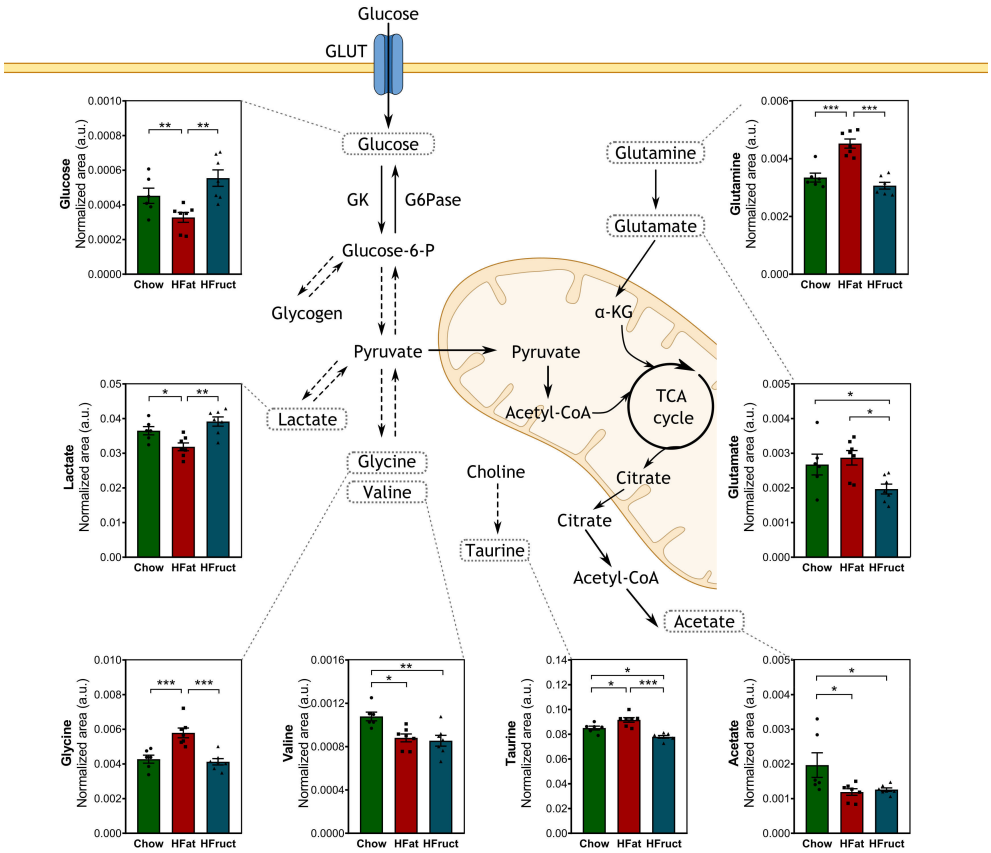


FIGURE 3
Effects of the HFat and HFruct diets on the muscle metabolism of C57Bl/6J male mice. Chow, high-fat (HFat), and high-fructose (HFruct) muscle levels of metabolites involved in glycolysis/gluconeogenesis and amino acid metabolism. α-KG, α-ketoglutarate; G6Pase, glucose-6-phosphatase; GK, glucokinase; GLUT, glucose transporter; IMP, inosine monophosphate; TCA, tricarboxylic acid. Data are presented as mean ± SEM for five to seven mice. *p<0.05, **p<0.01; ***p<0.001.

and the most affected pathways in WAT by HFat (Supplementary Figure 3B).

Brown adipose tissue metabolome is modulated by high-fat or high-fructose feeding

Although it has been disregarded for years, studies about the presence of BAT in adults and the discovery of its crosstalk with important metabolic organs, namely, the muscle, has brought BAT to the spotlight (37). The BAT metabolome was assessed, and the graphical diagram in Figure 5 discloses the most affected metabolites, along with the graphs for each metabolite, and a heat map is depicted in Supplementary Figure 4A and the most affected pathways in BAT by HFat (Supplementary Figure 4B) or HFruct feeding (Supplementary Figure 4C). HFruct led to an increase in betaine, taurine, glutamine, and leucine. In contrast, the same diet led to a decrease in 3-hydroxybutyrate when compared to Chow-diet-fed mice (Figure 5). On the other hand, HFat diet led to a decrease in glutathione (GSH), which was already reported to be inversely correlated with the activation of thermogenesis, the hallmark function of BAT (38). Moreover, HFat feeding resulted in decreased levels of glycerol, when compared to Chow-diet-fed mice. On the other hand, HFat caused an increase in glutamine and acetate compared with Chow diet, and the latter was already associated with upregulation of mitochondrial biogenesis, contributing to increased thermogenesis (39).

HFat decreases n-6 PUFA while HFruct diets increase MUFA in brown adipose tissue

BAT fatty acid methyl esters levels were measured upon HFat and HFruct feeding for 12 weeks. We observed that HFat diet leads to an increase in relative abundance of saturated fatty

10.3389/fendo.2022.898471

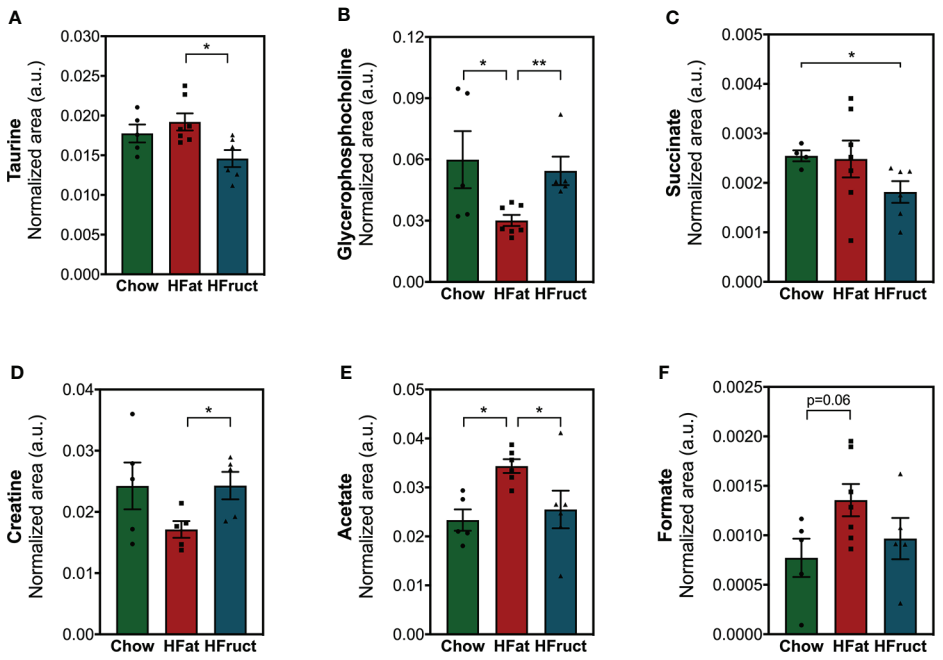


FIGURE 4
Effects of the HFat and HFruct diets on the epididymal white adipose tissue (WAT) metabolism of C57Bl/6J male mice. **(A)** Taurine; **(B)** glycerophosphocholine; **(C)** succinate; **(D)** creatine; **(E)** acetate; **(F)** formate relative areas in WAT after 12 weeks of normal chow (Chow), high-fat (HFat), or high-fructose (HFruct) feeding. Data are presented as mean ± SEM for four to seven mice. *p<0.05, **p<0.01.

acids (SFAs; 44.27 ± 0.45) when compared to Chow-fed mice and HFruct-fed mice (25.58 ± 0.75 and 23.62 ± 0.96, respectively; Table 2). On the contrary, n-6 PUFA were decreased in HFat (7.95 ± 0.13) fed mice, when compared to both Chow- (24.65 ± 1.72) and HFruct (19.63 ± 0.42)-fed mice, mainly due to a decrease in linoleic acid (C18:2n6). MUFA were increased in HFruct-fed mice (56.10 ± 0.82) in comparison with both Chow- (48.82 ± 1.11) and HFat (47.45 ± 0.48)-fed mice, reflecting an increase in elaidic acid (C18:1n9).

Discussion

The sedentary lifestyle and poor nutritional habits have led to an increase in the prevalence of metabolic disorders, such as obesity, T2DM and NAFLD. Importantly, the increased consumption of ultra-processed food resulted in increased intake of simple carbohydrates and saturated fat (40). Although it is already known that the enrichment in lipids and in carbohydrates, particularly fructose, has different pathophysiological effects, the consequence of these specific components of the diet on the metabolome of important metabolic organs such as the liver, muscle, and adipose tissue depots remains to be fully understood.

HFat diet, alone or combined with sucrose as is the case of the present study (D12331, ResearchDiets), is known to lead to obesity and insulin resistance in mice, originating a similar metabolic imbalance to what is observed in humans (41–43). On the other hand, fructose alone does not cause a significant increase in body weight (44). This effect is also seen in humans, and, in comparison with glucose, fructose has less effects on body weight gain (45), despite fructose deleterious effects in inflammatory processes (46).

The current study confirmed that, at 18 weeks of age and after 12 weeks of feeding, HFat-diet-fed mice had higher caloric intake, body weight, and hyperglycemia than Chow-diet-fed mice. Moreover, HFat-diet-fed mice were glucose intolerant and insulin resistant. Conversely, HFruct-diet-fed mice presented lower body weight gain than the Chow group and normal glucose tolerance. Although these animals had a decreased food intake, compared to the Chow group, the caloric intake was similar. Both food, water, and caloric intake results are supported by previous studies, although with a slightly different percentage of fructose (44, 47).

Nutritional imbalances promoting ectopic lipid accumulation are particularly striking in the liver (48, 49). Our results are in line with what is already described in the literature (50, 51). Indeed, HFat mice developed NAFL, with increased triglycerides and cholesterol. HFruct-diet-fed mice developed mild hepatic degeneration mainly in periportal areas. This impact of an HFruct diet on liver histology may be due to the activation of a lipogenic pathway and the absence of a regulatory

10.3389/fendo.2022.898471

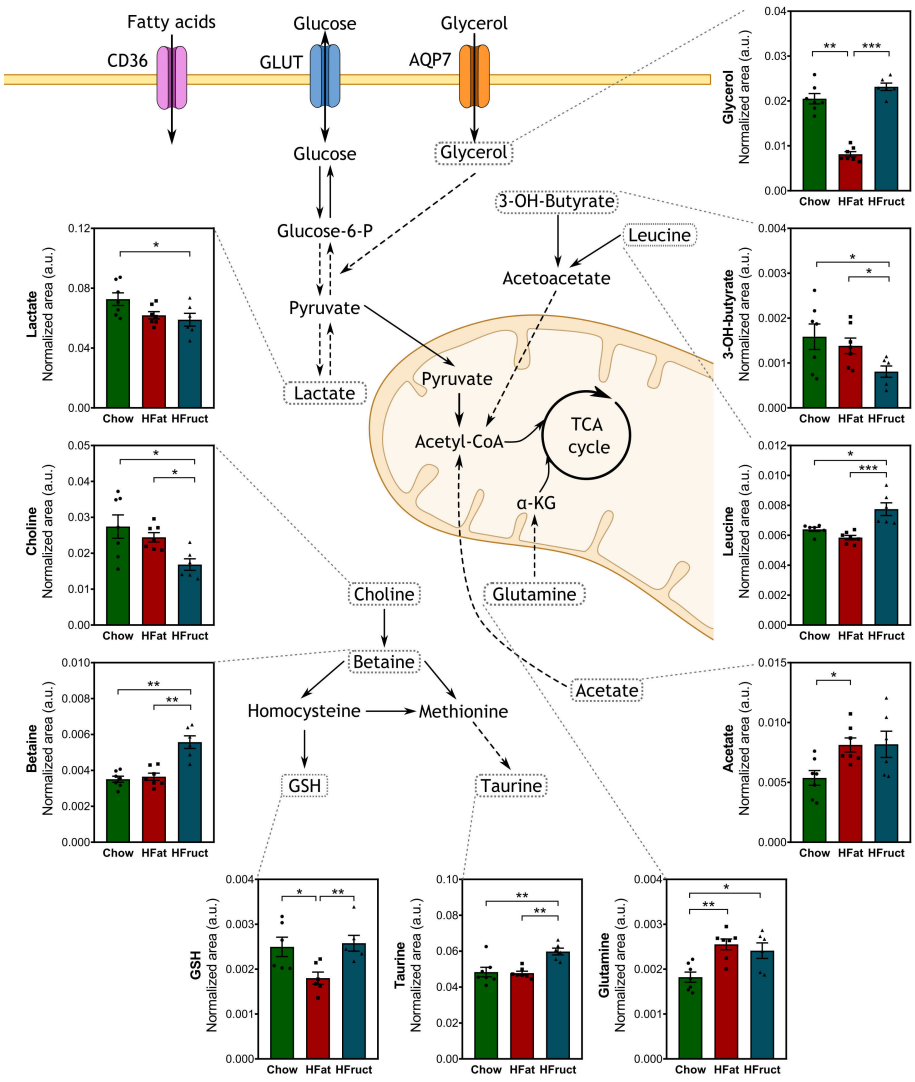


FIGURE 5
Effects of the HFat and HFruct diets on the brown adipose tissue (BAT) metabolism of C57Bl/6J male mice. Chow, high-fat (HFat), and high-fructose (HFruct) BAT levels of metabolites involved in glycolysis/gluconeogenesis, amino acid metabolism, and methionine cycle. α-KG, α-ketoglutarate; AQP7, aquaporin 7; CD36, cluster of differentiation 36; G6Pase, glucose-6-phosphatase; GK, glucokinase; GLUT, glucose transporter; GSH, glutathione; IMP, inosine monophosphate; TCA, tricarboxylic acid. Data are presented as mean ± SEM for five to seven mice. *p<0.05, **p<0.01; ***p<0.001.

mechanism of fructose metabolism, contrarily to what happens with glucose (52, 53). Consequently, fructolysis will continue to occur, even when glycolysis is inhibited due to positive cellular energy balance. In fact, this effect was already been observed in studies with as low as 10% of fructose in drinking water (50). A proper glucose usage depends on a functional insulin signaling pathway. Indeed, hepatic insulin signaling was severely affected by both diets. A profound decrease in Akt phosphorylation at both phosphorylation sites was found, demonstrating that these animals are insulin resistant due to an impairment of the insulin signaling pathway, upstream of Akt, but downstream of the InsR (54). These results are supported by previous studies where insulin signaling is severely affected by fructose intake, namely, through a decrease in Akt phosphorylation (53, 55).

The liver is the most important metabolic organ, regulating carbohydrate, lipid, and protein metabolism (56). While in the fed state, liver takes up glucose that is either stored as glycogen or converted into fatty acids; in the fasted state, liver produces and releases glucose through glycogenolysis and gluconeogenesis (56). Subsequently, substrates as glucose and triglycerides go into the bloodstream and are metabolized by peripheral organs. Adipose tissue, in turn, releases non-esterified fatty acids and glycerol. Together with alanine and lactate, which are released by the muscle, these metabolites are used as precursors for

10.3389/fendo.2022.898471

TABLE 2 Fatty acid composition of brown adipose tissue (BAT) obtained from normal chow diet (Chow), high-fat diet (HFat), and high-fructose (HFruct)-fed male C57Bl6/J mice after 12 weeks of diet.

Fatty Acid	Chow	HFat	HFruct
C10:0	0.013 ± 0.005	0.210 ± 0.013	0.023 ± 0.012
C12:0	0.390 ± 0.155	9.767 ± 0.330[a]	1.010 ± 0.384[b]
C14:0	1.896 ± 0.137	11.261 ± 0.141[a]	2.137 ± 0.358[b]
C15:0	0.074 ± 0.004	0.066 ± 0.003	0.046 ± 0.008
C16:0	18.601 ± 0.761	19.777 ± 0.134[a]	14.823 ± 0.461[a,b]
C16:1n7	5.709 ± 0.560	9.066 ± 0.279[a]	4.369 ± 0.213[a,b]
C18:0	4.314 ± 0.100	2.947 ± 0.111[a]	4.804 ± 0.158[b]
C18:1n9	39.277 ± 0.550	35.859 ± 0.474[a]	47.856 ± 0.854[a,b]
C18:1n7	2.976 ± 0.165	2.109 ± 0.049	2.853 ± 0.088
C18:2n6	23.689 ± 1.650	7.397 ± 0.121[a]	18.016 ± 0.403[a,b]
C18:3n3	0.569 ± 0.027	0.211 ± 0.006	0.326 ± 0.019
C20:0	0.256 ± 0.028	0.239 ± 0.019	0.727 ± 0.053
C20:1n9	0.861 ± 0.041	0.417 ± 0.013	1.027 ± 0.042
C20:3n6	0.184 ± 0.008	0.084 ± 0.003	0.186 ± 0.009
C20:4n6	0.703± 0.060	0.433 ± 0.025	1.369 ± 0.104
C22:0	0.030 ± 0.009	0.000 ± 0.000	0.050 ± 0.012
C22:4n6	0.051 ± 0.010	0.019 ± 0.003	0.036 ± 0.007
C22:5n6	0.024 ± 0.005	0.020 ± 0.004	0.023 ± 0.004
C22:5n3	0.046 ± 0.009	0.024 ± 0.003	0.027 ± 0.005
C22:6n3	0.336 ± 0.023	0.097 ± 0.006	0.297 ± 0.009
Σ MUFA	48.823 ± 1.112	47.449 ± 0.479	56.104 ± 0.823[a,b]
Σ n3 PUFA	0.950 ± 0.047	0.334 ± 0.005[a]	0.649 ± 0.018[a,b]
Σ n6 PUFA	24.650 ± 1.719	7.951 ± 0.134[a]	19.630 ± 0.424[a,b]
Σ SFA	25.577 ± 0.750	44.267 ± 0.447[a]	23.617 ± 0.961[b]

Values represent mean ± SEM for five to seven mice per experimental group. [a]$p<0.05$ vs. Chow; [b]$p<0.05$ vs. HFat. Monounsaturated fatty acids (MUFAs) correspond to C16:1n7, C18:1n9, C18:1n7, and C20:1n9. n-3 polyunsaturated fatty acids (PUFA) correspond to C18:3n3, C22:5n3, and C22:6n3; n-6 PUFA correspond to C18:2n6, C20:3n6, C20:4n6, C22:4n6, and C22:5n6. Saturated fatty acids (SFAs) correspond to C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C20:0, and C22:0.

gluconeogenesis. Hepatic glycogen levels were decreased in HFat-diet-fed mice. Interestingly, glucokinase expression levels were increased in the liver, while G6Pase levels were unchanged. These results point towards a metabolic dysregulation; as in the fasted state, it is expected that glucokinase transcription decreases in favor of G6Pase expression. In addition, glycerate-3-phosphate produced during glycolysis can be converted to serine, which is interconvertible with glycine. While serine was decreased in the liver of HFat-diet-fed mice, glycine was increased. In fact, decreased hepatic levels of serine, due to a downregulation of phosphoglycerate dehydrogenase, contribute to the development of fatty liver disease (57). One of the pathological features of fatty liver disease is hepatocyte swelling, which was observed in our study in HFat-diet-fed mice. Indeed, glutamine, a potent osmoregulator that contributes to cell swelling (28), was increased in HFat-diet-fed mice compared to Chow-fed mice. Moreover, glutamine, is known for promoting stimulation of canalicular bile salt excretion (58). On the other hand, glycine is involved in the enterohepatic cycle of bile acids, key players in lipid absorption and regulation of cholesterol homeostasis (27), which were also increased in the HFat group. Aside from glycine, taurine, which is also conjugated with cholesterol for the synthesis of bile acids, was increased in the liver of HFat-diet-fed mice. All these results point towards an increased production of bile acids. In fact, bile acids are crucial for an effective digestion of lipids (59). As these amino acids and cholesterol were increased only in HFat-diet-fed mice, it suggests a possible response to the increased lipid intake. Consistently, we also observe an increase in hepatic Srebp2 and Scd1 expression, suggesting that the lipogenic pathway may be active as a response to increased levels of hepatic fatty acid levels. Moreover, glycerol was increased in the liver of HFat-diet-fed mice, pointing towards an increase in adipose tissue lipolysis, which goes in line with previous studies in adipocytes (60). Additionally, dietary composition had repercussions on hepatic lipid composition, and we observed an increase in hepatic SFA in HFat-fed mice compared to Chow. SFAs are potent lipotoxic species and induce apoptosis and proinflammatory pathways through several mechanisms (61). Although this increase was not so prominent, there was also a decrease in PUFA, known to play a protective role against cell injury by clearing fat from hepatocytes and improving liver

histology and lipid profile in patients with NASH (62). Altogether, this imbalance in the lipid species might contribute to NAFL development in the HFat group. On the other hand, the HFruct group also presents a decrease in hepatic glycogen levels, without changes in the expression of any of the analyzed glucose- and lipid-metabolism-related genes. However, there was an increase in MUFA and a decrease in n-6 PUFA in comparison with Chow. In fact, there have been contradictory results in the literature regarding MUFA levels and Scd1 mRNA expression (63); however, the animals were fed with 60% (w/v) fructose for a longer period than in the present study, which indicates that the effects of fructose on the activation of lipogenic pathways are time and dose dependent. Interestingly, we found an increase in alanine levels, in concurrence with the data obtained in a recent study with HFruct-fed rats (64). This increase in alanine levels may be a consequence of an increase in the release of alanine from the muscle that is then delivered to the liver through Cahill cycle or to its conversion from pyruvate in the liver.

The skeletal muscle is a major site for glucose metabolism. Insulin resistance in the muscle is known to cause glycogen depletion, lipid accumulation, impairment of the tissue's normal functions, ultimately leading to sarcopenia (29). Muscle stores glucose in the form of glycogen, which facilitates the rapid initiation of energy production for contraction, even when glucose is not readily available from circulation (29). In this study, HFat diet promoted a decrease in glucose and lactate pools in the muscle, pointing towards the use of other substrates to obtain energy. In the need for other substrates, amino acids may be metabolized. Skeletal muscles are the most relevant site of glutamine stock, synthesis, and release (65). In this study, glutamine levels were increased in HFat-fed mice, and glutamate levels remained similar to the levels detected in the muscle of Chow-diet-fed mice. Glutamate levels can be used to produce glutamine that will be then delivered to the liver where it may have several functions (65, 66). Indeed, and as there was no glycogen pool in the liver, gluconeogenesis may use glutamine as a substrate for glucose production after an overnight fasting (32), as was the case of the present study. On the contrary, HFruct diet promoted a decrease in muscular glutamate levels, which might negatively affect glutathione synthesis and/or purine nucleotide cycle (67). Moreover, a decrease in valine was observed in both HFat- and HFruct-fed groups. Valine is used to obtain energy with the resulting ammonia by-products participating in the conversion of glutamate to glutamine, thus sustaining the previously mentioned hypothesis. Although it has already been described that diet-induced obese animals have a decrease in branched-chain amino acids (BCAAs) (68), there are studies describing a positive correlation between valine circulating levels and insulin resistance in men (69, 70).

WAT has a crucial role for lipid homeostasis, namely, by regulating triglyceride levels, and for fatty acid availability by lipolysis, also generating substrates for energy metabolism *via* β-oxidation. In fact, we observed an increase in the WAT levels of acetate in HFat-fed mice compared to Chow, which indicates increased β-oxidation (71). On the contrary, creatine was decreased in HFat-fed mice compared to HFruct-fed mice. As this metabolite enhances energy expenditure (35), the finding goes in accordance with the larger fat depots in this group. Although taurine promotes thermogenesis in the BAT and muscle, it does not have the same effect in WAT (72), which was also not observed in the present study. In fact, taurine supplementation was shown to prevent obesity in female mice after 18 weeks of high-fat feeding (36). In this study, male mice fed with HFat diet for 12 weeks presented no differences in taurine compared to the Chow group, which might indicate that decreases in taurine may be more noticeable in females or only appear with prolonged HFat feeding.

Organ crosstalk is mediated by signaling factors and ensures whole-body metabolic homeostasis. However, in case of disease or metabolic dysfunction, metabolic impairment in one organ will lead to dysregulation of others. Although this is known, the interplay and metabolic crosstalk between the different organs remain obscure. In recent years, BAT was rediscovered, and very recently, the existence of a crosstalk with the liver and the muscle was unveiled (73). HFat diet led to a decrease in GSH levels in the BAT compared with both Chow and HFruct diets. GSH was already reported to be inversely correlated with the activation of thermogenesis in white adipocytes due to its action on forkhead box O1 (74). Consequently, decreased GSH levels might have the same effect in BAT and increase thermogenesis in the BAT of HFat-diet-fed mice (38). Moreover, HFat-diet-fed mice had increased levels of glutamine and acetate in BAT. Acetate upregulates mitochondrial biogenesis, which is the key organelle for thermogenesis (39). Importantly, HFruct feeding led to an increase in betaine and taurine, probably at the expense of decreased choline. In fact, betaine protects against fructose-induced inflammation in astrocytes (75). HFruct-diet-fed animals also presented increased leucine. This amino acid is an activator of the mammalian target of rapamycin (mTOR) pathway, leading to reduced thermogenesis (76, 77). Moreover, the levels of 3-OH-butyrate, considered an alternative carbon source for thermogenesis (78), were decreased in HFruct-fed mice. Thus, high-fat feeding seems to be an activator of thermogenesis, while HFruct leads to a decrease in this process. Moreover, it was already reported that in HFat conditions, BAT functions largely like WAT in its role as a depot for excess energy (79). In these cases, adipocytes suffer a remodeling due to its storage needs and, consequently, the need of the membrane to be more fluid (80). In fact, we observed a completely different fatty acid distribution in HFat-fed mice compared to both Chow- and HFruct-fed mice that were lean. This difference might represent the need of the adipocytes to remodel and be able to store more fat.

In summary, our data provide novel evidence that, aside from the extensively described effects of high-fat and high-

fructose diets on glucose and insulin homeostasis, these diets differentially and significantly affect liver, muscle, WAT, and BAT metabolic profiles. Interestingly, our data suggests a crosstalk between the liver, muscle, and BAT, specifically through alanine, glutamine, and lactate.

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 animal study was reviewed and approved by DGAV - Portuguese General Directorate of Food and Veterinary Medicine and by NOVA Medical School Ethics Committee.

Author contributions

MJM: experimental work, animal studies, data analysis and interpretation, and writing of the manuscript. IS-L: experimental work, animal studies, and data analysis and interpretation. IJ: NMR and data analysis. JFR: data interpretation. MGA: conceptualization, data interpretation, and writing of manuscript. MPM: conceptualization, supervision, data interpretation, and writing of the manuscript. All authors read and agree with the final version of the manuscript.

Funding

This work was supported by "Fundação para a Ciência e a Tecnologia"—FCT MJM (PD/BD/114256/2016), MPM (PTDC/BIM-MET/4265/2014 and PTDC/MEC-MET/29314/2017), MGA (PTDC/BIM-MET/4712/2014), iNOVA4Health (UIDB/Multi/04462/2020), by the European Commission Marie Skłodowska-Curie Action H2020 (mtFOIE GRAS, grant agreement n. 734719), by the Sociedade Portuguesa de Diabetologia, and by the research infrastructure CONGENTO, project LISBOA-01-0145-FEDER-022170, co-financed by Lisboa Regional Operational Programme (Lisboa2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund and by Fundação para a Ciência e Tecnologia (Portugal). NMR data were collected at the UC-NMR facility which is supported in part by FEDER – European Regional Development Fund through the COMPETE Programme (Operational Programme for Competitiveness) and by National Funds through FCT – Fundação para a Ciência e a Tecnologia (Portuguese Foundation for Science and Technology) through grants REEQ/481/QUI/2006, RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012, and Rede Nacional de Ressonância Magnética Nuclear (RNRMN).

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.

The reviewer RS declared a shared affiliation with the author MA to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.898471/full#supplementary-material

References

1. Fan J-G, Kim S-U, Wong VW-S. New trends on obesity and NAFLD in Asia. *J Hepatol* (2017) 67(4):862–73. doi: 10.1016/j.jhep.2017.06.003

2. NCD Risk Factor Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* (2016) 387 (10026):1377–96. doi: 10.1016/S0140-6736(16)30054-X

3. Grarup N, Sandholt CH, Hansen T, Pedersen O. Genetic susceptibility to type 2 diabetes and obesity: from genome-wide association studies to rare variants and beyond. *Diabetologia* (2014) 57(8):1528–41. doi: 10.1007/s00125-014-3270-4

4. Eng JM, Estall JL. Diet-induced models of non-alcoholic fatty liver disease: Food for thought on sugar, fat, and cholesterol. *Cells* (2021) 10(7):1805. doi: 10.3390/cells10071805

5. Stott NL, Marino JS. High fat rodent models of type 2 diabetes: From rodent to human. *Nutrients* (2020) 12(12):3650. doi: 10.3390/nu12123650

6. Nonaka H, Ohue-Kitano R, Masujima Y, Igarashi M, Kimura I. Dietary medium-chain triglyceride decanoate affects glucose homeostasis through GPR84-mediated GLP-1 secretion in mice. *Front Nutr* (2022) 9:848450. doi: 10.3389/fnut.2022.848450

7. Tchernof A, Després J-P. Pathophysiology of human visceral obesity: An update. *Physiol Rev* (2013) 93(1):359. doi: 10.1152/physrev.00033.2011

8. Merino J. Precision nutrition in diabetes: when population-based dietary advice gets personal. *Diabetologia* (2022) Ahead of print. doi: 10.1007/s00125-022-05721-6

9. Pascot A, Lemieux I, Prud'homme D, Tremblay A, Nadeau A, Couillard C, et al. Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity. *J Lipid Res* (2001) 42(12):2007–14. doi: 10.1016/S0022-2275(20)31529-7

10. Muzurović E, Peng CC-H, Belanger MJ, Sanoudou D, Mikhailidis DP, Mantzoros CS. Nonalcoholic fatty liver disease and cardiovascular disease: a review of shared cardiometabolic risk factors. *Hypertension* (2022) 79(7):1319–26. doi: 10.1161/HYPERTENSIONAHA.122.17982

11. Cusi K, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, et al. American Association of clinical endocrinology clinical practice guideline for the diagnosis and management of nonalcoholic fatty liver disease in primary care and endocrinology clinical settings: Co-sponsored by the American association for the study of liver diseases (AASLD). *Endocr Pract* (2022) 28(5):528–62. doi: 10.1016/j.eprac.2022.03.010

12. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int* (2017) 37(Suppl 1):81–4. doi: 10.1111/liv.13299

13. Bosserhoff A, Hellerbrand C. Obesity and fatty liver are 'grease' for the machinery of hepatic fibrosis. *Digest Dis (Basel Switzerland)* (2011) 29(4):377–83. doi: 10.1159/000329800

14. Scheja L, Heeren J. Metabolic interplay between white, beige, brown adipocytes and the liver. *J Hepatol* (2016) 64(5):1176–86. doi: 10.1016/j.jhep.2016.01.025

15. De Bandt JP, Jegatheesan P, Tennoune-El-Hafaia N. Muscle loss in chronic liver diseases: The example of nonalcoholic liver disease. *Nutrients* (2018) 10 (9):1195. doi: 10.3390/nu10091195

16. Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* (2008) 295(6): E1323–32. doi: 10.1152/ajpendo.90617.2008

17. Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J Lipid Res* (2008) 49(5):1137–46. doi: 10.1194/jlr.D700041-JLR200

18. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* (2001) 29(9):e45–5. doi: 10.1093/nar/29.9.e45

19. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, et al. HMDB: the human metabolome database. *Nucleic Acids Res* (2007) 35(suppl_1):D521–6. doi: 10.1093/nar/gkl923

20. Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, et al. Proposed minimum reporting standards for chemical analysis chemical analysis working group (CAWG) metabolomics standards initiative (MSI). *Metabolomics* (2007) 3(3):211–21. doi: 10.1007/s11306-007-0082-2

21. Savorani F, Tomasi G, Engelsen SB. Icoshift: A versatile tool for the rapid alignment of 1D NMR spectra. *J Magn Reson* (2010) 202(2):190–202. doi: 10.1016/j.jmr.2009.11.012

22. Xia J, Wishart DS. Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr Protoc Bioinf* (2016) 55:14.10.11–14.10.91. doi: 10.1002/cpbi.11

23. Patti M-E. Gene expression in humans with diabetes and prediabetes: what have we learned about diabetes pathophysiology? *Curr Opin Clin Nutr Metab Care* (2004) 7(4):383–90. doi: 10.1097/01.mco.0000134359.23288.72

24. Patsouris D, Mandard S, Voshol PJ, Escher P, Tan NS, Havekes LM, et al. PPARα governs glycerol metabolism. *J Clin Invest* (2004) 114(1):94–103. doi: 10.1172/JCI200420468

25. Sivanesan S, Taylor A, Zhang J, Bakovic M. Betaine and choline improve lipid homeostasis in obesity by participation in mitochondrial oxidative demethylation. *Front Nutr* (2018) 5. doi: 10.3389/fnut.2018.00061

26. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care* (2007) 30(1):89–94. doi: 10.2337/dc06-1519

27. Alves A, Bassot A, Bulteau AL, Pirola L, Morio B. Glycine metabolism and its alterations in obesity and metabolic diseases. *Nutrients* (2019) 11(6):1356. doi: 10.3390/nu11061356

28. Häussinger D, Graf D, Weiergräber OH. Glutamine and cell signaling in liver. *J Nutr* (2001) 131(9 Suppl):2509S–2514S; discussion 2523S-2504S. doi: 10.1093/jn/131.9.2509S

29. Rachek LI. Free fatty acids and skeletal muscle insulin resistance. *Prog Mol Biol Transl Sci.* (2014) 121:267–92. doi: 10.1016/B978-0-12-800101-1.00008-9

30. Moro T, Ebert SM, Adams CM, Rasmussen BB. Amino acid sensing in skeletal muscle. *Trends Endocrinol Metab* (2016) 27(11):796–806. doi: 10.1016/j.tem.2016.06.010

31. Jepson MM, Bates PC, Broadbent P, Pell JM, Millward DJ. Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. *Am J Physiol Endocrinol Metab* (1988) 255(2):E166–72. doi: 10.1152/ajpendo.1988.255.2.E166

32. Stumvoll M, Perriello G, Meyer C, Gerich J. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. *Kidney Int* (1999) 55(3):778–92. doi: 10.1046/j.1523-1755.1999.055003778.x

33. Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. *Acta Physiol (Oxf)* (2012) 205(2):194–208. doi: 10.1111/j.1748-1716.2012.02409.x

34. Sahuri-Arisoylu M, Brody LP, Parkinson JR, Parkes H, Navaratnam N, Miller AD, et al. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int J Obes (Lond)* (2016) 40 (6):955–63. doi: 10.1038/ijo.2016.23

35. Kazak L, Chouchani ET, Lu GZ, Jedrychowski MP, Bare CJ, Mina AI, et al. *et al*: Genetic depletion of adipocyte creatine metabolism inhibits diet-induced thermogenesis and drives obesity. *Cell Metab* (2017) 26(4):660–71.e663. doi: 10.1016/j.cmet.2017.08.009

36. Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, et al. Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. *Endocrinology* (2006) 147(7):3276–84. doi: 10.1210/en.2005-1007

37. Cypess AM. A new connection between muscle and brown fat. *J Pediatr* (2011) 158(5):696–8. doi: 10.1016/j.jpeds.2011.01.001

38. Mercer SW, Trayhurn P. Effect of high fat diets on the thermogenic activity of brown adipose tissue in cold-acclimated mice. *J Nutr* (1984) 114(6):1151–8. doi: 10.1093/jn/114.6.1151

39. Hu J, Kyrou I, Tan BK, Dimitriadis GK, Ramanjaneya M, Tripathi G, et al. Short-chain fatty acid acetate stimulates adipogenesis and mitochondrial biogenesis *via* GPR43 in brown adipocytes. *Endocrinology* (2016) 157(5):1881–94. doi: 10.1210/en.2015-1944

40. Rippe JM, Angelopoulos TJ. Sucrose, high-fructose corn syrup, and fructose, their metabolism and potential health effects: what do we really know? *Adv Nutr* (2013) 4(2):236–45. doi: 10.3945/an.112.002824

41. Wang ZQ, Zhang XH, Yu Y, Poulev A, Ribnicky D, Floyd ZE, et al. Bioactives from bitter melon enhance insulin signaling and modulate acyl carnitine content in skeletal muscle in high-fat diet-fed mice. *J Nutr Biochem* (2011) 22(11):1064–73. doi: 10.1016/j.jnutbio.2010.09.004

42. Small L, Brandon AE, Turner N, Cooney GJ. Modeling insulin resistance in rodents by alterations in diet: what have high-fat and high-calorie diets revealed? *Am J Physiol Endocrinol Metab* (2018) 314(3):E251–e265. doi: 10.1152/ajpendo.00337.2017

43. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, et al. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metab: Clin Exp* (1995) 44(5):645–51. doi: 10.1016/0026-0495(95)90123-X

44. Bergheim I, Weber S, Vos M, Krämer S, Volynets V, Kaserouni S, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. *J Hepatol* (2008) 48(6):983–92. doi: 10.1016/j.jhep.2008.01.035

45. Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, Mirrahimi A. Fructose vs. glucose and metabolism: do the metabolic differences matter? *Curr Opin Lipidol* (2014) 25(1):8–19. doi: 10.1097/MOL.0000000000000042

46. Jegatheesan P, De Bandt J-P. Fructose and NAFLD: The multifaceted aspects of fructose metabolism. *Nutrients* (2017) 9(3):230. doi: 10.3390/nu9030230

47. Lindqvist A, Baelemans A, Erlanson-Albertsson C. Effects of sucrose, glucose and fructose on peripheral and central appetite signals. *Regul Peptides* (2008) 150(1-3):26–32. doi: 10.1016/j.regpep.2008.06.008

48. Parry SA, Hodson L. Influence of dietary macronutrients on liver fat accumulation and metabolism. *J Invest Med Off Publ Am Fed Clin Res* (2017) 65 (8):1102–15. doi: 10.1136/jim-2017-000524

49. Bosy-Westphal A, Braun W, Albrecht V, Muller MJ. Determinants of ectopic liver fat in metabolic disease. *Eur J Clin Nutr* (2019) 73(2):209–14. doi: 10.1038/s41430-018-0323-7

50. Yang Y, Li J, Wei C, He Y, Cao Y, Zhang Y, et al. Amelioration of nonalcoholic fatty liver disease by swertiamarin in fructose-fed mice. *Phytomed Int J Phytother Phytopharmacol* (2019) 59:152782. doi: 10.1016/j.phymed.2018.12.005

51. Jahn D, Kircher S, Hermanns HM, Geier A. Animal models of NAFLD from a hepatologist's point of view. *Biochim Biophys Acta (BBA) Mol Basis Dis* (2019) 1865(5):943–53. doi: 10.1016/j.bbadis.2018.06.023

52. Herman MA, Samuel VT. The sweet path to metabolic demise: Fructose and lipid synthesis. *Trends Endocrinol Metab* (2016) 27(10):719–30. doi: 10.1016/j.tem.2016.06.005

53. Softic S, Gupta MK, Wang G-X, Fujisaka S, O'Neill BT, Rao TN, et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J Clin Invest* (2017) 127(11):4059–74. doi: 10.1172/JCI94585

54. Guo S, Copps KD, Dong X, Park S, Cheng Z, Pocai A, et al. The Irs1 branch of the insulin signaling cascade plays a dominant role in hepatic nutrient homeostasis. *Mol Cell Biol* (2009) 29(18):5070–83. doi: 10.1128/MCB.00138-09

55. Sumlu E, Bostanci A, Sadi G, Alcigir ME, Akar F. Lactobacillus plantarum improves lipogenesis and IRS-1/AKT/eNOS signalling pathway in the liver of high-fructose-fed rats. *Arch Physiol Biochem* (2020) 2020:1–9. doi: 10.1080/13813455.2020.1727527

56. Rui L. Energy metabolism in the liver. *Compr Physiol* (2014) 4(1):177–97. doi: 10.1002/cphy.c130024

57. Sim WC, Lee W, Sim H, Lee KY, Jung SH, Choi YJ, et al. *et al*: Downregulation of PHGDH expression and hepatic serine level contribute to the development of fatty liver disease. *Metab: Clin Exp* (2020) 102:154000. doi: 10.1016/j.metabol.2019.154000

58. Hallbrucker C, Lang F, Gerok W, Häussinger D. Cell swelling increases bile flow and taurocholate excretion into bile in isolated perfused rat liver. *Biochem J* (1992) 281(Pt 3):593–5. doi: 10.1042/bj2810593

59. McGlone ER, Bloom SR. Bile acids and the metabolic syndrome. *Ann Clin Biochem* (2019) 56(3):326–37. doi: 10.1177/0004563218817798

60. Rotondo F, Ho-Palma AC, Remesar X, Fernández-López JA, Romero M, Alemany M. Glycerol is synthesized and secreted by adipocytes to dispose of excess glucose, *via* glycerogenesis and increased acyl-glycerol turnover. *Sci Rep* (2017) 7(1):8983. doi: 10.1038/s41598-017-09450-4

61. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology* (2006) 147(2):943–51. doi: 10.1210/en.2005-0570

62. El-Badry AM, Graf R, Clavien PA. Omega 3 - omega 6: What is right for the liver? *J Hepatol* (2007) 47(5):718–25. doi: 10.1016/j.jhep.2007.08.005

63. Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim H-J, et al. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms *. *J Biol Chem* (2004) 279(24):25164–71. doi: 10.1074/jbc.M402781200

64. Morrell A, Tripet BP, Eilers BJ, Tegman M, Thompson D, Copie V, et al. Copper modulates sex-specific fructose hepatoxicity in nonalcoholic fatty liver disease (NALFD) wistar rat models. *J Nutr Biochem* (2020) 78:108316. doi: 10.1016/j.jnutbio.2019.108316

65. Cruzat V, Rogero MM, Keane KN, Curi R, Newsholme P. Glutamine: Metabolism and immune function, supplementation and clinical translation. *Nutrients* (2018) 10(11):1564. doi: 10.3390/nu10111564

66. Haüussinger D, Graf D. Weiergraüber OH: Glutamine and cell signaling in liver. *J Nutr* (2001) 131(9):2509S–14S. doi: 10.1093/jn/131.9.2509S

67. Rutten EP, Engelen MP, Schols AM, Deutz NE. Skeletal muscle glutamate metabolism in health and disease: state of the art. *Curr Opin Clin Nutr Metab Care* (2005) 8(1):41–51. doi: 10.1097/00075197-200501000-00007

68. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* (2012) 15(5):606–14. doi: 10.1016/j.cmet.2012.01.024

69. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, et al. *et al*: Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* (2010) 53(4):757–67. doi: 10.1007/s00125-009-1637-8

70. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. *et al*: A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* (2009) 9(4):311–26. doi: 10.1016/j.cmet.2009.02.002

71. Yamashita H, Kaneyuki T, Tagawa K. Production of acetate in the liver and its utilization in peripheral tissues. *Biochim Biophys Acta* (2001) 1532(1-2):79–87. doi: 10.1016/S1388-1981(01)00117-2

72. Kim KS, Doss HM, Kim HJ, Yang HI. Taurine stimulates thermoregulatory genes in brown fat tissue and muscle without an influence on inguinal white fat tissue in a high-fat diet-induced obese mouse model. *Foods* (2020) 9(6):688. doi: 10.3390/foods9060688

73. Scheele C, Wolfrum C. Brown adipose crosstalk in tissue plasticity and human metabolism. *Endocr Rev* (2019) 41(1):53–65. doi: 10.1210/endrev/bnz007

74. Lettieri Barbato D, Tatulli G, Maria Cannata S, Bernardini S, Aquilano K, Ciriolo MR. Glutathione decrement drives thermogenic program in adipose cells. *Sci Rep* (2015) 5(1):13091. doi: 10.1038/srep13091

75. Li JM, Ge CX, Xu MX, Wang W, Yu R, Fan CY, et al. Betaine recovers hypothalamic neural injury by inhibiting astrogliosis and inflammation in fructose-fed rats. *Mol Nutr Food Res* (2015) 59(2):189–202. doi: 10.1002/mnfr.201400307

76. Cheng Y, Meng Q, Wang C, Li H, Huang Z, Chen S, et al. Leucine deprivation decreases fat mass by stimulation of lipolysis in white adipose tissue and upregulation of uncoupling protein 1 (UCP1) in brown adipose tissue. *Diabetes* (2010) 59(1):17–25. doi: 10.2337/db09-0929

77. Wang Y, Li Z, Zhang X, Xiang X, Li Y, Mulholland MW, et al. Nesfatin-1 promotes brown adipocyte phenotype. *Sci Rep* (2016) 6(1):34747. doi: 10.1038/srep34747

78. Srivastava S, Kashiwaya Y, King MT, Baxa U, Tam J, Niu G, et al. Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. *FASEB J* (2012) 26(6):2351–62. doi: 10.1096/fj.11-200410

79. Hageman RS, Wagener A, Hantschel C, Svenson KL, Churchill GA, Brockmann GA. High-fat diet leads to tissue-specific changes reflecting risk factors for diseases in DBA/2J mice. *Physiol Genomics* (2010) 42(1):55–66. doi: 10.1152/physiolgenomics.00072.2009

80. Pietiläinen KH, Róg T, Seppänen-Laakso T, Virtue S, Gopalacharyulu P, Tang J, et al. Association of lipidome remodeling in the adipocyte membrane with acquired obesity in humans. *PLoS Biol* (2011) 9(6):e1000623. doi: 10.1371/journal.pbio.1000623

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 September 2022
DOI 10.3389/fendo.2022.942412

OPEN ACCESS

EDITED BY
Sonia Michael Najjar,
Ohio University, United States

REVIEWED BY
Xingchun Wang,
Tongji University, China
Jie Yang,
Cleveland Clinic, United States

*CORRESPONDENCE
Mei-Fang Li
15821955054@126.com
Lian-Xi Li
lilx@sjtu.edu.cn

†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 12 May 2022
ACCEPTED 16 August 2022
PUBLISHED 05 September 2022

CITATION
Wang J-W, Jin C-H, Ke J-F, Ma Y-L, Wang Y-J, Lu J-X, Li M-F and Li L-X (2022) Serum iron is closely associated with metabolic dysfunction-associated fatty liver disease in type 2 diabetes: A real-world study.
Front. Endocrinol. 13:942412.
doi: 10.3389/fendo.2022.942412

Serum iron is closely associated with metabolic dysfunction-associated fatty liver disease in type 2 diabetes: A real-world study

Jun-Wei Wang[1†], Chun-Hua Jin[2†], Jiang-Feng Ke[1†], Yi-Lin Ma[1], Yu-Jie Wang[1], Jun-Xi Lu[1], Mei-Fang Li[3*] and Lian-Xi Li[1*]

[1]Department of Endocrinology and Metabolism, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, Shanghai, China, [2]Department of Endocrinology and Metabolism, Shanghai Songjiang District Central Hospital, Songjiang Hospital Affiliated to Shanghai Jiaotong University School of Medicine (Preparatory Stage), Shanghai, China, [3]Department of Emergency, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Aims: There is still a debate about the relationship between serum iron and metabolic dysfunction-associated fatty liver disease (MAFLD). Furthermore, few relevant studies were conducted in type 2 diabetes mellitus (T2DM). Therefore, this study aimed to explore the association of serum iron levels with MAFLD in Chinese patients with T2DM.

Methods: This cross-sectional, real-world study consisted of 1,467 Chinese T2DM patients. MAFLD was diagnosed by abdominal ultrasonography. Based on serum iron quartiles, the patients were classified into four groups. Clinical characteristics were compared among the four groups, and binary logistic analyses were used to assess the associations of serum iron levels and quartiles with the presence of MAFLD in T2DM.

Results: After adjusting for gender, age, and diabetes duration, significantly higher prevalence of MAFLD was found in the second (45.7%), third (45.2%), and fourth (47.0%) serum iron quartiles than in the first quartiles (26.8%), with the highest MAFLD prevalence in the fourth quartile ($p < 0.001$ for trend). Moreover, increased HOMA2-IR ($p = 0.003$ for trend) and decreased HOMA2-S ($p = 0.003$ for trend) were observed across the serum iron quartiles. Fully adjusted binary logistic regression analyses indicated that both increased serum iron levels (OR: 1.725, 95% CI: 1.427 to 2.085, $p < 0.001$) and quartiles ($p < 0.001$ for trend) were still closely associated with the presence of MAFLD in T2DM patients even after controlling for multiple confounding factors.

Conclusions: There is a positive correlation between the presence of MAFLD and serum iron levels in T2DM patients, which may be attributed to the close association between serum iron and insulin resistance. Serum iron levels may

10.3389/fendo.2022.942412

act as one of the indicators for evaluating the risk of MAFLD in T2DM individuals.

KEYWORDS

serum iron, non-alcoholic fatty liver disease, metabolic dysfunction-associated fatty liver disease, type 2 diabetes, insulin resistance

Introduction

In addition to oxygen transport, iron also has a vital role in many metabolic processes with the potential to cause oxidative damage when in excess (1). Specifically, serum ferric iron is carried by transferrin and transported into the cell, where it is reduced to ferrous iron (2). Ferrous iron facilitates peroxidation of membrane-bound, PUFA-containing lipids and triggers propagation of lipid peroxidation, which cause damage to mitochondria and other organelles and finally lead to the development and progression of metabolic disorders (2). Therefore, iron overload may be linked to multiple metabolic disorders such as obesity, hyperlipidemia, hyperglycemia, and insulin resistance (1, 3, 4). For example, a recent study indicated increased serum iron levels in patients with type 2 diabetes mellitus (T2DM) (4). Moreover, the risk of developing diabetes induced by iron was probably close to the relative risk generated by obesity (1). Additionally, it was also noted that iron metabolism disorders were remarkably correlated with insulin resistance and obesity (3).

As one of the metabolic disorders, metabolic dysfunction-associated fatty liver disease (MAFLD), formerly named non-alcoholic fatty liver disease (NAFLD), is referred to as the "hepatic manifestation of the metabolic syndrome" and causes a progressive liver disease group including steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma (5). MAFLD is centered on hepatic fat accumulation and comorbid with obesity, T2DM, or evidence of metabolic dysregulation, with a prevalence of 25.9%–38.0% in the general population (6). Moreover, a recent meta-analysis estimated that T2DM patients had a higher risk developing MAFLD than the general population, with a prevalence of 55.5%–70% (7, 8). In our recent studies, the prevalence of MAFLD was 39.4%–52.6% in patients with T2DM, which was also higher than that in the general population (9–11). Given the high prevalence of MAFLD in T2DM, early identification and intervention of risk factors associated with MAFLD will be beneficial in reducing the occurrence of MAFLD in T2DM subjects.

Currently, some studies have identified several iron-related serum markers such as serum ferritin, hepcidin, and serum transferrin saturation, which are closely associated with MAFLD (12–14). However, the relationship between serum iron and MAFLD is infrequently studied and probably not explicit in general and diabetic populations. For example, in a small sample study of mostly biopsy-proven NAFLD, only two (3%) patients had high serum iron levels (15). Additionally, a negative correlation between serum iron and NAFLD prevalence in the general population was found, as the U.S. National Health and Nutrition Examination Survey (NHANES) data indicated (16). However, several studies pointed to the presence of unchanged serum iron levels in patients with NAFLD (17–19). Moreover, a previous study emphasized an elevated serum iron level in NAFLD patients in comparison to patients without NAFLD (20).

Notably, the relationship between iron status and MAFLD remained controversial in T2DM subjects, as there were few studies and clinical trials. A study including T2DM subjects highlighted that the T2DM prevalence increased in the NAFLD group compared with the non-NAFLD group, but without an increase in serum iron (21). Likewise, in a study containing nearly half of NAFLD patients with T2DM, histological iron in liver was not associated with NAFLD severity (22). However, a previous study including patients with T2DM endorsed an increase in the NAFLD prevalence with elevated serum iron levels (23).

Therefore, our aim was to investigate the correlation between serum iron levels and MAFLD diagnosed by abdominal ultrasonography in Chinese patients with T2DM.

Abbreviations: T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; MAFLD, metabolic dysfunction fatty liver disease; DD, diabetes duration; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; LLD, lipid-lowering drugs; BMI, body mass index; HbA1C, glycated hemoglobin A1c; IIAs, insulin or insulin analogue; WHR, waist-to-hip ratio; FPG, fasting plasma glucose; 2-h PPG, 2-h postprandial plasma glucose; 2-h C-P, 2-h postprandial C-peptide; HOMA2-IR, HOMA of insulin resistance; TG, total triglycerides; TC, total cholesterol; FCP, fasting C-peptide; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase; Cr, creatinine; SUA, serum uric acid; UAE, urinary albumin excretion; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

Materials and methods

Subjects and study design

This cross-sectional, real-world study included T2DM patients hospitalized in the Department of Endocrinology and

Metabolism, the Sixth People's Hospital of Shanghai Jiao Tong University from January 2006 to December 2012, and some of the patient data were from our recent studies (11, 24–26). The hospital ethical review committee approved this study [approved number: 2018-KY-018(K)], with written consent obtained from all participants. Inclusion criteria incorporated T2DM diagnosed in accordance with the WHO criteria, age ≥ 17 years old, complete clinical information and biochemical parameters, and available abdominal ultrasound findings (25). After excluding the patients with disorders related to iron metabolism such as hemochromatosis, iron-deficiency anemia, menstruation within a week, and blood transfusion or donation recently; those with liver diseases caused by drugs, viral hepatitis, and other reasons excluding alcohol; and those with other serious systemic diseases or infectious diseases, 1,467 patients were classified into four groups according to the serum iron quartiles.

Physical examination and laboratory tests

The following data were collected at admission as previously described: hypertension history, diabetes duration (DD), alcohol intake, smoking habits, use of lipid-lowering drugs (LLDs), metformin, insulin sensitizers, insulin or insulin analogs (IIAs), and physical data including height, waist and hip circumference, weight, and blood pressure (10, 11). Specifically, the definitions of hypertension, obesity, smoking, and alcohol status were described in our previous studies (11, 25).

After fasting overnight and 2 h after breakfast on the second day of admission, blood samples were collected. Serum alanine transaminase (ALT) was measured by an enzymatic rate method with the definition of elevated ALT more than 65 U/L according to our previous study (10). Serum iron was determined using colorimetric assay by a LAbOSPECT 008AS automatic biochemical analyzer (Hitachi, Japan) (27), and serum ferritin level was measured using chemiluminescence immunoassay by a cobas e 602 module (Roche Diagnostics, Germany) (28). Other laboratory parameters such as blood glucose, lipids, insulin, C-peptide, kidney function, and urine tests were measured as described previously (10, 11, 25). The homeostasis model assessment of insulin resistance (HOMA2-IR) and the homeostasis model assessment of sensitivity (HOMA2-S) were estimated using HOMA2 Calculator version 2.2.3 (11). The estimated glomerular filtration rate (eGFR) was calculated according to the formula recommended for the Chinese population [$175\times(\text{serum creatinine})^{-1.234}\times(\text{age})^{-0.179}(\times 0.79, \text{if female})$] (25).

Abdominal ultrasonography and diagnostic criteria

The hepatic ultrasound examinations and diagnosis of hepatic steatosis were in accordance with our previous studies (10, 11). Since T2DM patients were selected as the target population in the present study, MAFLD was diagnosed as ultrasonographically verified hepatic steatosis in addition to the presence of T2DM, which was proposed by an international expert panel from 22 countries (29).

Statistical analysis

Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Normality was assessed for continuous variables and then expressed as mean ± standard deviation or median and interquartile range. In particular, the differences between the two groups were evaluated by the *t*-test or the Mann–Whitney *U* test, while the differences between multiple groups were assessed using one-way ANOVA or the Kruskal–Wallis *H* test. Chi-square tests were used to analyze categorical variables. When gender and/or age was considered as confounders, categorical variables were corrected with logistic regression, and continuous variables were adjusted with univariate linear regression models. After non-normally distributed variables were transformed by normal score transformation, binary logistic regression was done for assessing the correlation of serum iron levels and quartiles with the MAFLD presence. Five models were constructed to evaluate the association of serum iron with MAFLD. Statistical significance was set at $p < 0.05$.

Results

Characteristics of the study subjects

This study consisted of 1,467 inpatients with T2DM. In accordance with the serum iron quartiles with cutoffs of <10.6, 10.6–14.0, 14.1–18.0, and >18.0 μmol/L, they were classified into four groups. The subjects' baseline characteristics grouped by serum iron quartiles are highlighted in Table 1. There was a significant age and sex difference among the four groups. After adjusting for sex and age, with ascending serum iron levels, IIAs usage, DD, and levels of urinary albumin excretion (UAE) and C-reactive protein (CRP) were obviously decreased, and levels of fasting plasma glucose (FPG), fasting C-peptide (FCP), 2-h postprandial C-peptide (2h C-P), low-density lipoprotein cholesterol (LDL-C), ALT, and eGFR were significantly increased (all $p < 0.05$). Additionally, there were obvious differences in LLD usage, waist circumference (WC), BMI, and values of SBP, DBP, 2-h postprandial plasma glucose (2-h PPG), creatine (Cr), total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and serum uric acid (SUA) among the serum iron quartiles in T2DM patients (all $p < 0.05$). However, no obvious difference was found in hypertension and obesity prevalence, smoking status, alcohol intake, metformin and insulin sensitizers use, WHR, SBP, DBP,

10.3389/fendo.2022.942412

TABLE 1 Characteristics of the subjects according to serum iron levels.

Variables	Q1 (n = 369)	Q2 (n = 363)	Q3 (n = 368)	Q4 (n = 367)	p-value	*p-value
Serum iron (μmol/L)	<10.6	10.6–14.0	14.1–18.0	>18.0	—	—
Male (n, %)	145 (39.3%)	158 (43.5%)	173 (47.0%)	251 (68.4%)	<0.001	<0.001
Age (years)	63 ± 13	61 ± 12	61 ± 12	56 ± 13	<0.001	<0.001
*DD (months)	120 (48–156)	96 (36–168)	84 (36–132)	60 (12–120)	<0.001	0.006
Hypertension (n, %)	201 (54.5%)	209 (57.6%)	216 (58.7%)	187 (51.0%)	0.146	0.205
Obesity (n, %)	156 (42.3%)	174 (47.9%)	166 (45.1%)	177 (48.2%)	0.327	0.461
Smoking (n, %)	63 (17.1%)	90 (24.8%)	101 (27.4%)	139 (37.9%)	<0.001	0.146
Alcohol (n, %)	36 (9.8%)	54 (14.9%)	54 (14.7%)	85 (23.3%)	<0.001	0.537
IIAs (n, %)	289 (78.3%)	246 (67.8%)	235 (63.9%)	226 (61.6%)	<0.001	<0.001
LLD (n, %)	71 (19.2%)	110 (30.3%)	106 (28.8%)	92 (25.1%)	0.003	0.004
Metformin (n, %)	182 (49.3%)	203 (55.9%)	199 (54.1%)	194 (52.9%)	0.331	0.334
Insulin sensitizers (n, %)	28 (7.6%)	45 (12.4%)	46 (12.5%)	33 (9.0%)	0.065	0.077
SBP (mmHg)	134 ± 18	133 ± 19	135 ± 18	131 ± 17	0.021	0.552
DBP (mmHg)	79 ± 10	80 ± 9	81 ± 10	81 ± 9	0.133	0.466
WC (cm)	87.95 ± 9.98	89.85 ± 10.74	89.65 ± 10.55	90.08 ± 10.17	0.043	0.039
WHR	0.91 ± 0.07	0.91 ± 0.07	0.92 ± 0.06	0.92 ± 0.07	0.257	0.253
BMI (kg/m^2)	24.34 ± 3.56	25.04 ± 3.62	24.85 ± 3.58	25.02 ± 3.61	0.033	0.028
*FPG (mmol/L)	7.40 (5.72–9.58)	7.51 (6.11–9.59)	7.83 (6.22–9.71)	8.12 (6.69–10.19)	0.001	0.001
*2-h PPG (mmol/L)	12.90 (9.42–16.34)	13.24 (9.94–16.39)	12.72 (9.70–15.99)	13.67 (10.47–17.12)	0.099	0.008
HbA1C (%)	9.06 ± 2.56	8.67 ± 2.25	8.91 ± 2.20	8.88 ± 2.15	0.164	0.091
*FCP (ng/ml)	1.60 (0.93–2.65)	1.79 (1.10–2.74)	1.89 (1.29–2.82)	1.93 (1.24–2.76)	0.006	0.002
*2h C-P (ng/ml)	3.25 (1.75–5.26)	4.17 (2.27–6.27)	4.35 (2.55–6.70)	4.57 (2.59–7.08)	<0.001	<0.001
*TG (mmol/L)	1.19 (0.88–1.74)	1.50 (1.04–2.36)	1.44 (1.00–2.13)	1.40 (0.99–2.00)	<0.001	<0.001
TC (mmol/L)	4.37 ± 1.01	4.78 ± 1.06	4.81 ± 1.15	4.76 ± 1.03	<0.001	<0.001
HDL-C (mmol/L)	1.10 ± 0.29	1.13 ± 0.30	1.16 ± 0.30	1.16 ± 0.29	0.019	<0.001
LDL-C (mmol/L)	2.70 ± 0.79	2.96 ± 0.83	3.00 ± 0.86	3.10 ± 0.91	<0.001	<0.001
*ALT (U/L)	16 (11–25)	19 (14–29)	22 (16–36)	25 (17–37)	<0.001	<0.001
*Cr (μmol/L)	68.0 (56.0–91.0)	66.0 (56.0–82.0)	66.0 (56.0–79.3)	69.0 (58.0–79.0)	0.057	<0.001
*SUA (μmol/L)	295 (240–358)	323 (262–380)	314 (262–371)	317 (265–380)	0.006	0.027
*UAE (mg/24 h)	17.95 (8.85–64.63)	12.45 (7.47–40.67)	12.30 (7.16–27.95)	11.10 (6.73–27.16)	<0.001	<0.001
*eGFR (ml/min/1.73 m^2)	98.8 (69.8–129.1)	102.2 (85.1–127.7)	105.6 (87.0–128.4)	110.9 (95.5–131.0)	<0.001	<0.001
*CRP (mg/L)	3.12 (0.98–8.83)	1.36 (0.57–3.42)	1.13 (0.47–2.69)	0.87 (0.40–1.85)	<0.001	<0.001

Values are expressed as the mean ± SD, or median with interquartile range, or percentages.
p-value: The p-values were not adjusted for age and sex for the trend.
p*-value: The p*-values were adjusted for sex and age for the trend.
*The Kruskal–Wallis H test was applied.

and glycated hemoglobin A1c (HbA1C) among the serum iron quartile groups.

Comparisons of MAFLD prevalence and serum iron levels stratified by sex, age, and DD

Figure 1 compares MAFLD prevalence and serum iron levels among the different gender, age, and DD groups. The MAFLD overall prevalence was 41.2%, with a higher prevalence in women (44.9%) than in men (37.4%) after adjusting for DD and age (p < 0.001, Figure 1A). However, serum iron levels were significantly lower in women than in men (p < 0.001, Figure 1D). In addition, a significant decrease in the prevalence of MAFLD was linked to increasing age (p < 0.001 for trend) and longer DD (p < 0.001 for trend) (Figures 1B, C). Likewise, there was a significant decline in serum iron levels with advancing age (p = 0.002 for trend) and prolonged DD (p = 0.022 for trend) (Figures 1E, F).

Comparisons of serum iron levels and MAFLD prevalence

Figure 2 compares the serum iron levels between T2DM patients with and without MAFLD and the prevalence of

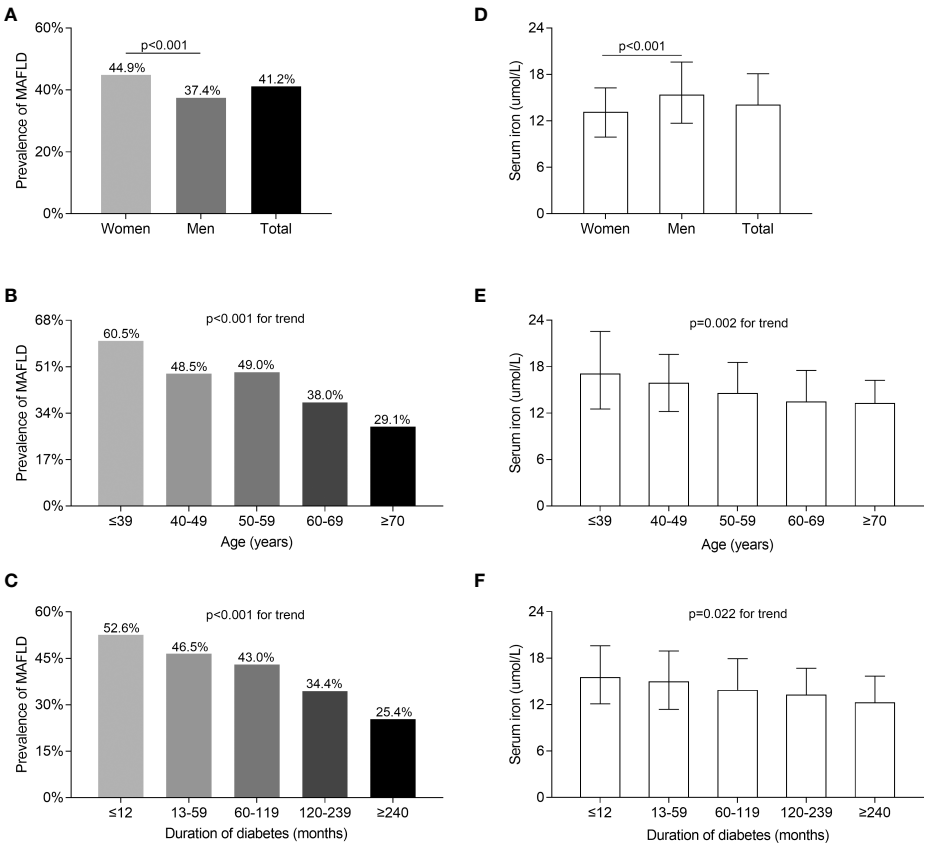


FIGURE 1
Comparisons of MAFLD prevalence and serum iron levels stratified by sex, age, and DD. **(A)** Overall prevalence of MAFLD and comparisons of the prevalence of MAFLD stratified by gender ($p < 0.001$). **(B)** Comparisons of the MAFLD prevalence among patients stratified by age ($p < 0.001$ for trend). **(C)** Comparisons of the MAFLD prevalence among patients stratified by DD ($p < 0.001$ for trend). **(D)** Overall serum iron levels and comparisons of serum iron levels stratified by gender ($p < 0.001$). **(E)** Comparisons of serum iron levels among patients stratified by age ($p = 0.002$ for trend). **(F)** Comparisons of serum iron levels among patients stratified by DD ($p = 0.022$ for trend).

MAFLD among the serum iron quartiles. After correcting for sex, age, and DD, serum iron and ferritin levels were significantly increased in T2DM individuals with MAFLD in comparison to those without MAFLD ($p < 0.001$, Figures 2A, C). Moreover, significantly higher prevalence of MAFLD was found in Q2 (45.7%), Q3 (45.2%), and Q4 (47.0%) compared to Q1 (26.8%), with the highest MAFLD prevalence in Q4 after adjusting for age, sex, and DD ($p < 0.001$ for trend) (Figure 2B). Additionally, there was an increased trend of serum ferritin across serum iron quartiles ($p < 0.001$ for trend) (Figure 2D).

Comparisons of serum ALT levels

The comparisons of serum ALT levels and the percentage of the patients with elevated ALT levels in different groups are displayed in Figure 3. After controlling for sex, age, and DD, serum ALT values and the percentage of the patients with elevated serum ALT levels were significantly greater in T2DM patients with MAFLD compared with those without MAFLD ($p < 0.001$, Figures 3A, C). Furthermore, both the percentage of the patients with elevated serum ALT levels (Q1: 1.90%; Q2: 4.40%; Q3: 6.00%; Q4: 11.70%; $p = 0.001$ for trend, Figure 3B) and serum ALT levels [Q1: 16 (11–25); Q2: 19 (14–29); Q3: 22 (16–36); Q4: 25 (17–37); $p < 0.001$ for trend, Figure 3D] rose with the increasing serum iron quartiles after adjusting for sex, age, and DD.

Comparisons of HOMA2-IR and HOMA2-S

Figure 4 illustrates the HOMA2-IR and HOMA2-S comparisons between T2DM patients with and without MAFLD as well as across serum iron quartile groups. After adjusting for sex, age, and DD, higher HOMA2-IR and lower HOMA2-S were observed in T2DM patients with MAFLD than in those without MAFLD (all $p < 0.001$, Figures 4A, C). Moreover, the significantly increased trend in HOMA2-IR ($p = 0.003$ for trend, Figure 4B)

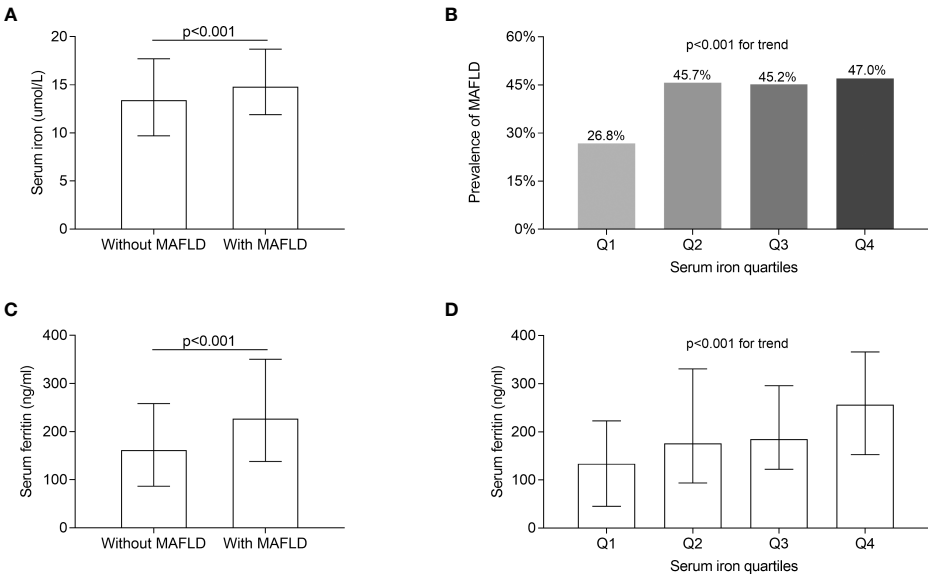


FIGURE 2
Comparisons of serum iron and ferritin levels, and MAFLD prevalence. **(A)** Comparisons of serum iron levels between T2DM patients with and without MAFLD ($p < 0.001$). **(B)** Comparisons of the prevalence of MAFLD across the serum iron quartile groups ($p < 0.001$ for trend). **(C)** Comparisons of serum ferritin between the patients with and without MAFLD ($p < 0.001$). **(D)** Comparisons of serum ferritin levels across the serum iron quartile groups ($p < 0.001$ for trend).

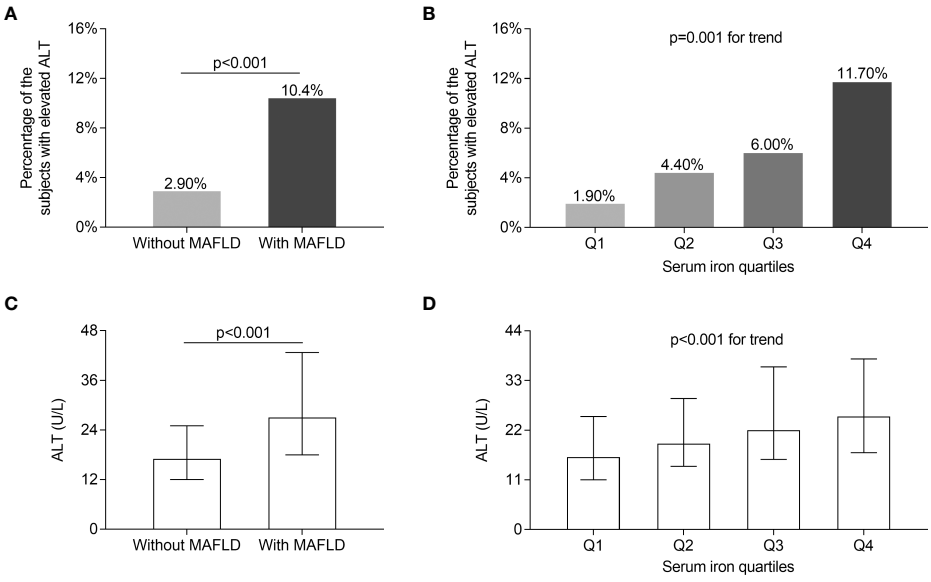


FIGURE 3
Comparisons of serum ALT levels. **(A)** Comparisons of the percentage of the subjects with elevated ALT levels between the patients with and without MAFLD ($p < 0.001$). **(B)** Comparisons of the percentage of the subjects with elevated ALT levels across the serum iron quartile groups ($p = 0.001$ for trend). **(C)** Comparisons of serum ALT levels between the patients with and without MAFLD ($p < 0.001$). **(D)** Comparisons of serum ALT levels across the serum iron quartile groups ($p < 0.001$ for trend).

and the obviously decreased trend in HOMA2-S (p = 0.003 for trend, Figure 4D) were observed across the serum iron quartiles.

Association of serum iron levels with MAFLD

Table 2 shows binary logistic analysis for the association of serum iron levels with MAFLD in T2DM patients. Before (Model 1) and after adjustment for age, sex, smoking, alcohol use, DD, obesity, and hypertension (Model 2), the patients with higher serum iron levels showed an increased risk for MAFLD comorbidity (p < 0.001). Further correcting for the treatment of LLD, metformin, IIAs and insulin sensitizers (Model 3), physical examination data (Model 4), and laboratory parameters (Model 5), serum iron levels continued to be positively associated with the presence of MAFLD (all p < 0.001).

Association of serum iron quartiles with MAFLD

Table 3 shows the association of serum iron quartiles with the presence of MAFLD in T2DM patients, which was analyzed by binary logistic regression. In unadjusted analysis, higher serum iron quartiles presented an obviously increased risk of MAFLD (p < 0.001 for trend) (Model 1). After further adjustment for age, sex, smoking, alcohol consumption, DD, obesity, and hypertension, higher serum iron quartiles remained related to a higher risk of MAFLD (p < 0.001 for trend) (Model 2). After further correction for variables regarding medication therapy (Model 3) and physical examination (Model 4), increased serum iron quartiles were stably correlated with the presence of MAFLD (all p < 0.001 for trend). Finally, after controlling for laboratory parameters, the correlation between serum iron quartiles and the presence of MAFLD stayed significantly positive (p < 0.001 for trend) (Model 5).

Discussion

The prevalence of MAFLD among T2DM patients was 41.2% in the present study, close to our previous findings of 39.4%–52.6% in T2DM, which was diagnosed based on NAFLD without alcohol users (9–11). Even though the criteria of NAFLD was replaced with MAFLD including patients with alcohol consumption in the current study, the difference of prevalence was comparatively insignificant. Therefore, it was suitable to choose MAFLD as the definition. Consistent with our previous study, the prevalence of MAFLD was higher in women, middle-aged patients, and patients with a short DD in this study (11). Notably, the median age of the enrolled patients was about 60 years old. Previous studies noted that women over the age of 60 had a higher prevalence of MAFLD than men, possibly influenced by menopause in women (30, 31). Moreover, the

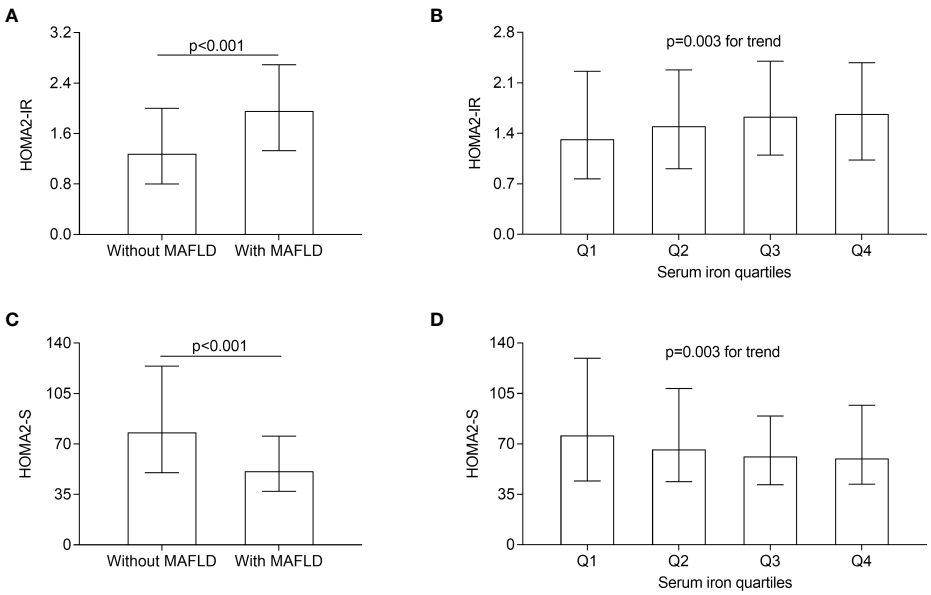


FIGURE 4
Comparisons of HOMA2-IR and HOMA2-S. **(A)** Comparisons of HOMA2-IR between the patients with and without MAFLD (p < 0.001). **(B)** Comparisons of HOMA2-IR across the serum iron quartile groups (p = 0.003 for trend). **(C)** Comparisons of HOMA2-S between the patients with and without MAFLD (p < 0.001). **(D)** Comparisons of HOMA2-S across the serum iron quartile groups (p = 0.003 for trend).

TABLE 2 Association of the prevalence of MAFLD with serum iron.

	B statistic	OR	95% CI	*p*-value
Model 1	0.311	1.365	1.223-1.523	<0.001
Model 2	0.329	1.390	1.224-1.578	<0.001
Model 3	0.313	1.368	1.202-1.556	<0.001
Model 4	0.345	1.412	1.220-1.633	<0.001
Model 5	0.545	1.725	1.427-2.085	<0.001

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, DD, smoking status, alcohol intake, obesity, and hypertension.
Model 3: Further adjustment for use of LLD, IIAs, metformin, and insulin sensitizers.
Model 4: Further adjustment for SBP, DBP, WC, WHR, and BMI.
Model 5: Further adjustment for TC, TG, HDL-C, LDL-C, eGFR, Cr, SUA, UAE, HbA1C, FCP, 2-h CP, HOMA2-IR, FPG, 2-h PPG, ferritin, and CRP.

peak prevalence of MAFLD was between the ages of 18 and 50 in other studies, which supported our suggestion that middle-aged people were more likely to develop MAFLD compared with older people (32, 33). Possible reasons for a higher MAFLD prevalence in younger patients were as follows: middle-aged people were more susceptible to sedentary lifestyles, obesity, and stressful socioeconomic status, which increased MAFLD risk; elderly patients experienced an increased overall mortality partially caused by fatty liver; and the deceased patients were excluded from the MAFLD population (34, 35). Additionally, the negative association between DD and MAFLD prevalence was partly explained by several studies, in which obesity and insulin resistance were involved in the early stages of T2DM as risk factors for MAFLD (11, 36, 37). Alternatively, as the duration of diabetes increased, the duration of glucose-lowering medication was correspondingly longer, some of which were thought to have a therapeutic effect on MAFLD and might lead to a reduction in MAFLD (38). Moreover, serum iron levels were higher in men than in women, but the MAFLD prevalence was lower in men than in women, which might be explained by the fact that MAFLD was influenced by multiple factors in addition to gender and serum iron. Additionally, serum iron levels decreased with increasing age and DD, which corresponded with a decrease in MAFLD prevalence with higher age and longer DD.

Currently, the correlation between serum iron and MAFLD remains unclear in the general population. For example, a cross-sectional study showed that the serum iron levels and the NAFLD prevalence had a negative correlation (16). Conversely, a recent study based on obese patients found that the severe NAFLD group had higher serum iron levels than the mild or moderate groups (39). Other studies suggested an irrelevant association between serum iron and NAFLD staging and liver fat content (17, 18). Likewise, besides few relevant investigations, there were conflicting opinions on the relationship between iron status and MAFLD in T2DM patients. For example, a previous study exhibited a positive correlation between iron store and the degree of NAFLD in patients with coexisting T2DM and NAFLD (40), whereas another study supported no correlation between NAFLD and hepatic iron in T2DM subjects (22). Therefore, we conducted the present study investigating the serum iron levels and MAFLD correlation in patients with T2DM.

Notably, the present study demonstrated that there was a positive correlation between serum iron levels and the prevalence of MAFLD. The risk of MAFLD increased nearly 1.73-fold with each 1 SD increase in serum iron levels. Consistent with our results, a study comprising subjects with T2DM noted 20% of patients with steatosis in the low serum iron group compared with 78.9% of patients with steatosis in

TABLE 3 Association of the prevalence of MAFLD with serum iron quartile groups.

	ORs (95% CI)				*p*-values for trend
	Q1	Q2	Q3	Q4	
Model 1	1	2.298 (1.687–3.131)	2.241 (1.646–3.051)	2.432 (1.787–3.310)	<0.001
Model 2	1	2.551 (1.805–3.607)	2.463 (1.740–3.487)	2.496 (1.745–3.570)	<0.001
Model 3	1	2.315 (1.627–3.295)	2.246 (1.577–3.199)	2.370 (1.651–3.402)	<0.001
Model 4	1	2.588 (1.736–3.856)	2.429 (1.640–3.596)	2.532 (1.684–3.807)	<0.001
Model 5	1	2.944 (1.808–4.794)	3.185 (1.961–5.170)	4.009 (2.375–6.766)	<0.001

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, DD, smoking status, alcohol intake, obesity, and hypertension.
Model 3: Further adjustment for use of LLD, IIAs, metformin, and insulin sensitizers.
Model 4: Further adjustment for SBP, DBP, WC, WHR, and BMI.
Model 5: Further adjustment for TC, TG, HDL-C, LDL-C, eGFR, Cr, SUA, UAE, HbA1C, FCP, 2-h CP, HOMA2-IR, FPG, 2-h PPG, ferritin, and CRP.

the high serum iron group (13). Similarly, patients with NAFLD had an average body iron of 1.6 g compared with 1.4 g without NAFLD in a clinical trial based on T2DM and prediabetes (41). Furthermore, a previous study supported that a 12-month glucose-lowering strategy for poorly controlled T2DM patients enabled the simultaneous reduction of serum ferritin from 223 μg/L to 121 μg/L, hepatic iron concentrations from 109.2 mg/100 mg to 89.7 mg/100 mg, and the prevalence of MAFLD from 80% to 25% (42), which indicates positive correlation between iron and MAFLD in T2DM patients. Additionally, the prevalence of MAFLD increased at least threefold when serum iron was greater than 10.6 μmol/L. Even though the prevalence of MAFLD was the highest in Q4, all other groups actually had higher prevalence of MAFLD compared with Q1 (serum iron < 10.6 μmol/L). It suggested that there might be a threshold value of serum iron, beyond which the risk of MAFLD increases significantly. Given that iron depletion ameliorated MAFLD and that iron deficiency was also associated with the increased risk of metabolic dysfunction (43, 44), it might be required to maintain serum iron in a suitable range for treatment and prevention of MAFLD.

Moreover, the present study also suggested increased liver enzymes with the increase of serum iron levels, which reflected the aggravation of hepatocyte damage. Therefore, rising serum iron might be closely related to the severity of liver damage caused by MAFLD in addition to the increased presence of MAFLD. Similar to our findings, several studies also displayed a positive association between serum or body iron and ALT levels in patients with or without T2DM (39, 41, 45). For example, the NHANES study including NAFLD subjects without T2DM indicated a 1.13-fold risk of elevated ALT levels with increasing deciles of serum iron concentration (45). Interestingly, after induction of iron depletion to near-iron deficiency, there was nearly half of the reduction in serum ALT levels in MAFLD patients with T2DM, without descent in non-MAFLD patients with T2DM treated equally (41). Therefore, a possibly more severe hepatocellular injury was related to elevated serum iron, which might suggest the progression of fatty liver.

The reason explaining the close correlation of serum iron with MAFLD may be attributed to insulin resistance induced by iron, which was a major mechanism in the MAFLD pathogenesis (3). Iron metabolism disorders aggravate oxidative damage to hepatocytes and thus lead to insulin resistance and subsequent compensatory hyperinsulinemia, which promote hepatic *de novo* lipogenesis and cholesterol synthesis as well as reduce free fatty acid catabolism by oxidation (3, 8, 44). Moreover, the significant relationship of high iron status with insulin resistance has been repeatedly confirmed in many studies (17, 39, 41). For example, HOMA-IR was positively linked to serum iron with a correlation coefficient of 0.189 in obese patients with or without T2DM (39).

A previous study underlined that in patients with obesity and metabolic syndrome, the increase of serum iron was accompanied by elevated insulin resistance evaluated by HOMA-IR (39). A subsequent study speculated that the elevated iron levels could impair the function of pancreatic β cells and cause systemic insulin resistance in T2DM subjects (46). Furthermore, previous studies also detected that iron removal improved both insulin sensitivity and β-cell function in patients comprising T2DM (17, 39, 41). Understandably, we also proved higher HOMA2-IR, FCP, 2h C-P, and lower HOMA2-S from the lowest to highest serum iron quartiles. Therefore, increased insulin resistance caused by high iron levels might contribute to the development and progression of MAFLD in T2DM.

Verified by other studies, we also observed the elevation of serum ferritin in T2DM patients with MAFLD compared to those without MAFLD (13, 47). Although serum ferritin levels increased with the rising of serum iron quartiles, serum ferritin was no longer an independent risk factor for MAFLD in the binary logistic regression analysis. A previous study supported the idea that serum ferritin levels failed to correlate with NAFLD after adjusting for multiple factors (48). The possible reason for inconsistency was that serum ferritin reflected the stored iron in cells, which might not be fully associated with iron that exerted function (49). Moreover, CRP levels decreased from lower to higher serum iron quartiles in the present study. Consistently, in a study based on a mouse model, MAFLD was exacerbated accompanied by elevated serum iron but reduced CRP levels (8). Another study indicated the lack of correlation between CRP and iron status in T2DM patients (50). Moreover, no appreciable difference in CRP between non-NAFLD and NAFLD in some cases might suggest multiple factors instead of merely inflammation influencing MAFLD (5, 51).

We faced some limitations during the current research. First, some cases with milder steatosis were probably missed by ultrasonography. Even so, there was a good concordance between ultrasound diagnosis and pathological diagnosis in NAFLD patients (52). Moreover, ultrasonography was recommended as the first-line imaging method by the clinical guidelines for MAFLD, which ensured accuracy and convenience of ultrasound diagnosis to detect steatosis (29, 35). Second, although various factors might influence insulin resistance, we adjusted as much factors as possible including DD, medication usage, lipids, and so on to reduce the impact on the results. Third, the present study was conducted in a specific population and further population expansion was needed to confirm the findings. Fourth, since this is a cross-sectional study, it is difficult to determine the causal relationship between iron and MAFLD, whereas we supposed that serum iron might be one of the causes of MAFLD based on previous studies (8, 39, 43). It has been suggested that iron overload aggravated hepatic insulin resistance (8, 39), while iron depletion therapy ameliorated NAFLD (43).

Conclusions

In conclusion, serum iron levels are independently and positively associated with MAFLD in patients with T2DM. Serum iron levels could be a biomarker to evaluate the risk of MAFLD for better screening and prevention in T2DM patients.

Data availability statement

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

Ethics statement

This study was reviewed and approved by Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

L-XL and M-FL provided the hypothesis, designed the study, and revised the manuscript. J-WW, C-HJ, and J-FK made contributions to the acquisition, analysis, or interpretation of data. J-WW drafted the manuscript. Y-LM, J-XL, and Y-JW participated in the revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from the National Key Research and Development Plan (2018YFC1314900 and 2018YFC1314905), the National Natural Science Foundation of China (81770813 and 82070866), the Translational Medicine National Key Science and Technology Infrastructure Open Project (TMSK-2021-116), the Exploratory Clinical Research Project of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (ynts202105), and the Shanghai Municipal Key Clinical Specialty.

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. Simcox JA, McClain DA. Iron and diabetes risk. *Cell Metab* (2013) 17:329–41. doi: 10.1016/j.cmet.2013.02.007

2. Stockwell BR. Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell* (2022) 185:2401–21. doi: 10.1016/j.cell.2022.06.003

3. Britton LJ, Subramaniam VN, Crawford DH. Iron and non-alcoholic fatty liver disease. *World J Gastroenterol* (2016) 22:8112–22. doi: 10.3748/wjg.v22.i36.8112

4. Altamura S, Kopf S, Schmidt J, Müdder K, da Silva AR, Nawroth P, et al. Uncoupled iron homeostasis in type 2 diabetes mellitus. *J Mol Med* (2017) 95:1387–98. doi: 10.1007/s00109-017-1596-3

5. Eslam M, Sanyal AJ, George JInternational Consensus Panel. MAFLD: A consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* (2020) 158:1999–2014.e1. doi: 10.1053/j.gastro.2019.11.312

6. Lin H, Zhang X, Li G, Wong GL-H, Wong VW-S. Epidemiology and clinical outcomes of metabolic (Dysfunction)-associated fatty liver disease. *J Clin Transl Hepatol* (2021) 9:972-82. doi: 10.14218/JCTH.2021.00201

7. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* (2019) 71:793–801. doi: 10.1016/j.jhep.2019.06.021

8. Altamura S, Müdder K, Schlotterer A, Fleming T, Heidenreich E, Qiu R, et al. Iron aggravates hepatic insulin resistance in the absence of inflammation in a novel db/db mouse model with iron overload. *Mol Metab* (2021) 51:101235. doi: 10.1016/j.molmet.2021.101235

9. Li T-T, Wang A-P, Lu J-X, Chen M-Y, Zhao C-C, Tang Z-H, et al. Prevalence and clinical characteristics of non-alcoholic fatty liver disease in newly diagnosed patients with ketosis-onset diabetes. *Diabetes Metab* (2018) 44:437–43. doi: 10.1016/j.diabet.2018.03.002

10. Zhao C-C, Wang A-P, Li L-X, Li T-T, Chen M-Y, Zhu Y, et al. Urine uric acid excretion is associated with nonalcoholic fatty liver disease in patients with type 2 diabetes. *J Diabetes Complications* (2016) 30:1074–80. doi: 10.1016/j.jdiacomp.2016.04.017

11. Zhang Z-H, Ke J-F, Lu J-X, Liu Y, Wang A-P, Li L-X. Serum retinol-binding protein levels are associated with nonalcoholic fatty liver disease in Chinese patients with type 2 diabetes mellitus: A real-world study. *Diabetes Metab J* (2022) 46:129–39. doi: 10.4093/dmj.2020.0222

12. Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* (2011) 55:920–32. doi: 10.1016/j.jhep.2011.05.008

13. Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic

severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatol Baltim Md* (2012) 55:77–85. doi: 10.1002/hep.24706

14. Pan X, Chen B, Liu W, Li Y, Hu Z, Lin X, et al. Circulating iron levels interaction with central obesity on the risk of nonalcoholic fatty liver disease: A case-control study in southeast China. *Ann Nutr Metab* (2019) 74:207–14. doi: 10.1159/000497228

15. Duseja A, Das R, Das A, Dhiman RK, Chawla YK, Garewal G. Serum iron levels and hepatic iron overload in patients with nonalcoholic steatohepatitis. *Dig Dis Sci* (2006) 51:1730–1. doi: 10.1007/s10620-005-9041-9

16. Yang H-H, Chen G-C, Li D-M, Lan L, Chen L-H, Xu J-Y, et al. Serum iron and risk of nonalcoholic fatty liver disease and advanced hepatic fibrosis in US adults. *Sci Rep* (2021) 11:10387. doi: 10.1038/s41598-021-89991-x

17. Datz C, Felder TK, Niederseer D, Aigner E. Iron homeostasis in the metabolic syndrome. *Eur J Clin Invest* (2013) 43:215–24. doi: 10.1111/eci.12032

18. Mendler M-H, Turlin B, Moirand R, Jouanolle A-M, Sapey T, Guyader D, et al. Insulin resistance–associated hepatic iron overload. *Gastroenterology* (1999) 117:1155–63. doi: 10.1016/S0016-5085(99)70401-4

19. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. *J Hepatol* (2007) 46:700–7. doi: 10.1016/j.jhep.2006.09.018

20. Park SH, Kim BI, Kim SH, Kim HJ, Park DI, Cho YK, et al. Body fat distribution and insulin resistance: Beyond obesity in nonalcoholic fatty liver disease among overweight men. *J Am Coll Nutr* (2007) 26:321–6. doi: 10.1080/07315724.2007.10719618

21. Lonardo A, Loria P, Leonardi F, Borsatti A, Neri P, Pulvirenti M, et al. Fasting insulin and uric acid levels but not indices of iron metabolism are independent predictors of non-alcoholic fatty liver disease. a case-control study. *Dig Liver Dis* (2002) 34:204–11. doi: 10.1016/S1590-8658(02)80194-3

22. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* (1999) 116:1413–9. doi: 10.1016/s0016-5085(99)70506-8

23. Puchakayala BK, Verma S, Kanwar P, Hart J, Sanivarapu RR, Mohanty SR. Histopathological differences utilizing the nonalcoholic fatty liver disease activity score criteria in diabetic (type 2 diabetes mellitus) and non-diabetic patients with nonalcoholic fatty liver disease. *World J Hepatol* (2015) 7:2610–8. doi: 10.4254/wjh.v7.i25.2610

24. Ke J-F, Wang J-W, Lu J-X, Zhang Z-H, Liu Y, Li L-X. 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

25. Ke J-F, Wang J-W, Zhang Z-H, Chen M-Y, Lu J-X, Li L-X. Insulin therapy is associated with an increased risk of carotid plaque in type 2 diabetes: A real-world study. *Front Cardiovasc Med* (2021) 8:599545. doi: 10.3389/fcvm.2021.599545

26. Liu F, Chen S, Zhao W, Chen M, Ke J, Zhang Z, et al. Urine uric acid excretion levels are positively associated with obesity and abdominal obesity in type 2 diabetes patients without chronic kidney disease. *Diabetes Metab Syndr Obes Targets Ther* (2021) 14:4691–703. doi: 10.2147/DMSO.S335558

27. Yang Y, Dai C, Chen X, Feng J. The relationship between serum trace elements and oxidative stress of patients with different types of cancer. *Oxid Med Cell Longev* (2021) 2021:1–13. doi: 10.1155/2021/4846951

28. Ayan D, Soylemez S. Measuring plasma ferritin levels with two different methods: A comparison of roche cobas E601 versus roche cobas C501 (integrated modular system roche cobas 6000). *J Med Biochem* (2020) 39:13–8. doi: 10.2478/jomb-2018-0048

29. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* (2020) 73:202–9. doi: 10.1016/j.jhep.2020.03.039

30. Hashimoto E, Taniai M, Tokushige K. Characteristics and diagnosis of NAFLD/NASH: Non-invasive alternatives to liver biopsy. *J Gastroenterol Hepatol* (2013) 28:64–70. doi: 10.1111/jgh.12271

31. Burra P, Bizzaro D, Gonta A, Shalaby S, Gambato M, Morelli MC, et al. Clinical impact of sexual dimorphism in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). *Liver Int* (2021) 41:1713–33. doi: 10.1111/liv.14943

32. Huang Y-P, Zhang S, Zhang M, Wang Y, Wang W-H, Li J, et al. Gender-specific prevalence of metabolic-associated fatty liver disease among government employees in tianjin, China: a cross-sectional study. *BMJ Open* (2021) 11:e056260. doi: 10.1136/bmjopen-2021-056260

33. Golabi P, Paik J, Hwang JP, Wang S, Lee HM, Younossi ZM. Prevalence and outcomes of non-alcoholic fatty liver disease (NAFLD) among Asian American adults in the united states. *Liver Int* (2019) 39:748–57. doi: 10.1111/liv.14038

34. Wilmot E, Idris I. Early onset type 2 diabetes: risk factors, clinical impact and management. *Ther Adv Chronic Dis* (2014) 5:234–44. doi: 10.1177/2040622314548679

35. Chen Y, Li H, Li S, Xu Z, Tian S, Wu J, et al. Prevalence of and risk factors for metabolic associated fatty liver disease in an urban population in China: a cross-sectional comparative study. *BMC Gastroenterol* (2021) 21:212. doi: 10.1186/s12876-021-01782-w

36. Gallwitz B, Kazda C, Kraus P, Nicolay C, Schernthaner G. Contribution of insulin deficiency and insulin resistance to the development of type 2 diabetes: nature of early stage diabetes. *Acta Diabetol* (2013) 50:39–45. doi: 10.1007/s00592-011-0319-4

37. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* (1999) 104:787–94. doi: 10.1172/JCI7231

38. Ferguson D, Finck BN. Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus. *Nat Rev Endocrinol* (2021) 17:484–95. doi: 10.1038/s41574-021-00507-z

39. Ma B, Sun H, Zhu B, Wang S, Du L, Wang X, et al. Hepatic steatosis is associated with elevated serum iron in patients with obesity and improves after laparoscopic sleeve gastrectomy. *Obes Facts* (2021), 14:64–71. doi: 10.1159/000511736

40. Yan J-X, Pan B-J, Zhao P-P, Wang L-T, Liu J-F, Fu S-B. Serum ferritin is correlated with non-alcoholic fatty liver disease in middle-aged and older patients with type 2 diabetes. *Endocr Connect* (2021) 10:1560–9. doi: 10.1530/EC-21-0367

41. Facchini FS, Hua NW, Stoohs RA. Effect of iron depletion in carbohydrate-intolerant patients with clinical evidence of nonalcoholic fatty liver disease. *Gastroenterology* (2002) 122:931–9. doi: 10.1053/gast.2002.32403

42. Viganò M, Vergani A, Trombini P, Paleari F, Piperno A. Insulin resistance influence iron metabolism and hepatic steatosis in type II diabetes. *Gastroenterology* (2000) 118:986–7. doi: 10.1016/s0016-5085(00)70193-4

43. Valenti L, Fracanzani AL, Dongiovanni P, Rovida S, Rametta R, Fatta E, et al. A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia. *World J Gastroenterol* (2014) 20:3002–10. doi: 10.3748/wjg.v20.i11.3002

44. Ahmed U, Latham PS, Oates PS. Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. *World J Gastroenterol* (2012) 18:4651–8. doi: 10.3748/wjg.v18.i34.4651

45. Ruhl CE, Everhart JE. Relation of elevated serum alanine aminotransferase activity with iron and antioxidant levels in the united states. *Gastroenterology* (2003) 124:1821–9. doi: 10.1016/s0016-5085(03)00395-0

46. Wang X, Fang X, Zheng W, Zhou J, Song Z, Xu M, et al. Genetic support of a causal relationship between iron status and type 2 diabetes: A mendelian randomization study. *J Clin Endocrinol Metab* (2021) 106:e4641–51. doi: 10.1210/clinem/dgab454

47. Amin RF, El Bendary AS, Ezzat SE, Mohamed WS. Serum ferritin level, microalbuminuria and non-alcoholic fatty liver disease in type 2 diabetic patients. *Diabetes Metab Syndr Clin Res Rev* (2019) 13:2226–9. doi: 10.1016/j.dsx.2019.05.030

48. Buzzetti E, Petta S, Manuguerra R, Luong TV, Cabibi D, Corradini E, et al. Evaluating the association of serum ferritin and hepatic iron with disease severity in non-alcoholic fatty liver disease. *Liver Int Off J Int Assoc Study Liver* (2019) 39:1325–34. doi: 10.1111/liv.14096

49. Brissot P, Ropert M, Le Lan C, Loréal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta* (2012) 1820:403–10. doi: 10.1016/j.bbagen.2011.07.014

50. Van Campenhout A, Van Campenhout C, Lagrou AR, Abrams P, Moorkens G, Van Gaal L, et al. Impact of diabetes mellitus on the relationships between iron-, inflammatory- and oxidative stress status. *Diabetes Metab Res Rev* (2006) 22:444–54. doi: 10.1002/dmrr.635

51. Mörwald K, Aigner E, Bergsten P, Brunner SM, Forslund A, Kullberg J, et al. Serum ferritin correlates with liver fat in Male adolescents with obesity. *Front Endocrinol* (2020) 11:340. doi: 10.3389/fendo.2020.00340

52. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatol Baltim Md* (2011) 54:1082–90. doi: 10.1002/hep.24452

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 09 September 2022
DOI 10.3389/fendo.2022.944991

OPEN ACCESS

EDITED BY
Maria João Meneses,
Universidade Nova de Lisboa, Portugal

REVIEWED BY
Li Chen,
Jilin University, China
Ines Sousa-Lima,
Universidade NOVA de Lisboa, Portugal

*CORRESPONDENCE
Yu Hua
37931208@qq.com
Yingxian Sun
yxsun@cmu.edu.cn

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

RECEIVED 16 May 2022
ACCEPTED 19 August 2022
PUBLISHED 09 September 2022

CITATION
Wang P, Guo X, Zhou Y, Li Z, Yu S, Sun Y and Hua Y (2022) Monocyte-to-high-density lipoprotein ratio and systemic inflammation response index are associated with the risk of metabolic disorders and cardiovascular diseases in general rural population. *Front. Endocrinol.* 13:944991. doi: 10.3389/fendo.2022.944991

Monocyte-to-high-density lipoprotein ratio and systemic inflammation response index are associated with the risk of metabolic disorders and cardiovascular diseases in general rural population

Pengbo Wang, Xiaofan Guo, Ying Zhou, Zhao Li, Shasha Yu, Yingxian Sun* and Yu Hua*

The Department of Cardiology, The First Hospital of China Medical University, Shenyang, China

Background: The present study aimed to clarify the effects of four inflammatory indicators (monocyte-to-high-density lipoprotein ratio [MHR], neutrophil-to-lymphocyte ratio [NLR], systematic immune-inflammation index [SII], and systemic inflammation response index [SIRI]) in evaluating the risk of metabolic diseases and cardiovascular disease (CVD), filling the gap of inflammation-metabolism system research in epidemiology.

Methods: We conducted a cross-sectional study and multivariable logistic regression analysis to elucidate the association between inflammatory indicators and metabolic diseases and CVD risk. Metabolic diseases were defined as metabolic disorders (MetDs) or their components, such as metabolic syndrome (MetS), dyslipidemia, and central obesity. We calculated the Framingham risk score (FRS) to evaluate 10-year CVD risk.

Results: Odds ratios for the third vs. the first tertile of MHR were 2.653 (95% confidence interval [CI], 2.142–3.286) for MetD, 2.091 (95% CI, 1.620–2.698) for MetS, 1.547 (95% CI, 1.287–1.859) for dyslipidemia, and 1.515 (95% CI, 1.389–1.652) for central obesity. Odds ratios for the third vs. the first tertile of SIRI were 2.092 (95% CI, 1.622–2.699) for MetD, 3.441 (95% CI, 2.917–4.058) for MetS, 1.417 (95% CI, 1.218–1.649) for dyslipidemia, and 2.080 (95% CI, 1.613–2.683) for central obesity. The odds ratio of a 10-year CVD risk of >30% for the third vs. the first tertile of MHR was 4.607 (95% CI, 2.648–8.017) and 3.397 (95% CI, 1.958–5.849) for SIRI.

Conclusions: MHR and SIRI had a significant association with MetD and its components, in which a higher level of MHR or SIRI tended to accompany a higher risk of metabolic diseases. Furthermore, they also correlated with CVD,

and the increment of these indicators caused a gradually evaluated risk of 10-year CVD risk.

KEYWORDS

monocyte-to-high-density lipoprotein ratio, systemic inflammation response index, metabolic disorders, metabolic syndrome, 10-year cardiovascular disease risk

Introduction

Metabolic diseases have been recognized as crucial risk factors and chronic pathology processes in the elderly population, and increasing evidence has confirmed that metabolic dysfunction is the basis of various chronic diseases, such as diabetes mellitus (DM), and cardiovascular disease (CVD) events (1–3). Various studies have revealed that multiple metabolic diseases in China were prevalent, and the prevalence was gradually increasing (3), with the prevalence of 31.1% for metabolic syndrome (MetS) (4), 33.8% for dyslipidemia (5), and 40.8% for central obesity (4). The northeast rural regions of China even had a higher prevalence of MetS (37.3% for men and 45.8% for women) than the general level due to multiple chronic disease-related lifestyles, such as a high-salt diet (6), which causes a heavy burden of chronic diseases and CVD. Therefore, it is critical to elucidate the potential mechanism and associated risk factors for metabolic diseases and further explore the possible intervention strategies.

The metabolic and immune systems are among the essential requirements for the homeostasis of the human body (7). Increasing evidence has shown a high-grade inflammatory response in adipose tissue with a prevalent infiltration of macrophages and other immune cells among the adipocytes, during which cells changed from anti-inflammatory into pro-inflammatory status under this infiltration of immune cells, suggesting that chronic inflammation could potentially be related to obesity (8). Additionally, cellular metabolic processes could also be regulated by inflammatory molecules or inflammatory pathways, such as cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6, and IL-1β), which could act in an autocrine or paracrine manner and interfere with the insulin signaling in peripheral tissues by activating the c-Jun N-terminal kinase (JNK) pathway or nuclear factor kappa B (NF-κB) pathway, inducing β-cell dysfunction and promoting insulin resistance (9, 10). In addition, multiple inflammatory factors are involved in the regulation of various metabolic products, such as lipids and glucose (11, 12), and high-grade inflammation could lead to extensive accumulation of abnormal lipids and glucose, eventually resulting in metabolic dysfunction.

Recent clinical research has indicated that salicylate sodium therapy or treatment with inflammatory cytokine inhibitors could significantly reduce the serum glucose of type 2 DM (T2DM) patients and decrease the risk of CVD events (12–14). However, most current studies focused on inflammation and metabolism have been conducted on adipose tissue or immune cells, and most diseases at the individual level have been limited to obesity and DM, representing only a limited aspect of metabolic diseases, especially in the elderly population in whom obesity is not a typical metabolic alteration, leading to a limitation of epidemiological studies on inflammation and metabolic disorders. Moreover, cellular metabolic dysfunction or abnormal metabolism of cellular products could be restored by multiple homeostatic mechanisms and compensatory behaviors, which might only manifest as a pre-metabolic imbalance status at the individual level and, thus, could not cause the symptoms of a metabolic disorder (MetD). Furthermore, previous studies have usually used inflammatory factors, such as IL-1/6 and TNFα, to reflect inflammation levels, which seemed more suitable for molecular research. Additionally, in clinical or large-scale population screening, the blood cell count has been more commonly measured to reflect the inflammatory status.

The composite inflammatory indicators such as monocyte-to-high-density lipoprotein ratio (MHR) (15), neutrophil-to-lymphocyte ratio (NLR) (15), systematic immune-inflammation index (SII) (16), and systemic inflammation response index (SIRI) (17) are a novel type of parameters based on the traditional peripheral blood cell count, calculated by combining different biochemical parameters to balance inflammation and immunity status (17). Previous studies have confirmed that these indicators could reflect inflammation levels and were widely used in evaluating the risk of various chronic diseases and the prognosis of tumors (18–22). Therefore, our study enrolled and screened these four simple-to-calculate and easily accessible systemic inflammatory indicators to assess their effect in evaluating the risk of metabolic diseases and CVD, filling the knowledge gap on the association between inflammation and metabolic diseases at the individual level.

Methods

Study population

The present study was based on the Northeast China Rural Cardiovascular Health Study (NCRCHS), aiming to further reveal the association between various inflammatory indicators and the risk of metabolic diseases. We conducted the baseline study from July 2012 to August 2013, and the detailed protocol was described in previous research (23, 24), which is summarized in Figure 1. In brief, we recruited 11,956 residents of rural regions (aged ≥35 years) from 26 villages in three countries in Northeastern China. However, there were 4,467 subjects who met the exclusion criteria, which included pregnancy, cancer, mental disorders, or failure to complete related research, such as those with incomplete data. We further excluded 69 subjects with extremely abnormal white blood cell (WBC) counts (>50 × 10^9/l or <1.0 × 10^9/l) to avoid the effects of the acute phase of infection on the inflammatory status. Ultimately, we obtained a target population of 7,420 people.

Data collection and ethics

We established a cardiologist team to conduct outpatient face-to-face interviews with participants and complete paper vision standard questionnaires to collect data. Before the project, we conducted a training course about project-related knowledge and ethical content. Only the staff who passed the related test could be authorized to conduct subsequent research.

The Ethics Committee of China Medical University approved our project (Shenyang, China, ethical approved project identification code: AF-SOP-07-1, 0-01). Every participant received and signed a paper-version informed consent after clarifying the relevant information on the study objectives, benefits, medical procedures, confidentiality agreement on personal information, and agreement on publication of related data research.

Lifestyle risk factors

Information, such as age, gender, or physical activity, was obtained from a standard questionnaire during the interview. We also asked the participants whether they were currently smoking or drinking. Physical activity level was considered to combine occupational workload and leisure-time exercise and was then reclassified into three levels: low, moderate, and high level. The salt intake was classified into three categories, low, medium, or high salt intake, defined as ≤6, 6–10, or >10 g/day, respectively. We used tea consumption to represent the caffeine intake of the population and divided the population into three subgroups: no tea consumption subgroup, rarely subgroup (one to two cups/day) or often subgroup (≥3 cups/day). All participants were asked whether they had suffered from CVD, stroke, or kidney diseases.

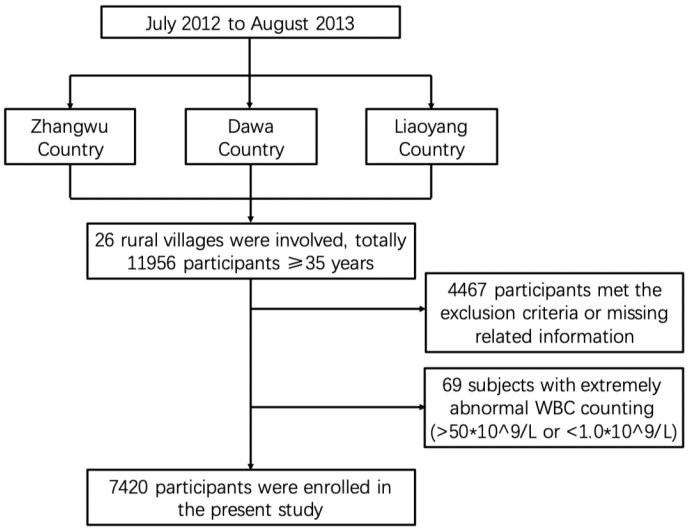


FIGURE 1
Flowchart of our selected study population protocol. We randomly selected 26 villages from three countries in northeastern China from July 2012 to August 2013. In total, 11,956 participants were enrolled in our study. After excluding people who did not meet the research criteria, such as those with cancer, pregnancy, and missing related information, and further excluding 69 subjects with extremely abnormal WBC counts, we finally got a study population of 7,420 subjects.

Variable measurement

All participants were told to fast for at least 12 h in advance, and blood samples were collected the next morning. The blood samples were added to vacutainer tubes containing an anticoagulant, and plasma was obtained by centrifugation. The final purpose of blood samples was to gather data on blood biochemistry and perform blood routine examinations. Fasting blood glucose (FPG), triglyceride (TG), plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and glycated hemoglobin (HbA1C) were obtained by enzymatic analysis on an Olympus AU640 automated analyzer (Olympus, Kobe, Japan). All laboratory equipment was calibrated, and samples were repeated using the blind method. The measurement of height and weight needed participants to keep a standing posture, wear lightweight clothes, and be without shoes.

The waist circumference (WC) was measured at the umbilicus level at the end of a normal expiration. The measurement results were accurate to 0.1 kg and 0.1 cm, respectively. The measurement of blood pressure was performed according to the American Heart Association protocol. Subjects were told to rest in a quiet room for at least 10 min, and an automatic electronic sphygmomanometer (HEM-741C; Omron, Tokyo, Japan) was used to measure blood pressure three times. The measurements were taken on the naked upper arm in a seated position with a 5-min interval between measurements. The average of three blood pressure measurements was selected and used for all subsequent analyses.

Definition

According to the 7th Joint National Committee guideline, we defined hypertension as blood pressure of ≥140/90 mmHg (systolic/diastolic blood pressure [SBP/DBP]) or being under medication treatment for hypertension in the last 2 weeks (25). DM was defined as FPG of ≥7.0 mmol/l or a previous diagnosis of DM (26). Hyperuricemia was diagnosed as serum uric acid (SUA) concentration of ≥420 μmol/l for men and ≥360 μmol/l for women (27). According to the International Diabetes Federation (IDF) definition (28), MetS was defined by central obesity (waist circumference of ≥90 cm for men and ≥80 cm for women) plus any two of the following factors: 1) TG of ≥1.7 mmol/l; 2) HDL-C level of <1.03 mmol/l in men or <1.29 mmol/l in women, or specific treatment for this lipid abnormality; 3) SBP of ≥130 or DBP of ≥85 mmHg or treatment of previously diagnosed hypertension; 4) FPG of ≥5.6 mmol/l or previously diagnosed T2DM. Dyslipidemia was defined by satisfying any of the following diseases (29): (1) hypercholesterolemia: plasma total cholesterol (TCH) of ≥5.2 mmol/l; (2) hypertriglyceridemia: TG of ≥1.7 mmol/l; (3) high LDL-C: LDL-C of ≥3.4 mmol/l; (4) low HDL-C: HDL-C of <1.03 mmol/l for men or <1.29 mmol/l for women. MetD was defined by satisfying any of MetS, dyslipidemia, and central obesity criteria. As for the estimated glomerular filtration rate (eGFR), we chose formulas including creatinine level suggested by the Chronic Kidney Disease Epidemiology Collaboration equations (CKD-EPI) (30). The body mass index (BMI) was calculated as weight (kg)/height (m^2). The 10-year CVD risk was determined by the overall score of the Framingham risk score (FRS), which included gender, age, HDL-C, TCH, SBP, and smoking (31). Furthermore, the inflammatory indicators were calculated by the following: 1) MHR = monocyte count/HDL-C (15); 2) NLR = neutrophil count/lymphocyte count (15); 3) SII = platelet count × neutrophil count/lymphocyte count (16); 4) SIRI = neutrophil count × monocyte count/lymphocyte count (17).

We also converted four inflammatory indicators into three levels by tertiles, and the lowest level was set as a reference. The detailed intervals of these indicators were as follows: 1) MHR, T1: ≤0.27; T2: 0.28–0.42; T3: ≥0.43; 2) NLR, T1: ≤1.47; T2: 1.48–2.08; T3: ≥2.09; 3) SII, T1: ≤282.63; T2: 282.64–427.34; T3: ≥427.38; 4) SIRI, T1: ≤0.61; T2: 0.62–1.03; T3: ≥1.04.

Statistical analysis

Overall, the data were normally distributed; thus, we described them by mean ± standard deviation (M ± SD) or frequency and percentage for continuous and categorical variables, respectively. The differences between continuous variables were compared by one-way analysis of variance (ANOVA) and χ^2-test analysis for categorical variables. We conducted a multivariable logistic regression model to calculate odd ratios (ORs) and 95% confidence interval (CI) to be able to assess the association between inflammatory indicators and various diseases. Statistical analyses were performed by SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). A P value of <0.05 under the two-tailed condition was considered statistically significant.

Results

The study subjects had a high prevalence of chronic diseases and metabolic diseases

Tables 1, 2 summarize the characteristic of participants in the present study. The subjects involved in the present study were predominantly middle-aged and elderly population with an average age of 53.7 years (54.1 years for men and 53.39 years for women). Our study subjects were exposed to multiple CVD risk factors, leading to prevalent chronic and metabolic diseases; for

10.3389/fendo.2022.944991

TABLE 1 Risk factor characteristics of the baseline population.

	Total N = 7,420 N (%)	Male N = 3,359 N (%)	Female N = 4,061 N (%)	pvalue
MetD	5,963 (80.4)	2,397 (71.4)	3,566 (87.8)	<0.001
MetS	2,317 (31.2)	680 (20.2)	1,637 (40.3)	<0.001
Dyslipidemia	5,613 (75.6)	2,261 (67.3)	3,352 (82.5)	<0.001
Hypercholesterolemia	3,781 (51.0)	1,595 (47.5)	2,186 (53.8)	<0.001
Hypertriglyceridemia	2,465 (33.2)	1,103 (32.8)	1,362 (33.5)	0.523
High LDL-C	1,827 (24.6)	729 (21.7)	1,098 (27.0)	<0.001
Low HDL-C	2,512 (33.9)	600 (17.9)	1,912 (47.1)	<0.001
Central obesity	3,040 (41.0)	842 (25.1)	2,198 (54.1)	<0.001
History of CVD	1,158 (15.6)	370 (11.0)	788 (19.4)	<0.001
History of stroke	692 (9.3)	330 (9.8)	362 (8.9)	0.180
History of nephrosis	150 (2.0)	55 (1.6)	95 (2.3)	0.032
Hypertension	3,494 (47.1)	1,699 (50.6)	1,795 (44.2)	<0.001
DM	842 (11.3)	363 (10.8)	479 (11.8)	0.182
Hyperuricemia	929 (12.5)	574 (17.1)	355 (8.7)	<0.001
Current smoking	2,560 (34.5)	1,938 (57.7)	622 (15.3)	<0.001
Current drinking	1,599 (21.5)	1,507 (44.9)	92 (2.3)	<0.001
Physical activity				<0.001
Low	2,722 (36.9)	943 (28.2)	1,779 (44.2)	
Medium	1,402 (19.0)	635 (19.0)	767 (19.0)	
High	3,247 (44.1)	1,765 (52.8)	1,482 (36.8)	
Salt intake				0.003
Low (<6 g/day)	86 (1.2)	27 (0.8)	27 (1.2)	
Medium (≥6 and <10 g/day)	1,109 (15.0)	470 (14.0)	639 (15.8)	
High (≥10 g/day)	6,203 (83.8)	2,851 (85.2)	3,352 (82.8)	
Tea consumption				<0.001
No	5,456 (73.5)	2,015 (60.0)	3,441 (84.7)	
Rarely (1–2 cups/day)	1,862 (25.1)	1,258 (37.5)	604 (14.9)	
Often (≥3 cups/day)	102 (1.4)	86 (2.6)	16 (3.9)	

Data are presented as N (%). Statistical significance was defined at $p < 0.05$ under two-tailed conditions. CVD, cardiovascular disease; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C. low-density lipoprotein cholesterol; MetD, metabolic disorders; MetS, metabolic syndrome.

example, 15.6% of the study population had CVD (19.4% for men and 11.0% for women), and 9.3% of participants suffered from stroke (9.8% for men and 8.9% for women). On the manifestation of chronic diseases, the subjects also had an obviously elevated BP level (138.54/82.24 mmHg averagely, 140.9/84.16 mmHg for men, and 136.6/80.65 mmHg for women) and FPG level (5.96 mmol/l averagely, 6.02 mmol/l for men, and 5.91 mmol/l for women), which consequentially caused the high prevalence of hypertension and DM in both genders (hypertension, 50.6% for men and 44.2% for women; DM, 10.81% for men and 11.8% for women). Most importantly, we observed that participants had prevalent metabolic disorders, where around 80.4% of participants suffered from it (71.4% for men and 87.8% for women).

Among the components of metabolic disorders, the prevalence of MetS was 31.2%, that of central obesity was 33.2%, and that of dyslipidemia was 75.6%. After the detailed classification of dyslipidemia, we found that 51% of participants had hypercholesterolemia, 33.2% had hypertriglyceridemia, and the prevalence of high LDL and low HDL was 24.6% and 33.9%, respectively. We also observed that female participants seemed more likely to suffer from metabolic disorders or various abnormal metabolic statuses than men. In terms of lifestyle, smoking and drinking accounted for 34.5% and 21.5% of participants, respectively, and were both more prevalent among the male population (57.7% vs. 15.3% for current smoking and 44.9% vs.2.3% for current drinking). Furthermore, the male population generally had a higher intensity of physical activity (52.8%), and female participants mostly had a low physical activity level (44.2%). In terms of diet, we noticed that male participants had a slight higher salt intake (≥10 g/day: 85.2% in men vs. 82.8% in women) and female participants presented a regular tea consumption (2.6% in men vs. 3.9% in women). We enrolled four inflammation parameters to systematically evaluate the inflammation status in the present study and found that male subjects had a lower inflammation

TABLE 2 The anthropometric and biochemical parameters of the baseline population.

	Total N = 7,420 M ± SD	Male N = 3,359 M ± SD	Female N = 4,061 M ± SD	pvalue
Age (years)	53.71 ± 8.68	54.10 ± 8.74	53.39 ± 8.62	0.42
BMI (kg/m^2)	24.58 ± 3.55	24.47 ± 3.43	24.67 ± 3.64	0.0012
WC (cm)	81.79 ± 9.61	82.93 ± 9.62	80.84 ± 9.49	<0.001
SBP (mmHg)	138.54 ± 21.62	140.90 ± 21.15	136.60 ± 21.81	<0.001
DBP (mmHg)	82.24 ± 11.63	84.16 ± 11.57	80.65 ± 11.45	<0.001
WBC (×10^9/L)	6.18 ± 2.08	6.43 ± 2.26	5.98 ± 1.90	<0.001
Monocyte (×10^9/L)	0.48 ± 0.28	0.50 ± 0.28	0.46 ± 0.28	<0.001
Neutrophil (×10^9/L)	3.74 ± 2.85	3.92 ± 2.84	3.58 ± 2.85	<0.001
Lymphocyte (×10^9/L)	2.66 ± 2.22	2.77 ± 2.25	2.57 ± 2.19	0.333
Platelet (×10^9/L)	206.37 ± 60.87	195.65 ± 58.36	215.23 ± 61.48	<0.001
BUN (mmol/L)	5.48 ± 2.19	5.81 ± 2.14	5.22 ± 2.19	<0.001
Scr (μmol/L)	75.45 ± 22.05	82.80 ± 20.31	69.37 ± 21.58	<0.001
eGFR (mL/min/1.73 m^2)	89.73 ± 14.59	91.80 ± 14.58	88.01 ± 14.38	<0.001
SUA (μmol/L)	299.15 ± 84.94	342.60 ± 84.33	263.20 ± 77.69	<0.001
FPG (mmol/L)	5.96 ± 1.55	6.02 ± 1.62	5.91 ± 1.50	0.002
TCH (mmol/L)	5.33 ± 1.10	5.25 ± 1.07	5.40 ± 1.13	<0.001
TG (mmol/L)	1.69 ± 1.49	1.70 ± 1.59	1.69 ± 1.41	0.672
HDL-C (mmol/L)	1.34 ± 0.32	1.33 ± 0.34	1.35 ± 0.30	0.019
LDL-C (mmol/L)	2.90 ± 0.80	2.85 ± 0.78	2.94 ± 0.81	<0.001
MHR	0.38 ± 0.25	0.36 ± 0.25	0.40 ± 0.24	<0.001
NLR	1.90 ± 0.99	1.83 ± 0.85	1.99 ± 1.13	<0.001
SII (*10^9/L)	390.19 ± 227.63	385.67 ± 233.48	393.92 ± 222.63	0.120
SIRI (*10^9/L)	0.93 ± 0.89	0.96 ± 0.87	1.21 ± 0.80	<0.001

Data are presented as M ± SD. Statistical significance was defined at $p < 0.05$ under two-tailed conditions. BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C. low-density lipoprotein cholesterol; M, mean; MHR, monocyte-to-high-density lipoprotein ratio; NLR, neutrophil-to-lymphocyte ratio; SBP, systolic blood pressure; Scr, serum creatinine; SD, standard deviation; SII, systematic immune-inflammation index; SIRI, systemic inflammation response index; SUA, serum uric acid; TCH, plasma total cholesterol; TG, triglyceride; WBC, white blood cells; WC, waist circumference.

degree than women (MHR, 0.36 vs. 0.40; NLR, 1.83 vs. 1.99; SIRI, 0.96 vs. 1.21), showing a similar difference in gender with the prevalence of various metabolic abnormalities.

The subjects with metabolic diseases had higher levels of inflammation status

We divided the subjects into various subgroups according to different metabolic diseases and compared the inflammation status difference between the normal subgroup and the corresponding disease subgroup to clarify whether inflammation had a potential association with metabolic status (Table 3). Overall, we observed that each indicator showed a significant increase in MetD patients. Additionally, in the components of MetD, we found that MetS patients had a higher level of all four inflammation parameters than non-MetS patients, but we only found that MHR, SII, and SIRI were significantly increased in dyslipidemia patients, and only MHR and SIRI were significantly elevated in central obesity patients. After further classification of dyslipidemia, we found that every four indicators in both hypercholesterolemia and hypertriglyceridemia patients showed a significant increment. The subjects with high LDL-C showed an overall increased inflammation marker levels except for MHR. We also found that MHR, SII, and SIRI were significantly increased in the subjects with low HDL-C. The above results suggested that abnormal metabolic status was usually accompanied by elevated inflammation parameters and showed higher inflammation levels than normal.

The prevalence of metabolic diseases was elevated with gradually aggravating inflammation status

We divided each inflammatory indicator into three levels by tertiles and compared the prevalence of metabolic diseases among different tertiles to clarify the changes in the risk of metabolic diseases under different inflammatory conditions (Figure 2). We observed that the general trend of alterations in MetD prevalence and its components were similar and consistent, showing a gradually elevated level with increased tertiles of four inflammatory parameters. Among these

10.3389/fendo.2022.944991

TABLE 3 The different levels of inflammation parameters divided by metabolic disorders or their components.

	MHR M ± SD	NLR M ± SD	SII M ± SD	SIRI M ± SD
MetD				
Yes	0.49 ± 0.25	1.96 ± 1.00	392.60 ± 225.82	1.24 ± 0.83
No	0.33 ± 0.22	1.89 ± 0.98	370.30 ± 234.70	0.82 ± 0.62
p	<0.001	0.017	<0.001	<0.001
MetS				
Yes	0.44 ± 0.28	1.92 ± 1.07	391.40 ± 238.51	0.97 ± 1.09
No	0.36 ± 0.23	1.27 ± 0.80	379.64 ± 201.63	0.92 ± 0.79
p	<0.001	0.027	0.042	0.018
Dyslipidemia				
Yes	0.40 ± 0.25	1.89 ± 0.97	394.10 ± 226.95	0.94 ± 0.83
No	0.34 ± 0.23	1.94 ± 1.07	378.03 ± 229.36	0.82 ± -.61
p	<0.001	0.051	0.009	<0.001
Central obesity				
Yes	0.42 ± 0.28	1.87 ± 1.06	389.20 ± 210.27	0.95 ± 1.06
No	0.36 ± 0.22	1.93 ± 0.86	390.87 ± 238.96	0.92 ± 0.75
p	<0.001	0.757	0.163	0.011
Hypercholesterolemia				
Yes	0.56 ± 0.24	1.96 ± 0.94	391.70 ± 230.29	1.39 ± 1.00
No	0.40 ± 0.20	1.55 ± 1.04	368.61 ± 224.85	0.94 ± 0.76
p	<0.001	<0.001	<0.001	<0.001
Hypertriglyceridemia				
Yes	0.45 ± 0.28	1.91 ± 1.06	394.32 ± 207.53	1.22 ± 1.00
No	0.35 ± 0.23	1.88 ± 0.85	378.13 ± 236.98	0.90 ± 0.76
p	<0.001	0.021	<0.001	<0.001
High LDL-C				
Yes	0.37 ± 0.26	1.92 ± 1.02	401.50 ± 216.32	1.39 ± 1.13
No	0.38 ± 0.24	1.54 ± 0.89	386.49 ± 231.10	0.94 ± 0.80
p	0.089	0.01	<0.001	<0.001
Low HDL-C				
Yes	0.47 ± 0.29	1.90 ± 0.84	400.04 ± 216.62	1.10 ± 0.95
No	0.34 ± 0.21	1.90 ± 1.06	385.15 ± 232.92	0.93 ± 0.76
p	<0.001	0.429	<0.001	<0.001

Data are presented as M ± SD. Statistical significance was defined at $p < 0.05$ under two-tailed conditions. HDL-C, high-density lipoprotein cholesterol; LDL-C. low-density lipoprotein cholesterol; M, mean; MetD, metabolic disorders; MetS, metabolic syndrome; MHR, monocyte-to-high-density lipoprotein ratio; NLR, neutrophil-to-lymphocyte ratio; SD, standard deviation; SII, systematic immune-inflammation index; SIRI, systemic inflammation response index.

indicators, we observed that the tertile increment of MHR and SIRI caused a stable and consistent elevation in the prevalence of various metabolic diseases. We also noticed that the magnitudes of increment in MHR were larger and more moderate in SIRI. The prevalence of MetD in the T3 subgroup of NLR had increased only by 0.4% based on the T2 subgroup, and there was only a 0.4% incremental prevalence of central obesity between the T1 and T2 subgroups of SII. Only the T3 subgroup had a significantly increased prevalence (by 3.0%). The abovementioned imbalanced alterations led to a statistically insignificant trend in their overall alterations. These alteration tendencies indicated that the subjects with a higher level of inflammatory parameters seemed more likely to have metabolic diseases, suggesting that there might be an association between inflammation and metabolic diseases.

High level of inflammatory parameters was associated with a higher risk of having metabolic diseases

To reveal the association between inflammation and metabolic diseases, we conducted multivariable logistic regression and adjusted different co-variables to guarantee the accuracy and reliability of the association (Table 4). For MetD and MetS, we adjusted the ORs of different inflammatory

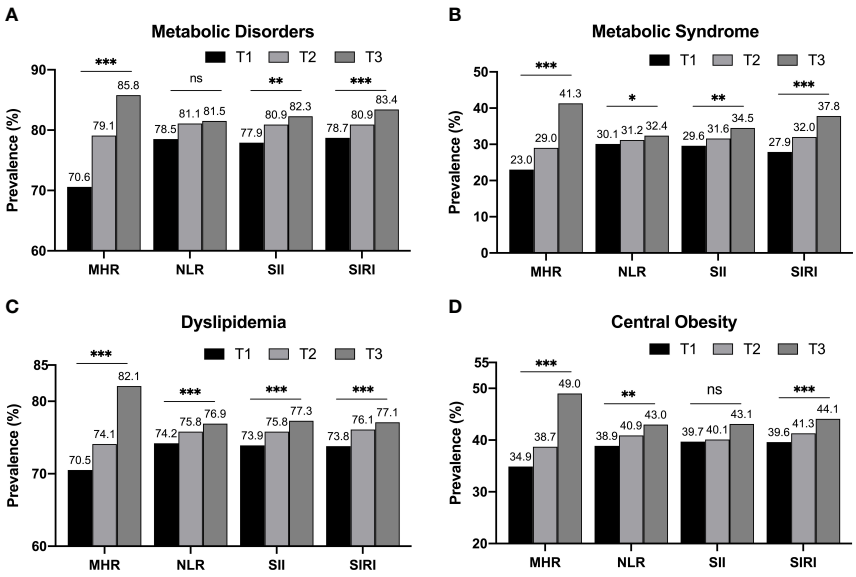


FIGURE 2
The prevalence of metabolic diseases in different inflammatory indicators grouped by tertiles. Regardless of the total MetD **(A)** or its detailed components, such as MetS **(B)**, dyslipidemia **(C)**, and central obesity **(D)**, they all showed a gradually increasing tendency with each tertile increment of various four inflammatory indicators. Statistical significance was defined under two-tailed condition. *p < 0.05; **p < 0.01; ***p < 0.001; ns, no significant.

parameters by model A, which included age, gender, a history of CVD, WBC counting, SUA concentration, eGFR, physical activity, salt intake, tea consumption, and a history of current smoking and drinking. For dyslipidemia, we added hypertension and DM based on model A and defined it as model B. For central obesity, we set up model C, which supplemented TCH, TG, LDL-C, and HDL-C into model B. Overall, we observed a significant association between inflammation and the risk of having metabolic diseases, which included MetD and various components of it, and the risk trended to elevate with increasing levels of inflammation. Compared with the lowest inflammatory indicator subgroup (T1 subgroups of all indicators), which was defined as a reference, the T3 subgroups of MHR had a 2.653-fold higher risk of MetD (the highest risk) (OR, 2.653; 95% CI, 2.142–3.286) and a 1.547-fold higher risk of having dyslipidemia (the highest risk) (OR, 1.547; 95% CI, 1.287–1.859) than reference.

The highest risk of MetS and central obesity was observed in the T3 subgroup of SIRI, with a risk of 3.441-fold (OR, 3.441; 95% CI, 2.917–4.058) for MetS and 2.080-fold (OR, 2.080; 95% CI, 1.613–2.683) for central obesity. By comparing the various indicators of the present study, we found that MHR and SIRI had a stable ability to evaluate the risk of metabolic diseases, showing a gradually elevated risk of having MetD or its components in every increased level of these two indicators, leading to a linear relationship between the levels of these two indicators and the risk of metabolic diseases. Each SD increment of MHR caused a 44.3% increase in risk for MetD, a 37.6% additional risk for MetS, a 47.2% additional risk for dyslipidemia, and a 41.8% additional risk for central obesity. Furthermore, each SD increment of SIRI caused a 37.8% extra risk for MetD, a 65.9% extra risk for MetS, a 19.7% extra risk for dyslipidemia, and a 45.4% extra risk for central obesity.

SII also showed good ability in risk evaluation of various metabolic diseases, such as MetS, dyslipidemia, and central obesity, and the tendencies of elevated risk also showed a linear association with per SD increment of SII. However, we did not observe the association between the T2 level of SII and the risk of MetD, leading to the non-linear risk association between SII and the risk of MetD. Lastly, NLR did not display an evaluation of the risk of various metabolic diseases at almost all levels.

High levels of inflammatory status predicted a higher risk of CVD

We calculated the FRS for each participant in these tertile subgroups and determined the 10-year CVD risk by their total score to further explain the applicability of these inflammatory indicators in predicting adverse outcomes (Table 5). We observed a progressive increment in CVD development risk with increasing inflammatory levels, and the T3 subgroups of all inflammatory indicators showed the highest risk of 10-year CVD, which was 35%–40% in the over 10%/11% (men/

TABLE 4 Inflammation states are associated with the risk of metabolic disorders and their components.

	Inflammation indicators tertiles			Continuous
	T1 Reference	T2 OR (95% CI)	T3 OR (95% CI)	per SD increment OR (95% CI)
MetD [a]				
MHR	Reference	1.385 (1.176-1.632) #	2.653 (2.142-3.286) #	1.443 (1.240-1.679) #
NLR	Reference	1.127 (0.926-1.372)	1.031 (0.783-1.358)	1.052 (0.811-1.366)
SII	Reference	1.105 (0.948-1.287)	1.299 (1.126-1.500) *	1.116 (1.049-1.188) *
SIRI	Reference	1.361 (1.174-1.577) #	2.092 (1.622-2.699) #	1.378 (1.047-1.815) *
MetS [a]				
MHR	Reference	1.424 (1.071-1.895) *	2.091 (1.620-2.698) #	1.376 (1.310-1.444) #
NLR	Reference	1.011 (0.837-1.220)	1.174 (0.954-1.445)	1.093 (0.849-1.407)
SII	Reference	1.267 (1.019,1.576) *	1.588 (1.324-1.904) #	1.235 (1.112-1.372) #
SIRI	Reference	1.592 (1.376-1.843) #	3.441 (2.917-4.058) #	1.659 (1.413-1.947) #
Dyslipidemia [b]				
MHR	Reference	1.131 (1.038-1.232) *	1.547 (1.287-1.859) #	1.472 (1.238-1.752) *
NLR	Reference	1.031 (0.830-1.281)	0.999 (0.992-1.007)	0.986 (0.967-1.005)
SII	Reference	1.221 (1.054-1.416) *	1.367 (1.176-1.591) #	1.112 (1.030-1.200) *
SIRI	Reference	1.237 (1.081-1.418) *	1.417 (1.218-1.649) #	1.197 (1.176-1.218) #
Central obesity [c]				
MHR	Reference	1.085 (0.855-1.377)	1.515 (1.389-1.652) #	1.418 (1.342-1.499) #
NLR	Reference	1.093 (0.849-1.407)	1.194 (0.957-1.490)	1.195 (0.950-1.504)
SII	Reference	1.120 (1.052-1.192) #	1.299 (1.190-1.418) #	1.216 (1.010-1.160) *
SIRI	Reference	1.445 (1.369-1.525) #	2.080 (1.613-2.683) #	1.454 (1.211-1.730) #

Statistical significance was defined at the following: *p < 0.05 under two-tailed conditions; #p < 0.001 under two-tailed conditions. a, the regression model that included age, gender, history of CVD, WBC counting, SUA concentration, eGFR, physical activity, salt intake, tea consumption, and current smoking and drinking. b, the regression model that included age, gender, history of CVD, WBC counting, SUA concentration, eGFR, physical activity, salt intake, tea consumption, current smoking and drinking, hypertension, and DM. c, the regression model that included age, gender, history of CVD, WBC counting, SUA concentration, eGFR, TCH level, TG level, LDL-C level, HDL-C level, physical activity, salt intake, tea consumption, current smoking and drinking, hypertension, and DM. The detailed intervals of these indicators were as the following: (1) MHR, T1: ≤0.27; T2: 0.28–0.42; T3: ≥0.43; (2) NLR, T1: ≤1.47; T2: 1.48–2.08; T3: ≥2.09; (3) SII, T1: ≤282.63; T2: 282.64–427.34; T3: ≥427.38; (4) SIRI, T1: ≤0.61; T2: 0.62–1.03; T3: ≥1.04. CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C. low-density lipoprotein cholesterol; M, mean; MetD, metabolic disorders; MetS, metabolic syndrome; MHR, monocyte-to-high-density lipoprotein ratio; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; SD, standard deviation; SII, systematic immune-inflammation index; SIRI, systemic inflammation response index; T, tertile.

women) risk subgroup, 10%–15% in the over 20%/22% (men/women) risk subgroup and approximately 3.0% in the over 30% risk subgroup. In addition, by comparing the alteration trend and per-level increment, we found that the inflammation level increase from T2 to T3 would bring a greater increment risk of CVD, especially in the over 20/22% and over 30% subgroups of FRS. These results demonstrated that the high inflammation levels indeed tended to indicate a higher risk of CVD or metabolic diseases or suggested that the subject was exposed to a high-risk status of diseases.

Chronic inflammatory status was accompanied by a higher risk of 10-year CVD

Previous results have already confirmed that high levels of inflammatory parameters tended to bring a higher CVD risk. Based on this hypothesis, we further conducted a logistic regression model of CVD risk, which included gender, current drinking, physical activity, salt intake, tea consumption, DM, hyperuricemia, WBC counting, BUN, Scr, TG, LDL-C, and inflammatory indicators, to confirm whether the high level of inflammation status was indeed associated with elevated CVD risk (Table 6). We observed that the subjects having a higher inflammation level were likely to have a higher risk of CVD, especially for the 10-year CVD risk in the over 10%/11% risk subgroup, in which the highest tertiles of all four indicators showed a significant association with it. Among these indicators, MHR and SIRI exhibited a stable correlation with CVD risk, and NLR and SII did not show a significant association with 10-year CVD risk and merely showed a weak association in some highest tertiles.

Compared with the lowest tertile, the subjects in the highest tertile of MHR had a 1.937-fold higher risk for 10-year CVD risk in the over 10%/11% risk subgroup, a 2.696-fold higher risk for 10-year CVD risk in the over 20%/22% risk subgroup, and a 4.607-fold higher risk for 10-year CVD risk in the over 30% risk

TABLE 5 Ten-year CVD risk reflected by FRS among different levels of multiple inflammation indicators.

	Ten-year CVD risk					
	Men >10%/women >11%		Men >20%/women >22%		Both >30%	
	N	%	N	%	N	%
MHR						
T1	632	25.7	156	6.4	22	0.9
T2	757	31.1	202	8.3	31	1.3
T3	1017	40.1	399	15.7	90	3.6
p for trend	<0.001		<0.001		<0.001	
NLR						
T1	682	27.9	198	8.1	32	1.3
T2	820	32.7	246	9.8	44	1.8
T3	904	36.6	313	12.7	67	2.7
p for trend	<0.001		<0.001		<0.001	
SII						
T1	782	31.6	232	9.4	36	1.5
T2	784	31.7	254	10.3	55	2.2
T3	840	34.0	321	13.0	79	3.2
p for trend	0.007		0.025		<0.001	
SIRI						
T1	597	24.2	156	6.3	21	0.9
T2	848	33.0	242	9.4	48	1.9
T3	961	40.4	359	15.1	74	3.1
p for trend	<0.001		<0.001		<0.001	

The detailed intervals of these indicators were as the following: (1) MHR, T1: ≤0.27; T2: 0.28–0.42; T3: ≥0.43; (2) NLR, T1: ≤1.47; T2: 1.48–2.08; T3: ≥2.09; (3) SII, T1: ≤282.63; T2: 282.64–427.34; T3: ≥427.38; (4) SIRI, T1: ≤0.61; T2: 0.62–1.03; T3: ≥1.04. CVD, cardiovascular disease; FRS, Framingham risk score; MHR, monocyte-to-high-density lipoprotein ratio; NLR, neutrophil-to-lymphocyte ratio; SII, systematic immune-inflammation index; SIRI, systemic inflammation response index; T, tertile.
Statistical significance was defined under two-tailed conditions.

subgroup. As for SIRI, the subjects in the highest tertile of SIRI had a 1.568-fold higher risk for 10-year CVD risk in the over 10%/11% risk subgroup, a 2.488-fold higher risk for 10-year CVD risk in the over 20%/22% risk subgroup, and a 3.397-fold higher risk for 10-year CVD risk in the over 30% risk subgroup compared to the lowest tertile. Furthermore, each SD increment of MHR caused a 29.3% additional risk for 10-year CVD risk in the over 10%/11% risk subgroup, a 42.3% extra risk for 10-year CVD risk in the over 20%/22% risk subgroup, and a 46.8% additional risk for 10-year CVD risk in the over 30% risk subgroup. In addition, SIRI displayed a more aggravated and stable elevated tendency in 10-year CVD risk, each SD increment of SIRI could bring a 22.0% additional risk in the over 10%/11% subgroup, 32.6% in the over 20%/22% subgroup, and 55.7% in the over 30% subgroup. These results suggested that a higher inflammatory status was associated with a higher CVD risk, confirming our hypothesis that long-term chronic inflammation seemed likely to cause CVD or elevate the CVD risk in the future.

Discussion

Most current studies focused on inflammation and metabolism have been conducted at the cellular level and indicated the regulation patterns and interaction effects between various cellular inflammatory factors and molecular metabolic behaviors. Our results, for the first time, revealed the epidemiological association between inflammation levels and the risk of metabolic diseases in a large-scale rural population in China. Our results filled a gap in the study of inflammation and metabolism at the individual and population levels, demonstrating that long-term chronic inflammatory states also affect metabolic status at the individual level, and higher levels of inflammation in the population were significantly associated with an elevated risk of various metabolic diseases, such as MetD and its components. We also compared and screened various inflammatory indicators and found that MHR and SIRI demonstrated a significant and stable effect in evaluating the risk of metabolic diseases in which a higher-level inflammation

TABLE 6 Chronic inflammation status was associated with 10-year CVD risk.

	Adjusted 10-year CVD risk					
	Men >10%/women >11%		Men >20%/women >22%		Both >30%	
	ORs (95% CI)	p value	ORs (95% CI)	p value	ORs (95% CI)	p value
MHR						
T1	–	–	–	–	–	–
T2	1.332 (1.090-1.628)	0.005	1.891 (1.490-2.400)	0.039	1.832 (0.984-3.413)	0.056
T3	1.937 (1.592-2.358)	<0.001	2.696 (2.119-3.431)	<0.001	4.607 (2.648-8.017)	<0.001
Per SD increment	1.293 (1.194–1.400)	<0.001	1.423 (1.305-1.551)	<0.001	1.468 (1.310-1.647)	<0.001
NLR						
T1	–	–	–	–	–	–
T2	1.119 (0.983-1.440)	0.075	1.098 (0.868-1.389)	0.435	1.218 (0.738-2.012)	0.440
T3	1.290 (1.063-1.565)	0.010	1.349 (1.074-1.695)	0.010	1.740 (0.827–2.779)	0.021
Per SD increment	1.022 (0.953–1.095)	0.544	1.074 (1.000-1.153)	0.051	1.094 (0.979-1.222)	0.113
SII						
T1	–	–	–	–	–	–
T2	1.130 (0.935-1.365)	0.207	1.123 (0.896-1.408)	0.314	1.528 (0.963-2.426)	0.072
T3	1.265 (1.046-1.531)	0.016	1.166 (0.933-1.456)	0.177	1.292 (1.206-1.383)	<0.001
Per SD increment	1.061 (0.983–1.146)	0.130	1.041 (0.858-1.263)	0.681	1.134 (0.980-1.311)	0.092
SIRI						
T1	–	–	–	–	–	–
T2	1.372 (1.129–1.667)	0.001	1.315 (1.014-1.704)	<0.001	2.071 (1.1623-3.692)	0.014
T3	1.568 (1.290–1.907)	<0.001	2.488 (2.212-2.797)	<0.001	3.397 (1.958-5.894)	<0.001
Per SD increment	1.220 (1.205–1.236)	<0.001	1.326 (1.255-1.401)	<0.001	1.557 (1.228-1.974)	<0.001

Logistic regression model of CVD risk: gender, current drinking, physical activity, salt intake, tea consumption, DM, hyperuricemia, WBC counting, BUN, Scr, TG, LDL-C, and inflammatory indicators, respectively. CI, confidence interval; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M, mean; MetD, metabolic disorders; MetS, metabolic syndrome; MHR, monocyte-to-high-density lipoprotein ratio; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; SD, standard deviation; SII, systematic immune-inflammation index; SIRI, systemic inflammation response index; T, tertile.
Statistical significance was defined under two-tailed conditions.

status was usually accompanied by a higher risk of having MetD and its components. Lastly, based on the metabolic diseases, we further clarified the association between inflammation and risk of CVD and found that a higher inflammatory status was indeed associated with an elevated 10-year CVD risk, suggesting that long-term chronic inflammation seemed likely to cause CVD or elevate the CVD risk in future. Thus, we believe that high inflammation levels tended to indicate a higher risk of CVD or metabolic diseases or suggested that the subject was exposed to a high-risk status of diseases.

In the present study, we used four parameters to reflect inflammatory status. MHR and NLR were traditional indicators to evaluate the inflammatory status. SII and SIRI were novel indicators that assessed the balance between systemic inflammation and immune response in the body and had a better effect on reflecting the inflammatory state (17). Previous studies have confirmed that these indicators were more comprehensive and effective in evaluating inflammation levels and correlated with the prognosis of multiple chronic diseases and adverse CVD events (18–20, 32). Among four indicators, we observed that MHR and SIRI expressed a significant association with metabolic diseases, both overall MetD and detailed MetS, dyslipidemia, and central obesity. We also found that the risk association between these two inflammatory indicators and various metabolic diseases was in a linear manner, in which the risk would gradually elevate with the increment of indices. We observed that SII failed to have a significant association with MetD under low-grade inflammatory status (T2 subgroup). Regarding other metabolic disease components, compared with MHR or SIRI, SII had a weak but significant association with the risk of having MetS, dyslipidemia, and central obesity. Consistent with our conclusion, previous studies have observed that MRH might have a potential linkage with metabolic status. Some studies have indicated that the MHR was associated with BMI and WC levels among MetS patients and observed that patients usually had a higher MHR level, although MetS patients showed low-grade inflammatory status (33). Moreover, the elevated MHR level has been correlated with dyslipidemia among patients with chronic obstructive pulmonary disease (34). SII and SIRI have recently become novel parameters, with which a sufficient number of studies in the metabolic yield have not been yet conducted, but a study focused on the rural population believed that SIRI was correlated with hyperuricemia and SIRI could optimize the risk stratification

of hyperuricemia (35), which is also a type of metabolic diseases, indirectly supporting our conclusion. Additionally, a study has revealed that SII had a significant positive linear correlation with increased BMI, which could predict the risk for obesity (36). Therefore, we believe that SIRI had a robust association with metabolic diseases and could assess the risk of MetD and its components, especially MetS.

We observed that NLR almost did not show any association with the risk of metabolic diseases, regardless of the variable (category or continuous). However, we found that NLR demonstrated a significant correlation with MetS or related metabolic outcomes in some studies of specific populations, such as obesity population, bipolar disorder patients, and hyperglycemia patients during pregnancy (37–41). Additionally, patients with multiple metabolic diseases tend to have higher levels of NLR (42). We compared the population characteristics of these studies and further compared the differences between the four different inflammatory indicators. Thus, we believe that there were two possible reasons for these various conclusions. First, we found that NLR was commonly used in multiple-malignancy research to reflect the correlation between inflammation status and the risk of adverse prognosis, in which the microenvironment of patients was already in an overactive or extremely imbalanced inflammatory status (21, 22, 43). We also noticed that the subjects of these previous studies on NLR and metabolic studies all had different levels of metabolic abnormalities, suggesting that NLR was more appropriate for the evaluation of inflammatory status in populations with high inflammation levels. Hence, we believe that NLR seemed to be more suitable for evaluating the risk of metabolic or other adverse events based on preexisting severe diseases, such as MetS, obesity, and DM. We noticed that the calculation of NLR only involved neutrophil and lymphocyte counts, which could only simply respond to the inflammatory state, but MHR and SIRI additionally involved monocyte counts, suggesting that neutrophil counting alone did not provide a good evaluation of metabolic diseases and the immune state represented by monocytes seemed to play an important role in metabolic dysfunction. Thus, we believe that the immune-inflammatory system might be the real participant in inflammatory factor-mediated metabolic alteration. Moreover, we found that although SII did not directly involve monocyte counts, the additional factor of platelet counting could provide a certain description of the immune status to this indicator, allowing us to observe a correlation between SII and multiple metabolic diseases. Due to the absence of monocyte counting, SII showed a weaker evaluation effect than that of MHR and SIRI, confirming our speculation about the role of monocytes in representing the immune status. For these reasons, we failed to observe a significant association between NLR and various metabolic diseases in our study.

Metabolic dysfunction and diseases are recognized as crucial risk factors for CVD (3, 44); thus, we identified the patients who were exposed to abnormal metabolic status to enable us to provide CVD-related protective interventions timely, reduce the risk of CVD in the future, and lessen the burden of CVD events. Several studies have confirmed a significant correlation of these inflammatory indicators in specific populations for certain specific CVD events, such as myocardial infarction and heart failure (45–47). Some cohort studies have revealed that long-term chronic inflammation could significantly increase the risk of adverse prognosis (48, 49). Our study focused on the natural population and demonstrated that MHR and SIRI had an evaluation effect on 10-year CVD risk, regardless of whether the risk was over 10%/11%, 20%/22%, or 30%, further supporting the previous conclusions. We also noticed that NLR and SII did not have an association with the 10-year CVD risk, and these results were in contrast with some previous studies in which these two inflammatory indicators also showed a good evaluation of the risk of CVD events (45, 47). Except for the fact that these two indicators had subject characteristic preferences and disease adaptation, which was led by the monocyte-mediated immune system, as we mentioned before, our study performed FRS to describe the risk of 10-year CVD, leading to the risk for 10-year CVD risk we derived based on the risk probability, whereas other studies defined various CVD events directly as the outcomes in their regression models. Additionally, the differences in the abovementioned aspects might lead to different results on the relationship between these two indicators and CVD risk in different studies.

The present study had some strengths. Our study first examined inflammation and metabolic abnormalities at the individual level, filling the gap in epidemiological research in this area of inflammation and metabolism. This study provided a detailed and comprehensive classification of metabolic diseases, which could accurately reflect the alteration of metabolic status. We measured MHR, NLR, SII, and SIRI, which used common peripheral blood counts and incorporated immunological effects rather than the traditional inflammatory cytokines, to reflect the inflammatory status. Last, we further assessed 10-year CVD risk by FRS based on metabolic diseases to refine the conclusions of previous studies. We also had some limitations. First, the present study was cross-sectional research, having a limitation in the causal description of inflammation and metabolic diseases. In addition, we did not perform further screening to identify the reason for inflammation and simply excluded the subjects with extremely elevated WBC counts due to recent infections, because of which we failed to clarify the primary reason for the hyper-inflammatory status. Additionally, the present regression models did not contain the consumption of various medicines such as anti-hypertension drugs, and we did not consider the effects of drug administration on inflammation and metabolism levels; the present study was conducted in the natural population and tried to screen and figure out the high-risk population from the natural population whether they used drugs or not, thus we believed our conclusions were still acceptable. Finally, our study population was a natural population, but it still displayed a high

prevalence of metabolic abnormalities; thus, it might have led our results to selection bias. In the following study, we will refine the questionnaire and further conduct propensity score matching to eliminate these confounding factors and strengthen the conclusion of the present study.

In conclusion, we screened MHR and SIRI, which had a significant association with MetD and its components, such as MetS, dyslipidemia, and central obesity, in which a higher inflammatory status tended to accompany a higher risk of metabolic diseases. Moreover, we confirmed that the increment of these two indicators could cause a gradually evaluated risk of 10-year CVD. Lastly, by comparing the evaluation effects of these four indicators, we believe it was the immune-inflammation system that was involved in the alteration process of metabolic status.

Data availability statement

The raw data of the present study will be made available after evaluation and permission by the subject principals. Requests to access the data should be directed to YH (37931208@qq.com) and YS (yxsun@cmu.edu.cn).

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of China Medical University (Shenyang, China, ethical approved project identification code: AF-SOP-07-1, 0-01). The patients/participants provided their written informed consent to participate in this study.

Author contributions

All co-authors participated in the primary research. Conceptualization: YH and YS; project administration: YS, ZL, and XG; methodology: XG and PW; investigation: YZ and SY; writing (original draft preparation): PW; writing (review and editing): YH and PW. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by the following fundings: Liaoning Association for Science and Technology (project grant #LNKX2021FH15); the National Key Research and Development Program from the Ministry of Science and Technology of China (project grant #2018YFC1312400, subproject grant #2018YFC1312403); and the Science and Technology Program of Liaoning Province, China (grant #2020JH1/10300002).

Acknowledgments

We thank cardiologists and the staff from CDCs of Fuxin City, Panjin City, and Liaoyang City in Liaoning Province, who worked hard to ensure the reliability and accuracy of 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

References

1. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation* (2005) 112(20):3066–72. doi: 10.1161/circulationaha.105.539528

2. Wang J, Ruotsalainen S, Moilanen L, Lepistö P, Laakso M, Kuusisto J. The metabolic syndrome predicts cardiovascular mortality: A 13-year follow-up study in elderly non-diabetic finns. *Eur Heart J* (2007) 28(7):857–64. doi: 10.1093/eurheartj/ehl524

3. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* (2010) 56(14):1113–32. doi: 10.1016/j.jacc.2010.05.034

4. Yao F, Bo Y, Zhao L, Li Y, Ju L, Fang H, et al. Prevalence and influencing factors of metabolic syndrome among adults in China from 2015 to 2017. *Nutrients* (2021) 13(12):4475. doi: 10.3390/nu13124475

5. Lu Y, Zhang H, Lu J, Ding Q, Li X, Wang X, et al. Prevalence of dyslipidemia and availability of lipid-lowering medications among primary health care settings in China. *JAMA Netw Open* (2021) 4(9):e2127573. doi: 10.1001/jamanetworkopen.2021.27573

6. Chen S, Guo X, Yu S, Zhou Y, Li Z, Sun Y. Metabolic syndrome and serum liver enzymes in the general Chinese population. *Int J Environ Res Public Health* (2016) 13(2):223. doi: 10.3390/ijerph13020223

7. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* (2006) 444 (7121):860–7. doi: 10.1038/nature05485

8. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol* (2011) 11(11):738–49. doi: 10.1038/nri3071

9. Matulewicz N, Karczewska-Kupczewska M. Insulin resistance and chronic inflammation. *Postepy Hig Med Dosw (Online)* (2016) 70(0):1245–58. doi: 10.5604/17322693.1226662

10. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* (2006) 116(7):1793–801. doi: 10.1172/jci29069

11. Yan J, Horng T. Lipid metabolism in regulation of macrophage functions. *Trends Cell Biol* (2020) 30(12):979–89. doi: 10.1016/j.tcb.2020.09.006

12. Serhan CN. Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* (2007) 25:101–37. doi: 10.1146/annurev.immunol.25.022106.141647

13. Goldfine AB, Conlin PR, Halperin F, Koska J, Permana P, Schwenke D, et al. A randomised trial of salsalate for insulin resistance and cardiovascular risk factors in persons with abnormal glucose tolerance. *Diabetologia* (2013) 56(4):714–23. doi: 10.1007/s00125-012-2819-3

14. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, et al. Effects of interleukin-1β inhibition with canakinumab on hemoglobin A1c, lipids, c-reactive protein, interleukin-6, and fibrinogen: A phase iib randomized, placebo-controlled trial. *Circulation* (2012) 126(23):2739–48. doi: 10.1161/circulationaha.112.122556

15. Adali MK, Buber I, Kilic O, Turkoz A, Yilmaz S. Ticagrelor improves systemic immune-inflammation index in acute coronary syndrome patients. *Acta Cardiol* (2021) 7:1–7. doi: 10.1080/00015385.2021.1973770

16. Yang YL, Wu CH, Hsu PF, Chen SC, Huang SS, Chan WL, et al. Systemic immune-inflammation index (Sii) predicted clinical outcome in patients with coronary artery disease. *Eur J Clin Invest* (2020) 50(5):e13230. doi: 10.1111/eci.13230

17. Jin Z, Wu Q, Chen S, Gao J, Li X, Zhang X, et al. The associations of two novel inflammation indexes, sii and siri with the risks for cardiovascular diseases and all-cause mortality: A ten-year follow-up study in 85,154 individuals. *J Inflamm Res* (2021) 14:131–40. doi: 10.2147/jir.S283835

18. Zhang X, Ding R, Li H, Liu Y, Ou W, Hu J, et al. An association between inflammation and cerebral venous thrombosis: A retrospective study. *J Stroke Cerebrovasc Dis* (2021) 30(11):106084. doi: 10.1016/j.jstrokecerebrovasdis.2021.106084

19. Liu Z, Fan Q, Wu S, Wan Y, Lei Y. Compared with the monocyte to high-density lipoprotein ratio (Mhr) and the neutrophil to lymphocyte ratio (Nlr), the neutrophil to high-density lipoprotein ratio (Nhr) is more valuable for assessing the inflammatory process in parkinson's disease. *Lipids Health Dis* (2021) 20(1):35. doi: 10.1186/s12944-021-01462-4

20. Zhang Y, Xing Z, Zhou K, Jiang S. The predictive role of systemic inflammation response index (Siri) in the prognosis of stroke patients. *Clin Interv Aging* (2021) 16:1997–2007. doi: 10.2147/cia.S339221

21. Kuzucu İ, Güler İ, Kum RO, Baklacı D, Özcan M. Increased neutrophil lymphocyte ratio and platelet lymphocyte ratio in malignant parotid tumors. *Braz J Otorhinolaryngol* (2020) 86(1):105–10. doi: 10.1016/j.bjorl.2019.02.009

22. Solak M, Kraljević I, Zibar Tomšić K, Kaštelan M, Kakarigi L, Kaštelan D. Neutrophil-lymphocyte ratio as a prognostic marker in adrenocortical carcinoma. *Endocr Res* (2021) 46(2):74–9. doi: 10.1080/07435800.2020.1870234

23. Guo X, Li Z, Zhou Y, Yu S, Yang H, Sun G, et al. The effects of transitions in metabolic health and obesity status on incident cardiovascular disease: Insights from a general Chinese population. *Eur J Prev Cardiol* (2021) 28(11):1250–8. doi: 10.1177/2047487320935550

24. Li G, Shi C, Li T, Ouyang N, Guo X, Chen Y, et al. A nomogram integrating non-ecg factors with ecg to screen left ventricular hypertrophy among hypertensive patients from northern China. *J Hypertens* (2022) 40(2):264–73. doi: 10.1097/hjh.0000000000003003

25. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JLJr., et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The jnc 7 report. *Jama* (2003) 289 (19):2560–72. doi: 10.1001/jama.289.19.2560

26. Chamberlain JJ, Rhinehart AS, Shaefer CFJr., Neuman A. Diagnosis and management of diabetes: Synopsis of the 2016 American diabetes association standards of medical care in diabetes. *Ann Intern Med* (2016) 164(8):542–52. doi: 10.7326/m15-3016

27. Wise CM, Agudelo CA. Gout and hyperuricemia. *Curr Opin Rheumatol* (1990) 2(5):783–8. doi: 10.1097/00002281-199002050-00016

28. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. a consensus statement from the international diabetes federation. *Diabetes Med* (2006) 23(5):469–80. doi: 10.1111/j.1464-5491.2006.01858.x

29. Third Report of the National Cholesterol Education Program (Ncep). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel iii) final report. *Circulation* (2002) 106(25):3143–421. doi: 10.1161/circ.106.25.3143

30. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* (2009) 150(9):604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

31. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* (1998) 97(18):1837–47. doi: 10.1161/01.cir.97.18.1837

32. Chen JH, Zhai ET, Yuan YJ, Wu KM, Xu JB, Peng JJ, et al. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. *World J Gastroenterol* (2017) 23(34):6261–72. doi: 10.3748/wjg.v23.i34.6261

33. Battaglia S, Scialpi N, Berardi E, Antonica G, Suppressa P, Diella FA, et al. Gender, bmi and fasting hyperglycaemia influence monocyte to-hdl ratio (Mhr) index in metabolic subjects. *PloS One* (2020) 15(4):e0231927. doi: 10.1371/journal.pone.0231927

34. Markelić I, Hlapčić I, Rogić D, Rako I, Samaržija M, Popović-Grle S, et al. Lipid profile and atherogenic indices in patients with stable chronic obstructive pulmonary disease. *Nutr Metab Cardiovasc Dis* (2021) 31(1):153–61. doi: 10.1016/j.numecd.2020.07.039

35. Chen MQ, Wang HY, Shi WR, Sun YX. Estimate of prevalent hyperuricemia by systemic inflammation response index: Results from a rural Chinese population. *Postgrad Med* (2021) 133(2):242–9. doi: 10.1080/00325481.2020.1809870

36. Furuncuoğlu Y, Tulgar S, Dogan AN, Cakar S, Tulgar YK, Cakiroglu B. How obesity affects the Neutrophil/Lymphocyte and Platelet/Lymphocyte ratio, systemic immune-inflammatory index and platelet indices: A retrospective study. *Eur Rev Med Pharmacol Sci* (2016) 20(7):1300–6.

37. Sanchez-Autet M, Arranz B, Sierra P, Safont G, Garcia-Blanco A, de la Fuente L, et al. Association between neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, and c-reactive protein levels and metabolic status in patients with a bipolar disorder. *World J Biol Psych* (2022) 12:1–11. doi: 10.1080/15622975.2021.2013089

38. Karakaya S, Altay M, Kaplan Efe F, Karadağ İ, Ünsal O, Bulur O, et al. The neutrophil-lymphocyte ratio and its relationship with insulin resistance in obesity. *Turk J Med Sci* (2019) 49(1):245–8. doi: 10.3906/sag-1804-68

39. Wang J, Zhu QW, Cheng XY, Sha CX, Cui YB. Clinical significance of neutrophil-lymphocyte ratio and monocyte-lymphocyte ratio in women with hyperglycemia. *Postgrad Med* (2020) 132(8):702–8. doi: 10.1080/00325481.2020.1764235

40. Buyukkaya E, Karakas MF, Karakas E, Akçay AB, Tanboga IH, Kurt M, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. *Clin Appl Thromb Hemost* (2014) 20(2):159–63. doi: 10.1177/1076029612459675

41. Shiny A, Bibin YS, Shanthirani CS, Regin BS, Anjana RM, Balasubramanyam M, et al. Association of neutrophil-lymphocyte ratio with glucose intolerance: An indicator of systemic inflammation in patients with type 2 diabetes. *Diabetes Technol Ther* (2014) 16(8):524–30. doi: 10.1089/dia.2013.0264

42. Surendar J, Indulekha K, Mohan V, Pradeepa R. Association of neutrophil-lymphocyte ratio with metabolic syndrome and its components in Asian indians (Cures-143). *J Diabetes Complications* (2016) 30(8):1525–9. doi: 10.1016/j.jdiacomp.2016.08.006

43. Gomes Dos Santos A, de Carvalho RF, de Morais A, Silva TM, Baylão VMR, Azevedo M, et al. Role of neutrophil-lymphocyte ratio as a predictive factor of glioma tumor grade: A systematic review. *Crit Rev Oncol Hematol* (2021) 163:103372. doi: 10.1016/j.critrevonc.2021.103372

44. Ma X, Zhu S. Metabolic syndrome in the prevention of cardiovascular diseases and diabetes–still a matter of debate? *Eur J Clin Nutr* (2013) 67(5):518–21. doi: 10.1038/ejcn.2013.24

45. Qiao S, Gao W, Guo S. Neutrophil-lymphocyte ratio (Nlr) for predicting clinical outcomes in patients with coronary artery disease and type 2 diabetes mellitus: A propensity score matching analysis. *Ther Clin Risk Manag* (2020) 16:437–43. doi: 10.2147/tcrm.S244623

46. Inonu Koseoglu H, Pazarli AC, Kanbay A, Demir O. Monocyte Count/Hdl cholesterol ratio and cardiovascular disease in patients with obstructive sleep apnea syndrome: A multicenter study. *Clin Appl Thromb Hemost* (2018) 24(1):139–44. doi: 10.1177/1076029616677803

47. Xu M, Chen R, Liu L, Liu X, Hou J, Liao J, et al. Systemic immune-inflammation index and incident cardiovascular diseases among middle-aged and elderly Chinese adults: The dongfeng-tongji cohort study. *Atherosclerosis* (2021) 323:20–9. doi: 10.1016/j.atherosclerosis.2021.02.012

48. Sasaki K, Shoji T, Kabata D, Shintani A, Okute Y, Tsuchikura S, et al. Oxidative stress and inflammation as predictors of mortality and cardiovascular events in hemodialysis patients: The dream cohort. *J Atheroscler Thromb* (2021) 28 (3):249–60. doi: 10.5551/jat.56069

49. Kamon T, Kaneko H, Itoh H, Kiriyama H, Mizuno Y, Morita H, et al. Possible gender difference in the association between abdominal obesity, chronic inflammation, and preclinical atherosclerosis in the general population. *Int Heart J* (2021) 62(4):837–42. doi: 10.1536/ihj.20-654

frontiers | Frontiers in Public Health

TYPE Systematic Review
PUBLISHED 03 October 2022
DOI 10.3389/fpubh.2022.924488

OPEN ACCESS

EDITED BY
Maria João Meneses,
Universidade Nova de Lisboa, Portugal

REVIEWED BY
Liao Peng,
Sichuan University, China
Wei Wang,
West China Hospital, China
Carlos Levi D'Ancona,
State University of Campinas, Brazil

*CORRESPONDENCE
Tao Zhou
taozhou123456@163.com

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

RECEIVED 20 April 2022
ACCEPTED 19 August 2022
PUBLISHED 03 October 2022

CITATION
Fu Z, Wang F, Dang X and Zhou T (2022) The association between diabetes and nocturia: A systematic review and meta-analysis. *Front. Public Health* 10:924488. doi: 10.3389/fpubh.2022.924488

The association between diabetes and nocturia: A systematic review and meta-analysis

Zhiwei Fu[1], Fang Wang[2], Xing Dang[3] and Tao Zhou[3]*

[1]Department of Pediatric Surgery, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Department of Nutrition, Dazhou Central Hospital, Dazhou, China, [3]Department of Pediatric Surgery, Dazhou Central Hospital, Dazhou, China

Background: Many studies have explored the association between diabetes and nocturia, but it remains unclear. This article systematically analyses existing evidence of the relationship between diabetes and nocturia, including subgroup analysis based on the number of voids, gender, and continent, in the hope of reaching more reliable clinical conclusions relating to diabetes and nocturia.

Methods: PubMed, Web of Science, and Cochrane Library were searched for identifying studies relating to diabetes and nocturia prior to July 2021. Literature quality evaluation was performed using the Newcastle Ottawa Scale. A random effect meta-analysis was used for pooled odds ratios (ORs) and confidence intervals (CIs) as a means of evaluating the relationship between diabetes and nocturia.

Results: In total, 29 of 781 potentially relevant studies were proven to be eligible. The overall pooled OR demonstrated that diabetes increases the risk of nocturia (OR: 1.49; 95% CI: 1.38, 1.61; $P < 0.00001$). The association was found to be more robust among subjects $\geq$ 1 void than $\geq$ 2 void (OR: 1.74; 95% CI: 1.41, 2.14; $P < 0.00001$ vs. OR: 1.45; 95% CI: 1.33, 1.59; $P < 0.00001$), in males than females (OR: 1.59; 95% CI: 1.41, 1.79; $P < 0.00001$ vs. OR: 1.41; 95% CI: 1.20, 1.66; $P < 0.0001$) and in Asia than Europe or North America (OR: 1.54; 95% CI: 1.36, 1.75; $P < 0.00001$ vs. OR: 1.43; 95% CI: 1.19, 1.72; $P = 0.0001$ vs. OR: 1.45; 95% CI: 1.22, 1.73; $P < 0.0001$).

Conclusions: Diabetes has an association with a 1.49-fold higher risk of nocturia. This association is more robust for Asian and male subjects or those at a lower nocturia cut-off.

KEYWORDS

nocturia, diabetes, risk, systematic review, meta-analysis

Introduction

Nocturia is an incredibly common and bothersome lower urinary tract symptom (1). The incidence of nocturia increases with age. Large-scale investigations have found the incidence of nocturia of $\geq$ 2 times per night in 60-year-old to be approximately 25% (2). In addition to sleep disruption and impaired quality of life, nocturia can also result in falls, fractures, and increased mortality among the elderly. High-quality meta-analysis has proven that nocturia increases the risk of falls by approximately 20% and that of fractures by 32% (3). In addition, another meta-analysis has demonstrated that nocturia has an association with a 1.27-fold risk of mortality (4). Therefore, identifying the risk factors of nocturia is of great importance.

Nocturia is closely related to age, but it has many influencing factors, namely, hypertension and diabetes (4). Recent studies have shown diabetes to be related to nocturia with a limited level of evidence. However, with the interference of age, gender, race, and other confounding factors, further research is required regarding whether diabetes is an independent risk factor for nocturia. Therefore, the aim of this article is to comprehensively analyze the relationship between diabetes and nocturia and reach a reliable conclusion for further guiding the clinical management of nocturia.

Materials and methods

Search strategy

Standard preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines were adhered to when conducting this review. PubMed, Web of Science, and Cochrane Library were searched in order to identify studies relating to diabetes and nocturia that were published before July 2021. Search terms included: "nocturia and (diabetes or hyperglycemia)." Only articles that were published in English were included in the meta-analysis.

Inclusion and exclusion criteria

Inclusion and exclusion criteria were utilized based on the PICOS (patient/population, intervention, control, outcome, systematic) methodology.

Inclusion criteria: Studies that investigated diabetes and nocturia; Articles that included odds ratios (ORs) and confidence intervals (CIs); All the included articles provided the definition of diabetes and nocturia.

Exclusion criteria: System reviews or case reports were excluded; Incomplete data or no OR and 95% CI were excluded; Data from repeatedly published articles was only included once.

Data extraction and quality assessment

Two authors independently searched and screened the literature based on the established inclusion and exclusion criteria. The following data was extracted: First name of author, publication year, patient country, study design, sample size, gender, the definition of diabetes, the minimum number of voids per night, and the number of patients with nocturia. The Newcastle Ottawa Scale (NOS) was used for evaluating the quality of the included studies (5). All the aforementioned work was independently performed by two authors and any differing opinions were resolved through a discussion with a third author.

Statistical analysis

The data was analyzed using RevMan 5.3. We pooled the OR and 95% CI to evaluate the effect of diabetes on nocturia, and z-test was used to assess for statistical significance. Computed values for Cochran's Q test were used to evaluate heterogeneity. Random effects model was performed for high heterogeneity among studies ($P < 0.05$ or $I^2 > 50\%$). Otherwise, the fixed effects model was used. A funnel plot across all studies was made for the evaluation of publication bias. Sensitivity analysis was performed through the removal of individual studies.

For accurately investigating the relationship between diabetes and nocturia, multiple subgroup analyses were conducted. Weighted ORs were pooled in different subgroups according to 1-void and 2-void, male and female, patient continent, single-factor and multi-factor analysis.

Results

Literature screening and quality assessment

We initially screened 781 abstracts, and 439 articles were deleted due to duplication. After reading the full text of 71 articles, 29 articles met the inclusion criteria and were included in this meta-analysis (6–34). The screening flowchart was shown in Figure 1.

In total, 29 articles were included in the analysis performed in this article and the quality scores of the included literature are shown in Table 1. A total of 197,809 subjects were incorporated into the meta-analysis. The basic characteristics of the literature, namely, gender, the definition of diabetes, the minimum number of voids per night, and the number of patients with nocturia, can be seen in Table 1.

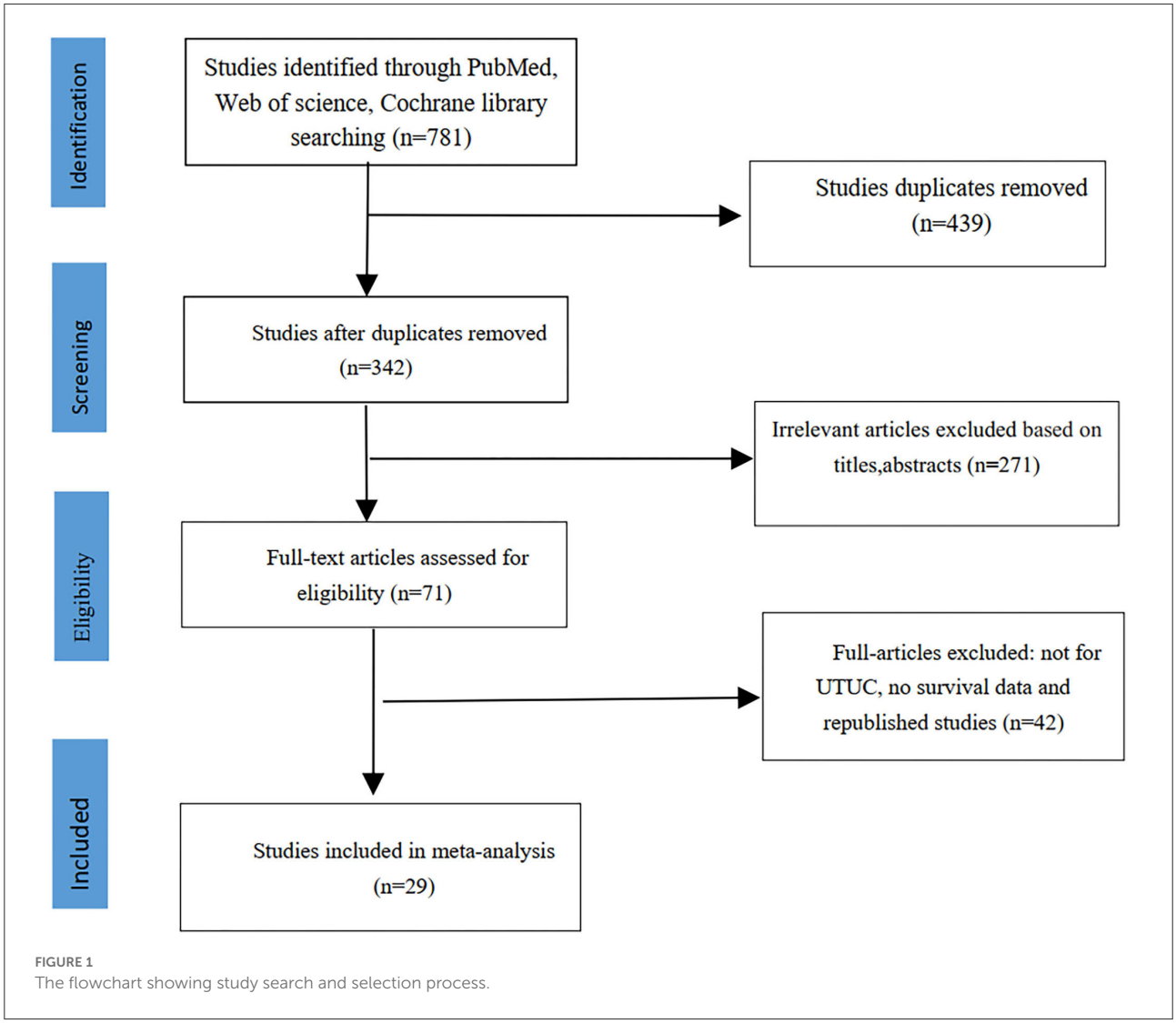


FIGURE 1
The flowchart showing study search and selection process.

Association of diabetes and nocturia

All 29 studies that were included explored the association between diabetes and nocturia (6–34). The heterogeneity among studies was found to be high and the random effect model was used ($P < 0.00001$, $I^2 = 72\%$). Pooled OR demonstrated that diabetes increases the risk of nocturia (OR: 1.49; 95% CI: 1.38, 1.61; $P < 0.00001$) (Figure 2). In subgroup analysis based on the number of voids, the association was found to be more robust in subjects ≥ 1 void than ≥ 2 void (OR: 1.74; 95% CI: 1.41, 2.14; $P < 0.00001$ vs. OR: 1.45; 95% CI: 1.33, 1.59; $P < 0.00001$).

Stratification by gender

For subgroup classification according to gender, 12 studies provided data relating to men (6, 8, 11, 16, 18, 20, 21, 27, 29, 31, 32, 34) and nine studies provided data relating to women (6, 8, 12, 14, 16, 21, 28, 31, 34). Pooled OR showed that diabetes increases the risk of nocturia for men (OR: 1.59; 95% CI: 1.41, 1.79; $P < 0.00001$) and women (OR: 1.41; 95% CI: 1.20, 1.66; $P < 0.0001$) (Figure 3). Heterogeneity among both men ($P = 0.006$, $I^2 = 58\%$) and women ($P = 0.009$, $I^2 = 61\%$) was found to be lower than heterogeneity for the overall cohort ($P < 0.0001$, $I^2 = 66\%$).

Stratification by country

In total, 5 studies provided data relating to Europe (7, 11, 21, 26, 28), 8 studies provided data relating to North America (10, 15, 16, 20, 22, 25, 27, 29), 1 study provided data relating to South America (6), and 15 studies provided data relating to Asia (8, 9, 12–14, 17–19, 23, 24, 30–34). Regardless of the continent,

TABLE 1 Basic characteristics and data of included articles.

Study	Year	Participants country	Study design	Sample size	Gender (Female/ Male)	Diabetes definition	Nocturia (minimum episodes)	Number of nocturia patients	NOS
Tikkinen et al. (28)	2009	Finnish	Questionnaires sent to subjects in the Population Register Center	3,307	N	History of diabetes	2	N	6
Liao et al. (18)	2011	Taiwan	Participants in health examinations at a Taiwan hospital	509	0/509	History of diabetes	2	N	7
Yoshimura et al. (32)	2004	Taiwan	Multistage health screening program in Taiwan	6,517	1,949/4,568	History of diabetes or fasting plasma glucose ≥ 126 mg/dL, or random glucose ≥200 mg/ dL.	2	1,856	8
Wen et al. (31)	2015	China	Multi-staged, stratified, random sampling of participants over 40 years in Zhengzhou City, China	9,637	6,621/3,016	History of diabetes	2	3,053	8
Gourova et al. (11)	2006	Netherlands	Questionnaires sent to elderly men in 21 general practices in Maastricht	2,934	0/2,934	History of diabetes	2	965	8
Hsieh et al. (12)	2008	Taiwan	Multistage selection of female participants over 60 older in Taiwan and neighboring islands	1,523	1,523/0	History of diabetes	1	1,120	8
Johnson II et al. (15)	2005	USA	Data from the Medical, Epidemiologic, and Social aspects of Aging (MESA) Study in Michigan	1,652	987/665	History of diabetes	2	520	8
Liew et al. (19)	2006	Singapore	A population-based cross-sectional survey was conducted in Singapore	2,273	1,134/1,139	History of diabetes	1	1,250	7
Obayashi et al. (24)	2015	Japan	A cross-sectional study of community-based elderly individuals	862	435/427	History of diabetes or fasting plasma glucose levels ≥ 7.0 mmol/L	2	262	8
Nakagawa et al. (23)	2010	Japan	Community sample ≥ 70 years in Japan	784	427/357	History of diabetes	2	359	7
Stone	2016	USA	Participants in USA health screening of men	30,500	0/30,500	History of diabetes	2	9,440	7
Kim et al. (16)	2018	USA	Participants aged ≥ 65 years were included from the NHANES dataset	4,698	2,323/2,375	History of diabetes or fasting plasma glucose ≥126 mg/dL, or random glucose ≥200 mg/ dL.	2	2,333	8
Azuero et al. (6)	2021	Colombia	A cross-sectional study conducted in five major cities in Colombia.	1,060	530/530	History of diabetes	1	593	7
Rembratt et al. (26)	2003	Sweden	Questionnaires sent to all inhabitants aged ≥ 65 years in Tierp, Sweden.	2,081	1,061/1,020	History of diabetes	2	603	7
Lightner et al. (20)	2012	USA	A random sample of men aged 40 – 79 years from Olmsted County, MN, USA	2,447	0/2,447	History of diabetes	2	440	7
Wang	2014	China	A cross-sectional survey for adults aged ≥18 in five geographical regions of China.	3,023	1,472/1,551	History of diabetes	2	747	8

(Continued)

10.3389/fpubh.2022.924488

TABLE 1 (Continued)

Study	Year	Participants country	Study design	Sample size	Gender (Female/Male)	Diabetes definition	Nocturia (minimum episodes)	Number of nocturia patients	NOS
Chow et al. (8)	2018	Taiwan	An internet-based study for subjects aged ≥ 40 years in China, South Korea, and Taiwan	8,284	4,208/4,076	History of diabetes	2	2,976	7
Huang et al. (13)	2012	Taiwan	Questionnaires sent to ≥ 40 years for the lower urinary tract symptoms in Taiwan	1,011	N	History of diabetes	2	385	7
Kim SY et al. (17)	2017	Korea	Data were collected by the Korean Centers for Disease Control and Prevention	92,626	N	History of diabetes	2	16,322	7
Madhu et al. (21)	2015	UK	A cross-sectional, population-representative survey involving 30,000 men and women from the USA, UK and Sweden evaluating lower urinary tract symptoms (LUTS)	30,000	15,810/14,107	History of diabetes	2	9,325	8
Victor et al. (29)	2019	USA	Data from a cluster-randomized trial of BP reduction in 52 black-owned barbershops in Los Angeles County, California (Clinicaltrials.gov, NCT02321618)	1,673	0/1,673	History of diabetes	2	485	7
Bing et al. (7)	2008	Denmark	Questionnaire was randomized sent to 4,000 individuals living in Copenhagen County	2,799	1,313/1,486	History of diabetes	2	1,022	8
Parthasarathy	2012	USA	Data from the Sleep Heart Health Study (SHHS) for middle-age and older adults	6,342	3,361/2,981	History of diabetes	1	3,625	7
Chung et al. (9)	2019	Korea	Data were prospective collected in Hanyang University Hospital	304	83/221	History of diabetes	2	83	7
Yow et al. (33)	2021	Malaya	A cross-sectional was conducted among community-dwelling Malaysian adults aged≥18 years old	4,616	2,634/1,982	History of diabetes	1	2,646	8
Fitzgerald	2006	USA	A multistage, stratified, cluster random sample were obtained from the Boston	5,506	3,205/2,301	History of diabetes	2	1,872	7
Zhang	2010	China	A cross-sectional survey of nocturia in several communities in northern China	1,198	592/606	History of diabetes	2	411	8
Ito et al. (14)	2019	Japan	Multiphasic health screening for 18 952 women in Fukui, Japan	18,952	0/18,952	History of diabetes	2	739	7
Mekki BS	2020	USA	A sample of 143 patients based on outpatient cardiology clinic	143	106/37	History of diabetes patients or fasting plasma glucose ≥126 mg/dL, or a recent HbA1c ≥6.5%	1	111	8

10.3389/fpubh.2022.924488

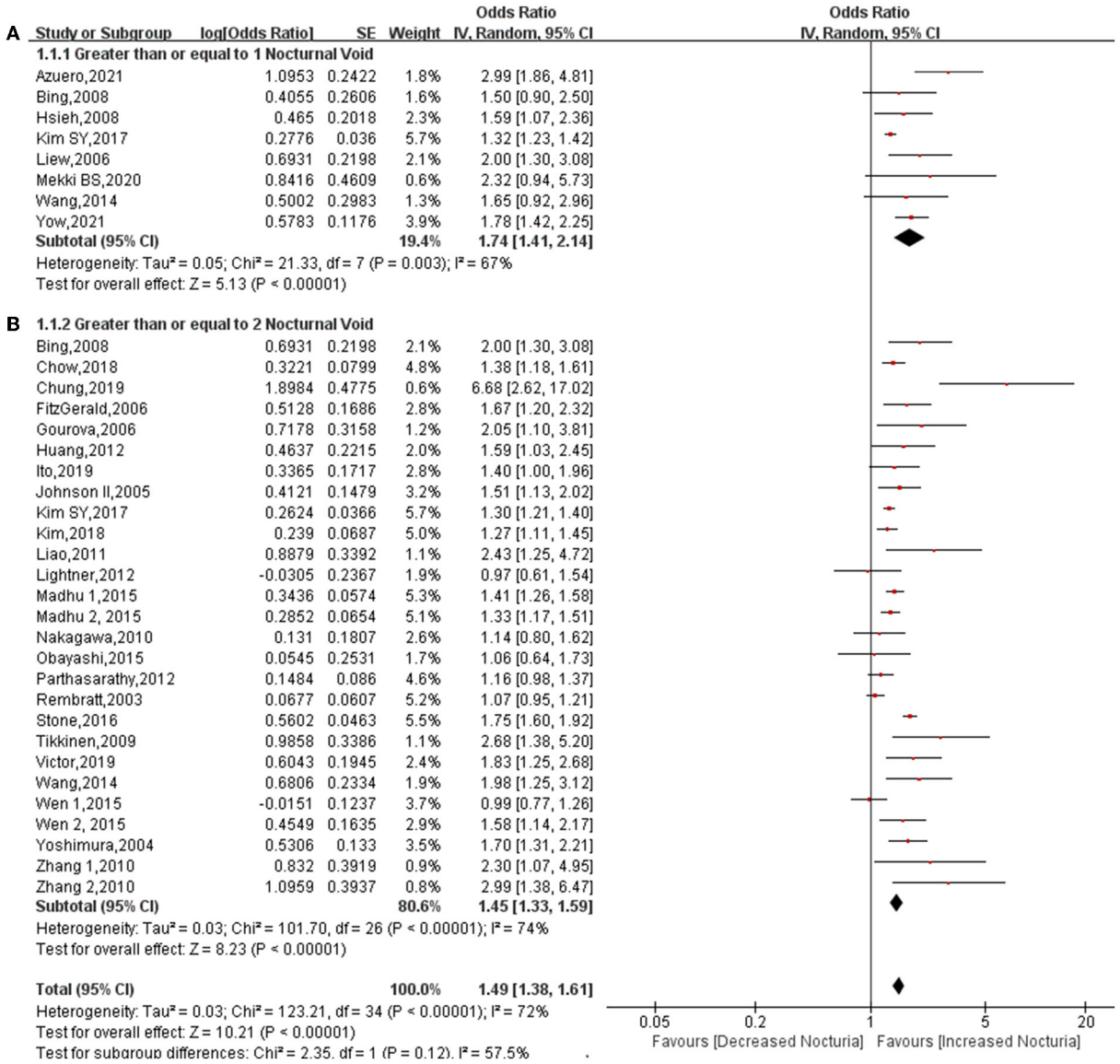


FIGURE 2
Forest plot for the association between diabetes and nocturia stratified by number of nocturia. Nocturia ≥1 void **(A)**, nocturia ≥2 void **(B)**.

diabetes increases the risk of diabetes. The pooled OR for the Asia subgroup was 1.54 (95% CI: 1.36, 1.75; $P < 0.00001$). The pooled OR for Asian participants was higher than for Europe subgroup (OR: 1.43; 95% CI: 1.19, 1.72; $P = 0.0001$) or North America (OR: 1.45; 95% CI: 1.22, 1.73; $P < 0.0001$) (Figure 4). A South American study showed that diabetes increases the risk of nocturia to a greatest extent (OR: 2.99; 95% CI: 1.86, 4.81; $P < 0.00001$). Heterogeneity among both Europe ($P = 0.0004$, $I^2 = 78\%$) and North America ($P < 0.0001$, $I^2 = 78\%$) was higher than the heterogeneity for the overall cohort ($P < 0.00001$, $I^2 = 74\%$). In contrast, the heterogeneity of the Asia participants ($P = 0.0001$, $I^2 = 65\%$) was lower than the overall cohort. High heterogeneity among subgroups ($P = 0.04$, $I^2 = 64.7\%$).

Stratification by univariate and multivariate analysis

A total of 14 studies provided data relating to univariate analysis (7–11, 15, 18, 20, 22–25, 29, 33) and 25 studies provided data relating to multivariate analysis (6–14, 16–21, 24, 26–34). The pooled results proved that diabetes significantly increases the risk of nocturia in univariate analysis (OR: 1.97; 95% CI: 1.54, 2.51; $P < 0.00001$). The pooled OR for univariate was found to be higher than the overall results (OR: 1.71; 95% CI: 1.54, 1.89; $P < 0.00001$), while the pooled OR for multivariate analysis (OR: 1.55; 95% CI: 1.41, 1.70; $P < 0.00001$) was lower than the overall results (Figure 5). Heterogeneity among univariate (P

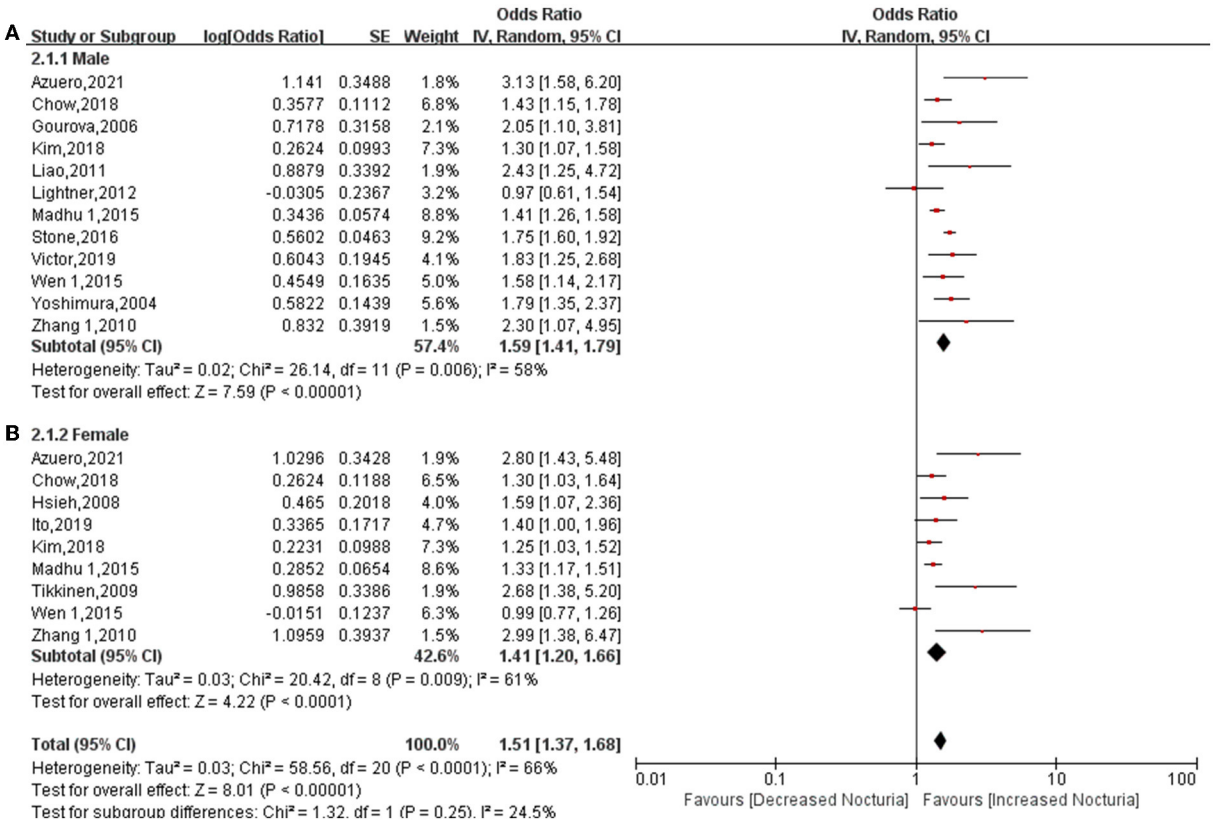


FIGURE 3
Forest plot for the association between diabetes and nocturia stratified by gender. Men **(A)**, Women **(B)**.

< 0.00001, $I^2 = 89\%$), multivariate ($P < 0.00001$, $I^2 = 76\%$), and overall analysis ($P < 0.00001$, $I^2 = 87\%$) was found to be higher. The heterogeneity among subgroups was high ($P = 0.07$, $I^2 = 69.3\%$). This indicates that multivariate analysis can weaken the interference other factors have on the results.

Sensitivity analysis and publication bias

We constructed a funnel plot to detect publication bias for diabetes and nocturia frequency. There is no publication bias for all studies (Supplementary Figure 1). When performed sensitivity analysis by removing individual studies, no sources of heterogeneity were found.

Discussion

This is believed to be the first meta-analysis that explores the relationship between diabetes and nocturia. The conclusions reached following this systematic review have significant guiding value for clinical practice. First, of the 197,809 subjects that were analyzed, diabetes increased the risk of nocturia by approximately 49%, the probability increasing to 1.74-fold for subjects $\geq$ 1 void nocturia. In addition, in subgroup classification based on gender, diabetes increased the risk of nocturia among males (OR: 1.59; 95% CI: 1.41, 1.79; $P < 0.00001$) and females (OR: 1.41; 95% CI: 1.20, 1.66; $P < 0.0001$). The association between diabetes and nocturia was found to be stronger in male subjects than in female subjects. In addition, the pooled OR for Asia (OR: 1.54) was found to be higher than Europe (OR: 1.43) and North America (OR: 1.45). There is a greater likelihood of diabetes being related to nocturia in Asians than in Europeans and Americans. Furthermore, diabetes significantly increased the risk of nocturia in univariate analysis (OR: 1.97), but OR dropped to 1.55 in the multivariate analysis. This demonstrates that many factors interfere with the effect of diabetes on nocturia, and these factors will be discussed later.

Most studies have found that after making adjustments for other factors, diabetes is an independent risk factor for nocturia (6, 18, 32). However, relatively few studies have reported that diabetes and nocturia are two independent diseases (20, 24). Diabetes is a common cause of nocturia for several reasons. Osmotic diuresis secondary to hyperglycemia can significantly increase the output of urine during the night (35). In addition,

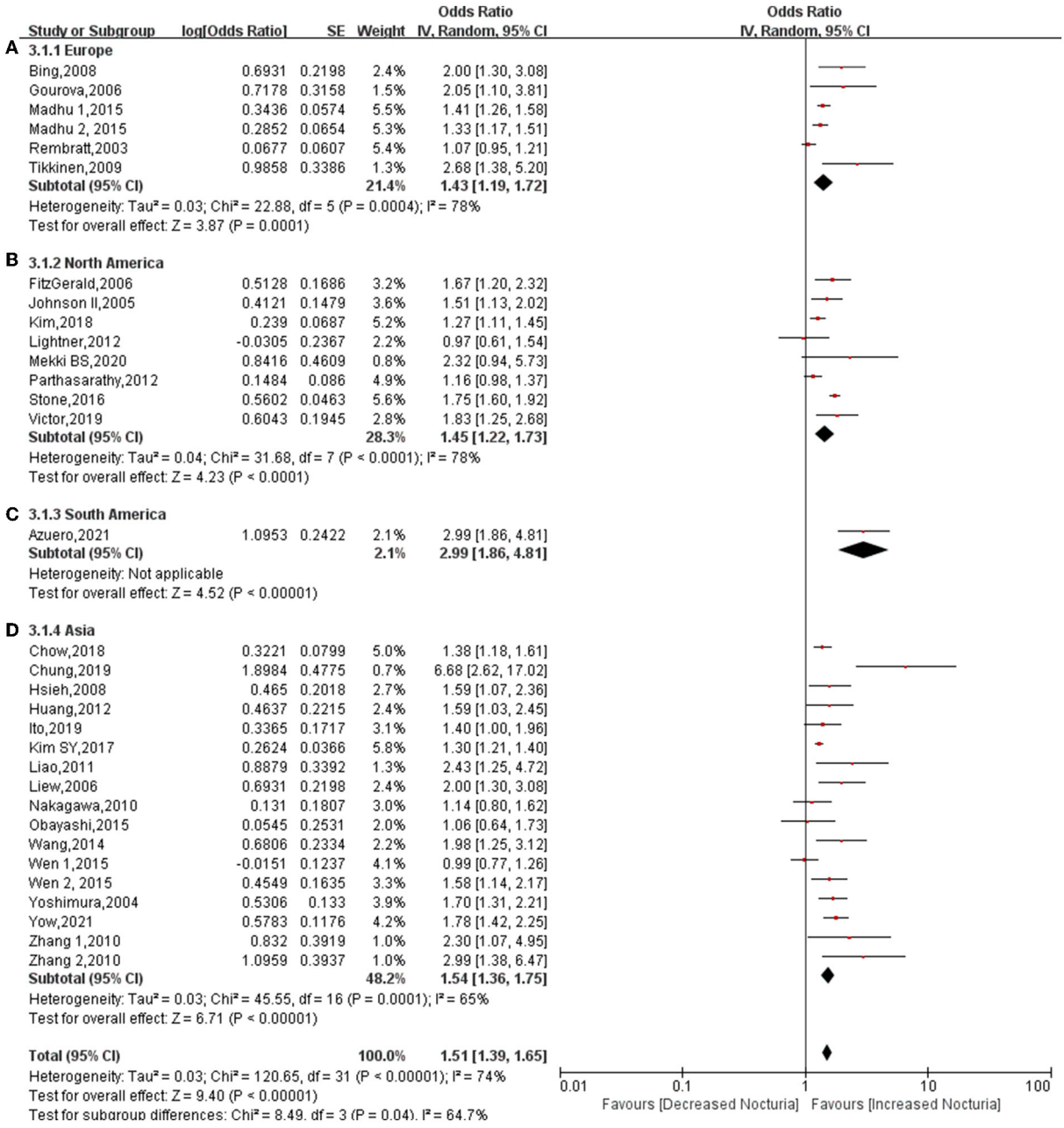


FIGURE 4
Forest plot for the association between diabetes and nocturia stratified by country. Europe **(A)**, North America **(B)**, South America **(C)**, Asia **(D)**.

diabetes-induced cerebrovascular disease or peripheral nerve stimulation resulting in bladder sensory dysfunction or detrusor overactivity may be a cause of overactive bladder (36). A survey that was conducted in Japan found that 25% of patients with diabetes also had bladder detrusor hyperreflexia (37).

Although a strong correlation exists between nocturia and age, the link between diabetes and nocturia appears to have no connection with age, potentially due to the fact that the prevalence of diabetes increases with age. Many studies have found that following adjustments for the effects of age, diabetes has a significant association with nocturia (18, 32). The results of this study are in accordance with previous studies that found diabetes to increase the risk of nocturia even following adjustments made for age, gender, and other factors in multivariate analysis (OR: 1.55).

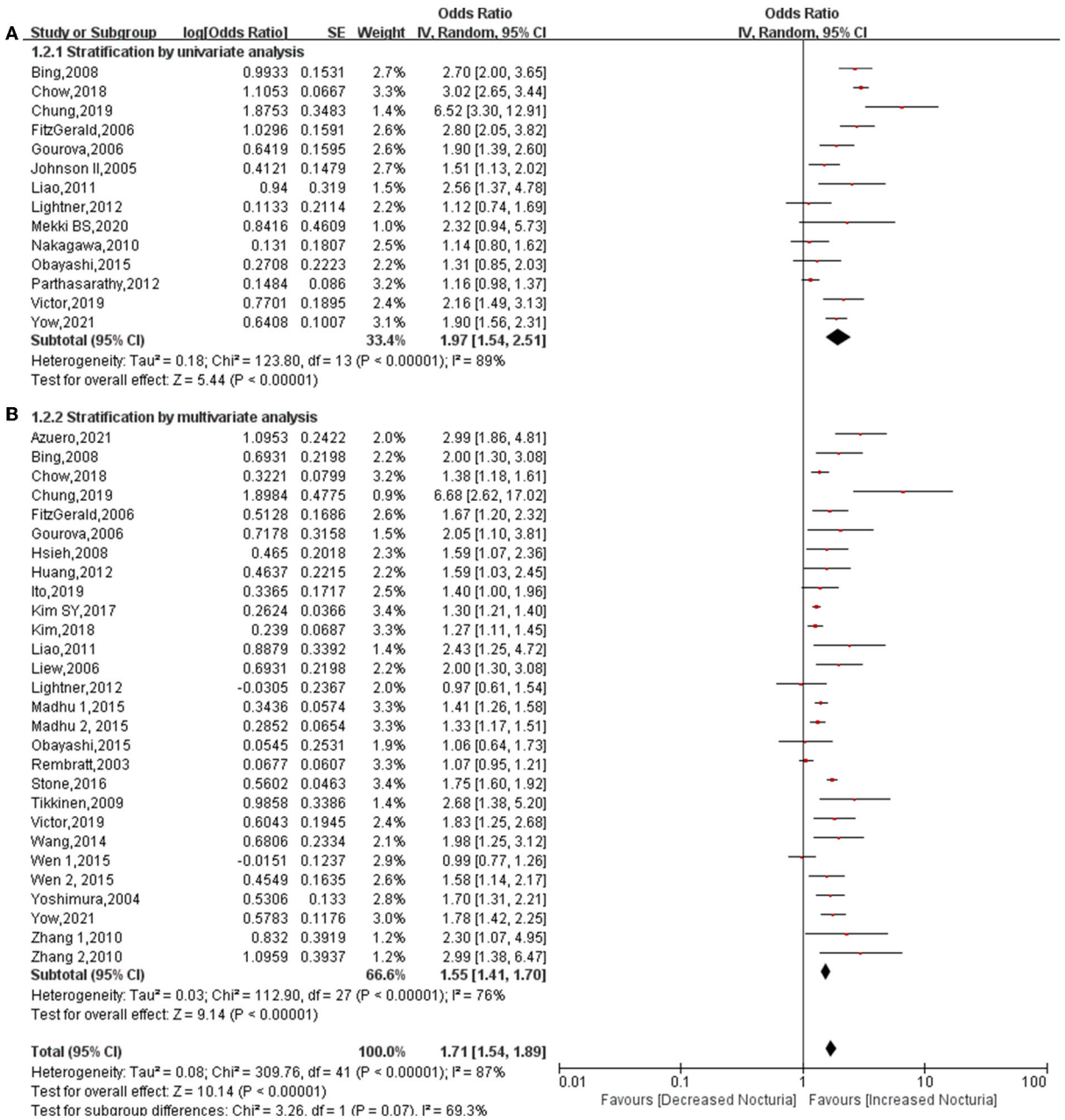


FIGURE 5
Forest plot for the association between diabetes and nocturia stratified by univariate analysis **(A)** and multivariate analysis **(B)**.

The more robust relationship between diabetes and nocturia remains controversial in men in comparison to women. In the subgroup classification based on gender in this article, the association between diabetes and nocturia was found to be slightly stronger among men (OR: 1.59) than women (OR: 1.41). However, a meta-analysis indicated that the correlation between hypertension and nocturia is stronger in women (OR: 1.45) than in men (OR: 1.28) (38). This demonstrates that the influence of gender on nocturia is interfered with by accompanying diseases. In addition, the influence gender has on nocturia and the influence diabetes has on bladder function are also interfered with by several confounding factors. In a study that was conducted by Tikinen et al. (39) women younger than 50 years were found to have a higher incidence of nocturia than men of the same age, but the increase rate of nocturia in men was observed as being twice as fast as that of older women. Bing

et al. noted that although there is a similar prevalence of nocturia in men and women, women have a higher tolerance to nocturia than men (7). A study found bladder dysfunction caused by diabetes to account for 59.26% of women and 74.07% of men (40). This proves that the degree of association between diabetes and nocturia differs between genders.

Many studies have reported the incidence of nocturia to vary between people of different races. Limited by several races included in one study, subgroup analysis could only be performed by continent. Only one study in South America has investigated the relationship between diabetes and nocturia, so the level of evidence for the results is incredibly low. A more robust association was found between diabetes and nocturia in Asia (OR: 1.54) than in Europe (OR: 1.43) or North America (OR: 1.45). The strong association between diabetes and nocturia in Asia may prove to be particularly useful, particularly considering the fact that Asians are more prone to organ damage resulting from diabetes. The inclusion of several races in individual studies has resulted in particularly high heterogeneity within the group. Therefore, the correlation between diabetes and nocturia in different continents warrants further study.

The strengths of this review include a contemporary search of studies published in English, duplicate assessment of inclusion criteria, and the quality of evidence and extracted data. This is believed to be the first meta-analysis that explores the relationship between diabetes and nocturia. Through an overall evaluation and subgroup analysis, the results of this study provide evidence of an association between diabetes and nocturia. However, there were inevitably some limitations with the meta-analysis in this study. First, many subjects were diagnosed with diabetes based on their medical histories rather than the current status of hyperglycemia. Second, the nocturia data that was obtained through questionnaires was found to be too subjective. Third, pertinent diseases such as hypertension, obesity, and other diseases that may strengthen the association between diabetes and nocturia were not examined. Fourth, the subgroup analysis by country was unable to provide an analysis based on race. At last, the significant difference in the number of cases that were included in the study may lead to biased results.

Conclusions

Diabetes has an association with a 1.49-fold higher risk of nocturia. This association is more robust for Asian and male subjects or those at a lower nocturia cut-off.

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

ZF wrote the manuscript. FW collected and analyzed the data. XD helped the review and revised the manuscript. TZ helped to design the study and revised the article. All authors have read and approved the manuscript.

Acknowledgments

We are grateful to the authors and patients of the included studies.

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.2022.924488/full#supplementary-material

References

1. Hashim H, Blanker MH. International Continence Society (ICS) report on the terminology for nocturia and nocturnal lower urinary tract function. *Neurourol Urodyn.* (2019) 38:499–508. doi: 10.1002/nau.23917

2. Bosch JL, Weiss JP. The prevalence and causes of nocturia. *J Urol.* (2013) 189:S86–92. doi: 10.1016/j.juro.2012.11.033

3. Pesonen JS, Vernooij RWM, Cartwright R, Aoki Y, Agarwal A, Mangera A, et al. The Impact of nocturia on falls and fractures: a systematic review and meta-analysis. *J Urol.* (2020) 203:674–83. doi: 10.1097/JU.0000000000000459

4. Pesonen JS, Cartwright R, Vernooij RWM, Aoki Y, Agarwal A, Mangera A, et al. The impact of nocturia on mortality: a systematic review and meta-analysis. *J Urol.* (2020) 203:486–95. doi: 10.1097/JU.0000000000000463

5. Wells G, Shea B, 0'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcasstle-Ottawa Scale (NOS) for Assessing the Quality of Non-Randomised Studies in Meta-Analysis.* Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed July 4, 2021).

6. Azuero J, Santander J, Trujillo CG, Caicedo JI, Zuluaga L, Becerra AM, et al. Potential associations of adult nocturia. Results from a national prevalence study. *Neurourol Urodyn.* (2021) 40:819–28. doi: 10.1002/nau.24624

7. Bing MH, Moller LA, Jennum P, Mortensen S, Lose G. Nocturia and associated morbidity in a Danish population of men and women aged 60–80 years. *BJU Int.* (2008) 102:808–14. doi: 10.1111/j.1464-410X.2008.07813.x

8. Chow PM, Liu SP, Chuang YC, Lee KS, Yoo TK, Liao L, et al. The prevalence and risk factors of nocturia in China, South Korea, and Taiwan: results from a cross-sectional, population-based study. *World J Urol.* (2018) 36:1853–62. doi: 10.1007/s00345-018-2329-0

9. Chung JH, Moon HS, Park SY, Kim KR, Cho SH, Kim YT. Effect of nocturnal hypoxia on nocturia in patients with obstructive sleep apnea. *Int Neurourol J.* (2019) 23:161–68. doi: 10.5213/inj.1938026.013

10. Fitzgerald MP, Litman HJ, Link CL, McKinlay JB. The association of nocturia with cardiac disease, diabetes, body mass index, age and diuretic use: results from the BACH survey. *J Urol.* (2007) 177:1385–9. doi: 10.1016/j.juro.2006.11.057

11. Gourova LW, van de Beek C, Spigt MG, Nieman FH, van Kerrebroeck PE. Predictive factors for nocturia in elderly men: a cross-sectional study in 21 general practices. *BJU Int.* (2006) 97:528–32. doi: 10.1111/j.1464-410X.2006.06029.x

12. Hsieh CH, Kuo TC, Hsu CS, Chang ST, Lee MC. Nocturia among women aged 60 or older in Taiwan. *Aust N Z J Obstet Gynaecol.* (2008) 48:312–6. doi: 10.1111/j.1479-828X.2008.00870.x

13. Huang MH, Chiu AF, Wang CC, Kuo HC. Prevalence and risk factors for nocturia in middle-aged and elderly people from public health centers in Taiwan. *Int Braz J Urol.* (2012) 38:818–24. doi: 10.1590/1677-553820133806818

14. Ito H, Aoki Y, Oe H, Taga M, Tsuchiyama K, Yokoyama O. Low and high body mass index values are associated with female nocturia. *Neurourol Urodyn.* (2019) 38:2250–54. doi: 10.1002/nau.24126

15. Johnson T M. 2nd, Sattin R W, Parmelee P, Fultz N H, Ouslander J G. Evaluating potentially modifiable risk factors for prevalent and incident nocturia in older adults. *J Am Geriatr Soc.* (2005) 53:1011–6. doi: 10.1111/j.1532-5415.2005.53321.x

16. Kim JS, Chung HS, Yu JM, Cho ST, Moon S. Analyzing the factors associated with nocturia in older people in the United States. *Ann Geriatr Med Res.* (2018) 22:184–88. doi: 10.4235/agmr.18.0044

17. Kim SY, Bang W, Kim MS, Park B, Kim JH, Choi HG. Analysis of the prevalence and factors associated with nocturia in adult Korean Men. *Sci Rep.* (2017) 7:41714. doi: 10.1038/srep41714

18. Liao CH, Chiang HS, Yu HJ. Serum testosterone levels significantly correlate with nocturia in men aged 40-79 years. *Urology.* (2011) 78:631–5. doi: 10.1016/j.urology.2011.05.033

19. Liew LC, Tiong HY, Wong ML, Png DC, Tan JK. A population study of nocturia in Singapore. *BJU Int.* (2006) 97:109–12. doi: 10.1111/j.1464-410X.2006.05867.x

20. Lightner DJ, Krambeck AE, Jacobson DJ, McGree ME, Jacobsen SJ, Lieber MM, et al. Nocturia is associated with an increased risk of coronary heart disease and death. *BJU Int.* (2012) 110:848–53. doi: 10.1111/j.1464-410X.2011.10806.x

21. Madhu C, Coyne K, Hashim H, Chapple C, Milsom I, Kopp Z. Nocturia: risk factors and associated comorbidities; findings from the EpiLUTS study. *Int J Clin Pract.* (2015) 69:1508–16. doi: 10.1111/ijcp.12727

22. Mekki P, Monaghan TF. Nocturia and electrocardiographic abnormalities among patients at an inner-city cardiology clinic. *Neurourol Urodyn.* (2021) 40:509–14. doi: 10.1002/nau.24590

23. Nakagawa H, Niu K, Hozawa A, Ikeda Y, Kaiho Y, Ohmori-Matsuda K, et al. Impact of nocturia on bone fracture and mortality in older individuals: a Japanese longitudinal cohort study. *J Urol.* (2010) 184:1413–8. doi: 10.1016/j.juro.2010.05.093

24. Obayashi K, Saeki K, Kurumatani N. Relationship between asymmetric dimethylarginine and nocturia in the general elderly population: The HEIJO-KYO cohort. *Neurourol Urodyn.* (2015) 34:769–73. doi: 10.1002/nau.22647

25. Parthasarathy S, Fitzgerald M, Goodwin JL, Unruh M, Guerra S, Quan S F. Nocturia, sleep-disordered breathing, and cardiovascular morbidity in a community-based cohort. *PLoS ONE.* (2012) 7:e30969. doi: 10.1371/journal.pone.0030969

26. Rembratt A, Norgaard J P, Andersson KE. Nocturia and associated morbidity in a community-dwelling elderly population. *BJU Int.* (2003) 92:726–30. doi: 10.1046/j.1464-410X.2003.04467.x

27. Stone BV, Shoag J, Halpern JA, Mittal S, Lewicki P, Golombos DM, et al. Prostate size, nocturia and the digital rectal examination: a cohort study of 30 500 men. *BJU Int.* (2017) 119:298–304. doi: 10.1111/bju.13613

28. Tikkinen KA, Auvinen A, Johnson TM. 2nd, Weiss JP, Keränen T, Tiitinen A, et al. A systematic evaluation of factors associated with nocturia–the population-based FINNO study. *Am J Epidemiol.* (2009) 170:361–8. doi: 10.1093/aje/kwp133

29. Victor RG, Li N, Blyler CA, Mason OR, Chang LC, Moy NPB, et al. Nocturia as an unrecognized symptom of uncontrolled hypertension in black men aged 35 to 49 years. *J Am Heart Assoc.* (2019) 8:e010794. doi: 10.1161/JAHA.118.010794

30. Wang Y, Hu H, Xu K, Zhang X, Wang X, Na Y, et al. Prevalence, risk factors, and symptom bother of nocturia: a population-based survey in China. *World J Urol.* (2015) 33:677–83. doi: 10.1007/s00345-014-1411-5

31. Wen L, Wen YB, Wang ZM, Wen JG, Li ZZ, Shang XP, et al. Risk factors of nocturia (two or more voids per night) in Chinese people older than 40 years. *Neurourol Urodyn.* (2015) 34:566–70. doi: 10.1002/nau.22623

32. Yoshimura K, Terada N, Matsui Y, Terai A, Kinukawa N, Arai Y. Prevalence of and risk factors for nocturia: analysis of a health screening program. *Int J Urol.* (2004) 11:282–7. doi: 10.1111/j.1442-2042.2004.00791.x

33. Yow HY, Tiong JJL, Mai CW, van der Werf E, Zainuddin ZM, Toh CC, et al. Prevalence of nocturia among community-dwelling adults: a population-based study in Malaysia. *BMC Urol. (*2021) 21:95. doi: 10.1186/s12894-021-00860-1

34. Zhang X, Zhang J, Chen J, Zhang C, Li Q, Xu T, et al. Prevalence and risk factors of nocturia and nocturia-related quality of life in the Chinese population. *Urol Int.* (2011) 86:173–8. doi: 10.1159/000321895

35. Weiss JP, Weinberg AC, Blaivas JG. New aspects of the classification of nocturia. *Curr Urol Rep.* (2008) 9:362–7. doi: 10.1007/s11934-008-0063-7

36. Barry MJ, Fowler FJ, O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK, et al. The American urological association symptom index for benign prostatic hyperplasia. The measurement committee of the American urological association. *J Urol.* (1992) 148:1549–57. doi: 10.1016/S0022-5347(17)36966-5

37. Ueda T, Yoshimura N, Yoshida O. Diabetic cystopathy: relationship to autonomic neuropathy detected by sympathetic skin response. *J Urol.* (1997) 157:580–4. doi: 10.1016/S0022-5347(01)65209-1

38. Rahman SN, Cao DJ, Monaghan TF, Flores VX, Vaysblat M, Moy MW, et al. Phenotyping the association between nocturia and hypertension: a systematic review and meta-analysis. *J Urol.* (2021) 205:1577–83. doi: 10.1097/JU.0000000000001433

39. Tikkinen KA, Tammela TL, Huhtala H, Auvinen A. Is nocturia equally common among men and women? A population based study in Finland. *J Urol.* (2006) 175:596–600. doi: 10.1016/S0022-5347(05)00245-4

40. Kebapci N, Yenilmez A, Efe B, Entok E, Demirustu C. Bladder dysfunction in type 2 diabetic patients. *Neurourol Urodyn.* (2007) 26:814–9. doi: 10.1002/nau.20422

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 07 October 2022
DOI 10.3389/fendo.2022.998499

OPEN ACCESS

EDITED BY
Sonia Michael Najjar,
Ohio University, United States

REVIEWED BY
Jason I. E. Bruce,
The University of Manchester,
United Kingdom
Maryam Zahedi,
Shahid Beheshti University of Medical Sciences, Iran

*CORRESPONDENCE
Wei Huang
dr_wei_huang@scu.edu.cn
Qing Xia
xiaqing@medmail.com.cn
Yin Zhu
ndyfy01977@ncu.edu.cn

†These authors have contributed equally to this work and share first authorship

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

RECEIVED 20 July 2022
ACCEPTED 20 September 2022
PUBLISHED 07 October 2022

CITATION
Yang X, Shi N, Yao L, He W, Zhu P, Li S, Li L, Li Y, Liu S, Deng L, Jin T, Liu T, Lu N, Windsor JA, Sutton R, Zhu Y, Xia Q and Huang W (2022) Impact of admission and early persistent stress hyperglycaemia on clinical outcomes in acute pancreatitis.
Front. Endocrinol. 13:998499.
doi: 10.3389/fendo.2022.998499

Impact of admission and early persistent stress hyperglycaemia on clinical outcomes in acute pancreatitis

Xinmin Yang[1†], Na Shi[1†], Linbo Yao[1], Wenhua He[2], Ping Zhu[1], Sheyu Li[3,4], Lan Li[1], Yuying Li[1], Shiyu Liu[1], Lihui Deng[1], Tao Jin[1], Tingting Liu[1], Nonghua Lu[2], John A. Windsor[5], Robert Sutton[6], Yin Zhu[2*], Qing Xia[1*] and Wei Huang[1*]

[1]West China Centre of Excellence for Pancreatitis, Institute of Integrated Traditional Chinese and Western Medicine, West China-Liverpool Biomedical Research Centre, West China Hospital, Sichuan University, Chengdu, China, [2]Department of Gastroenterology, First Affiliated Hospital of Nanchang University, Nanchang, China, [3]Department of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu, China, [4]Department of Guideline and Rapid Recommendation, Cochrane China Center, MAGIC China Centre, Chinese Evidence-Based Medicine Center, West China Hospital, Sichuan University, Chengdu, China, [5]Applied Surgery and Metabolism Laboratory, School of Biological Sciences, University of Auckland, Auckland, New Zealand, [6]Liverpool Pancreatitis Research Group, Liverpool University Hospitals National Health Service (NHS) Foundation Trust and Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Background: To determine the impact of glucose levels at admission and during first week (early phase) on clinical outcomes in patients with acute pancreatitis (AP) and to investigate the relationship between stress hyperglycaemia (SHG) and hypertriglyceridaemia (HTG).

Methods: Two independent and prospective databases were retrospectively analysed (n = 1792). Patients admitted with pain of less than 48 hours and confirmed AP were included. SHG was defined as admission blood glucose ≥ 10.00 mmol/L (non-diabetic) or ≥ 16.67 mmol/L (diabetic). Blood glucose records for the first week were inspected to determine whether SHG lasted ≥ 48 hours (persistent) or < 48 hours (transient). Clinical outcomes were compared between designated patient groups using multivariate and trend analyses. The correlation between SHG and HTG (serum triglyceride ≥ 5.65 mmol/L) was also analysed.

Results: On admission, SHG was present in 27.8% (499/1792) patients; during the first 48 hours of admission, transient and persistent SHG was found in 31% (556/1792) and 8.0% (144/1792) patients, respectively. Admission SHG was associated with higher incidence of persistent organ failure, acute necrotic collection, major infection, and mortality as well as prolonged length of hospital stay (all $P < 0.05$). Duration of SHG was also associated with worsened clinical outcomes (all $P < 0.05$). In HTG-AP patients, more severe clinical outcomes were observed in those who concomitantly had SHG ($P < 0.05$).

Conclusions: Admission and persistent SHG during the first week of admission worsens clinical outcomes of AP patients. These effects are more pronounced when admission HTG co-existed.

KEYWORDS

acute pancreatitis, blood glucose, stress hyperglycaemia, hypertriglyceridaemia, clinical outcomes

Introduction

Acute pancreatitis (AP) is one of the leading acute gastrointestinal diseases which has no effective and targeted drug treatment (1) and causes a significant social-economic burden (2). The global incidence of AP is increasing (3) with gallstones and alcohol excess being the most common aetiologies (4). Hypertriglyceridaemia (HTG) has become more common worldwide (5) and has become one of the leading causes in China (6–8). The sequelae of AP, including diabetes mellitus (DM) has a serious impact on quality of life (9, 10). About 20% of patients with AP will develop DM within 3 years of discharge from hospital and the risk increases over time (11, 12). Early diagnosis of hyperglycaemia and optimisation of in-hospital management may help prevent AP-related DM inferred from strong evidence of critically illness (13).

It is now well known that acute illness or injury can result in hyperglycaemia, insulin resistance and glucose intolerance, collectively termed stress hyperglycaemia (SHG) (14). SHG is a key risk factor for incident DM in survivors of critical illness (13). It is plausible, however, that critical illness uncovers latent insulin resistance and/or impaired pancreatic β-cell function, such that SHG identifies patients at increased risk of subsequently developing DM (15). Evidence to date also demonstrates that prolonged severe SHG is associated with a significantly elevated risk of mortality in patients in intensive care unit (ICU) (16). While a pilot study from New Zealand suggested elevated admission fasting blood glucose (BG) might be associated with worse clinical outcomes in AP (17), the relationship between SHG and AP warrants further study. Furthermore, the relationship between SHG and HTG is uncertain. DM, dyslipidaemia and their treatment are highly linked (18, 19). Insulin treatment has been frequently used in the management of HTG-associated AP (HTG-AP), in patients both with and without diabetes (20). And while the overall clinical outcomes are worse with HTG-AP than other aetiologies (6), it is not known whether this is also attributed by SHG.

We have recently investigated the specific BG levels that define SHG in AP patients with or without pre-existing DM (21). It was found that BG ≥ 10.00 mmol/L (180 mg/dL; in non-diabetic patients) or ≥ 16.67 (300 mg/dL; in diabetic patients) were independently associated with persistent organ failure. These findings have not been validated and SHG was only investigated at the time admission without knowing the impact of SHG duration during the early phase of AP on the clinical outcomes. The aims of this study were to (1) validate and explore the impact of admission SHG and persistent SHG during first week, respectively; and (2) investigate the relationship between SHG and HTG and impact on clinical outcomes in AP patients.

Methods

Study design and patient population

The present study was based on the retrospective analysis using the STROBE guidelines (22) of two large Chinese AP prospective databases of consecutively enrolled AP patients. The two cohorts included patients admitted to the West China Hospital of Sichuan University (Chengdu) from January 2016 to August 2017 and First Affiliated Hospital of Nanchang University (Nanchang) from January 2011 to December 2018 (23, 24), respectively. The institutional review boards at both centres (database approval number: Chengdu, No. 2015[247]; Nanchang, No. 2011[001]) approved the study. Data of 1792 patients with AP were used in the study, 688 patients were contributed by Chengdu, and 1104 by Nanchang, respectively (Supplementary Figures 1A, B).

Data collection

All patients followed uniform diagnostic criteria for AP according to Revised Atlanta Criteria (RAC) (25). Comprehensive clinical data were prospectively recorded on admission, within 24 hours, 24-48 hours, day 3 and if still being hospitalised, then on days 5, 7 and once a week then as previously reported (6, 8, 21, 23, 24). The data collected included demographics of age, sex, body mass index (BMI), date of admission, time from abdominal pain onset to hospital admission, referral status, and co-morbidities. Clinical data

included vital signs, haematology, biochemistry, blood gas analysis, clinical severity scores, CT pancreatic imaging. Treatment data included drugs, drainage, debridement, organ failure support. Outcome data included clinical outcomes (below), date of discharge or death. Patients were managed according to the International Association of Pancreatology/ American Pancreatic Association (IAP/APA) (26).

Inclusion and exclusion criteria

The inclusion criteria were adult patients (18-80 years) who had pain for 48 hours or less prior to admission, including those referred from other hospitals. The BG levels were determined at the time of admission and during the first week of admission at a frequency of at least every 48 hours.

The exclusion criteria were admission hypoglycaemia (BG level < 3.9 mmol/L) (27); use of glucocorticoids before admission; pregnancy or lactation; AP aetiologies of trauma, chronic pancreatitis or neoplasia; advanced comorbidities (congestive heart failure 3-4 or unstable coronary heart disease, end stage lung diseases, chronic kidney disease stage 4-5, liver cirrhosis with modified Child-Pugh grade 2-3, malignancy or immune deficiency); and incomplete data.

Definitions

Blood glucose: admission BG data used in this study were derived from the first blood biochemistry analysis of patients who presented at emergence department and very few were obtained at general ward. Subsequent daily BG levels during the first week were from the biochemistry analysis of blood drawn prior to any potential food intake in the morning (fasted at least for 8 hours overnight).

Stress hyperglycaemia: defined as BG ≥ 10.00 mmol/L or ≥ 16.67 mmol/L for non-diabetic and diabetic patients, respectively (21), regardless of insulin treatment status.

Persistent SHG: defined as SHG not resolved after 48 hours of treatment. Patients with early fulminant pancreatitis who died within 48 hours after admission and hence were not able to develop SHG lasting > 48 hours, were included in the group of patients with persistent SHG (28, 29).

Transient SHG: defined as SHG of less than 48 hours duration regardless of insulin treatment.

Pre-existing DM: diagnosed based on disease and medicine history, or serum glycated haemoglobin (HbA1c; ≥ 6.5% or 48 mmol/mol) as per American Diabetes Association (ADA) criteria (27).

HTG-AP: defined as AP with serum triglyceride (TG) levels ≥ 5.65 mmol/L on admission after ruling out common aetiologies (6, 21). Definitions for other aetiologies were as previously described (6, 8, 21, 23, 24).

Outcomes

The primary clinical outcome was persistent organ failure, defined as at least one of the systems (respiratory, circulatory, or renal) having modified Marshall organ failure score ≥ 2 and lasting ≥ 48 hours (25). Secondary outcome measures included multiple organ dysfunction syndrome (MODS) (2 or more systems), acute necrotic collection (25), major infection (presence of infected pancreatic necrosis, sepsis and/or pneumonia) with microbiological and/or imaging evidences (30), mortality followed up for 3 months, and length of hospital stay (LOHS).

Statistical analysis

Continuous data are displayed as medians with 25^{th}-75^{th} percentile and compared using Mann–Whitney *U* test, Kruskal-Wallis *H* test, or Cuzick's trend test analyses. Categorical data are expressed as number with percentages and compared using Chi-square test (or Fisher's test), linear trend test or proportional trend test analyses.

Multivariate logistic regression analysis was used to report categorical outcome measures and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Cox proportional hazards analysis was used to report LOHS and expressed as hazard ratios (HRs) with 95% CI (days for deceased patients were considered as truncated data). In both multivariate logistic regression and Cox proportional hazards analyses, baseline factors including age, or those of important clinical significance were adjusted. To quantify the effect of unmeasured potential confounding factors, we report the E-value, which represents the minimum strength of association on the risk ratio scale that an unmeasured confounder would need to have with both the exposure (with SHG) and the outcomes to fully explain away an association between the two (31). Survival differences between the duration of SHG were performed using the log rank test and plotted on Kaplan–Meier curve and adjusted using the methods of marginal balancing in groups. A two-sided $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS® 26.0 (IBM, Armonk, New York, USA). The E-value and 95% CI were calculated using an online calculator: https://www.evalue-calculator.com/evalue/ (31, 32).

Results

The demographics and clinical outcomes are shown in Supplementary Table 1. Of the included 1792 patients, the median age was 47 years (38–57), with 1135 (63.3%) were male and 245 (13.7%) had pre-existing DM. Persistent organ

failure developed in 373 (20.8%) and 81 (4.5%) were MODS. Of 1510 patients had CT scan, 419 (27.7%) had acute necrotic collection. Major infection was diagnosed in 141 (7.9%) of all patients and 43 died (2.4%), all occurred in those who had persistent organ failure/MODS. The overall median LOHS was 9 days (6–14). We found these two cohorts had the same distribution of the primary outcome persistent organ failure and other important clinical outcomes such as MODS, acute necrotic collection, major infection and mortality (all $P > 0.05$) of the present study design, albeit with the demographic, aetiologies, and admission clinical severity scoring systems varied.

SHG on admission and impact on clinical outcomes

SHG on admission was present in 499 (27.8%) patients (Table 1). There were no statistical differences in age, gender, pre-existing DM and time to admission between those with and without SHG. There were significant differences in BMI, Charlson comorbidity index, tertiary cases, admission TG, HTG-associated aetiology (37.3% vs 23.2%), and clinical severity scores (all $P < 0.05$) between patients with and without SHG.

Results for comparing clinical outcomes between patients with and without admission SHG are shown in Table 2. These were adjusted for baseline parameters including age, gender, BMI, Charlson comorbidity index, time to admission, referral status, biliary aetiology, admission TG levels and APACHE II score. Patients with admission SHG had significantly worse clinical outcomes: persistent organ failure (OR 2.00, 95% CI 1.51-2.65), acute necrotic collection (OR 1.78, 95%CI 1.39-2.29), major infection (OR 2.22, 95%CI 1.52-3.24), and mortality (OR 2.11, 95%CI 1.04-4.29) (all adjusted $P < 0.05$), corresponded to E-values of 2.18, 2.00, 3.87 and 3.64, respectively; the respective E-values for the low 95% CIs were 1.76, 1.64, 2.41 and 1.24. Admission SHG was also significant associated with increased LOHS (HR 0.77, 95%CI 0.68-0.85) when compared with those without SHG, E-value with the high 95% CI were 1.92 and 1.63.

Duration of SHG and impact on clinical outcomes

Transient SHG was found in 556 (31%) and persistent SHG in 144 (8.0%) patients (139 were non-diabetic) and comparison between these two groups is demonstrated in Supplementary Table 2. There were no significant differences between these groups for age, gender, and alcohol aetiology (all $P > 0.05$). In

TABLE 1 Baseline characteristics of patients with or without stress hyperglycaemia on admission.

	Total (n = 1792)	No stress hyperglycaemia (n = 1293)	Stress hyperglycaemia (n = 499)	*P*
Age, years, median (25^{th}-75^{th} percentile)	47 (38-57)	47 (39-58)	46 (38-54)	0.094
Sex, male, n (%)	1135 (63.3)	807 (62.4)	328 (65.7)	0.191
BMI, median (25^{th}-75^{th} percentile)	24.24 (22.03-26.86)	24.03 (21.8-26.37)	25.16 (22.77-27.68)	**<0.001**
Charlson comorbidity index, median (25^{th}-75^{th} percentile)	0 (0-1)	0 (0-1)	0 (0-2)	**0.003**
Pre-existing DM, n (%)	245 (13.7)	176 (13.6)	69 (13.8)	0.905
Referral, n (%)	907 (50.6)	627 (48.5)	280 (56.1)	**0.004**
Time to admission, days, median (25^{th}-75^{th} percentile)	1 (1-2)	1 (1-2)	1 (1-2)	0.17
Aetiology, n (%)				
Biliary	696 (38.8)	543 (42.0)	153 (30.7)	**<0.001**
HTG-associated	486 (27.1)	300 (23.2)	186 (37.3)	**<0.001**
Alcohol excess	161 (9.0)	118 (9.1)	43 (8.6)	0.736
Others or unknown	449 (25.1)	332 (25.7)	117 (23.4)	0.329
Admission glucose and lipid levels, median (25^{th}-75^{th} percentile)				
Blood glucose, mmol/L	8.06 (6.38-11.61)	7.08 (5.9-8.5)	12.99 (11.24-17.25)	**<0.001**
Triglycerides, mmol/L	2.65 (1.04-10.84)	2.77 (0.95-8.24)	6.05 (1.52-14.82)	**<0.001**
Admission clinical severity scores, median (25^{th}-75^{th} percentile)				
SIRS	2 (1-2)	1 (1-2)	2 (1-3)	**<0.001**
APACHE II	6 (3-8)	5 (3-8)	7 (4-10)	**<0.001**

P for Mann–Whitney U test or Chi-square test of comparison between no stress hyperglycaemia and stress hyperglycaemia groups.
BMI, body mass index; DM, diabetes mellitus; HTG, hypertriglyceridaemia; SIRS, Systemic Inflammatory Response Syndrome; APACHE II, Acute Physiology and Chronic Health Evaluation II.
Bold values indicates that the P value is statistically significant.

TABLE 2 Comparison of clinical outcomes with or without stress hyperglycaemia on admission.

Parameters	No stress hyperglycaemia (n=1293)	Stress hyperglycaemia (n=499)	*P*	Estimate, OR or HR (95% CI)	Adjusted *P*
Persistent organ failure, n (%)	203 (15.7)	170 (34.1)	**<0.001**	2.00 (1.51-2.65)[a]	**<0.001**
MODS, n (%)	40 (3.1)	41 (8.2)	**<0.001**	1.18 (0.68-2.06)[a]	0.558
Acute necrotic collection, n (%)	245 (18.9)	174 (34.9)	**<0.001**	1.78 (1.39-2.29)[a]	**<0.001**
Major infection, n (%)	69 (5.3)	72 (14.4)	**<0.001**	2.22 (1.52-3.24)[a]	**<0.001**
Mortality, n (%)	17 (1.3)	26 (5.2)	**<0.001**	2.11 (1.04-4.29)[a]	**0.039**
LOHS, days, median (25^{th}-75^{th} percentile)	8 (6-13)	11 (7-17)	**<0.001**	0.77 (0.68-0.85)[b]	**<0.001**

[a]Logistic regression with OR after adjusting baseline variates.
[b]Cox proportional hazards model with HR (deceased patients removed) after adjusting baseline variates. These variates included age, gender, body mass index, Charlson comorbidity index, time to admission, referral status, biliary aetiology, admission triglyceride levels and APACHE II that each with considerable clinical importance.
OR, odds ratio; HR, hazard ratio; CI, confidence interval; MODS, Multiple Organ Dysfunction Syndrome; LOHS, length of hospital stays.
Bold values indicates that the P value is statistically significant.

patients with increased duration of SHG there was a significant increase in BMI, HTG aetiology, admission BG and TG levels, and clinical severity scores (all $P < 0.001$).

Persistent SHG was associated with more severe AP compared with transient SHG or no SHG groups (both $P < 0.001$). There were no significant differences between transient SHG and no SHG groups (Figure 1A). There were 10 (0.9%), 20 (3.6%) and 13 (9.0%) deaths in no, transient and persistent SHG patients, respectively, followed a significant step-wise increase with duration of hyperglycaemia (all Log rank $P < 0.001$; Figure 1B), even after adjusting for baseline parameters including age, gender, BMI, Charlson comorbidity index, time to admission, referral status, biliary aetiology, and admission TG levels (all $P < 0.001$; Figure 1C).

The comparison of clinical outcomes between patients with no, transient, and persistent SHG was adjusted age, gender, BMI, Charlson comorbidity index, time to admission, referral status, biliary aetiology, admission TG levels, and APACHE II using multivariate analysis (adjusted OR or HR). Across these 3 groups there was a step-wise increase in the incidence of persistent organ failure, MODS, acute necrotic collection, major infection, and mortality with corresponding prolonged LOHS (all $P_{trend} < 0.05$; Figure 2). In addition, the clinical outcomes were worse in patients with persistent SHG compared with those with admission SHG (Supplementary Figure 2).

Relationship between SHG and HTG and impact on clinical outcomes

There was a significant positive association between admission BG and TG levels ($r_s = 0.297$, $P < 0.001$; Supplementary Figure 3A). The incidence of SHG in AP patients with HTG was significantly higher than those with non-HTG (39.2% vs 21.3%, $P < 0.001$; Supplementary Figure 3B).

Subgroup analyses compared the impact of admission SHG on clinical outcomes between non-HTG and HTG-associated AP patients. The clinical outcomes were worse in non-HTG-AP patients with admission SHG than those without SHG (Table 3, *upper panel*). The same was true for HTG-AP patients (Table 3, *lower panel*). These results were confirmed after adjusting for age, gender, BMI, Charlson comorbidity index, time to admission, and referral status (Table 3).

Discussion

In this study, we have validated the newly defined SHG in AP patients showing a step-wise relationship between increased admission glucose levels and worsened clinical outcomes. This study also demonstrates that AP patients can be classified according to glycaemic status during the disease early phase (first week) into no, transient, and persistent SHG which were associated with escalating disease severity and adverse clinical outcomes; clinical outcomes were worse in patients with persistent SHG compared with those with admission SHG. Furthermore, the association of SHG with adverse clinical outcomes remained robust in HTG-AP patients.

A large number of studies have focused on the probability of DM after pancreatitis and its possible mechanism (11, 12, 33–35). Few have studied on BG changes under stress status and its impact on the severity or outcomes of AP patients (21). The pathogenesis of SHG during critical illness is complex, including increased release of counter-regulatory hormones, altered insulin receptor signalling due to inflammation, pancreatic beta-cell inhibition and interventions such as administration of glucocorticoids or parenteral nutrition (36, 37). How glucose metabolism is affected by derangement of adrenaline, glucagon, cortisol, and insulin remains to be elusive due to lack of comparable studies, it is relatively clearer that hyperglycaemia in AP mainly due to both impairment of beta-cells resulting a decrease in insulin secretion and the production of cytokines. This causes the appearance/worsening of insulin resistance and subsequently induces hyperglycaemia, which, in turn, may

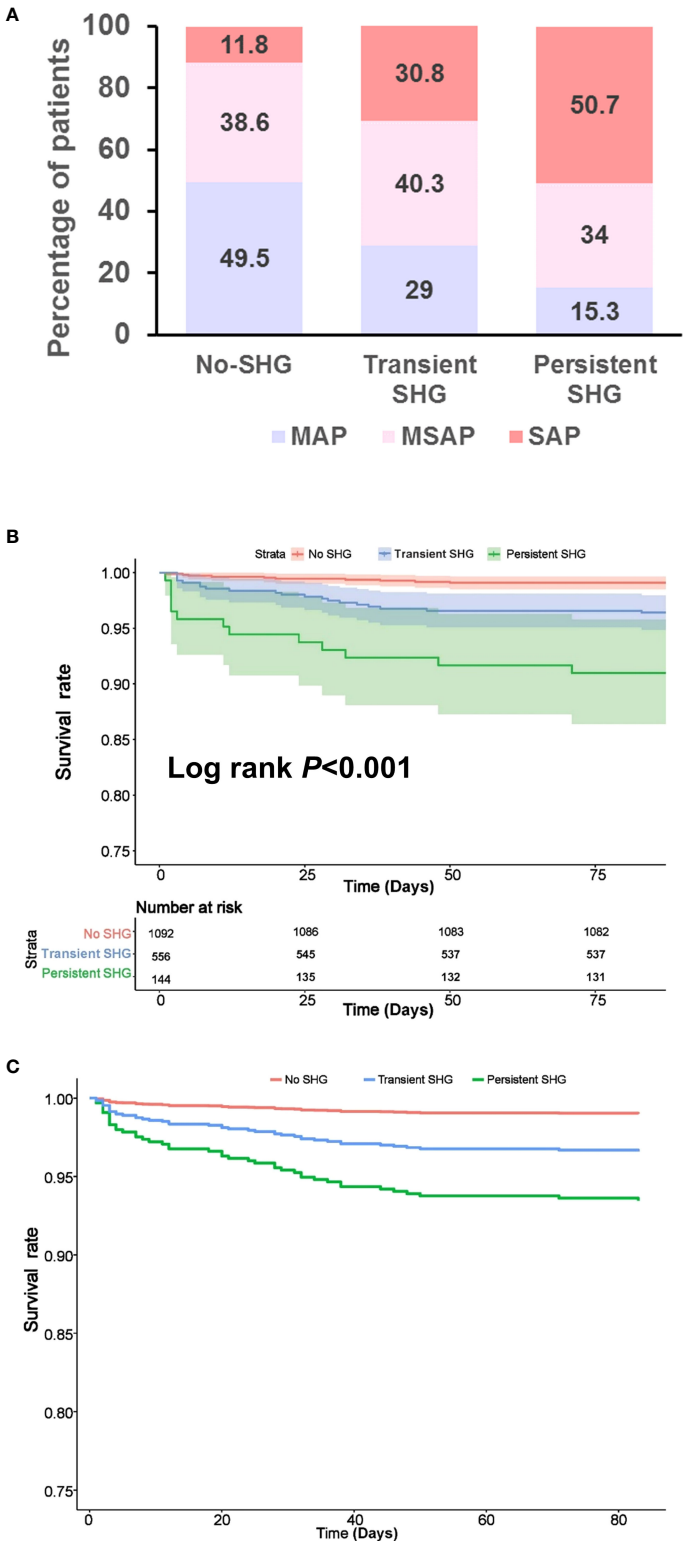
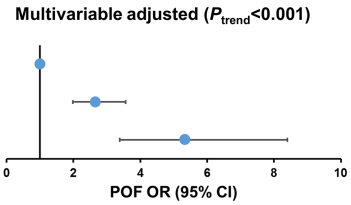
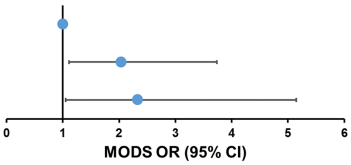


FIGURE 1
Features of patients stratified by duration of stress hyperglycaemia during the first week of admission. **(A)** Severity classification. **(B)** Kaplan–Meier survival curve. **(C)** Survival curve after adjusting for age, gender, BMI, Charlson comorbidity index, time to admission, referral status, biliary aetiology and admission TG levels. SHG, stress hyperglycaemia; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis.

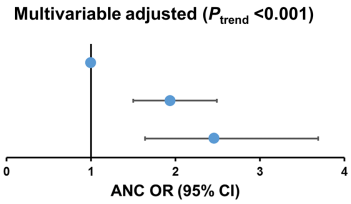
Duration of stress hyperglycemia	POF n/n total (%)	Estimate, OR (95% CI)	Adjusted *P*
No	129/1092 (11.8)	1 (reference)	-
Transient (<48 hours)	171/556 (30.8)	2.66 (1.99-3.56)	<0.001
Persistent (≥48 hours)	73/144 (50.7)	5.34 (3.39-8.40)	<0.001



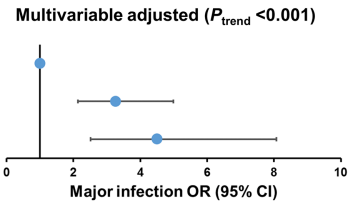
Duration of stress hyperglycemia	MODS n/n total (%)	Estimate, OR (95% CI)	Adjusted *P*
No	22/1092 (2.0)	1 (reference)	-
Transient (<48 hours)	38/556 (6.8)	2.04 (1.11-3.74)	0.021
Persistent (≥48 hours)	21/144 (14.6)	2.33 (1.05-5.15)	0.037



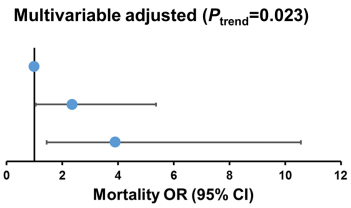
Duration of stress hyperglycemia	ANC n/n total (%)	Estimate, OR (95% CI)	Adjusted *P*
No	182/1092 (16.7)	1 (reference)	-
Transient (<48 hours)	176/556 (31.7)	1.94 (1.50-2.49)	<0.001
Persistent (≥48 hours)	61/144 (42.4)	2.46 (1.64-3.69)	<0.001



Duration of stress hyperglycemia	Major infection n/n total (%)	Estimate, OR (95% CI)	Adjusted *P*
No	40/1092 (3.7)	1 (reference)	-
Transient (<48 hours)	72/556 (12.9)	3.27 (2.14-4.99)	<0.001
Persistent (≥48 hours)	29/144 (20.1)	4.50 (2.51-8.07)	<0.001



Duration of stress hyperglycemia	Mortality n/n total (%)	Estimate, OR (95% CI)	Adjusted *P*
No	10/1092 (0.9)	1 (reference)	-
Transient (<48 hours)	20/556 (3.6)	2.36 (1.04-5.37)	0.041
Persistent (≥48 hours)	13/144 (9.0)	3.91 (1.44-10.57)	0.007



Duration of stress hyperglycemia	LOHS (day)	Estimate, OR (95% CI)	Adjusted *P*
No	8 (6-12)	1 (reference)	-
Transient (<48 hours)	11 (7-17)	0.65 (0.59-0.73)	<0.001
Persistent (≥48 hours)	13 (8-20)	0.58 (0.48-0.70)	<0.001

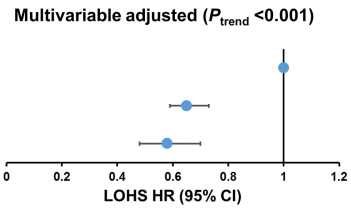


FIGURE 2
Trend analysis for clinical outcomes stratified by duration of stress hyperglycaemia. POF, persistent organ failure; MODS, Multiple Organ Dysfunction Syndrome; ANC, acute necrotic collection; LOHS, length of hospital stays.

TABLE 3 Clinical outcomes of patients stratified by stress hyperglycaemia in non-HTG and HTG groups on admission.

Non-HTG patients (n = 1139)					
Parameters	**No stress hyperglycaemia (n = 896)**	**Stress hyperglycaemia (n = 243)**	***P***	**Estimate, OR or HR (95% CI)**	**Adjusted *P***
Persistent organ failure, n (%)	136 (15.2)	78 (32.1)	**<0.001**	2.47 (1.76-3.47)[a]	**<0.001**
MODS, n (%)	25 (2.8)	9 (3.7)	0.458	1.18 (0.53-2.63)[a]	0.695
Acute necrotic collection, n (%)	156 (17.4)	87 (35.8)	**<0.001**	2.54 (1.84-3.51)[a]	**<0.001**
Major infection, n (%)	45 (5.0)	38 (15.6)	**<0.001**	3.28 (2.06-5.23)[a]	**<0.001**
Mortality, n (%)	10 (1.1)	11 (4.5)	**<0.001**	3.76 (1.52-9.31)[a]	**0.004**
LOHS, days, median (25^{th}-75^{th} percentile)	8 (6-12)	10 (6-16)	**0.001**	0.77 (0.61-0.83)[b]	**<0.001**
HTG-associated patients (n = 653)					
Parameters	**No stress hyperglycaemia (n = 397)**	**Stress hyperglycaemia (n = 256)**	***P***	**Estimate, OR or HR (95% CI)**	**Adjusted *P***
Persistent organ failure, n (%)	67 (16.9)	92 (35.9)	**<0.001**	2.51 (1.71-3.68)[a]	**<0.001**
MODS, n (%)	15 (3.8)	32 (12.5)	**<0.001**	2.88 (1.48-5.60)[a]	**0.002**
Acute necrotic collection, n (%)	89 (22.4)	87 (34.0)	**0.001**	1.55 (1.07-2.22)[a]	**0.019**
Major infection, n (%)	24 (6.0)	34 (13.3)	**0.002**	2.12 (1.21-3.72)[a]	**0.009**
Mortality, n (%)	7 (1.8)	15 (5.9)	**0.005**	2.76 (1.08-7.04)[a]	**0.034**
LOHS, days, median (25^{th}-75^{th} percentile)	9 (7-13)	12 (8-19)	**<0.001**	0.72 (0.61-0.85)[b]	**<0.001**

[a]Logistic regression with OR after adjusting baseline variates.
[b]Cox proportional hazards model with HR (deceased patients removed) after adjusting baseline variates. These variates included age, gender, body mass index, Charlson comorbidity index, time to admission and referral status that each with considerable clinical importance.
HTG, hypertriglyceridaemia; OR, odds ratio; HR, hazard ratio; CI, confidence interval; MODS, Multiple Organ Dysfunction Syndrome; LOHS, length of hospital stay.
Bold values indicates that the P value is statistically significant.

further damage beta-cells and worsen insulin resistance observed in critical illness (16). In this study, we also further demonstrate that AP patients with SHG often had higher BMI and TG levels, suggesting that well accepted risk factors for DM also contribute to the development of SHG.

While the definition of SHG varied among studies (36, 38), the strength of the association between each glucose parameter and outcome emphasises the significance of this relationship. SHG is associated with a significantly elevated risk of mortality in ICU patients (16). A recently meta-analysis demonstrated that COVID-19 patients with hyperglycaemia (BG ≥ 7, 7.7, 10, or 11 mmol/L) also had a higher risk of developing severe or critical illness compared with normoglycaemia patients regardless of prior DM conditions (39). Similarly, we optimised SHG as BG ≥ 10.00 mmol/L and ≥ 16.67 mmol/L for AP patients without pre-existing diabetes and those with pre-existing diabetes, respectively, according to multivariate logistic regression and ROC curves) on admission (21). Here, we further verified these admission BG cut-off values and confirmed a step-wise relationship between increased glucose levels and worsened clinical outcomes in AP patients, consistent with our previous findings. Sensitivity analyses revealed that it would take very strong confounding to negate the associations observed in this study. These observations are the same when analysing the two composition cohorts of varied baseline characteristics and aetiologies separately (data not shown).

Persistent hyperglycaemia is a common parameter used to evaluate blood glucose fluctuations, and our finding of their association with adverse clinical outcomes in AP patients is similar to that in acute ischaemic stroke, acute myocardial infarction, intracerebral haemorrhage, and other critical illness (28, 29, 40, 41). On the other hand, the early phase (first week) in severe AP patients is often accompanied by persistent SIRS which develops to persistent organ failure/MODS, serving as the predominate cause of death (8, 42). Duration of organ failure during the first week had proved to be strongly associated with the risk of local complications and death (8, 43). Therefore, in the current study, we also studied dynamic nature of glucose changes and their association with clinical outcomes during the first week in AP patients. Persistent SHG was defined as the SHG persisted over 48 hours as other disease definitions (28, 29), and we newly defined transient SHG for those SHG presented but less than 48 hours referred to the definition time of whether the organ failure in AP persists or not (25). There was a stepwise increase of adverse clinical outcomes in patients with no, transient, and persistent SHG. And the increase was more pronounced with persistent SHG than with admission SHG. This means that once SHG occurred during the first week of admission, the risk of all adverse clinical outcomes increased, and the longer the duration of SHG, the more serious the clinical outcome would be. However, we cannot directly establish the cause-effect relationship claiming that SHG aggravated the severity of AP in the current study.

Whether SHG worsens clinical outcomes of AP patients warrant further basic and clinical research.

HTG has been reported as the aetiology of AP in more than a third of cases in some large Chinese AP cohorts (6–8). This may be due to an increasing prevalence of central obesity (44) and/or metabolic syndrome in Chinese populations (45). We and others have previously shown that admission TG levels are associated with worse clinical outcomes in AP patients (6, 46), but these analyses did not account for co-existing SHG. We took a subgroup analysis for HTG-AP and found the similar adverse effect of SHG on the outcomes as for the whole cohort of AP. These findings highlight a close interaction between glucose haemostasis and lipid metabolism (47, 48). This is an important finding because it provides justification for strategies to lower both glucose and TG in the acute management of AP. A recent meta-analysis of observational studies supported the use of insulin for the early management of HTG-AP patients (49). Recently, a compelling experimental study suggests that endogenous insulin protected pancreatic acinar cells during AP by preserving glycolytic ATP supply to calcium pumps (50). Therefore, insulin can protect against AP by both acting on acinar cells and hormone-sensitive lipase (preventing release of free fatty acids from TG) and this warrants clinical trials.

Our study has several limitations. Firstly, as the nature of *post hoc* analysis, the BG data were not available for every day (data were mainly missing on day 4 and day 6), which may cause the lower proportion of transient SHG than it actually was. Secondly, only more than half of the patients had HbA1c measured on admission and thus we most likely underestimate the prevalence of pre-existing DM in out cohorts. Thirdly, we could not determine from both databases what the timing of and which treatment strategies affected the glucose levels. Therefore, we did not try to perform an analysis of glycaemic variability. Further research with individual-level data on treatment type and timing may help clarify these questions. Finally, we did not investigate the impact of admission and duration of SHG on the probability of developing post-pancreatitis DM, which will comprise a separate study.

Conclusion

In conclusion, we demonstrate that the admission and duration of SHG had important impact on development adverse clinical outcomes in AP patients. Screening, monitoring, and targeting AP patients with high-risk of developing SHG may have beneficial clinical implication.

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.

Author contributions

XY and NS contributed equally to this study. XY, NS, WHe, LL, YL, LY, LD, TJ, NL, and YZ: acquisition of data. XY, NS, and WHu: drafting of manuscript. XY, NS, WHe, PZ, TL, LD, TJ, and WHu: analysis and interpretation of data. SheL and PZ: statistical analysis supervision. ShiL, RS, and JW: important intelligence input. RS, JW, YZ, and QX: critical revision of the manuscript. WHu, QX, and YZ: study concept and design, obtained funding, study supervision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by National Natural Science Foundation of China (No. 81973632, No. 82270672, WHu; No. 81774120, QX; No. 81960128, YZ; No. 82100682, NS); Key Research and Development Programme of Science and Technology Department of Sichuan Province (2022YFS0406, XY; 2020YFS0235, NS); China Postdoctoral Science Foundation (2022M712285, XY; 2022T150453, XY); NIHR Senior Investigator Award (RS).

Acknowledgments

These authors thank all the staff from the pancreas multidisciplinary teams at West China Hospital of Sichuan University and First Affiliated Hospital of Nanchang University for their continuous support.

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.2022.998499/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Patient selection flow chart. **(A)** Chengdu cohort. **(B)** Nanchang cohort. AP, acute pancreatitis; CP, chronic pancreatitis.

SUPPLEMENTARY FIGURE 2
Adverse clinical outcomes in patients with admission and persistent stress hyperglycaemia. SHG, stress hyperglycaemia; POF, persistent organ failure; MODS, multiple organ dysfunction syndrome; ANC, acute necrotic collection.

SUPPLEMENTARY FIGURE 3
Glucose and lipid metabolism disorder on admission in AP patients. **(A)** Correlation between glucose and triglycerides. **(B)** Admission stress hyperglycaemia in HTG and non-HTG AP patients. TG, triglycerides; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; No-SHG, no stress hyperglycaemia; SHG, stress hyperglycaemia.

References

1. Moggia E, Koti R, Belgaumkar AP, Fazio F, Pereira SP, Davidson BR, et al. Pharmacological interventions for acute pancreatitis. *Cochrane Database Syst Rev* (2017) 4:CD011384. doi: 10.1002/14651858.CD011384.pub2

2. Peery AF, Crockett SD, Murphy CC, Lund JL, Dellon ES, Williams JL, et al. Burden and cost of gastrointestinal, liver, and pancreatic diseases in the united states: Update 2018. *Gastroenterology* (2019) 156(1):254–72 e11. doi: 10.1053/j.gastro.2018.08.063

3. Cho J, Petrov MS. Pancreatitis, pancreatic cancer, and their metabolic sequelae: Projected burden to 2050. *Clin Transl Gastroenterol* (2020) 11(11): e00251. doi: 10.14309/ctg.0000000000000251

4. Boxhoorn L, Voermans RP, Bouwense SA, Bruno MJ, Verdonk RC, Boermeester MA, et al. Acute pancreatitis. *Lancet* (2020) 396(10252):726–34. doi: 10.1016/S0140-6736(20)31310-6

5. Hines OJ, Pandol SJ. Management of severe acute pancreatitis. *BMJ* (2019) 367:l6227. doi: 10.1136/bmj.l6227

6. Zhang R, Deng L, Jin T, Zhu P, Shi N, Jiang K, et al. Hypertriglyceridaemia-associated acute pancreatitis: Diagnosis and impact on severity. *HPB (Oxford)* (2019) 21(9):1240–9. doi: 10.1016/j.hpb.2019.01.015

7. Ding Y, Zhang M, Wang L, Yin T, Wang N, Wu J, et al. Association of the hypertriglyceridemic waist phenotype and severity of acute pancreatitis. *Lipids Health Dis* (2019) 18(1):93. doi: 10.1186/s12944-019-1019-2

8. Shi N, Liu T, de la Iglesia-Garcia D, Deng L, Jin T, Lan L, et al. Duration of organ failure impacts mortality in acute pancreatitis. *Gut* (2020) 69(3):604–5. doi: 10.1136/gutjnl-2019-318241

9. Petrov MS, Yadav D. Global epidemiology and holistic prevention of pancreatitis. *Nat Rev Gastroenterol Hepatol* (2019) 16(3):175–84. doi: 10.1038/s41575-018-0087-5

10. Hart PA, Bradley D, Conwell DL, Dungan K, Krishna SG, Wyne K, et al. Diabetes following acute pancreatitis. *Lancet Gastroenterol Hepatol* (2021) 6 (8):668–75. doi: 10.1016/S2468-1253(21)00019-4

11. Das SL, Singh PP, Phillips AR, Murphy R, Windsor JA, Petrov MS. Newly diagnosed diabetes mellitus after acute pancreatitis: A systematic review and meta-analysis. *Gut* (2014) 63(5):818–31. doi: 10.1136/gutjnl-2013-305062

12. Zhi M, Zhu X, Lugea A, Waldron RT, Pandol SJ, Li L. Incidence of new onset diabetes mellitus secondary to acute pancreatitis: A systematic review and meta-analysis. *Front Physiol* (2019) 10:637. doi: 10.3389/fphys.2019.00637

13. Ali Abdelhamid Y, Kar P, Finnis ME, Phillips LK, Plummer MP, Shaw JE, et al. Stress hyperglycaemia in critically ill patients and the subsequent risk of diabetes: A systematic review and meta-analysis. *Crit Care* (2016) 20(1):301. doi: 10.1186/s13054-016-1471-6

14. Marik PE, Bellomo R. Stress hyperglycemia: An essential survival response! *Crit Care Med* (2013) 41(6):e93–4. doi: 10.1097/CCM.0b013e318283d124

15. Smith FG, Sheehy AM, Vincent JL, Coursin DB. Critical illness-induced dysglycaemia: Diabetes and beyond. *Crit Care* (2010) 14(6):327. doi: 10.1186/cc9266

16. Badawi O, Waite MD, Fuhrman SA, Zuckerman IH. Association between intensive care unit-acquired dysglycemia and in-hospital mortality. *Crit Care Med* (2012) 40(12):3180–8. doi: 10.1097/CCM.0b013e3182656ae5

17. Kennedy JI, Askelund KJ, Premkumar R, Phillips AR, Murphy R, Windsor JA, et al. Leptin is associated with persistence of hyperglycemia in acute pancreatitis: A prospective clinical study. *Med (Baltimore)* (2016) 95(6):e2382. doi: 10.1097/MD.0000000000002382

18. Berglund L, Brunzell JD, Goldberg AC, Goldberg IJ, Sacks F, Murad MH, et al. Evaluation and treatment of hypertriglyceridemia: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2012) 97(9):2969–89. doi: 10.1210/jc.2011-3213

19. Li J, Du H, Wang Y, Aertgeerts B, Guyatt G, Hao Q, et al. Safety of proprotein convertase subtilisin/kexin 9 inhibitors: A systematic review and meta-analysis. *Heart* (2022) 108(16):1296–302. doi: 10.1136/heartjnl-2021-320556

20. Simha V. Management of hypertriglyceridemia. *Bmj* (2020) 371:m3109. doi: 10.1136/bmj.m3109

21. Yang X, Zhang R, Jin T, Zhu P, Yao L, Li L, et al. Stress hyperglycemia is independently associated with persistent organ failure in acute pancreatitis. *Dig Dis Sci* (2021) 67(5):1879–89. doi: 10.1007/s10620-021-06982-8

22. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. Strengthening the reporting of observational studies in epidemiology (Strobe) statement: Guidelines for reporting observational studies. *BMJ* (2007) 335 (7624):806–8. doi: 10.1136/bmj.39335.541782.AD

23. Li L, Jin T, Wen S, Shi N, Zhang R, Zhu P, et al. Early rapid fluid therapy is associated with increased rate of noninvasive positive-pressure ventilation in hemoconcentrated patients with severe acute pancreatitis. *Dig Dis Sci* (2020) 65 (9):2700–11. doi: 10.1007/s10620-019-05985-w

24. Yu B, He WH, Lu N. Risk factors for acute kidney injury in acute pancreatitis: A 7-year retrospective analysis of patients in a Large tertiary hospital. *Pancreas* (2020) 49(8):1057–62. doi: 10.1097/MPA.0000000000001613

25. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis–2012: Revision of the Atlanta classification and definitions by international consensus. *Gut* (2013) 62(1):102–11. doi: 10.1136/gutjnl-2012-302779

26. Iap/Apa evidence-based guidelines for the management of acute pancreatitis. *Pancreatology* (2013) 13(4 Suppl 2):e1–15. doi: 10.1016/j.pan.2013.07.063

27. American Diabetes A. 15. diabetes care in the hospital: Standards of medical care in diabetes-2019. *Diabetes Care* (2019) 42(Suppl 1):S173–S81. doi: 10.2337/dc19-S015

28. van der Horst IC, Nijsten MW, Vogelzang M, Zijlstra F. Persistent hyperglycemia is an independent predictor of outcome in acute myocardial infarction. *Cardiovasc Diabetol* (2007) 6:2. doi: 10.1186/1475-2840-6-2

29. Fuentes B, Ortega-Casarrubios MA, Sanjose B, Castillo J, Leira R, Serena J, et al. Persistent hyperglycemia >155 Mg/Dl in acute ischemic stroke patients: How well are we correcting it?: implications for outcome. *Stroke* (2010) 41(10):2362–5. doi: 10.1161/STROKEAHA.110.591529

30. Bakker OJ, van Brunschot S, van Santvoort HC, Besselink MG, Bollen TL, Boermeester MA, et al. Early versus on-demand nasoenteric tube feeding in acute pancreatitis. *N Engl J Med* (2014) 371(21):1983–93. doi: 10.1056/NEJMoa1404393

31. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: Introducing the e-value. *Ann Intern Med* (2017) 167(4):268–74. doi: 10.7326/M16-2607

32. Mathur MB, Ding P, Riddell CA, VanderWeele TJ. Web site and r package for computing e-values. *Epidemiology* (2018) 29(5):e45–e7. doi: 10.1097/EDE.0000000000000864

33. Shen HN, Yang CC, Chang YH, Lu CL, Li CY. Risk of diabetes mellitus after first-attack acute pancreatitis: A national population-based study. *Am J Gastroenterol* (2015) 110(12):1698–706. doi: 10.1038/ajg.2015.356

34. Bharmal SH, Cho J, Alarcon Ramos GC, Ko J, Stuart CE, Modesto AE, et al. Trajectories of glycaemia following acute pancreatitis: A prospective longitudinal cohort study with 24 months follow-up. *J Gastroenterol* (2020) 55(8):775–88. doi: 10.1007/s00535-020-01682-y

35. Pendharkar SA, Singh RG, Bharmal SH, Drury M, Petrov MS. Pancreatic hormone responses to mixed meal test in new-onset Prediabetes/Diabetes after non-necrotizing acute pancreatitis. *J Clin Gastroenterol* (2020) 54(2):e11–20. doi: 10.1097/MCG.0000000000001145

36. Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet* (2009) 373(9677):1798–807. doi: 10.1016/S0140-6736(09)60553-5

37. Deane AM, Horowitz M. Dysglycaemia in the critically ill - significance and management. *Diabetes Obes Metab* (2013) 15(9):792–801. doi: 10.1111/dom.12078

38. Inzucchi SE. Clinical practice. management of hyperglycemia in the hospital setting. *N Engl J Med* (2006) 355(18):1903–11. doi: 10.1056/NEJMcp060094

39. Lee MH, Wong C, Ng CH, Yuen DCW, Lim AYL, Khoo CM. Effects of hyperglycaemia on complications of covid-19: A meta-analysis of observational studies. *Diabetes Obes Metab* (2021) 23(1):287–9. doi: 10.1111/dom.14184

40. Faustino EV, Apkon M. Persistent hyperglycemia in critically ill children. *J Pediatr* (2005) 146(1):30–4. doi: 10.1016/j.jpeds.2004.08.076

41. Wu TY, Putaala J, Sharma G, Strbian D, Tatlisumak T, Davis SM, et al. Persistent hyperglycemia is associated with increased mortality after intracerebral hemorrhage. *J Am Heart Assoc* (2017) 6(8):e005760. doi: 10.1161/JAHA.117.005760

42. Garg PK, Singh VP. Organ failure due to systemic injury in acute pancreatitis. *Gastroenterology* (2019) 156(7):2008–23. doi: 10.1053/j.gastro.2018.12.041

43. Johnson CD, Abu-Hilal M. Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. *Gut* (2004) 53(9):1340–4. doi: 10.1136/gut.2004.039883

44. Zhang L, Wang Z, Wang X, Chen Z, Shao L, Tian Y, et al. Prevalence of abdominal obesity in China: Results from a cross-sectional study of nearly half a million participants. *Obes (Silver Spring)* (2019) 27(11):1898–905. doi: 10.1002/oby.22620

45. Li R, Li W, Lun Z, Zhang H, Sun Z, Kanu JS, et al. Prevalence of metabolic syndrome in mainland China: A meta-analysis of published studies. *BMC Public Health* (2016) 16:296. doi: 10.1186/s12889-016-2870-y

46. Nawaz H, O'Connell M, Papachristou GI, Yadav D. Severity and natural history of acute pancreatitis in diabetic patients. *Pancreatology* (2015) 15(3):247–52. doi: 10.1016/j.pan.2015.03.013

47. Guilherme A, Henriques F, Bedard AH, Czech MP. Molecular pathways linking adipose innervation to insulin action in obesity and diabetes mellitus. *Nat Rev Endocrinol* (2019) 15(4):207–25. doi: 10.1038/s41574-019-0165-y

48. Kopchick JJ, Berryman DE, Puri V, Lee KY, Jorgensen JOL. The effects of growth hormone on adipose tissue: Old observations, new mechanisms. *Nat Rev Endocrinol* (2020) 16(3):135–46. doi: 10.1038/s41574-019-0280-9

49. He W, Cai W, Camilleri G, Yao L. Insulin versus blood purification in the early management of hypertriglyceridaemia-associated acute pancreatitis: Systemic review and meta-analysis. 2020 EPC/IAP S1424-3903(22):00469-0 abstract. doi: 10.1016/j.pan.2020.07.108

50. Bruce JIE, Sanchez-Alvarez R, Sans MD, Sugden SA, Qi N, James AD, et al. Insulin protects acinar cells during pancreatitis by preserving glycolytic atp supply to calcium pumps. *Nat Commun* (2021) 12(1):4386. doi: 10.1038/s41467-021-24506-w

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 13 October 2022
DOI 10.3389/fendo.2022.961762

OPEN ACCESS

EDITED BY
Maria João Meneses,
Universidade Nova de Lisboa,
Portugal

REVIEWED BY
Lorena Mardones Leiva,
Universidad Católica de la Santísima
Concepción, Chile
Jiaqi Li,
Sichuan University, China
Yuli Huang,
Southern Medical University, China

*CORRESPONDENCE
Wei Wang
wangw253@mail.sysu.edu.cn

†These authors have contributed
equally to this work and share
first authorship

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

RECEIVED 05 June 2022
ACCEPTED 28 September 2022
PUBLISHED 13 October 2022

CITATION
Zheng D, Zhang X, You L, Li F, Lin D,
Sun K, Ren M, Yan L and Wang W
(2022) The association of liver
enzymes with diabetes mellitus risk
in different obesity subgroups:
A population-based study.
Front. Endocrinol. 13:961762.
doi: 10.3389/fendo.2022.961762

The association of liver enzymes with diabetes mellitus risk in different obesity subgroups: A population-based study

Dinghao Zheng†, Xiaoyun Zhang†, Lili You, Feng Li,
Diaozhu Lin, Kan Sun, Meng Ren, Li Yan and Wei Wang*

Department of Endocrinology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China

Background: Numerous observational studies have shown that liver enzymes correlated with diabetes mellitus (DM) risk significantly, but limited studies showed whether different obesity subgroups present the same correlation. Our objective was to evaluate the association of liver enzymes with DM risk in different obesity subgroups based on a middle-aged Chinese population.

Methods: We conducted a population-based cross-sectional study and surveyed 9,916 people aged 40 years and above. A two-slope linear regression model was used to analyze the cutoff points of obesity in DM risk. Restricted cubic splines were used to analyze the correlation between liver enzymes and DM risk in different obesity categories. The odds ratios and 95% confidence intervals (CIs) were calculated using the logistic regression model.

Results: The cutoff points of body mass index (BMI) and waist circumference were 30.55 kg/m^2 and 98.99 cm for DM risk, respectively. The serum gamma-glutamyl transferase (GGT) concentration was positively correlated with DM risk in the subgroups with waist circumference <98.99 cm [OR = 1.04, 95% CI (1.03–1.05)], BMI <30.55 kg/m^2 [OR = 1.04, 95% CI (1.03–1.05)], and BMI ≥30.55 kg/m^2 [OR = 1.18, 95% CI (1.04–1.39)], but not in the subgroup with waist circumference ≥98.99 cm. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations have no significant correlation with the risk of diabetes in all groups.

Conclusion: The results showed that serum GGT concentration was correlated with DM risk but not with AST or ALT in the middle-aged population. However, the correlation disappeared when waist circumference was over 98.99 cm, and serum GGT concentration had a limited value for DM risk in waist circumference over 98.99 cm.

KEYWORDS

GGT, AST, ALT, diabetes mellitus, obesity

Introduction

According to the International Diabetic Federation diabetes atlas (10th edition, 2021), the global prevalence of diabetes has reached 10.5% and is expected to rise to 11.3% in 2030 and 12.2% in 2045. Approximately 6.7 million people (20–79 years old) died of diabetes or its complications in 2021, accounting for approximately 12.2% of all deaths worldwide (1, 2). According to an epidemiological survey, the prevalence of diabetes mellitus in mainland China was 12.8% in 2017, indicating that diabetes mellitus has become an important public health problem in the world, especially in China (3). Therefore, identifying the high-risk population of diabetes is important to solving the public diabetes burden.

Numerous observational studies indicated that liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were positively correlated with the risk of diabetes mellitus and metabolic syndrome, especially serum GGT (4–11). The liver plays an important role in maintaining the homeostasis of glucose metabolism, and liver enzyme concentrations are positively correlated with liver damage. Serum ALT and AST are released from the injured hepatocytes. GGT widely exists on the surface of cell membranes and is a key participant in the metabolism of glutathione. Glutathione is an important cellular antioxidant, which is closely related to inflammation and oxidative stress in tissues (12, 13), and oxidative stress and inflammation play an important role in the development of insulin resistance (14).

Obesity refers to a state that is obviously overweight, caused by the excessive accumulation of body fat (15). Obesity can promote global inflammation and peripheral insulin resistance, increasing the prevalence of various metabolic diseases, such as diabetes and non-alcoholic fatty liver disease (16, 17). According to a 2015–2017 national cross-sectional study in China, the incidence of overweight and obesity is 30.1% and 11.9%, respectively, and the proportion of the population with a body mass index (BMI) ≥30 kg/m^2 is 6.3%. The prevalence of diabetes mellitus (DM) in BMI <25 kg/m^2, 25 kg/m^2 ≤ BMI < 30 kg/m^2, and BMI ≥30 kg/m^2 is 8.8%, 13.8%, and 20.1%, respectively (3).

To better assess the correlation between liver enzymes and DM risk, obesity classification should be considered. However, limited studies showed whether obesity plays a role in the correlation between liver enzymes and the risk of diabetes mellitus. So, we conducted a large population cross-sectional study in Guangzhou, China, to clarify the relationship and provided guidance for identifying a high-risk population of diabetes mellitus.

Method

Study populations

From June to November 2011, we performed a community-based cross-sectional research in Guangzhou, China. The participants in this study were selected from the Risk Evaluation of Cancers in Chinese Diabetic Individuals: A longitudinal (REACTION) research project, which has established a multicenter prospective observational study to evaluate chronic diseases in the Chinese population (18). The study population, design, and protocol have been previously described (19). Briefly, through inspection notices or home visits, a total of 10,104 residents aged 40 or over were invited to participate. A total of 9,916 individuals accepted to participate in the poll after signing the permission form, and the participation rate was 98.1%. Among these participants, individuals who failed to provide BMI (n = 278), waist circumference (WC) (n = 229), liver enzymes (n = 2,127, one or more among the AST, ALT, GGT), blood glucose (n = 230), and diabetes history information (n = 47) were excluded, and individuals with diabetes history were also excluded (n = 691) (Figure 1). Finally, a total of 6,434 qualified individuals were included in the final data analysis.

The research protocol has been approved by the Institutional Review Committee of Sun Yat-sen Memorial Hospital affiliated to Sun Yat-Sen University and complies with the principles of the Declaration of Helsinki II. Each participant gave written informed consent before data were collected.

Measurements

We collected information about lifestyle factors, sociodemographic characteristics, education information, marital status, and family history of diabetes using standard questionnaires. Lifestyle factors include smoking and drinking. Smoking or drinking habits are classified as “never,” “current” (smokers or drinks regularly in the past 6 months), or “never” (stops smoking or drinking for more than 6 months).

All participants used standard procedures to complete the anthropometric measurements with the assistance of trained personnel. With automatic electronic equipment (OMRON, Omron Company, China), blood pressure measurements were performed three consecutive times by the same observer at 5-min intervals. The analysis was performed using the average of three blood pressure measurements. Participants wore light clothing and had no shoes, and their height and weight were measured to be within 0.1 cm and 0.1 kg, respectively. BMI was computed by multiplying body weight (kg) by height (square meter) (kg/m^2). WC was measured at the level of the umbilical cord when the participant is standing and at the end of a mild exhalation.

After an overnight fast for at least 10 h, a venous blood sample was collected for laboratory testing. Measurement of fasting blood glucose (FPG), fasting serum insulin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), GGT, AST, and ALT was performed using an automatic analyzer (Beckman CX-7 Biochemical Autoanalyzer, Brea, CA, USA). Hemoglobin A1c (HbA1c) was evaluated by

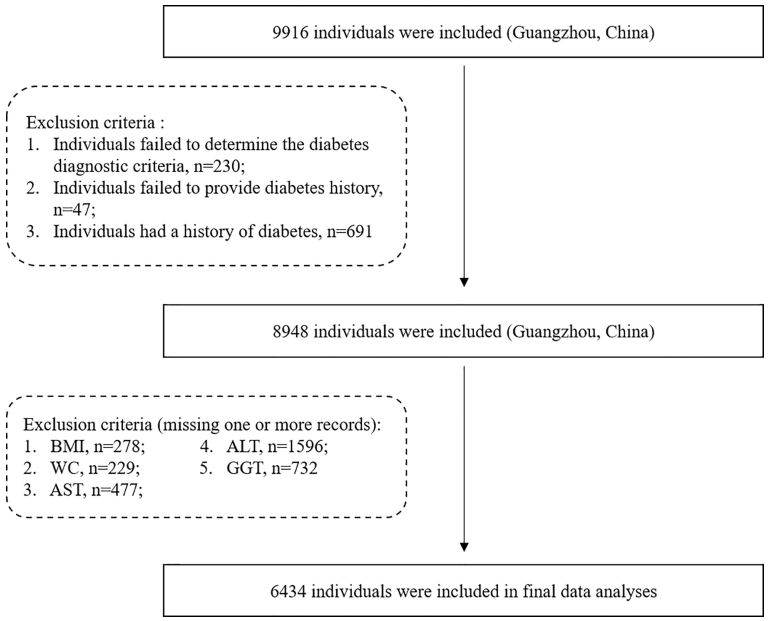


FIGURE 1
Study population flowchart.

high-performance liquid chromatography (Bio-Rad, Hercules, CA, USA). Diabetes is diagnosed according to the 1999 World Health Organization's diagnostic criteria, including fasting blood glucose greater than or equal to 7.0 mmol/L and/or OGTT 2 h greater than or equal to 11.1 mmol/L.

Statistical analysis

Continuous variables were presented as means ± standard deviation (SD). Skewed variables were presented as medians (interquartile ranges). Categorical variables were expressed as proportions. Differences among groups were tested by one-way ANOVA, and *post-hoc* comparisons were performed by using Bonferroni correction. Comparisons between categorical variables were performed with the χ^2 test.

We performed the two-slope regression model to determine the relationship between BMI or WC and the risk of diabetes to analyze the cutoff points for DM risk.

Linear regression and logistic regression were performed to calculate the odds ratios (ORs) of diabetes and 95% confidence intervals (95% CIs) after adjusting for age, gender, BMI, SBP, TG, and HDL. Restricted cubic splines were performed to visualize the shape of the dose–response association among the AST, ALT, GGT, and odds ratio of diabetes, respectively. All statistical analyses were performed using RStudio version 3.6.1. A two-tailed p <0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

The clinical characteristics of the study population are shown in Table 1. The mean age of the diabetes group versus the non-diabetes group was 57.6 (7.00) vs. 54.7 (6.68). The diabetes group also had higher BMI, WC, DBP, SBP, CHOL, TG, and LDL and lower HDL (all p for trend <0.001). In addition, compared with the non-diabetes group, the diabetes group had higher serum GGT levels (24.0 vs. 19.0 U/L, $p < 0.001$) and higher serum ALT levels (13.0 vs. 12.0 U/L, $p < 0.001$), but with no significant difference for AST levels (19.0 vs. 18.0 U/L, $p = 0.163$).

The effect of BMI and WC on the risk of diabetes

In order to explore the appropriate cutoff points of BMI and WC for the risk of diabetes, we performed the two-slope regression model to visualize the association of BMI or WC on the risk of diabetes. The cutoff points for BMI and WC were 30.55 kg/m^2 and 98.99 cm, respectively (Figure 2). The results showed that when BMI or WC exceeds 30.55 kg/m^2 or 98.99 cm, respectively, the risk of diabetes will increase significantly.

10.3389/fendo.2022.961762

TABLE 1 Characteristics of the study population.

Characteristics	Group		
	Non-diabetes	Diabetes	*p*
Male, *n* (%)	1,511 (27.2%)	238 (27.1%)	0.989
Age, mean (SD)	54.7 (6.68)	57.6 (7.00)	< 0.001
Status of marriage, *n* (%)	4,987 (90.3%)	772 (88.6%)	0.046
Elementary school and below, *n* (%)	564 (10.4%)	145 (17.0%)	< 0.001
Smoking, *n* (%)	514 (9.38%)	73 (8.48%)	0.622
Drinking, *n* (%)	154 (2.82%)	27 (3.13%)	0.280
Family history of diabetes, *n* (%)	853 (15.6%)	187 (21.8%)	< 0.001
Height, mean (SD)	158 (7.20)	158 (6.74)	0.007
Weight, mean (SD)	58.1 (8.54)	60.5 (8.34)	< 0.001
BMI, mean (SD)	23.2 (2.83)	24.4 (2.92)	< 0.001
WC, mean (SD)	80.3 (8.40)	84.3 (8.39)	< 0.001
HC, mean (SD)	93.4 (6.13)	94.7 (6.19)	< 0.001
SBP, mean (SD)	125 (16.0)	133 (16.8)	< 0.001
DBP, mean (SD)	74.8 (9.77)	77.6 (9.77)	< 0.001
HR, mean (SD)	80.4 (10.3)	82.9 (10.7)	< 0.001
CHOL, mean (SD)	5.25 (1.06)	5.44 (1.10)	< 0.001
TG, median (IQR)	1.19 [0.89; 1.62]	1.49 [1.10; 1.97]	< 0.001
HDL, mean (SD)	1.36 (0.33)	1.27 (0.32)	< 0.001
LDL, mean (SD)	3.18 (0.84)	3.31 (0.90)	< 0.001
AST, median (IQR)	18.0 [16.0; 21.0]	19.0 [16.0; 22.0]	0.163
ALT, median (IQR)	12.0 [9.00; 16.0]	13.0 [9.00; 17.0]	< 0.001
GGT, median (IQR)	19.0 [14.0; 25.0]	24.0 [18.0; 31.0]	< 0.001

The curve correlation between liver enzymes and DM risk

We explored the correlation between liver enzymes and the risk of DM in different BMI and WC subgroups, according to the cutoff point above, using restricted cubic spline graphs. The results showed that in the population with BMI <30.55 kg/m^2, ALT and AST have no curvilinear correlation with the risk of DM (p = 0.362 and p = 0.840, respectively), and GGT has a curvilinear correlation with the risk of DM (p < 0.001) (Figures 3A–C). In the population with BMI ≥30.55 kg/m^2, liver enzymes have no curvilinear correlation with the risk of DM (all p > 0.05) (Figures 3D–F). According to WC, in the population with WC <98.99 cm, our study found that ALT and AST have no curvilinear correlation with the risk of DM (p = 0.382 and p = 0.935, respectively), and GGT has a curve correlation with the risk of DM (p < 0.001) (Figures 3G–I). It is particularly worth noting that in the population with a WC ≥98.99 cm, although the statistical analysis of the curve relationship has no significance (all p > 0.05), the restricted

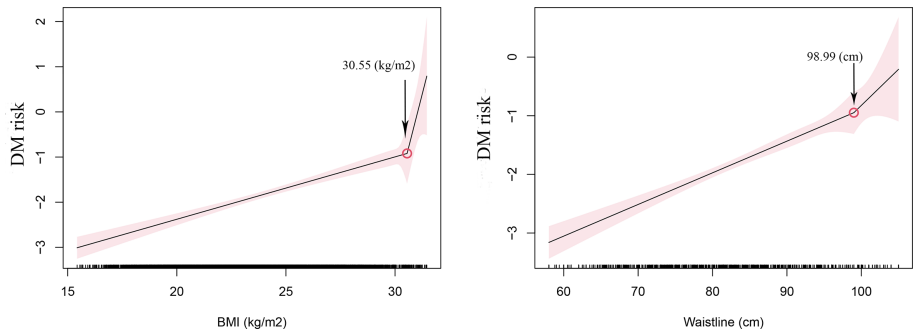


FIGURE 2
The effect of body mass index (BMI) and waist circumference (WC) on the risk of diabetes.

10.3389/fendo.2022.961762

cubic spline graph showed a U-shaped relationship between liver enzymes and DM risk (Figures 3J–L).

The association of liver enzymes with DM risk in different BMI subgroups

In our study, we grouped the subjects according to the cutoff point of BMI and explored the relationship between liver enzymes and the risk of diabetes in different groups. The results of the study showed that only GGT levels have a significant correlation with the DM risk (Table 2). After adjusting for confounding factors, the OR (95% CI) is 1.04 (1.03–1.05, $p < 0.001$) in the population with BMI <30.55 kg/m^2, and the OR (95% CI) is 1.18 (1.04–1.39, $p < 0.024$) in the population with BMI ≥30.55 kg/m^2. As for AST and ALT, after adjusting for confounding factors, the ORs (95% CI) were 0.98 (0.96–1.01, $p = 0.094$) and 1.00 (0.99–1.02, $p = 0.573$) in the

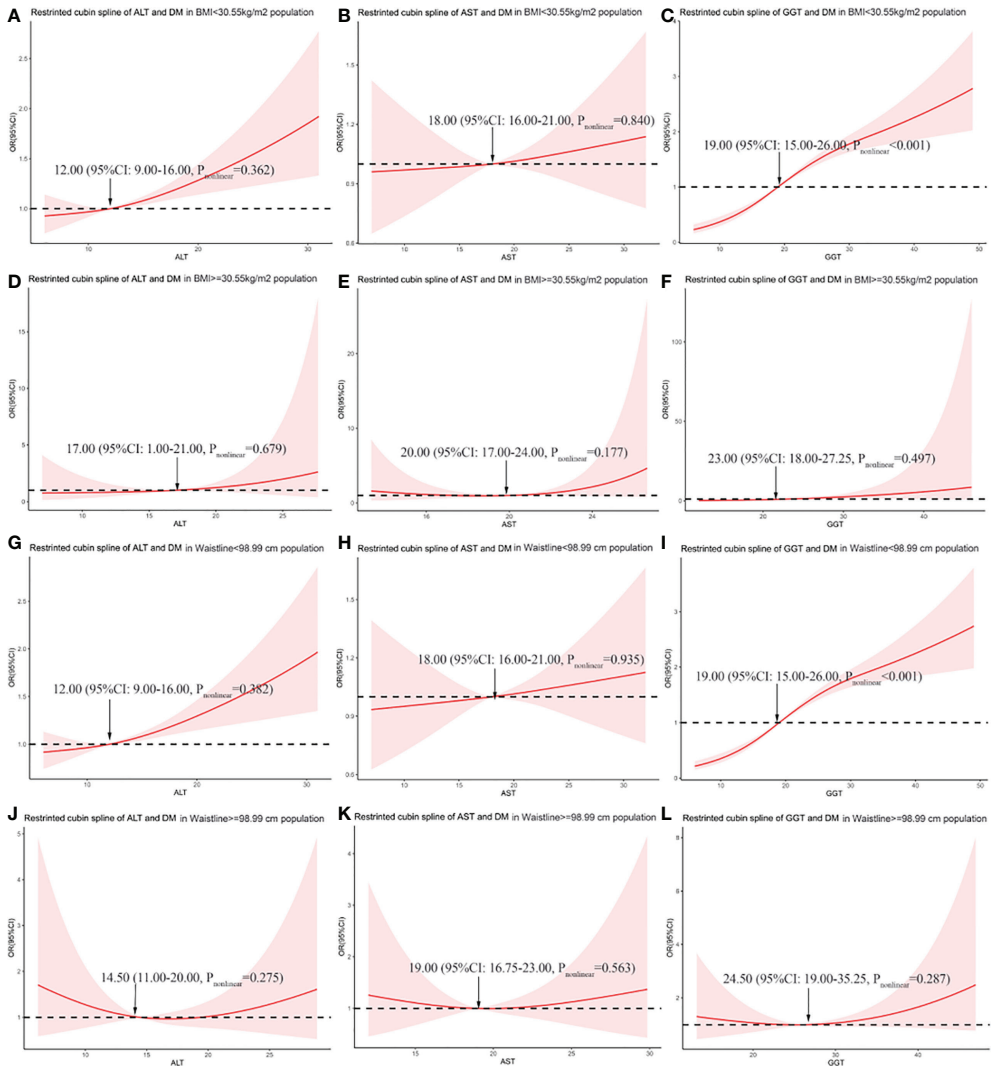


FIGURE 3
The association between liver enzymes and the risk of diabetes in different BMI and WC subgroups. **(A)** Association between alanine aminotransferase (ALT) and odds ratio (OR) in the subgroup with BMI <30.55 kg/m^2. **(B)** Association between aspartate aminotransferase (AST) and OR in the subgroup with BMI <30.55 kg/m^2. **(C)** Association between gamma-glutamyl transferase (GGT) and OR in the subgroup with BMI <30.55 kg/m^2. **(D)** Association between ALT and OR in the subgroup with BMI ≥30.55 kg/m^2. **(E)** Association between AST and OR in the subgroup with BMI ≥30.55 kg/m^2. **(F)** Association between GGT and OR in the subgroup with BMI ≥30.55 kg/m^2. **(G)** Association between ALT and OR in the subgroup with WC <98.99 cm. **(H)** Association between AST and OR in the subgroup with WC <98.99 cm. **(I)** Association between GGT and OR in the subgroup with WC <98.99 cm. **(J)** Association between ALT and OR in the subgroup with WC ≥98.99 cm. **(K)** Association between AST and OR in the subgroup with WC ≥98.99 cm. **(L)** Association between GGT and OR in the subgroup with WC ≥98.99 cm.

subgroup with BMI <30.55 kg/m^2, respectively. Moreover, the ORs (95% CI) were 1.16 (0.98–1.39, p = 0.095) and 1.05 (0.94–1.20, p = 0.412) in the subgroup with BMI ≥30.55 kg/m^2.

The association of liver enzymes with DM risk in different WC subgroups

Subjects were grouped according to the cutoff points of WC. We explored the association between liver enzymes and DM in different WC subgroups (Table 3). After adjusting for confounding factors, the results showed that GGT only has a positive correlation with DM risk in the population with WC <98.99 cm, and the OR (95% CI) is 1.04 (1.03–1.05, p < 0.001). In the population with WC ≥98.99 cm, the serum GGT has no significant correlation with DM risk, and the OR (95% CI) is 1.02 (0.98–1.06, p = 0.365). As for ASL and ALT, they had no significant correlation with DM risk in both WC subgroups.

According to the restricted cubic spline graph (Figures 3J–L), in the population with WC ≥98.99 cm, liver enzymes show a U-shaped relationship with DM risk. Therefore, we divided them into three categories according to the distribution of liver enzymes. AST was divided into three categories according to 16.75 and 23.00 U/L, and ALT was divided according to 11.00 and 20.00 U/L. GGT was divided based on 19.00 and 32.25 U/L (Table 3). For all liver enzymes, the middle range group was used as the reference group (the ALT reference category was 16.75–23.00 U/L, the AST reference category was 11.00–20.00 U/L, and the GGT reference category was 19.00–32.25 U/L). The results showed that liver enzymes have no correlation with the risk of diabetes significantly in the population with WC ≥98.99 cm (all p > 0.05).

Discussion

In this manuscript, we focused on the relationship between liver enzymes and DM risk in different obesity subgroups in a cross-sectional study. We got the cutoff points of BMI and WC according to the two-slope linear regression model, and restricted cubic splines were used to analyze the correlation between liver enzymes and DM risk in different classes of the obese population divided according to cutoff points. Our results showed that only serum GGT level was correlated with DM risk instead of ALT and AST. It is worth noting that the correlation disappeared in the subgroup with WC ≥98.99 cm.

For obesity categories, previous studies conventionally grouped individuals according to the WHO criterion or percentile (20, 21). Our obesity classifications were not based on these criteria, considering that the criteria cannot reflect DM risk accurately. We conducted a two-slope regression model to find out the cutoff point where the DM risk increased faster. The cutoff points were 98.99 cm and 30.55 kg/m^2, respectively. Interestingly, the BMI cutoff point was similar to the WHO obesity classification (BMI ≥ 30 kg/m^2); however, the Chinese always use the Asian obesity classification (BMI > 28 kg/m^2). Moreover, the WC cutoff point was much higher than the abdominal obesity classification (WC > 90 cm for men and WC > 85 cm for women). We used the common criteria as the cutoff point to repeat the analysis, and the results showed a significant association between GGT and DM risk in all groups (Table S1). The results implied that different regional populations with different ages may need more special criteria for DM risk and other obesity complications.

Our study showed that after grouping by BMI or WC, there was no significant correlation between ALT or AST and DM risk, and only GGT was correlated in some subgroups. The results were consistent with previous studies (4, 22–24). The results suggested that GGT levels were more significant for evaluating DM risk than other liver enzymes. However, there were other studies that showed conflicting results. A population study in Europe showed that ALT was significantly correlated with impaired glucose tolerance (IGT) but not with GGT or AST (25), suggesting that ALT

TABLE 2 The linear regression model of the relationship between liver enzymes and DM risk in BMI subgroups.

Liver enzymes category	Fold change (95% CI) of DM risk per unit increase in liver enzymes			
	Unadjusted model		Adjusted model[a]	
BMI < 30.55 kg/m^2 (n = 6,386)				
AST	1.01 (0.99–1.02)	p = 0.398	0.98 (0.96–1.01)	p = 0.094
ALT	1.03 (1.01–1.04)	p < 0.001	1.00 (0.99–1.02)	p = 0.573
GGT	1.05 (1.04–1.06)	p < 0.001	1.04 (1.03–1.05)	**p < 0.001**
BMI ≥ 30.55 kg/m^2 (n = 48)				
AST	1.08 (0.95–1.24)	p = 0.263	1.16 (0.98–1.39)	p = 0.095
ALT	1.06 (0.97–1.16)	p = 0.202	1.05 (0.94–1.20)	p = 0.412
GGT	1.13 (1.05–1.26)	p = 0.001	1.18 (1.04–1.39)	**p = 0.024**

[a]Covariates in the adjusted model: gender, age, BMI, SBP, TG, and HDL.
Bold value are statistically significant.

10.3389/fendo.2022.961762

TABLE 3 Regression model of the relationship between liver enzymes and DM risk in WC subgroups.

Liver enzymes category	Fold change (95% CI) of DM risk per unit or class increase in liver enzymes			
	Unadjusted model		Adjusted model[a]	
Waistline < 98.99cm[b] (n = 6,290)				
AST	1.01 (0.99–1.02)	p = 0.369	0.99 (0.97–1.01)	p = 0.161
ALT	1.03 (1.02–1.04)	p < 0.001	1.01 (0.99–1.02)	p = 0.388
GGT	1.05 (1.04–1.06)	p < 0.001	1.04 (1.03–1.05)	**p < 0.001**
Waistline ≥ 98.99 cm[b] (n = 144)				
AST	1.01 (0.94–1.08)	p = 0.891	1.00 (0.92–1.09)	p = 0.958
ALT[b]	1.00 (0.95–1.05)	p = 0.984	0.98 (0.92–1.04)	p = 0.558
GGT[b]	1.02 (0.99–1.05)	p = 0.260	1.02 (0.98–1.06)	p = 0.365
Waistline ≥ 98.99 cm[c]				
AST				
≤16.75	1.42 (0.58–3.40)	p = 0.436	1.29 (0.47–3.47)	p = 0.618
16.75–23.00	1.00	–	1.00	–
≥23.00	1.97 (0.85–4.57)	p = 0.111	2.16 (0.80–5.88)	p = 0.128
ALT				
≤11.00	1.57 (0.68–3.65)	p = 0.288	1.78 (0.67–4.82)	p = 0.249
11.00–20.00	1.00	–	1.00	–
≥20.00	1.67 (0.69–4.07)	p = 0.254	1.29 (0.46–3.57)	p = 0.627
GGT				
≤19.00	1.09 (0.45–2.55)	p = 0.845	1.67 (0.60–4.72)	p = 0.326
19.00–35.25	1.00	–	1.00	–
≥32.25	1.56 (0.66–3.65)	p = 0.305	2.07 (0.75–5.78)	p = 0.127

[a]Covariates in the adjusted model: gender, age, BMI, SBP, TG, and HDL.
[b]Linear regression models for liver enzymes.
[c]Logistic regression models for the liver enzyme category.
Bold value are statistically significant.

and prediabetes are closely related. However, the study had limitations. The sample size of that study was 157, and it only adjusted for age and gender. A population study in China showed that GGT levels were not significantly associated with HOMA-IR in non-alcoholic fatty liver disease patients (NAFLD). The study only included 212 patients. The sample size may not be powerful and the HOMA-IR was not equal to DM risk (26). Another prospective study in Korea that included 548 patients showed that GGT levels were associated with DM risk only in women but not in men. Our study population mainly consisted of women, which may lead to conflicting results (4). A Mendelian randomization study showed that genetically higher ALT was associated with a higher DM risk but not GGT (27). However, the Mendelian randomization study was based on genes, not considering environment function and compensation function, and we cannot exclude the possibility that GGT was associated with DM risk. GGT is a protein that exists widely on the cell membrane and is closely related to the metabolism of glutamate in cells. GGT regulates the level of oxidative stress in cells and tissues, which is closely related to diabetes mellitus risk (14). In this respect, GGT has a more reasonable metabolic relevance, which partly explains why GGT in liver enzymes has a more significant diabetes risk association than ALT or AST.

We observed that GGT was associated with DM risk in different subgroups, but this association disappeared in the group with WC ≥98.99 cm. The result showed that the association will be disturbed for severe abdominal obesity individuals. A 15,792 middle-aged community-based prospective cohort study in the USA showed that GGT was associated with DM risk, after adjusting for body mass index, WC, and other confounding factors (28). The result was inconsistent with ours to some extent, and for this reason, we cannot get the association in the group with WC ≥98.99 cm. The contradiction may rely on WC stratification. The possible mechanism underlying the phenomenon was unclear and may be proposed as follows. Severe abdominal obesity may be a

confounding factor for GGT and DM risk, which was similar to other points (29). Abdominal obesity with increased visceral fat is closely related to systemic inflammation and oxidative stress, and abnormal oxidative stress usually leads to increased serum GGT concentrations (12). So, patients with severe abdominal obesity, who are at high DM risk, may be accompanied by elevated serum GGT concentration raised from abnormal oxidative stress and led to the confounding associations between GGT and DM. The other explanation was that the risk factors for the development of diabetes mellitus in the population with severe abdominal obesity were not similar in other groups. Furthermore, the risk of DM reflected by GGT may not play a major role in a population with severe abdominal obesity, and GGT will also show different associations with DM risk in a population with different pathophysiological states.

Our research also has certain limitations. The major limitation is that the data of the ultrasonic diagnosis of fatty liver were incomplete, considering that the liver enzymes were highly associated with fatty liver and we could not exclude the confounding factor. Secondly, our study has a cross-sectional design. Causal inferences could not be drawn between serum GGT and DM risk among different subgroups, so more in-depth prospective studies may be needed to prove it. Thirdly, in the subgroup of people with WC ≥98.99 cm, the disappearance of the relationship may lie in the sample size of the subgroups (n = 144), and we should get more samples to ensure the associations. Lastly, the population in this study consists of middle-aged individuals aged >40 years old in South China and cannot represent the younger population and the North China population. Furthermore, the population mainly consists of women, partially because we invited residents over the age of 40 years and women are predominant in this age range in China. The pathophysiological mechanism of this research still needs to be further clarified.

In summary, in this cross-sectional study of a large population, we found that increased serum GGT levels are correlated with the risk of diabetes, and showed different effects in subgroups with different BMI or WC values. Serum GGT may be a better reference marker for predicting the risk of diabetes mellitus than AST or ALT in clinical practice. Individuals with elevated liver enzymes, especially GGT, should be alert to the risk of diabetes mellitus. However, GGT has limitations on DM risk in a population with severe abdominal obesity. For this population, even elevated GGT cannot reflect the risk of diabetes mellitus, and other biomarkers should be considered.

Conclusion

Our study suggests that serum GGT levels have greater reference significance than AST or ALT for the risk of diabetes in the middle-aged population. Moreover, GGT levels correlate with DM risk except for those with severe abdominal obesity. In clinical practice, GGT should be combined with WC to determine DM risk.

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 Institutional Review Committee of Sun Yat-sen Memorial Hospital affiliated to Sun Yat-Sen University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Funding

This study was supported by grants from the National Science Foundation of China (U20A20352) and Guang Dong Clinical Research Center for Metabolic Diseases (2020B1111170009). The funders were not involved in research design, data collection and analysis, preparation of the manuscript, or decision to publish.

Acknowledgments

We are indebted to the participants in this study for their continued and excellent support and our colleagues for their valuable 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

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/fendo.2022.961762/full#supplementary-material

References

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

2. International Diabetes Federation. *IDF diabetes atlas*. Brussels, Belgium: International Diabetes Federation (2021).

3. 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

4. Ahn H-R. The association between liver enzymes and risk of type 2 diabetes: the namwon study. *Diabetol Metab Syndr* (2014) 6(1):14. doi: 10.1186/1758-5996-6-14

5. Bonnet F, Ducluzeau PH, Gastaldelli A, Laville M, Anderwald CH, Konrad T, et al. Liver enzymes are associated with hepatic insulin resistance, insulin secretion, and glucagon concentration in healthy men and women. *Diabetes* (2011) 60:1660–7. doi: 10.2337/db10-1806

6. Cho HC. The association between serum GGT concentration and diabetic peripheral polyneuropathy in type 2 diabetic patients. *Korean Diabetes J* (2010) 34:111–8. doi: 10.4093/kdj.2010.34.2.111

7. Doi Y. Liver enzymes as a predictor for incident diabetes in a Japanese population: the hisayama study. *Obes (Silver Spring)* (2007) 15:1841–50. doi: 10.1038/oby.2007.218

8. Ford ES, Schulze MB, Bergmann MM, Thamer C, Joost HG, Boeing H. Liver enzymes and incident diabetes: Findings from the European prospective investigation into cancer and nutrition (EPIC)-potsdam study. *Diabetes Care* (2008) 31:1138–43. doi: 10.2337/dc07-2159

9. Kawamoto R, Tabara Y, Kohara K, Miki T, Ohtsuka N, Kusunoki T, et al. Serum gamma-glutamyl transferase within its normal concentration range is related to the presence of impaired fasting glucose and diabetes among Japanese community-dwelling persons. *Endocr Res* (2011) 36:64–73. doi: 10.3109/07435800.2010.534756

10. Lee YS, Cho Y, Burgess S, Davey Smith G, Relton CL, Shin SY, et al. Serum gamma-glutamyl transferase and risk of type 2 diabetes in the general Korean population: A mendelian randomization study. *Hum Mol Genet* (2016) 25:3877–86. doi: 10.1093/hmg/ddw226

11. Nano J, Muka T, Ligthart S, Hofman A, Darwish Murad S, Janssen HLA, et al. Gamma-glutamyltransferase levels, prediabetes and type 2 diabetes: A mendelian randomization study. *Int J Epidemiol* (2017) 46:1400–9. doi: 10.1093/ije/dyx006

12. Hogg N. S-nitrosoglutathione as a substrate for gamma-glutamyl transpeptidase. *Biochem J* (1997) 323:477–81. doi: 10.1042/bj3230477

13. Wickham S, West MB, Cook PF, Hanigan MH. Gamma-glutamyl compounds: substrate specificity of gamma-glutamyl transpeptidase enzymes. *Anal Biochem* (2011) 414:208–14. doi: 10.1016/j.ab.2011.03.026

14. Yan Y, Li S, Liu Y, Bazzano L, He J, Mi J, et al. Temporal relationship between inflammation and insulin resistance and their joint effect on hyperglycemia: the bogalusa heart study. *Cardiovasc Diabetol* (2019) 18:109. doi: 10.1186/s12933-019-0913-2

15. Gonzalez-Muniesa P, Martinez-Gonzalez MA, Hu FB, Despres JP, Matsuzawa Y, Loos RJF, et al. Obesity. *Nat Rev Dis Primers* (2017) 3:17034. doi: 10.1038/nrdp.2017.34

16. Sjöström L. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* (2004) 351:2689–93. doi: 10.1056/NEJMoa035622

17. Malnick SD, Beergabel M, Knobler H. Non-alcoholic fatty liver: A common manifestation of a metabolic disorder. *QJM* (2003) 96:699–709. doi: 10.1093/qjmed/hcg120

18. Ning G, Reaction Study G. Risk evaluation of cAncers in Chinese diabeTic individuals: A lONgitudinal (REACTION) study. *J Diabetes* (2012) 4:172–3. doi: 10.1111/j.1753-0407.2012.00182.x

19. Lin D, Sun K, Li F, Qi Y, Ren M, Huang C, et al. Association between habitual daytime napping and metabolic syndrome: A population-based study. *Metabolism* (2014) 63:1520–7. doi: 10.1016/j.metabol.2014.08.005

20. German CA, Laughey B, Bertoni AG, Yeboah J. Associations between BMI, waist circumference, central obesity and outcomes in type II diabetes mellitus: The ACCORD trial. *J Diabetes Complications* (2020) 34:107499. doi: 10.1016/j.jdiacomp.2019.107499

21. Wang Y, Shirore RM, Ramakrishnan C, Tan NC, Jafar TH. Adiposity measures and pre-diabetes or diabetes in adults with hypertension in Singapore polyclinics. *J Clin Hypertens (Greenwich)* (2019) 21:953–62. doi: 10.1111/jch.13587

22. Hozawa A. Relation of gamma-glutamyltransferase and alcohol drinking with incident diabetes: the HIPOP-OHP study. *J Atheroscler Thromb* (2010) 17:195–202. doi: 10.5551/jat.3202

23. Lee DH, Silventoinen K, Jacobs DRJr., Jousilahti P, Tuomileto J. Gamma-glutamyltransferase, obesity, and the risk of type 2 diabetes: observational cohort study among 20,158 middle-aged men and women. *J Clin Endocrinol Metab* (2004) 89:5410–4. doi: 10.1210/jc.2004-0505

24. Oberlinner C, Zober A, Nawroth PP, Humpert PM, Morcos M. Alanine-aminotransferase levels predict impaired glucose tolerance in a worksite population. *Acta Diabetol* (2010) 47:161–5. doi: 10.1007/s00592-009-0148-x

25. Shiraishi M, Tanaka M, Okada H, Hashimoto Y, Nakagawa S, Kumagai M, et al. Potential impact of the joint association of total bilirubin and gamma-glutamyltransferase with metabolic syndrome. *Diabetol Metab Syndr* (2019) 11:12. doi: 10.1186/s13098-019-0408-z

26. Sheng X, Che H, Ji Q. The relationship between liver enzymes and insulin resistance in type 2 diabetes patients with nonalcoholic fatty liver disease. *Horm Metab Res* (2018) 50(5):397–402. doi: 10.1055/a-0603-7899

27. Liu J, Au Yeung SL, Lin SL, Leung GM, Schooling CM. Liver enzymes and risk of ischemic heart disease and type 2 diabetes mellitus: A mendelian randomization study. *Sci Rep* (2016) 6:38813. doi: 10.1038/srep38813

28. Schneider ALC, Lazo M, Ndumele CE, Pankow JS, Coresh J, Clark JM, et al. Liver enzymes, race, gender and diabetes risk: The atherosclerosis risk in communities (ARIC) study. *Diabetic Med* (2013) 30:926–33. doi: 10.1111/dme.12187

29. Wen J, Liang Y, Wang F, Sun L, Guo Y, Duan X, et al. C-reactive protein, gamma-glutamyltransferase and type 2 diabetes in a Chinese population. *Clin Chim Acta* (2010) 411:198–203. doi: 10.1016/j.cca.2009.11.002

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 25 October 2022
DOI 10.3389/fendo.2022.1032814

OPEN ACCESS

EDITED BY
Sonia Michael Najjar,
Ohio University, United States

REVIEWED BY
Sivarajan Kumarasamy,
Ohio University, United States
Assaad Antoine Eid,
American University of Beirut, Lebanon

*CORRESPONDENCE
Jiayu Duan
jyduan@zzu.edu.cn
Jiancheng Dong
dongjc@ntu.edu.cn
Zhangsuo Liu
zhangsuoliu@zzu.edu.cn

†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 31 August 2022
ACCEPTED 03 October 2022
PUBLISHED 25 October 2022

CITATION
Yan Q, Zhao Z, Liu D, Li J, Pan S, Duan J, Dong J and Liu Z (2022) Integrated analysis of potential gene crosstalk between non-alcoholic fatty liver disease and diabetic nephropathy. *Front. Endocrinol.* 13:1032814. doi: 10.3389/fendo.2022.1032814

Integrated analysis of potential gene crosstalk between non-alcoholic fatty liver disease and diabetic nephropathy

Qianqian Yan[1,2†], Zihao Zhao[1,2†], Dongwei Liu[1,2,3,4], Jia Li[1,2,3,4], Shaokang Pan[1,2,3,4], Jiayu Duan[1,2,3,4*], Jiancheng Dong[2*] and Zhangsuo Liu[1,2,3,4*]

[1]Department of Integrated Traditional and Western Nephrology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [2]Research Institute of Nephrology, Zhengzhou University, Zhengzhou, China, [3]Henan Province Research Center for Kidney Disease, Zhengzhou, China, [4]Key Laboratory of Precision Diagnosis and Treatment for Chronic Kidney Disease in Henan Province, Zhengzhou, China

Background: Growing evidence indicates that non-alcoholic fatty liver disease (NAFLD) is related to the occurrence and development of diabetic nephropathy (DN). This bioinformatics study aimed to explore optimal crosstalk genes and related pathways between NAFLD and DN.

Methods: Gene expression profiles were downloaded from Gene Expression Omnibus. CIBERSORT algorithm was employed to analyze the similarity of infiltrating immunocytes between the two diseases. Protein–protein interaction (PPI) co-expression network and functional enrichment analysis were conducted based on the identification of common differentially expressed genes (DEGs). Least absolute shrinkage and selection operator (LASSO) regression and Boruta algorithm were implemented to initially screen crosstalk genes. Machine learning models, including support vector machine, random forest model, and generalized linear model, were utilized to further identify the optimal crosstalk genes between DN and NAFLD. An integrated network containing crosstalk genes, transcription factors, and associated pathways was developed.

Results: Four gene expression datasets, including GSE66676 and GSE48452 for NAFLD and GSE30122 and GSE1009 for DN, were involved in this study. There were 80 common DEGs between the two diseases in total. The PPI network built with the 80 common genes included 77 nodes and 83 edges. Ten optimal crosstalk genes were selected by LASSO regression and Boruta algorithm, including *CD36, WIPI1, CBX7, FCN1, SLC35D2, CP, ZDHHC3, PTPN3, LPL*, and *SPP1*. Among these genes, LPL and SPP1 were the most significant according to NAFLD-transcription factor network. Five hundred twenty-nine nodes and 1,113 edges comprised the PPI network of activated pathway-gene. In addition, 14 common pathways of these two diseases were recognized using

Gene Ontology (GO) analysis; among them, regulation of the lipid metabolic process is closely related to both two diseases.

Conclusions: This study offers hints that NAFLD and DN have a common pathogenesis, and LPL and SPP1 are the most relevant crosstalk genes. Based on the common pathways and optimal crosstalk genes, our proposal carried out further research to disclose the etiology and pathology between the two diseases.

KEYWORDS

non-alcoholic fatty liver disease, diabetic nephropathy, crosstalk, LPL, SPP1, bioinformatics

Introduction

Diabetic nephropathy (DN) is a rigorous microvascular complication primarily associated with both type 1 and type 2 diabetes mellitus (T2DM) and has been the leading cause of end-stage renal disease (ESRD) worldwide (1–3). Both morbidity and mortality of DN have promptly increased around the world (1, 2, 4). Non-alcoholic fatty liver disease (NAFLD) has become pyramidally ordinary in parallel with the adding popularity of obesity and other components of the metabolic syndrome (5, 6). NAFLD is distinguished as the existence of fat storage ≥5% of liver weight with the absence of excessive alcohol consumption or secondary cause of liver diseases such as autoimmune hepatitis, hemochromatosis, and Wilson's disease (3, 7–9). Being metabolic diseases, factors that contribute to NAFLD, such as diabetes, chronic inflammation, insulin resistance, and obesity, are also associated with the development of DN.

Several observational studies reported an impressive proportion that there were 70%–86% of patients with NAFLD also suffering from T2DM (6, 8, 10–12). Jia et al. (13) found that the cumulative incidence of DN in patients with NAFLD was much higher than those without it and that the liver fat content was positively correlated with increased occurrence of albuminuria and decreased glomerular filtration rate (GFR). Targher et al. (14) also found that the prevalence of diabetic retinopathy and chronic kidney disease (CKD) was significantly higher in patients with NAFLD. Previous epidemiological studies further suggested several contributors including metabolic syndrome, dysbiosis, unhealthy diets, platelet activation, and processes acting as the linking factors between NAFLD and CKD, which implied the potential correlations involved in the pathogenesis of liver and kidney disease (15). That NAFLD might be a risk factor for DN had been analyzed by some researchers (13). The relationship between NAFLD and DN seems rational and of clinical interest to some extent.

Based on the results of current observational studies, the potential contributions of genetic factors and protein–protein interactions (PPIs) on the correlation of NAFLD and DN should be further analyzed. In this study, bioinformatics analysis was used to disclose the crosstalk mechanisms between NAFLD and DN at the transcriptomic level. The mutual transcription characteristics would offer new insights into the common pathogenesis of NAFLD and DN. The purpose of this study is to recognize optimal crosstalk genes, participant pathways, and transcription factors (TFs). We hypothesize the existence of crosstalk genes between NAFLD and DN, then employed comprehensive bioinformatics and enrichment analyses to identify the common differentially expressed genes (DEGs) and the functional pathways of NAFLD and DN. At last, we identified 10 crosstalk genes, favoring the similarity between these two diseases.

Materials and methods

Study design and data collection

We acquired microarray data from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). After earnest review, four gene expression profiles (GSE66676 and GSE48452 were NAFLD datasets, and GSE30122 and GSE1009 were diabetic human kidney disease datasets, with no other complications) were selected. Figure 1 shows the schematic of the research.

Data procession and differentially expressed gene analysis

We combined two datasets for each disease to increase the sample size. R software (version 4.1.1; https://www.r-project.org/) and "BiocManager" packages were applied to analyze the data. The expression data from different datasets were normalized using the

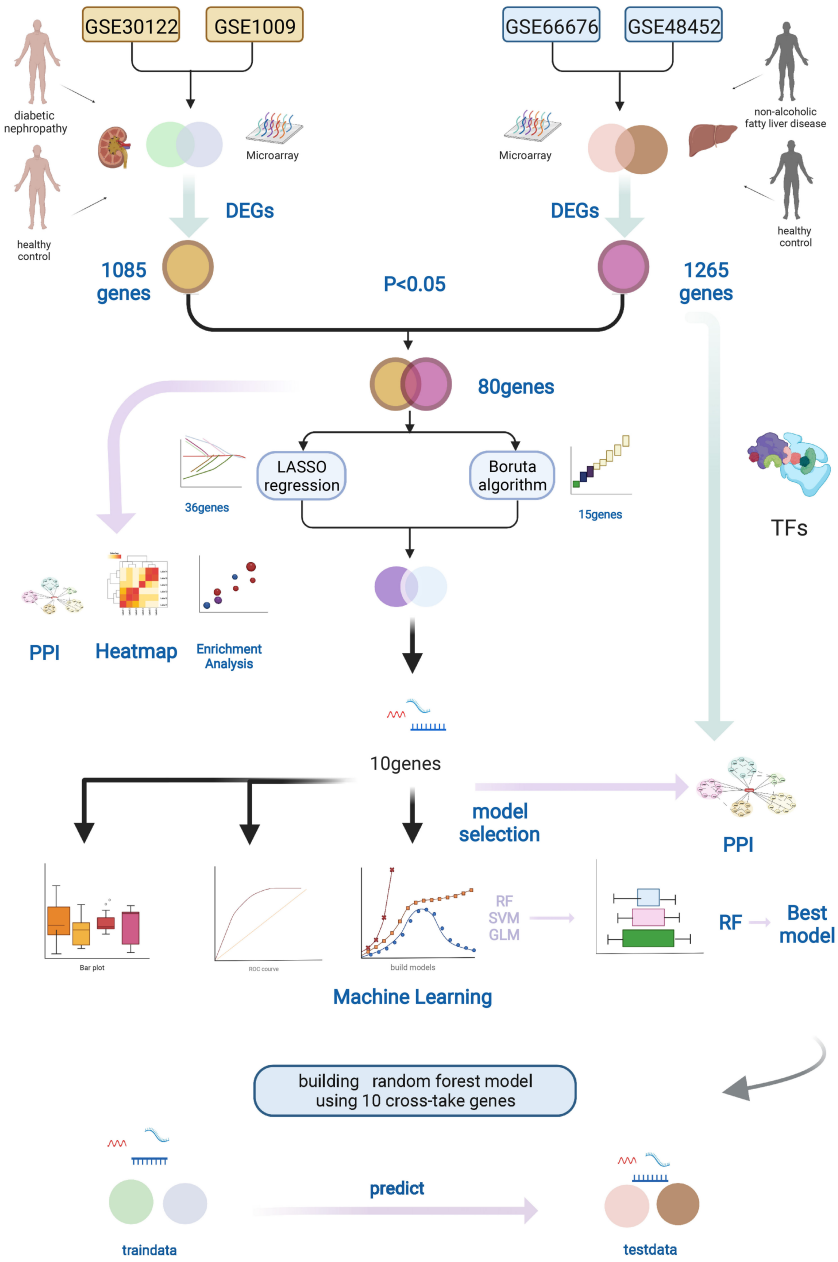


FIGURE 1
Workflow of this study. We downloaded the gene expression profiles of NAFLD and DN from the GEO database, including two NAFLD datasets (GSE66676 and GSE48452) and two DN datasets (GSE30122 and GSE1009). Datasets were merged, and DEGs were found by R software. The LASSO regression and Boruta algorithm were used to select the optional crosstalk genes. RF model was considered the best model to predict DN using the 10 crosstalk genes. In addition, protein–protein interaction (PPI) and functional enrichment analyses of the DEGs were performed. Graphic created with BioRender.com. NAFLD, nonalcoholic fatty liver disease; DN, diabetic nephropathy; GEO, Gene Expression Omnibus, DEGs, different expression genes.

robust multi-array average and merged together, and the "sva" library was used for combating batch correction to remove batch effects. We then used the Linear Models for microarray data ("limma" package) to identify DEGs by comparing the expression values between NAFLD patients and control cases. Genes with $P < 0.05$ were considered DEGs. The same way was done in the diabetic kidney disease dataset. The "pheatmap" package was used to draw the heatmap of the DEGs in R software.

Gene set enrichment analysis

In order to understand and interpret coordinate pathway-level changes in transcriptomics experiments represented under different conditions, the gene set enrichment analysis (GSEA) was used to determine whether there are statistically significant differences between the two groups as to a defined set of genes (16). R package "clusterProfiler" was used to perform GSEA. Gene sets "c7.all.v7.5.1.entrez" were downloaded from "Downloads (gsea-msigdb.org)" website, and then R software was employed to retrieve systematic functional annotation information. $P < 0.05$ was the cutoff criterion. We examined the pathway-level changes for all DEGs in NAFLD and DN to find out whether there were reduplicative pathways.

Immune infiltration analysis

The proportions of 22 kinds of immune cells, including naive B cells, memory B cells, plasma cells, CD8 T cells, naive CD4 T cells, CD4 resting memory T cells, activated memory CD4 T cells, helper follicular T cells, regulatory T cells (Tregs), delta gamma T cells, resting natural killer (NK) cells, activated natural killer (NK) cells, monocytes, macrophages M0, macrophages M1, macrophages M2, resting dendritic cells (DCs), active DCs, resting mast cells, activated mast cells, eosinophils, and neutrophils, were obtained, and CIBERSORT algorithm was utilized to analyze the gene expression data between NAFLD and DN.

We brought in all genes that were expressed in both NALFD and DN patients to explore the common ground based on 22 kinds of immune cells between the two diseases. The percentage of each kind of immune cell in the samples was calculated. Single-sample gene set enrichment analysis (ssGSEA) was employed to calculate the degree of penetration of 28 immune cell types on the grounds of the expression levels of genes in 28 published gene sets for immune cells (17).

Identification of potential crosstalk genes and functional enrichment analysis

The potential crosstalk genes were identified as DN-related DEGs overlapped with the NAFLD-related ones. These crosstalk genes could have the potential ability of linking the pathogeneses of NAFLD and DN.

To further determine the biological features of potential crosstalk genes, Gene Ontology (GO) analysis was accomplished by "clusterProfiler" of R Bioconductor packages. A classification method is offered by the "clusterProfiler" packages to classify genes based on their projection at a specific level of the GO corpus and provide functions to calculate enrichment values for GO terms. The enriched function with $P < 0.05$ was considered a significant pathway. Based on this analysis method, we selected the top 20 GO biological processes. To identify the most significant clusters of the crosstalk genes, PPI network of crosstalk genes was constituted by STRING (STRING 11.5; Search Tool for the Retrieval of Interaction Gene; https://string-db.org/). Cytoscape (version 3.8.0) was used to visualize the PPI network.

Identification of optimal diagnostic crosstalk genes

To better screen the risk crosstalk genes between NAFLD and DN, Boruta algorithm and least absolute shrinkage and selection operator (LASSO) regression were performed in R project. The LASSO regression was used to filter the best predictive features while fitting a generalized linear model (GLM) and avoiding overfitting. The Boruta employed a wrapper approach, built around a random forest (RF) classifier. After merging two NAFLD datasets, the expression values of potential crosstalk genes were extracted. The DEGs between NAFLD patients and healthy controls were reserved for feature selection, and the optimal crosstalk genes were initially recognized using the Boruta algorithm and LASSO regression. To narrow it down further, we combined the results of the two algorithms.

On the grounds of the optimal diagnostic crosstalk gene expression on the NAFLD merged dataset, we created the RF model, support vector machine (SVM) model, and GLM to pick out the best model. The response variable was the diagnosis of NAFLD or not, and the DEGs were used as explanatory variables. We then used the explain feature of "DALEX" package in R to find out which was the finest model among these three models aforementioned based on the plot of residual distribution.

Development of the random forest model using optimal diagnostic crosstalk genes

After extracting the gene expression values of the filtered crosstalk genes that constitute the merged gene expression profile, the RF model with the gene expression value and sample type was built (NAFLD and healthy) to further confirm the diagnostic value of these crosstalk genes. The R package "randomForest" was applied to build the RF model. The "ComBat" method of "sva" packages in R project was performed to eliminate the batch effect. It is worth noting that the gene sample expression values were changed after a series of operations that are mentioned above, comparing previous gene expressions. Therefore, the primitive expression profile of the two datasets GSE66676 and GSE48452 was obtained. Afterward,

the optimal crosstalk genes were confirmed by Boruta algorithm and LASSO regression, and then the expression values of the optimal crosstalk genes from the merged data were confirmed. We select the gene expression values of the filtered optimal genes to form the merged gene expression profile, and the RF models with the gene expression profile value and sample type were set up (NAFLD or healthy). The NAFLD merged data were input as training data, and the DN merged data were imported as testing data. The prediction effectiveness was determined by the accuracy rate of the test set.

Transcription factor-adjusted and pathway analysis of the crosstalk genes

We downloaded TFs that regulate the target genes from TRRUST and ChEA3 databases, taking the intersection of TFs from these two databases. Based on the TF–target relationship, the NAFLD-related TF–target pairs were picked out, and the Cytoscape software was used to set up and visualize the TF–target gene interaction network.

In order to pick out activated pathways, the remarkably enriched pathways by the DEGs of NAFLD were screened. We selected the potential crosstalk pathways that may be the bridge of NAFLD and DN and obtained the genes functioning in each pathway. Finally, the Cytoscape software was used to construct the pathway–gene crosstalk network. For the purpose of confirming the functional TFs, which adjusted the crosstalk genes in the activated pathways, we picked out the crosstalk genes in the pathway–gene pairs and identified the NAFLD-related TFs and DN-related TFs. Moreover, 10 crosstalk genes were also included. Finally, the network of these four parts was created.

Results

Identification of differentially expressed genes and functional pathways by gene set enrichment analysis

A total of 215 study subjects were included in the current study. The mean age with standard deviation was 63.29 ± 14.61 years (DN patients) and 52.44 ± 12.90 years (healthy control) for the DN group and 45.92 ± 11.29 years (NAFLD patients) and 33.52 ± 8.82 years (healthy control) for the NAFLD group. The proportion of women was 66.5% and 78.9% for the DN and NAFLD groups, respectively. To identify DEGs between NAFLD and healthy controls, we recruited microarray expression profiles of GSE66676 and GSE48452 from the GEO database. After merging and normalizing the microarray data, 1,265 DEGs between NAFLD and healthy controls were selected by "limma" package ($P < 0.05$). Two DN datasets were also picked out from the GEO website, which was done in the same way. Finally, we got 1,265 DEGs in the NAFLD merged dataset and 1,085 DEGs in the DN merged dataset (heatmap shown in Supplementary Figures S1A, B). GSEA was implemented to reveal the functional similarity between the two diseases. All DEGs of each disease were contained in the GSEA using gene set "c7.all.v7.5.1.entrez." As a result, three common pathways were identified in NAFLD and DN (Supplementary Figures S2A, B).

Immune infiltration analysis

By employing the CIBERSORT algorithm, we investigated the similarity in immune infiltration between NAFLD patients and DN patients in 22 subpopulations of immune cells. The results acquired from NAFLD patients and DN patients were summarized by R software (Supplementary Figure S3A). The samples were screened according to $P < 0.05$, and the percentage of each kind of immune cell in the samples was calculated. As shown in Supplementary Figure S3B, there are no significant differences between NAFLD and DN tissue in most immune cells, such as macrophage M1, which were considered to be proinflammatory and promote inflammation (18). However, the DN tissue generally included a high ratio of naive CD4 T cells, delta gamma T cells, activated NK cells, and resting mast cells, while resting NK cells had the opposite trend of expression. In the ssGSEA (Supplementary Figure S3C), 17 immune cell subtypes, including activated B cell, NK T cell, immature B cell, effector memory CD8 T cell, and central memory CD4 T cell, demonstrated no significant expression differences between NAFLD and DN. However, Myeloid-derived suppressor cells (MDSC), memory B cells, regulatory T cells, T follicular helper cells, and Type 1 T helper cells showed higher expression in DN patients, while immature DCs were highly expressed in NAFLD patients. The consequences of the CIBERSORT algorithm and ssGSEA manifest that the two diseases are likely to have a similar immune infiltration environment, which laid the theoretical foundation to link them.

Identifying crosstalk genes, Gene Ontology analysis, and construction of the protein–protein interaction network

After overlapping the DEGs of the two datasets, we finally got 80 crosstalk genes. The Venn diagram for the DEGs was given in Figure 2A. The heatmaps of common DEGs between NAFLD and DN were represented in Figures 2B, C. The GO analysis found that common DEGs were most intensively related to neutrophil-related pathways such as neutrophil degranulation, neutrophil activation involved in immune response, neutrophil-mediated immunity, and neutrophil activation. The detailed biological pathways in which DEGs were involved were shown in Figures 3A–D.

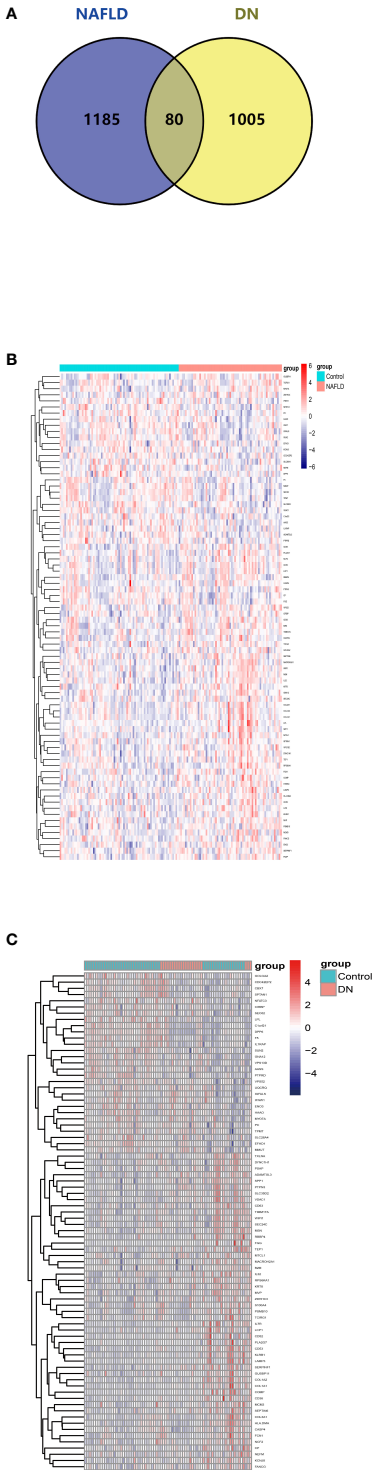


FIGURE 2
Venn diagram and expression level of common DEGs. **(A)** The intersection of DEGs in the NAFLD merged dataset and DN merged dataset from GEO contains 80 optimal crosstalk genes. The expression level of 80 common DEGs in the NAFLD merged dataset **(B)** and DN merged dataset **(C)**.

Seventy-seven nodes and 283 edges comprised the constructed PPI network of common DEGs (Figure 4A). The most significant module (score = 5.846) was recognized by MCODE, a plug-in of Cytoscape. CytoHubba was used to identify the hub genes (Figure 4B). SPP1 may be the key gene that contacts NAFLD and CKD, since it had the highest score in the biological network.

Prediction of optimal crosstalk genes and building the machine learning model

We then extracted the expression data of the 80 genes from the NAFLD gene expression profile. Gene biomarkers were identified with the LASSO and Boruta algorithms. A total of 15 genes were finally selected (Figures 5A–C; Supplementary Table S2). Furthermore, the optimal crosstalk genes were identified by overlapping biomarkers derived from these two algorithms. We got 10 optimal crosstalk genes in the end.

To select and create the optimal prediction model, three models including SVM, RF, and GLM were created in light of the training NAFLD merged dataset. After that, the "DALEX" package's explanatory feature in R was utilized to analyze the three aforementioned models. As shown in Figures 6A–C, which revealed the residual distribution, the RF model was confirmed as the best suitable model because it possesses the least sample residual. Ultimately, the expression of the 10 optimal crosstalk genes in NAFLD [*CD36, WIPI1, CBX7, FCN1, SLC35D2, CP, ZDHHC3, PTPN3*, lipoprotein lipase (LPL), and *SPP1*] was input to create the RF model. The gene expression profile of the 10 feature genes was also extracted from the DN merged dataset. Treating the NAFLD merged dataset as training data and the DN merged dataset as validation data, the predicted outcome of the RF model was shown in Supplementary Table S1. Supplementary Figure S4 shows the importance of 10 genes in the RF model. The forecast performance of each gene in both NAFLD and DN was shown in Supplementary Figure S5. The area under the curve (AUC) values of LPL and SPP1 in DN were 86% and 80.1%, and the AUC values of LPL and SPP1 in NAFLD were 72.5% and 64.3%, respectively. Supplementary Figure S6 showed the expression of the 10 genes in the two diseases.

Transcription factor–gene regulation network

We got a total of 35 mutual TFs, and the TF–target network was established as shown in Figure 7A. The optimal crosstalk genes with the highest degree were SPP1 and LPL and therefore potentially played a significant role in the TF–target network. Supplementary Figure S7 showed the network between NAFLD TF–target pairs and DN–genes.

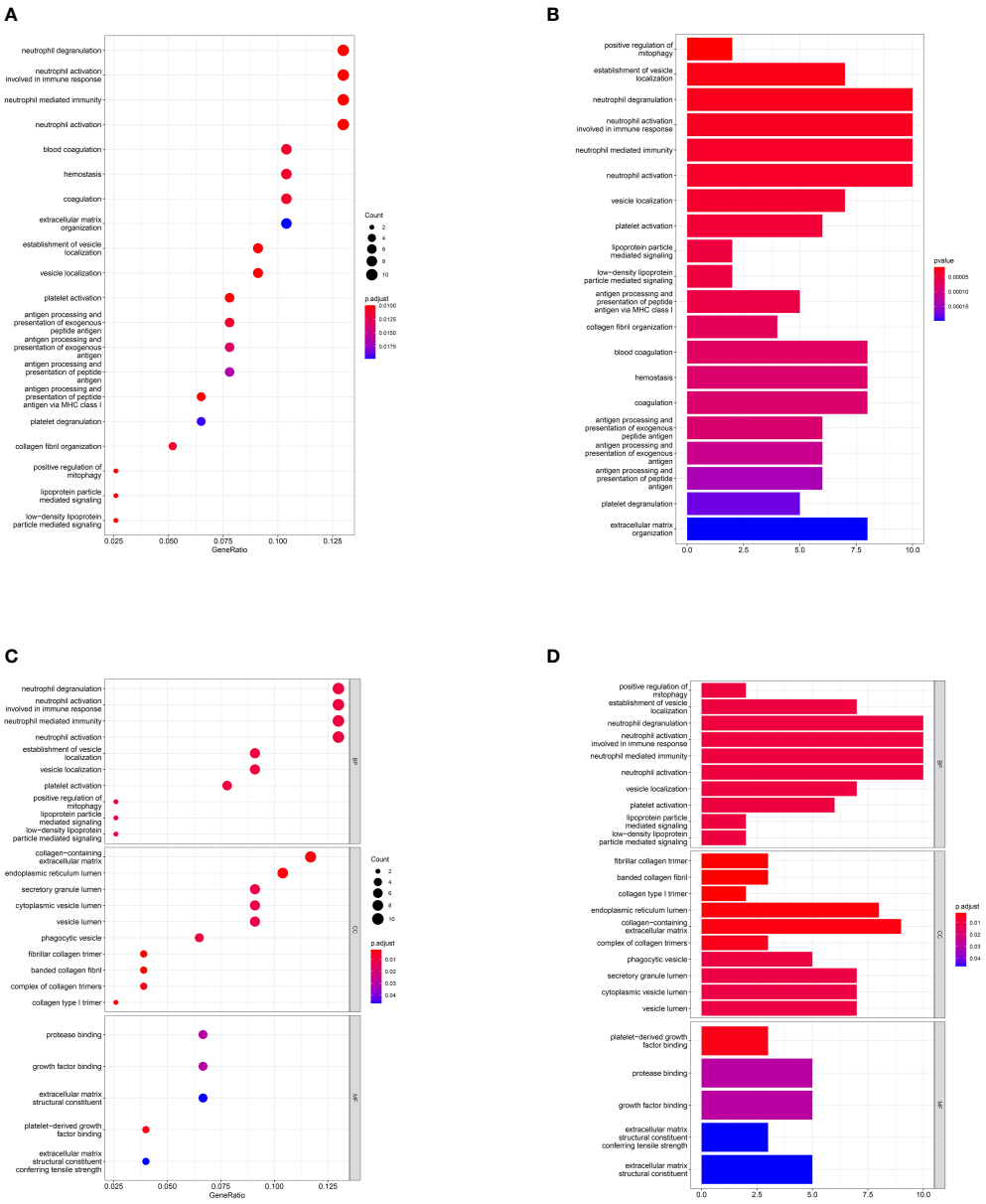


FIGURE 3
Gene Ontology pathway enrichment analysis. Result of GO pathway enrichment analysis of the 80 common DEGs, the dot plot **(A)** and bar plot **(B)**. Biological process (BP), cellular component (CC), and molecular function (MF) analysis results of 80 common DEGs, the dot plot **(C)** and bar plot **(D)**. X-axis represents the proportion of DEGs and Y-axis on behalf of the different categories. The size of the circle manifests the number of genes enriched in each category and different properties are denoted by the color of the circle.

Ultimately, 14 crucial pathways, which may play a key role in the progress of NAFLD, were selected. In order to recognize the pathway crosstalk between NAFLD and DN, we established the pathway–gene crosstalk network. Five hundred twenty-nine nodes and 1,113 edges were included in the activated pathway–gene network (Figure 7B). To further explore the relationship between NAFLD and DN, the DN-related TF–target pairs and NAFLD-related TF–target pairs were extracted and the PPI was built. Meanwhile, the PPI between 10 crosstalk genes was obtained, then the activated TF–crosstalk gene network was established (Figure 7C). Consequently, we discovered that crosstalk genes were regulated by many TFs. The closeness of their relationship was indicated by the size of the circle. The highest degree among the 10 crosstalk genes remained to be SPP1 and LPL. DN-related TF–target pairs had a closer relationship with crosstalk genes.

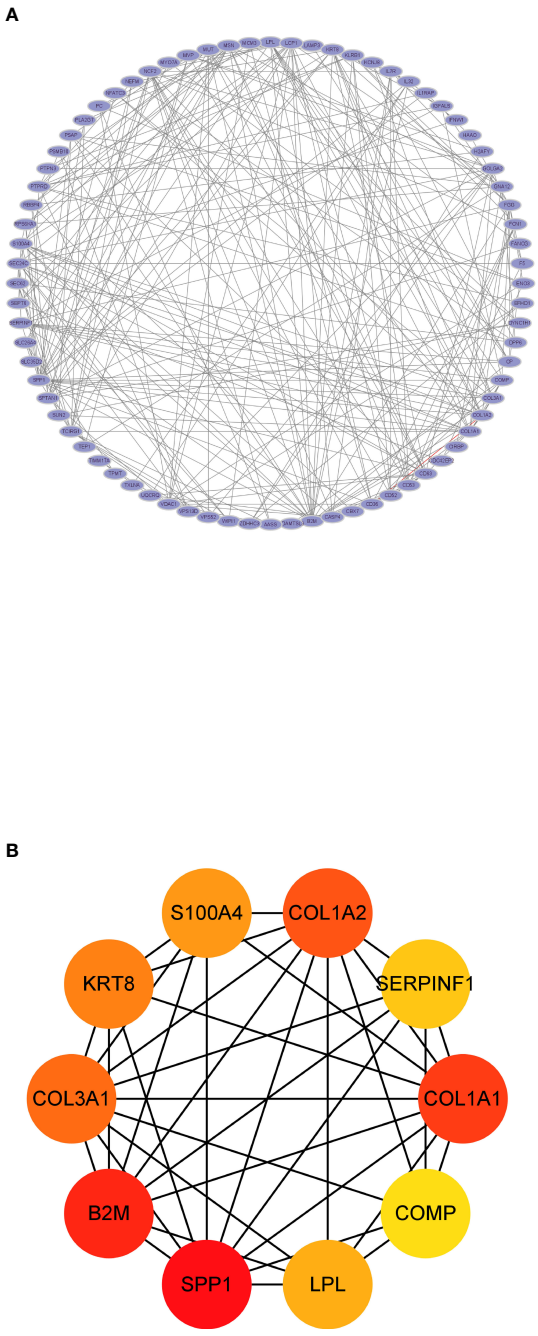


FIGURE 4
The protein–protein interaction analysis. **(A)** The PPI network analysis of the 80 common DEGs. **(B)** The hub genes identified by CytoHubba.

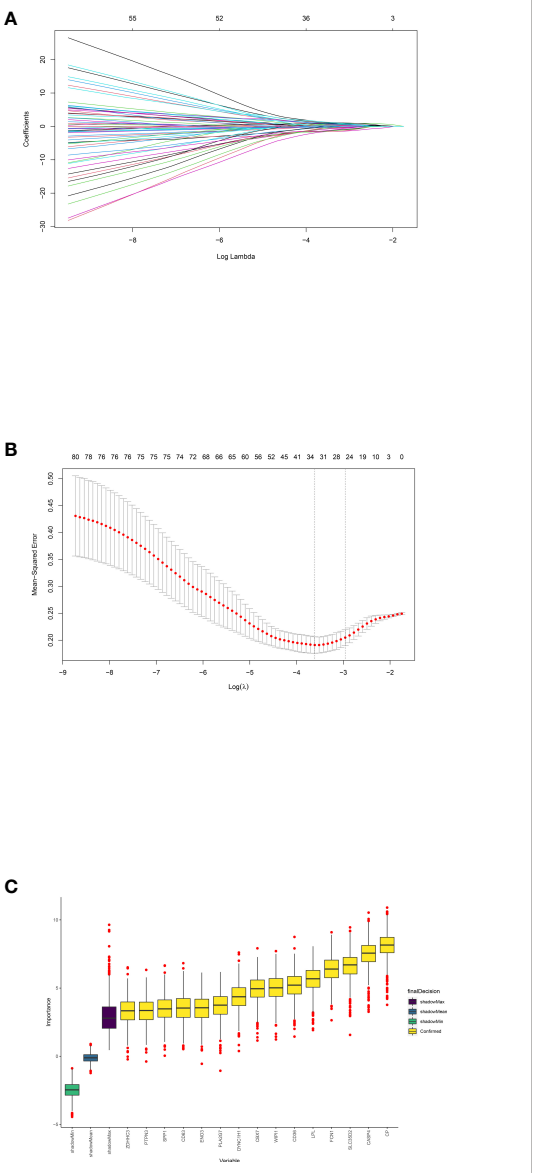


FIGURE 5
Feature selection. Crosstalk gene selection by LASSO regression **(A, B)** and Boruta algorithm **(C)**. **(A)** By using LASSO model to confirm the optimal genes, the partial likelihood deviance curve was plotted vs. log(lambda). Based on 1 SE of the minimum criteria (the 1-SE criteria) to draw dotted vertical lines. **(B)** Confirmed 34 genes with non-zero coefficients by optimal lambda. Panel **(A)** shows the coefficient profile plot produced against the log(lambda) sequence. **(C)** The 15 confirmed genes were indicated by the yellow box. X-axis represents selected genes. Y-axis represents the score of each gene.

Discussion

The major outcome of this study was that bioinformatics analysis could expose crosstalk genes between NAFLD and DN. Accordingly, LPL and SPP1 were identified to be the most concerned genes; meanwhile, some participant pathways were identified. According to their expression values in each patient and forecast ability, the latent correlation of these genes was confirmed. The areas under the ROC curve of these two genes are higher than those of most genes (Supplementary Figure S3);

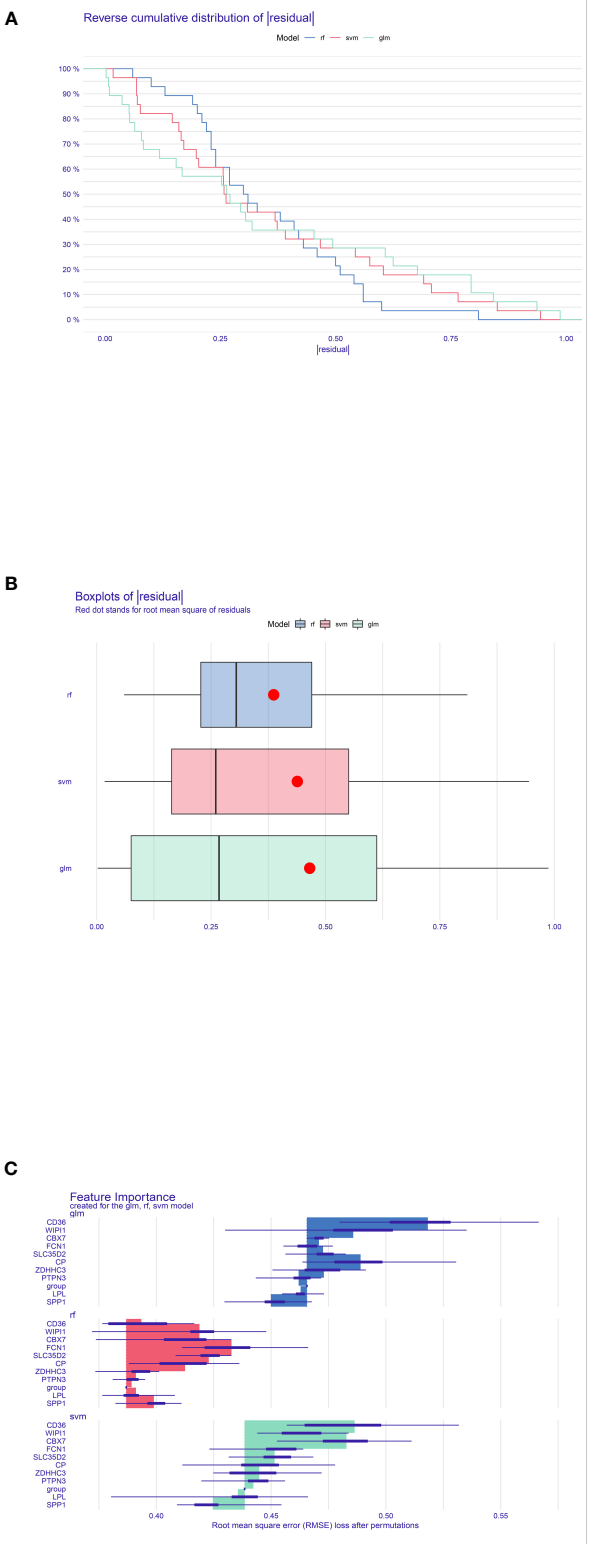


FIGURE 6
Construction and evaluation of RF model, SVM model, and GLM. **(A)** Accumulated residual distribution picture of the sample. **(B)** Boxplot of the residuals of the sample. The root mean square of the residuals was indicated by a red dot. **(C)** The significance of the variables in the three models.

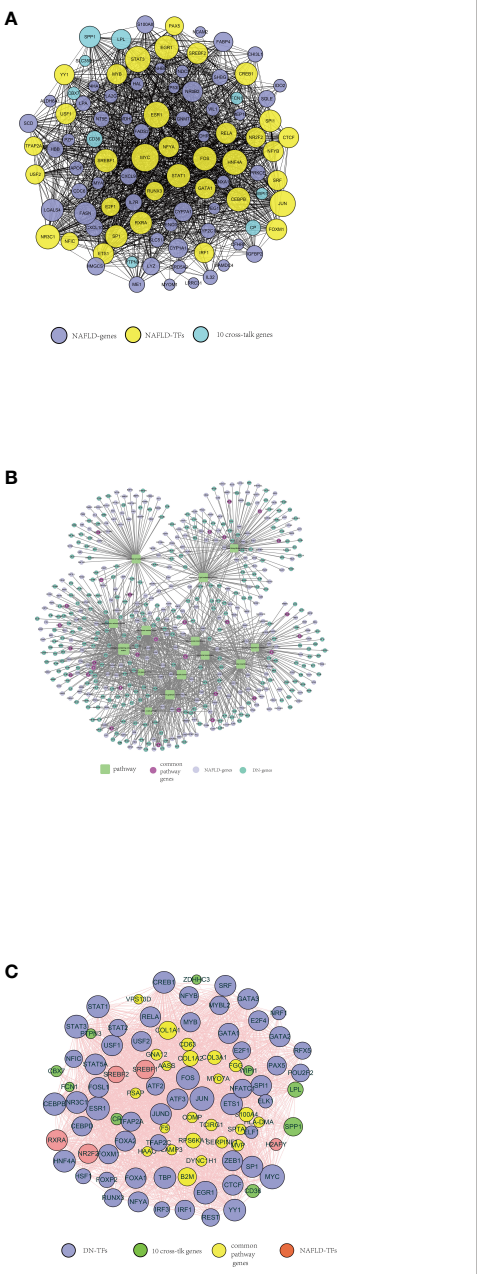


FIGURE 7
A series of protein–protein interaction (PPI) networks. **(A)** NAFLD-related TFs selected by TRRUST and ChEA3, indicated by yellow circles. The purple circles represent the top 50 significant differentially expressed genes in the NAFLD merged dataset. The optimal 10 crosstalk genes are indicated by the green circle. **(B)** Fourteen crucial pathways functioning in both NAFLD and DN, indicated by green squares. The purple-red circles represent the genes from each pathway both NAFLD-related and DN-related. The lilac circles represent the significant differentially expressed genes in the NAFLD merged dataset. The green circle represents the significant differentially expressed genes in the DN merged dataset. **(C)** DN-related TFs selected by TRRUST and ChEA3, indicated by purple circles. The green circles represent 10 optimal crosstalk genes. The genes from each pathway both NAFLD-related and DN-related were indicated by yellow circles. The pink circles represent NAFLD-related TFs.

furthermore, they have the highest degree in the TF–target network.

According to our results, the aberrant lipid and glucose metabolism plays an important role in the crosstalk between NAFLD and DN. The LPL gene, which belongs to the lipase gene family including hepatic lipase, endothelial lipase, and pancreatic lipase, could combine with lipoproteins and cell surface proteins concurrently, resulting in accumulation and uptake of lipoproteins (19, 20). In 2019, Teratani et al. (21) found that the expression of LPL was changed in hepatic stellate cells in NAFLD patients. Serum obesity-related factors, including interleukin-6, leptin, and free fatty acid (FA), could further affect its circulating level (21). NAFLD would even evolve to hepatocellular carcinoma (HCC) due to the aberrant activation of LPL, since it had great impact on HCC cell proliferation and lipid deposition (19).

On the other hand, LPL had also been proven to be associated with the development and progression of DN (20). A previous study has shown that DN rats have elevated levels of total cholesterol, triglycerides (TGs), and low-density lipoproteins (LDL), accompanied by significant changes in plasma LPL activity (22). When the activity of LPL is affected, it would consequently result in hypertriglyceridemia, which is a pivotal trait of nephrotic syndrome (23). Our previous studies also found that dyslipidemia was one of major risk factors for diabetic kidney disease (24, 25). In 2019, Al Shawaf et al. (23) found that the level of circulating ANGPTL4, an inhibitor of LPL, was significantly higher in DN patients compared with those in T2DM patients and healthy controls. Its expression was also positively correlated with serum creatinine and urinary albumin-to-creatinine ratio (23). These findings indicated that the suppressing efforts on LPL were increasing during the progression from DM to DN, which suggested an intervention target for the early prevention of the development of DN in DM patients.

Another crucial crosstalk gene we found was SPP1, which encodes osteopontin and is expressed in a variety of cells and tissues including endothelial cells, DCs, macrophages, and kidney (26, 27). Osteopontin is known as a regulator of hepatic stellate cell activation. Zhu et al. (28) found that the contribution of hepatocyte-derived osteopontin in NAFLD was capable of altering the liver microenvironment to potentiate fibrosis *via* a Notch-activated pathway. Notch-mediated osteopontin secretion in hepatocytes could directly activate hepatic stellate cells and cause excessive collagen deposition, despite hepatocellular injury. Furthermore, by performing chronic γ-secretase inhibitor treatment, liver Notch activity was decreased and hepatic stellate cell activation and liver fibrosis were reduced (28). In the progression of DN, Notch signaling pathway was activated following long-term exposure to hyperglycemia (29). The expression of constitutively active Notch intracellular domain in mature podocytes caused podocyte dedifferentiation, glomerulosclerosis, and apoptosis that substantially caused albuminuria and progressive renal failure (30, 31). By treating with γ-secretase inhibitors, the diabetes-induced glomerulosclerosis and podocyte injury could be prevented, which suggested that inhibiting the overactive Notch pathway in renal cells could be a potential plausible therapeutic approach (32).

Previous studies indicated that hyperglycemia would increase the expression of SPP1, which caused an elevated exposure of cells to proinflammatory cytokines and inflammation indicators including tumor necrosis factor α, transforming growth factor β, and interleukin-1 (33–36). In our current study, the immune filtration analysis also found that there were common expressions of macrophages and DCs between the NAFLD and DN groups, which suggested that inflammatory activations were involved in the crosstalk of the two diseases. It has been proven that in high-glucose conditions, the transcriptional activity of SPP1 was enhanced in proximal tubular epithelial cells (PTECs), which means that when T2DM occurred, the expression of SPP1 will increase and SPP1 promotes the occurrence and development of both diseases (37). Zhang et al. (38) also found that the SPP1 was negatively correlated with GFR in diabetic kidney disease patients. Some researchers believed that SPP1 could be the core target to treat diabetic kidney disease by using traditional Chinese medicine (39). SPP1 is also conjectured to function in the transformation of non-alcoholic steatohepatitis (NASH) to HCC like LPL (40).

In the enrichment analysis part, we found that 14 pathways were involved in the crosstalk between NAFLD and DN, one of which was "regulation of lipid metabolic process." Several previous studies also demonstrated that the progression of DN was linked to serum lipid abnormalities and renal ectopic lipid accumulation (18, 41, 42). The proportion of kidney-absorbed LDL would be different when the activity of the LDL receptor changed; meanwhile, the expression of LDL receptor would be remarkably suppressed by cholesterol in podocytes (42). Lipid loading facilitates the phenotypic conversion of podocytes, which results in the disappearance of its epithelial features (43–45). Most DN patients performed albuminuria or macroproteinuria during the progression of disease. The albumin also acted as a vehicle for FAs in urine. Consequently, albuminuria may cause extensive accumulation of FAs and accelerate kidney injury in DN patients (46). By analyzing 34 DN patients and 12 healthy controls, Herman-Edelstein et al. (47) found a high degree of correlation between lipid metabolism and GFR. In the situation of continuing hyperglycemia in diabetic patients, TGs and FAs were accumulated (47). Ectopic lipid accumulation in non-adipose tissues, such as liver, kidney, heart, and pancreas, occurs because of raised serum TGs, FFAs, and modified cholesterol (41, 48–51), which appear to play a part in the pathogenesis of DN (52–54). This condition also seems to result in NAFLD. This suggests that diabetes also acts as a link between these two diseases. Consistent with this, obvious neutral lipid accumulation was found both in glomeruli and

tubulointerstitium in diabetic kidneys (47). Two of the features of DN in electron microscope podocyte process effacement, interestingly, lipotoxicity and lipid cumulation, can lead to podocyte malfunction and apoptosis (55). Hence, it is reasonable for DN to see heavy lipid deposition (47).

A previous study found that the total counts of lipid droplets (LDs) decreased when kidney tissue was seriously fibrosed. This process was similar to the progression of NASH (56). Liver is the central organ of lipoprotein metabolism, since it takes part in the production of lipoprotein particles in all categories. It also plays a central role in the metabolism of TGs and cholesterol. Serum TGs and remnant cholesterol would be elevated when liver function is impaired. Then, it comes with altered glucose metabolism and insulin resistance, which are believed to be hallmarks of NAFLD (57). NAFLD occurs at the time of excessive intake of FAs and TGs from the circulation. Unbalanced lipid metabolism is also related to NAFLD advancement from steatosis to NASH; moreover, alterations in liver and serum lipidomic signatures are excellent indicators of NAFLD's development and progression (58).

Both NAFLD and DN were considered to be affected by chronic inflammation progression, especially in individuals with abnormal serum glucose and lipid concentration (7, 12, 59). In our current study, we also found that there were aberrant proportions and expression levels of immune indicators in both diseases. There were a total of 7 and 17 kinds of immune cells performing higher proportion and expression levels, respectively. Most of them showed no significant differences between NAFLD and DN groups, which indicated the mutual mechanism in the two diseases. Enrichment analysis further demonstrated that several common DEGs were enriched in immune-related functions, including neutrophil activation, neutrophil-mediated immunity, and positive regulation of mitophagy.

Liver-mediated lipid changes are associated with the severity of proteinuria (60). Similarly, the diagnosis of DN refers to the appearance of specific pathologic structural alongside functional changes in the kidney of patients with DM, one of which is proteinuria (61). Renal injury is generally non-reversible on the condition that albuminuria persistently occurs (13). Various mechanisms, such as poor plasma glucose control, activation of sympathetic nervous system, and insulin resistance both in liver and kidney contribute coordinately to the advancement of kidney diseases (62, 63). Metabolic syndrome is a significant contributor to the evolution of chronic kidney dysfunction (64, 65). It has already been confirmed that lipid accumulation is strongly associated with inflammatory stress in the kidney. Through perturbing the LDL receptor pathway and induced phenotypic change and dysfunction in podocytes, inflammation induces lipid accumulation (42). Obesity is closely associated with these two diseases, which have been considered as a risk factor for both NAFLD and DN (9, 66, 67). Obesity, T2DM, and NAFLD can not only facilitate systemic insulin resistance but also boost the accumulation of hepatic fat and impairment of glucose metabolism (3, 66); in the meantime, insulin resistance can stimulate hepatic macrophages. T2DM often causes chronic hyperinsulinemia, which plays a vital role in liver metabolism abnormalities (41). The possibility that kidney dysfunction occurs was remarkably higher in patients with NAFLD based on two cross-sectional studies (14, 68). There is an assumption that along with the advancement of NAFLD into NASH, diabetic kidney diseases would occur (69). With the progression of NASH, metabolic disorders, for example, dyslipidemia, insulin resistance, and glucose intolerance, would collectively advance the renin-angiotensin (RAS) system system and influence nitric oxide formation (70–72), which can facilitate the progression of DN. Simultaneously, a mechanism in NASH adjusted by liver-derived inflammatory mediators and oxidative stress, which boosts the free proinflammatory, procoagulant, pro-oxidant, and profibrogenic factors from the liver, participates in the development of DN (69, 73–75). Renal hemodynamics may be influenced because of activation of the sympathetic nervous system, hence conducing to the onset or deterioration of kidney diseases (3, 63). Therefore, based on these common risk factors, it is reasonable to conjecture the interlink between NAFLD and DN, and early detection and treatment of NAFLD may be of clinical significance for the intervention of DN.

Altogether, this study identified two major crosstalk genes and relevant shared pathways, which emphasize the comparability and underlying relationship between NAFLD and DN. It is reasonable to consider that NAFLD patients are vulnerable to get and develop kidney dysfunction caused by T2DM and insulin insistence. Despite the credibility of such a probability interlink, more research needs to be done to reveal the potential mechanism of these two diseases. Nevertheless, there exist several limitations in our study. Firstly, data on survival times and outcomes are lacking. Therefore, the effect of these crosstalk genes on survival could not be tested. Secondly, the integration of different gene expression datasets might be biased because of discrepancies in the experimental setting for each dataset. Ideally, unnecessary bias should be avoided by making sure all gene expression data have the same experimental settings. In addition, it was a retrospective study. For the purpose of averting analysis bias associated with retrospective studies, a prospective study is recommended to be conducted. Lastly, the current study is entirely ground on computer analyses, thus the validation analysis based on wet-lab will be encouraged to confirm these crosstalk genes we found.

Conclusion

To the best of our knowledge, this is the first study of crosstalk mechanisms between NAFLD and DN using bioinformatics analysis, identifying common immune and TF-related mechanisms. LPL and SPP1 are the most relevant crosstalk genes in our study, which suggest that NAFLD and DN may have a common pathogenesis.

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.

Author contributions

QY, ZZ, JYD, and DL designed the study, collected the data, analyzed the data, and wrote the manuscript. ZL, JCD, SP, and JL reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (Grant No. 82103916), General Program of the National Science Foundation of China General Project (No. 81970633).

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/fendo.2022.1032814/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The functional similarity between NAFLD and DN. **(A)** Comparison of functional enrichment of DN and healthy controls. **(B)** Comparison of functional enrichment of NAFLD and healthy controls.

SUPPLEMENTARY FIGURE 2
The expression level of all DEGs The expression level of all DEGs in NAFLD merged dataset **(A)** and DN merged dataset **(B)**.

SUPPLEMENTARY FIGURE 3
Immune characteristics of NAFLD and DN Comparison of immune characteristics between NAFLD and DN. **(A)** An intuitive picture of the percentage of the 22 immune cells in NAFLD patients and DN patients. **(B)** The proportion of 22 immune cells in two diseases. **(C)** The expression of 28 immune cells in two diseases.

SUPPLEMENTARY FIGURE 4
Rank the importance of the RF model.

SUPPLEMENTARY FIGURE 5
The predictive efficiency of the 10 crosstalk genes in NAFLD **(A)** and DN **(B)**.

SUPPLEMENTARY FIGURE 6
The expression of the 10 crosstalk genes in NAFLD **(A)** and DN **(B)**.

SUPPLEMENTARY FIGURE 7
The protein-protein interaction network of the NAFLD related TFs and the top 180 significant differentially expressed genes in DN merged dataset.

References

1. Kato M, Natarajan R. Diabetic nephropathy–emerging epigenetic mechanisms. *Nat Rev Nephrol* (2014) 10(9):517–30. doi: 10.1038/nrneph.2014.116

2. Xiong Y, Zhou L. The signaling of cellular senescence in diabetic nephropathy. *Oxid Med Cell Longev* (2019) 2019:7495629. doi: 10.1155/2019/7495629

3. Wang T-Y, Wang R-F, Bu Z-Y, Targher G, Byrne CD, Sun D-Q, et al. Association of metabolic dysfunction-associated fatty liver disease with kidney disease. *Nat Rev Nephrol* (2022) 18(4):259–68. doi: 10.1038/s41581-021-00519-y

4. Bell S, Fletcher EH, Brady I, Looker HC, Levin D, Joss N, et al. End-stage renal disease and survival in people with diabetes: a national database linkage study. *QJM* (2015) 108(2):127–34. doi: 10.1093/qjmed/hcu170

5. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. *BMC Med* (2017) 15(1):45. doi: 10.1186/s12916-017-0806-8

6. Arslan MS, Turhan S, Dincer I, Mizrak D, Corapcioglu D, Idilman R. A potential link between endothelial function, cardiovascular risk, and metabolic syndrome in patients with non-alcoholic fatty liver disease. *Diabetol Metab Syndr* (2014) 14(6):109. doi: 10.1186/1758-5996-6-109

7. Wijarnpreecha K, Thongprayoon C, Boonpheng B, Panjawatanan P, Sharma K, Ungprasert P, et al. Nonalcoholic fatty liver disease and albuminuria: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* (2018) 30 (9):986–94. doi: 10.1097/MEG.0000000000001169

8. Heidari Z, Gharebaghi A. Prevalence of non alcoholic fatty liver disease and its association with diabetic nephropathy in patients with type 2 diabetes mellitus. *J Clin Diagn Res* (2017) 11(5):OC04–OC7. doi: 10.7860/JCDR/2017/25931.9823

9. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* (2011) 43(8):617–49. doi: 10.3109/07853890.2010.518623

10. Targher G, Chonchol M, Bertolini L, Rodella S, Zenari L, Lippi G, et al. Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease. *J Am Soc Nephrol* (2008) 19(8):1564–70. doi: 10.1681/ASN.2007101155

11. Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care* (2007) 30(3):734–43. doi: 10.2337/dc06-1539

12. Targher G, Tessari R, Bertolini L. Prevalence of Nonalcoholic fatty liver disease and its association wuth cardiovascular disease among type 2 diabetic patients. *Diabetes Care* (2007) 30: 734–43. doi: 10.2337/dc06-porting

13. Jia G, Di F, Wang Q, Shao J, Gao L, Wang L, et al. Non-alcoholic fatty liver disease is a risk factor for the development of diabetic nephropathy in patients with type 2 diabetes mellitus. *PloS One* (2015) 10(11):e0142808. doi: 10.1371/journal.pone.0142808

14. Targher G, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, et al. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. *Diabetologia* (2008) 51(3):444–50. doi: 10.1007/s00125-007-0897-4

15. Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. *J Hepatol* (2020) 72(4):785–801. doi: 10.1016/j.jhep.2020.01.013

16. Zeng M, Liu J, Yang W, Zhang S, Liu F, Dong Z, et al. Multiple-microarray analysis for identification of hub genes involved in tubulointerstial injury in diabetic nephropathy. *J Cell Physiol* (2019). doi: 10.1002/jcp.28313

17. Yan G, An Y, Xu B, Wang N, Sun X, Sun M. Potential impact of ALKBH5 and YTHDF1 on tumor immunity in colon adenocarcinoma. *Front Oncol* (2021) 11:670490. doi: 10.3389/fonc.2021.670490

18. Yuan F, Zhang Q, Dong H, Xiang X, Zhang W, Zhang Y, et al. Effects of des-acyl ghrelin on insulin sensitivity and macrophage polarization in adipose tissue. *J Transl Int Med* (2021) 9(2):84–97. doi: 10.2478/jtim-2021-0025

19. Wu Z, Ma H, Wang L, Song X, Zhang J, Liu W, et al. Tumor suppressor ZHX2 inhibits NAFLD-HCC progression *via* blocking LPL-mediated lipid uptake. *Cell Death Differ* (2020) 27(5):1693–708. doi: 10.1038/s41418-019-0453-z

20. Mead JR, Irvine SA, Ramji DP. Lipoprotein lipase: structure, function, regulation, and role in disease. *J Mol Med (Berl)* (2002) 80(12):753–69. doi: 10.1007/s00109-002-0384-9

21. Teratani T, Tomita K, Furuhashi H, Sugihara N, Higashiyama M, Nishikawa M, et al. Lipoprotein lipase up-regulation in hepatic stellate cells exacerbates liver fibrosis in nonalcoholic steatohepatitis in mice. *Hepatol Commun* (2019) 3 (8):1098–112. doi: 10.1002/hep4.1383

22. Kandasamy N, Ashokkumar N. Renoprotective effect of myricetin restrains dyslipidemia and renal mesangial cell proliferation by the suppression of sterol regulatory element binding proteins in an experimental model of diabetic nephropathy. *Eur J Pharmacol* (2014) 743:53–62. doi: 10.1016/j.ejphar.2014.09.014

23. Al Shawaf E, Abu-Farha M, Devarajan S, Alsairafi Z, Al-Khairi I, Cherian P, et al. ANGPTL4: A predictive marker for diabetic nephropathy. *J Diabetes Res* (2019) 2019:4943191. doi: 10.1155/2019/4943191

24. Duan JY, Duan GC, Wang CJ, Liu DW, Qiao YJ, Pan SK, et al. Prevalence and risk factors of chronic kidney disease and diabetic kidney disease in a central Chinese urban population: a cross-sectional survey. *BMC Nephrol* (2020) 21 (1):115. doi: 10.1186/s12882-020-01761-5

25. Duan J, Wang C, Liu D, Qiao Y, Pan S, Jiang D, et al. Prevalence and risk factors of chronic kidney disease and diabetic kidney disease in Chinese rural residents: a cross-sectional survey. *Sci Rep* (2019) 9(1):10408. doi: 10.1038/s41598-019-46857-7

26. Ashizawa N, Graf K, Do YS, Nunohiro T, Giachelli CM, Meehan WP, et al. Osteopontin is produced by rat cardiac fibroblasts and mediates a II -induced DNA synthesis and collagen gel contraction. *J Clin Invest* (1996) 98(10):2218–27. doi: 10.1172/JCI119031

27. Murry CE, Giachelli CM, Schwartz SM, Vrackot R. Macrophages express osteopontin during repair of myocardial necrosis. *Am J Pathol* (1994) 145(6):1450–62.

28. Zhu C, Kim K, Wang X, Bartolome A, Salomao M, Dongiovanni P, et al. Hepatocyte notch activation induces liver fibrosis in nonalcoholic steatohepatitis. *Sci Transl Med* (2018) 10(468):eaat0344. doi: 10.1126/scitranslmed.aat0344

29. Lin CL, Wang FS, Hsu YC, Chen CN, Tseng MJ, Saleem MA, et al. Modulation of notch-1 signaling alleviates vascular endothelial growth factor-mediated diabetic nephropathy. *Diabetes* (2010) 59(8):1915–25. doi: 10.2337/db09-0663

30. Niranjan T, Bielesz B, Gruenwald A, Ponda MP, Kopp JB, Thomas DB, et al. The notch pathway in podocytes plays a role in the development of glomerular disease. *Nat Med* (2008) 14(3):290–8. doi: 10.1038/nm1731

31. Waters AM, Wu MYJ, Onay T, Scutaru J, Liu J, Lobe CG, et al. Ectopic notch activation in developing podocytes causes glomerulosclerosis. *J Am Soc Nephrol* (2008) 19(6):1139–57. doi: 10.1681/ASN.2007050596

32. Bonegio R, Susztak K. Notch signaling in diabetic nephropathy. *Exp Cell Res* (2012) 318(9):986–92. doi: 10.1016/j.yexcr.2012.02.036

33. Guo H, Cai CQ, Schroeder RA, Kuo PC. Osteopontin is a negative feedback regulator of nitric oxide synthesis in murine macrophages. *J Immunol* (2001) 166 (2):1079–86. doi: 10.4049/jimmunol.166.2.1079

34. Ricardo SD, Franzoni DF, Roesener CD, Crisman JM, Diamond JR. Angiotensinogen and AT1 antisense inhibition of osteopontin translation in rat proximal tubular cells. *Am J Physiol Renal Physiol* (2000) 278(5):F708–16. doi: 10.1152/ajprenal.2000.278.5.F708

35. Hullinger TG, Pan Q, Viswanathan HL, Somerman MJ. TGFbeta and BMP-2 activation of the OPN promoter: roles of smad- and hox-binding elements. *Exp Cell Res* (2001) 262(1):69–74. doi: 10.1006/excr.2000.5074

36. Sodhi CP, Phadke SA, Batlle D, Sahai A. Hypoxia and high glucose cause exaggerated mesangial cell growth and collagen synthesis: role of osteopontin. *Am J Physiol Renal Physiol* (2001) 280(4):F667–74. doi: 10.1152/ajprenal.2001.280.4.F667

37. Shirakawa K, Sano M. Sodium-glucose Co-transporter 2 inhibitors correct metabolic maladaptation of proximal tubular epithelial cells in high-glucose conditions. *Int J Mol Sci* (2020) 21(20). doi: 10.3390/ijms21207676

38. Zhang Y, Li W, Zhou Y. Identification of hub genes in diabetic kidney disease *via* multiple-microarray analysis. *Ann Transl Med* (2020) 8(16):997. doi: 10.21037/atm-20-5171

39. Xing L, Xing W, Guo H. [Exploring the therapeutic mechanism of longqi fang for diabetic kidney disease based on network pharmacology and verification in rats]. *Nan Fang Yi Ke Da Xue Xue Bao* (2022) 42(2):171–80. doi: 10.12122/j.issn.1673-4254.2022.02.02

40. Wang Z, Zhao Z, Xia Y, Cai Z, Wang C, Shen Y, et al. Potential biomarkers in the fibrosis progression of nonalcoholic steatohepatitis (NASH). *J Endocrinol Invest* (2022) 45(7):1379–92. doi: 10.1007/s40618-022-01773-y

41. Opazo-Rios L, Mas S, Marin-Royo G, Mezzano S, Gomez-Guerrero C, Moreno JA, et al. Lipotoxicity and diabetic nephropathy: Novel mechanistic insights and therapeutic opportunities. *Int J Mol Sci* (2020) 21(7). doi: 10.3390/ijms21072632

42. Zhang Y, Ma KL, Liu J, Wu Y, Hu ZB, Liu L, et al. Inflammatory stress exacerbates lipid accumulation and podocyte injuries in diabetic nephropathy. *Acta Diabetol* (2015) 52(6):1045–56. doi: 10.1007/s00592-015-0753-9

43. Reidy K, Susztak K. Epithelial-mesenchymal transition and podocyte loss in diabetic kidney disease. *Am J Kidney Dis* (2009) 54(4):590–3. doi: 10.1053/j.ajkd.2009.07.003

44. Li Y, Kang YS, Dai C, Kiss LP, Wen X, Liu Y. Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria. *Am J Pathol* (2008) 172(2):299–308. doi: 10.2353/ajpath.2008.070057

45. Yamaguchi Y, Iwano M, Suzuki D, Nakatani K, Kimura K, Harada K, et al. Epithelial-mesenchymal transition as a potential explanation for podocyte depletion in diabetic nephropathy. *Am J Kidney Dis* (2009) 54(4):653–64. doi: 10.1053/j.ajkd.2009.05.009

46. Weinberg JM. Lipotoxicity. *Kidney Int* (2006) 70(9):1560–6. doi: 10.1038/sj.ki.5001834

47. Herman-Edelstein M, Scherzer P, Tobar A, Levi M, Gafter U. Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy. *J Lipid Res* (2014) 55(3):561–72. doi: 10.1194/jlr.P040501

48. Sharma S, Adrogue JV, Golfman L, Uray I, Lemm J, Youker K, et al. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* (2004) 18(14):1692–700. doi: 10.1096/fj.04-2263com

49. Schulze PC. Myocardial lipid accumulation and lipotoxicity in heart failure. *J Lipid Res* (2009) 50(11):2137–8. doi: 10.1194/jlr.R001115

50. Marfella R, Di Filippo C, Portoghese M, Barbieri M, Ferraraccio F, Siniscalchi M, et al. Myocardial lipid accumulation in patients with pressure-overloaded heart and metabolic syndrome. *J Lipid Res* (2009) 50(11):2314–23. doi: 10.1194/jlr.P900032-JLR200

51. Belopolsky Y, Khan MQ, Sonnenberg A, Davidson DJ, Fimmel CJ. Ketogenic, hypocaloric diet improves nonalcoholic steatohepatitis. *J Transl Int Med* (2020) 8(1):26–31. doi: 10.2478/jtim-2020-0005

52. Proctor G, Jiang T, Iwahashi M, Wang Z, Li J, Levi M. Regulation of renal fatty acid and cholesterol metabolism, inflammation, and fibrosis in akita and OVE26 mice with type 1 diabetes. *Diabetes* (2006) 55(9):2502–9. doi: 10.2337/db05-0603

53. Kim HJ, Moradi H, Yuan J, Norris K, Vaziri ND. Renal mass reduction results in accumulation of lipids and dysregulation of lipid regulatory proteins in the remnant kidney. *Am J Physiol Renal Physiol* (2009) 296(6):F1297–306. doi: 10.1152/ajprenal.90761.2008

54. Bobulescu IA. Renal lipid metabolism and lipotoxicity. *Curr Opin Nephrol Hypertens* (2010) 19(4):393–402. doi: 10.1097/MNH.0b013e32833aa4ac

55. Nosadini R, Tonolo G. Role of oxidized low density lipoproteins and free fatty acids in the pathogenesis of glomerulopathy and tubulointerstitial lesions in type 2 diabetes. *Nutr Metab Cardiovasc Dis* (2011) 21(2):79–85. doi: 10.1016/j.numecd.2010.10.002

56. Nagaya T, Tanaka N, Suzuki T, Sano K, Horiuchi A, Komatsu M, et al. Down-regulation of SREBP-1c is associated with the development of burned-out NASH. *J Hepatol* (2010) 53(4):724–31. doi: 10.1016/j.jhep.2010.04.033

57. Deprince A, Haas JT, Staels B. Dysregulated lipid metabolism links NAFLD to cardiovascular disease. *Mol Metab* (2020) 42:101092. doi: 10.1016/j.molmet.2020.101092

58. Mato JM, Alonso C, Noureddin M, Lu SC. Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. *World J Gastroenterol* (2019) 25(24):3009–20. doi: 10.3748/wjg.v25.i24.3009

59. Lv WS, Sun RX, Gao YY, Wen JP, Pan RF, Li L, et al. Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. *World J Gastroenterol* (2013) 19(20):3134–42. doi: 10.3748/wjg.v19.i20.3134

60. Vaziri ND. Disorders of lipid metabolism in nephrotic syndrome: mechanisms and consequences. *Kidney Int* (2016) 90(1):41–52. doi: 10.1016/j.kint.2016.02.026

61. Umanath K, Lewis JB. Update on diabetic nephropathy: Core curriculum 2018. *Am J Kidney Dis* (2018) 71(6):884–95. doi: 10.1053/j.ajkd.2017.10.026

62. Targher G, Byrne CD. Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. *Nat Rev Nephrol* (2017) 13(5):297–310. doi: 10.1038/nrneph.2017.16

63. Spoto B, Pisano A, Zoccali C. Insulin resistance in chronic kidney disease: a systematic review. *Am J Physiol Renal Physiol* (2016) 311(6):F1087–F108. doi: 10.1152/ajprenal.00340.2016

64. Chen J, Muntner P, Hamm LL, Fonseca V, Batuman V, Whelton PK, et al. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. *J Am Soc Nephrol* (2003) 14(2):469–77. doi: 10.1097/01.ASN.0000046029.53933.09

65. Chen J. The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med* (2004) 140(3):167–74. doi: 10.7326/0003-4819-140-3-200402030-00007

66. Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut* (2021) 70(5):962–9. doi: 10.1136/gutjnl-2020-322572

67. Wan H, Wang Y, Xiang Q, Fang S, Chen Y, Chen C, et al. Associations between abdominal obesity indices and diabetic complications: Chinese visceral adiposity index and neck circumference. *Cardiovasc Diabetol* (2020) 19. doi: 10.1186/s12933-020-01095-4

68. Targher G, Bertolini L, Chonchol M, Rodella S, Zoppini G, Lippi G, et al. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and retinopathy in type 1 diabetic patients. *Diabetologia* (2010) 53(7):1341–8. doi: 10.1007/s00125-010-1720-1

69. Saito H, Tanabe H, Kudo A, Machii N, Higa M, Yamaguchi S, et al. High FIB4 index is an independent risk factor of diabetic kidney disease in type 2 diabetes. *Sci Rep* (2021) 11(1):11753. doi: 10.1038/s41598-021-88285-6

70. Cohen DE, Fisher EA. Lipoprotein metabolism, dyslipidemia, and nonalcoholic fatty liver disease. *Semin Liver Dis* (2013) 33(4):380–8. doi: 10.1055/s-0033-1358519

71. Goh GB, Pagadala MR, Dasarathy J, Unalp-Arida A, Sargent R, Hawkins C, et al. Renin-angiotensin system and fibrosis in non-alcoholic fatty liver disease. *Liver Int* (2015) 35(3):979–85. doi: 10.1111/liv.12611

72. Targher G. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *Massachusetts Med Soc* (2010) 363(14):1341–50. doi: 10.1056/NEJMra0912063

73. Anders HJ, Huber TB, Isermann B, Schiffer M. CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat Rev Nephrol* (2018) 14 (6):361–77. doi: 10.1038/s41581-018-0001-y

74. Musso G, Cassader M, Cohney S, De Michieli F, Pinach S, Saba F, et al. Fatty liver and chronic kidney disease: Novel mechanistic insights and therapeutic opportunities. *Diabetes Care* (2016) 39(10):1830–45. doi: 10.2337/dc15-1182

75. Fukazawa K, Lee HT. Updates on hepato-renal syndrome. *J Anesth Clin Res* (2013) 4(9):352. doi: 10.4172/2155-6148.1000352

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 03 November 2022
DOI 10.3389/fendo.2022.1032164

OPEN ACCESS

EDITED BY
Sonia Michael Najjar,
Ohio University, United States

REVIEWED BY
Lauren Koch,
University of Toledo, United States
Graciela Muniz-Terrera,
Ohio University, United States
Giulia Quinto,
University of Padua, Italy

*CORRESPONDENCE
Adriaan G. Holleboom
a.g.holleboom@amsterdamumc.nl

†These authors share first authorship

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

RECEIVED 30 August 2022
ACCEPTED 10 October 2022
PUBLISHED 03 November 2022

CITATION
Houttu V, Bouts J, Vali Y, Daams J, Grefhorst A, Nieuwdorp M and Holleboom AG (2022) Does aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? A systematic literature review and meta-analysis. *Front. Endocrinol.* 13:1032164. doi: 10.3389/fendo.2022.1032164

Does aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? A systematic literature review and meta-analysis

Veera Houttu[1,2†], Julia Bouts[1,2†], Yasaman Vali[3], Joost Daams[4], Aldo Grefhorst[2], Max Nieuwdorp[1,2] and Adriaan G. Holleboom[1,2*]

[1]Department of Vascular Medicine, Amsterdam Gastroenterology, Endocrinology Metabolism, Amsterdam UMC, Location AMC at University of Amsterdam, Amsterdam, Netherlands, [2]Department of Experimental Vascular Medicine, Amsterdam Gastroenterology, Endocrinology Metabolism, Amsterdam UMC, Location AMC at University of Amsterdam, Amsterdam, Netherlands, [3]Department of Epidemiology and Data Science, Amsterdam UMC, Location AMC at University of Amsterdam, Amsterdam, Netherlands, [4]Medical Library, Amsterdam UMC, Location AMC at University of Amsterdam, Amsterdam, Netherlands

Background: Exercise is an effective strategy for the prevention and regression of hepatic steatosis in patients with non-alcoholic fatty liver disease (NAFLD), but it is unclear whether it can reduce advanced stages of NAFLD, i.e., steatohepatitis and liver fibrosis. Furthermore, it is not evident which modality of exercise is optimal to improve/attenuate NAFLD.

Objectives: The aim is to systematically review evidence for the effect of aerobic exercise (AE) on NAFLD, in particular non-alcoholic steatohepatitis (NASH) and liver fibrosis.

Methods: A systematic literature search was conducted in Medline and Embase. Studies were screened and included according to predefined criteria, data were extracted, and the quality was assessed by Cochrane risk of bias tools by two researchers independently according to the protocol registered in the PROSPERO database (CRD42021270059). Meta-analyses were performed using a bivariate random-effects model when there were at least three randomized intervention studies (RCTs) with similar intervention modalities and outcome.

Results: The systematic review process resulted in an inclusion a total of 24 studies, 18 RCTs and six non-RCTs, encompassing 1014 patients with NAFLD diagnosed by histological or radiological findings. Studies were grouped based on the type of AE: moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT). A total of twelve meta-analyses were

10.3389/fendo.2022.1032164

conducted. Compared to controls, MICT resulted in a mean difference (MD) in the NAFLD biomarkers alanine transaminase (ALT) and aspartate aminotransferase (AST) of -3.59 (CI: -5.60, -1.59, p<0.001) and -4.05 (CI: -6.39, -1.71, p<0.001), respectively. HIIT resulted in a MD of -4.31 (95% CI: -9.03, 0.41, p=0.07) and 1.02 (95% CI: -6.91, 8.94, p=0.8) for ALT and AST, respectively. Moreover, both AE types compared to controls showed a significantly lower magnetic resonance spectroscopy (MRS) determined liver fat with a MD of -5.19 (95% CI: -7.33, -3.04, p<0.001) and -3.41 (95% CI: -4.74, -2.08, p<0.001), for MICT and HIIT respectively. MICT compared to controls resulted in a significantly higher cardiorespiratory fitness (MD: 4.43, 95% CI: 0.31, 8.55, p=0.03).

Conclusion: Liver fat is decreased by AE with a concomitant decrease of liver enzymes. AE improved cardiorespiratory fitness. Further studies are needed to elucidate the impact of different types of AE on hepatic inflammation and fibrosis.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier (CRD42021270059).

KEYWORDS

non-alcoholic fatty liver disease, aerobic exercise, high-intensity interval training, moderate-intensity continuous training, systematic review, meta-analysis

1 Introduction

The prevalence of obesity has strongly increased, driving an increase in the prevalence of non-alcoholic fatty liver disease (NAFLD) (1, 2). Consequently, NAFLD is now the most common liver disease globally, affecting 30–40% of adult men and 15–20% of adult women (3). The latest findings show an alarming number of children who are developing NAFLD in their early childhood (4). NAFLD is intertwined/associated with multiple metabolic diseases, i.e., metabolic syndrome and type 2 diabetes mellitus (T2DM). At least half of the patients with T2DM have NAFLD. Moreover, atherosclerotic cardiovascular disease (asCVD) is the main cause of mortality among patients with NAFLD (5–7).

The disease spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) and fibrosis, of which the latter often results in liver-related mortality and morbidity (8, 9). NAFLD can even lead to cirrhosis and hepatocellular carcinoma (HCC), and patients with NAFLD might ultimately require liver transplantation (10–12). The pathophysiology of NAFLD is complex, but insulin resistance seems to be a crucial driving factor (13). Hyperalimentation (14), total parental nutrition (6), or sedentary lifestyle in combination with genetic heritability have also been recognized as important drivers of NAFLD (2, 15).

Despite the magnitude of the clinical problems of NAFLD and its burden on public health, pharmacotherapy for advanced stages of NAFLD has not yet been developed (16). Therefore, lifestyle interventions are still cornerstone management elements for NAFLD (17–19). Current guidelines targeting NAFLD recommend lifestyle therapies, including exercise and dietary modifications. Aerobic exercise (AE) is a type of physical activity when increase in the heart rate and breath are maintained over a period of time (20). Patients with hepatic steatosis are recommended to perform moderate-intensive or vigorous-intensive AE for 150–300 or 75–150 minutes/week, respectively (17–19). In addition to the direct liver related benefits of AE, AE reduces the risk for asCVD in patients with NAFLD (21). However, the evidence for the effect of AE on advanced stages of NAFLD, including fibrosis and NASH, is scarce. Furthermore, which type or modality of exercise intervention is optimal for patients with NAFLD is not yet evident (22, 23).

To address these knowledge gaps, we systematically reviewed the scientific literature to explore the effects of AE without dietary adjustments on NAFLD and NASH, and its associated markers. In this study, we focused on different modalities of AE, namely high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and sprint-interval training (SIT).

2 Methods and materials

This study was reported using the Preferred Reporting Items of Systematic Reviews and Meta-analysis (PRISMA) statement guidelines (24). The protocol of the systematic review is available in PROSPERO (CRD42021270059) (25).

2.1 Search strategy and data sources

A sensitive systematic literature search was conducted in Medline (*via* OVID) and Embase (*via* OVID) in close collaboration with an information specialist (JD) in February 2021. Also, a scoping search was conducted in SPORTDiscuss in response to the reviewer suggestion yielding four articles in population other than patients with NAFLD being irrelevant to this systematic review. The search was limited to articles published in English. The full search strategy is described in detail in Supplementary Material 1.

2.2 The eligibility criteria

2.2.1 Inclusion and exclusion criteria

Studies were eligible if they fulfilled the following inclusion criteria (1): adults patients (≥ 18 years of age) with NAFLD/NASH (with or without fibrosis) diagnosed by histology (liver biopsy) or by non-invasive methods such as magnetic resonance imaging (MRI), ultrasonography (US) or vibration-controlled transient elastography (VCTE; FibroScan) (2); the main outcomes of interest were changes from baseline to the follow up on intrahepatic lipids (IHL), liver stiffness, fibrosis, steatohepatitis and/or inflammation (3); study intervention designed with at least one AE arm without dietary intervention. Studies that contained a dietary intervention combined with exercise were excluded. Studies were excluded if they were animal studies, case reports, case series, conference abstracts and letter/commentary studies. Studies were also excluded if they included subjects with excessive alcohol use, viral hepatitis or autoimmune hepatitis, Wilson's disease or hemochromatosis, or when the subjects were children (< 18 years of age).

2.3 Screening process

After deduplication, the remaining titles and abstracts of the articles were screened independently by two reviewers (JB and VH) using the Rayyan QCRI program (26). In case of any disagreement, consensus was reached by discussion between the reviewers. In next screening phase, full texts were judged independently by the same two reviewers. A third reviewer (AH or YV) was consulted in case of disagreements.

2.4 Assessment of methodological quality

The Cochrane risk of bias tool for RCTs (RoB2) (27), and the Cochrane risk of bias tool (ROBINS-I) for non-RCT (28) were used to assess the risk of bias, which was performed by JB and VH independently. The risk of bias tool RoB2 contains five different domains that were used to assess the risk of bias, namely those 1) arising from the randomizing process; 2) due to deviations from the intended intervention; 3) due to missing outcome data; 4) in measurement outcome; and 5) in selection of the reported results. The overall judgment of the bias was classified based on the domains in RoB2 as a) low risk of bias, b) some concerns about bias, and c) high risk of bias.

ROBINS-I assessed 1) the risk of bias due to confounding effects; 2) the risk of bias in selection of participants into the study; 3) the risk of bias in classification of the intervention. The risk of bias tool ROBINS-I contains categories of low, moderate, serious and critical risk of bias, or no information.

2.5 Data extraction

The following study characteristics were extracted: title, author, country, year of publication, study design, diagnostic test features, study group characteristics, and the characteristics of exercise intervention (type of exercise, intensity and duration). Primary outcomes were NAFLD activity score (NAS) and individual histological scores for inflammation, ballooning and fibrosis, liver stiffness measurement (LSM) on FibroScan, liver fat (IHL based on MRI, score/grade based on US, steatosis based on controlled attenuation parameter (CAP) on FibroScan), and liver function markers (alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (γGT)). Additional outcomes were glucose metabolism markers (glucose, insulin, hemoglobin A1c (HbA1c), and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)), plasma lipid profile markers (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG)), body composition (body weight, fat mass, body mass index (BMI), and (relative) amounts of visceral adipose tissue (VAT) and subcutaneous visceral tissue (SAT)) and cardiorespiratory fitness (peak or maximal oxygen uptake (VO2 peak) or (VO2 max)). Data extraction was conducted by one author and cross-checked by the other author (JB/VH). Study authors were contacted in case of the absence of reported values.

2.6 Statistical analysis

Included studies were categorized based on the type of the AE intervention (HIIT, MICT and SIT), the measurement technique

of liver outcome (histology, MRI, US, FibroScan) and the type of control group. A meta-analysis was performed when there were at least three studies with similar intervention modalities and outcomes. In addition, outcome data were grouped based on intensity, type of AE (HIIT vs. MICT), and type of control group. When articles could not be included for meta-analysis, a narrative synthesis was used to summarize the findings.

Data per marker was unified by using appropriate conversion factors. In case HOMA-IR was not reported and data on glucose and insulin were available, HOMA-IR was calculated by using the formula HOMA-IR = *[fasting insulin x fasting glucose]/22.5*. Data expressed as mean with 95% confidence interval (CI) were calculated to standard deviations (SD) using the formula of the Cochrane Handbook for Systematic Reviews of Interventions (29). Changes after the intervention were calculated for each parameter in intervention and control groups according to the same Cochrane Handbook (29).

Meta-analyses were performed using the Cochrane Review Manager (RevMan version 5.4, the Cochrane Collaboration, 2020) (30). The extracted data were input as mean ± SD. Heterogeneity was checked by using the chi-square and I^2 tests, and 95% CI was calculated using a random effects model. Pearson correlations of mean differences of two outcomes were performed by using IBM SPSS Statistics (version 26.0, Chicago, USA). Sensitivity analyses were conducted to investigate the influence of exercise duration, by removing a study with considerably longer intervention than other studies from meta-analysis (31).

2.7 Publication bias

Publication bias was reduced by searching in different electronic databases, checking abstracts for any further missing reports, checking references from other reviews and contacting experts and authors. Funnel plots were not constructed since the meta-analyses in this review do not have a required minimum of 10 studies per subgroup (29).

3 Results

3.1 Database search and article selection

The database search resulted in a total of 1420 articles. After screening the titles and abstracts, 73 remained for full-text assessment. According to the eligibility criteria, 24 studies, of which 18 were RCTs and six were non-RCTs, were included in this systematic review. In total, ten studies were included in the meta-analysis. The whole selection process is presented in the flow chart (Figure 1).

3.2 Study characteristics

This systematic review included 18 RCTs (Table 1) and six non-RCTs (Table 2), including a total of 815 and 199 subjects with diagnosed NAFLD, respectively. The sample size of the studies varied from 11 (43) to 209 (49). The mean age ranged between 39.7 ± 6.7 (39) and 60 ± 3.4 years (49), and most of the studies included both men and women. The method used for diagnosis of NAFLD and/or NASH varied: six studies used histology (n=121) (37, 41, 44, 46, 53, 55), nine MRI (n=399) (32, 33, 35, 43, 47–49, 51, 56), eight US (n=386) (34, 38, 42, 45, 46, 50, 52, 53), and one FibroScan (n=48) (39). Included studies reported NAFLD in different stages; histologically assessed NAS score varied from 3.6 (37) to 5 (41), while the patients' steatotic status ranged from 10.3 ± 4.4% (40) to 31.3 ± 4.8% of liver fat (33) based on MRS. Liver stiffness was assessed by LSM FibroScan in three studies (n=68) (34, 42, 44), while two studies scored liver histology to assess hepatic fat and fibrosis (n=25), as well as liver inflammation and ballooning (37, 54).

3.3 Characteristics of exercise interventions

The AE interventions of RCTs and non-RCTs with varying types of sports, intensities and durations are presented in Tables 1, 2. Of the 18 RCTs, four conducted HIIT, 10 MICT and four did both types of AE. There were three HIIT and one MICT non-RCTs. In two non-RCTs a sprint interval training intervention (SIT) was performed (53, 56). Altogether six studies executed a RCT-HIIT either by bicycle/ergometer with three training sessions per week with a duration per session varying from 13-min (42) to 40–60 min for 12 (40, 41) or eight weeks (32), or by treadmill training with four sessions per week for four weeks (48) or six months (49). Control groups received standard care or nothing, except for the resistance training (RT) control group of Oh et al. (42). Intensity of the training intervals was based either on VO2max (32), VO2peak (42, 48), Borg rating of perceived exertion (40, 41), maximum predicted heart rate (MHR) (49), heart rate reserve (HRR) (54) or maximum heart rate (HRmax) (51, 52).The duration of RCT-MICT interventions varied from eight weeks (32, 34, 45) up to six months (37, 38, 44, 49), and were conducted on a cycle ergometer (32, 34, 42) or a treadmill (33, 36, 37), or the intervention was treadmill or brisk walking (35, 44, 45, 47, 49). Interventions were performed at 60–80% (32–34, 38, 39, 42, 45) or between 30% and 60% of heart rate (HR) (36, 43, 46–49). Cardiorespiratory fitness was evaluated by ergospirometry or treadmill spirometry test in eight studies (32, 41–44, 46–48).

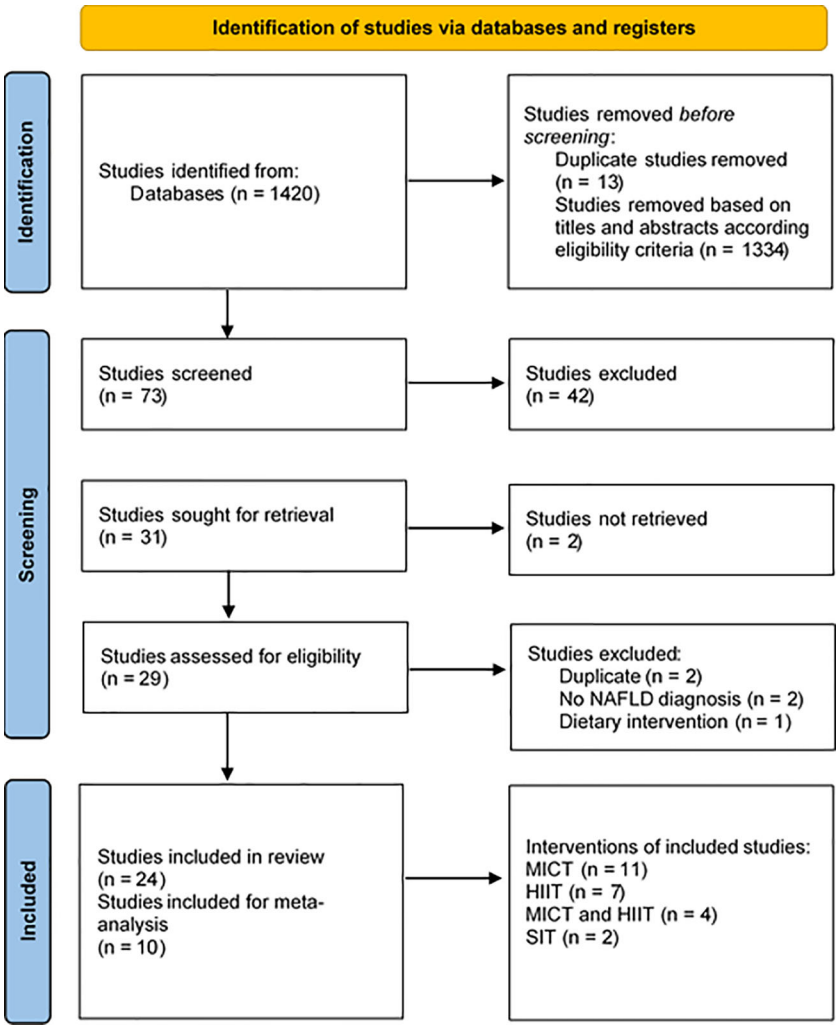


FIGURE 1
Preferred reporting items of systematic reviews and meta-analysis (PRISMA) flow chart [16]. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; SIT, sprint interval training.

TABLE 1 The characteristics of the included randomized controlled trials (RCT).

First author, year of publication and country	Intervention	Sample size (F/M)	Age (mean ± SD)	Disease stage	Diagnosis technique	Outcome assessment technique	Intervention details	Control	Duration
Abdelbasset et al., 2020, Saudi Arabia (32)	MICT and HIIT	I (HIIT): 16 (6/10) I (MICT): 15 (7/8) C: 16 (7/9)	I (HII): 54.4 ± 5.8 I (MIC): 54.9 ± 4.7 C: 55.2 ± 4.3	IHL % I (HII): 12.4 ± 4.5% I (MIC): 12.9 ± 4.2% C: 11.2 ± 5.1%	Diagnostic guidelines for NAFLD in the Asia-Pacific region	MRI-PDFF	3 x week, 40-50 min Cycling: 5-min warm up, continuous intensity at 60-70% of the max HR, 5-min cooling down 3 x week, 40 min Cycling: 5-min warm up, 4-min interval x 3 at 80-85% VO2max, 2-min rest intervals	No exercise program, standard care	8 weeks

(Continued)

TABLE 1 Continued

First author, year of publication and country	Intervention	Sample size (F/M)	Age (mean ± SD)	Disease stage	Diagnosis technique	Outcome assessment technique	Intervention details	Control	Duration
							at 50% VO2max, 5-min cooling down		
Bacchi, E. et al., 2013, Italy (33)	MICT	I: 14 (4/10) C: 17 (5/12)	I: 55.6 ± 2.0 C: 56.0 ± 1.9	IHL % I: 25.7 ± 3.7% C: 31.3 ± 4.8%	H-MRS	H-MRS	3 x week 60 min Treadmill, cycle or elliptical machines at 60-65% of heart rate	RT: 9 exercises on weight machines, 3 series of 10 repetitions at 70-80%	16 weeks
Cevik, T. et al., 2020, Turkey (34)	MICT	I: 16 (10/6) C: 15 (9/6)	I: 43.75 ± 8.62 C: 45.07 ± 9.11	–	US	FibroScan	4x week 40 min Cycle ergometer at 60-80% of HR	AE + whole body vibration: Vertical-sinusoidal vibration platforms, 15 min	8 weeks
Cheng, S. et al., 2017, China (35)	MICT	I: 22 (17/5) C: 18 (14/4)	I: 59 ± 4.4 C: 60 ± 3.4	NAFLD with impaired FG	H-MRS	H-MRS	2-3 x week, 30-60 min Nordic brisk walking + other group exercises at 60-75% of VO2max	No exercise program, Advised to maintain their current level of PA	12 weeks
Cuthbertson, D. et al., 2016, UK (36)	MICT	I: 30 (7/23) C: 20 (4/16)	I: 50 [46, 58]* C: 52 [46, 59]*	IHL % I: 19.4% [14.6, 36.1]* C: 16.0% [9.6, 32.5]*	Clinically by hepatologist	H-MRS	3-5 x week, 30-45 min Treadmill, cross-trainer, bike ergometer, rower at 30-60% of HRR	No exercise program, Advice about the health benefits of exercise in NAFLD	16 weeks
Eckard, C. et al., 2013, Italy (37)	MICT	I: 9 (3/6) C: 11 (4/7)	I: 51 ± 11 C: 52 ± 10	NAS I: 3.7 ± 1.1 C: 3.6 ± 1.1	Histology	Histology	4-7 x week, 20-60 min Exercise bicycle, treadmill	No exercise program, standard care	24 weeks
Franco, I. et al., 2019, Italy (38)	MICT	I: 42 (6/36) C: 52 (10/42)	Data not reported	Moderate to severe	US	US	4 x week, 45 min 65-75% of VO2	Combined exercise	24 weeks
Franco, I. et al., 2021, Italy (39)	MICT	I: 25 (11/14) C: 23 (6/17)	I: 50.45 ± 9.45 C: 46.23 ± 9.39	Moderate to severe	FibroScan	FibroScan	3 x week, 50-60 min Treadmill, cycling, cross-training and rowing at 60-75% MHR	Combined exercise	12 weeks
Hallsworth, K. et al., 2015 (40)	HIIT	I: 12 C: 11	I: 54 ± 10 C: 52 ± 12	IHL % I: 10.6 ± 4.9 C: 10.3 ± 4.4	Clinically	H-MRS	3 x week, 30-40 min Cycling: 5-min warm up (very light/somewhat hard), 2-min interval (very hard) x 5 + cumulative 10 sec increase per interval per week, 3-min rest, 3-min cooling down Intensity based on Borg rating of perceived exertion	No exercise program, standard care	12 weeks
Houghton, D. et al., 2017, Australia (41)	HIIT	I: 12 C: 12	I: 54 ± 12 C: 51 ± 16	NAS I: 5 [3, 7]* C: 5 [2, 7]*	Histology	H-MRS	3 x week, 45-60 min Cycling: 5-min warm up, 2-min interval x 3, 1-min rest intervals, Intensity based on Borg rating of perceived exertion (16-20, very hard)	No exercise program, standard care	12 weeks

(Continued)

TABLE 1 Continued

First author, year of publication and country	Intervention	Sample size (F/M)	Age (mean ± SD)	Disease stage	Diagnosis technique	Outcome assessment technique	Intervention details	Control	Duration
							Resistant training: hip and knee extension, horizontal row, chest press, vertical row, and knee extension, Intensity based on Borg rating of perceived exertion (14-16, hard)		
Oh, S. et al., 2017, Japan (42)	MICT and HIIT	I (HIIT): 20 (0/20) I (MICT): 13 (0/13) C (RT): 19 (0/19)	I (HIAT): 48.6 ± 1.8 I (MICT): 48.2 ± 2.3 C (RT): 51.2 ± 1.9	–	Diagnostic guidelines for NAFLD in the Asia-Pacific region	FibroScan CEUS	3 x week, 13 min Cycling: 2-min warm up (30 W, 60 rpm), 3-min interval x 3 at 80-85% VO2max (70-80 rpm), 2-min rest interval x 2 at 50% VO2max (60 rpm), 3-min cooling down (30 W, 60 rpm) 3 x week, 13 min Cycling: 2-min warm up (30 W, 60 rpm), 3-min interval x 3 at 80-85% VO2max (70-80 rpm), 2-min rest interval x 2 at 50% VO2max (60 rpm), 3-min cooling down (30 W, 60 rpm)	Resistance exercise: Push-ups, sit ups, leg press, leg extensions/ curls, chest press, pull downs Intensity based on 1-RM strength test	12 weeks
Pugh, C. et al., 2013, UK (43)	MICT	Inclusion 13 (6/7) I: 6 C: 5	I: 50 [44, 56]* C: 48 [38, 57]*	–	US and elevated ALT, and H-MRS	H-MRS	3-5 x week, 30-45 min 30-60% HRR*	Conventional care	16 weeks
Rezende, R. et al., 2016, Brazil (44)	MICT	I: 19 (19/0) C: 21 (21/0)	I: 56 ± 7.8 C: 54.4 ± 8.9	–	Histology	FibroScan	2 x week, 30-50 min Treadmill	No exercise program	24 weeks
Shamsoddini, A. et al., 2015, Iran (45)	MICT	I (MICT): 10 (0/10) I (RT): 10 (0/10) C: 10 (0/10)	I: 39.7 ± 6.7 C: 45.8 ± 7.3	Moderate to severe	US	US	3 x week, 45 min Treadmill at 60-75% MHR (max HR)	No exercise program	8 weeks
Shojaee-Moradie, F. et al., 2016, UK (46)	MICT	I: 15 (0/15) C: 12 (0/12)	I: 52.4 ± 2.2 C: 52.8 ± 3.0	IHL % I: 19.6% [14.8, 30.0]* C: 12.5% [6.9, 32.9]*	US or histology	H-MRS	4-5 x week Gym-based aerobic exercise at 40-60%	Conventional lifestyle advice	16 weeks
Sullivan, S. et al., 2012, USA (47)	MICT	I: 12 (8/4) C: 6 (5/1)	I: 47.5 ± 2.2 C: 47.5 ± 3.1	IHL % I: raw data missing C:	H-MRS	H-MRS	5 x week, 30-60 min Treadmill at 45-55% VO2peak	No exercise program	16 weeks
Winn, N. et al., 2018, USA (48)	MICT and HIIT	I (HIIT): 8 I (MICT): 8 C: 5	I (HIIT): 41 ± 14 I (MICT): 46 ± 19 C: 51 ± 13	–	H-MRS	H-MRS	4 x week, duration was calculated as the ratio of 80 L/O2 by the average VO2 (L/min/O2) per each session Treadmill at 55% VO2peak 4 x week, Treadmill: 4-min intervals at	No exercise program	4 weeks

(Continued)

TABLE 1 Continued

First author, year of publication and country	Intervention	Sample size (F/M)	Age (mean ± SD)	Disease stage	Diagnosis technique	Outcome assessment technique	Intervention details	Control	Duration
							80% VO2peak, 3-min rest intervals at 50% VO2peak		
Zhang, H. et al., 2016, China (49)	MICT and HIIT	I (MICT): baseline 73 (37/36) completed 69 (52/21) I (MICT-HIIT): baseline 73 (38/35) completed 66 (51/22) C: 74 (46/28)	I (MICT): 54.4 ± 7.4 I (MICT-HIIT): 53.2 ± 7.1 C: 54.9 ± 6.8	IHL % I (mod): 18.0 ± 9.9 I (mod-vig): 18.4 ± 9.9 C: 17.5 ± 11.0	H-MRS	H-MRS	5 x week, brisk walking 30 min (in total 150 min/week) at 45-55% MHR** Ttreadmill 30-min at 65-80% of MHR**	No exercise program	26 weeks

C, control group; CT, computed tomography; F, female; FG, fasting glucose; HIIT, high-intensity interval training; I, intervention group; M, male; MICT, moderate-intensity continuous training; MRS, magnetic resonance spectroscopy; Mri-PDFF, magnetic resonance imaging proton density fat fraction; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PA, physical activity; RT, resistance training; SD, standard deviation; US, ultrasonography.
*95% CI.
**MHR, maximum predicted heart rate, calculated as 220/min for men and 210/min for women minus the participant's age.

3.4 Meta-analysis and narrative review

The meta-analysis was performed for the liver outcomes (IHL, ALT, AST, γGT), glucose metabolism (glucose, HOMA-IR) and the plasma lipid profile parameters (TC, LDL-C, HDL-C, TG), as well as the cardiorespiratory fitness level (VO_2max and VO_2peak) and the total body weight from RCTs. Eight studies could not be integrated into the meta-analysis due to unsuitable study design or incomparable assessment techniques. Therefore, these eight studies are reported narratively. Among these studies is the one by Cevik et al. (34), in which two active arms, one AE intervention with and one without whole-body vibration, were used. Bacchi et al. (33) also performed a RCT with two active arms. Also, the 2019 and 2020 studies of Franco et al. (38, 39) had multiple active arms. The first study was conducted with two active arms and the latter with six active intervention arms. The studies by Oh et al. (42) and Winn et al. (48) had three and two active arms, respectively. For the studies of Eckard et al. (37) and Shamsoddini et al. (45) assessment techniques were not comparable with other studies. Additionally, all RCTs do not report/study of an outcome of interest, and therefore, are neither integrated in the meta-analysis nor reported narratively. All non-RCTs with the outcome of interest were narratively reviewed.

3.4.1 Liver related outcomes

Various measures as a proxy of NAFLD/NASH have been used in the included studies. While most studies report plasma transaminases and use MRS to quantify IHL, some scored histology to assess inflammation and fibrosis, or performed FibroScan as a proxy for liver fibrosis.

3.4.1.1 Inflammation and fibrosis

FibroScan using LSM was used only in two studies with a MICT intervention (n=35) (34, 44), thus meta-analysis of this method was not possible. In general, MICT did not result in a significant effect on LSM. In the HIIT study that employed LSM, the intervention led to a significant reduction in hepatic stiffness (-16.8%, n=20, p<0.005) (42). In another study, HIIT reduced LSM as well as histological assessed hepatocyte ballooning and fibrosis (54). A rather long 6-month MICT did not significantly reduce the histological-determined NAS (37).

3.4.1.2 Liver transaminases

With respect to liver transaminases, a meta-analysis of seven studies shows that subjects in AE (n=283) had significantly lower plasma ALT concentrations compared to controls (n=280) (MD: -3.78, 95% CI: -5.58, -1.98, p<0.001) (Figure 2A). However, the plasma ALT concentrations were only significantly lower in MICT subjects but not in HIIT subjects. Yet, there is no significant subgroup difference (I2 = 0%, Chi2 = 0.08, df=1, p=0.78). While plasma AST concentrations were not lower in AE compared to control, MICT subjects (n=161) had significant lower plasma AST concentrations than control subjects (n=150) (MD: -4.05, 95% CI: -6.39, -1.71, p=0.0007) (Figure 2B). No significant differences were observed between the groups in plasma γGT concentrations (Figure 2C).

TABLE 2 The characteristics of the included non-randomized controlled trials (non-RCT).

First author, year of publication and country	Intervention	Sample size (F/M)	Age (mean ± SD)	Disease stage	Diagnosis technique	Outcome assessment technique	Intervention details	Control	Duration
Abd El-Kader, M. et al., 2014, Saudi Arabia (50)	MICT	50 (24/26)	I (AE): 50.87 ± 5.93 I (RT): 51.12 ± 5.58	–	US	NA	3 x week, 40 min 5 min warm-up, 30 min treadmill at 60%-80% of HRmax (increased weekly), 5 min cooling down	40 min, resistance machines, 10 min stretching, 30 min RT at 60 and 80% of their one maximal repetition weight	12 weeks
Haus, M.J. et al., 2013, USA (51)	HIIT	I: 14 (sex distribution not reported)	I: 55.6 ± 2.0 C: 56.0 ± 1.9	–	MRS	MRS	60 min for 7 days, treadmill at 85% of HRmax	–	7 days
Khaoshbaten, M. et al., 2013, Iran (52)	HIIT	I: 45 (16/29) C: 45 (17/28)	I: 35.6 ± 9.2 I: 39.5 ± 6.9	Grade I-III	US	US	30 min for 3 times x week, at HRmax	Medical therapy, 1000 mg vitC + 400 units vitE	3 months
MacLean, C. et al., 2018, UK (52)	SIT	1: 2 (2/10)	I: 45 ± 8	Steatosis to NASH	Histology or US	FIB-4	2 times x week (5–10 × 6-s 'all-out' cycle sprints)	NA	6 weeks
O'Gorman, P. et al., 2020, Ireland (53)	HIIT	I: 16 (12/4) C: 8 (5/3)	I: 61 ± 15 C: 58 ± 23	Steatosis to fibrotic NASH	Histology	Histology and FibroScan	2 supervised, 3 unsupervised times x week, 40-75% of HRR	Standard care	12 weeks
Sargeant, J. et al., 2018, Germany (54)	SIT	I: 9 (0/9)	I: 41 ± 8	IHL % 15.6 ± 8.4	H-MRS	H-MRS	3 x week, 4 intervals of max sprint cycling per session, increasing interval every 2 weeks (total of 90 intervals)	–	6 weeks

C, control group; CT, computed tomography; F, female; HIIT, high-intensity interval training; HRmax, maximum heart rate; HIIT, high-intensity interval training; HRR, heart rate reserve; I, intervention group; M, male; MICT, moderate-intensity continuous training; MRS, magnetic resonance spectroscopy; MRS-PDFF, magnetic resonance spectroscopy; NA, not applicable; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NA, not available; RT, resistance training; SD, standard deviation; SIT, sprint interval training; US, ultrasonography.

Four RCTs that report transaminases were not included in the meta-analysis due to incomparable study designs. These studies showed varying results on the ALT concentrations upon AE. The study by Cevik et al. (34) reported a significant decrease in ALT and AST concentrations after an AE with and without whole body vibration from baseline. In turn, Bacchi et al. (33) did not show a significant reduction in ALT after an AE intervention (nor after RT intervention). In line, Winn et al. (48) did not find significant changes in transaminases from baseline after HIIT or MICT, but unfortunately, they did not report the change in ALT of the control group for comparison. Oh et al. (42) reported no change in ALT or AST concentrations after HIIT, but there was a significant change after MICT. In non-RCTs, there is a significant reduction of ALT and AST concentrations after the 3-month HIIT intervention in the study by Khaoshbaten et al. (52), as well as after a 12-week MICT intervention by Abd El Kader et al. (50). The SIT intervention by MacLean et al. (53) did affect transaminases. Houghton et al. (41) show close to significant reduction in γGT after 12-week HIIT. However, another 12-week HIIT did not results change in the concentrations (40), nor did AE with whole-body vibration (34).

3.4.1.3 Intrahepatic lipids

In total, eight studies were included in the IHL meta-analysis (Figure 3): five MICT studies (35, 36, 43, 46, 47), two HIIT studies (40, 41), and two studies with both HIIT and MICT (32, 49). There was a significant lower IHL upon the HIIT intervention (n=108) compared to the control (n=114) (-3.41; 95% CI: -4.74, -2.08, p<0.001). The IHL was also lower in the MICT subjects (n=169) than in the controls (n=151) (-5.19; 95% CI: -7.33, -3.04, p<0.001). The overall effect of AE (n=277), irrespective of the type, was significantly lower IHL than in controls (n=265) with a mean difference of -4.10 (95% CI: -5.33, -2.87, p<0.001).

Among the three RCTs that were not included in the meta-analysis due to large differences in the study design/intervention arms, Bacchi et al. (33) reported a 32.8% reduction of MRI-determined hepatic fat in AE arm compared to the baseline; another intervention arm was a resistance training showing a

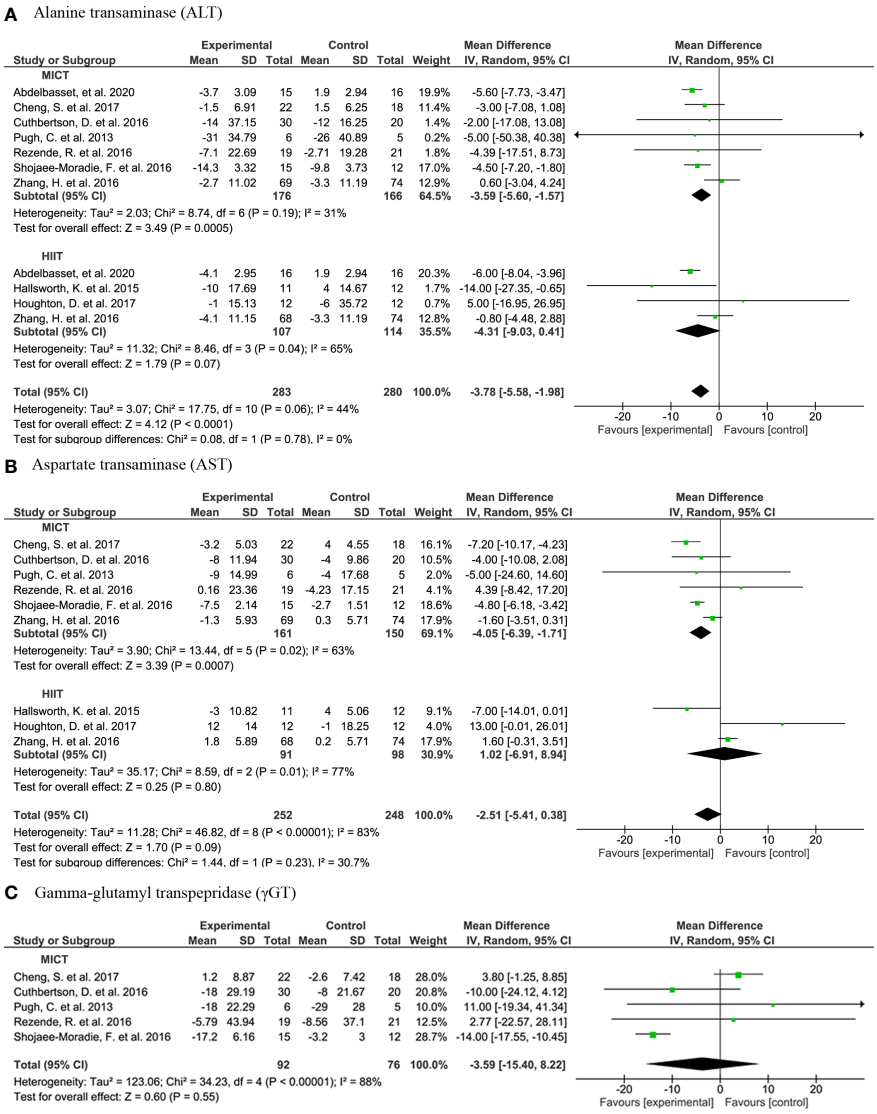


FIGURE 2
Forrest plot for the effect of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on liver transaminases: **(A)** alanine transaminase (ALT); **(B)** aspartate transaminase (AST); **(C)** Forrest plot for the effect of MICT on gamma-glutamyl transpeptidase (γGT).

relative reduction of -25.9%. Moreover, Oh et al. (42) reported a reduction of hepatic fat after both HIIT and RT interventions of -16.6% and -47.2%, respectively. Also, Winn et al. (48) reported reductions in IHL of -37.0% or -20.0% after both HIIT or MICT, respectively. In non-RCTs, the SIT intervention by Sergeant et al. (56) led to 12.4% reduction of hepatic fat. O'Gorman and colleagues (54) do not report regression in steatosis assessed by histology after a 12-week HIIT. However, they observed a significant decrease in CAP measured by FibroScan (51). FibroScan was also used to assess liver steatosis measured using the CAP in three MICT studies (34, 42, 44). CAP was not significantly affected after an 8-week MICT intervention without whole-body vibration (34). In contrast, MICT with whole-body vibration showed significant decrease in CAP. The study by Oh et al. (42) showed a significant reduction in steatosis assessed using CAP after baseline in all three intervention groups (RT, HIIT and MICT) in accordance with MRI-determined IHL results.

3.4.2 Body weight

The body weight was lower upon AE compared to control with a MD of -1.90 (95% CI: -2.45, -1.35, n=245, p<0.001) without heterogeneity (I2 = 0, τ2 = 0.00, p=0.83). With respect to the AE subgroups, the MDs for body weight were -1.80 (95% CI: -2.15, -1.08, n=154, p<0.001) and -2.06 (95% CI: -2.93, -1.18, n=91, p<0.001) for MICT and HIIT, respectively. No significant

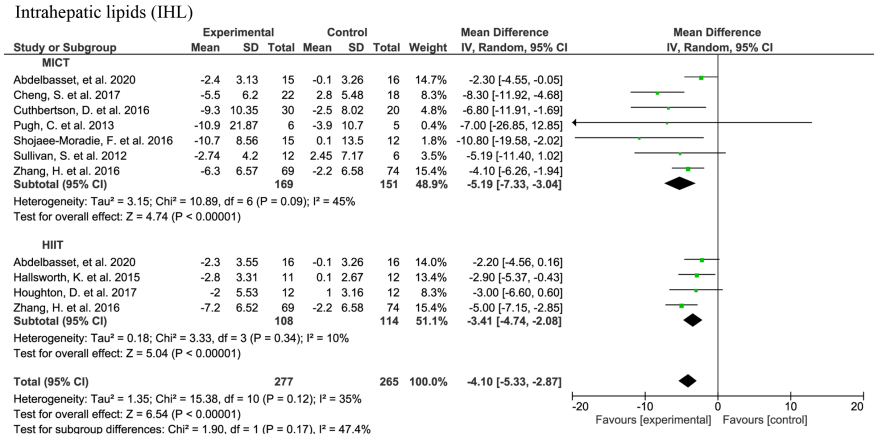


FIGURE 3
Forrest plot for effect of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on intrahepatic lipids (IHL) measured by magnetic resonance spectroscopy (MRS).

subgroup difference was observed (Chi2 = 0.20, df=1, I2 = 0%, p=0.65) (Figure 4).

Studies that were not part of the meta-analysis used varying metrics to measure body compositional changes or did not have a comparable study design to be included in the meta-analysis. Among these the study of Abdelbasset et al. (32) reported a significant decrease in BMI after both HIIT and MICT. A MICT intervention by Rezende et al. (44) did not lead to a significant change in BMI, but it did decrease waist circumference, albeit that this was not significantly different from the decrease in the control group. Bacchi et al. (33) observed a significant decrease in BMI, total body fat mass, MRI-determined VAT and SAT, thickness of superficial subcutaneous adipose tissue layer and sagittal abdominal diameter after both MICT and RT interventions. Similarly, fat mass decreased upon a MICT intervention by Oh et al. (42). In this latter study, no significant changes in body weight, visceral and subcutaneous adipose tissue area were observed upon HIIT. The 6-month MICT intervention of Eckard et al. (37) did not induce significant changes in body weight. In non-RCTs, there was a significant change in weight and BMI upon three month HIIT intervention in the study of Khaoshbaten et al. (52). In line, O'Gorman et al. (54) found a significant change in BMI and waist circumference upon HIIT. In two SIT interventions no effects on body weight and composition were seen (53, 56), albeit that a decrease in VAT mass was reported in the latter study (56).

In order to study whether the significant reductions in weight are associated with the reductions in liver fat, we performed a Pearson correlation analysis with all studies. This

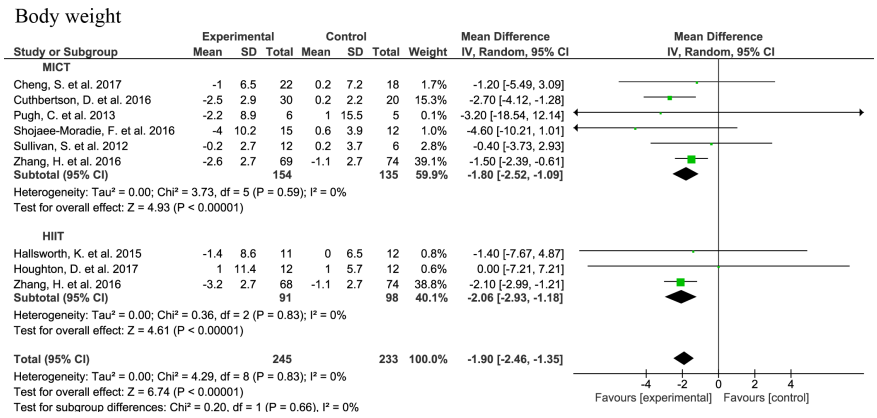


FIGURE 4
Forrest plot for the effect of moderate-interval continuous training (MICT) and high-intensity interval training (HIIT) on total body weight.

showed that a reduction of IHL upon AE intervention correlated significantly with the weight reduction (r=0.714, p=0.031) (Figure 5). Of interest, reductions of IHL and weight were not correlated with the duration of the intervention or with any other measured parameter.

3.4.3 Cardiorespiratory fitness

The meta-analysis for cardiorespiratory fitness was performed with MICT intervention studies since this outcome was not reported in the other interventions. Four studies assessing cardiorespiratory fitness either by VO2max (46) or VO2peak (43, 44, 47) upon 16- (43, 46, 47) or 24-week (44) MICT interventions are integrated in the meta-analysis. There is a significantly higher cardiorespiratory fitness in the MICT groups (n=52) compared to the controls (n=44) with a MD of 4.43 (95% CI: 0.31, 8.55, p=0.03) with a considerable heterogeneity (I2 = 96%, τ2 = 15.61, p<0.00001) (Figure 6).

In non-RCTs, a significant increase in VO2max was observed after a HIIT intervention of seven consecutive days (51). Also, VO2max is increased significantly after HIIT exercise, as well as when compared to the control group (54). There were significant increases in VO2peak and VO2max after SIT programs (51, 53).

In order to study whether the significant increase in cardiorespiratory fitness compared to controls are associated with the reductions in liver fat, we performed a Pearson correlation analysis with the studies. Cardiorespiratory fitness did not show significant correlation with liver fat (r=-0.04, p=0.98), or with liver transaminases (ALT r=88, p=0.32; AST r=0.94, p=0.22). Yet, these analyses were conducted with a very limited number of studies (n=3) due to the limited availability of the outcomes of interest and the independent variables.

3.4.4 Glucose metabolism

The meta analysis showed that there was no significant change in glucose concentrations upon MICT (MD: -0.04, 95% CI: -0.23, 0.15, n=161, p=0.70), HIIT (MD: 0.07, 95% CI: -0.07, 0.21, n=91, p=0.35), or AE in general (MD: -0.01, 95% CI: -0.15, 0.13, n=252,

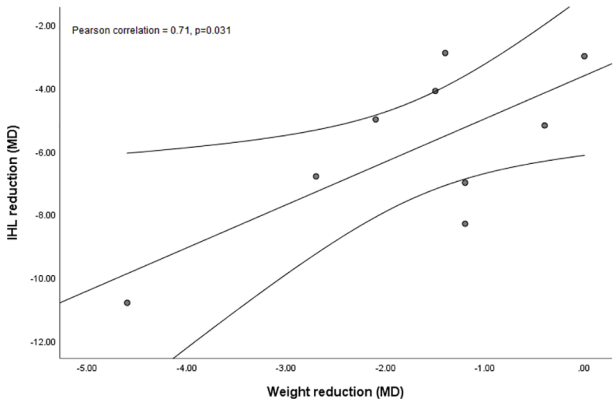


FIGURE 5
Simple scatter plot with linear fit line and 95% CI of intrahepatic lipids (IHL, %) reduction mean difference (MD) against weight reduction (kg; MD) upon aerobic exercise (AE) including both modalities, high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) interventions.

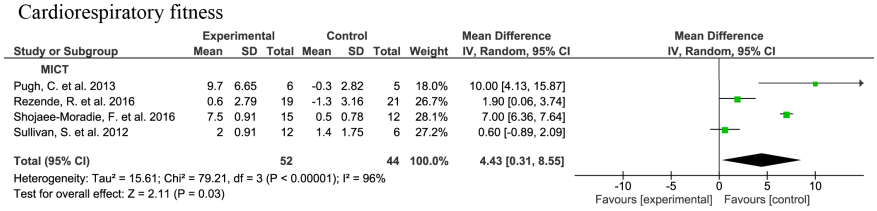


FIGURE 6
Forrest plot for the effect of moderate-intensity continuous training (MICT) on cardiorespiratory fitness level assessed as maximum or peak oxygen uptake (VO2max or VO2peak).

p=0.88) (Figure 7A). Yet, when compared to controls (n=40), HIIT subjects (n=39) had a lower HOMA-IR with a MD of -0.42 (95% CI: -0.76, -0.07, p=0.02) (Figure 7B).

Other proxies of glucose metabolism were investigated in a subset of the articles that were not part of the meta-analysis due to an incomparable study design. For instance, Bacchi et al. (33) reported a modest significant increase in the glucose disposal rate after AE intervention. Cevik et al. (34) did not show a significant change in glucose after the interventions. However, HOMA-IR was significantly decreased after AE with whole-body vibration but not in those without the vibration (34). Winn et al. (48) did not find a significant change in HOMA-IR upon HIIT or MICT. In non-RCTs, HOMA-IR decreased after a SIT program by Sergeant et al. (56), as well as by MacLean et al. (53). Haus et al. (51) reported a significant reduction in fasting plasma glucose concentrations after a 7-day HIIT.

3.4.5 Plasma lipids

In the meta analysis, the plasma total cholesterol concentration was significantly lower upon AE in general and upon MICT than in controls, with a MD of -0.19 (95% CI: -0.29, -0.09, n=261, p<0.001) and -0.20 (95% CI: -0.31, -0.09, n=154, p<0.001) (Figure 8A) for AE and MICT, respectively. In line, plasma LDL-C concentration was lower upon MICT, and HDL-C concentration was higher upon AE, MICT and HIIT, compared to controls (Figures 8B, C). When compared to controls, AE subjects had lower plasma TG concentrations (MD: -0.26, 95% CI: -0.39, -0.14, n=284, p<0.0001) with a considerable heterogeneity of 81% ($\tau 2$ = 0.02, p<0.0001) (Figure 8D).

The meta-analysis of lipid concentrations did not include the following studies due to non-comparable study designs. In the study of Winn et al. (48), plasma TC and HDL-C concentrations were unchanged upon MICT and HIIT (48). Bacchi et al. (33) and Cevik et al. (34) did not report a significant change in LDL-C after a HIIT intervention (34). Yet, plasma TG concentrations are decreased after MICT in the study by Bacchi et al. (33), but not in the study by Cevik et al. (34). In non-RCTs, there is a significant increase in plasma HDL-C concentrations upon a SIT (56). Also, Khaoshbaten et al. (52) observe a significant increase in HDL-C concentrations and a decrease in TG concentrations upon 3-month HIIT.

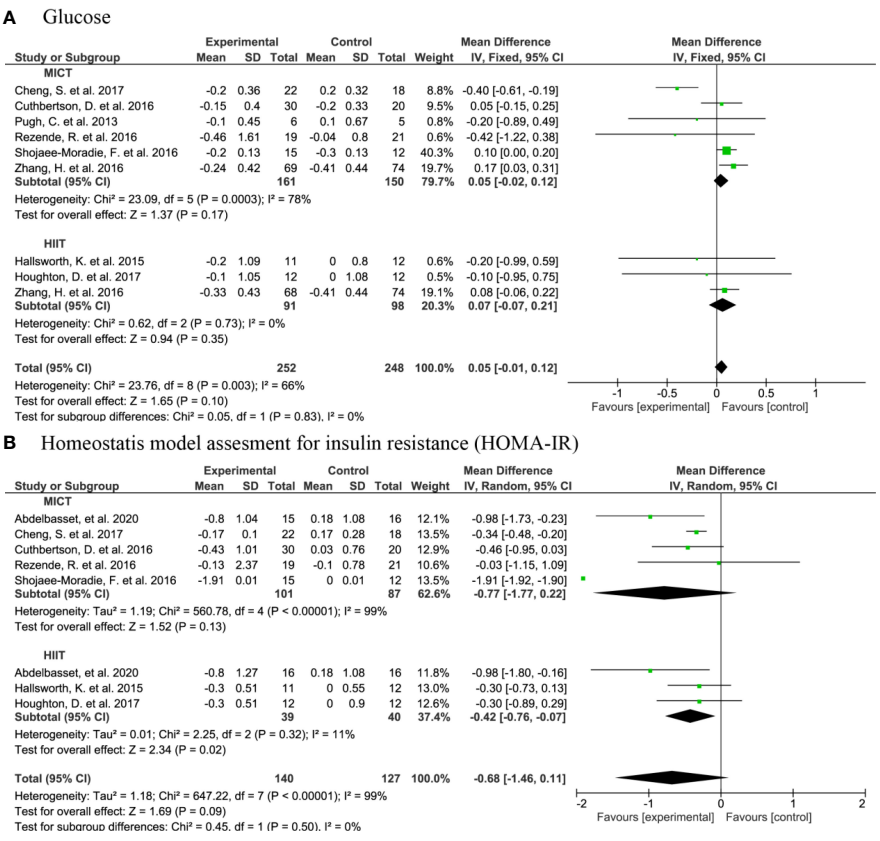


FIGURE 7
Forrest plot for the effect of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on: **(A)** glucose; **(B)** homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

10.3389/fendo.2022.1032164

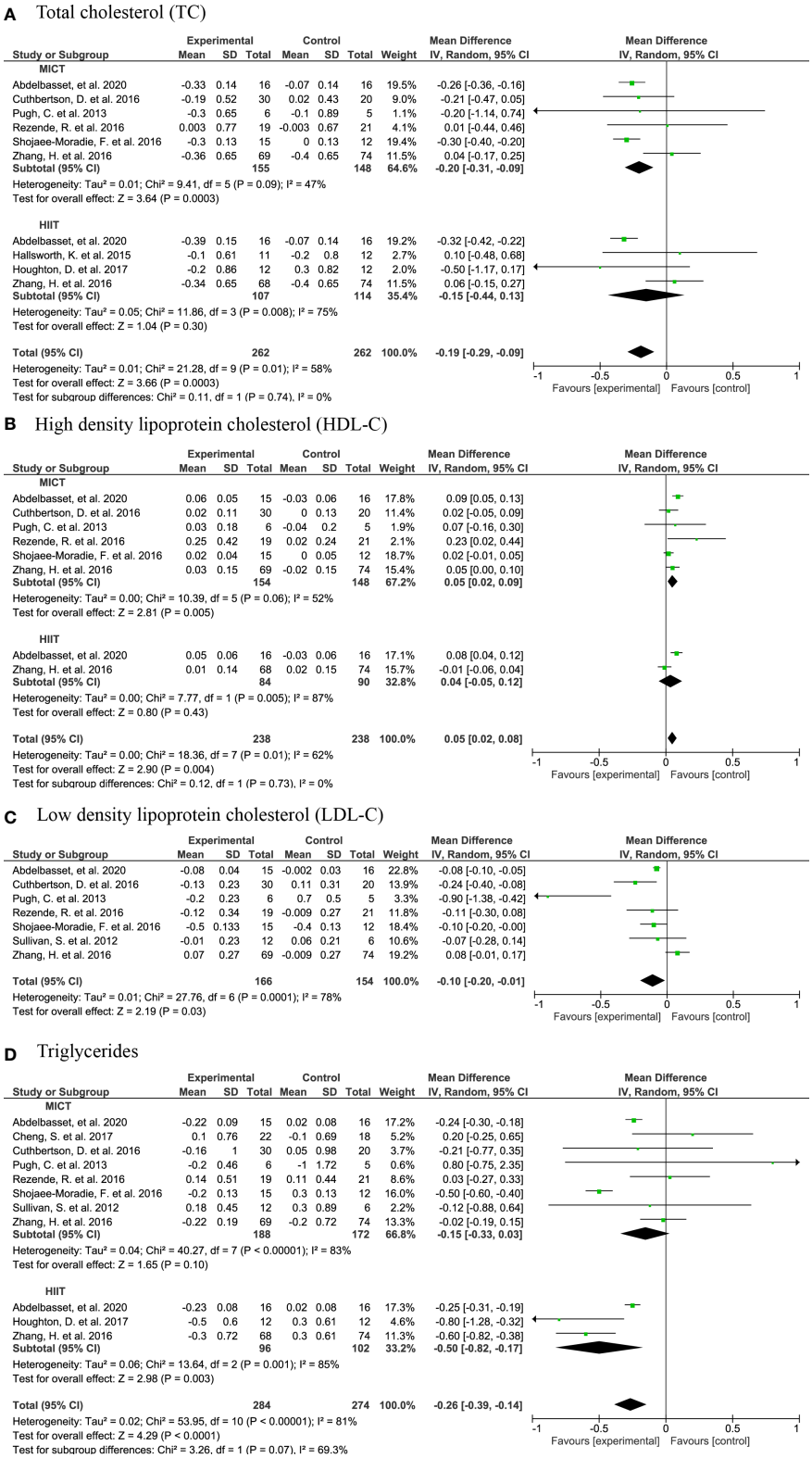


FIGURE 8
Forrest plot for the effect of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on plasma lipids: **(A)** total cholesterol (TC); **(B)** high-density lipoprotein cholesterol (HDL-C); **(C)** Forrest plot for the effect of MICT on low-density lipoprotein cholesterol (LDL-C); **(D)** triglycerides (TG).

10.3389/fendo.2022.1032164

3.5 Sensitivity analysis

In order to examine the impact of the intervention duration on the meta-analyses, we conducted sensitivity analysis by removing Rezende et al. (n=19) (44) with a 24-week intervention, which did not lead to any significant changes. Furthermore, we performed a sensitivity analysis by removing Zhang et al. (n=135) (49) with a 26-week intervention from the analysis (Table 3). The analysis did not result in a significant difference in the IHL; however, the plasma AST results changed significantly before and after removing Zhang et al. (MD: -2.51, p=0.09 vs. MD: -4.43, p=0.002). In regard to the plasma TC, there was a significant change, after removing the data of Zhang et al. for HIIT (MD: -0.15 vs. -0.30) as well as for the HIIT for TG (MD: -0.50 vs. -0.47). After removing both Rezende et al. (44) and Zhang et al. (49), in addition to the significant changes mentioned by removing Zhang et al., TG for MICT changed significantly (MD: -0.15, p=0.10 vs. MD: -0.23, p=0.03) (Table 4).

TABLE 3 Results of meta-analyses before and after sensitivity analysis including mean difference (MD), 95% CI and significance level, as well as heterogeneity (I2) and significance level per intervention type, consisting of studies with similar intervention duration excluding study by Zhang et al.

Outcome	AE				MICT				HIIT			
	MD 95% CI	p-val	I2	p-val	MD 95% CI	p-val	I2	p-val	MD 95% CI	p-val	I2	p-val
IHL (%) Before	-4.10 [-5.33, -2.87]	<0.00001	35%	0.12	-5.19, [-7.33, -3.04]	<0.00001	45%	0.09	-3.41 [-4.74, -2.08]	<0.00001	10%	0.34
IHL (%) After	-4.08 [-5.77, -2.39]	<0.00001	43%	0.08	-5.92 [-9.01, -2.82]	0.0002	53%	0.06	-2.62 [-4.16, -1.08]	0.0009	0%	0.90
ALT (UI) Before	-3.78 [-5.58, -1.98]	<0.0001	44%	0.06	-3.59 [-5.60, -1.57]	0.0005	31%	0.19	-4.31 [-9.03, -.41]	0.07	65%	0.04
ALT (UI) After	-5.29 [-6.51, -4.08]	<0.00001	0%	0.78	-4.83 [-6.36, -3.30]	<0.00001	0%	0.92	-6.41 [-11.15, -1.66]	0.008	14%	0.31
AST (UI) Before	**-2.51 [-5.41, 0.38]**	**0.09**	**83%**	**<0.00001**	-4.05 [-6.39, -1.71]	0.0007	63%	0.02	1.02 [-6.91, 8.94]	0.80	77%	0.01
AST (UI) After	**-4.43 [-7.30, -1.57]**	**0.002**	**50%**	**0.06**	-5.16 [-6.67, -3.65]	<0.00001	8%	0.36	2.22 [-17.32, 21.76]	0.82	86%	0.008
Glucose (mmol/L) Before	0.05 [-0.01, 0.12]	0.10	66%	0.003	0.05 [-0.02, 0.12]	0.17	78%	0.0003	0.07 [-0.07, 0.21]	0.35	0%	0.73
Glucose (mmol/L) After	0.01 [-0.07, 0.09]	0.88	69%	0.003	0.01 [-0.07, 0.09]	0.82	79%	0.0007	-0.15 [-0.73, 0.42]	0.60	0%	0.87
TC (mmol/L) Before	-0.19 [-0.29, -0.09]	0.0003	58%	0.01	-0.20 [-0.31, -0.09]	0.0003	47%	0.09	**-0.15 [-0.44, 0.13]**	**0.30**	**75%**	**0.008**
TC (mmol/L) After	-0.28 [-0.34, -0.23]	<0.00001	0%	0.68	-0.27 [-0.33, -0.20]	<0.00001	0%	0.72	**-0.30 [-0.47, -0.12]**	**0.001**	**12%**	**0.32**
LDL-C (mmol/L) Before	–	–	–	–	-0.10 [-0.20, -0.01]	0.03	78%	0.0001	–	–	–	–
LDL-C (mmol/L) After	–	–	–	–	-0.14 [-0.24, -0.05]	0.003	68%	0.009	–	–	–	–
HDL-C (mmol/L) Before	0.05 [0.02, 0.08]	0.0004	62%	0.01	0.05 [0.02, 0.09]	0.005	52%	0.06	0.04 [-0.05, 0.12]	0.004	62%	0.001
HDL-C (mmol/L) After	0.06 [0.02, 0.10]	0.002	58%	0.04	0.06 [0.01, 0.11]	0.03	61%	0.03	0.08 [0.04, 0.12]	0.0002	NA	NA
Triglycerides (mmol/L) Before	-0.26 [-0.39, -0.14]	<0.0001	81%	<0.00001	-0.15 [-0.33, 0.03]	0.10	83%	<0.00001	**-0.50 [-0.82, -0.17]**	**0.003**	**85%**	**0.001**
Triglycerides (mmol/L) After	-0.26 [-0.39, -0.14]	<0.0001	78%	<0.00001	-0.19 [-0.38, 0.01]	0.07	81%	<0.0001	**-0.47 [-1.00, 0.06]**	**0.08**	**80%**	**0.03**
Weight (kg) Before	-1.90 [-2.46, -1.35]	<0.00001	0%	0.83	-1.80 [-2.52, -1.09]	<0.00001	0%	0.59	-2.06 [-2.93, -1.18]	<0.00001	0%	0.83
Weight (kg) After	-2.26 [-3.43, -1.08]	0.0002	0%	0.82	-2.35 [-3.57, -1.14]	0.0001	0%	0.65	-0.80 [-5.53, 3.94]	0.74	0%	0.77

ALT, alanine transferase; AST, aspartate transferase; HIIT, high-intensity interval training; HOMA-IR, homeostasic assessment for insulin resistance; HDL-C, high density lipoprotein cholesterol; IHL, intrahepatic lipids; kg, kilogram; LDL-C, low density lipoprotein cholesterol; MICT, moderate-intensity continuous training; mmol/l, milligram per deciliter; UI, international unit; VO2max, maximum oxygen consumption. NA; not applicable.
Results of sensitivity analyses that differ in significance from the original meta-analysis are marked in bold.

TABLE 4 Results of meta-analyses before and after sensitivity analysis including mean difference (MD), 95% CI and significance level, as well as heterogeneity (I2) and significance level per intervention type, consisting of studies with similar intervention duration excluding study by Zhang et al. and Rezende et al.

Outcome	AE				MICT				HIIT			
	MD 95% CI	p-val	I2	p-val	MD 95% CI	p-val	I2	p-val	MD 95% CI	p-val	I2	p-val
IHL (%) Before	-4.10 [-5.33, -2.87]	<0.00001	35%	0.12	-5.19, [-7.33, -3.04]	<0.00001	45%	0.09	-3.41 [-4.74, -2.08]	<0.00001	10%	0.34
IHL (%) After	-4.08 [-5.77, -2.39]	<0.00001	43%	0.08	-5.92 [-9.01, -2.82]	0.0002	53%	0.06	-2.62 [-4.16, -1.08]	0.0009	0%	0.90
ALT (UI) Before	-3.78 [-5.58, -1.98]	<0.0001	44%	0.06	-3.59 [-5.60, -1.57]	0.0005	31%	0.19	-4.31 [-9.03, -.41]	0.07	65%	0.04
ALT (UI) After	-5.30 [-6.52, -4.08]	<0.00001	0%	0.69	-4.84 [-6.37, -3.30]	<0.00001	0%	0.83	-6.41 [-11.15, -1.66]	0.008	14%	0.31
AST (UI) Before	**-2.51 [-5.41, 0.38]**	**0.09**	**83%**	**<0.00001**	-4.05 [-6.39, -1.71]	0.0007	63%	0.02	1.02 [-6.91, 8.94]	0.80	77%	0.01
AST (UI) After	**-4.91 [-7.69, -2.12]**	**0.0005**	**50%**	**0.08**	-5.17 [-6.40, -3.95]	<0.00001	0%	0.53	2.22 [-17.32, 21.76]	0.82	86%	0.008
Glucose (mmol/L) Before	0.05 [-0.01, 0.12]	0.10	66%	0.003	0.05 [-0.02, 0.12]	0.17	78%	0.0003	0.07 [-0.07, 0.21]	0.35	0%	0.73
Glucose (mmol/L) After	0.01 [-0.07, 0.09]	0.80	73%	0.002	0.01 [-0.07, 0.09]	0.74	84%	0.0004	-0.15 [-0.73, 0.42]	0.60	0%	0.87
TC (mmol/L) Before	-0.19 [-0.29, -0.09]	0.0003	58%	0.01	-0.20 [-0.31, -0.09]	0.0003	47%	0.09	**-0.15 [-0.44, 0.13]**	**0.30**	**75%**	**0.008**
TC (mmol/L) After	-0.29 [-0.34, -0.23]	<0.00001	0%	0.78	-0.27 [-0.34, -0.21]	<0.00001	0%	0.90	**-0.30 [-0.47, -0.12]**	**0.001**	**12%**	**0.32**
LDL-C (mmol/L) Before	–	–	–	–	-0.10 [-0.20, -0.01]	0.03	78%	0.0001	–	–	–	–
LDL-C (mmol/L) After	–	–	–	–	-0.15 [-0.27, -0.04]	0.007	74%	0.004	–	–	–	–
HDL-C (mmol/L) Before	0.05 [0.02, 0.08]	0.0004	62%	0.01	0.05 [0.02, 0.09]	0.005	52%	0.06	0.04 [-0.05, 0.12]	0.004	62%	0.001
HDL-C (mmol/L) After	0.06 [0.02, 0.09]	0.002	57%	0.05	0.05 [0.00, 0.09]	0.05	61%	0.05	0.08 [0.04, 0.12]	0.0002	NA	NA
Triglycerides (mmol/L) Before	-0.26 [-0.39, -0.14]	<0.0001	81%	<0.00001	**-0.15 [-0.33, 0.03]**	**0.10**	**83%**	**<0.00001**	**-0.50 [-0.82, -0.17]**	**0.003**	**85%**	**0.001**
Triglycerides (mmol/L) After	-0.30 [-0.43, -0.17]	<0.0001	79%	<0.0001	**-0.23 [-0.45, -0.02]**	**0.03**	**81%**	**<0.0001**	**-0.47 [-1.00, 0.06]**	**0.08**	**80%**	**0.03**
Weight (kg) Before	-1.90 [-2.46, -1.35]	<0.00001	0%	0.83	-1.80 [-2.52, -1.09]	<0.00001	0%	0.59	-2.06 [-2.93, -1.18]	<0.00001	0%	0.83
Weight (kg) After	-2.26 [-3.43, -1.08]	0.0002	0%	0.82	-2.35 [-3.57, -1.14]	0.0001	0%	0.65	-0.80 [-5.53, 3.94]	0.74	0%	0.77

ALT, alanine transferase; AST, aspartate transferase; HIIT, high-intensity interval training; HOMA-IR, homeostasic assessment for insulin resistance; HDL-C, high density lipoprotein cholesterol; IHL, intrahepatic lipids; kg, kilogram; LDL-C, low density lipoprotein cholesterol; MICT, moderate-intensity continuous training; mmol/l, milligram per deciliter; UI, international unit; VO2max, maximum oxygen consumption. NA; not applicable.
Results of sensitivity analyses that differ in significance from the original meta-analysis are marked in bold.

3.6 Quality assessment

The quality assessment resulted in overall low risk of bias in all domains for three RCTs (35, 42, 47), while ten articles showed some concerns in the overall judgment since they had a moderate risk in one domain (32–34, 37, 39, 43–46, 49) (Figure 9A). One study showed a high risk of classification of interventions as the intervention groups were not clearly defined, also the information used to define the intervention groups was not mentioned at the start (52). Two studies were scored high risk of selection bias, since they did not use random sequence (38, 48). Studies that raised concerns in the quality are included in the narrative review, however, were not integrated in the meta-analysis. Quality assessment of non-RCTs is shown in Figure 9B. Non-RCTs are included in the narrative review.

4 Discussion

This systematic review assessed the effects of two types of AE (MICT and HIIT) without dietary changes on NAFLD and related metabolic parameters. Our findings showed that both

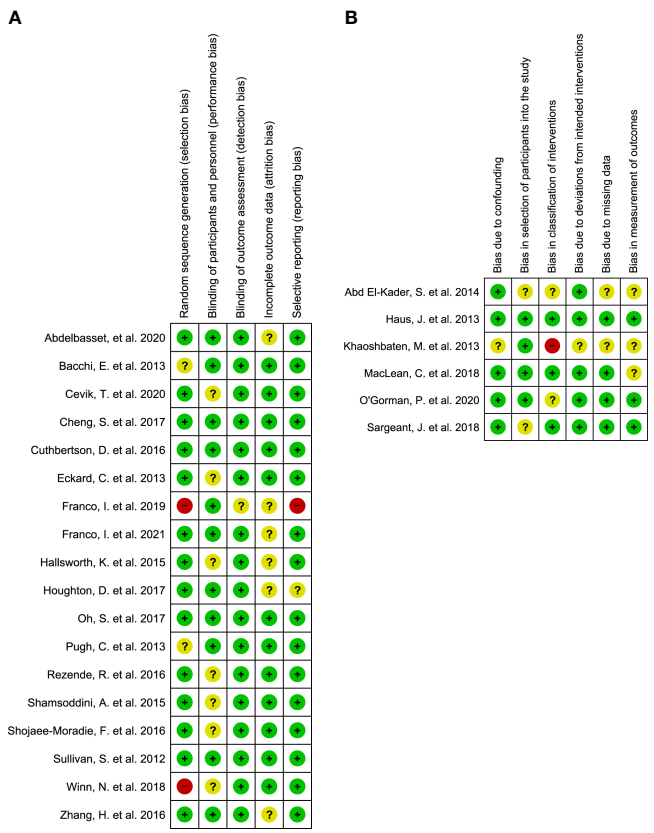


FIGURE 9
The results of the quality assessment of **(A)** randomized controlled trials (RCTs); **(B)** non-randomized controlled trials (non-RCTs).

HIIT and MICT significantly reduced IHL, an effect significantly associated with reductions in body weight. In addition, MICT but not HIIT reduced liver transaminases and both AE types improved the plasma lipid profile. Although AE did not affect blood glucose concentrations, HIIT improved HOMA-IR.

In general, we observed no effect of AE on fibrosis, related varying results on liver stiffness. While MICT did not lead to improvements in liver fibrosis in the included studies (34, 44), HIIT did significantly improve liver fibrosis (42, 54). Previously, fibrosis regression has been associated with a dose-dependent increase in physical activity (51). In line, insufficient physical activity is an independent predictor of fibrosis in NAFLD (21). Despite the fact that liver fibrosis is the most important determinant of the risk of liver-related mortality (57), most studies invest in detecting steatosis and not fibrosis. Like fibrosis, liver inflammation as an endpoint in lifestyle interventions has been overlooked. In contrast, plasma concentrations of the liver enzymes AST, ALT and γGT as proxy of liver functioning are often measured. Increased plasma concentrations of these liver enzymes are associated with an increased risk for the progression to advanced liver disease (58), characterized by inflammation and fibrosis. Yet, non-invasive NAFLD tests that often include the plasma transaminase concentrations are not optimal (59). Thus, in order to more firmly draw conclusions on the effects of exercise on NASH and liver fibrosis, well controlled exercise intervention studies with sufficient sample sizes and elucidative endpoints in patients with advanced stages of NAFLD are called for.

Nearly all included studies showed lower IHL after MICT and HIIT. This is in line with a recent systematic review in which the effects of MICT and HIIT were studied in a wide range of patients, i.e., those with obesity and T2DM, without information on NAFLD (60). We found a significant positive correlation between the reductions in body weight and IHL upon AE. Of interest, it has already been suggested that effects of AE on IHL might be mediated by alterations in body composition, e.g., loss of body weight and adipose tissue mass, or an increased muscle mass (61). In line, another meta-analysis reported similar association of body weight loss and decrease in IHL (62). Based on these earlier studies and our present results, we can conclude that AE-induced weight loss and changes in body composition are crucial in reducing IHL. It also underscores that weight loss *per se* is beneficial for the liver.

We did not detect a differential effect of MICT and HIIT on IHL. This is in line with previous studies in which HIIT and MICT were compared (32, 36, 42, 48), underscoring that neither was superior in ameliorating steatosis. However, some studies reported that moderate-intensity training is better in reducing liver fat than low-intensity training (63). This notion was supported by a prospective study in which vigorous but not moderate physical activity lowered the risk for NAFLD (64). Yet, current guidelines state that any level of physical activity or exercise can be beneficial for patients with NAFLD (17). Perhaps, practically, the paramount aim may be to engage sedentary patients to increase their activity at any level (22). In order to increase the engagement for long-term adherence to exercise, physical activity and exercise programs should be personalized according to the limitations and preferences (i.e. comorbidities, clinical characteristics, personal goals) of the individual patient with NAFLD (22, 54, 65). More studies need to investigate the different modalities of AE in patients with different stages of NAFLD to direct these personalized guidelines.

We did not find a significant change in blood glucose concentrations upon AE, which was in line with a previous study (66). Yet, a modest, but a significant decrease was found in HOMA-IR upon HIIT. Patients with NASH often have a higher HOMA-IR than healthy controls (67) and thus, are insulin resistant (68). Insulin resistance plays a central role in hepatic lipid accumulation (69). Consequently, lipid metabolism is also often altered in patients with NAFLD (70), and therefore patients with NAFLD present an increased asCVD related mortality (5). Evidently, elevated LDL-C concentrations, promoting the development of thrombus and plaque (71, 72), and elevated concentrations of plasma TG (73) increase the risk of asCVD. Simultaneously, an increase of HDL-C concentrations is associated with a significant risk reduction (7, 71). Our meta-analysis showed significant higher plasma HDL-C concentrations and lower plasma LDL-C and TC concentrations upon the MICT compared to controls. Plasma TG concentrations were only lower upon HIIT compared to controls. Another systematic review about AE-induced changes in NAFLD, however, did not report significant changes in plasma lipids, except for a reduction in plasma TG concentrations (66). Physical activity is associated with lower asCVD related mortality in patients with NAFLD (21), which is probably partly due to the improvements in lipid profile, and partly due to improved cardiorespiratory fitness (74). In fact, we observed higher cardiorespiratory fitness upon MICT compared to control in our meta-analyses. These metabolic targets, cardiorespiratory fitness together with plasma lipid profile by exercise should be viewed as part of holistic treatment of patients with NAFLD who present an increased risk for asCVD.

Most of the included studies performed supervised exercise sessions, regardless of the modality (32, 33, 35–37, 40, 42, 44–47, 49, 51, 54, 56). In addition to the supervised sessions, some of the studies prescribed home-based/unsupervised training to the study subjects (40, 54, 75). Yet, this does have a major impact to our meta-analyses. The compliance to the exercise intervention was determined either by cardiopulmonary exercise test (43, 46–48, 54, 56, 75) or by physical activity accelerometer (45, 47, 75) or/and questionnaire (40, 75). Given that limited number of studies determined the cardiorespiratory fitness at the follow-up, the meta-analysis for cardiorespiratory fitness could be only conducted with four comparable studies. In future studies, it would be ideal to assess the compliance to the intervention by the exercise test for reliable results, and simultaneously, study the efficacy of the aerobic exercise intervention.

Regarding limitations, lack of sufficient data impeded conducting meta-analysis for all interventions and outcomes, while relatively small number of studies were included in each conducted meta-analysis. In addition, the small number of studies precluded the detection of publication bias on a funnel plot. Publication bias may also arise from the grey literature that the systematic review of the literature do not consider. To minimize the risk of publication bias, we used a comprehensive and sensitive search strategy for finding published full texts and conference abstracts as well as backward reference searching of the included studies. Furthermore, we detect variable degrees of heterogeneity in the meta-analyses. In general, meta-analyses with high heterogeneity should be interpreted cautiously. High heterogeneity may be driven from statistical and methodological heterogeneity since clinical heterogeneity has been kept minimum by including well characterized patient group and interventions (31). Different methods that an outcome is measured may lead to differential intervention effect sizes resulting in a high heterogeneity, for example the meta-analyses of HOMA-IR have high heterogeneity. HOMA-IR was calculated by using the reported values of glucose and insulin that in the first place are measured in different laboratories, possibly by different methods, leading to high heterogeneity in the meta-analyses.

In conclusion, the evidence available for exercise interventions in patients with NAFLD clearly indicates that hepatic fat is decreased by AE. It can be mediated by reduction of body weight, regardless of exercise modality, with a concomitant decrease of liver enzymes upon MICT, and improvements in plasma lipids. In addition, HIIT may improve HOMA-IR. Yet, there is a striking lack of studies with liver histology, precluding any firm conclusions pertaining to the effect of AE on NASH or liver fibrosis, the main clinically relevant disease parameters in NAFLD. In order to draw conclusions on the effects of exercise on NASH and liver fibrosis and determine the optimal modality of AE, well-controlled exercise intervention studies with elucidative endpoints in patients with advanced stages of NAFLD are called for.

Data availability statement

Publicly available datasets were analyzed in this study.

Author contributions

VH and JB contributed equally to the protocol of the systematic review, and design and organization of the manuscript, as well as writing, reviewing, editing of the manuscript. VH and JB performed together the quality assessment, data extraction, statistical analysis, and meta-analysis. YV contributed to the protocol of the systematic review as well as reviewing and editing of the manuscript. JD designed and conducted the search. AG, MN, and JD contributed to reviewing and editing of the manuscript. AH supervised the work and contributed to the protocol of the systematic review, as well as reviewing and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work has received funding from an ITN Marie Curie BestTreat – Building a Gut Microbiome Engineering Toolbox for *In-Situ* Therapeutic Treatments for Non-alcoholic Fatty Liver Disease No. 813781 ITN BestTreat (on which VH is appointed). AH was supported by the Amsterdam UMC Fellowship, Health–Holland TKI-PPP grants, grants for the Dutch Gastroenterology Foundation and by a research grant from Novo Nordisk. MN was supported by a personal ZONMW VICI grant 2020 [09150182010020].

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.2022.1032164/full#supplementary-material

References

1. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* (2017) 23(47):8263–76. doi: 10.3748/wjg.v23.i47.8263

2. Bluher M. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol* (2019) 15(5):288–98. doi: 10.1038/s41574-019-0176-8

3. Ress C, Kaser S. Mechanisms of intrahepatic triglyceride accumulation. *World J Gastroenterol* (2016) 22(4):1664–73. doi: 10.3748/wjg.v22.i4.1664

4. Mohamed RZ, Jalaludin MY, Anuar Zaini A. Predictors of non-alcoholic fatty liver disease (NAFLD) among children with obesity. *J Pediatr Endocrinol Metab* (2020) 33(2):247–53. doi: 10.1515/jpem-2019-0403

5. Golabi P, Fukui N, Paik J, Sayiner M, Mishra A, Younossi ZM. Mortality risk detected by atherosclerotic cardiovascular disease score in patients with nonalcoholic fatty liver disease. *Hepatol Commun* (2019) 3(8):1050–60. doi: 10.1002/hep4.1387

6. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* (2018) 15(1):11–20. doi: 10.1038/nrgastro.2017.109

7. Ahmed HM, Miller M, Nasir K, McEvoy JW, Herrington D, Blumenthal RS, et al. Primary low level of high-density lipoprotein cholesterol and risks of coronary heart disease, cardiovascular disease, and death: Results from the multi-ethnic study of atherosclerosis. *Am J Epidemiol* (2016) 183(10):875–83. doi: 10.1093/aje/kwv305

8. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology* (2020) 158 (6):1611–25.e12. doi: 10.1053/j.gastro.2020.01.043

9. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology* (2017) 65(5):1557–65. doi: 10.1002/hep.29085

10. Amarapurka DN, Amarapurkar AD, Patel ND, Agal S, Baigal R, Gupte P, et al. Nonalcoholic steatohepatitis (NASH) with diabetes: predictors of liver fibrosis. *Ann Hepatol* (2006) 5(1):30–3. doi: 10.1016/S1665-2681(19)32036-8

11. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* (2013) 57(2):601–9. doi: 10.1002/hep.26093

12. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* (2015) 62(1 Suppl):S47–64. doi: 10.1016/j.jhep.2014.12.012

13. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* (1999) 107(5):450–5. doi: 10.1016/S0002-9343(99)00271-5

14. Sheldon GF, Peterson SR, Sanders R. Hepatic dysfunction during hyperalimentation. *Arch Surg* (1978) 113(4):504–8. doi: 10.1001/archsurg.1978.01370160162028

15. Meroni M, Longo M, Tria G, Dongiovanni P. Genetics is of the essence to face NAFLD. *Biomedicines* (2021) 9(10):1359. doi: 10.3390/biomedicines9101359

16. Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* (2021) 184(10):2537–64. doi: 10.1016/j.cell.2021.04.015

17. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the

management of non-alcoholic fatty liver disease. *J Hepatol* (2016) 64(6):1388–402. doi: 10.1016/j.jhep.2015.11.004

18. Younossi ZM, Corey KE, Lim JK. AGA clinical practice update on lifestyle modification using diet and exercise to achieve weight loss in the management of nonalcoholic fatty liver disease: Expert review. *Gastroenterology* (2021) 160(3):912–8. doi: 10.1053/j.gastro.2020.11.051

19. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American association for the study of liver diseases. *Hepatology* (2018) 67(1):328–57. doi: 10.1002/hep.29367

20. Patel H, Alkhawam H, Madanieh R, Shah N, Kosmas CE, Vittorio TJ. Aerobic vs anaerobic exercise training effects on the cardiovascular system. *World J Cardiol* (2017) 9(2):134–8. doi: 10.4330/wjc.v9.i2.134

21. Kim D, Konyn P, Cholankeril G, Ahmed A. Physical activity is associated with nonalcoholic fatty liver disease and significant fibrosis measured by FibroScan. *Clin Gastroenterol Hepatol* (2021) 20(6):e1438–e1455. doi: 10.1016/j.cgh.2021.06.029

22. Hallsworth K, Adams LA. Lifestyle modification in NAFLD/NASH: Facts and figures. *JHEP Rep* (2019) 1(6):468–79. doi: 10.1016/j.jhepr.2019.10.008

23. Berger D, Desai V, Janardhan S. Con: Liver biopsy remains the gold standard to evaluate fibrosis in patients with nonalcoholic fatty liver disease. *Clin Liver Dis* (2019) 13(4):114–6. doi: 10.1002/cld.740

24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj* (2021) 372:n71. doi: 10.1136/bmj.n71

25. Houttu VBJ, Vali J, Daams J, Grefhorst A, Nieuwdorp M, Holleboom AG. *Can aerobic exercise reduce NASH and liver fibrosis in patients with non-alcoholic fatty liver disease? a systematic review of the literature and meta-analysis PROSPERO CRD42021270059: International prospective register of systematic reviews* (2022). Available at: https://www.crd.york.ac.uk/prospero/#myprospero.

26. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* (2016) 5(1):210. doi: 10.1186/s13643-016-0384-4

27. Sterne JAC SJ, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* (2019) 28 (366):l4898. doi: 10.1136/bmj.l4898

28. Sterne JAC HM, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, et al. ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions. *BMJ* (2016) 355:i4919. doi: 10.1136/bmj.i4919

29. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. *Cochrane handbook for systematic reviews of interventions version 6.3 (updated February 2022)*. Cochrane (2022). Available at: www.training.cochrane.org/handbook.

30. The Cochrane Collaboration. *Review manager web (RevMan web)*. (2020) 2020, RW.

31. Deeks JJ HJ, Altman DG. Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editors. *Cochrane handbook for systematic reviews of interventions version 6.3 (updated February 2022)*. Cochrane (2022). Available at: www.training.cochrane.org/handbook.

32. Abdelbasset WK, Tantawy SA, Kamel DM, Alqahtani BA, Soliman GS. A randomized controlled trial on the effectiveness of 8-week high-intensity interval exercise on intrahepatic triglycerides, visceral lipids, and health-related quality of life in diabetic obese patients with nonalcoholic fatty liver disease. *Medicine* (2019) 98(12):e14918. doi: 10.1097/MD.0000000000014918

33. Bacchi E, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, et al. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). *Hepatology* (2013) 58(4):1287–95. doi: 10.1002/hep.26393

34. Çevik Saldiran T, Mutluay FK, Yağci İ, Yilmaz Y. Impact of aerobic training with and without whole-body vibration training on metabolic features and quality of life in non-alcoholic fatty liver disease patients. *Ann Endocrinol* (2020) 81 (5):493–9. doi: 10.1016/j.ando.2020.05.003

35. Cheng S, Ge J, Zhao C, Le S, Yang Y, Ke D, et al. Effect of aerobic exercise and diet on liver fat in pre-diabetic patients with non-alcoholic-fatty-liver-disease: A randomized controlled trial. *Sci Rep* (2017) 7(1):15952. doi: 10.1038/s41598-017-16159-x

36. Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, Jones H, Pugh CJ, Richardson P, et al. Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homoeostasis in obese patients with non-alcoholic fatty liver disease. *Clin Sci* (2016) 130(2):93–104. doi: 10.1042/CS20150447

37. Eckard C, Cole R, Lockwood J, Torres DM, Williams CD, Shaw JC, et al. Prospective histopathologic evaluation of lifestyle modification in nonalcoholic fatty liver disease: a randomized trial. *Therap Adv Gastroenterol* (2013) 6(4):249–59. doi: 10.1177/1756283X13484078

38. Franco I, Bianco A, Diaz MDP, Bonfiglio C, Chiloiro M, Pou SA, et al. Effectiveness of two physical activity programs on non-alcoholic fatty liver disease. a randomized controlled clinical trial. *Rev Fac Cien Med Univ Nac Cordoba* (2019) 76(1):26–36. doi: 10.31053/1853.0605.v76.n1.21638

39. Franco I, Bianco A, Mirizzi A, Campanella A, Bonfiglio C, Sorino P, et al. Physical activity and low glycemic index Mediterranean diet: Main and modification effects on NAFLD score. results from a randomized clinical trial. *Nutrients* (2020) 13(1):66. doi: 10.3390/nu13010066

40. Hallsworth K, Thoma C, Hollingsworth KG, Cassidy S, Anstee QM, Day CP, et al. Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. *Clin Sci (Lond)* (2015) 129(12):1097–105. doi: 10.1042/CS20150308

41. Houghton D, Thoma C, Hallsworth K, Cassidy S, Hardy T, Burt AD, et al. Exercise reduces liver lipids and visceral adiposity in patients with nonalcoholic steatohepatitis in a randomized controlled trial. *Clin Gastroenterol Hepatol* (2017) 15(1):96–102.e3. doi: 10.1016/j.cgh.2016.07.031

42. Oh S, So R, Shida T, Matsuo T, Kim B, Akiyama K, et al. High-intensity aerobic exercise improves both hepatic fat content and stiffness in sedentary obese men with nonalcoholic fatty liver disease. *Sci Rep* (2017) 7:43029. doi: 10.1038/srep43029

43. Pugh CJ, Cuthbertson DJ, Sprung VS, Kemp GJ, Richardson P, Umpleby AM, et al. Exercise training improves cutaneous microvascular function in nonalcoholic fatty liver disease. *Am J Physiol Endocrinol Metab* (2013) 305(1): E50–8. doi: 10.1152/ajpendo.00055.2013

44. Rezende RE, Duarte SM, Stefano JT, Roschel H, Gualano B, de Sa Pinto AL, et al. Randomized clinical trial: benefits of aerobic physical activity for 24 weeks in postmenopausal women with nonalcoholic fatty liver disease. *Menopause* (2016) 23 (8):876–83. doi: 10.1097/GME.0000000000000647

45. Shamsoddini A, Sobhani V, Ghamar Chehreh ME, Alavian SM, Zaree A. Effect of aerobic and resistance exercise training on liver enzymes and hepatic fat in Iranian men with nonalcoholic fatty liver disease. *Hepat Mon* (2015) 15(10): e31434. doi: 10.5812/hepatmon.31434

46. Shojaee-Moradie F, Cuthbertson DJ, Barrett M, Jackson NC, Herring R, Thomas EL, et al. Exercise training reduces liver fat and increases rates of VLDL clearance but not VLDL production in NAFLD. *J Clin Endocrinol Metab* (2016) 101 (11):4219–28. doi: 10.1210/jc.2016-2353

47. Sullivan S, Kirk EP, Mittendorfer B, Patterson BW, Klein S. Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology* (2012) 55(6):1738–45. doi: 10.1002/hep.25548

48. Winn NC, Liu Y, Rector RS, Parks EJ, Ibdah JA, Kanaley JA. Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity - a randomized trial. *Metabolism* (2018) 78:128–40. doi: 10.1016/j.metabol.2017.08.012

49. Zhang HJ, He J, Pan LL, Ma ZM, Han CK, Chen CS, et al. Effects of moderate and vigorous exercise on nonalcoholic fatty liver disease: A randomized clinical trial. *JAMA Intern Med* (2016) 176(8):1074–82. doi: 10.1001/jamainternmed.2016.3202

50. Abd El-Kader SM, Al-Jiffri OH, Al-Shreef FM. Markers of liver function and inflammatory cytokines modulation by aerobic versus resisted exercise training for nonalcoholic steatohepatitis patients. *Afr Health Sci* (2014) 14(3):551–7. doi: 10.4314/ahs.v14i3.8

51. Haus JM, Solomon TP, Kelly KR, Fealy CE, Kullman EL, Scelsi AR, et al. Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* (2013) 98(7):E1181–8. doi: 10.1210/jc.2013-1229

52. Khaoshbaten M, Gholami N, Sokhtehzari S, Monazami AH, Nejad MR. The effect of an aerobic exercise on serum level of liver enzymes and liver echogenicity in patients with non alcoholic fatty liver disease. *Gastroenterol Hepatol Bed Bench* (2013) 6(Suppl 1):S112–6. doi: 10.22037/ghfbb.v6i0.482

53. MacLean C, Dillon J, Babraj JA, Vollaard NBJ. The effect of low volume sprint interval training in patients with non-alcoholic fatty liver disease. *Physician Sportsmedicine* (2018) 46(1):87–92. doi: 10.1080/00913847.2018.1411171

54. O'Gorman P, Naimimohasses S, Monaghan A, Kennedy M, Melo AM, NF D, et al. Improvement in histological endpoints of MAFLD following a 12-week aerobic exercise intervention. *Aliment Pharmacol Ther* (2020) 52(8):1387–98. doi: 10.1111/apt.15989

55. O'Gorman P, Monaghan A, McGrath M, Naimimohasses S, Gormley J, Norris S. Determinants of physical activity engagement in patients with nonalcoholic fatty liver disease: The need for an individualized approach to lifestyle interventions. *Phys Ther* (2021) 101(2):pzaa 195. doi: 10.1093/ptj/pzaa195

56. Sargeant JA, Gray LJ, Bodicoat DH, Willis SA, Stensel DJ, Nimmo MA, et al. The effect of exercise training on intrahepatic triglyceride and hepatic insulin sensitivity: a systematic review and meta-analysis. *Obes Rev* (2018) 19(10):1446–59. doi: 10.1111/obr.12719

57. Heyens LJM, Busschots D, Koek GH, Robaeys G, Francque S. Liver fibrosis in non-alcoholic fatty liver disease: From liver biopsy to non-invasive biomarkers in diagnosis and treatment. *Front Med* (2021) 8:615978. doi: 10.3389/fmed.2021.615978

58. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* (2006) 44(4):865–73. doi: 10.1002/hep.21327

59. Balkhed W, Åberg FO, Nasr P, Ekstedt M, Kechagias S. Repeated measurements of non-invasive fibrosis tests to monitor the progression of non-alcoholic fatty liver disease: A long-term follow-up study. *Liver Int* (2022) 42 (7):1545–56. doi: 10.1111/liv.15255

60. Sabag A, Barr L, Armour M, Armstrong A, Baker CJ, Twigg SM, et al. The effect of high-intensity interval training vs moderate-intensity continuous training on liver fat: A systematic review and meta-analysis. *J Clin Endocrinol Metab* (2022) 107(3):862–81. doi: 10.1210/clinem/dgab795

61. Bellicha A, van Baak MA, Battista F, Beaulieu K, Blundell JE, Busetto L, et al. Effect of exercise training on weight loss, body composition changes, and weight maintenance in adults with overweight or obesity: An overview of 12 systematic reviews and 149 studies. *Obes Rev* (2021) 22(S4):e13256. doi: 10.1111/obr.13256

62. Baker CJ, Martinez-Huenchullan SF, D'Souza M, Xu Y, Li M, Bi Y, et al. Effect of exercise on hepatic steatosis: Are benefits seen without dietary intervention? a systematic review and meta-analysis. *J Diabetes* (2021) 13(1):63–77. doi: 10.1111/1753-0407.13086

63. Nath P, Panigrahi MK, Sahu MK, Narayan J, Sahoo RK, Patra AA, et al. Effect of exercise on NAFLD and its risk factors: Comparison of moderate versus low intensity exercise. *J Clin Transl Hepatol* (2020) 8(2):120–6. doi: 10.14218/JCTH.2019.00012

64. Kerr CJ, Waterworth SP, Brodie D, Sandercock GRH, Ingle L. The associations between physical activity intensity, cardiorespiratory fitness, and non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* (2021) 36(12):3508–14. doi: 10.1111/jgh.15672

65. McPherson S, Armstrong MJ, Cobbold JF, Corless L, Anstee QM, Aspinall RJ, et al. Quality standards for the management of non-alcoholic fatty liver disease (NAFLD): consensus recommendations from the British association for the study of the liver and British society of gastroenterology NAFLD special interest group. *Lancet Gastroenterol Hepatol* (2022) 7(8):755–69. doi: 10.1016/S2468-1253(22)00061-9

66. Babu AF, Csader S, Lok J, Gómez-Gallego C, Hanhineva K, El-Nezami H, et al. Positive effects of exercise intervention without weight loss and dietary changes in NAFLD-related clinical parameters: A systematic review and meta-analysis. *Nutrients* (2021) 13(9):3135. doi: 10.3390/nu13093135

67. Chatterjee A, Basu A, Das K, Singh P, Mondal D, Bhattacharya B, et al. Hepatic transcriptome signature correlated with HOMA-IR explains early nonalcoholic fatty liver disease pathogenesis. *Ann Hepatology* (2020) 19(5):472–81. doi: 10.1016/j.aohep.2020.06.009

68. Vogeser M, König D, Frey I, Predel H-G, Parhofer KG, Berg A. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. *Clin Biochem* (2007) 40 (13):964–8. doi: 10.1016/j.clinbiochem.2007.05.009

69. Arab JP, Arrese M, Trauner M. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. *Annu Rev Pathol* (2018) 13:321–50. doi: 10.1146/annurev-pathol-020117-043617

70. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res* (2009) 48(1):1–26. doi: 10.1016/j.plipres.2008.08.001

71. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. *Framingham Study Am J Med* (1977) 62(5):707–14. doi: 10.1016/0002-9343(77)90874-9

72. Bhargava S, de la Puente-Secades S, Schurgers L, Jankowski J. Lipids and lipoproteins in cardiovascular diseases: a classification. *Trends Endocrinol Metab* (2022) 33(6):409–23. doi: 10.1016/j.tem.2022.02.001

73. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. *Am J Cardiol* (1998) 81(4a):7b–12b. doi: 10.1016/s0002-9149(98)00031-9

74. Myers J, McAuley P, Lavie CJ, Despres J-P, Arena R, Kokkinos P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: Their independent and interwoven importance to health status. *Prog Cardiovasc Diseases* (2015) 57(4):306–14. doi: 10.1016/j.pcad.2014.09.011

75. Keating SE, Hackett DA, Parker HM, O'Connor HT, Gerofi JA, Sainsbury A, et al. Effect of aerobic exercise training dose on liver fat and visceral adiposity. *J Hepatol* (2015) 63(1):174–82. doi: 10.1016/j.jhep.2015.02.022

frontiers | Frontiers in Endocrinology

TYPE Mini Review
PUBLISHED 15 December 2022
DOI 10.3389/fendo.2022.1058101

OPEN ACCESS

EDITED BY
Ruben Nogueiras,
University of Santiago de Compostela, Spain

REVIEWED BY
María Jesús González Rellán,
University of Toronto, Canada
Marcos F. Fondevila,
University of Santiago de Compostela, Spain

*CORRESPONDENCE
Teresa C. Delgado
TERESADEJESUS.CARDOSODELGADO@osakidetza.eus

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

RECEIVED 30 September 2022
ACCEPTED 21 November 2022
PUBLISHED 15 December 2022

CITATION
Delgado TC, de las Heras J and Martínez-Chantar ML (2022) Understanding gut-liver axis nitrogen metabolism in Fatty Liver Disease. *Front. Endocrinol.* 13:1058101. doi: 10.3389/fendo.2022.1058101

Understanding gut-liver axis nitrogen metabolism in Fatty Liver Disease

Teresa C. Delgado[1,2]*, Javier de las Heras[2,3,4] and María L. Martínez-Chantar[1,5]

[1]Liver Disease Lab, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain, [2]Congenital Metabolic Disorders, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain, [3]Division of Pediatric Metabolism, Department of Pediatrics, CIBERer, Cruces University Hospital, Barakaldo, Spain, [4]Department of Pediatrics, University of the Basque Country (UPV/EHU), Leioa, Spain, [5]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Carlos III National Health Institute, Madrid, Spain

The homeostasis of the most important nitrogen-containing intermediates, ammonia and glutamine, is a tightly regulated process in which the gut-liver axis plays a central role. Several studies revealed that nitrogen metabolism is altered in Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), a consensus-driven novel nomenclature for Non-Alcoholic Fatty Liver Disease (NAFLD), the most common chronic liver disease worldwide. Both increased ammonia production by gut microbiota and decreased ammonia hepatic removal due to impaired hepatic urea cycle activity or disrupted glutamine synthetase activity may contribute to hepatic ammonia accumulation underlying steatosis, which can eventually progress to hyperammonemia in more advanced stages of steatohepatitis and overt liver fibrosis. Furthermore, our group recently showed that augmented hepatic ammoniagenesis *via* increased glutaminase activity and overexpression of the high activity glutaminase 1 isoenzyme occurs in Fatty Liver Disease. Overall, the improved knowledge of disrupted nitrogen metabolism and metabolic miscommunication between the gut and the liver suggests that the reestablishment of altered gut-liver axis nitrogenous balance is an appealing and attractive therapeutic approach to tackle Fatty Liver Disease, a growing and unmet health problem.

KEYWORDS

Gut-liver axis, nitrogen metabolism, Fatty Liver Disease, ammonia, glutamine, glutaminase, urea cycle

Introduction

Ammonia, a central element in whole-body nitrogen metabolism

Ammonia is an inorganic nitrogen waste product metabolized and produced in all tissues and the principal culprit of hepatic encephalopathy (HE), a spectrum of neuropsychiatric abnormalities derived from liver dysfunction (1). Elevated blood ammonia, hyperammonemia, results from an imbalance between the ammonia produced and the body capacity to metabolize or remove it. In the human body under healthy conditions, ammonia is produced mostly in the gut by three main mechanisms: hydrolysis of urea by bacterial urease, bacterial protein deamination, and intestinal mucosal glutamine metabolism. Although the colon is conventionally assumed to be the major site of gut-ammonia production, recent evidence indicates that the stomach and small intestine are also involved (2). In the opposite, hepatic urea cycle is a well-described metabolic pathway for ammonia detoxification. Five urea cycle enzymes (UCEs) [carbamoyl phosphate synthetase I (CPS-1), Ornithine transcarbamoylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase 1 (ARG1)] and 2 membranes transporters mediate the conversion of toxic ammonia into non-toxic urea that is excreted in the urine, ureagenesis (3). Hepatic ammonia metabolism is a highly zonated process being ureagenesis exclusively restricted to the periportal zone where the portal blood that carries ammonia from the gut first passes. Hepatic ammonia escaping ureagenesis is further excreted by means of the glutamine synthetase (GS) enzyme that catalyzes the ATP-dependent condensation of ammonia and glutamate to glutamine and solely expressed in the pericentral zone of the liver lobule. Failures in any of the gene coding for UCEs gives rise to a series of inherited life-threatening conditions overall characterized by aberrant ammonia accumulation, the urea cycle disorders (UCDs) (4). Also, the liver-specific GS-deficient mice present hyperammonemia and show increased locomotion, impaired fear memory, and a slightly reduced life span (5). By using liver specific and whole-body GS-KO mice together with stepwise increments of enterally or intravenously administered ammonium carbonate to challenge ammonia detoxification, Hakvoort and colleagues established that urea cycle and GS contribute equally to hepatic ammonia detoxification (6).

Glutamine, a major nontoxic interorgan nitrogen carrier, and hepatic glutamine metabolism

Glutamine, the most abundant amino acid in the body, much like ammonia is a major player in whole-body nitrogen metabolism. In the liver, glutamine metabolism is zonated, where glutamine is usually converted to glutamate and ammonia by the periportal mitochondrial enzyme glutaminase. Glutamate is further converted to α-ketoglutarate (αKG) by glutamate dehydrogenase (GDH) to enter the tricarboxylic acids (TCA) cycle. Ammonia produced during glutamine breakdown by glutaminase in periportal hepatocytes, together with ammonia derived for the gut entering liver through the portal vein, is partially removed by the urea cycle activity. Unremoved ammonia is delivered *via* the blood stream to pericentral hepatocytes for efficient hepatic ammonia detoxification through GS activity that converts ammonia into glutamine completing the intrahepatic cycling of glutamine (Figure 1A). *In vivo* experiments suggest that changes in net hepatic glutamine balance are mainly regulated by glutaminase activity, with the flux through glutamine synthetase being relatively constant (7). The glutaminase family consists of two isoenzymes, the kidney-type glutaminase (*GLS*, also known as *GLS1*) and the liver-type glutaminase (*GLS2*) genes. Healthy adult intestine and kidney express the *GLS* gene whereas the *GLS2* gene is highly expressed in the healthy adult liver (8).

Metabolic dysfunction-Associated or Non-Alcoholic Fatty Liver Disease (MAFLD/NAFLD)

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), a consensus driven novel nomenclature for Non-Alcoholic Fatty Liver Disease (NAFLD) (9, 10)*, and from now on referred to as Fatty Liver Disease, comprehends a spectrum of conditions characterized by hepatic fat accumulation which can progress to inflammation, fibrosis and eventually leading to cirrhosis and hepatocellular carcinoma. Fatty Liver Disease is the most common type of chronic liver disease worldwide with an updated estimated prevalence of nearly 40%* (11)*, the leading cause of liver-related morbidity and mortality* (12)*, and often associated with obesity, insulin resistance, and diabetes* (13, 14).

Ammonia in fatty liver disease

Disturbed interorgan trafficking of ammonia is a feature of Fatty Liver Disease. Indeed, our group and others showed that hepatic ammonia is increased in mouse models of diet-induced steatohepatitis as well as in Fatty Liver Disease patients' liver biopsies (15–18). On the other hand, hyperammonemia, a distinguishable feature in advanced stages of cirrhosis and liver failure, was solely reported in mouse models of advanced fibrosis whereas it remains normal in animal models of steatosis and early stages of steatohepatitis (17, 19). In agreement, Felipo et al. described

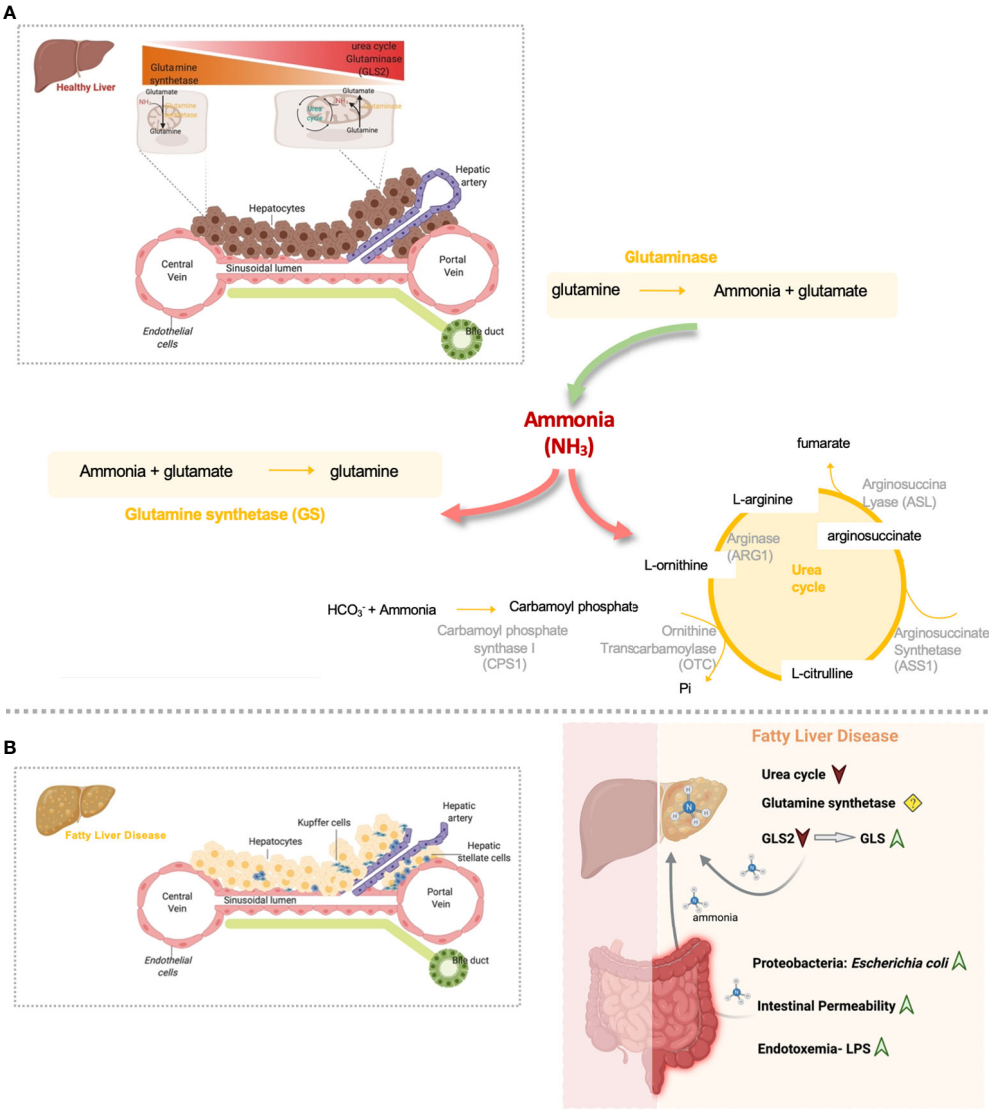


FIGURE 1
Overview of hepatic nitrogen metabolism homeostasis and disrupted gut-liver nitrogen metabolism in Fatty Liver Disease. **(A)** Both ammonia and glutamine hepatic metabolism are highly zonated processes. Hepatic ammonia enters in the liver from the gut where it can be eliminated by the urea cycle activity. In alternative, periportal hepatic glutaminase can convert glutamine to ammonia. Glutaminase and gut-derived ammonia that is not excreted in the periportal urea cycle, escapes to the pericentral region where is further eliminated by means of the glutamine synthetase (GS) enzyme. This enzyme converts ammonia back to glutamine and closing the glutamine cycle in order to maintain glutamine homeostasis. **(B)** Nitrogen metabolism homeostasis is disrupted in Fatty Liver Disease. Overall, inflamed gut in Fatty Liver Disease is characterized by the accumulation of proteobacteria Escherichia coli, very active in the production of ammonia. Gut-derived ammonia efflux through the portal vein can damage the liver directly or in alternative can exert detrimental effects on gut permeability and might indirectly contribute to NAFLD facilitating toxic molecules drainage into the portal blood. Additionally, it is possible that endotoxin and inflammation may contribute to increased uptake of ammonia from the gut into the bloodstream and thereby contribute to the latter's toxic effect on the liver. In addition, Fatty liver Disease is characterized by diminished hepatic urea cycle activity and a switch from the low activity GLS2 to the high activity glutaminase, GLS, that together induce the accumulation of ammonia content in the liver. Ammonia can further drive fibrosis by promoting hepatic stellate cells activation, the main fibrogenic cell type. (Created by Biorender.com).

that patients with liver cirrhosis are hyperammonemic, Fatty Liver Disease patients have normal blood ammonia and patients with advanced steatohepatitis present mild hyperammonemia (20). Of interest, *in vivo* experiments in mouse models of early Fatty Liver Disease have shown that blood ammonia concentrations are significantly elevated in the portal vein blood (21), which suggests that a still functional hepatic ammonia extraction by the liver may reestablish normal blood ammonia levels.

Gut-derived ammonia in fatty liver disease

Compelling evidence links the gut microbiome, intestinal barrier integrity, and the accumulation of fat in the liver. Indeed, dietary factors may alter the gut microbiota and intestinal barrier function, favoring the occurrence of metabolic endotoxemia and low-grade inflammation, contributing to the development of obesity and Fatty Liver Disease (revised in (22)). Gut-derived ammonia efflux through the portal vein can damage the liver directly or in alternative can exert detrimental effects on gut permeability and might indirectly contribute to Fatty Liver Disease facilitating toxic molecules drainage into the portal blood. Additionally, it is possible that endotoxin and inflammation, hallmarks of Fatty Liver Disease, may contribute to increased gut ammonia uptake into the bloodstream and accounting for the latter's toxic effect on the liver (23). *In vitro* studies unraveled that the largest amount of ammonia is generated by gram-negative anaerobes, clostridia, enterobacteria such as *E. coli*, and *Bacillus spp* (24–26). Noteworthy, enterobacteria such *as E. Coli* are consistently enriched in steatosis and steatohepatitis (22). Shotgun metagenomic sequencing revealed a positive association between increased abundance of *E. coli* and advanced fibrosis in Fatty Liver Disease patients (27). Although these results suggest an accumulation of gut ammonia-producing bacteria in Fatty Liver Disease, the precise amount of ammonia generated by gut microbiota and its contribution to the accumulation of hepatic ammonia or hyperammonemia during steatohepatitis remains to be explored.

Deregulated hepatic urea cycle activity in fatty liver disease

Ureagenesis through the urea hepatic cycle is impaired in animal models and patients with Fatty Liver Disease (16, 28, 29). Under these circumstances, reduced ureagenesis is related to decreased regulation of the UCEs at the gene level *via* hypermethylation of promoter regions of these genes (16, 30). However, reduction in the functional capacity for ureagenesis is more pronounced in simple steatosis than in steatohepatitis (29). Though post-transcriptional changes of the urea cycle genes cannot be ruled out, this difference is most probably a result of increased glucagon levels or inflammatory mediators, known to stimulate ureagenesis, that are augmented in steatohepatitis and not in simple steatosis. In the last years, steatosis-driven downregulation of urea cycle enzymes genes has been associated to the detrimental effects of long-chain fatty acids (31) or, in alternative, changes in the methylation index and methionine cycle rates observed in Fatty Liver Disease (32).

Glutamine synthetase activity in fatty liver disease

Glutamine synthetase expression is decreased in cirrhosis independently of the etiology of the patients (33) and in drug-induced liver injury (34). In the opposite, GS is increased in regenerative states such as chronic hepatitis, focal nodular hyperplasia, peritumoral hyperplasia and some hepatocellular neoplasms (33). In pre-clinical mouse models of Fatty Liver Disease, GS protein expression was shown to be augmented (19, 21). Likewise, Eriksen et al. described that GS gene expression is augmented in patients both in simple steatosis and steatohepatitis compared to healthy liver lean and obese controls (29). In agreement, increased protein GS was also reported by our group in patients with early steatohepatitis (19). However, other studies have shown that hepatic protein GS expression decreased progressively in patients with steatosis and almost disappeared in steatohepatitis (16). Discrepancies reported in literature can reflect a lack of specificity of some antibodies used for the immunohistochemical staining or in alternative a mismatch between the regulation at the transcriptionally or post-transcriptional level of GS. Indeed, the GS gene is transcriptionally activated by glucocorticoid hormones in a tissue-specific fashion. However, at the ultimate level, the GS enzyme expression if governed by a post-transcriptional mechanism where GS protein turnover dependent of 26S proteosome is increased by a product feedback mechanism after glutamine stimulation (35). Further studies are necessary to better understand the spatiotemporal regulation of GS expression in the progression of Fatty Liver Disease.

Glutamine and hepatic glutaminase activity in fatty liver disease

Glutamine metabolism homeostasis is disrupted in Fatty Liver Disease. Although serum glutamine is not significantly altered between healthy controls and patients with steatohepatitis, serum glutamate, a metabolite which metabolic pathways are closely related to glutamine, is augmented in patients with steatohepatitis (19). Similar findings were reported by Kuo et al., where serum glutamine remains constant as fibrosis severity worsens, but glutamate, the glutamate/glutamine ratio, the TCA cycle intermediate α-KG, and their downstream metabolites in the TCA cycle all increase as fibrosis severity worsened. Other study has shown that glutamine concentrations were significantly elevated in the hepatic vein and heart blood of Western diet-fed mice, a mouse model of steatohepatitis, but not in the portal vein blood (21). Differences in glutamine concentrations between

the hepatic and portal veins are consistent with increased hepatic GS expression in Fatty Liver Disease (21).

Hepatic glutamine metabolism is mostly regulated by the actions of the glutaminase enzyme (7). Eriksen et al. described that *GLS2* expression, the liver type glutaminase, is decreased in steatohepatitis (29). In addition, recent findings from our group and others unraveled that the high-activity isoenzyme 1 of glutaminase (GLS) is overexpressed in mouse models and patients with steatohepatitis, which translates into an overall increase of hepatic glutaminase activity (19, 36) (Figure 1B). Importantly, the overexpression of GLS was detected in steatotic hepatocytes in pre-clinical and clinical samples of Fatty Liver Disease, with its transcriptional regulation still not fully understood (19). The relevance of glutamine metabolism in other types of hepatic cells during Fatty Liver Disease has also been addressed. Indeed, activation of HSCs, the main fibrogenic cell type, is highly dependent on glutaminolysis and GLS (36), suggesting that glutaminolysis is a potential diagnostic marker and therapeutic target during the progression from steatohepatitis to fibrosis (37). In addition, glutamine is one of the main sources of energy for Kupffer and endothelial cells (38), with GLS stimulating the proliferation, migration, and survival of the latter (39). However, to our knowledge the relevance of glutaminase alterations in Kupffer and endothelial cells underlying Fatty Liver Disease has not been specifically addressed.

Modulation of nitrogen metabolism in fatty liver disease: Therapeutic approaches

Deregulated ammonia and glutamine homeostasis are hallmarks of Fatty Liver Disease. Therefore, is not surprising that therapeutic approaches modulating the gut-liver nitrogen metabolism are potentially relevant for the treatment of Fatty Liver Disease.

Targeting gut microbiota in fatty Liver disease therapy

The gut is a potential source of systemic ammonia in Fatty Liver Disease; thus, capturing part of the gut ammonia may mitigate disease symptoms. Even though the beneficial effects of targeting ammonia-producing gut microbiota in advanced stages of chronic liver disease, such as cirrhosis, have been previously explored, studies addressing the effects of the specific targeting of ammonia-producing microbiota in Fatty Liver Disease are scarce.

Non-absorbable disaccharides, such as lactulose, are the first-line therapy for patients with hyperammonemia underlying UCDs and cirrhosis related HE (40–42). It has been shown that lactulose treatment ameliorated hepatic inflammation in animal models with steatohepatitis but could not completely prevent the development of steatohepatitis (43, 44). The antibiotic rifaximin, often used in combination with lactulose, has become the most effective antibiotic of choice in the treatment of hyperammonemia (45). Whereas some studies have shown that Rifaximin therapy appears to be effective and safe in modifying steatohepatitis through reduction of serum endotoxin and improvement of insulin resistance, proinflammatory cytokines, CK-18, and liver fat score (46, 47), other studies do not indicate a clear beneficial effect of rifaximin in patients with Fatty Liver Disease (48).

Probiotics can also reduce the total amount of ammonia in the portal blood by inhibiting bacterial urease activity. As most probiotics produce acids that reduce the pH in the intestine, ammonia absorption also decreases (49). In addition, probiotics reduce inflammation and oxidative stress in liver cells which further leads to increased hepatic clearance of ammonia (50–53). Even though several studies and clinical trials have encouraged the use of probiotic supplementation as promising and safe therapeutic approach in Fatty Liver Disease, nowadays the efficacy of probiotics in the management of these conditions remains limited to hypotheses.

Fecal microbiota transplantation (FMT) is an emerging treatment approach that is aimed at rebuilding intestinal microbiota to treat diseases and has been shown to attenuate hyperammonemia in HE animal models (54, 55). FMT may reduce ammonia synthesis by altering the gut microbiota composition to a taxon low in urease, diminish uptake of ammonia by reestablishing the integrity of the intestinal barrier and increase ammonia clearance by improving liver function. Shen et al. depleted animals of their preexisting gut microbiota and then inoculated with altered Schaedler flora (ASF), a defined consortium of 8 bacteria with minimal urease gene content. This protocol resulted in establishment of a persistent new community that promoted a long-term reduction in fecal urease activity and ammonia production. In a murine model of hepatic injury, ASF transplantation was associated with decreased morbidity and mortality (56). In another study, Kurtz et al. modified the oral probiotic *Escherichia coli nissle 1917* to create a strain (*SYNB1020*) that produces L-arginine and consumes ammonia. *SYNB1020* was shown to decrease systemic hyperammonemia in a mouse model of thioacetamide (TAA)-induced liver injury and phase I clinical trial showed a significant clinical effect and safety (57).

Finally, supplementation with Yaq-001, a non-absorbable synthetic carbon with high adsorptive capacity for bacterial products including LPS and pro-inflammatory cytokines, can reduce the transintestinal migration of gut microbiota and related metabolites, such as ammonia, bacteria-derived products, acetaldehyde, hydrophobic bile acids, and inflammation factors, including TNF-α and IL-6 in cirrhotic rats (58). This compound has also been shown to be safe and well tolerated in decompensated cirrhotic patients (59) and

further studies in animal models and patients with Fatty liver Disease can provide insightful results.

Therapeutic strategies to restore hepatic ammonia homeostasis either increasing ureagenesis or inhibiting glutaminase in fatty liver disease

Therapeutic strategies to reestablish ammonia content by diminishing urea cycle activity in patients with UCDs, cirrhosis and liver failure, have been developed in the last years. In fact, treatment with sodium benzoate to UCDs patients effectively decreases the blood ammonia level by reducing glycine metabolism in the liver, kidney, and brain. Other therapeutic option is sodium phenylacetate/phenylbutyrate that is rapidly oxidized to phenylacetate (PA) which conjugates to glutamine in the liver and is excreted as phenylacetylglutamine (PAGN) by the kidneys. In alternative, ornithine phenylacetate (OP) that on one hand uses PA to condensate to hepatic glutamine and excreted as PAGN, and on the other hand uses ornithine to boost urea cycle activity, effectively reduced ammonia levels in bile duct ligated rats, a mouse model of hepatic cholestasis, and in cirrhosis (60, 61). For the treatment of Fatty liver Disease, OP has been shown to reduce hyperammonemia whilst ameliorating steatohepatitis and fibrosis in mouse models (16, 17). In addition, hepatic ammonia content can be potentially rescued by inhibiting the activity of hepatic glutaminase. On this basis, our group and others have recently shown that both the specific silencing of hepatic GLS isoenzyme by using molecular approaches based on small interference RNA technology or its inhibition by using small chemical inhibitors can ameliorate steatohepatitis and fibrosis in pre-clinical mouse models of Fatty Liver Disease (19, 36). Finally, in the last years some *in vivo* studies have been carried out to address the effects of glutamine supplementation in the treatment of Fatty Liver Disease, with some of them showing a certain protective effect (62–64). Table 1 summarizes the current therapeutic approaches that have been used to date for the modulation of nitrogen metabolism in Fatty Liver Disease.

Concluding remarks

Fatty Liver Disease is characterized by increased hepatic ammonia content in early stages of steatosis followed by mild hyperammonemia in more advanced stages of steatohepatitis and fibrosis (15–18, 20). In addition, glutamine homeostasis is altered in Fatty Liver Disease as shown by increased serum glutamate/glutamine ratio in these patients (19, 36, 37). Fatty Liver Disease is characterized by gut dysbiosis and accumulation of the enterobacteria *E. coli*, very active in the production of ammonia (27). In addition, hepatic ammonia accumulation underlying Fatty Liver Disease is associated both with impaired liver ureagenesis through downregulation of the expression of UCEs (16, 28–30), and augmented hepatic glutaminase activity (19, 29, 36). Whether Fatty Liver Disease impairs nitrogen metabolism, or if these alterations in ammonia metabolism, ureagenesis ad glutaminase activity occur first and are the root of the appearance or progression of Fatty Liver Disease remains to be uncovered. However, the fact that UCDs patients seldom develop fatty liver as a consequence of disturbed ammonia metabolism (65), highlights the relevance of disturbed nitrogen metabolism as a potential driving mechanism in the onset of Fatty liver Disease.

TABLE 1 Current therapeutic approaches modulating nitrogen metabolism in pre-clinical and clinical studies in Fatty Liver Disease.

		Phase of the study	Main findings	Refs
Modulation of gut microbiota	Lactulose	Pre-clinical: Animal models	Improve hepatic inflammation Cannot prevent steatohepatitis development	(43, 44)
	Rifaximin	Clinical trials and observational studies	Safe and effective in modifying steatohepatitis No beneficial effect	(46–48)
	Fecal microbiota transplantation (FMT)	Pre-clinical: Animal models	Stools derived from healthy rodents could correct gut microbiota in steatohepatitis mice	(56)
	Engineering/FMT (SYNB1020)	Clinical trial: phase 1	Proven to be safe and effective in lowering ammonemia.	(57)
	Yaq-001	Clinical trial: programmed	A safety and efficacy study for the therapy of Fatty Liver Disease is being conducted	(58, 59)
Targeting hepatic ureagenesis/glutaminase	Ornithine phenylacetate (OP)	Pre-clinical: Animal models	Reduced hyperammonemia. Improved steatohepatitis and fibrosis	(16, 17)
	Glutaminase inhibitor: BPTES	Pre-clinical: Animal models	Reduced fibrosis in mouse models of CCl_4-induced liver fibrosis	(36)
	GLS silencing	Pre-clinical: Animal models	Ameliorated steatohepatitis in mouse models of diet-induced steatohepatitis without significant fibrosis	(19)
Glutamine supplementation	Glutamine supplement in diet	Pre-clinical: Animal models	Glutamine has a certain protective effect in Fatty Liver Disease	(62–64)

Even though in the last years an effort has been carried out to define and understand the miscommunication between the gut and the liver in the deregulation of nitrogen metabolism in Fatty Liver Disease, there are still open questions. First, the fractional contribution of gut ammonia production, disturbed urea cycle function, glutamine synthetase expression or enhanced hepatic glutaminase activity contribution to altered ammonia and glutamine homeostasis in the context of Fatty Liver Disease remains to be addressed. We acknowledge that these pathways may contribute unequally and vary depending on several factors such as age, gender, body mass index, dietary regimen, stage of the disease and others. For example, the liver is a sexual dimorphic organ, and at least in healthy rats, significant sex-related changes in urea cycle were found (66). Therefore, studies addressing the discovery of organ-specific biomarkers panels to be used as surrogates of the main metabolic pathways underlying deregulated nitrogen metabolism in Fatty Liver Disease are relevant. These biomarkers will allow the classification and grouping in sub-types of patients presenting similar characteristics with respect to their gut-liver nitrogen metabolism. Secondly, the term MAFLD was coined, among other things, to avoid diagnosis exclusion based on alcohol consumption (9). Alcohol consumption is clearly under-reported in NAFLD (67) which precludes that many of the clinical studies presenting impaired nitrogen metabolism in the context of NAFLD may also include patients undergoing alcohol consumption. In agreement, earlier studies suggest that patients with acute and chronic alcoholic liver disease present hyperammonemia and decreased capacity for urea synthesis (68, 69). Thereby, it would be interesting to understand how alcohol modulates the gut-liver nitrogen metabolism independent of other dietary factors which can be successfully performed in *in vivo* mouse models. Also, although the gut and the liver are central in the regulation of whole-body nitrogen metabolism, other organs cannot be obliviated. In fact, Elfeki and Singal recently proposed that chronic kidney disease may contribute to the higher levels of ammonia among patients with Fatty Liver Disease (70). And finally, is crucial to find novel targets and more effective pharmacological agents or therapeutical approaches to modulate nitrogen metabolism.

In summary, the liver-gut axis nitrogen metabolism is disturbed in Fatty Liver Disease with significant changes in ammonia and glutamine homeostasis and this has led to the hypothesis that therapeutic strategies that modulate nitrogen metabolism can potentially be used for the clinical managements of Fatty Liver Disease.

Author contributions

Writing and correction of the manuscript: TCD, JdH and MLM-C. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from Ministerio de Ciencia, Innovación y Universidades (MICINN) PID2020-117116RB-I00 integrated in Plan Estatal de Investigación Científica y Técnica y Innovación (to MLM-C), and co-funded with Fondos FEDER (to MLM-C), Subprograma Retos Colaboración RTC2019-007125-1 (to MLM-C), La Caixa Foundation Program HR17-00601 (to MLM-C), Proyectos Investigación en Salud DTS20/00138 (to MLM-C), Departamento de Industria del Gobierno Vasco (to MLM-C), and Ciberehd_ISCIII_MINECO, which is funded by the Instituto de Salud Carlos III. This work was partially funded by the Basque Department of Education (IT1281-19) (JdH), "Investigador AECC" (INVES18050CARD) and Ayuda RYC2020-029316-I financiada por MCIN/AEI/10.13039/501100011033 y por El FSE invierte en tu futuro (to TCD).

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. Bosoi CR, Rose CF. Identifying the direct effects of ammonia on the brain. *Metab Brain Dis* (2009) 24(1):95–102. doi: 10.1007/s11011-008-9112-7

2. Levitt DG, Levitt MD. A model of blood-ammonia homeostasis based on a quantitative analysis of nitrogen metabolism in the multiple organs involved in the production, catabolism, and excretion of ammonia in humans. *Clin Exp Gastroenterol* (2018) 11:193–215. doi: 10.2147/CEG.S160921

3. Meijer AJ, Lamers WH, Chamuleau RA. Nitrogen metabolism and ornithine cycle function. *Physiol Rev* (1990) 70(3):701–48. doi: 10.1152/physrev.1990.70.3.701

4. Walker V. Ammonia toxicity and its prevention in inherited defects of the urea cycle. *Diabetes Obes Metab* (2009) 11(9):823–35. doi: 10.1111/j.1463-1326.2009.01054.x

5. Qvartskhava N, Lang PA, Gorg B, Pozdeev VI, Ortiz MP, Lang KS, et al. Hyperammonemia in gene-targeted mice lacking functional hepatic glutamine synthetase. *Proc Natl Acad Sci USA* (2015) 112(17):5521–6. doi: 10.1073/pnas.1423968112

6. Hakvoort TB, He Y, Kulik W, Vermeulen JL, Duijst S, Ruijter JM, et al. Pivotal role of glutamine synthetase in ammonia detoxification. *Hepatology* (2017) 65(1):281–93. doi: 10.1002/hep.28852

7. Watford M. Glutamine and glutamate metabolism across the liver sinusoid. *J Nutr* (2000) 130(4S Suppl):983S–7S. doi: 10.1093/jn/130.4.983S

8. Mates JM, Segura JA, Martin-Rufian M, Campos-Sandoval JA, Alonso FJ, Marquez J. Glutaminase isoenzymes as key regulators in metabolic and oxidative stress against cancer. *Curr Mol Med* (2013) 13(4):514–34. doi: 10.2174/1566524011313040005

9. Eslam M, Sanyal AJ, George J, International Consensus P. MAFLD: A consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* (2020) 158(7):1999–2014.e1. doi: 10.1053/j.gastro.2019.11.312

10. Mendez-Sanchez N, Bugianesi E, Gish RG, Lammert F, Tilg H, Nguyen MH, et al. Global multi-stakeholder endorsement of the MAFLD definition. *Lancet Gastroenterol Hepatol* (2022) 7(5):388–90. doi: 10.1016/S2468-1253(22)00062-0

11. Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* (2022) 7(9):851–61. doi: 10.1016/S2468-1253(22)00165-0

12. Cotter TG, Rinella M. Nonalcoholic fatty liver disease 2020: The state of the disease. *Gastroenterology* (2020) 158(7):1851–64. doi: 10.1053/j.gastro.2020.01.052

13. Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. *Lancet Diabetes Endocrinol* (2022) 10(4):284–96. doi: 10.1016/S2213-8587(22)00003-1

14. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism* (2019) 92:82–97. doi: 10.1016/j.metabol.2018.11.014

15. Gutierrez-de-Juan V, Lopez de Davalillo S, Fernandez-Ramos D, Barbier-Torres L, Zubiete-Franco I, Fernandez-Tussy P, et al. A morphological method for ammonia detection in liver. *PLoS One* (2017) 12(3):e0173914. doi: 10.1371/journal.pone.0173914

16. De Chiara F, Heeboll S, Marrone G, Montoliu C, Hamilton-Dutoit S, Ferrandez A, et al. Urea cycle dysregulation in non-alcoholic fatty liver disease. *J Hepatol* (2018) 69(4):905–15. doi: 10.1016/j.jhep.2018.06.023

17. De Chiara F, Thomsen KL, Habtesion A, Jones H, Davies N, Gracia-Sancho J, et al. Ammonia scavenging prevents progression of fibrosis in experimental nonalcoholic fatty liver disease. *Hepatology* (2020) 71(3):874–92. doi: 10.1002/hep.30890

18. Rojas A, Garcia-Lozano MR, Gil-Gomez A, Romero-Gomez M, Ampuero J. Glutaminolysis-ammonia-urea cycle axis, non-alcoholic fatty liver disease progression and development of novel therapies. *J Clin Transl Hepatol* (2022) 10(2):356–62. doi: 10.14218/JCTH.2021.00247

19. Simon J, Nunez-Garcia M, Fernandez-Tussy P, Barbier-Torres L, Fernandez-Ramos D, Gomez-Santos B, et al. Targeting hepatic glutaminase 1 ameliorates non-alcoholic steatohepatitis by restoring very-Low-Density lipoprotein triglyceride assembly. *Cell Metab* (2020) 31(3):605–22.e10. doi: 10.1016/j.cmet.2020.01.013

20. Felipo V, Urios A, Montesinos E, Molina I, Garcia-Torres ML, Civera M, et al. Contribution of hyperammonemia and inflammatory factors to cognitive impairment in minimal hepatic encephalopathy. *Metab Brain Dis* (2012) 27(1):51–8. doi: 10.1007/s11011-011-9269-3

21. Ghallab A, Myllys M, Friebel A, Duda J, Edlund K, Halilbasic E, et al. Spatio-temporal multiscale analysis of Western diet-fed mice reveals a translationally relevant sequence of events during NAFLD progression. *Cells* (2021) 10(10). doi: 10.3390/cells10102516

22. Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* (2020) 17(5):279–97. doi: 10.1038/s41575-020-0269-9

23. Thomsen KL, De Chiara F, Rombouts K, Vilstrup H, Andreola F, Mookerjee RP, et al. Ammonia: A novel target for the treatment of non-alcoholic steatohepatitis. *Med Hypotheses* (2018) 113:91–7. doi: 10.1016/j.mehy.2018.02.010

24. Vince AJ, Burridge SM. Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. *J Med Microbiol* (1980) 13(2):177–91. doi: 10.1099/00222615-13-2-177

25. Richardson AJ, McKain N, Wallace RJ. Ammonia production by human faecal bacteria, and the enumeration, isolation and characterization of bacteria capable of growth on peptides and amino acids. *BMC Microbiol* (2013) 13:6. doi: 10.1186/1471-2180-13-6

26. Vince A, Dawson AM, Park N, O'Grady F. Ammonia production by intestinal bacteria. *Gut* (1973) 14(3):171–7. doi: 10.1136/gut.14.3.171

27. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* (2017) 25(5):1054–62.e5. doi: 10.1016/j.cmet.2017.04.001

28. Thomsen KL, Gronbaek H, Glavind E, Hebbard L, Jessen N, Clouston A, et al. Experimental nonalcoholic steatohepatitis compromises ureagenesis, an essential hepatic metabolic function. *Am J Physiol Gastrointest Liver Physiol* (2014) 307(3):G295–301. doi: 10.1152/ajpgi.00036.2014

29. Eriksen PL, Vilstrup H, Rigbolt K, Suppli MP, Sorensen M, Heeboll S, et al. Non-alcoholic fatty liver disease alters expression of genes governing hepatic nitrogen conversion. *Liver Int* (2019) 39(11):2094–101. doi: 10.1111/liv.14205

30. Gallego-Duran R, Ampuero J, Pastor-Ramirez H, Alvarez-Amor L, Del Campo JA, Maya-Miles D, et al. Liver injury in non-alcoholic fatty liver disease is associated with urea cycle enzyme dysregulation. *Sci Rep* (2022) 12(1):3418. doi: 10.1038/s41598-022-06614-9

31. Tomomura M, Tomomura A, Dewan MA, Saheki T. Long-chain fatty acids suppress the induction of urea cycle enzyme genes by glucocorticoid action. *FEBS Lett* (1996) 399(3):310–2. doi: 10.1016/S0014-5793(96)01344-0

32. Mato JM, Martinez-Chantar ML, Lu SC. Methionine metabolism and liver disease. *Annu Rev Nutr* (2008) 28:273–93. doi: 10.1146/annurev.nutr.28.061807.155438

33. Fleming KE, Wanless IR. Glutamine synthetase expression in activated hepatocyte progenitor cells and loss of hepatocellular expression in congestion and cirrhosis. *Liver Int* (2013) 33(4):525–34. doi: 10.1111/liv.12099

34. Rodriguez-Agudo R, Goikoetxea-Usandizaga N, Serrano-Macia M, Fernandez-Tussy P, Fernandez-Ramos D, Lachiondo-Ortega S, et al. Methionine cycle rewiring by targeting miR-873-5p modulates ammonia metabolism to protect the liver from acetaminophen. *Antioxidants (Basel)* (2022) 11(5). doi: 10.3390/antiox11050897

35. Labow BI, Souba WW, Abcouwer SF. Mechanisms governing the expression of the enzymes of glutamine metabolism–glutaminase and glutamine synthetase. *J Nutr* (2001) 131(9 Suppl):2467S–74S. doi: 10.1093/jn/131.9.2467S

36. Du K, Hyun J, Premont RT, Choi SS, Michelotti GA, Swiderska-Syn M, et al. Hedgehog-YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. *Gastroenterology.* (2018) 154(5):1465–79.e13. doi: 10.1053/j.gastro.2017.12.022

37. Du K, Chitneni SK, Suzuki A, Wang Y, Henao R, Hyun J, et al. Increased glutaminolysis marks active scarring in nonalcoholic steatohepatitis progression. *Cell Mol Gastroenterol Hepatol* (2020) 10(1):1–21. doi: 10.1016/j.jcmgh.2019.12.006

38. Spolarics Z, Lang CH, Bagby GJ, Spitzer JJ. Glutamine and fatty acid oxidation are the main sources of energy for kupffer and endothelial cells. *Am J Physiol* (1991) 261(2 Pt 1):G185–90. doi: 10.1152/ajpgi.1991.261.2.G185

39. Peyton KJ, Liu XM, Yu Y, Yates B, Behnammanesh G, Durante W. Glutaminase-1 stimulates the proliferation, migration, and survival of human endothelial cells. *Biochem Pharmacol* (2018) 156:204–14. doi: 10.1016/j.bcp.2018.08.032

40. Wang JY, Bajaj JS, Wang JB, Shang J, Zhou XM, Guo XL, et al. Lactulose improves cognition, quality of life, and gut microbiota in minimal hepatic encephalopathy: A multicenter, randomized controlled trial. *J Dig Dis* (2019) 20(10):547–56. doi: 10.1111/1751-2980.12816

41. Yang N, Liu H, Jiang Y, Zheng J, Li DM, Ji C, et al. Lactulose enhances neuroplasticity to improve cognitive function in early hepatic encephalopathy. *Neural Regener Res* (2015) 10(9):1457–62. doi: 10.4103/1673-5374.165516

42. Montagnese S, Russo FP, Amodio P, Burra P, Gasbarrini A, Loguercio C, et al. Hepatic encephalopathy 2018: A clinical practice guideline by the Italian association for the study of the liver (AISF). *Dig Liver Dis* (2019) 51(2):190–205. doi: 10.1016/j.dld.2018.11.035

43. Fan JG, Xu ZJ, Wang GL. Effect of lactulose on establishment of a rat non-alcoholic steatohepatitis model. *World J Gastroenterol* (2005) 11(32):5053–6. doi: 10.3748/wjg.v11.i32.5053

44. Gokcen P, Ozturk O, Adali G, Tosun I, Dogan HO, Kara H, et al. A novel therapeutic approach to NASH: Both polyethylene glycol 3350 and lactulose reduce hepatic inflammation in C57BL/6J mice. *Adv Clin Exp Med* (2021) 30(11):1167–74. doi: 10.17219/acem/140506

45. Kaji K, Takaya H, Saikawa S, Furukawa M, Sato S, Kawaratani H, et al. Rifaximin ameliorates hepatic encephalopathy and endotoxemia without affecting the gut microbiome diversity. *World J Gastroenterol* (2017) 23(47):8355–66. doi: 10.3748/wjg.v23.i47.8355

46. Abdel-Razik A, Mousa N, Shabana W, Refaey M, Elzehery R, Elhelaly R, et al. Rifaximin in nonalcoholic fatty liver disease: Hit multiple targets with a single shot. *Eur J Gastroenterol Hepatol* (2018) 30(10):1237–46. doi: 10.1097/MEG.0000000000001232

47. Gangarapu V, Ince AT, Baysal B, Kayar Y, Kilic U, Gok O, et al. Efficacy of rifaximin on circulating endotoxins and cytokines in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* (2015) 27(7):840–5. doi: 10.1097/MEG.0000000000000348

48. Cobbold JFL, Atkinson S, Marchesi JR, Smith A, Wai SN, Stove J, et al. Rifaximin in non-alcoholic steatohepatitis: An open-label pilot study. *Hepatol Res* (2018) 48(1):69–77. doi: 10.1111/hepr.12904

49. Liu J, Lkhagva E, Chung HJ, Kim HJ, Hong ST. The pharmabiotic approach to treat hyperammonemia. *Nutrients* (2018) 10(2). doi: 10.3390/nu10020140

50. Lunia MK, Sharma BC, Sharma P, Sachdeva S, Srivastava S. Probiotics prevent hepatic encephalopathy in patients with cirrhosis: A randomized controlled trial. *Clin Gastroenterol Hepatol* (2014) 12(6):1003–8.e1. doi: 10.1016/j.cgh.2013.11.006

51. Pratap Mouli V, Benjamin J, Bhushan Singh M, Mani K, Garg SK, Saraya A, et al. Effect of probiotic VSL3 in the treatment of minimal hepatic encephalopathy: A non-inferiority randomized controlled trial. *Hepatol Res* (2015) 45(8):880–9. doi: 10.1111/hepr.12429

52. Solga SF. Probiotics can treat hepatic encephalopathy. *Med Hypotheses* (2003) 61(2):307–13. doi: 10.1016/S0306-9877(03)00192-0

53. Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. *J Hepatol* (2003) 38(5):681–7. doi: 10.1016/S0168-8278(03)00097-7

54. Kang DJ, Hylemon PB, Bajaj JS. Fecal transplant to mitigate hyperammonemia and hepatic encephalopathy in animal models. *Ann Hepatol* (2015) 14(5):762–3. doi: 10.1016/S1665-2681(19)30774-4

55. Wang WW, Zhang Y, Huang XB, You N, Zheng L, Li J. Fecal microbiota transplantation prevents hepatic encephalopathy in rats with carbon tetrachloride-induced acute hepatic dysfunction. *World J Gastroenterol* (2017) 23(38):6983–94. doi: 10.3748/wjg.v23.i38.6983

56. Shen TC, Albenberg L, Bittinger K, Chehoud C, Chen YY, Judge CA, et al. Engineering the gut microbiota to treat hyperammonemia. *J Clin Invest* (2015) 125(7):2841–50. doi: 10.1172/JCI79214

57. Kurtz CB, Millet YA, Puurunen MK, Perreault M, Charbonneau MR, Isabella VM, et al. An engineered e. coli nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. *Sci Transl Med* (2019) 11(475):eaau7975. doi: 10.1126/scitranslmed.aau7975

58. De Chiara FL, Lu W, Davies N, Mookerjee RP, Jalan R, Macnaughtan JR, et al. Characterization of the protective effects of yaq-001 on organ injury in cirrhosis. *J Hepatol* (2018) 68(Suppl.):S463–5. doi: 10.1016/S0168-8278(18)31173-5

59. Macnaughtan JR, Soeda J, Sawhney R, Oben J, Davies N, Mookerjee R, et al. Oral therapy with non-absorbable carbons of controlled porosity (YAQ-100) selectively modulates stool microbiome and its function and this is associated with restoration of immune function and inflasomme activation. *J Hepatol* (2015) 62(Suppl.):S240. doi: 10.1016/S0168-8278(15)30110-0

60. Jalan R, Wright G, Davies NA, Hodges SJ. L-ornithine phenylacetate (OP): A novel treatment for hyperammonemia and hepatic encephalopathy. *Med Hypotheses* (2007) 69(5):1064–9. doi: 10.1016/j.mehy.2006.12.061

61. Jover-Cobos M, Noiret L, Lee K, Sharma V, Habtesion A, Romero-Gomez M, et al. Ornithine phenylacetate targets alterations in the expression and activity of glutamine synthase and glutaminase to reduce ammonia levels in bile duct ligated rats. *J Hepatol* (2014) 60(3):545–53. doi: 10.1016/j.jhep.2013.10.012

62. Lin Z, Cai F, Lin N, Ye J, Zheng Q, Ding G. Effects of glutamine on oxidative stress and nuclear factor-kappaB expression in the livers of rats with nonalcoholic fatty liver disease. *Exp Ther Med* (2014) 7(2):365–70. doi: 10.3892/etm.2013.1434

63. Sellmann C, Baumann A, Brandt A, Jin CJ, Nier A, Bergheim I. Oral supplementation of glutamine attenuates the progression of nonalcoholic steatohepatitis in C57BL/6J mice. *J Nutr* (2017) 147(11):2041–9. doi: 10.3945/jn.117.253815

64. Sellmann C, Jin CJ, Degen C, De Bandt JP, Bergheim I. Oral glutamine supplementation protects female mice from nonalcoholic steatohepatitis. *J Nutr* (2015) 145(10):2280–6. doi: 10.3945/jn.115.215517

65. Strong A, Gold J, Gold NB, Yudkoff M. Hepatic manifestations of urea cycle disorders. *Clin Liver Dis (Hoboken)* (2021) 18(4):198–203. doi: 10.1002/cld.1115

66. Leskanocova AC, Babincak M, Verboova L, Benetinova Z, Macekova D, Kostolny J, et al. Sexual dimorphism in energy metabolism of wistar rats using data analysis. *Molecules* (2020) 25(10):2353. doi: 10.3390/molecules25102353

67. Staufer KH-S, Strebinger G, Pimigstorfer P, Suesse S, Scherzer T-M, Paulweber B, et al. Trauner, m. ethyl glucuronide in hair detects a high rate of harmful alcohol consumption in presumed non-alcoholic fatty liver disease. *J Hepatol* (2022) 77:918–30. doi: 10.1016/j.jhep.2022.04.040

68. Ravi S, Bade KS, Hasanin M, Singal AK. Ammonia level at admission predicts in-hospital mortality for patients with alcoholic hepatitis. *Gastroenterol Rep (Oxf).* (2017) 5(3):232–6.

69. Glavind E, Aagaard NK, Gronbaek H, Moller HJ, Orntoft NW, Vilstrup H, et al. Alcoholic hepatitis markedly decreases the capacity for urea synthesis. *PLoS One* (2016) 11(7):e0158388. doi: 10.1371/journal.pone.0158388

70. Elfeki MA, Singal AK. The possible link between higher ammonia levels associated with non-alcoholic fatty liver related cirrhosis and diabetes: Are we missing chronic kidney disease? *J Hepatol* (2022). doi: 10.1016/j.jhep.2022.09.018

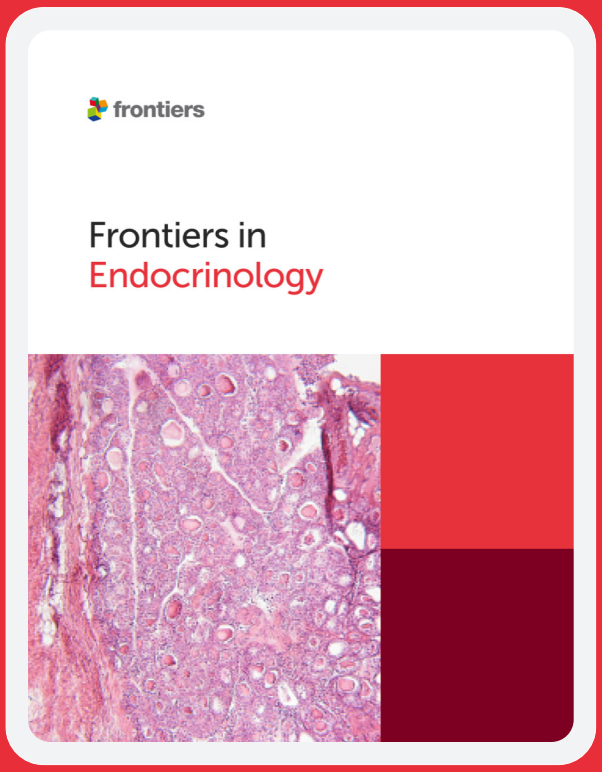
Frontiers in Endocrinology

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