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Insulin resistance, hyperinsulinemia, and associated diseases

Edited by
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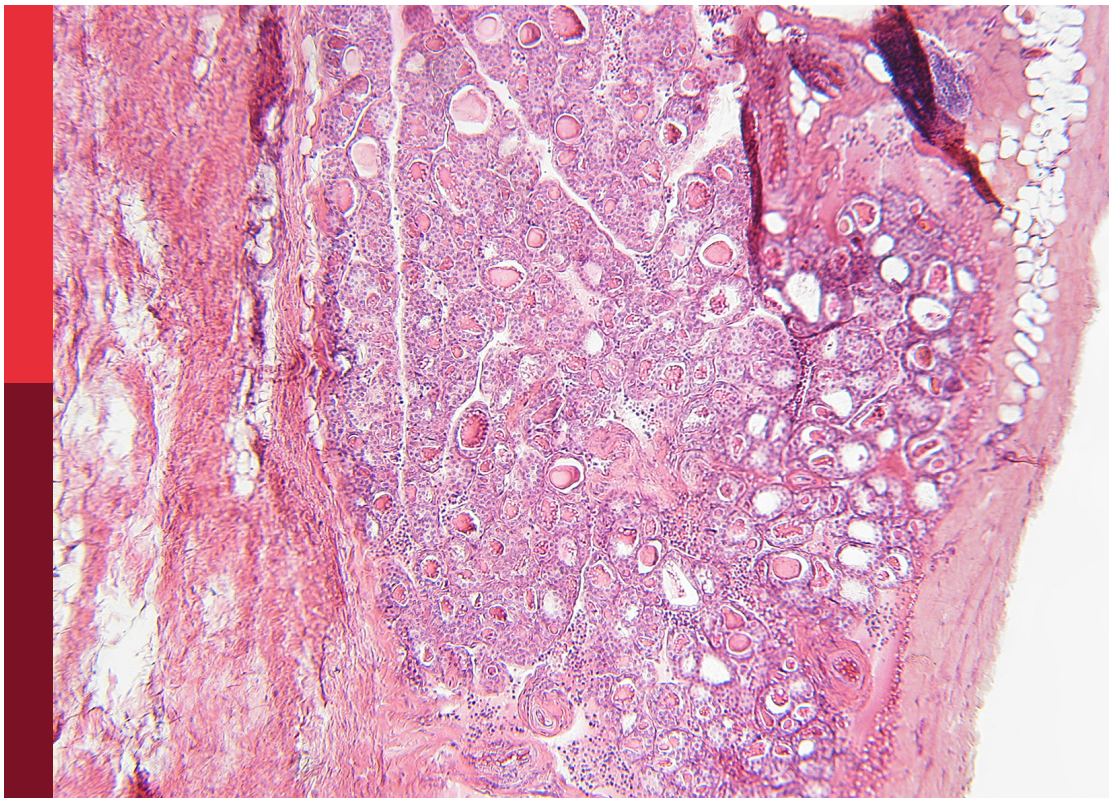

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Insulin resistance, hyperinsulinemia, and associated diseases

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The relationship between polycystic ovary syndrome and insulin resistance from 1983 to 2022: A bibliometric analysis

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Background: Polycystic ovary syndrome (PCOS) is a common clinical disease often associated with insulin resistance (IR). The interaction between PCOS and IR will promote the progress of PCOS and the risk of related complications, harm women's physical and mental health, and increase the social and economic burden.

Materials and methods: PCOS IR-related works of literature were retrieved through the Web of Science Core Collection (WoSCC) Database and imported into VOSviewer and CiteSpace, respectively, in plain text format to conduct the literature visualization analysis of authors, countries, institutions, highly cited works of literature, and keywords, aiming to reveal the hot spots and trends of PCOS IR fields.

Results: A total of 7,244 articles were retrieved from 1900 to 2022. Among them, the United States has made the largest contribution. Diamanti-Kandarakis E was the author with the most publications, and the University of Athens was the institution with most publications. Keyword analysis showed that PCOS interacts with IR mainly through sex-hormone binding globulin, luteinizing hormone, insulin-like growth factor, oxidative stress, and other mechanisms. In addition, the complications of PCOS complicated with IR are also the focus of researchers' attention.

Conclusions: Through bibliometric analysis, this paper obtains the research hotspot and trend of PCOS IR fields, which can provide a reference for subsequent research.

KEYWORDS

polycystic ovary syndrome, insulin resistance, bibliometric analysis, CiteSpace, VOSviewer

Introduction

Polycystic ovary syndrome (PCOS) is a complex disease with highly heterogeneous clinical manifestations, which affects about 6–10% of women of reproductive age worldwide, making them prone to infertility, adverse pregnancy outcomes, endometrial cancer, and other diseases (1, 2). Studies have shown that about 35–80% of PCOS patients have insulin resistance (IR) (3, 4), which means researchers are paying more and more attention to the role of IR in PCOS. IR can not only aggravate the hormone disorder and ovulation disorder of PCOS but also increase the incidence of type 2 diabetes and cardiovascular disease (5–7), which greatly increases the social and economic burden and endangers women's physical and mental health. Therefore, it is of great significance to clarify the pathological mechanism between PCOS and IR and to prevent and treat it as soon as possible.

Bibliometrics analysis is a comprehensive knowledge system integrating mathematics, statistics, and philology with an emphasis on quantification (8). It was first defined by Pritchard in 1969 (9) and has developed rapidly in recent years, providing great convenience for literature reading. The CiteSpace V developed by Chaomei Chen of Drexel University and VOSviewer developed by Van Eck NJ of Leiden University are commonly used in bibliometrics. Through qualitative and quantitative analysis of existing database literature, the contribution of different countries, authors, and institutions, as well as hotspots and trends in the research field can be explored. By using the bibliometrics method, combined with VOSviewer and CiteSpace software, this study has conducted a visual analysis of the literature in the field of PCOS IR, tracking research hot spots and trends, to provide a reference for researchers.

Materials and methods

Data sources and search strategy

The data in this paper are retrieved through the Web of Science Core Collection (WoSCC) Database, and the retrieval strategy is as follows: TS = ("Polycystic Ovary Syndrome" OR "Ovary Syndrome, Polycystic" OR "Syndrome, Polycystic Ovary" OR "Stein-Leventhal Syndrome" OR "Stein Leventhal Syndrome" OR "Syndrome, Stein-Leventhal" OR "Sclerocystic Ovarian Degeneration" OR "Ovarian Degeneration, Sclerocystic" OR "Sclerocystic Ovary Syndrome" OR "Polycystic Ovarian Syndrome" OR "Ovarian Syndrome, Polycystic" OR "Sclerocystic Ovaries" OR "Ovary, Sclerocystic" OR "Sclerocystic Ovary") AND TS = ("Insulin resistance"). The time was set to 1900–2022, and the language chosen was English. Review and journal articles were included in this study. The retrieved articles were exported to a plain text file.

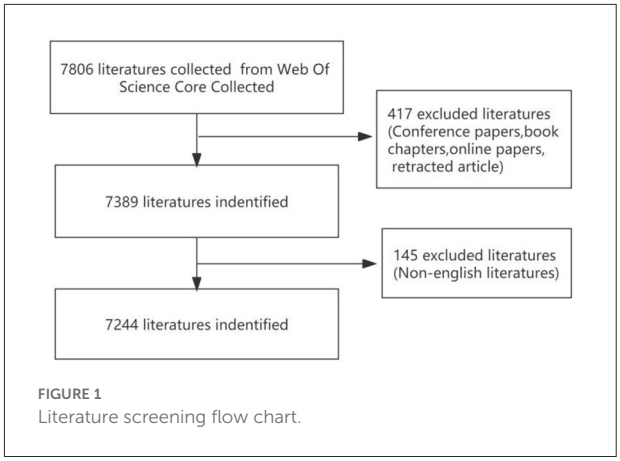

FIGURE 1
Literature screening flow chart.

Bibliometric software

In this study, VOSviewer and CiteSpace were used for bibliometric analysis of PCOS IR. VOS Viewer's strong graphical display ability can clearly show the cooperative relationship between projects (10). In VOSviewer, the node size was proportional to the co-occurrence times and the color represents the cluster. Compared with VOSviewer, CiteSpace has a stronger keyword outburst ability and highlights the trend and change of research hotspots (8).

Data analysis

Plain text files were imported into VOSviewer software for visual analysis of authors, institutions, and countries and were also imported into the Citespace V 5.8 software for keyword visualization analysis. The Citespace software was used to set the following parameters: Time slicing (from 1983 to 2022), Node types (keywords), Pruning (Pathfinder, pruning sliced networks, pruning the merged networks), Selection Criteria (the value of K in g-index is changed to 5), and other parameter settings follow the initial software settings.

Results

Analysis of article numbers and trends

Through screening, a total of 7,244 articles were included in this study (Figure 1). The first article on the PCOS IR field was published in 1983. Since 1991, the number of articles published in the PCOS IR field has continued to increase, reaching a peak of 597 articles in 2021 (Figure 2). As of May 2022, there are 157 articles in the PCOS IR field, and this number will continue to increase.

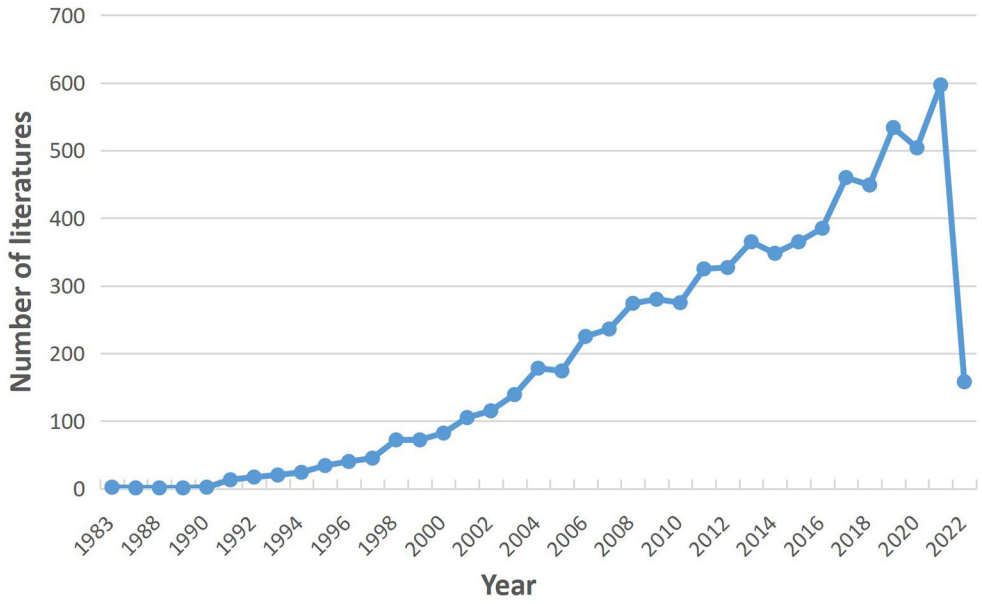

FIGURE 2
Distribution trend of PCOS IR from 1983 to 2022.

Analysis of national cooperation

Through visual analysis, articles on PCOS IR came from 102 countries (Figure 3). The United States contributed the most, with 1,630 articles (22.5%), followed by China (12.7%) and Turkey (7.1%). The national cooperation visualization map shows that the three countries have very little cooperation, with the United States conducting in-depth cooperation with Poland, France, and other Western countries.

Analysis of author cooperation

A total of 25,113 authors participated in PCOS IR publications (Figure 4). Diamanti-Kandarakis E ranked first with 64 publications, followed by Escobar-Morreale HF (61) and Legro RS (59). Although Azziz R ranked sixth in the number of articles published, Azziz R's group was at the heart of the network, working closely with other groups.

Analysis of institutional cooperation

A total of 4,991 institutions participated in PCOS IR, and the top 10 institutions published 942 articles in total. The University of Athens ranked first with 146 articles, followed by Monash University and Aristotle University Thessaloniki (Figure 5). University of Athens, Aristotle University Thessaloniki, University of Chile, and the University of Belgrade have established close cooperation.

Top cited publications

The top 10 highly cited literature types include Guideline (6), review (3), and clinical research (1) (Table 1). Due to the heterogeneity of the clinical manifestations of PCOS, the guidelines formulated by various medical associations are not uniform. The high citation of the guidelines represents the demand of researchers for consensus formulation of PCOS.

Analysis of keyword

As shown in the keyword visualization map (Figure 6), "insulin resistance" was the most frequent keyword, followed by "women," "polycystic ovary syndrome," "pco," "prevalence," "obesity," "risk," "metabolic syndrome," "impaired glucose tolerance," and so on. LLR algorithm in Citespace is used to cluster keywords. The top five keywords clusters are "sex-hormone-binding globulin," "luteinizing hormone," "diabetes mellitus," "quality of life," and "metformin." (Figure 7). Keywords burst detection map shows that recent focuses of researchers are "oxidative stress," "consensus," criteria," and "supplementation" (Figure 8).

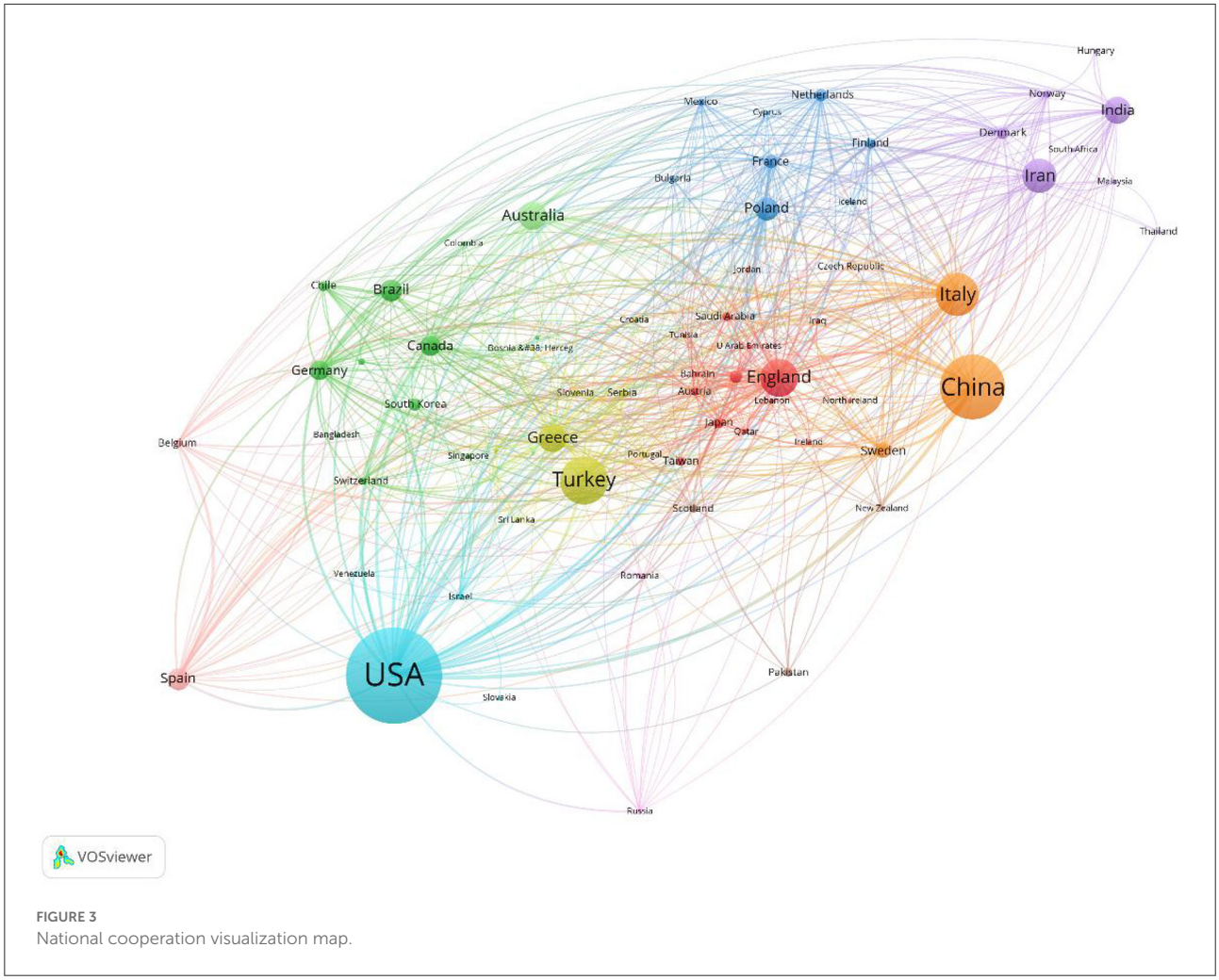

FIGURE 3
National cooperation visualization map.

Discussion

Polycystic ovary syndrome is a reproductive endocrine disease with menstrual disorder, androgen excess, and polycystic ovarian changes as the main clinical manifestations and is the main cause of ovulation dysfunction and infertility. IR and PCOS have a mutually promoting relationship, which not only affects the physical and mental health of PCOS patients but also increases the risk of type 2 diabetes and cardiovascular disease and the social and economic burden. Therefore, it is of great significance to clarify the pathological mechanism between PCOS and IR, and early intervention and treatment for the treatment of PCOS. Bibliometrics analysis can excavate research hotspots in a certain field and display them in the form of network co-occurrence maps, enabling researchers to intuitively and quickly understand the data. In this study, literature bibliometric analysis was conducted on the research field of PCOS IR to explore its development.

The number of PCOS IR papers has shown a steady increase to 2021, which indicates that IR, as an important pathological link in the development of PCOS, continues to receive the attention of researchers. In the first half of 2022, 157 articles were published in the PCOS IR field, which may indicate the number of publications in 2022 will be lower than before. This may be due to incomplete publication or research bottlenecks. While all countries are working together on PCOS IR, the United States made the biggest contribution. This may be related to the high incidence of PCOS in the United States. According to statistics, the incidence of PCOS in the United States is about 5–20%. The annual economic burden caused by PCOS is as high as 3.7 billion dollars (11), and this figure will be much higher if combined with insulin resistance and other long-term complications (12). In addition, it should be noted that the United States, China, Turkey, and other countries with a large number of publications conduct in-depth cooperation to promote the development of this field. Among the authors, Diamanti-Kandarakis E is the author who has published the

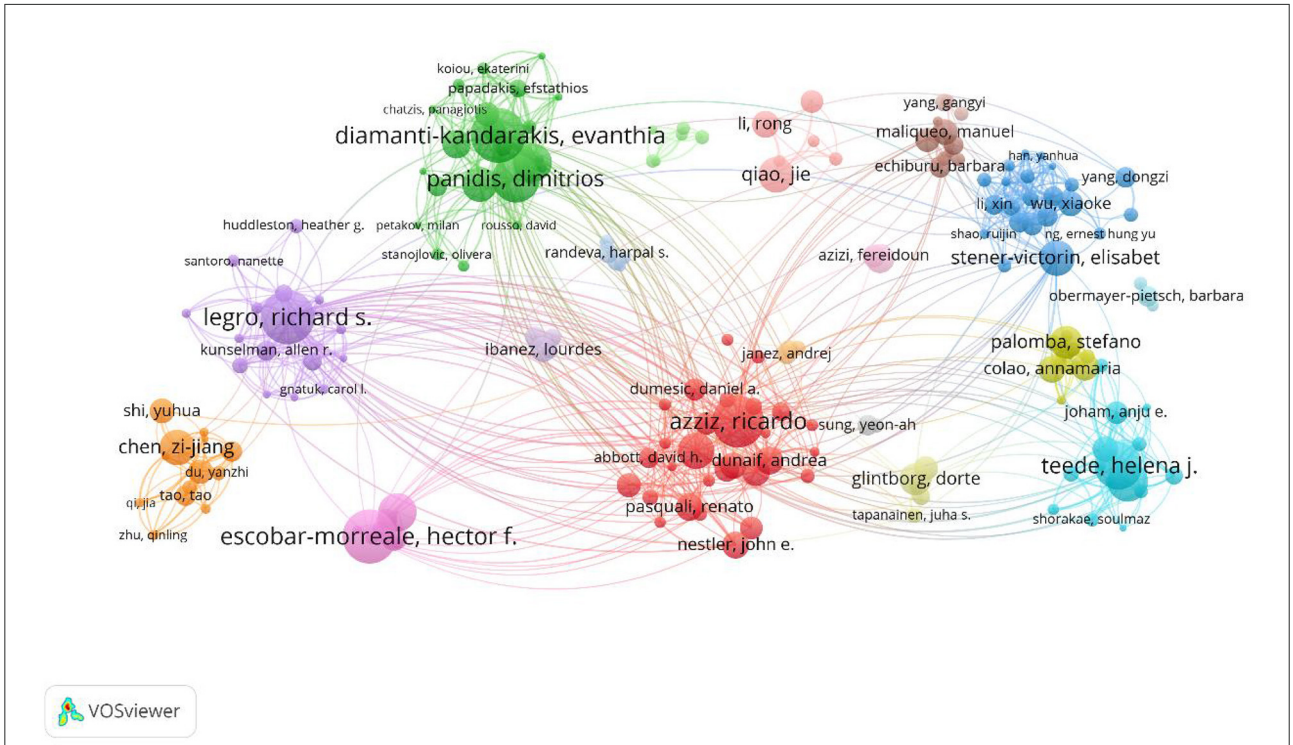

FIGURE 4
Author cooperation visualization map.

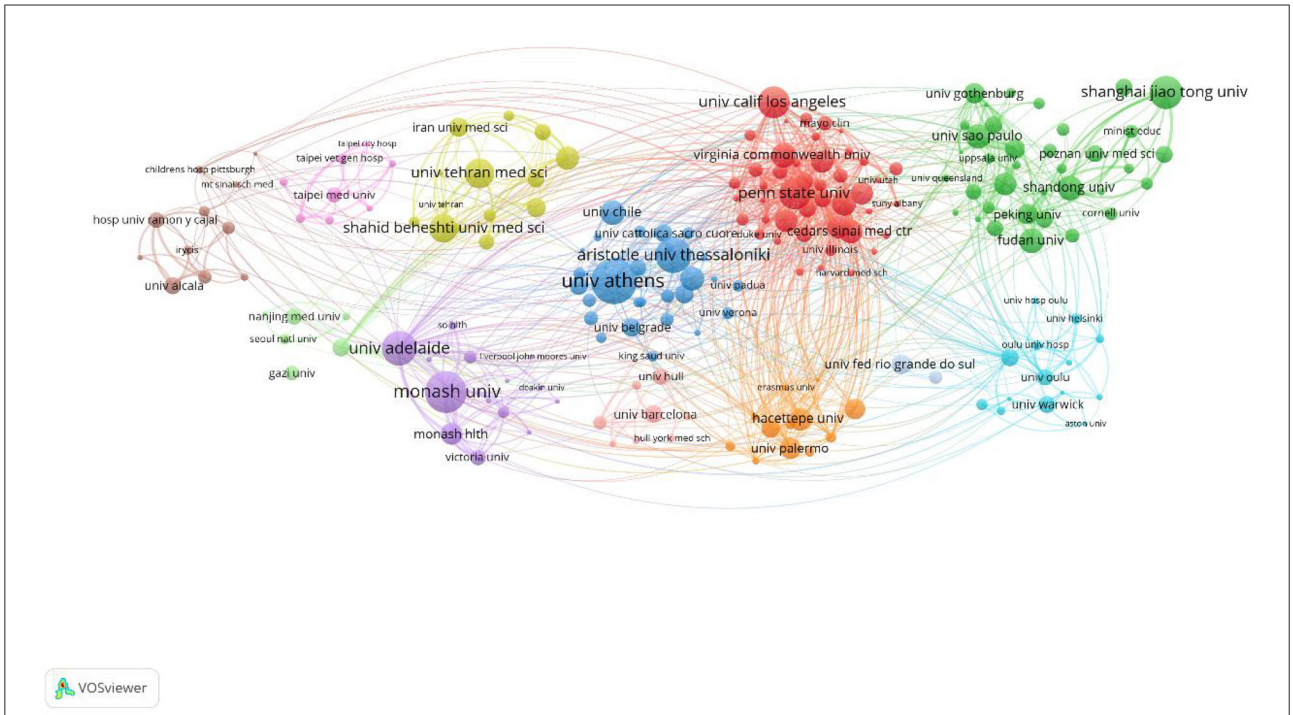

FIGURE 5
Institution cooperation visualization map.

TABLE 1 Top 20 cited references of PCOS IR fields.

Ranking	Counts	Centrality	Publication year	Author	Journal	Vol	Page
1	338	0	2004	Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group	Hum. Reprod	19	41
2	306	0	2004	Chang, J	Fertil. Steril	81	19
3	235	0.02	2012	Diamanti-Kandarakis, E	Endocr. Rev	33	981
4	216	0.02	2013	Legro, R.S	J. Clin. Endocr. Metab	98	4565
5	203	0.06	2012	Fauser, B.C.J.M	Fertil. Steril	97	28
6	191	0	2005	Ehrmann, D.A	New. Engl. j. Med	352	1223
7	180	0	2004	Azziz, R	J. Clin. Endocr. Metab	89	2745
8	179	0.02	2010	Wild, R.A	J. Clin. Endocr. Metab	95	2038
9	168	0	2018	Escobar-Morreale, H.F	Nat. Rev. Endocrinol	14	270
10	164	0.02	2009	Azziz, R	Fertil. Steril	91	456
11	158	0	2016	Azziz, R	Nat. Rev. Dis. Primers	2	16057
12	153	0.03	2016	Rosenfield, R.L	Endocr. Rev	37	467
13	153	0.09	2010	March, W.A	Hum. Reprod	25	544
14	151	0.02	2006	Azziz, R	J. Clin. Endocr. Metab	91	4237
15	147	0	1997	Dunaif, A	Endocr. Rev	18	774
16	147	0.02	2013	Stepto, N.K	Hum. Reprod	28	777
17	146	0.01	2016	Bozdag, G	Hum. Reprod	31	2841
18	141	0.01	2005	Apridonidze T	J. Clin. Endocr. Metab	90	1929
19	140	0.01	1999	Legro, R.S	J. Clin. Endocr. Metab	84	165
20	134	0.01	2011	Goodarzi, M.O	Nat. Rev. Endocrinol	7	219

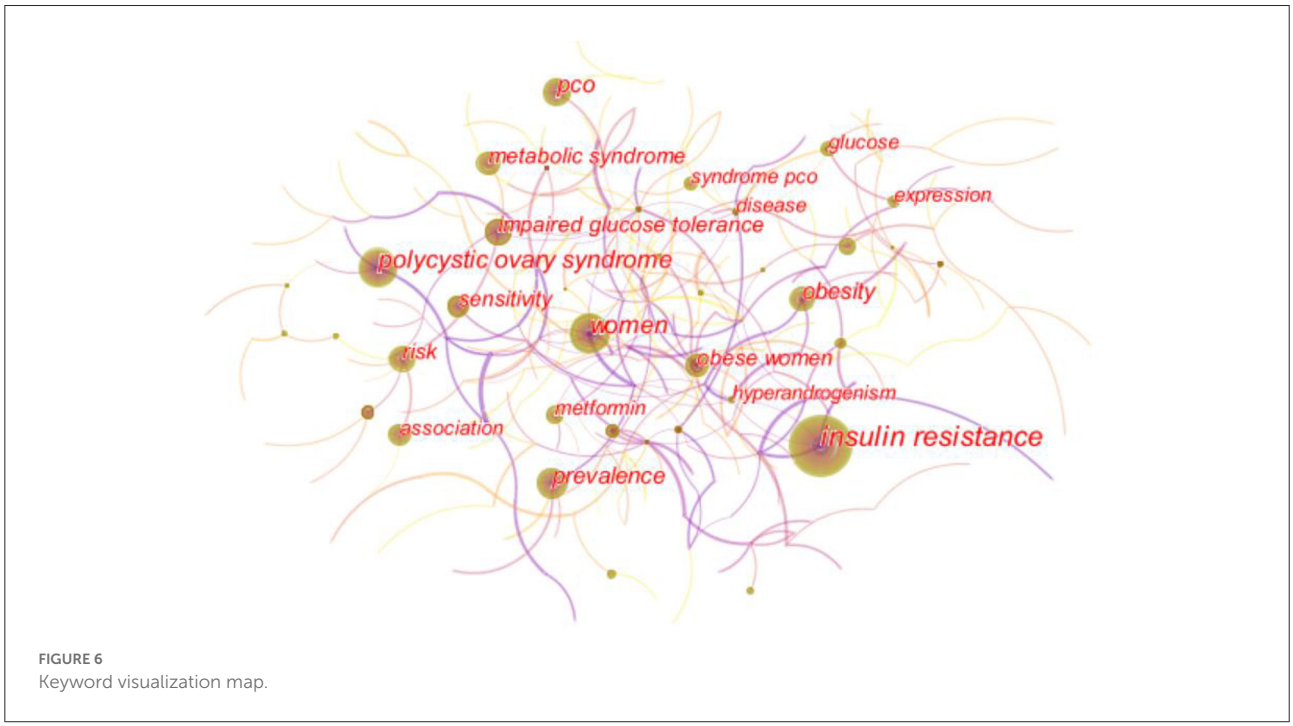

FIGURE 6
Keyword visualization map.

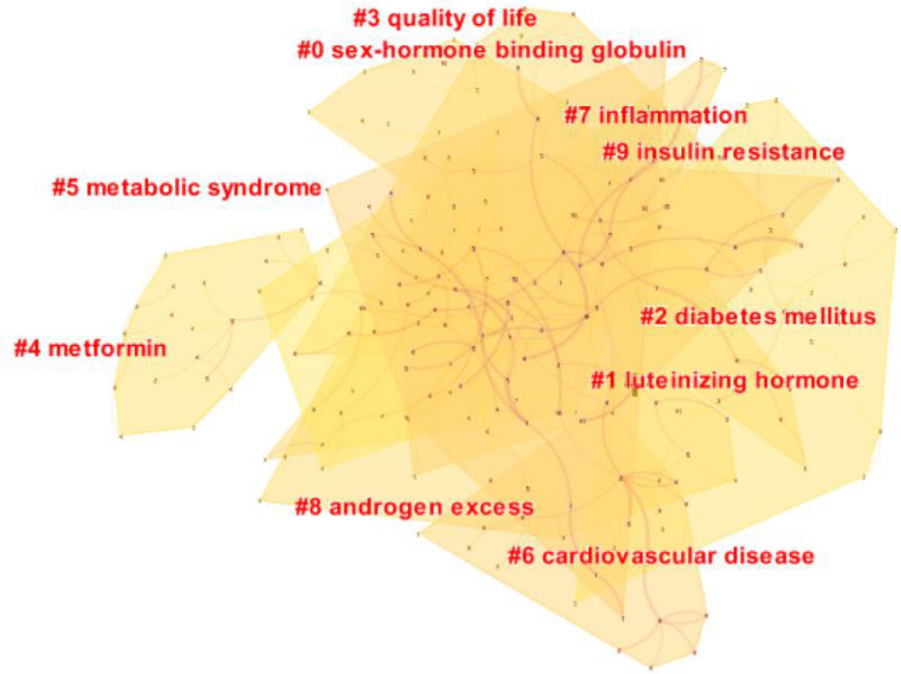

FIGURE 7
Keywords cluster map.

most, while Azziz R has cooperated the most with other author groups and was in the core position. Surprisingly, educational institutions such as universities are more interested in PCOS IR research than hospitals.

Among the top 10 cited works of literature, there are six works of literature about guidelines that were formulated by Rotterdam European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM)-Sponsored PCOS consensus workshop group, Endocrine Society, Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. ESHRE/ASRM group thinks two Oligo—and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries to be diagnostic of PCOS, but the role of IR in PCOS has not been emphasized. Endocrine Society endorses ESHRE/ASRM group's diagnostic criteria for PCOS; the AE-PCOS Society has a different opinion, it thinks clinical and/or biochemical signs of hyperandrogenism are the necessary criterion for diagnosis. In addition, both the AE-PCOS Society and the Endocrine Society pay attention to the role of insulin resistance in PCOS and recommend routine screening of glucose tolerance and early treatment. In addition, the only clinical study with top 10 cited references was published by Azziz R in 2004 (13). This clinical study included 400 women aged 18–45 who participated in pre-employment physical examination; the research found that the cumulative prevalence of PCOS was 6.6%, which provided an epidemiological basis for PCOS.

Keyword analysis showed that the pathological mechanism between PCOS and IR was one of the hot spots of researchers' attention. "Sex-hormone-binding globulin," and "luteinizing hormone" are the top two clusters, representing two common mechanisms of IR increasing PCOS free testosterone level (14, 15): First, insulin receptors in the pituitary gland are triggered to release luteinizing hormone, and second, the synthesis of sex hormone-binding globulin (SHBG) in the liver is inhibited. Increased androgen can also promote the decomposition of adipose tissue, increase the production of free fatty acids and inflammatory factors, and further aggravate IR, causing a vicious cycle (16). According to Figures 6, 8, "Obesity" and "body fat distribution" are also the focus of researchers. Obesity aggravates hyperandrogenemia and insulin resistance, which is the intermediate link between PCOS and IR (17). It is worth noting that thin PCOS is also accompanied by insulin resistance, and the occurrence of IR resistance may be mainly related to visceral fat accumulation (18, 19). According to Figure 8, the heat of "growth factor i" lasted from 1991 to 2006, and the burst intensity was 37.73. The research (20, 21) showed that the serum level of free insulin-like growth factor-1(IGF-1) increases, and insulin-like growth factor-binding protein-1(IGFBP-1) is low in PCOS IR patients; such alternation may drive PCOS follicles to

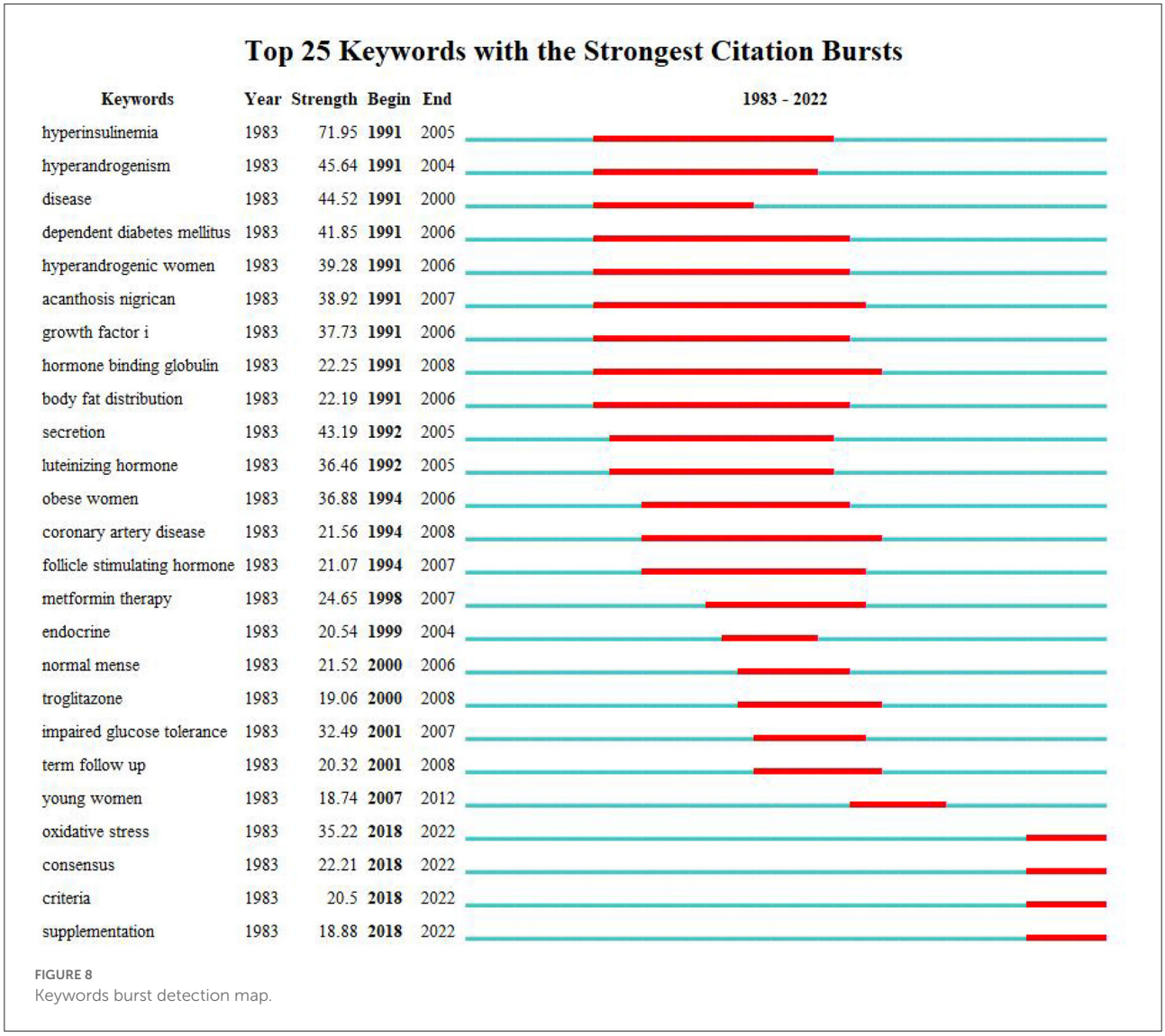

FIGURE 8
Keywords burst detection map.

produce excessive sheath androgen. The heat of "oxidative stress" lasted from 2018 to now, and the burst intensity is 35.22. Oxidative stress is a common pathological mechanism of PCOS and IR. Oxidative stress can reduce glucose uptake in musculoskeletal muscle and insulin secretion in pancreatic beta cells to induce IR (22, 23), increase androgen level (24), destroy follicular microenvironment (25), and promote the progress of PCOS and IR. Researchers found that antioxidant therapy can significantly improve the reproductive metabolic disorder of PCOS (26, 27). Metformin has been recommended for these pathological mechanisms, not only because of its ability to increase insulin sensitivity (28), but also because it increases IGF-1 (21, 29), reduces oxidative stress, and partially restores PCOS metabolic and hormonal disorders. Certainly, the pathological mechanism and treatment of PCOS and IR are still worthy of further exploration.

Metabolic disorders and complications secondary to PCOS IR are another hot topic of researchers' attention. "Risk," "metabolic syndrome," and "impaired glucose tolerance" in Figure 6, and "diabetes mellitus" and "cardiovascular disease" in Figure 7 are all associated with disease risk. Due to the mutual promotion and crosstalk between IR and androgen overload, patients with PCOS IR have significantly increased risks of diabetes and cardiovascular diseases. It is well known that chronic pancreatic stress under IR can cause impaired glucose tolerance and damage to islet β cells, leading to the occurrence of type 2 diabetes (30, 31). Excessive androgen of PCOS can lead to abnormal vasoconstriction and relaxation function (32, 33), resulting in vascular endothelial dysfunction and aggravating the occurrence of cardiovascular diseases. Guidelines developed by some medical associations began to pay attention to the harm caused by PCOS complications, suggesting

screening of glucose tolerance tests, glycated hemoglobin, cardiometabolic risk factors, etc. (34–36). In addition, infertility, irregular menstruation, acne, hirsuteness, obesity, and other manifestations are more common in PCOS IR patients, which makes patients easily complicated with anxiety and depression, resulting in decreased quality of life (37, 38), which should also be given importance by researchers. According to Figure 8, there are also "consensus," "criteria," and "supplementation" that have continued since 2018, which reminds researchers of the urgent need for unified standards and consensus development in the field of PCOS IR.

In conclusion, current research focuses on the pathological mechanism between PCOS and IR and the prevention and treatment of long-term risks. The mechanism of PCOS IR is the mutual promotion of hyperandrogenemia and IR, the increase of IGF-1 and the enhancement of oxidative stress, etc. Among them, oxidative stress may be the focus of future research on the mechanism between PCOS and IR. The long-term complications of PCOS IR include diabetes, cardiovascular disease, metabolic syndrome, etc. Early administration of metformin may have positive effects on the treatment of PCOS and the prevention and treatment of its complications, which requires more rigorous clinical trials. It is still the goal of future research to further explore the mechanism between PCOS and IR and to develop therapeutic drugs that can take into account both reproductive and metabolic disorders and psychological abnormalities of PCOS. In addition, it is very necessary to strengthen the cooperative relationship between authors and regions and develop uniform standards and consensus recognized by the industry, which has also been the focus of research in the recent 4 years.

Through bibliometrics, this paper presents the cooperative relationships among authors, countries, and institutions, as well as research hotspots and trends in the field of PCOS IR research, which provides benefits for many researchers. Researchers should optimize their research according to research hotspots, continue to explore the pathological mechanism of PCOS IR, and make continuous efforts to block the disease progression and related complications of patients, maintain the physical and mental health of patients, and reduce the social and economic burden.

However, some limitations of this article have to be considered. First, Since WoS has the most complete citation information, this study only included works of literature in the WoSCC database, which made the literature collection incomplete. Second, there is no unified standard for CiteSpace and VOSviewer software settings, which may cause some deviations in visual analysis.

Author contributions

TC and YY designed the manuscript. FJ and PL drafted part of the manuscript. XL reviewed the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest

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

Publisher's note

References

1. Mills G, Badeghiesh A, Suarthana E, Baghlaf H. Dahan MH. Associations between polycystic ovary syndrome and adverse obstetric and neonatal outcomes: a population study of 91 million births. *Hum Reprod.* (2020) 35:1914–21. doi: 10.1093/humrep/deaa144

2. Hardiman P, Pillay OC, Atiomo W. Polycystic ovary syndrome and endometrial carcinoma. *Lancet.* (2003) 361:1810–2. doi: 10.1016/S0140-6736(03)13409-5

3. Amisi CA. Markers of insulin resistance in polycystic ovary syndrome women: an update. *World J Diabetes.* (2022) 13:129–49. doi: 10.4239/wjd.v13.i3.129

4. Carmina E, Lobo RA. Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. *Fertil Steril.* (2004) 82:661–5. doi: 10.1016/j.fertnstert.2004.01.041

5. Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN. Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* (2005) 90:66–71. doi: 10.1210/jc.2004-0229

6. Gomez JMD, VanHise K, Stachenfeld N, Chan JL, Merz NB, Shufelt C. Subclinical cardiovascular disease and polycystic ovary syndrome. *Fertil Steril.* (2022) 117:912–23. doi: 10.1016/j.fertnstert.2022.02.028

7. Bogari NM. Genetic construction between polycystic ovarian syndrome and type 2 diabetes. *Saudi J Biol Sci.* (2020) 27:2539–43. doi: 10.1016/j.sjbs.2020.05.004

8. Shan M, Dong Y, Chen J, Su Q, Wan Y. Global Tendency and Frontiers of research on myopia from 1900 to 2020: a bibliometrics analysis. *Front Public Health.* (2022) 10:846601. doi: 10.3389/fpubh.2022.846601

9. Khalil GM. Gotway Crawford CA. A bibliometric analysis of US-based research on the behavioral risk factor surveillance system. *Am J Prev Med.* (2015) 48:50–7. doi: 10.1016/j.amepre.2014.08.021

10. van Eck NJ, Waltman L. Citation-based clustering of publications using CitNetExplorer and VOSviewer. *Scientometrics.* (2017) 111:1053–70. doi: 10.1007/s11192-017-2300-7

11. Riestenberg C, Jagasia A, Markovic D, Buyalos RP, Azziz R. Health care-related economic burden of polycystic ovary syndrome in the United States: pregnancy-related and long-term health consequences. *J Clin Endocrinol Metab.* (2022) 107:575–85. doi: 10.1210/clinem/dgab613

12. Blonde L. Epidemiology, costs, consequences, and pathophysiology of type 2 diabetes: an American epidemic. *Ochsner J.* (2001) 3:126–31.

13. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab.* (2004) 89:2745–9. doi: 10.1210/jc.2003-032046

14. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: relevance to mechanism of anovulation. *J Clin Endocrinol Metab.* (1998) 83:3984–91. doi: 10.1210/jc.83.11.3984

15. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev.* (2015) 36:487–525. doi: 10.1210/er.2015-1018

16. He FF Li YM. Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: a review. *J Ovarian Res.* (2020) 13:73. doi: 10.1186/s13048-020-00670-3

17. Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: etiology, treatment, and genetics. *Metabolism.* (2019) 92:108–20. doi: 10.1016/j.metabol.2018.11.002

18. Satyaraddi A, Cherian KE, Kapoor N, Kunjummen AT, Kamath MS, Thomas N, et al. Body composition, metabolic characteristics, and insulin resistance in obese and nonobese women with polycystic ovary syndrome. *J Hum Reprod Sci.* (2019) 12:78–84. doi: 10.4103/jhrs.JHRS_2_19

19. Romualdi D, Versace V, Tagliaferri V, De Cicco S, Immediata V, Apa R, et al. The resting metabolic rate in women with polycystic ovary syndrome and its relation to the hormonal milieu, insulin metabolism, and body fat distribution: a cohort study. *J Endocrinol Invest.* (2019) 42:1089–97. doi: 10.1007/s40618-019-01029-2

20. Vasiljević M, Prorocić M, Dragojević S, Tasić L, Ganović R, Stanimirović B. [The role of insulin-like growth-factor binding proteins in normal and polycystic ovaries]. *Srp Arh Celok Lek.* (1998) 126:488–94.

21. Berker B, Emral R, Demirel C, Corapcioglu D, Unlu C, Kose K. Increased insulin-like growth factor-I levels in women with polycystic ovary syndrome, and beneficial effects of metformin therapy. *Gynecol Endocrinol.* (2004) 19:125–33. doi: 10.1080/09513590400007309

22. Takeda E, Arai H, Yamamoto H, Okumura H, Taketani Y. Control of oxidative stress and metabolic homeostasis by the suppression of postprandial hyperglycemia. *J Med Invest.* (2005) 52 Suppl: 259–65. doi: 10.2152/jmi.52.259

23. Mancini A, Bruno C, Vergani E. d'Abate C, Giacchi E, Silvestrini A. Oxidative Stress and low-grade inflammation in polycystic ovary syndrome: controversies and new insights. *Int J Mol Sci.* (2021) 22:10. doi: 10.3390/ijms22041667

24. Yilmaz M, Bukan N, Ayvaz G, Karakoç A, Törüner F, Cakir N, et al. The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. *Hum Reprod.* (2005) 20:3333–40. doi: 10.1093/humrep/dei258

25. Naigaonkar A, Dadachanji R, Hinduja I, Mukherjee S. Altered redox status may contribute to aberrant folliculogenesis and poor reproductive outcomes in women with polycystic ovary syndrome. *J Assist Reprod Genet.* (2021) 38:2609–23. doi: 10.1007/s10815-021-02241-x

26. Kazemi M, Lalooha F, Nooshabadi MR, Dashti F, Kavianpour M, Haghighian HK. Randomized double blind clinical trial evaluating the Ellagic acid effects on insulin resistance, oxidative stress and sex hormones levels in women with polycystic ovarian syndrome. *J Ovarian Res.* (2021) 14:100. doi: 10.1186/s13048-021-00849-2

27. Pourteymour Fard Tabrizi F, Hajizadeh-Sharafabad F, Vaezi M, Jafari-Vayghan H, Alizadeh M, Maleki V. Quercetin and polycystic ovary syndrome, current evidence and future directions: a systematic review. *J Ovarian Res.* (2020) 13:11. doi: 10.1186/s13048-020-0616-z

28. Hernández-Jiménez JL, Barrera D, Espinoza-Simón E, González J, Ortíz-Hernández R, Escobar L, et al. Polycystic ovarian syndrome: signs and feedback effects of hyperandrogenism and insulin resistance. *Gynecol Endocrinol.* (2022) 38:2–910. doi: 10.1080/09513590.2021.2003326

29. Pawelczyk L, Spaczynski RZ, Banaszewska B, Duleba AJ. Metformin therapy increases insulin-like growth factor binding protein-1 in hyperinsulinemic women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol.* (2004) 113:209–21310. doi: 10.1016/j.ejogrb.2003.09.031

30. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev.* (2012) 33:981–1030. doi: 10.1210/er.2011-1034

31. Pani A, Gironi I, Di Vieste G, Mion E, Bertuzzi F, Pintaudi B. From prediabetes to type 2 diabetes mellitus in women with polycystic ovary syndrome: lifestyle and pharmacological management. *Int J Endocrinol.* (2020) 2020:6276187. doi: 10.1155/2020/6276187

32. Usselman CW, Yarovinsky TO, Steele FE, Leone CA, Taylor HS, Bender JR, et al. Androgens drive microvascular endothelial dysfunction in women with polycystic ovary syndrome: role of the endothelin B receptor. *J Physiol.* (2019) 597:2853–65. doi: 10.1113/JP277756

33. Alves JV, da Costa RM, Pereira CA, Fedoce AG, Silva CAA, Carneiro FS, et al. Supraphysiological levels of testosterone induce vascular dysfunction via activation of the NLRP3 Inflammasome. *Front Immunol.* (2020) 11:1647. doi: 10.3389/fimmu.2020.01647

34. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab.* (2010) 95:2038–49. doi: 10.1210/jc.2009-2724

35. Dokras A, Saini S, Gibson-Helm M, Schulkin J, Cooney L, Teede H. Gaps in knowledge among physicians regarding diagnostic criteria and management of polycystic ovary syndrome. *Fertil Steril.* (2017) 107:1380–6.e138110. doi: 10.1016/j.fertnstert.2017.04.011

36. Dokras A. Heart health in polycystic ovary syndrome: time to act on the data. *Fertil Steril.* (2022) 117:885–6. doi: 10.1016/j.fertnstert.2022.03.014

37. Gibson-Helm M, Teede H, Dunaif A, Dokras A. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* (2017) 102:604–12.

38. Douglas KM, Fenton AJ, Eggleston K, Porter RJ. Rate of polycystic ovary syndrome in mental health disorders: a systematic review. *Arch Womens Ment Health.* (2022) 25:9–19. doi: 10.1007/s00737-021-01179-4

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Case report: Successful combination therapy with double-filtration plasmapheresis and rituximab under the condition of the use of a sensor-augmented pump for type B insulin resistance syndrome

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Type B insulin resistance syndrome (TBIR) is a rare disease characterized by refractory diabetes due to severe insulin resistance caused by anti-insulin receptor autoantibodies, and a standard treatment regimen for TBIR has not been established, leading to therapeutic difficulties and high mortality. Since TBIR is known to be associated with autoimmune diseases such as systemic lupus erythematosus (SLE), glucocorticoids are often used as key immunosuppressive agents. However, glucocorticoids have the potential to exacerbate the pathophysiology of TBIR by worsening insulin sensitivity, which leads to hyperglycemia and muscle wasting. Here, we report a case history of a 66-year-old man who was diagnosed as having TBIR in combination with SLE and Sjögren's syndrome with marked hyperglycemia, ketosis, and muscle wasting. He was successfully treated with combination therapy of double-filtration plasmapheresis (DFPP) and administration of the anti-CD20 monoclonal antibody rituximab without induction of glucocorticoid therapy while using a sensor-augmented insulin pump (SAP) to prevent hypoglycemia. Remission of diabetes was achieved without severe hypoglycemic events and his circulating insulin receptor antibodies became negative after seven months of initiation of these treatments. Based on the successful clinical courses of this

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case, our report suggests the possibility of an effective therapeutic regimen with DFPP and rituximab under the condition of the use of an SAP for a patient with TBIR without induction of glucocorticoids.

KEYWORDS

type B insulin resistance (TBIR), double-filtration plasma apheresis, rituximab, sensor-augmented pump (SAP), ketosis, catabolism, systemic lupus erythematosus, Sjögren's syndrome

Introduction

Type B insulin resistance syndrome (TBIR) is a disorder that was originally reported by Kahn et al. in 1976 as three cases with marked insulin resistance, melanosis nigricans, and the presence of anti-insulin receptor antibodies in blood (1). TBIR is known to be associated with underlying autoimmune diseases such as systemic lupus erythematosus (SLE), Sjögren's syndrome, various connective tissue diseases, interstitial lung disease, and skin-, blood-, and liver-associated disorders (2). The exact prevalence of TBIR and the involvement of genetic abnormalities remain unknown because of its extreme rarity (3). However, it has been reported that the prognosis is poor if no remission is achieved, resulting in uncontrolled glycemia, muscle wasting and weight loss (4, 5).

A standard treatment for TBIR has not yet been established, although spontaneous remission has been reported in some cases (4). The presence of circulating anti-insulin receptor antibodies underlies the pathogenesis of TBIR, and immunosuppressive therapy is therefore essential for the treatment. Indeed, it has been reported that combination therapy with immunosuppressants such as rituximab, glucocorticoids, cyclophosphamide, and azathioprine contributes to reduction in mortality in patients with TBIR (5). Glucocorticoid is the most commonly used immunosuppressant; however, glucocorticoid can exacerbate insulin resistance, leading to hyperglycemia, which are central to the pathophysiology of TBIR (6). Furthermore, long-term maintenance therapy with glucocorticoids may be needed because most reports have indicated that it takes 3 to 12 months to achieve remission defined as amelioration of hyperglycemia and discontinuation of insulin (5, 7) and even more than 2 years in some cases (8). Glycemic control in the acute phase of TBIR is also important for preventing uncontrolled glycemia-induced complications, muscle wasting, and weight loss. Since it has been reported that insulin-like growth factor 1 (IGF-1) treatment (9) and plasmapheresis (10, 11) were effective for controlling blood glucose levels in patients with TBIR, these treatments may be promising for the acute phase of TBIR until immunosuppressive therapies exert effects. It should be noted that in addition to refractoriness to the treatment for hyperglycemia, hypoglycemia is another important reason why treatment is difficult in patients with TBIR. It has been reported that anti-insulin receptor antibodies can act not only as antagonists at high titers but also as agonists at low titers (3). Therefore, caution is needed for preventing hypoglycemia during the treatment of TBIR, but no method to prevent treatment-induced hypoglycemia with a reduced titer in anti-insulin receptor antibodies has been established.

Considering the above-described facts, the following points are required in a therapeutic strategy for TBIR: 1) immunosuppressive therapies that can achieve rapid remission without worsening insulin resistance, 2) management for glycemia-induced complications such as acute muscle wasting and weight loss by controlling glycemia, and 3) prevention of both hyperglycemia and hypoglycemia events by monitoring blood glucose. Here, we describe a history of a patient with newly diagnosed TBIR with hyperglycemia, hyperinsulinemia and ketosis who was successfully treated with double-filtration plasmapheresis (DFPP) and administration of rituximab while using a recently developed sensor-augmented pump (SAP), which is a device that combines a continuous insulin infusion pump and real-time continuous glucose monitoring (rtCGM). Compared to simple plasma exchange, DFPP, a semi-selective blood-purification modality derived from plasma exchange, has advantages in selectively removing immunoglobulin fractions, minimizing substitution fluid such as fresh frozen plasma or albumin solution, and reducing cost (12). Since fresh frozen plasma, the major substitution fluid in simple plasma exchange, is known to contain high concentrations of glucose (13), we hypothesized that DFPP would be more suitable for the treatment of TBIR than simple plasma exchange. Notably, there was no need to use glucocorticoid, which can exacerbate insulin resistance, until the TBIR was in remission in this case. Our treatment regimen used in this patient may open the way for the establishment of an effective therapeutic strategy for TBIR.

Case report

A 66-year-old Japanese man was referred to Sapporo Medical University Hospital for treatment of newly developed TBIR. He had suffered from discoid lupus with leucopenia, hypocomplementemia, positive anti-nuclear antibody and anti-Smith antibody. He was diagnosed with SLE at the age of 48 years and had been stable on only topical therapy for discoid lupus. At the age of 65 years, his regular medical checkup did not reveal any glucose intolerance. However, six months later, he developed both polyuria and thirst and visited his local doctor. He was diagnosed as having diabetes mellitus with blood glucose of 300 mg/dl and HbA1c of 9.1%, and he was admitted to his local hospital. He was initially treated with metformin and multiple daily injections of insulin, but his hyperglycemia did not improve even with a total insulin dose of 120 units/day (insulin degludec of 30 units and insulin aspart of 30 units per each meal). Addition of sulfonylureas and dulaglutide, a weekly glucagon-like peptide 1 (GLP-1) receptor agonist, at 0.75 mg/week also did not improve his hyperglycemia. In addition to marked elevation in fasting serum insulin and C-peptide levels, serum anti-insulin receptor antibody test was positive, leading to the diagnosis of TBIR. He was transferred to our hospital for further treatment of TBIR.

On admission, his body weight was 70.0 kg, body mass index (BMI) was 24.2 kg/m^2, blood pressure was 85/64 mmHg, pulse rate was 107 bpm, and body temperature was 36.1°C. Since he was suffering from hyperglycemia and anorexia, decreased blood pressure with tachycardia on admission was considered to be due to intravascular dehydration caused by osmotic diuresis. There was no skin rash including melanosis nigricans, erythema spheroids or discoid lupus. Achilles tendon reflex was not decreased, but bilateral lower extremity vibratory sensation was decreased. Results of laboratory tests on admission revealed severe insulin resistance and ketosis: fasting blood glucose level was 225 mg/dl, fasting serum insulin level was 455 μIU/ml, fasting serum C-peptide level was 5.45 ng/ml, and β-hydroxybutyrate level was 1.8 mM (Table 1). The activity of SLE was assessed by Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) (14) and it was 4 points for hypocomplementemia (2 points), thrombocytopenia (1 point), and leukopenia (1 point). In addition, serum anti-Sjögren's-syndrome-related antigen A (anti-SS-A) and B (anti-SS-B) antibodies were positive and the Schirmer test was positive (right 3 mm/5 min, left 1 mm/5 min) together with positive ocular surface staining by fluorescein, indicating a new diagnosis of Sjögren's syndrome (15). There was no diabetic retinopathy on his eyes. Since pancytopenia was observed, bone marrow aspirations and biopsies were performed and revealed fatty marrow, resulting in a diagnosis of pancytopenia secondary to autoimmune diseases. No malignancy was found in his body.

His clinical course after admission is shown in Figure 1A. Results of intermittently-scanned CGM (isCGM, FreeStyle Libre, Abbott Diabetes Care Inc., CA, U.S.A.) revealed severe hyperglycemia throughout the day without evidence of hypoglycemia (Figure 2A). In addition to persistent ketosis, his body weight was progressively reduced, suggesting catabolic state. Therefore, we decided to perform combination therapy with DFPP (twice a week) and infusion of the anti-CD20 antibody rituximab (375 mg/m^2, once weekly for 4 weeks) as previously reported (16), followed by administration of hydroxychloroquine for reducing SLE activity. Furthermore, an SAP (MiniMed 640G system, Medtronic, Dublin, Ireland), which can detect rapid decline in interstitial glucose levels, was introduced instead of isCGM (Figure 2B) due to early detection of hypoglycemic event. Following these combined treatment strategies, hyperglycemia and ketosis improved, and he was discharged on his 71st admission day. Thereafter, his HbA1c gradually improved and no severe hypoglycemia occurred due to glycemic monitoring and fine-tuning of insulin dosage using an SAP. Additional rituximab administration (375 mg/m^2/week, 1 time) was performed six months after initial infusion. Anti-insulin receptor antibodies became negative after seven months of initiation of the therapy. Representative glucose profiling nine months after these treatments is shown in Figure 2C. Soft lean mass evaluated by bioelectrical impedance analysis was increased from 46.5 kg at baseline to 50.7 kg after his anti-insulin receptor antibodies became negative, suggesting an improvement in the catabolic state (Figure 1B). Platelets counts and hypocomplementemia were also recovered with an improvement of glycemic control and SLEDAI-2K became 1 point seven months after the initiation of therapy (Figure 1B). There were no remarkable adverse events including infusion reaction, opportunistic infections, severe infections, gastrointestinal symptoms, retinopathy and hemorrhagic events throughout his clinical course.

Discussion

There are two salient points in this report: 1) A combination therapy including DFPP and rituximab, followed by hydroxychloroquine administration, led to successful remission of TBIR without use of glucocorticoid therapy in a TBIR patient complicated with SLE and Sjögren's syndrome. 2) SAP was useful to the monitoring and control of glucose levels during aggressive TBIR therapy.

Since the patient had progressive weight loss and muscle wasting, DFPP was used as an acute-phase therapy to prevent catabolism by restoring insulin sensitivity *via* removal of anti-insulin receptor antibodies. Actually, DFPP has been applied to various conditions including autoimmune diseases and organ transplantations (12, 17, 18). Since TBIR has been reported to be

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TABLE 1 Laboratory findings on admission.

<Complete blood count>			<Diabetes-related and Endocrinology measurements>			<Immunological tests>		
WBC	2200	/μL	β-hydroxybutyrate	1.8	μM	CRP	<0.10	mg/dL
Neutrocyte	68	%	Glucose	226	mg/dL	IgG	1843	mg/dL
Lymphocyte	20	%	HbA1c	10.8	%	IgA	435	mg/dL
Monocyte	5	%	Insulin	452.7	μIU/mL	IgM	44	mg/dL
Eosinocyte	5	%	C-peptide	5.45	mg/dL	C3	57	mg/dL
Basocyte	2	%	HOMA-IR	252.6		C4	12	mg/dL
RBC	400	$x10^4$/μL	HOMA-β	999	%	CH50	38	/mL
Hb	12.1	g/dL	C-peptide index	2.41		ANA	160	folds
Ht	36.3	%	Anti-GAD antibody	(-)	U/mL	RF	(-)	
Plt	9.8	$x10^4$/μL	Anti-IA-2 antibody	(-)	U/mL			
<Biochemistry measurements>			Anti-insulin antibody	(-)	%	Anti-dsDNA antibody	(-)	IU/mL
TP	7.0	g/dL	Anti-insulin receptor antibody	(+)		Anti-SS-A antibody **	16	folds
Alb	3.6	g/dL				Anti-SS-B antibody **	8	folds
T-bil	0.7	mg/dL	TC	145	mg/dL	Anti-CCP antibody	0.7	U/mL
AST	20	IU	TG	40	mg/dL	Anti-Sm antibody **	(-)	folds
ALT	18	IU	HDL-C	47	mg/dL	Anti-RNPantibody **	(-)	folds
ALP*	80	IU	LDL-C	90	mg/dL	Anti-CL IgG antibody	< 2.6	IK/mL
LDH	151	IU	TSH	1.42	μIU/mL	Anti-CL IgM antibody	2.2	IJ/mL
BUN	13	mg/dL	FT3	2.4	pg/mL	Anti-β2GPI IgG antibody	12.6	IJ/mL
Cr	0.5	mg/dL	FT4	1.22	ng/dL	Anti-β2GPI IgM antibody	< 1.1	IJ/mL
UA	4.0	mg/dL	ACTH	13.4	pg/mL	PA-IgG	48.6	ng/10^7cells
Na	137	mEq/L	Cortisol	9.76	μg/dL	Lupus anticoagulant test	(-)	
K	3.8	mEq/L				Urine anti-HP antibody	(+)	

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; TP, total protein; Alb, albumin; T-bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment - Insulin Resistance; HOMA-β, Homeostasis Model Assessment for β-cell function; Anti-GAD antibody, anti glutamic acid decarboxylase antibody; Anti-IA-2 antibody, anti islet antigen 2 antibody; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol-cholesterol; TSH, thyroid-stimulating hormone; FT3, free thyroxine 3; FT4, free thyroxine 4; ACTH, adrenocorticotropic hormone; CRP, C-reactive protein; ANA, antinuclear antibody; RF, rheumatoid factor; Anti-dsDNA antibody, double-stranded DNA antibody; Anti-SS-A antibody, anti Sjögren's-syndrome-related antigen A; Anti-SS-B antibody, anti Sjögren's-syndrome-related antigen B; Anti-CCP antibody, anti-cyclic citrullinated peptide antibody; Anti-RNP antibody, anti-ribonucleoprotein antibody; Anti-Sm antibody, anti-Smith antibody; Anti-CL antibody, anti-caldiolipin antibody; Anti-β2GPI antibody, anti-β2 glycoprotein I antibody; PA-IgG, platelet-associated IgG; Urine anti-HP antibody, urine anti-Helicobacter pylori antibody.*, Tested by Japan Society of Clinical Chemistry method. **, Tested by Ouchterlony method.

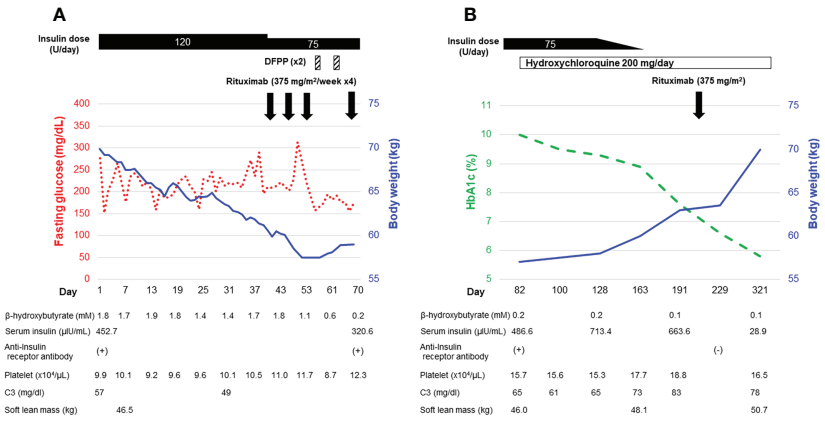

FIGURE 1
Clinical course of the patient during hospitalization **(A)** and in the outpatient setting **(B)**.

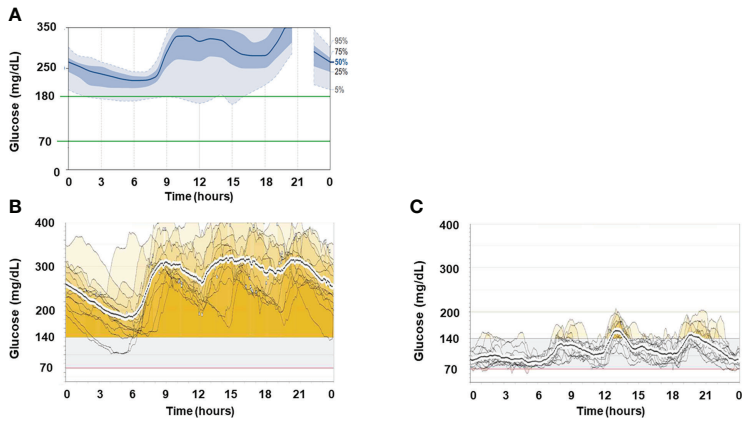

FIGURE 2
Representative glucose profiles of CGM recordings. **(A)** Representative ambulatory glucose profile recorded by isCGM before treatment. **(B)** Two-week glucose profiles recorded by rtCGM one month after the initiation of treatment. **(C)** Two-week glucose profiles recorded by rtCGM nine months after the initiation of treatment.

an autoimmune disorder caused by polyclonal autoantibodies (3), mechanical removal of such autoantibodies using DFPP is a reasonable therapeutic approach. Indeed, some previous case reports have shown rapid improvement in hyperglycemia after plasmapheresis, suggesting the effectiveness in this acute phase treatment (10, 11). Since albumin solution, rather than fresh frozen plasma containing high concentrations of glucose (13), is usually used as the substitution fluid in DFPP, DFPP might be an optimal plasmapheresis for the treatment of TBIR, which is characterized by severe insulin resistance. In contrast, we acknowledge that DFPP has a possible disadvantage in loss of high molecular weight coagulation factors that can lead to hemorrhagic events (19). To avoid this side effect of DFPP, we took the following actions in the present case: 1) levels of serum fibrinogen, one of the high molecular weight coagulation factors, were measured before DFPP and 2) only two DFPP sessions were performed. Fortunately, in the present case, the levels of fibrinogen never reached below 100 mg/dl, which we had set as a criterion for discontinuation of DFPP. In addition, no hemorrhagic events were found throughout his clinical course. Thus, monitoring serum fibrinogen levels and performing fewer DFPP sessions may lead to early detection or prevention of coagulation factor depletion in DFPP, thereby preventing hemorrhagic events.

One interesting aspect of this case is that blood β-hydroxybutyrate became negative despite persistent hyperglycemia after treatment with DFPP (Figure 1). Although the precise reason for the differential effects of DFPP on blood glucose and ketone metabolism in TBIR remains unknown, insulin signaling is known to be different in organs or types of cells including adipocytes, hepatocytes, and myocytes (20) and it is possible that the temporary decrease in anti-insulin receptor antibodies caused by DFPP preferentially improved insulin signaling only in adipocytes or hepatocytes. It should also be noted that DFPP does not suppress the production of anti-insulin receptor antibodies from abnormal B cells; therefore, its efficacy is temporal. Taken together, DFPP may be specifically effective for patients with TBIR who are suffering from extremely high insulin resistance and progressive catabolism.

As an alternative to DFPP, treatment with IGF-1 has been reported to be an option for acute treatment of TBIR (9). IGF-1 can promote glucose uptake into cells *via* binding to IGF receptors or insulin receptors. Thus, administration of recombinant IGF-1 could theoretically activate intracellular glucose uptake pathways *via* an alternative pathway rather than insulin signaling that is inhibited by the anti-insulin receptor antibody. However, a recent report indicated that the effectiveness of IGF-1 for TBIR may be limited (21). This limitation is due to the possibility that anti-insulin receptor antibodies also have an affinity for the IGF-1 receptor (22) or that IGF-1 itself is not effective for removing the anti-insulin receptor antibodies that are the causative molecules of TBIR. Of course, IGF-1 treatment may contribute to better outcomes for TBIR by improving hyperglycemia, but we thought that DFPP was likely to surpass the beneficial effects of administration of recombinant IGF-1, and we chose DFPP as the treatment of choice for the acute phase in the present case.

One of the important factors contributing to the successful outcome of this case was that the patient achieved remission with negative anti-insulin receptor antibody by rituximab without glucocorticoid therapy. The effects of rituximab for TBIR are controversial (7, 23, 24) and rituximab was administered with concomitant use of glucocorticoids in previous cases. In general, rituximab takes several weeks after administration to exert its effect for suppression of antibody production. Rapid remission induction is important in

autoimmune diseases such as anti-neutrophil cytoplasmic antibody-associated vasculitis or immune thrombocytopenic purpura, and concomitant use of glucocorticoids and rituximab is needed to achieve rapid remission induction. In our case, we used DFPP instead of IGF-1 or glucocorticoids to avoid acute phase complications and it worked well in our case. Other immunosuppressants such as cyclophosphamide have also been reported to be effective for TBIR, but glucocorticoids were used in combination in those cases (7). In this case, administration of cyclophosphamide was avoided due to pancytopenia. It is still unclear if hydroxychloroquine improved TBIR as an immunomodulatory drug; however, hydroxychloroquine was added as a standard-of-care treatment for SLE.

Autoantigens that may trigger the development of TBIR also remain unknown. Imai et al. previously reported the association between TBIR and *Helicobacter pylori* infection (25). Indeed, the patient's urine anti-*Helicobacter pylori* antibody was positive on admission (Table 1), although he achieved remission without *Helicobacter pylori* eradication treatment. Additionally, systemic autoimmune diseases such as SLE and Sjögren's syndrome are known to be associated with TBIR. In the present case as well, the patient was complicated with SLE and Sjögren's syndrome. The disease severity of SLE was mild at the onset of TBIR, but platelet counts and hypocomplementemia recovered along with a decrease in the titer of anti-insulin receptor antibodies, suggesting that TBIR may be associated with concomitant autoimmune diseases. Since TBIR is an extremely rare disease, its detailed etiology remains unknown. Further studies will be needed to clarify the detailed pathogenesis of TBIR.

The pattern of glycemic variability in TBIR has been reported to include only hyperglycemia and a mixture of hyperglycemia and hypoglycemia, although a recent report recommended that a case showing hypoglycemia alone should be excluded from the diagnosis of TBIR (26). Hyperinsulinemia induced by increased endogenous insulin secretion in response to severe insulin resistance is elicited in most cases of TBIR. Thus, the development of significant hypoglycemia, whether treatment-related or not, requires careful attention. Therefore, an SAP may be the best tool for both treating TBIR and monitoring blood glucose to prevent hypoglycemia as it allows adjustment of insulin dosing while monitoring blood glucose with rtCGM. In this case, because of increased endogenous insulin secretion due to consistent severe insulin resistance, the dose of insulin infusion was not increased according to blood glucose levels, and a strategy that prioritizes the avoidance of hypoglycemia was chosen. Whether increasing doses of insulin infusion or using a closed-loop insulin delivery system (27) such as a system that optimizes insulin doses according to blood glucose would result in better outcomes in TBIR is unknown and requires further studies.

In summary, we described a case history of a patient with TBIR who presented hyperglycemia, hyperinsulinemia, and ketosis and who was successfully treated with an SAP in combination with DFPP and rituximab. Our therapeutic approach may provide an effective and novel treatment strategy that could become a new insight for the care of TBIR.

Ethics statements

Ethical review and approval was not required because this is not a clinical study but a case report in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

Author contributions

AO, MK, and TS designed the structure of the manuscript and wrote the original manuscript. AO and TS prepared figures and tables. CA, SB, HK, and KM analyzed the patient data and revised the manuscript. TomY and TosY supervised the patient's clinical course and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

TS: Honoraria (lecture fee) from Abbott Japan LLC., Novo Nordisk Pharma Ltd.

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References

1. Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM, et al. The syndromes of insulin resistance and acanthosis nigricans. insulin-receptor disorders in man. *N Engl J Med* (1976) 294:739–45. doi: 10.1056/NEJM197604012941401

2. Martins LM, Fernandes VO, Carvalho MMD, Gadelha DD, Queiroz PC, Montenegro Junior RM. Type b insulin resistance syndrome: a systematic review. *Arch Endocrinol Metab* (2020) 64:337–48. doi: 10.20945/2359-3997000000257

3. Angelidi AM, Filippaios A, Mantzoros CS. Severe insulin resistance syndromes. *J Clin Invest* (2021) 131:e142245. doi: 10.1172/JCI142245.s

4. Arioglu E, Andewelt A, Diabo C, Bell M, Taylor SI, Gorden P. Clinical course of the syndrome of autoantibodies to the insulin rece.ptor (type b insulin resistance): a 28-year perspective. *Med (Baltimore)* (2002) 81:87–100. doi: 10.1097/00005792-200203000-00001

5. Klubo-Gwiezdzinska J, Lange M, Cochran E, Semple RK, Gewert C, Brown RJ, et al. Combined immunosuppressive therapy induces remission in patients with severe type b insulin resistance: A prospective cohort study. *Diabetes Care* (2018) 41:2353–60. doi: 10.2337/dc18-0884

6. Li JX, Cummins CL. Fresh insights into glucocorticoid-induced diabetes mellitus and new therapeutic directions. *Nat Rev Endocrinol* (2022) 18:1–18. doi: 10.1038/s41574-022-00683-6

7. Iseri K, Iyoda M, Shikida Y, Inokuchi T, Morikawa T, Hara N, et al. Rituximab for the treatment of type b insulin resistance syndrome: a case report and review of the literature. *Diabetes Med* (2017) 34:1788–91. doi: 10.1111/dme.13524

8. Malek R, Chong AY, Lupsa BC, Lungu AO, Cochran EK, Soos MA, et al. Treatment of type b insulin resistance: a novel approach to reduce insulin receptor autoantibodies. *J Clin Endocrinol Metab* (2010) 95:3641–7. doi: 10.1210/jc.2010-0167

9. Hirano T, Adachi M. Insulin-like growth factor 1 therapy for type b insulin resistance. *Ann Intern Med* (1997) 127:245–6. doi: 10.7326/0003-4819-127-3-199708010-00024

10. Eriksson JW, Bremell T, Eliasson B, Fowelin J, Fredriksson L, Yu ZW. Successful treatment with plasmapheresis, cyclophosphamide, and cyclosporin a in type b syndrome of insulin resistance. case report. *Diabetes Care* (1998) 21 (8):1217–20. doi: 10.2337/diacare.21.8.1217

11. Page KA, Dejardin S, Kahn CR, Kulkarni RN, Herold KC, Inzucchi SE. A patient with type b insulin resistance syndrome, responsive to immune therapy. *Nat Clin Pract Endocrinol Metab* (2007) 3:835–40. doi: 10.1038/ncpendmet0693

12. Hirano R, Namazuda K, Hirata N. Double filtration plasmapheresis: Review of current clinical applications. *Ther Apher Dial* (2021) 25:145–51. doi: 10.1111/1744-9987.13548

13. Ewalenko P, Deloof T, Peeters J. Composition of fresh frozen plasma. *Crit Care Med* (1986) 14:145–6. doi: 10.1097/00003246-198602000-00015

14. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* (2002) 29:288–91.

15. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American college of Rheumatology/European league against rheumatism classification criteria for primary sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* (2017) 76:9–16. doi: 10.1136/annrheumdis-2016-210571

16. Coll AP, Thomas S, Mufti GJ. Rituximab therapy for the type b syndrome of severe insulin resistance. *N Engl J Med* (2004) 350:310–1. doi: 10.1056/NEJM200401153500324

17. Choi AY, Manook M, Olaso D, Ezekian B, Park J, Freischlag K, et al. Emerging new approaches in desensitization: Targeted therapies for HLA sensitization. *Front Immunol* (2021) 12:694763. doi: 10.3389/fimmu.2021.694763

18. Tanabe K. Double-filtration plasmapheresis. *Transplantation* (2007) 84: S30–2. doi: 10.1097/01.tp.0000296103.34735.b8

19. Seishima M, Shibuya Y, Kato G, Aoki T. Decreased factor XIII activity in a patient with subcutaneous bleeding after double filtration plasmapheresis. *Ther Apher Dial* (2009) 13:229–31. doi: 10.1111/j.1744-9987.2009.00688.x

20. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev* (2018) 98:2133–223. doi: 10.1152/physrev.00063.2017

21. Hirota Y, Suwanai H, Yamauchi T, Kadowaki T. Clinical features of type b insulin resistance in Japanese patients: Case report and survey-based case series study. *J Diabetes Res* (2020) 2020, 2020:4359787. doi: 10.1155/2020/4359787

22. Soos MA, Siddle K. Immunological relationships between receptors for insulin and insulin-like growth factor i. evidence for structural heterogeneity of insulin-like growth factor I receptors involving hybrids with insulin receptors. *Biochem J* (1989) 263:553–63. doi: 10.1042/bj2630553

23. Takei M, Ishii H, Kawai Y, Kato K, Sekido T, Sato Y, et al. Efficacy of oral glucocorticoid and cyclosporine in a case of rituximab-refractory type b insulin resistance syndrome. *J Diabetes Investig* (2015) 6:734–8. doi: 10.1111/jdi.12337

24. Concepción-Zavaleta MJ, Ildefonso-Najarro SP, Plasencia-Dueñas EA, Quispe-Flores MA, Armas-Flórez CD, Luna-Victorio LE. Successful remission of type b insulin resistance syndrome without rituximab in an elderly male. *Endocrinol Diabetes Metab Case Rep* (2020) 2020:20–0110. doi: 10.1530/EDM-20-0110

25. Imai J, Yamada T, Saito T, Ishigaki Y, Hinokio Y, Kotake H, et al. Eradication of insulin resistance. *Lancet* (2009) 374:264. doi: 10.1016/S0140-6736(09)60872-2

26. Ogawa W, Araki E, Ishigaki Y, Hirota Y, Maegawa H, Yamauchi T, et al. New classification and diagnostic criteria for insulin resistance syndrome. *Diabetol Int* (2022) 13:337–43. doi: 10.1007/s13340-022-00570-5

27. Sophie T. Closed-loop insulin delivery systems: Past, present, and future directions. *Front Endocrinol* (2022) 13:919942. doi: 10.3389/fendo.2022.919942 Available at: https://www.frontiersin.org/articles/10.3389/fendo.2022.919942/full.

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Complex impacts of gallstone disease on metabolic syndrome and nonalcoholic fatty liver disease

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Background: Patients with gallstone disease (GSD) often have highly co-occurrence with metabolic syndrome (MetS) and Nonalcoholic fatty liver disease (NAFLD) both associated with insulin resistance (IR). Meanwhile, highly prevalence of NAFLD was found in patients who received cholecystectomy. However, the associations of GSD with MetS, NAFLD is inconsistent in the published literature. And risk of cholecystectomy on NAFLD is unclear.

Methods: We searched the Medline EMBASE and WOS databases for literature that met our study topic. To be specific, studies with focus on associations between GSD and MetS/NAFLD, and risk evaluation on cholecystectomy and NAFLD incidence were enrolled for further analysis. The random effect model was used to calculate the combined relative ratio (RR) and odds ratio (OR)and 95% confidence interval (CI).

Results: Seven and six papers with focus on connections between GSD and NAFLD/MetS prevalence. Correspondingly, seven papers with focus on risk of cholecystectomy on NAFLD occurrence were also enrolled into meta-analysis. After pooling the results from individual study, patients with GSD had higher risk of MetS (OR:1.45, 95%CI: 1.23-1.67, I^2 = 41.1%, P=0.165). Risk of GSD was increased by 52% in NAFLD patients (pooled OR:1.52, 95%CI:1.24-1.80). And about 32% of increment on NAFLD prevalence was observed in patients with GSD (pooled OR: 1.32, 95%CI:1.14-1.50). With regard to individual MetS components, patients with higher systolic blood pressure were more prone to develop GSD, with combined SMD of 0.29 (96%CI: 0.24-0.34, P<0.05). Dose-response analysis found the GSD incidence was significantly associated with increased body mass index (BMI) (pooled OR: 1.02, 95%CI:1.01-1.03) in linear trends. Patients who received cholecystectomy had a higher risk of post-operative NAFLD (OR:2.14, 95%CI: 1.43-2.85), P<0.05). And this impact was amplified in obese patients (OR: 2.51, 95%CI: 1.95-3.06, P<0.05).

Conclusion: Our results confirmed that controls on weight and blood pressure might be candidate therapeutic strategy for GSD prevention. And concerns should be raised on *de-novo* NAFLD after cholecystectomy.

KEYWORDS

gallstone disease, metabolic syndrome, nonalcoholic fatty liver disease, cholecystectomy, insulin resistance, meta-analysis

1 Introduction

Gallstone disease (GSD) is a significant burden in health care around the world (1). GSD is the second largest digestive disease after gastroesophageal reflux disease in the United States (2). GSD caused great pain to adults (3). Although the incidence was much higher than that of children, it tended to be younger (4). Its incidence is also high in the worldwide population with a prevalence of 5-25% in Westerners (5) and 3-15% in Asians (6). In spite of lower mortality, much payment should be listed from medical insurance for hospitalization and treatment for GSD patients (7). Cholecystectomy is the most common surgical procedure for the treatment of cholelithiasis and its complications in the world, where laparoscopic surgery was used in about 90% of cases (5). Risk factors for GSD such as cholecystitis (acute/chronic), symptomatic cholelithiasis, biliary dyskinesia, acalculous cholecystitis, gallstone pancreatitis and gallbladder masses/polyps can be treated by cholecystectomy (8). In addition to common bile duct injury, bile leakage (9), bleeding, indigestion and vague non-colic abdominal pain (10), cholecystectomy can further cause a series of metabolic changes such as increased serum triglyceride, rising very-low-density-lipoprotein levels (11, 12) and metabolic syndrome in cardiovascular diseases like type 2 diabetes and hypertension.

Clinically, insulin resistance (IR) is defined as the inability of insulin to keep blood glucose levels in a healthy range (13). However, apart from regulating glucose metabolism, insulin was also involved in other metabolic activities in the body (14). IR played a crucial role in metabolic disorders such as metabolic syndrome (MetS) and hepatic steatosis (14, 15). MetS and GSD have common risk factors, and the greatest correlation is abdominal obesity and insulin resistance (16). Nonalcoholic fatty liver disease (NAFLD) represents an excessive accumulation of adipocytes in the liver as presentation of IR in liver. It often coexisted with GSD (17). Current research showed that insulin resistance and GSD can influence each other (18). That is, IR promoted GSD, and GSD in turn aggravated IR (18). Results from large cohort of non-diabetic Korean men found systemic IR as independent predictor for GSD (19). The most important way insulin resistance affected GSD was to disrupt the metabolism of cholesterol in the body (20). A study shown that both MetS and NAFLD can accelerate the increase of cholesterol synthesis in the body, and the excessive secretion of bile cholesterol was related to the increase of bile lithogenicity (20).

Conversely, systemic glucose and lipid metabolism can be regulated by gallbladder (21). The gallbladder helps to maintain glucose, lipids and homeostasis (21). When GSD occurred, cholesterol in bile was increased with lowered phospholipids and bile acid (21). Both cholecystectomy and GSD had adverse effects on insulin sensitivity (22). Moreover, there were persistent defects in the regulation of liver lipid metabolism in patients undergoing cholecystectomy (22). Therefore, cholecystectomy particularly influenced the occurrence and development of NAFLD.

In view of the tight relationship between GSD and metabolic derangements, many studies were performed with topics on associations between GSD and MetS/NAFLD occurrence (23–36). Otherwise, the impacts of cholecystectomy on post-operative NAFLD were also assessed in previous studies (12, 31, 37–41). However, the above relationships were still controversial with difference across individual studies. Several EBM papers were published to illustrate the associations between GSD and metabolic derangements (42, 43). After careful evaluation, we found several defects for these reviews. To be specific, in literature by Veeravich (42) and Jiang (43) etc, authors only calculated the quantitative correlations between GSD and NAFLD/MetS without considerations on direction of these two covariates, which can't avoid potential bias inevitably. Otherwise, Jiang et al. (42) only referred patients with higher BMI had higher susceptibility to develop GSD. But in-depth dose-response analysis was not performed in prior EBM study to illustrate the continuous effects of BMI variations on GSD incidence. Hence, to timely update assessment, literature

Abbreviations: BMI, body mass index; CDS, Chinese Diabetes Society; CI, confidence interval; FFA, free fatty acids; FXR, farnesoid X receptor; GB, gallbladder; GSD, gallstone disease; HR: hazard ratio; IR, insulin resistance; WOS, Web of Science; MetS: metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Group III; NOS, Newcastle-Ottawa Scale; OR, odds ratio; RR, risk ratio; SMD, standardized mean differences; T2DM, type 2 diabetes mellitus; USG, Ultrasonogram.

involved on GSD and NAFLD/MetS need to be categorized by directions to analyze the bidirectional relationship between GSD and NAFLD/MetS. And in-depth dose-response analysis should be performed to show the continuous risk of quantitative metabolic variables on GSD risk.We conducted systematic review and meta-analysis based on the existing literature for more effective evidence on prevention of GSD and post-cholecystectomyic metabolic complications.

2 Materials and methods

2.1 Search strategy

A Meta-analysis was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (see checklist S1, flow diagram S1 and abstract checklist S1) (44). A relevant literature search was conducted using Medline, Embase, and Web of Science (WOS) databases from the date of inception to 24 July 2022 (without language restrictions). The following terms were used to search literatures: "gallstones disease"; "cholelithiasis"; "Metabolic syndrome"; "syndrome X"; "insulin resistance syndrome;" "MetS"; "Nonalcoholic fatty liver disease;" "nonalcoholic Steatohepatitis"; "NAFLD"; "NASH"; "cholecystectomy"; and "Laparoscopic cholecystectomy." If relevant literatures were omitted, additional manual retrieval was performed. The search strategy for the database is available in Table S1.

2.2 Study selection and data extraction

Eligibility criteria: 1) Published retrospective, prospective cohort studies and cross-sectional studies. 2) GSD / METS / NAFLD was the testing group's endpoint. 3) MetS diagnostic criteria for the study were given and the diagnosis of cholelithiasis needed to be confirmed by imaging or surgery. 4) The odds ratio (OR) / relative ratio (RR) / Hazard Ratio (HR) and corresponding 95% confidence intervals (CI) may be derived from studies or can be calculated. Studies were excluded if 1) literature was not the above research type, or the unpublished; 2) The subjects were not related to GSD/METS/NAFLD (no interest of subjects); 3) The disease outcomes were not just GSD/METS/NAFLD but with organic lesions, liver dysfunction or viral hepatitis in the hepatobiliary system (no interest of outcomes).

The study characteristics were extracted from all the literatures: first author; publication year; country and ethnicity of data origin; study design type; enrolled study population (including number of cases and total number); disease outcome; definition of MetS and NAFLD; risk of disease; the mean ± SD of MetS components such as body mass index (BMI), blood pressure (systolic pressure, diastolic pressure), waist circumference (WC), triglyceride (TG), fasting blood glucose (FBG), high density lipoprotein cholesterol (HDL-C) in case and control group; the calculation method of origin data; and adjusted covariates (the risks with the most extensive covariate adjusted were included to avoid potential bias).

2.3 Quality assessment

A preliminary assessment of the quality of each study was evaluated by two authors (JTL and QHL), respectively using the Newcastle-Ottawa Scale (NOS) (45). NOS consists of three main components, including participant selection, interstudy comparability and outcome assessment, corresponding to four, three and two stars (Table S2). Nine stars represent the highest quality paper, and a score of six stars is considered high-quality research. Meanwhile, we also carried out GRADE evaluation to assess our results quality (Figures S2, S3). When there are differences between the two authors, the original paper is re-evaluated by a third author (ZLX).

2.4 Statistical analysis

We initially combined the results of the included studies with a random effect model (Inverse variance), and chose OR (for cross-sectional study) and RR (for prospective cohort study))and 95% confidence intervals to quantify the relationship between Gallstone and MetS, gallstone and NAFLD, and cholecystectomy and NAFLD. If heterogeneity is significant, the random-effects model will be used, and heterogeneity will be assessed by I^2 statistics (low, high and medium heterogeneity of I^2 are defined as 25%, 50% and 75%, respectively (46).

To explore the relationship between GSD and MetS/NAFLD, we first compared the combined OR of end-stage (MetS/NAFLD) with or without GSD and the combined OR of end-stage (GSD) with or without GSD. Secondly, according to the different BMI intervals provided in the two reports (29, 30), the midpoints of each interval's upper and lower bounds were set as approximate median or mean. When the highest category was available, 1.2 times the category's low value was allocated (47). Using generalized least squares (GLST) calculations, BMI levels were estimated for each 1kg/m^2 increase, and then OR increments from both studies were combined to reflect the linear dose-response risk for GSD incidence. On the basis of information from reviewed studies, pooled standardized mean differences (SMD) of specific individual MetS components connected to the GSD occurrence were analyzed. In addition, a subgroup analysis was performed to determine the impact of potential confounding factors. Sensitivity analysis was carried out to look into the effects that a single study could have had on the outcomes.

When discussing the relationship between cholecystectomy and NAFLD, the combined RR and RR of NAFLD patients with or without cholecystectomy were also compared. Subsequently,

subgroup analysis and meta-regression were performed to explore the heterogeneity of potential sources further.

To calculate any potential publication bias, Egger's test was employed. P<0.05 was deemed significance. All of the statistical analyses were performed using Stata 15.0 version software (Stata Corp, College Station, TX, USA).

Our study flow diagram was shown in Figure S1.

3 Results

3.1 Search results and quality assessment results

The flow diagram of the meta-analysis registration study was shown in Figure 1. After excluding 894 duplicates from three databases (Medline, Embase, and WOS), we screened 3210 potentially relevant articles. The number of eligible articles finally registered was 19 (seven for GSD-MetS, six for GSD-NAFLD and six for cholecystectomy-NAFLD) with a high degree of uniformity among the evaluators (Cohen's Kappa =0.766). A manual search of references and related reviews of published studies revealed no additional studies. According to the NOS rating system, all included studies were assessed to be of good quality. Participants' NOS scores ranged from 6 to 9, with an average score of 7.84. The results of NOS quality assessment scores are shown in Table S3. The results of GRADE evaluation reported low level of our enrolled articles because they were observational studies (Figures S2, S3).

3.2 Characteristics of the enrolled studies

The study characteristics are presented in Table 1. Seven articles on the relationship between GSD and MetS were included in the meta-analysis, of which two prospective cohort studies reported the effect of MetS on the incidence of GS. Four cross-sectional and one prospective cohort studies reported the effect of GSD on the incidence of MetS. East Asians, Hispanics and Caucasians participated in five, one and one studies. Sample size varies widely between studies, with some fewer than 300 people and some as many as 200,000, for a total of 246,006 participants. Among the participants, 3,802 had GSD, with a prevalence ranging from 1.41% to 4.77%, and 2,034 had MetS, with a prevalence ranging from 6.09% to 35.02%. Three reported whether a single MetS component would affect the incidence of GSD and presented by standardized mean differences (SMD), we merge the results as shown in Figure 2. Regarding to the diagnostic criteria of MetS, the definition provided by the National Cholesterol Education Program Adult treatment Group III (NCEPATPIII) (48) in four studies and one used modified NCEP-ATP III. Alternatively, Chinese Diabetes Society (CDS) (49), the International Diabetes Federation (IDF) (50), and Taiwan National Health Department criteria (51) were used for the enrolled studies (Table S4). Four, one, and one studies' results were calculated using multivariate logistic regression, the Chi-Square test, multiple GEE, and Cox proportional hazard models.

There are six articles focusing on the relationship between GSD and NAFLD, two articles were prospective cohort studies, and four were cross-sectional studies. All patients were diagnosed with GS and NAFLD using Ultrasonogram (USG) Diagnosis. The total number of participants was as high as 322,630, including 215,497 patients with GSD, whose prevalence ranged from 4.44% to 76.66%, and 5926 patients with NAFLD, whose prevalence ranged from 1.86% to 34.43%. East Asians, Hispanics and Caucasians participated in four, one and one studies.

Six articles were included to explore the occurrence of NAFLD after cholecystectomy. A total of 261002 people

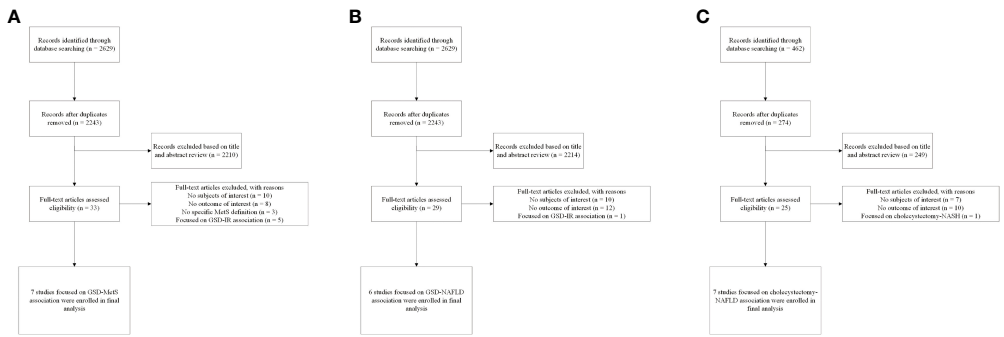

FIGURE 1
Flow diagram of eligible literature selection **(A)** Literature on association between GSD and MetS; **(B)** Literature on association between GSD and NAFLD; **(C)** Literature on association between cholecystectomy and NAFLD. GSD, gallstone disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; IR, insulin resistance.

TABLE 1 Characteristics of the nineteen studies included in meta-analysis.

First author, publication year [ref]	Country	Ethnicity	Study design	Enrolled study population(case/total)	Outcome	MetS definition	NAFLD diagnose	MetS components	OR/RR/HR	Mean ± SD (case/control)	Calculation method	Adjusted covariates
Chen et al. 2012 (25)	China	East Asian	Cross-sectional	918/7570 without MetS	MetS	NCEP-ATP-III on the Asia			OR: 1.29 (1.09-1.52)		Multivariate logistic regression model	Age
								BMI		26.3 ± 3.0 25.2 ± 3.4		
								WC		91.6 ± 9.4 87.8 ± 10.7		
								SBP		123.6 ± 14.3 119.8 ± 14.5		
								DBP		74.1 ± 9.8 72.2 ± 10.4		
								FBG		5.39 ± 1.37 5.11 ± 1.04		
								TG		201.1 ± 183.3 183.7 ± 182.7		
Nahum et al. 2005 (29)	USA	Hispanics	Cross-sectional	65/245 without MetS	MetS	NCEP-ATPIII			OR: 2.79(1.46-5.33)		Multivariate logistic regression model	Age and sex
								WC	OR: 3.61(1.95-6.71)			
								BMI		28.4 ± 5.7 26.3 ± 4.8		
								SBP		15.7 ± 2.2 14.4 ± 1.9		
Lin et al. 2014 (27)	China	East Asian	Cross-sectional	734/12050 without MetS	MetS	Taiwan criteria			OR: 1.61 (1.336-1.898)		Multivariate logistic regression model	Age and sex
								WC		84.4 ± 10.1 81.2 ± 9.9		
								SBP		127.8 ± 17.8 122.6 ± 17.1		
								DBP		81.0 ± 11.1 78.8 ± 10.6		

(Continued)

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TABLE 1 Continued

First author, publication year [ref]	Country	Ethnicity	Study design	Enrolled study population(case/total)	Outcome	MetS definition	NAFLD diagnose	MetS components	OR/RR/HR	Mean ± SD (case/control)	Calculation method	Adjusted covariates
								HDL-C		46.1 ± 12.1 49.2 ± 12.8		
								TG		126.8 ± 111.9 115.1 ± 10.5		
Naim et al. 2011 (28)	Turkey	Caucasian	Cross-sectional	217/217 without MetS	MetS	NCEP-ATPIII			OR: 1.434 (1.222-1.846)		Multivariate logistic regression model	Age, MetS, DM, large WC, HOMA-IR, gallstone size and BMI
Amit et al. 2019 (24)	India	East Asian	Prospective cohort	100/200 without MetS	MetS	NCEP-ATPIII			RR: 1.313 (1.107-1.556)		Chi- Square test model	
Kim et al. 2021 (23)	Korea	East Asian	Prospective cohort	2929/207850 without GSD	GSD	IDF			HR: 1.39(1.05-1.85)		Cox proportional hazard model	Age, sex, eGFR, GGT, smoking, alcohol intake and physical activity
Zhu et al. 2016 (26)	China	East Asian	Prospective cohort	873/18291 without GSD	GSD	CDS			RR: 1.25(1.06-1.49)		Multiple GEE model	Age
Kim et al. 2009 (30)	Korea	East Asian	Cross-sectional	6085/34574 without MetS	MetS	NCEP-ATPIII		BP	OR: 1.67(1.58-2.00)		Multivariate logistic regression model	Age, MetS, DM, large WC, HOMA-IR, gallstone size and BMI
Koller et al 2012 (32)	Slovakia	Northern European	Cross-sectional	198/482 without GSD	GSD		USG		OR: 1.78(1.16-2.73)		Multivariate logistic regression model	Age, gender, BMI, smoking, alcohol drinking, regular exercise, and DM, hypertension, eGFR and HDL-C
Koller et al 2012 (9)	Slovakia	Northern European	Cross-sectional	166/482 without NAFLD	NAFLD		USG		OR: 1.92(1.24-2.96)		Multivariate logistic regression model	Age, gender, BMI, smoking, alcohol drinking, regular exercise, and DM, hypertension, eGFR, total cholesterol, triglyceride, and HDL-C
Chang et al 2018 (31)	Korea	East Asian	Prospective cohort	214446/283446 without GSD	GSD		USG		HR: 1.26(1.17-1.35)		Multivariate logistic regression model	BMI, smoking, alcohol intake, exercise, total calorie intake, hypertension,

(Continued)

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TABLE 1 Continued

First author, publication year [ref]	Country	Ethnicity	Study design	Enrolled study population(case/total)	Outcome	MetS definition	NAFLD diagnose	MetS components	OR/RR/HR	Mean ± SD (case/control)	Calculation method	Adjusted covariates
												and diabetes, dyslipidemia, LDL-C, HDL-C, triglycerides and HOMA-IR
				4073/218519 without NAFLD	NAFLD		USG		HR: 1.14(1.07-1.22)			
Young et al. 2019 (36)	Korea	East Asian	Cross-sectional	355/7886 without GSD	GSD		USG		OR: 1.48 (0.875-1.485)		Binary logistic regression model	Age, sex, grade of fatty liver disease, BMI, fasting blood glucose, and total cholesterol, LDLs, HDLs, triglycerides
Liu et al. 2014 (34)	China	East Asian	Prospective cohort	498/11200 without GSD	GSD		USG		RR: 1.33(1.00-1.53)		Multiple GEE model	Age, BMI, SBP, ALB, GLO, TG and GLU
Qiao et al. 2017 (35)	China	East Asian	Cross-sectional	919/7583 without NAFLD	NAFLD		USG		OR: 1.28(1.07-1.52)		Multivariate logistic regression model	BMI≥24, hyperlipidaemia, and hypertension
Lee et al. 2014 (33)	China	East Asian	Cross-sectional	768/12033 without NAFLD	NAFLD		USG		OR: 1.32(1.04-1.69)		Binary logistic regression model	Age, gender, and BMI, smoking, alcohol drinking, DM and HDL-C
Yun et al. 2016 (41)	USA	Hispanics	Cross-sectional	50/82 without NAFLD	NAFLD		USG		OR: 2.4(1.8–3.3)		Multivariate logistic regression model	
Kwak et al. 2015 (12)	China	East Asian	Cross-sectional	149/17612 without NAFLD	NAFLD		USG		OR: 1.35(1.03-1.77)		Multivariate logistic regression model	Age, sex, hypertension, diabetes, BMI, smoking, physical activity, total cholesterol, triglycerides and HDL-C cholesterol
Yue et al. 2019 (40)	Britain	Hispanics	Cross-sectional	772/10074 without NAFLD	NAFLD		USG		OR: 2.61(1.89-3.61)		Cox proportional hazard models	Age, sex, ethnicity, smoking and drinking status, SBP, total cholesterol, and HDL-C
Ruh et al. 2012 (38)	USA	Hispanics	Cross-sectional	265/12232 without NAFLD	NAFLD		USG		OR: 2.4(1.8-3.3)		Cox proportional hazard models	Age, sex, ethnicity, BMI, WC, diabetes, HDL-C, SBP, DBP, smoking, smoking, alcohol intake and physical activity
Carmen et al. 2009 (37)	USA	Hispanics	Prospective cohort	795/4307without NAFLD	NAFLD		USG		OR: 1.04(0.62-1.77)		Multivariate logistic regression model	Age, sex, education level, physical activity, total energy intake, hypertension, DM and BMI

(Continued)

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TABLE 1 Continued

First author, publication year [ref]	Country	Ethnicity	Study design	Enrolled study population(case/total)	Outcome	MetS definition	NAFLD diagnose	MetS components	OR/RR/HR	Mean ± SD (case/control)	Calculation method	Adjusted covariates
Chang et al. 2018 (31)	Korea	East Asian	Prospective cohort	33506/94865 Men without NAFLD	NAFLD		USG		HR: 1.29(1.10-1.52)		Multivariate logistic regression model	Age, sex, BMI, smoking, alcohol intake, exercise, total calorie intake, and history of hypertension, history of diabetes, and medication for dyslipidemia
Chang et al. 2018 (31)	Korea	East Asian	Prospective cohort	15795/121830 Women without NAFLD	NAFLD		USG		HR: 1.05(0.86-1.28)		Multivariate logistic regression model	Age, sex, BMI, smoking, alcohol intake, exercise, total calorie intake, and history of hypertension, history of diabetes, and medication for dyslipidemia

GSD, gallstone disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; IR, insulin resistance; RR, relative ratio; OR: odds ratio; HR: hazard ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference TG, triglyceride; FBG, fasting blood glucose; HDL-C, high density lipoprotein cholesterol; NCEP ATPIII, National Cholesterol Education Progrm Adult treatment Group III; CDS, Chinese Diabetes Society; USG, Ultrasonogram; DM, diabetes mellitus; HOMA-IR, homeostasis model of assessment-insulin resistance; eGFR, estimated glomerular filtration rate; GGT, γ-glutamyltransferase; GLO, serum globulins; ALB, serum albumin; BUN, blood urea nitrogen; GLU, total glucose.

participated, and the probability of suffering from NAFLD after the operation was as high as 60.97% Those who were East Asian or Hispanics engaged in just two and four studies respectively. Alcohol intake and physical activity were adjusted as covariates in three studies and only two study excluded patients with type 1 diabetes.

3.3 Quantitative analysis

3.3.1 Risk of GSD on MetS occurrence

The pooled OR of incident MetS was 1.45 (95%CI: 1.23-1.67) with low heterogeneity (I^2=41.1%, P=0.165; Figure 3A) for the yes versus no category of GSD in four enrolled cross-sectional studies.

3.3.1.1 Subgroup, Sensitivity Analyses and Publication bias analysis

We attempt to assess the probable causes of heterogeneity using subgroup analysis due to the considerable variability in the overall study. Subgroup analysis was classified according to sample size, ethnicity, GSD incidence and calculation method. Among them, the subgroup analysis of GSD incidence, sample size and ethnicity could change heterogeneity. The merger OR of high GSD incidence is 1.76 (95%CI:0.62-2.90), which is the same as that of Westerners and low sample size (Table 2). Furthermore, the combined SMD of a single MetS component was specifically analyzed in four studies. Of the five components, hypertension was the only potential MetS component associated with increased prevalence of MetS. The combined SMD of 0.29 (95%CI:0.24-0.34) (Figure 2).

In order to further explore the causes of overall heterogeneity, we carried out a sensitivity analysis. After omitting one study in turn and re-evaluating the summary OR of other studies, it is found that the heterogeneity is eliminated by excluding Chen's study (Figure 4A). The larger sample size is the cause of this phenomenon.

Each study's SE of the log OR was placed against the log OR for visual examination on the Egger's funnel plot (Figure 5A). Egger's test did not reveal any publication bias despite the funnel plot's minor asymmetry (P = 0.148).

3.3.2 Risk of MetS on GSD occurrence

The results of comprehensive analysis suggested that patients with MetS have an increased risk of developing GSD. The two studies included in the analysis are highly heterogeneous. (RR:1.28; 95%CI: 1.09-1.47; I^2 = 0%, P=0.546) (Figure 3B).

3.3.2.1 Dose-response analysis

The original data of OR value and 95% confidence interval of GS disease in different intervals of BMI were given by Nahum

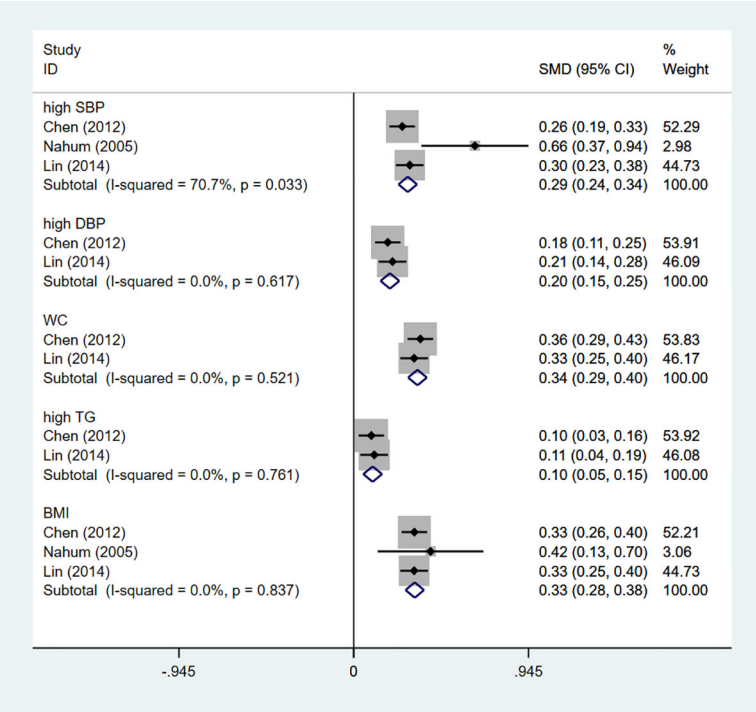

FIGURE 2
The influence of single MetS component on GSD risk. GSD, gallstone disease; MetS, metabolic syndrome; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; TG, triglyceride; BMI, body mass index.

et al. and Jonguk et al. When calculating and analyzing a single study, the results of both studies showed that the risk increase rate for every 1kg/m^2 up in BMI was from 1% to 4%. We tried to combine the results of the two calculations and found that for every 1kg/m^2 growth in BMI, the risk of GSD increased by 2%. If we specified the upper limit range of 35 and the lower limit range of 18.5, it could be seen that there is a positive linear relationship between them (P=0.0091, Figure 6).

3.3.3 Risk of NAFLD on GSD occurrence

Two prospective cohort and two cross-sectional studies reported the risk of NAFLD associated with elevated GSD.

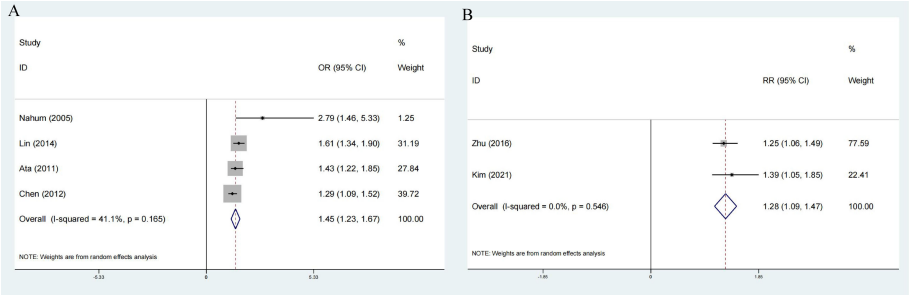

FIGURE 3
Forest plot on association between GSD and MetS **(A)** Pooled odds ratios of comparing the prevalence of MetS between GSD and non-GSD population (cross-sectional studies); **(B)** Pooled odds ratios comparing the prevalence of GSD between MetS and non-MetS population(cross-sectional studies); Pooled relative ratios of comparing the prevalence of GSD between NAFLD and non-NAFLD population; Pooled relative ratios of comparing the prevalence of NAFLD between GSD and non-GSD population. GSD, gallstone disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease.

TABLE 2 Subgroup analysis assessing different variables for the risk of metabolic syndrome caused by gallstone disease in included cross-sectional studies.

Variables	Number	OR [95% Conf. Interval]	Heterogeneity	
			I-squared (%)	P
sample size				
<300	2	1.76 (0.62-2.90)	45.6%	0.175
>300	2	1.44 (1.12-1.75)	68.2%	0.076
P=0.165				
incidence rate(%)				
>15	2	1.76 (0.62-2.90)	45.6%	0.175
<15	2	1.44 (1.12-1.75)	68.2%	0.076
P<0.05				
Ethnicity				
Others (Caucasian, Hispanics)	2	1.76 (0.62-2.90)	45.6%	0.175
East Asian	2	1.44 (1.12-1.75)	68.2%	0.076
P=0.165				
calculation method				
Multivariate logistic regression	3	1.55 (1.31-1.78)	12.4%	0.319
Chi- Square test	1	1.29 (1.07-1.78)	NA	NA
P=0.165				

NA, Not applicable.

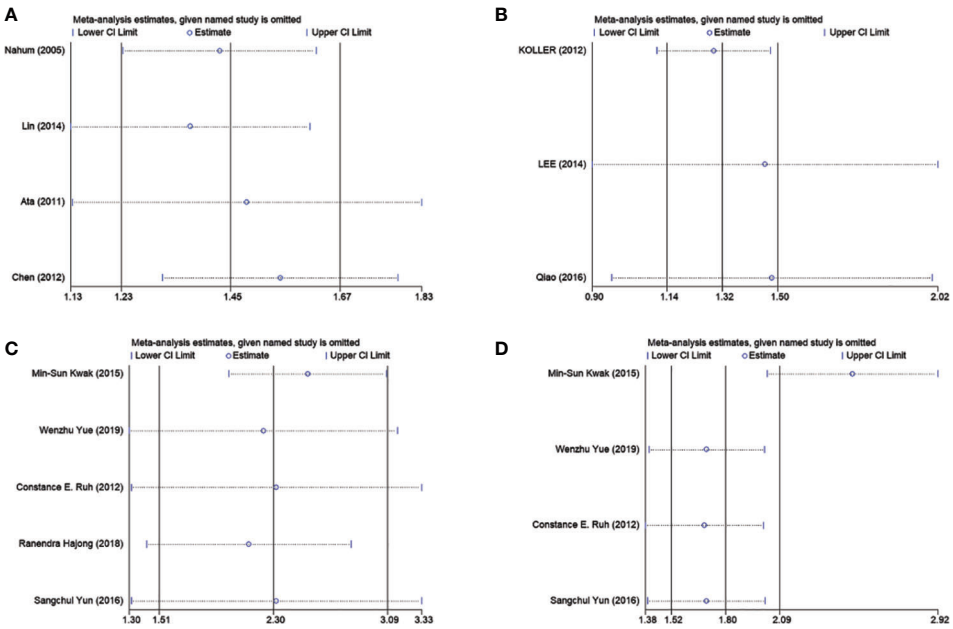

FIGURE 4
Sensitivity analyses of association between GSD and MetS as well as cholecystectomy and NAFLD. **(A)** Represents eliminated heterogeneity excluding a study of association between GSD and MetS in cross-sectional studies. **(B)** Represent eliminated heterogeneity excluding studies of association between GSD and NAFLD in cross-sectional studies. **(C, D)** Represent eliminated heterogeneity excluding studies of association between cholecystectomy and NAFLD in cross-sectional studies. GSD, gallstone disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease.

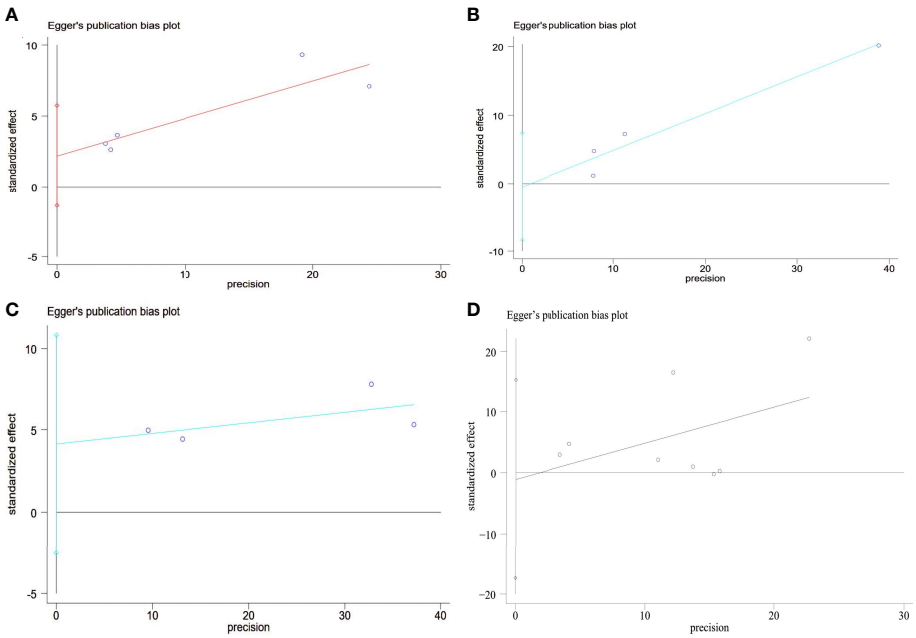

FIGURE 5
Egger's funnel plot analysis of publication bias **(A)** Prevalence of MetS between GSD and non-GSD population, Egger's test: P = 0.148. **(B)** Prevalence of GSD between NAFLD and non-NAFLD population, Egger's test: P = 0.117. **(C)** Prevalence of NAFLD between GSD and non-GSD population, Egger's test: P = 0.813. **(D)** Prevalence of NAFLD after cholecystectomy, Egger's test: P = 0.873. GSD, gallstone disease; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease.

The pooled risk effects between groups with and without NAFLD was RR=1.27 (1.18-1.35) without heterogeneity, P=0.624 (Figure 7B) and OR=1.52 (95%CI: 1.24-1.80) without heterogeneity, P=0.485 (Figure 7A).

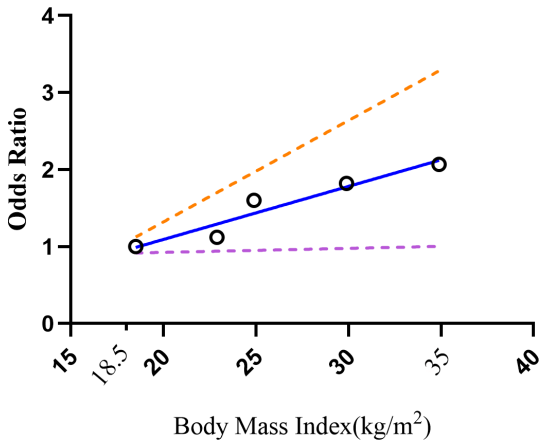

FIGURE 6
Dose-response relations between BMI levels and risk of GSD. GSD, gallstone disease; BMI, body mass index.

3.3.3.1 Publication bias analysis

To assess the publication bias, Egger's test was used. No significant publication bias was observed (Egger's P = 0.813; Figure 5B).

3.3.4 Risk of GSD on NAFLD occurrence

Three cross-sectional studies showed that NAFLD was associated with 1.3 times GSD risk (OR:1.32;95%CI:1.14-1.50), without heterogeneity, P=0.370 (Figure 7C).

Subgroup, Sensitivity Analyses and Publication bias analysis

Subgroup analysis was classified by sample size, ethnicity, NAFLD incidence and calculation method. No subgroup caused significant heterogeneity (Table 3).

We did sensitivity analysis by deleting one research at a time from the meta-analysis. Koller et al. 's study could significantly change the OR and heterogeneity of the aggregate. It may be related to the low sample size (Figure 4B). Use of Egger's test was made. There was no evidence of publication bias (P =0.873; Figure 5C).

3.3.5 Risk of NAFLD after cholecystectomy

Comprehensive analysis of all cross-sectional literature manifested that the risk of NAFLD events after cholecystectomy was higher than that without cholecystectomy intervention (OR:2.14;95%CI:1.43-2.85), with high heterogeneity (I^2=79.1%,

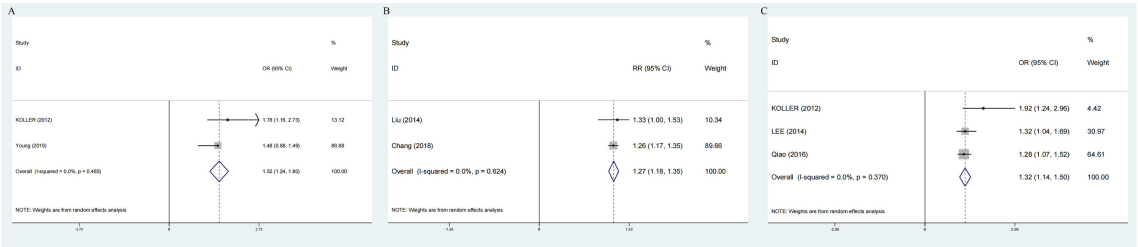

FIGURE 7
Forest plot on association between GSD and NAFLD. **(A)** Pooled odds ratios of comparing the prevalence of GSD between NAFLD and non-NAFLD population (cross-sectional studies); **(B)** Pooled relative ratios of comparing the prevalence of GSD between NAFLD and non-NAFLD population (prospective cohort studies); **(C)** Pooled relative ratios of comparing the prevalence of NAFLD between GSD and non-GSD population (cross-sectional studies). GSD, gallstone disease; NAFLD, nonalcoholic fatty liver disease.

P < 0.05; Figure 8A). But the prospective cohort literature indicated an opposite result (RR:0.96,95%CI:0.56-1.36) (Figure 8B).

3.3.5.1 Subgroup, meta-regression analyses, Sensitivity Analyses and Publication bias analysis

Subcomponent analysis included ethnicity, sample size, adjusted alcohol intake, adjusted physical activity, BMI and Excluded 1 diabetes in cross-sectional study. Hispanics, BMI>25kg/m^2, adjusted physical activity, excluded 1diabetes and adjusted alcohol intake lowered the heterogeneity (Table 4).

Meta-regression analyses showed that study design and ethnicity were the two causes of high heterogeneity (P < 0.05) (Figure 9).

Sensitivity analysis showed that the heterogeneity of cross-sectional study was affected by Kwak et al.'s study and Carmen et al. 's study could significantly change the RR and heterogeneity of the aggregate (Figures 4C, D). It may be related to the high sample size. Egger's test was used. No significant publication bias was observed (P =0.873; Figure 5D).

TABLE 3 Subgroup analysis assessing different variables for the risk of nonalcoholic fatty liver disease caused by gallstone disease in included cross-sectional studies.

Variable	Number	OR [95% Conf. Interval]	Heterogeneity	
			I-squared (%)	P
Ethnicity				
East Asian	2	1.29 (1.11-1.48)	0.00%	0.843
Northern Europe	1	1.92 (1.06-2.78)	NA	NA
P=0.0370				
sample size				
<8000	2	1.46 (0.92-2.02)	49.8%	0.158
>8000	1	1.32 (1.00-1.64)	NA	NA
P=0.370				
incidence rate				
<30	2	1,29 (1.11-1.48)	0.00%	0.843
>30	1	1.92 (1.06-2.78)	NA	NA
P=0.370				
calculation method				
multivariate logistic regression	2	1.46 (0.92-2.02)	49.8%	0.03
Binary logistic regression	1	1.32 (1.00-1.64)	NA	NA
P=0.370				

NA, Not applicable.

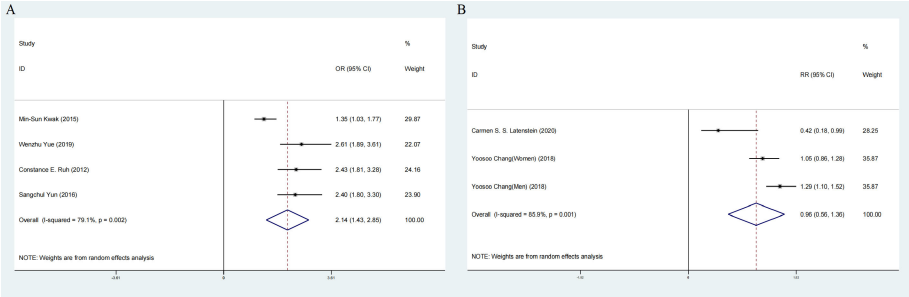

FIGURE 8
Forest plot of association between cholecystectomy and NAFLD. **(A)** Pooled odds ratios of comparing the prevalence of NAFLD between cholecystectomy and non-cholecystectomy population (cross-sectional studies). **(B)** Pooled odds ratios of comparing the prevalence of NAFLD between cholecystectomy and non-cholecystectomy population (prospective cohort studies).NAFLD, nonalcoholic fatty liver disease.

4 Discussion

Long-term research was conducted on both the MetS/NAFLD and GSD connection, as well as the relationship between cholecystectomy and NAFLD. The purpose of this meta-analysis was to conduct a complete examination of all the currently available data and to integrate that information to arrive at conclusive findings about this possible link. There were two primary outcomes. Firstly, there was a complex association between GSD and metabolic disorders including NAFLD and

TABLE 4 Subgroup analysis assessing different variables for the risk of nonalcoholic fatty liver disease after cholecystectomy in included cross-sectional studies.

Variables	Number	OR [95% Conf. Interval]	Heterogeneity	
			I-squared (%)	P
BMI				
<25	2	1.82 (0.80-2.85)	83.5%	0.014
>25	2	2.51 (1.95-3.06)	0.00%	<0.05
P <0.05				
ethnicity				
Asian	1	1.35 (0.98-1.72)	NA	NA
Hispanics	4	2.47 (2.02-2.92)	0.00%	0.929
P <0.05				
Sample size				
<10000	1	2.40 (1.65-3.15)	NA	NA
>10000	3	2.07 (1.18-2.96)	82.7%	<0.05
P <0.05				
Adjusted-alcohol intake				
No	1	1.35 (0.98-1.72)	NA	NA
Yes	3	2.47 (2.02-2.92)	0.00%	0.929
P <0.05				
Adjusted-Physical activity				
No	2	2.49 (1.93-3.06)	0.00%	0.718
Yes	2	1.84 (0.79-2.90)	84.9%	<0.05
P <0.05				
Excluded 1 diabetes				
No	2	2.49 (1.93-3.06)	0.00%	0.718
Yes	2	1.84 (0.79-2.90)	84.9%	<0.05
P <0.05				

NA, Not applicable.

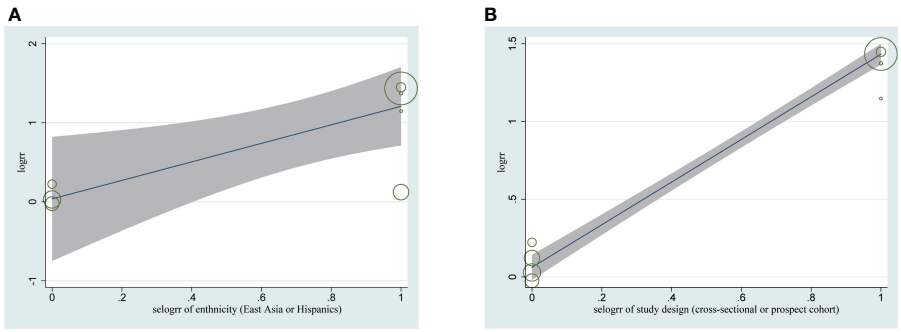

FIGURE 9
Meta-regression analyses assessing the heterogeneity of between cholecystectomy and NAFLD **(A)** Impact of ethnicity on associations between NAFLD between cholecystectomy. **(B)** Impact of study design on associations between NAFLD and cholecystectomy. NAFLD, nonalcoholic fatty liver disease.8

MetS. Secondly, NAFLD was probably related to cholecystectomy. After we performed subgroup analyses, meta-regression analyses and dose-response analyses, some new results were obtained to support the primary outcomes. To be specific, Hypertension may increase the incidence of GSD. And approximate 2% increment was observed on the GSD incidence per 1 kg/m^2 of BMI elevation. Furthermore, obese GSD patients who have undergone cholecystectomy were more likely to develop NAFLD than non-obese GSD patients.

After pooling previous studies, we found that MetS could cause GSD (without heterogeneity). And GSD was a risk factor of MetS with low heterogeneity. However, we still performed subgroup analyses and the results showed that the heterogeneity was strengthened by sample size, GSD incidence and ethnicity. So we supposed that the epidemiological evidence of MetS could make sense. Prevalence among white adults in developed countries was as high as 10% to 15% (52). And The National Health and Nutrition Examination Survey in the United States pointed to an overall MetS prevalence of 23.7% (53). Nevertheless, the popularity of GSD in China did not exceed 15% (54). By 2000, the prevalence of MetS was 15.1% (54), significantly lower than that in the United States. The results of the subgroup analysis did not confirmed that Hispanics and Caucasians could affect MetS incidence and we think it was caused by low enrolled population.

A liver condition known as nonalcoholic fatty liver disease (NAFLD) can range from moderate hepatic steatosis to nonalcoholic steatohepatitis (NASH) (55). NASH can subsequently develop into advanced liver fibrosis, cirrhosis, or hepatocellular carcinoma (55). The prevalence of NAFLD in the general population worldwide is as high as 20% (56). We found that NAFLD patients were 15 times more likely to develop GSD than non-NAFLD patients (without heterogeneity), whereas GSD patients were 1.3 times more likely to develop NAFLD than non-GSD patients (without heterogeneity). Some studies have demonstrated a bidirectional and independent association between GSD and NAFLD (57). A longitudinal cohort Asian study followed 11200 participants for 6 years and found NAFLD was an independent risk factor for GSD (RR=1.2381, 95% CI:1.003-1.528), especially in women (RR=1.707,95CI%: 1.245-2.341) (34). Similar results were observed by Loria et al. (58) in a cohort with a greater frequency of GSD than the general community. Despite adjusting the factors significantly related to GSD for NAFLD patients in their investigation, Yilmaz et al. (59) could not find a correlation between the diagnosis of GSD and nonalcoholic steatohepatitis. (OR=1.03; 95% CI 0.5-2.1), indicating that GSD was not an independent risk factor for NAFLD. However, a “chicken and egg” dispute is now going on over the temporal association between NAFLD and GSD, and there is no explicit agreement on the topic (60). Our results only demonstrated a temporal association of NAFLD affecting GSD.

Given the analysis of individual MetS components, only high systolic blood pressure was significantly associated with high GSD prevalence and it raised the heterogeneity (Figure 2). Perhaps due to insufficient data in the included studies, it was inconsistent with previous meta-analyses that all components of the metabolic syndrome were positively associated with GSD prevalence (43). Recently, Zhang et al. (61)conducted a cross-sectional research in a Chinese population from Liaoning Province with the purpose of examining the effects of systolic and diastolic blood pressure on GSD. They achieved findings that were comparable with ours. As one of the diagnostic criteria for MetS, its association with gallstones can be explained by insulin resistance (62). Worsening insulin resistance can trigger mechanisms that increase renal sodium reabsorption and sympathetic nervous system activity (62, 63), ultimately

leading to hypertension in patients with MetS. In addition, high blood pressure may also be inseparable from obesity according to our results. Liew et al. (55) put forward that Asian obese patients had higher diastolic blood pressure with cholelithiasis. But the mechanism is unclear, and perhaps it is related to insulin resistance. Furthermore, Hsu et al. (64)discovered that obesity represented by high waist circumference and BMI is the main risk factor for GSD. But few studies compared its detailed degree of influence. We hypothesized that BMI might be a useful marker for predicting and screening for GSD based on the positive linear dose-response relationship. It is well known that BMI is a specific parameter for overweight and obesity (65). On the one hand, high BMI incurred larger gallbladder and higher cholesterol synthase activity (66). On the other hand, mature adipocytes, a bridge between obesity and GSD, could secrete leptin (67). Such fat factor played an irreplaceable role in regulating the motility of gallbladder (GB) (68) and promoting the secretory function of stone formation (69). For this, obese patients often had the phenomenon of insufficient contractile ability of GB and supersaturated cholesterol in bile (69). And IR could promote stone formation in normal and overweight people (70). It is worth noting that obesity related to MetS was more about highlighting abdominal obesity caused by high waists (71). Tsai et al. (71) proved that the abdominal circumference and waist-to-hip ratio are related with an increased risk of cholecystectomy, irrespective of BMI in Western women. For men, using BMI alone may mask excess fat (72). And it is easier to measure waist circumference than BMI. However, none of the registered studies reported a dose-response of high waist circumference in our meta-analysis. Accordingly, it is essential to strengthen the study on the incidence of GSD in the degree of high waist circumference.

NAFLD has traditionally been considered the hepatic manifestation of the metabolic syndrome because NAFLD is often associated with repertoire of MetS features (73). Leite et al. (74) found that about two-thirds of obese and type 2 diabetic patients had hepatic steatosis. About 50% of patients with hyperlipidemia (75) and 50% of patients with essential hypertension (76) also had hepatic steatosis. That's why experts emphasized changing NAFLD to MAFLD in recent years (77). MAFLD more accurately reflects the current understanding of fatty liver disease associated with metabolic dysfunction (77). According to the findings of epidemiological research, the rise in the prevalence of obesity was the primary cause of the increase in the death rate from NAFLD (78). Although the increased incidence of NAFLD was often attributed to the obesity epidemic, NAFLD was detected in non-obese individuals (79). So it is a more complex disease process. The relationship between abnormal glucose metabolism and fatty liver disease has been agreed upon (36). Fasting blood glucose levels were proven to be wholly associated with the presence of gallstones in NAFLD patients in a research comparing those with simple NAFLD to those with NAFLD complicated by GSD (36). That is, NAFLD might promote GSD through metabolic syndrome factors. However, Lu et al. (80) highlighted that type 2 diabetes mellitus (T2DM) predisposed to GSD more than NAFLD. Meanwhile, T2DM can aggravate the course of NAFLD (81). Therefore, the diagnosis and treatment of NAFLD cannot be ignored in patients with both diabetes and GSD.

A growing number of studies suggested in multivariate adjustment analyses that gallstones were no longer independently associated with NAFLD but cholecystectomy was the independent risk factor for NAFLD (12, 42). Though the combined results of the cross-sectional studies showed cholecystectomy is related to NAFLD but the heterogeneity was high. Furthermore, we can't confirm their causality after combining from the results from prospective studies. Otherwise, potential defects of these two enrolled prospective studies (31, 37) on GSD and NAFLD should be considered. To be specific, Carmen et al. only mentioned one ultrasound during the follow up which was not qualified to prove that the NAFLD is posterior to the surgery because at least two ultrasounds are necessary. And Chang et al. showed a slight independent relationship between GSD and NAFLD in their multivariate analysis. But this association was only observed in males but not in females. All in all, more prospective studies are worthy on further investigation to explore whether cholecystectomy could cause NAFLD independently.

High heterogeneity can be caused by racial factors. According to our subgroup analysis and regression analysis, the phenomenon in Hispanics is about twice as common as than risks in Asians. Global figures showed that while NAFLD prevalence in Asia is only 27% (82), it is approximately 30% in the United States (83). According to research by Golabi et al. (84), the prevalence of NAFLD among Asian American adults was almost three times lower than it is among Hispanic Americans (47 vs. 26%, respectively). And obesity also might be a reasonable explanation for potential heterogeneity. We discovered that obese patients with gallstones had a greater chance of developing NAFLD following cholecystectomy than non-obese patients did when we compared the degree of BMI as a confounding variable. Non-obesity individuals did not have gallstones (Figure 8B). It suggested that cholecystectomy may aggravate the disorder of lipid distribution in some way and promote the accumulation of fat in the liver. The fact that Hispanic patients who undergo non-obesity cholecystectomy had significantly higher levels of NAFLD than non-Hispanic patients supports this conclusion (85). A study by Amigo et al. (86) found that cholecystectomy in mice increased bile cholesterol and energy consumption, leading to an increase in triglyceride and very low density lipoprotein levels and

worsening NAFLD in mice. It provided strong evidence for the effect of cholecystectomy on lipid metabolism (86). In addition, Kakati et al. (87) found that the median time to diagnosis of NAFLD after cholecystectomy was approximately 6.2 years. But the timing of cholecystectomy was not associated with disease progression in patients with preoperatively diagnosed NAFLD (87). It indicated that it was indispensable to check NAFLD regularly after cholecystectomy. And in the future, more clinical studies should be put into this direction.

In fact, IR provides a key link between MetS, NAFLD, increased susceptibility to gallstones, and cholecystectomy (88). The core of lipid metabolism disorder is insulin resistance (89). Lipolysis could be induced by peripheral insulin resistance (90). A large amount of free fatty acids (FFA) entered the liver from the peripheral tissue to produce more fat (89). Meanwhile, lowering activity of the peripheral lipoprotein lipase predisposed an increase in chylomicron (89). The process affected the regulation of triglycerides in the liver, resulting in the accumulation of triglycerides, which further aggravated liver insulin resistance (89). The increase of triglycerides accelerated the synthesis of endogenous cholesterol. It may have something to do with the obesity (91). However, Scragg et al. (92) explained that the phenomenon that the increase of plasma insulin concentration aggravates the incidence of GSD is independent of obesity but is related to women and age. At present, some researchers also showed that obesity is neither necessary nor sufficient for the pathogenesis of GSD (18). Therefore, there may be an indirect relationship between obesity and insulin resistance to regulate GSD jointly. Other studies had explained the molecular mechanism (93, 94). When the liver develops insulin resistance on its own, the nuclear heterodimeric receptor farnesoid X receptor (FXR) gene was down-regulated and the corresponding receptor expression decreased (93). And then bile acid transporter protein Abcb11 and phospholipid transporter Abcb4 reduced (93). Finally, bile acid could not be transferred and accumulated, and the content of bile acid in bile decreased (93). In another pathway, up-regulated cholesterol secretion genes ABCG5 and ABCG8 promoted more expression of cholesterol transporters and finally increased cholesterol secretion (94). Inhibition of 7α -hydroxylase contributed to the conversion of cholesterol to bile acids, resulting in cholesterol supersaturation (95). Combined with our data, Figure 10 summarizes and quantitatively demonstrates the underlying mechanisms of IR and GSD association.

It is important to note the reliability and usefulness of our findings. We evaluated the association between GSD and NAFLD/MetS by consideration of risk direction. To be specific, the pooled GSD risk in subjects with/without NAFLD/MetS, and risk of metabolic derangements based on GSD status were evaluated respectively in different models. Based on the positive linear dose-response relationship, using BMI to predict GSD has a high cost-effectiveness (64). In the future, a perfect and standardized prediction model (96) can be made for clinical use and even help people to perform self-prevention. This model allows patients to compare their risk of GSD based on BMI measurements when they are in the hospital or at home. Weight loss treatments, such as more activity and a restricted diet, should be used in patients with higher risk of GSD (97). Due to the potential causal relationship between IR and GSD, reducing modifiable risk factors for MetS and NAFLD is expected to be a future target for drug design (98). For example, try coming up with some medicines that can boost transporter efficiency and enzyme activity (98). We found that

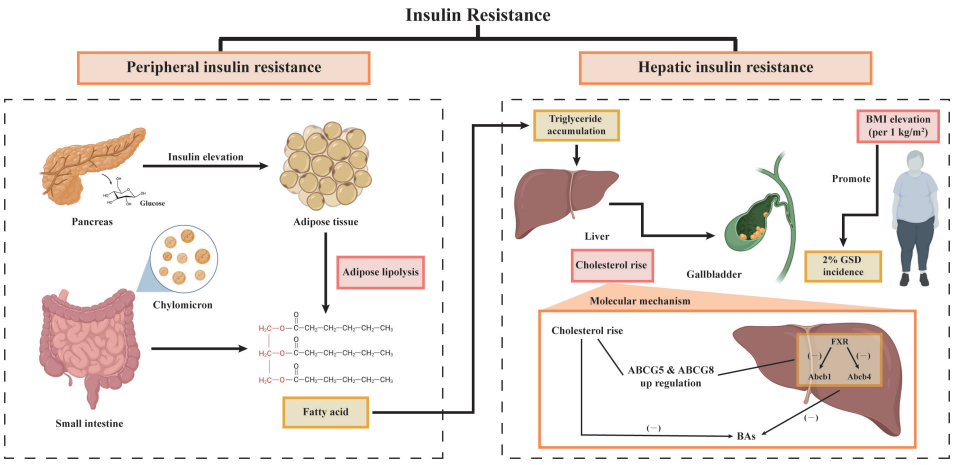

FIGURE 10
Potential mechanisms between GSD and IR. GSD, gallstone disease; BMI, body mass index; FXR, farnesoid X receptor.

obesity may increase the incidence of NAFLD in GSD patients who have undergone cholecystectomy. So we presumed that subsequent studies should investigate whether GB-preserving cholecystolithotomy is preferable to cholecystectomy from the standpoint of metabolic regulation (99). And it would improve the prognosis, reduce the risk of postoperative complications, and lower the cost of medical insurance.

We noted that there were certain limitations even though the majority of the included research were of high quality. First of all, we were unable to conclude with certainty that the observed outcomes were not attributable to NAFLD/MetS itself or to any of the other possible confounding variables. Second, only a small number of prospective cohort studies were included in each analysis, which limited the ability to obtain more conclusive evidence and the conclusions need to be confirmed in more and larger cohort studies. Third, it is necessary to state the drawbacks of combining data from different recruited research, such as the lack of consistency in risk measurements and statistical methodologies. RR and OR from different statistical models exhibited discrepant meaning, suggesting the presence of heterogeneities if the two were combined. Fourth, very few included studies could support the dose-response analysis of BMI, so the relationship between BMI and GSD would be compared later. Fifth, this study is not a mechanism study, so the potential mechanism of IR affecting GSD has not been well described. We will follow up on animal experiments to explore this process. Sixth, due to the lack of data on relevant risk factors provided in the registered articles, we cannot probe into the detailed biological interaction between NAFLD and BMI after cholecystectomy. Therefore, we are planning to collect more information based on information from our center to evaluate the quantitative relationship between cholecystectomy and NAFLD incidence in patients with different BMI categories (100, 101). In addition, omic data played crucial roles in exploring the mechanism of complex disease (102). And multi-omics data was confirmed to disclose the function of genes based on network analysis (103). Actually, the temporal relationship between GSD and NAFLD is an interesting study topic and we are planning to clarify this causal-effect interaction between these two covariates based on cohort study. Currently, we are collecting gallbladder samples from sample who received cholecystectomy which might provide more reliable evidence to reveal the mechanism of complex associations between GSD and insulin resistance.

5 Conclusion

This meta-analysis provided evidence that the close relationship between GSD and MetS/NAFLD, or insulin resistance, and the close relationship between cholecystectomy and NAFLD. No matter what kind of disease, geographical differences in the risks are greater in the America, compared to Europe and Asia. We also observed that calculating BMI might be a useful and customized technique for determining the likelihood of developing GSD. Paying attention to the control of blood pressure and blood sugar is helpful in alleviating GSD. In the future, well-designed and high-quality prospective studies are needed to confirm these effects and to further study cholecystolithotomy through metabonomics.

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

ZL conceived and designed the study. JL and QL performed experiment and extracted information. JL and QL analyzed the data. JL wrote the manuscript. ZL reviewed the manuscript. ZL provided the funding support. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1032557/full#supplementary-material

References

1. Bodmer M, Brauchli YB, Krähenbühl S, Jick SS, Meier CR. Statin use and risk of gallstone disease followed by cholecystectomy. *Jama* (2009) 302(18):2001–7. doi: 10.1001/jama.2009.1601

2. Marschall HU, Einarsson C. Gallstone disease. *J Internal Med* (2007) 261 (6):529–42. doi: 10.1111/j.1365-2796.2007.01783.x

3. Kaechele V, Wabitsch M, Thiere D, Kessler AL, Haenle MM, Mayer H, et al. Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. *J Pediatr Gastroenterol Nutr* (2006) 42(1):66–70. doi: 10.1097/01.mpg.0000187816.31213.06

4. Walker SK, Maki AC, Cannon RM, Foley DS, Wilson KM, Galganski LA, et al. Etiology and incidence of pediatric gallbladder disease. *Surgery* (2013) 154 (4):927–33. doi: 10.1016/j.surg.2013.04.040

5. Lammert F, Gurusamy K, Ko CW, Miquel J-F, Méndez-Sánchez N, Portincasa P, et al. Gallstones. *Nat Rev Dis Primers* (2016) 2(1):1–17. doi: 10.1038/nrdp.2016.24

6. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* (2006) 368(9531):230–9. doi: 10.1016/S0140-6736(06)69044-2

7. Tran BX, Tran TD, Nathan N, Ngo CQ, Nguyen LT, Nguyen LH, et al. Catastrophic health expenditure of Vietnamese patients with gallstone diseases - a case for health insurance policy revaluation. *Clinicoecon Outcomes Res* (2019) 11:151–8. doi: 10.2147/ceor.S191379

8. Portincasa P, Di Ciaula A, De Bari O, Garruti G, Palmieri V, Wang D-H. Management of gallstones and its related complications. *Expert Rev Gastroenterol hepatology*. (2016) 10(1):93–112. doi: 10.1586/17474124.2016.1109445

9. Chinnery GE, Krige JE, Bornman PC, Bernon MM, Al-Harethi S, Hofmeyr S, et al. Endoscopic management of bile leaks after laparoscopic cholecystectomy surgery. *South Afr J Surgery* (2013) 51(4):116–21. doi: 10.10520/EJC146022

10. Schreuder AM, Busch OR, Besselink MG, Ignatavicius P, Gulbinas A, Barauskas G, et al. Long-term impact of iatrogenic bile duct injury. *Digestive Surgery* (2020) 37(1):10–21. doi: 10.1159/000496432

11. Chavez-Tapia NC, Mac Kinney-Novelo I, Sifuentes-Rentería SE, Torres-Zavala M, Castro-Gastelum G, Sánchez-Lara K, et al. Association between cholecystectomy for gallstone disease and risk factors for cardiovascular disease. *Ann Hepatology* (2012) 11(1):85–9. doi: 10.1016/S1665-2681(19)31490-5

12. Kwak M-S, Kim D, Chung GE, Kim W, Kim YJ, Yoon J-H. Cholecystectomy is independently associated with nonalcoholic fatty liver disease in an Asian population. *World J Gastroenterology: WJG*. (2015) 21(20):6287. doi: 10.3748/wjg.v21.i20.6287

13. Haas JT, Biddinger SB. Dissecting the role of insulin resistance in the metabolic syndrome. *Curr Opin Lipidology* (2009) 20(3):206. doi: 10.1097/MOL.0b013e32832b2024

14. Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* (2018) 19(1):31–44. doi: 10.1038/nrm.2017.89

15. Polyzos SA, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* (2009) 9 (3):299–314. doi: 10.2174/156652409787847191

16. Di Ciaula A, Wang DQ, Portincasa P. An update on the pathogenesis of cholesterol gallstone disease. *Curr Opin Gastroenterol* (2018) 34(2):71–80. doi: 10.1097/mog.0000000000000423

17. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* (2017) 66(6):1138–53. doi: 10.1136/gutjnl-2017-313884

18. Cortés VA, Barrera F, Nervi F. Pathophysiological connections between gallstone disease, insulin resistance, and obesity. *Obes Rev* (2020) 21(4):e12983. doi: 10.1111/obr.12983

19. Chang Y, Sung E, Ryu S, Park Y-W, Jang YM, Park M. Insulin resistance is associated with gallstones even in non-obese, non-diabetic Korean men. *J Korean Med Science* (2008) 23(4):644–50. doi: 10.3346/jkms.2008.23.4.644

20. Kim SS, Lee JG, Kim DW, Kim BH, Jeon YK, Kim MR, et al. Insulin resistance as a risk factor for gallbladder stone formation in Korean postmenopausal women. *Korean J Internal Med* (2011) 26(3):285. doi: 10.3904/kjim.2011.26.3.285

21. Arrese M, Cortés V, Barrera F, Nervi F. Nonalcoholic fatty liver disease, cholesterol gallstones, and cholecystectomy: new insights on a complex relationship. *Curr Opin Gastroenterol* (2018) 34(2):90–6. doi: 10.1097/mog.0000000000000416

22. Roda E, Aldini R, Mazzella G, Roda A, Sama C, Festi D, et al. Enterohepatic circulation of bile acids after cholecystectomy. *Gut* (1978) 19(7):640–9. doi: 10.1136/gut.19.7.640

23. Kim Y, Oh CM, Ha E, Park SK, Jung JY, Ryoo JH. Association between metabolic syndrome and incidence of cholelithiasis in the Korean population. *J Gastroenterol Hepatol* (2021) 36(12):3524–31. doi: 10.1111/jgh.15568

24. Peswani AR, Sequeira VJ, D'silva M, Ghanwat S, Shah PP, Pinto AC. Association between gallstone disease and metabolic syndrome. *IJCMR* (2019) 6 (10):J1–5. doi: 10.21276/ijcmr.2019.6.10.13

25. Chen LY, Qiao QH, Zhang SC, Chen YH, Chao GQ, Fang LZ. Metabolic syndrome and gallstone disease. *World J Gastroenterol* (2012) 18(31):4215–20. doi: 10.3748/wjg.v18.i31.4215

26. Zhu Q, Sun X, Ji X, Zhu L, Xu J, Wang C, et al. The association between gallstones and metabolic syndrome in urban han Chinese: a longitudinal cohort study. *Sci Rep* (2016) 6:29937. doi: 10.1038/srep29937

27. Lin IC, Yang YW, Wu MF, Yeh YH, Liou JC, Lin YL, et al. The association of metabolic syndrome and its factors with gallstone disease. *BMC Fam Pract* (2014) 15:138. doi: 10.1186/1471-2296-15-138

28. Ata N, Kucukazman M, Yavuz B, Bulus H, Dal K, Ertugrul DT, et al. The metabolic syndrome is associated with complicated gallstone disease. *Can J Gastroenterol* (2011) 25(5):274–6. doi: 10.1155/2011/356761

29. Méndez-Sánchez N, Chavez-Tapia NC, Motola-Kuba D, Sanchez-Lara K, Ponciano-Rodríguez G, Baptista H, et al. Metabolic syndrome as a risk factor for gallstone disease. *World J Gastroenterol* (2005) 11(11):1653–7. doi: 10.3748/wjg.v11.i11.1653

30. Kim J, Lee K. Relationship between metabolic syndrome and gallbladder stone. *Korean J Family Med* (2009) 30(8):610–6. doi: 10.4082/kjfm.2009.30.8.610

31. Chang Y, Noh YH, Suh BS, Kim Y, Sung E, Jung HS, et al. Bidirectional association between nonalcoholic fatty liver disease and gallstone disease: A cohort study. *J Clin Med* (2018) 7(11):458. doi: 10.3390/jcm7110458

32. Koller T, Kollerova J, Hlavaty T, Huorka M, Payer J. Cholelithiasis and markers of nonalcoholic fatty liver disease in patients with metabolic risk factors. *Scand J Gastroenterol* (2012) 47(2):197–203. doi: 10.3109/00365521.2011.643481

33. Lee YC, Wu JS, Yang YC, Chang CS, Lu FH, Chang CJ. Moderate to severe, but not mild, nonalcoholic fatty liver disease associated with increased risk of gallstone disease. *Scand J Gastroenterol* (2014) 49(8):1001–6. doi: 10.3109/00365521.2014.920912

34. Liu J, Lin H, Zhang C, Wang L, Wu S, Zhang D, et al. Non-alcoholic fatty liver disease associated with gallstones in females rather than males: a longitudinal cohort study in Chinese urban population. *BMC Gastroenterol* (2014) 14:213. doi: 10.1186/s12876-014-0213-y

35. Qiao QH, Zhu WH, Yu YX, Huang FF, Chen LY. Nonalcoholic fatty liver was associated with asymptomatic gallstones in a Chinese population. *Med (Baltimore)* (2017) 96(38):e7853. doi: 10.1097/md.0000000000007853

36. Kim YK, Kwon OS, Her KH. The grade of nonalcoholic fatty liver disease is an independent risk factor for gallstone disease: An observational study. *Med (Baltimore)* (2019) 98(27):e16018. doi: 10.1097/md.0000000000016018

37. Latenstein CSS, Alferink LJM, Darwish Murad S, Drenth JPH, van Laarhoven C, de Reuver PR. The association between cholecystectomy, metabolic syndrome, and nonalcoholic fatty liver disease: A population-based study. *Clin Transl Gastroenterol* (2020) 11(4):e00170. doi: 10.14309/ctg.0000000000000170

38. Ruhl CE, Everhart JE. Relationship of non-alcoholic fatty liver disease with cholecystectomy in the US population. *Am J Gastroenterol* (2013) 108(6):952–8. doi: 10.1038/ajg.2013.70

39. Hajong R, Dhal MR, Naku N, Kapa B. Incidence of nonalcoholic fatty liver disease in patients undergoing laparoscopic cholecystectomy. *J Family Med Prim Care* (2018) 7(6):1375–8. doi: 10.4103/jfmpc.jfmpc_193_18

40. Yue W, Sun X, Du T. Cholecystectomy versus central obesity or insulin resistance in relation to the risk of nonalcoholic fatty liver disease: the third US national health and nutrition examination survey. *BMC Endocr Disord* (2019) 19(1):95. doi: 10.1186/s12902-019-0423-y

41. Yun S, Choi D, Lee KG, Kim HJ, Kang BK, Kim H, et al. Cholecystectomy causes ultrasound evidence of increased hepatic steatosis. *World J Surg* (2016) 40(6):1412–21. doi: 10.1007/s00268-015-3396-7

42. Jaruvongvanich V, Sanguankeo A, Upala S. Significant association between gallstone disease and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Digestive Dis Sci* (2016) 61(8):2389–96. doi: 10.1007/s10620-016-4125-2

43. Jiang P, Ni Z, Huang S, Li X, Li Y, Huang H. The association between gallstone disease and metabolic syndrome related abnormalities: a systematic review and meta-analysis. *Int J Diabetes Developing Countries* (2021) 41(2):196–204. doi: 10.1007/s13410-020-00890-9

44. 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

45. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses*. Ottawa, ON: Ottawa Hospital Research Institute (2000).

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

47. Berlin JA, Longnecker MP, Greenland S. Meta-analysis of epidemiologic dose-response data. *Epidemiology* (1993) 4(3):218–28. doi: 10.1097/00001648-199305000-00005

48. Expert Panel on Detection E. Executive summary of the 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). *Jama* (2001) 285(19):2486–97. doi: 10.1001/jama.285.19.2486

49. Lu Y, Lu J, Wang S, Li C, Liu L, Zheng R, et al. Comparison of the diagnostic criteria of metabolic syndrome by international diabetes federation and that by Chinese medical association diabetes branch. *Zhonghua yi xue za zhi* (2006) 86(6):386–9. doi: 10.3760/j:issn:0376-2491.2006.06.007

50. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. *Circulation* (2009) 120(16):1640–5. doi: 10.1161/circulationaha.109.192644

51. Tsai T-Y, Cheng J-F, Lai Y-M. Prevalence of metabolic syndrome and related factors in Taiwanese high-tech industry workers. *Clinics* (2011) 66:1531–5. doi: 10.1590/S1807-59322011000900004

52. Stinton LM, Myers RP, Shaffer EA. Epidemiology of gallstones. *Gastroenterol Clinics* (2010) 39(2):157–69. doi: 10.1016/j.gtc.2010.02.003

53. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third national health and nutrition examination survey. *Jama* (2002) 287(3):356–9. doi: 10.1001/jama.287.3.356

54. Gu D, Gupta A, Muntner P, Hu S, Duan X, Chen J, et al. Prevalence of cardiovascular disease risk factor clustering among the adult population of China: results from the international collaborative study of cardiovascular disease in Asia (InterAsia). *Circulation* (2005) 112(5):658–65. doi: 10.1161/CIRCULATIONAHA.104.515072

55. Liew P-L, Wang W, Lee Y-C, Huang M-T, Lin Y-C, Lee W-J. Gallbladder disease among obese patients in Taiwan. *Obes Surgery* (2007) 17(3):383–90. doi: 10.1007/s11695-007-9068-4

56. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. *Hepatology* (2012) 55(6):2005–23. doi: 10.1002/hep.25762

57. Fracanzani AL, Valenti L, Russello M, Miele L, Bertelli C, Bellia A, et al. Gallstone disease is associated with more severe liver damage in patients with non-alcoholic fatty liver disease. *PloS One* (2012) 7(7):e41183. doi: 10.1371/journal.pone.0041183

58. Loria P, Lonardo A, Lombardini S, Carulli L, Verrone A, Ganazzi D, et al. Gallstone disease in non-alcoholic fatty liver: prevalence and associated factors. *J Gastroenterol hepatology* (2005) 20(8):1176–84. doi: 10.1111/j.1440-1746.2005.03924.x

59. Yilmaz Y, Ayyildiz T, Akin H, Colak Y, Ozturk O, Senates E, et al. Gallstone disease does not predict liver histology in nonalcoholic fatty liver disease. *Gut Liver* (2014) 8(3):313. doi: 10.5009/gnl.2014.8.3.313

60. Ahmed MH, Ali A. Nonalcoholic fatty liver disease and cholesterol gallstones: which comes first? *Scandinavian J Gastroenterology* (2014) 49(5):521–7. doi: 10.3109/00365521.2014.894119

61. Zhang X, Guan L, Tian H, Li Y. Prevalence and risk factors of gallbladder stones and polyps in liaoning, China. *Front Med (Lausanne)* (2022) 9:865458. doi: 10.3389/fmed.2022.865458

62. DeFronzo RA, Cooke CR, Andres R, Faloona G, Davis P. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest* (1975) 55(4):845–55. doi: 10.1172/JCI107996

63. Tuck M. Obesity, the sympathetic nervous system, and essential hypertension. *Hypertension* (1992) 19(1_supplement):I67. doi: 10.1161/01.HYP.19.1_Suppl.I67

64. Hsu H-Y, Huang C-Y, Hwang L-C. Sex difference of the predictive value of BMI, waist circumference and percentage body fat mass for gallstone disease. *Br J Nutr* (2019) 121(8):955–60. doi: 10.1017/S000711451900028X

65. Emerenziani S, Pier Luca Guarino M, Trillo Asensio LM, Altomare A, Ribolsi M, Balestrieri P, et al. Role of overweight and obesity in gastrointestinal disease. *Nutrients* (2019) 12(1):111. doi: 10.3390/nu12010111

66. Liu T, Wang W, Ji Y, Wang Y, Liu X, Cao L, et al. Association between different combination of measures for obesity and new-onset gallstone disease. *PloS One* (2018) 13(5):e0196457. doi: 10.1371/journal.pone.0196457

67. Ruhl CE, Everhart JE. Relationship of serum leptin concentration and other measures of adiposity with gallbladder disease. *Hepatology* (2001) 34(5):877–83. doi: 10.1053/jhep.2001.29005

68. Goldblatt MI, Swartz-Basile DA, Svatek CL, Nakeeb A, Pitt HA. Decreased gallbladder response in leptin-deficient obese mice. *J Gastrointestinal Surgery* (2002) 6(3):438–44. doi: 10.1016/S1091-255X(01)00046-4

69. Hyogo H, Roy S, Paigen B, Cohen DE. Leptin promotes biliary cholesterol elimination during weight loss in ob/ob mice by regulating the enterohepatic circulation of bile salts. *J Biol Chem* (2002) 277(37):34117–24. doi: 10.1074/jbc.M203912200

70. Diehl AK. Cholelithiasis and the insulin resistance syndrome. *Hepatology* (2000) 31(2):528–30. doi: 10.1002/hep.510310238

71. Tsai C-J, Leitzmann M, Willett WC, Giovannucci E. Central adiposity, regional fat distribution, and the risk of cholecystectomy in women. *Gut* (2006) 55(5):708–14. doi: 10.1136/gut.2005.076133

72. Heart N, Lung, Institute B, Diabetes NIo, Diseases K, et al. Clinical guidelines on the identification, evaluation, and treatment of overweight and

obesity in adults: the evidence report. In: *National institutes of health, national heart, lung, and blood institute* (1998).

73. Cortez-Pinto H, Camilo M, Baptista A, De Oliveira A, De Moura M. Non-alcoholic fatty liver: another feature of the metabolicsyndrome? *Clin Nutr* (1999) 18 (6):353–8. doi: 10.1016/S0261-5614(99)80015-6

74. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* (2009) 29(1):113–9. doi: 10.1111/j.1478-3231.2008.01718.x

75. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Digestive Dis Sci* (2000) 45(10):1929–34. doi: 10.1023/A:1005661516165

76. López-Suárez A, Guerrero JMR, Elvira-González J, Beltrán-Robles M, Cañas-Hormigo F, Bascuñana-Quirell A. Nonalcoholic fatty liver disease is associated with blood pressure in hypertensive and nonhypertensive individuals from the general population with normal levels of alanine aminotransferase. *Eur J Gastroenterol Hepatology* (2011) 23(11):1011–7. doi: 10.1097/MEG.0b013e32834b8d52

77. Eslam M, Sanyal AJ, George J, Sanyal A, Neuschwander-Tetri B, Tiribelli C, et al. 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

78. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* (2018) 67(1):123–33. doi: 10.1002/hep.29466

79. Albhaisi S, Chowdhury A, Sanyal AJ. Non-alcoholic fatty liver disease in lean individuals. *JHEP Rep* (2019) 1(4):329–41. doi: 10.1016/j.jhepr.2019.08.002

80. Lu Y, Hu L, Song J, Wan J, Chen H, Yin J. Gallstone disease and nonalcoholic fatty liver disease in patients with type 2 diabetes: a cross-sectional study. *BMC Endocrine Disord* (2021) 21(1):1–8. doi: 10.1186/s12902-021-00899-z

81. Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: a call to action. *Diabetes Care* (2017) 40(3):419–30. doi: 10.2337/dc16-1787

82. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease–meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* (2016) 64(1):73–84. doi: 10.1002/hep.28431

83. Zou B, Yeo Y, Nguyen V, Cheung R, Ingelsson E, Nguyen M. Prevalence, characteristics and mortality outcomes of obese, nonobese and lean NAFLD in the united states, 1999–2016. *J Internal Med* (2020) 288(1):139–51. doi: 10.1111/joim.13069

84. 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(4):748–57. doi: 10.1111/liv.14038

85. Cortés V, Quezada N, Uribe S, Arrese M, Nervi F. Effect of cholecystectomy on hepatic fat accumulation and insulin resistance in non-obese Hispanic patients: a pilot study. *Lipids Health disease* (2017) 16(1):1–7. doi: 10.1186/s12944-017-0525-3

86. Amigo L, Husche C, Zanlungo S, Lütjohann D, Arrese M, Miquel JF, et al. Cholecystectomy increases hepatic triglyceride content and very-low-density lipoproteins production in mice. *Liver Int* (2011) 31(1):52–64. doi: 10.1111/j.1478-3231.2010.02361.x

87. Kakati D, Kumar U, Russ K, Shoreibah M, Kuo Y-F, Jackson B, et al. Cholecystectomy does not worsen progression or outcomes in non-alcoholic fatty liver disease. *Trans Gastroenterol hepatology* (2020) 5:3–3. doi: 10.21037/tgh.2019.09.03

88. Sakurai Y, Kubota N, Yamauchi T, Kadowaki T. Role of insulin resistance in MAFLD. *Int J Mol Sci* (2021) 22(8):4156. doi: 10.3390/ijms22084156

89. Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. *Nat Rev Endocrinology* (2011) 7(8):456–65. doi: 10.1038/nrendo.2011.72

90. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased *de novo* lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* (2014) 146(3):726–35. doi: 10.1053/j.gastro.2013.11.049

91. Grundy SM. Cholesterol gallstones: a fellow traveler with metabolic syndrome. *Oxford Univ Press;* (2004) . p:1–2. doi: 10.1093/ajcn/80.1.1

92. Scragg R, Calvert GD, Oliver JR. Plasma lipids and insulin in gall stone disease: a case-control study. *Br Med J (Clin Res Ed)* (1984) 289(6444):521–5. doi: 10.1136/bmj.289.6444.521

93. Biddinger SB, Haas JT, Yu BB, Bezy O, Jing E, Zhang W, et al. Hepatic insulin resistance directly promotes formation of cholesterol gallstones. *Nat Med* (2008) 14(7):778–82. doi: 10.1038/nm1785

94. Uppal H, Zhai Y, Gangopadhyay A, Khadem S, Ren S, Moser JA, et al. Activation of liver X receptor sensitizes mice to gallbladder cholesterol crystallization. *Hepatology* (2008) 47(4):1331–42. doi: 10.1002/hep.22175

95. Donepudi AC, Ferrell JM, Boehme S, Choi HS, Chiang JYL. Deficiency of cholesterol 7α-hydroxylase in bile acid synthesis exacerbates alcohol-induced liver injury in mice. *Hepatol Commun* (2018) 2(1):99–112. doi: 10.1002/hep4.1129

96. Zhou S-N, Lu S-S, Ju D-W, Yu L-X, Liang X-X, Xiang X, et al. A new prognostic model covering all stages of intrahepatic cholangiocarcinoma. *J Clin Trans Hepatology* (2022) 10(2):254. doi: 10.14218/JCTH.2021.00099

97. Kistler KD, Brunt EM, Clark JM, Diehl AM, Sallis JF, Schwimmer JB. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol* (2011) 106(3):460–8. doi: 10.1038/ajg.2010.488

98. Raza S, Rajak S, Upadhyay A, Tewari A, Anthony Sinha R. Current treatment paradigms and emerging therapies for NAFLD/NASH. *Front Biosci (Landmark Ed)* (2021) 26(2):206–37. doi: 10.2741/4892

99. Swarne E, Srikanth M, Shreyas A, Desai S, Mehdi S, Gangadharappa H, et al. Recent advances, novel targets and treatments for cholelithiasis; a narrative review. *Eur J Pharmacol* (2021) 908:174376. doi: 10.1016/j.ejphar.2021.174376

100. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. *Eur J Epidemiol* (2005) 20(7):575–9. doi: 10.1007/s10654-005-7835-x

101. Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. *Eur J Epidemiol* (2011) 26(6):433–8. doi: 10.1007/s10654-011-9554-9

102. Liu Z, Xu J, Que S, Geng L, Zhou L, Mardinoglu A, et al. Recent progress and future direction for the application of multiomics data in clinical liver transplantation. *J Clin Trans Hepatology* (2022) 10(2):363. doi: 10.14218/JCTH.2021.00219

103. Liu Z, Zhao J, Wang W, Zhu H, Qian J, Wang S, et al. Integrative network analysis revealed genetic impact of pyruvate kinase L/R on hepatocyte proliferation and graft survival after liver transplantation. *Oxid Med Cell Longevity* (2021) 2021:1–31. doi: 10.1155/2021/7182914

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Visit-to-visit variability in triglyceride-glucose index and diabetes: A 9-year prospective study in the Kailuan Study

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Instruction/Aims: It is unknown whether variability in the triglyceride-glucose index (TyG-index) is associated with the risk of diabetes. Here, we sought to characterize the relationship between TyG-index variability and incident diabetes.

Methods: We performed a prospective study of 48,013 participants in the Kailuan Study who did not have diabetes. The TyG-index was calculated as ln [triglyceride (TG, mg/dL) concentration × fasting blood glucose concentration (FBG, mg/dL)/2]. The TyG-index variability was assessed using the standard deviation (SD) of three TyG-index values that were calculated during 2006/07, 2008/09, and 2010/11. We used the Cox proportional hazard models to analyze the effect of TyG-index variability on incident diabetes.

Results: A total of 4,055 participants were newly diagnosed with diabetes during the study period of 8.95 years (95% confidence interval (CI) 8.48–9.29 years). After adjustment for confounding factors, participants in the highest and second-highest quartiles had significantly higher risks of new-onset diabetes *versus* the lowest quartile, with hazard ratios (95% CIs) of 1.18 (1.08–1.29) and 1.13 (1.03–1.24), respectively (*P* trend< 0.05). These higher risks remained after further adjustment for the baseline TyG-index.

Conclusions: A substantial fluctuation in TyG-index is associated with a higher risk of diabetes in the Chinese population, implying that it is important to maintain a normal and consistent TyG-index.

KEYWORDS

triglyceride-glucose index, variability, diabetes mellitus, cohort study, insulin resistance

Introduction

Owing to socioeconomic advances and rising standards of living, the prevalence of diabetes mellitus in China has risen sharply over the past four decades, from 0.67% in 1980 to 12.8% in 2018 (1, 2). There were 140.9 million people in China with diabetes in 2019, and in 2045, the number is predicted to reach 174.4 million (3). Furthermore, diabetes is a risk factor for cardiocerebrovascular events, renal dysfunction, and overall morality (4–7), which have a major impact on society and the economy. Insulin resistance is a key pathogenetic feature of diabetes (8, 9), which is characterized by various metabolic disorders, including hyperglycemia and hypertriglyceridemia (10). Thus, it is essential to identify and control insulin resistance early to prevent diabetes.

The assessment of insulin resistance in the clinical setting is challenging because the gold standard method of the euglycemic clamp is expensive and relatively complex (11). Instead, the triglyceride-glucose (TyG) index, which is the product of the fasting blood glucose (FBG) and the fasting triglyceride (TG) concentration, has become established as reliable surrogate marker of insulin resistance (12, 13). Several studies have shown a link between a high TyG-index and diabetes (14–16). Furthermore, cohort studies conducted in European, Korean, and Chinese populations have revealed that a high TyG-index level is also associated with subsequent incident cardiovascular disease (CVD) (17–19). Although most previous studies of this index considered single measurements, it can be affected by several factors, such as age, diet, and exercise (20). The variability of the TyG index can reflect the long-term level of fluctuation (21). Therefore, in the present study, we aimed to test the hypothesis that high TyG-index variability is associated with the risk of diabetes-related outcomes in the Chinese population.

Materials and methods

Study sample

We studied data from the Kailuan Study, an ongoing prospective cohort study (22). This comprised information regarding 101,150 individuals who were enrolled to participate in a biennial questionnaire-based interview, which covered their demographic characteristics, medical history, and lifestyle; to undergo clinical examinations; and to undergo the measurement of laboratory parameters between 2006 and 2007. For the present study, the participants were required to have undergone two consecutive medical examinations during 2008/09 and 2010/11 to be eligible. Participants were excluded if they had diabetes in or prior to 2010, or if their FBG or TG data were missing for any of the examinations. After the application of these criteria, 48,013 participants remained for enrollment in the present study (Figure 1). The first survey, during 2006/07, was defined as the baseline survey, and the third survey (2010/11) as the starting point of the follow-up period.

All the participants gave their written informed consent and the study protocol was approved by the Ethics Committee of the Kailuan General Hospital (approval number: 2006-05).

TyG index and the calculation of TyG index variability

The TyG index was calculated as ln [TG (mg/dL) × FBG (mg/dL)/2] (23). TyG index variability was defined as the intra-individual variability of the TyG index, calculated using data collected during the three physical examinations. Four indices of variability were used:

(1) standard deviation (SD): $SD = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \overline{x})^2}$;

(2) coefficient of variation (CV): CV = (SD/mean × 100%);

(3) variation independent of the mean (VIM) (24, 25): VIM = $SD/mean^{\chi}$, where "mean" is the average of the mean TyG index values for the participants, and χ is derived from non-linear regression analysis in the PROC NLIN procedure of the SAS package (SAS Institute Inc., Cary, NC, USA);

(4) average real variability (ARV) (21): $ARV = \frac{1}{N-1}\sum_{K=1}^{N-1}|Value_{K+1} - Value_k|$.; and

(5) Slope of the TyG index change: regression lines were created using the three sets of TyG index data, and the slope of this regression line represented the overall trend in TyG index. This was used as an index of the long-term change in the TyG index. In the present study, a slope of the change in TyG index > 0 indicated overall positive variation, and a slope ≤0 indicated overall negative variation.

As previously described (26, 27), we placed the participants into four groups according to quartiles of the baseline SD of the

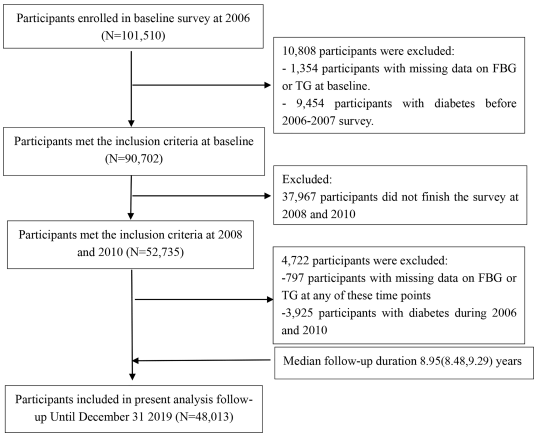

FIGURE 1
Flow chart for the inclusion of participants in the study.

TyG index: a Q1 group,<0.18; a Q2 group, 0.18–0.30; a Q3 group, 0.30–0.44; and a Q4 group, ≥0.44.

Outcome events

The outcome of the present study was new-onset diabetes, which has been defined previously in detail (28). Briefly, diabetes (29) was defined using an FBG of ≥7.0 mmol/L, the use of glucose-lowering drugs, or a self-reported history of diabetes. Participants were followed from their third examination, during 2010/11, to the first of the date on which diabetes was first diagnosed, the date of death or December 31, 2019.

Assessment of covariates

The demographic data (e.g., age, sex, and educational background), lifestyle (smoking, alcohol consumption, and physical activity habits) and medical history (hypertension and diabetes) of the participants were collected using questionnaires completed at face-to-face interviews. BMI was calculated as body mass (kg) divided by the square of height (m). Height, body mass, and blood pressure were measured by trained physicians using a standardized protocol.

Participants were instructed to visit the testing site in the morning after at least 8 hours of fasting and blood samples were collected from a cephalic vein by a trained laboratory technician. An automatic biochemical analyzer (7600-020, Hitachi, Tokyo, Japan) was used to measure the FBG, TG, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and high-sensitivity C-reactive protein (hs-CRP) concentrations. A current smoker was defined as someone who had smoked a mean of ≥ 1 cigarette per day during the preceding year, and participants were categorized as non-smokers or current smokers. An alcohol consumer was defined as someone who drank a mean of ≥ 100 mL of alcohol per day for at least the preceding year, and participants were categorized as non-drinkers or current drinkers. Participants were categorized as undertaking physical exercise if they performed exercise ≥ 3 times per week for ≥ 30 min on each occasion (30). Education was classified as high school or above *vs.* below high school level. Hypertension (31) was defined as a blood pressure ≥140/90 mmHg, the use of antihypertensive medication, or a self-reported history of hypertension.

Statistical analysis

Normally distributed, continuous data are expressed as mean ± standard deviation ($\bar{x} \pm s$) and non-normally distributed data as median (25%, 75% percentile), and were analyzed using one-way ANOVA or the Kruskal-Wallis rank sum test, respectively. Categorical data are expressed as absolute number and percentage and were analyzed using the chi-square test.

We used the Kaplan–Meier method to calculate the cumulative incidence of the primary outcome in each group and then compared the groups using the log-rank test. We also used univariate and multivariate Cox regression models to identify potential risk factors for diabetes. The relationship between TyG index variability and diabetes was characterized using Cox proportional hazards regression models. In model 1, we adjusted for age (continuous) and sex (categorical) at baseline. In model 2, we further adjusted for LDL-C (continuous), HDL-C (continuous), hs-CRP (continuous), BMI (continuous), smoking status (categorical, yes/no), alcohol consumption status (categorical, yes/no), physical exercise habits (categorical, yes/no), educational level (categorical), hypertension (categorical, yes/no), and the use of lipid-lowering medication (categorical, yes/no) at the start of the follow-up period. In model 3, we further adjusted for the TyG index at baseline.

We further conducted stratified analyses by the sex, age, and slope of the change in the TyG index of the participants. Several sensitivity analyses were conducted as follows: (1) after the exclusion of participants in whom diabetes developed within the first year of follow-up; (2) after the exclusion of participants who were taking lipid-lowering or antihypertensive medication; (3) after the exclusion of participants with a TG concentration ≥ 2.3 mmol/L at baseline; (4) adjusting for the baseline TG and FBG concentrations and without the inclusion of the baseline TyG-index; and (5) using other indices of TyG-index variability (ARV, CV, and VIM) instead of SD. We also repeated the analyses using Cox proportional hazards models. A two-sided $P< 0.05$ was considered to be statistically significant. We used SAS (version 9.4, SAS Institute Inc.) for the statistical analyses.

Results

Baseline characteristics of the study sample

A total of 48,013 participants were selected for the study. Their mean age was 48.78 ± 12.05 years and 36,356 (75.72%) were male. Compared with the Q1 group, the Q2 and Q3 groups had much higher BMI, SBP, DBP, TG, FBG, hs-CRP; and had higher prevalences of smoking, drinking, and hypertension ($P<0.01$; Table 1).

Results of the univariate and multivariate Cox regression analyses to identify risk factors for diabetes

Univariate Cox proportional-hazards regression showed that TyG index variability, age, sex, SBP, DBP, TyG-index, LDL-C, HDL-C, hs-CRP, BMI, smoking status, educational level,

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TABLE 1 Baseline characteristics of participants by TyG index variability quartiles (SD).

	Total	Q1	Q2	Q3	Q4	*P*
Participants	48013	12003	12003	12004	12003	
Age(years)	48.78 ± 12.05	50.09 ± 12.31	49.36 ± 12.09	48.85 ± 12.05	46.82 ± 11.51	<.01
Male, N (%)	36356 (75.72)	8805(73.36)	8978(74.80)	8989 (74.88)	9584 (79.85)	<.01
BMI (kg/m^2)	24.84 ± 3.12	24.76 ± 3.15	24.81 ± 3.18	24.87 ± 3.15	24.92 ± 3.00	<.01
SBP (mmHg)	128.55 ± 16.75	128.36 ± 16.93	128.44 ± 16.91	128.66 ± 16.92	128.73 ± 16.22	0.07
DBP (mmHg)	83.19 ± 9.18	82.82 ± 9.15	82.94 ± 9.15	83.25 ± 9.25	83.77 ± 9.15	<.01
HDL-C (mmol/L)	1.54 ± 0.32	1.55 ± 0.32	1.55 ± 0.32	1.54 ± 0.31	1.53 ± 0.32	<.01
LDL-C (mmol/L)	2.48 ± 0.63	2.50 ± 0.63	2.50 ± 0.63	2.48 ± 0.64	2.44 ± 0.63	<.01
FBG (mmol/L)	5.24 ± 0.52	5.09 ± 0.32	5.14 ± 0.60	5.35 ± 0.38	5.49 ± 0.47	<.01
TG (mmol/L)	1.30 (0.96-1.87)	1.21(0.88-1.58)	1.27(0.95-1.81)	1.32 (1.01–1.75)	1.69 (1.14-2.48)	<0.01
Hs-CRP (mg/L)	1.42 (0.76-2.83)	1.37 (0.73-2.70)	1.40 (0.76-2.71)	1.43 (0.77-2.92)	1.46 (0.78-3.06)	<0.01
TyG index$_{2006}$	8.55 ± 0.63	8.49 ± 0.50	8.52 ± 0.54	8.52 ± 0.60	8.71 ± 0.77	<.01
TyG index$_{2008}$	8.57 ± 0.62	8.51 ± 0.50	8.49 ± 0.53	8.56 ± 0.60	8.69 ± 0.80	<.01
TyG index$_{2010}$	8.61 ± 0.61	8.50 ± 0.51	8.56 ± 0.53	8.64 ± 0.59	8.90 ± 0.75	<.01
Smoking, N (%)	18360 (38.24)	4296.0 (35.79)	4423 (36.85)	4680 (38.99)	4961 (41.33)	<.01
Drinking, N (%)	16957 (35.32)	3948 (32.89)	4101 (34.17)	4188 (34.89)	4720 (39.32)	<.01
Physical activity, N (%)	6906 (14.38)	1944 (16.20)	1817 (15.14)	1697 (14.14)	1448 (12.06)	<.01
Hypertension, N (%)	22072 (45.97)	5391 (44.91)	5444 (45.36)	5563 (46.34)	5674 (47.27)	<.01
Antihypertensive drugs, N (%)	6944 (14.46)	1654 (13.78)	1715 (14.29)	1738 (14.48)	1837 (15.30)	<.01
Lipid-lowering drugs, N (%)	728 (1.52)	186 (1.55)	178 (1.48)	165 (1.37)	199 (1.66)	0.33
High school or above, N (%)	6217 (12.95)	1599 (13.32)	1606 (13.38)	1583 (13.19)	1429 (11.91)	<.01

P, comparison of baseline characteristics between different TyG index variability groups.
TyG index, triglyceride-glucose index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; hs-CRP, high-sensitivity C reactive protein.

physical activity habits, hypertension, and the use of lipid-lowering drugs were significantly associated with diabetes ($P<0.05$, Table 2).

Relationship between TyG-index variability and incident diabetes

During the mean follow-up period of 8.95 years (95% confidence interval (CI) 8.48–9.29 years), 4,055 (8.45%) of the participants developed diabetes. The incidence of diabetes increased with increasing TyG-index variability quartile, from 8.80 in Q1 to 11.70 per 1,000 person-years in Q4 (Tables 2, 3). Figure 2 shows that the participants in Q4 had a higher cumulative incidence of diabetes than those in Q1 (log-rank test, $P<0.01$). Tables 2, 3 shows the risk of incident diabetes according to the category of TyG-index variability, and the hazard ratio (HR) (95% CI) for Q4 *versus* Q1 was 1.34 (1.23–1.47) after adjustment for potential confounding factors. This association remained even after adjustment for the baseline TyG-index (HR 1.18, 95% CI 1.08–1.29). Each 1-SD increase in the SD of TyG-index variability was associated with a 4% higher risk of diabetes (HR 1.04, 95% CI, 1.01–1.07). In addition, similar results were obtained when the variability in the TyG-index was assessed using the ARV, CV, and VIM (Figure 3).

Results of the stratified and sensitivity analyses

Table 4 shows the results of the stratified analyses. In general, high TyG-index variability (group Q4) was significantly associated with a higher risk of diabetes across the various stratified groups. There were no significant effects of age, sex, or the slope of the change in the TyG index on the association between TyG-index variation and incident diabetes.

With respect to the sensitivity analyses, the results of excluding outcome events occurring within the first year of follow-up, individuals taking lipid-lowering or antihypertensive medication, or individuals with TG ≥ 2.3 mmol/L at baseline were consistent with the results of the principal analysis. Because the SD may depend upon the mean value for each person, we also reanalyzed the data using other indices of TyG-index variability (ARV, CV, and VIM) in place of SD, but the findings were unaffected (Table 5).

Discussion

In the present study, we have shown that high variability in the TyG-index is an independent risk factor for incident diabetes, even in individuals who are not taking

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TABLE 2 Risk factors for diabetes were analyzed by univariate and multivariate Cox regression analysis.

	Univariate Cox regression analyses		Multivariate Cox regression analyses	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
TyG index variability	1.08 (1.07,1.10)	<0.01	1.06 (1.03,1.09)	<0.01
Age	1.01 (1.01,1.02)	<0.01	1.01 (1.00,1.01)	<0.01
Gender	1.22 (1.13,1.32)	<0.01	0.98 (0.90,1.07)	0.68
BMI	1.16 (1.15,1.17)	<0.01	1.12 (1.10,1.13)	<0.01
SBP	1.04 (1.04,1.05)	<0.01	/	/
DBP	1.06 (1.05,1.06)	<0.01	/	/
HDL-C	0.63 (0.56,0.70)	<0.01	0.82 (0.74,0.92)	<0.01
LDL-C	1.24 (1.19,1.30)	<0.01	1.11 (1.06,1.16)	<0.01
hs-CRP	1.03 (1.02,1.03)	<0.01	1.01 (1.00,1.02)	<0.01
TyG $index_{2006}$	1.95 (1.87,2.05)	<0.01	1.82 (1.72,1.91)	<0.01
Current smoking	1.06 (1.00,1.13)	<0.01	1.03 (0.95,1.11)	0.50
Current drinker	1.02 (0.95,1.08)	0.12	0.96 (0.89,1.04)	0.31
Physical activity	0.94 (0.90,0.98)	<0.01	0.94 (0.86,1.03)	0.17
Hypertension	1.90 (1.79,2.02)	<0.01	1.29 (1.21,1.38)	<0.01
education	0.74 (0.69,0.78)	<0.01	0.80 (0.75,0.85)	<0.01
Lipid-lowering drugs	1.85 (1.53,2.24)	<0.01	1.22 (1.00,1.48)	0.04

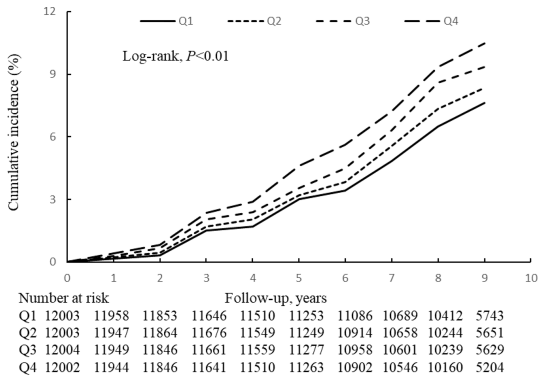

FIGURE 2
Kaplan-Meier incidence rate of diabetes by TyG-index variability (SD).

antihypertensive or lipid-lowering medication and do not have a TG concentration ≥ 2.3 mmol/L by means of a longitudinal cohort study.Several previous studies have evaluated the relationship of a single TyG-index value with diabetes in the general population (32–36). For example, a 9-year follow-up study showed that individuals with the highest TyG indexes were at a 2.30-fold higher risk of developing diabetes (37). In the China Health and Retirement Longitudinal Study, which involved 3.4 years of follow-up, every 1-SD increase in TyG index was associated with a 22% increase in the risk of developing diabetes (HR 1.22, 95% CI 1.14–1.31) in Chinese people of 45 years or above (36). In addition, TyG-index is positively associated with CVD in patients with diabetes (38). The results of the present study extend these findings by showing that visit-to-visit fluctuation in TyG-index is positively associated with the incidence of diabetes in the general population, independent of conventional risk factors for diabetes and the baseline TyG index. This implies that both the absolute value and the fluctuation in the TyG-index influence the risk of incident diabetes in the general population.

We have previously shown that the risk of diabetes is lower after antihypertensive and lipid-lowering therapy (39, 40). Therefore, we repeated the analysis after excluding individuals who were taking antihypertensive or lipid-lowering drugs, but this did not affect the findings. In addition, because metabolic abnormalities, including a high circulating TG concentration, increase the risk of diabetes (41), we excluded participants with

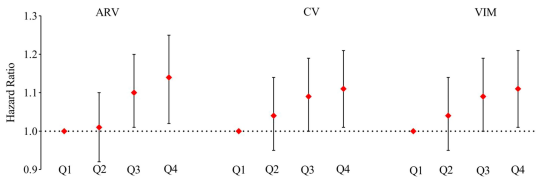

FIGURE 3
Sensitivity analysis of the association of TyG index variability with incident diabetes according to other indices of TyG-index variability (Average Real Variability, Coefficient of Variation, Variability Independent of the Mean) replacing Standard Deviation the in all the models. Model adjusted for age, sex, LDL-C, HDL-C, hs-CRP, BMI, smoking status, alcohol consumption status, physical exercise habits, educational level, hypertension, the use of lipid-lowering drugs, and TyG index.

TABLE 3 Hazard ratios and 95% Confidence intervals of incident diabetes of TyG index variability (SD).

	Case/Total	Incidence rate, per 1000 person-years	Model 1	Model 2	Model 3
Q1	891/12003	8.80	1.00	1.00	1.00
Q2	933/12003	9.18	1.05(0.96,1.15)	1.04(0.95,1.14)	1.04(0.95,1.14)
Q3	1042/12004	10.28	1.19(1.09,1.30)	1.16(1.06,1.27)	1.13(1.03,1.24)
Q4	1189/12003	11.70	1.39(1.27,1.51)	1.34(1.23,1.47)	1.18(1.08,1.29)
1-SD increase (0.22)			1.20(1.09,1.15)	1.11(1.08,1.15)	1.04(1.01,1.07)
P for Trend			<0.0001	<0.0001	<0.0001

Model 1: adjusted for age (continuous variable, years) and sex (categorical variable, men or women) in 2010.
Model2: included variables in model 1 and further LDL-C (as a continuous variable), HDL-C (as a continuous variable), hs-CRP (as a continuous variable), BMI (as a continuous variable), smoking status (as a categorical variable, yes or no), alcohol consumption status (as a categorical variable, yes or no), physical exercise habits (as a categorical variable, yes or no), educational level (as a categorical variable, high school or above vs. below high school level), hypertension (as a categorical variable, yes or no), and the use of lipid-lowering drugs (as a categorical variable, yes or no) in 2010.
Model 3: included variables in model 2 and further the TyG index (continuous variable) in baseline.

TABLE 4 Hazard ratios and 95% Confidence intervals of incident of TyG index in subgroup ratio variability (SD).

	Age (*P* for interaction 0.33)		Sex (*P* for interaction 0.16)		TyG index change slop (*P* for interaction 0.74)	
	<45 years	≥ 45 years	Female	Male	>0	≤0
Quartiles						
Q1	1.00	1.00	1.00	1.00	1.00	1.00
Q2	1.09 (0.92,1.30)	1.02 (0.93,1.12)	1.07 (0.88,1.30)	1.03 (0.93,1.15)	0.93 (0.81,1.05)	1.16 (1.01,1.32)
Q3	1.23 (1.06,1.50)	1.07 (0.96,1.19)	1.18 (0.98,1.43)	1.11 (1.01,1.24)	0.98 (0.94,1.01)	1.28 (1.13,1.46)
Q4	1.25 (1.06,1.46)	1.11 (1.00,1.24)	1.36 (1.12,1.65)	1.14 (1.02,1.25)	1.06 (1.01,1.11)	1.50 (1.32,1.70)
P for trend	<0.01	0.04	0.02	<0.01	<0.01	<0.01

Adjusted for age (continuous variable, years), sex (categorical variable, men or women), LDL-C (as a continuous variable), HDL-C (as a continuous variable), hs-CRP (as a continuous variable), BMI (as a continuous variable), smoking status (as a categorical variable, yes or no), alcohol consumption status (as a categorical variable, yes or no), physical exercise habits (as a categorical variable, yes or no), educational level (as a categorical variable, high school or above vs. below high school level), hypertension (as a categorical variable, yes or no), the use of lipid-lowering drugs (as a categorical variable, yes or no) in 2010, and TyG index (continuous variable) in baseline.

TABLE 5 Sensitivity analysis of the association of TyG index variability with incident diabetes.

	Sensitivity analysis 1	Sensitivity analysis 2	Sensitivity analysis 3	Sensitivity analysis 4
Q1	1.00	1.00	1.00	1.00
Q2	1.04 (0.95,1.14)	1.02 (0.93,1.12)	1.11 (0.98,1.26)	1.03 (0.94,1.13)
Q3	1.13 (1.03,1.24)	1.13 (1.03,1.24)	1.17 (1.03,1.33)	1.13 (1.03,1.23)
Q4	1.18 (1.08,1.29)	1.19 (1.06,1.28)	1.42 (1.25,1.62)	1.22 (1.12,1.34)
P for Trend	<0.0001	0.0002	<0.0001	<0.0001

Adjusted for age (continuous variable, years), sex (categorical variable, men or women), LDL-C (as a continuous variable), HDL-C (as a continuous variable), hs-CRP (as a continuous variable), BMI (as a continuous variable), smoking status (as a categorical variable, yes or no), alcohol consumption status (as a categorical variable, yes or no), physical exercise habits (as a categorical variable, yes or no), educational level (as a categorical variable, high school or above vs. below high school level), hypertension (as a categorical variable, yes or no), the use of lipid-lowering drugs (as a categorical variable, yes or no, except sensitivity analysis 2), and TyG index (continuous variable) in baseline.
Sensitivity analysis 1: the exclusion of participants in whom diabetes developed within the first year of follow-up.
Sensitivity analysis 2: the exclusion of participants who were taking lipid-lowering or antihypertensive medication.
Sensitivity analysis 3: the exclusion of participants with TG ≥ 2.3 mmol/L in baseline.
Sensitivity analysis 4: adjusting for the baseline TG and FBG and without the inclusion of the baseline TyG-index.

TG ≥ 2.3 mmol/L, but the results obtained were similar. Therefore, our findings emphasize the importance of regular monitoring and the maintenance of an appropriate TyG-index to prevent diabetes in the general population, even in individuals who are not taking antihypertensive or lipid-lowering medication and in those who do not have a TG concentration ≥ 2.3 mmol/L.

Although the mechanism linking high TyG-index variability with the development of diabetes has not been identified, there are several possible candidates. First, TyG is an index created using the fasting TG concentration and FBG (17, 23); therefore, high variability in TyG may be derived from large fluctuations in serum TG and/or FBG, which are associated with vascular

endothelial cell dysfunction, oxidative stress, and inflammation (42–45), all of which are key pathophysiological features of diabetes (46). In addition, β-cell dysfunction is a key defect in the pathogenesis of diabetes (47), and aberrant glucose and lipid metabolism can lead to the apoptosis of β cells (48), which causes a deterioration of glycemic control and ultimately the development of diabetes.

The strengths of the present study include that it represents the first assessment of the relationship between the fluctuation in TyG index between clinic visits and the risk of developing diabetes, performed using data from a large, prospective cohort study. However, the study also had some limitations. First, we did not distinguish type 1 and type 2 diabetes mellitus in the present study. However, the Chinese diabetes guidelines state that type 2 diabetes currently accounts for 95% of all cases of diabetes (29) and that type 2 diabetes is more common in older people. Given that the mean age of the study participants was 48.78 years, the present findings are likely to be largely representative of the risk type 2 diabetes. Second, the observational design of the study prevents the confirmation of a causal relationship between the variability in TyG index and diabetes. However, when we excluded individuals who developed diabetes within a year, the results were similar. Third, we did not assess the changes in blood glucose using other methods, such as the measurement of glycated hemoglobin or continuous blood glucose monitoring. Fourth, despite adjusting for potential risk factors for cardiovascular disease, because the study was an observational cohort study, other sources of residual or unmeasured confounding may still have existed, such as differences in diet.

In conclusion, we have shown that TyG-index variability is an independent risk factor for new-onset diabetes, which implies that TyG-index should be maintained to prevent the development of diabetes.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Kailuan General Hospital Ethics Committee (approval number: 2006-05). The patients/participants provided their written informed consent to participate in this study.

Author contributions

XW, and YaC wrote the main manuscript text and conceived and designed the study. ZH analyzed the data. ZCa, and XY carried out literature search. ZCh and GC were responsible for developing the first draft of the manuscript. LL, KW, and HZ were responsible for developing the second draft of the manuscript. SW and YoC performed the manuscript review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

References

1. Zhong XL. Diabetes mellitus survey in China. *Chin Med J (Engl)* (1982) 95 (6):423–30.

2. 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

3. International Diabetes Federation. IDF Diabetes Atlas te, 2019 (2019). Available at: https://www.diabetesatlas.org/en/ (Accessed 18 November 2019).

4. Bragg F, Holmes MV, Iona A, Guo Y, Du H, Chen Y, et al. Association between diabetes and cause-specific mortality in rural and urban areas of China. *Jama* (2017) 317(3):280–9. doi: 10.1001/jama.2016.19720

5. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* (2010) 375 (9733):2215–22. doi: 10.1016/s0140-6736(10)60484-9

6. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *Jama* (2002) 287(19):2570–81. doi: 10.1001/jama.287.19.2570

7. Rawshani A, Rawshani A, Franzén S, Eliasson B, Svensson AM, Miftaraj M, et al. Mortality and cardiovascular disease in type 1 and type 2 diabetes. *N Engl J Med* (2017) 376(15):1407–18. doi: 10.1056/NEJMoa1608664

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

9. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia* (2003) 46 (1):3–19. doi: 10.1007/s00125-002-1009-0

10. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* (2016) 126(1):12–22. doi: 10.1172/JCI77812

11. Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J, Triplitt CL. Assessment of pancreatic β-cell function: review of methods and clinical applications. *Curr Diabetes Rev* (2014) 10(1):2–42. doi: 10.2174/1573399810666140214093600

12. Abbasi F, Reaven GM. Comparison of two methods using plasma triglyceride concentration as a surrogate estimate of insulin action in nondiabetic subjects: triglycerides × glucose versus triglyceride/high-density lipoprotein cholesterol. *Metabolism* (2011) 60(12):1673–6. doi: 10.1016/j.metabol.2011.04.006

13. Toro-Huamanchumo CJ, Urrunaga-Pastor D, Guarnizo-Poma M, Lazaro-Alcantara H, Paico-Palacios S, Pantoja-Torres B, et al. Triglycerides and glucose index as an insulin resistance marker in a sample of healthy adults. *Diabetes Metab Syndr* (2019) 13(1):272–7. doi: 10.1016/j.dsx.2018.09.010

14. Park B, Lee HS, Lee YJ. Triglyceride glucose (TyG) index as a predictor of incident type 2 diabetes among nonobese adults: a 12-year longitudinal study of the Korean genome and epidemiology study cohort. *Transl Res* (2021) 228:42–51. doi: 10.1016/j.trsl.2020.08.003

15. Lee SH, Kwon HS, Park YM, Ha HS, Jeong SH, Yang HK, et al. Predicting the development of diabetes using the product of triglycerides and glucose: the chungju metabolic disease cohort (CMC) study. *PloS One* (2014) 9(2):e90430. doi: 10.1371/journal.pone.0090430

16. Lee JW, Lim NK, Park HY. The product of fasting plasma glucose and triglycerides improves risk prediction of type 2 diabetes in middle-aged koreans. *BMC Endocr Disord* (2018) 18(1):33. doi: 10.1186/s12902-018-0259-x

17. Wang X, Feng B, Huang Z, Cai Z, Yu X, Chen Z, et al. Relationship of cumulative exposure to the triglyceride-glucose index with ischemic stroke: a 9-year prospective study in the kailuan cohort. *Cardiovasc Diabetol* (2022) 21(1):66. doi: 10.1186/s12933-022-01510-y

18. Hong S, Han K, Park CY. The triglyceride glucose index is a simple and low-cost marker associated with atherosclerotic cardiovascular disease: a population-based study. *BMC Med* (2020) 18(1):361. doi: 10.1186/s12916-020-01824-2

19. Sánchez-Íñigo L, Navarro-González D, Fernández-Montero A, Pastrana-Delgado J, Martínez JA. The TyG index may predict the development of cardiovascular events. *Eur J Clin Invest* (2016) 46(2):189–97. doi: 10.1111/eci.12583

20. Wang A, Tian X, Zuo Y, Chen S, Meng X, Wu S, et al. Change in triglyceride-glucose index predicts the risk of cardiovascular disease in the general population: a prospective cohort study. *Cardiovasc Diabetol* (2021) 20 (1):113. doi: 10.1186/s12933-021-01305-7

21. Mena L, Pintos S, Queipo NV, Aizpúrua JA, Maestre G, Sulbarán T. A reliable index for the prognostic significance of blood pressure variability. *J Hypertens* (2005) 23(3):505–11. doi: 10.1097/01.hjh.0000160205.81652.5a

22. Wu S, Huang Z, Yang X, Zhou Y, Wang A, Chen L, et al. Prevalence of ideal cardiovascular health and its relationship with the 4-year cardiovascular events in a northern Chinese industrial city. *Circ Cardiovasc Qual Outcomes* (2012) 5(4):487–93. doi: 10.1161/CIRCOUTCOMES.111.963694

23. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab* (2010) 95 (7):3347–51. doi: 10.1210/jc.2010-0288

24. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* (2010) 375(9718):895–905. doi: 10.1016/S0140-6736(10)60308-X

25. Asayama K, Kikuya M, Schutte R, Thijs L, Hosaka M, Satoh M, et al. Home blood pressure variability as cardiovascular risk factor in the population of ohasama. *Hypertension* (2013) 61(1):61–9. doi: 10.1161/HYPERTENSIONAHA.111.00138

26. Li W, Huang Z, Fang W, Wang X, Cai Z, Chen G, et al. Remnant cholesterol variability and incident ischemic stroke in the general population. *Stroke* (2022) 53 (6):1934–41. doi: 10.1161/STROKEAHA.121.037756

27. Lau KK, Wong YK, Chang RS, Teo KC, Hon SF, Chan KH, et al. Visit-to-visit systolic blood pressure variability predicts all-cause and cardiovascular mortality after lacunar infarct. *Eur J Neurol* (2014) 21(2):319–25. doi: 10.1111/ene.12310

28. Zheng M, Zhang X, Chen S, Song Y, Zhao Q, Gao X, et al. Arterial stiffness preceding diabetes: A longitudinal study. *Circ Res* (2020) 127(12):1491–8. doi: 10.1161/CIRCRESAHA.120.317950

29. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* (2003) 26(11):3160–7. doi: 10.2337/diacare.26.11.3160

30. Zhao M, Song L, Sun L, Wang M, Wang C, Yao S, et al. Associations of type 2 diabetes onset age with cardiovascular disease and mortality: The kailuan study. *Diabetes Care* (2021) 44(6):1426–32. doi: 10.2337/dc20-2375

31. deRuiter W, Faulkner G. Tobacco harm reduction strategies: the case for physical activity. *Nicotine Tob Res* (2006) 8(2):157–68. doi: 10.1080/14622200500494823

32. Liu EQ, Weng YP, Zhou AM, Zeng CL. Association between triglyceride-glucose index and type 2 diabetes mellitus in the Japanese population: A secondary analysis of a retrospective cohort study. *BioMed Res Int* (2020), 2020:2947067. doi: 10.1155/2020/2947067

33. Pranata R, Huang I, Irvan Lim MA, Vania R. The association between triglyceride-glucose index and the incidence of type 2 diabetes mellitus-a systematic review and dose-response meta-analysis of cohort studies. *Endocrine* (2021) 74 (2):254–62. doi: 10.1007/s12020-021-02780-4

34. Sánchez-García A, Rodríguez-Gutiérrez R, Saldívar-Rodríguez D, Guzmán-López A, Mancillas-Adame L, González-Nava V, et al. Early triglyceride and glucose index as a risk marker for gestational diabetes mellitus. *Int J Gynaecol Obstet* (2020) 151(1):117–23. doi: 10.1002/ijgo.13311

35. Kim JA, Kim J, Roh E, Hong SH, Lee YB, Baik SH, et al. Triglyceride and glucose index and the risk of gestational diabetes mellitus: A nationwide population-based cohort study. *Diabetes Res Clin Pract* (2021) 171:108533. doi: 10.1016/j.diabres.2020.108533

36. Chen CL, Liu L, Lo K, Huang JY, Yu YL, Huang YQ, et al. Association between triglyceride glucose index and risk of new-onset diabetes among Chinese adults: Findings from the China health and retirement longitudinal study. *Front Cardiovasc Med* (2020) 7:610322. doi: 10.3389/fcvm.2020.610322

37. Park HM, Lee HS, Lee YJ, Lee JH. The triglyceride-glucose index is a more powerful surrogate marker for predicting the prevalence and incidence of type 2 diabetes mellitus than the homeostatic model assessment of insulin resistance. *Diabetes Res Clin Pract* (2021) 180:109042. doi: 10.1016/j.diabres.2021.109042

38. Wang L, Cong HL, Zhang JX, Hu YC, Wei A, Zhang YY, et al. Triglyceride-glucose index predicts adverse cardiovascular events in patients with diabetes and acute coronary syndrome. *Cardiovasc Diabetol* (2020) 19(1):80. doi: 10.1186/s12933-020-01054-z

39. Opie LH, Schall R. Old antihypertensives and new diabetes. *J Hypertens* (2004) 22(8):1453–8. doi: 10.1097/01.hjh.0000133732.24501.9e

40. Valensi P, Picard S. Lipids, lipid-lowering therapy and diabetes complications. *Diabetes Metab* (2011) 37(1):15–24. doi: 10.1016/j.diabet.2010.10.001

41. Samuelsson O, Pennert K, Andersson O, Berglund G, Hedner T, Persson B, et al. Diabetes mellitus and raised serum triglyceride concentration in treated hypertension–are they of prognostic importance? observational study. *Bmj* (1996) 313(7058):660–3. doi: 10.1136/bmj.313.7058.660

42. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Jama* (2006) 295(14):1681–7. doi: 10.1001/jama.295.14.1681

43. Horváth EM, Benko R, Kiss L, Murányi M, Pék T, Fekete K, et al. Rapid 'glycaemic swings' induce nitrosative stress, activate poly(ADP-ribose) polymerase and impair endothelial function in a rat model of diabetes mellitus. *Diabetologia* (2009) 52(5):952–61. doi: 10.1007/s00125-009-1304-0

44. Lee LL, Aung HH, Wilson DW, Anderson SE, Rutledge JC, Rutkowsky JM. Triglyceride-rich lipoprotein lipolysis products increase blood-brain barrier transfer coefficient and induce astrocyte lipid droplets and cell stress. *Am J Physiol Cell Physiol* (2017) 312(4):C500–c516. doi: 10.1152/ajpcell.00120.2016

45. Chen L, Chen R, Wang H, Liang F. Mechanisms linking inflammation to insulin resistance. *Int J Endocrinol* (2015) 2015, 508409. doi: 10.1155/2015/508409

46. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* (2013) 93(1):137–88. doi: 10.1152/physrev.00045.2011
47. Ashcroft FM, Rorsman P. Diabetes mellitus and the β cell: the last ten years. *Cell* (2012) 148(6):1160–71. doi: 10.1016/j.cell.2012.02.010
48. El-Assaad W, Joly E, Barbeau A, Sladek R, Buteau J, Maestre I, et al. Glucolipotoxicity alters lipid partitioning and causes mitochondrial dysfunction, cholesterol, and ceramide deposition and reactive oxygen species production in INS832/13 ss-cells. *Endocrinology* (2010) 151(7):3061–73. doi: 10.1210/en.2009-1238

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Study on insulin resistance and ischemic cerebrovascular disease: A bibliometric analysis *via* CiteSpace

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Background: It is reported that insulin resistance widely exists in non-diabetic patients with a recent history of transient ischemic attack (TIA) or ischemic stroke. There is currently strong evidence to prove the bidirectional effect of glucose metabolism disorders and stroke events. Therefore, it is necessary to retrospectively tease out the current status, hotspots, and frontiers of insulin resistance and ischemic cerebrovascular disease through CiteSpace.

Materials and methods: We searched the Web of Science (WOS) for studies related to insulin resistance and ischemic cerebrovascular disease from 1999 to April 2022, then downloaded the data into CiteSpace to generate a knowledge visualization map.

Results: A total of 1,500 publications relevant to insulin resistance and ischemic cerebrovascular disease were retrieved. The USA had the most articles on this topic, followed by PEOPLES R CHINA and JAPAN. WALTER N KERNAN was the most prolific author, whose research mainly focused on insulin resistance intervention after stroke (IRIS) trial. The most common keywords were myocardial ischemia, metabolic syndrome, ischemic stroke, cerebral ischemia, association, oxidative stress, inflammation, and adipose tissue. Major ongoing research trends include three aspects: (1) the association between insulin resistance and ischemic cerebrovascular disease in non-diabetic patients, (2) the intrinsic pathological mechanism between insulin resistance and ischemic cerebrovascular disease, and (3) early intervention of insulin resistance to improve the prognosis of stroke.

Conclusion: The results of this bibliometric study provide the current status and trends of clinical research publications in the field of insulin resistance and ischemic cerebrovascular disease. Insulin resistance is strongly associated with the occurrence of ischemic stroke, early neurological deterioration in stroke patients, post-stroke depression, and cerebral small vessel disease. Early treatment of insulin resistance can be an effective way to prevent the onset of ischemic stroke and improve stroke prognosis. This study may help researchers to identify hot topics and explore new research directions.

KEYWORDS

insulin resistance, ischemic cerebrovascular disease, association, oxidative stress, inflammation, CiteSpace

1. Introduction

Stroke is the second leading cause of death and the third leading cause of disability worldwide (1). An estimated 6.6 million Americans over the age of 20 have a stroke, occurring on average every 40 s and dying every 4 min (2, 3). In China, the mortality rate of stroke is as high as 149.49 per 100,000 (about 1.57 million deaths), accounting for 22.33% of the total deaths (4). Over the past decade, great advances have been made for cerebrovascular disease, many risk factors such as hypertension, diabetes, atrial fibrillation, smoking, alcohol abuse, obesity, and carotid stenosis have been found (5), and many effective methods have emerged for prevention and treatment, which include controlling blood pressure, blood lipids, blood sugar, anti-thrombotic therapy, smoking cessation, regular physical activity, etc. It does reduce the incidence, recurrence, and mortality rate of stroke events. However, about 790,000 individuals in the United States still have stroke events every year, with ischemic stroke accounting for 87% of all stroke events. Projections show that by 2030, an additional 3.4 million people aged ≥18 years will have had a stroke, a 20.5% increase in prevalence from 2012 (2, 3). This reminds us that some unrecognized and unappreciated vascular risk factors still need to be intervened early.

Insulin resistance is clinically defined as a decrease in glucose uptake and utilization capacity by exogenous or endogenous insulin when compared to the normal population (6). There is currently strong evidence to prove the bidirectional effect of glucose metabolism disorders and stroke events. Insulin resistance can be used as an early predictor and independent risk factor of cardiovascular and cerebrovascular events (7, 8), and stroke can aggravate glucose metabolism disorders and lead to insulin resistance (9). Some researchers have observed that rats with middle cerebral artery occlusion show decreased insulin secretory capacity and insulin sensitivity after 1 day of cerebral ischemia, accompanied by elevated fasting glucose and fasting insulin levels (10). The American Heart Association/American Stroke Association guidelines for the Prevention of Stroke in Patients with Stroke and Transient Ischemic Attack (TIA) published in 2021 state that ∼ 30% of ischemic stroke patients are pre-diabetic and 50% of non-diabetic ischemic stroke patients have insulin resistance (11–13). Another study has shown that impaired insulin sensitivity is very prevalent in non-diabetic patients with a recent TIA or non-disabling ischemic stroke (12). Such abnormalities in glycemic traits persist even after the stressful state has disappeared and can exacerbate neurological damage, leading to a poor prognosis. This reminds us that combing the relationship between insulin resistance and ischemic cerebrovascular disease can provide more therapeutic approaches for early intervention and reduce the incidence of stroke, as well as post-stroke adverse events. Therefore, it is necessary to analyze the current status, hotspots, and frontiers of insulin resistance and ischemic cerebrovascular disease visually by using CiteSpace (14).

CiteSpace is an information visualization software developed by Professor Chaomei Chen using Java language (15, 16), which is mainly used to explore the frontiers of discipline development and research status. This study included the literature related to insulin resistance and ischemic cerebrovascular disease, and analyze the research direction and hotspots knowledge by using CiteSpace software.

2. Materials and methods

2. 1. Search strategy

We searched for relevant studies using the following terms: (Lacunar infarction*) OR (Ischemic cerebrovascular disease*) OR (Ischemic*) OR (Brain Ischemia*) OR (Ischemic Encephalopathy*) OR (Cerebral Ischemia*) OR (Ischemic Stroke*) OR (Cryptogenic Stroke*) OR TIA OR (Cerebral Infarctions*) OR (Subcortical Infarction*) OR Transient Ischemic Attack and (Insulin resistance*). The type of literature is limited to "article" or "review," the language is limited to English, and the citation index is selected from the Web of Science Core Collection for the period 1999 to April 2022. Finally, a total of 1,905 articles were obtained.

2.2. Analysis tool–CiteSpace

Set up the CiteSpace folder with four subfolders: input, output, data, and project. The complete records and references of 1,905 articles retrieved from WOS were exported in plain text format, named "download_XX.txt," and then imported into the input folder for analysis in CiteSpace 5.8 R3 software. After eliminating the duplicate literature, 1,500 articles were left. Time slicing was performed from January 1999 to April 2022, years per slice was set to one, selection criteria top N was set to 50, pruning selected pathfinder, pruning sliced networks simplified atlas, node types selected author, institution, country, keywords, and reference.

The output results mainly include the annual publication analysis, countries and institutions cooperation analysis, co-authors analysis, co-occurring keywords and cluster analysis, keywords with citation bursts, and co-cited reference analysis. Relevant contents are visualized as knowledge maps, and key nodes and links in different maps are interpreted and analyzed (17). The node represents the analyzed research object. The higher the frequency of occurrence, the larger the range of nodes. The color and thickness of the node inner circle represent the frequency of occurrence in different periods. If the node has a purple outer circle, it represents a high betweenness centrality. Centrality reflects the role of the nodes in the knowledge network, nodes with high centrality (>0.1) were usually considered turning points or pivotal points in a field (15, 16). The links between the nodes represent the co-occurrence relationship, and the thickness of the links represents the strength of the co-occurrence. The more the links, the thicker, and the closer relationship between the nodes. CiteSpace provides module value (Q value) and average silhouette value (silhouette, S value) based on the network structure and clustering results. It is generally believed that a Q value > 0.3 (empirical value) means that the cluster structure is significant, an S value at 0.7 clusterings has certain significance and credibility, and above 0.5 that clustering is generally reasonable. The larger the cluster structure, the smaller the cluster ID (18).

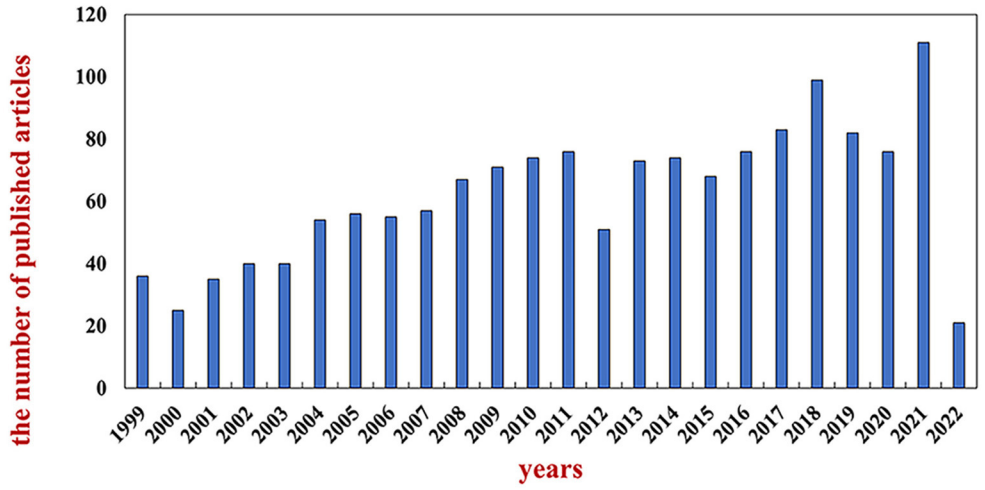

FIGURE 1
Annual trend chart of publications.

3. Results

3.1. Annual publications analysis

In this study, 1,500 included documents (publications in 2022 were not fully included) were statistically analyzed according to the published time, and the annual publication trends are shown in Figure 1. The overall number of documents showed a fluctuating upward trend. In the initial stage, the publication volume increased slowly from 1999 to 2011; from 2011 to 2015, there were temporary fluctuations, with a slight continued upward trend in the overall amount of publications; from 2015 to 2021, the growth rate of publications increased significantly and at a faster pace, reaching two peaks in 2018 and 2021.

3.2. Countries and institutions analysis

Generate co-occurrence network analysis of countries and institutions (Figure 2). Node $N = 708$, link $E = 2{,}299$, module value $Q = 0.5984$, S value $= 0.878$. Each node represents a country or institution. The top 10 countries are the United States, China, Japan, England, Italy, Canada, South Korea, Germany, Australia, and France, and those with high centrality (>0.10) are the United States, China, England, Italy, Germany, and Australia, which indicate that these countries have a certain influence in the study of the correlation between insulin resistance and ischemic cerebrovascular disease. The top three countries contributed 850 articles, accounting for 56.6% of all the included documents. The top five institutions are mainly universities, including Harvard University, Capital Medical University, Brown University, Brigham and Women's Hospital, and Boston University (Table 1). There is active cooperation between various countries and institutions, especially in the USA, China, and Japan.

3.3. Co-authors analysis

Generate co-occurrence network analysis of authors (Figure 3). The results show the number of nodes $N = 753$, link $E = 1{,}043$, $Q = 0.9449$, $S = 1$, representing a total of 753 authors included and 1,043 collaborations between authors. The top 10 authors can be grouped into two main categories (Table 2). The first category is a collaboration cluster diagram consisting of WALTER N KERNAN (21 articles), CATHERINE M VISCOLI (18 articles), SILVIO E INZUCCHI (13 articles), KAREN L FURIE (12 articles), LAWRENCE H YOUNG (11 articles), MARK GORMAN (10 articles), and ROBIN CONWIT (eight articles). The second category is the cooperative cluster diagram composed of YONGJUN WANG (10 articles), XINGQUAN ZHAO (eight articles), and YILONG WANG (seven articles).

In the first cooperative relationship cluster map, the author with the most publications was WALTER N KERNAN (21 articles). From 2003 to 2005, WN KERNAN's research team recognized the association between insulin resistance and increased risk of ischemic vascular events, that decreased insulin sensitivity and impaired glucose tolerance widely existed in patients with a recent history of TIA or ischemic stroke, using pioglitazone intervention can improve post-stroke insulin resistance (19–21). Subsequently, they initiated an international, multicenter, randomized, double-blind, placebo-controlled study IRIS trial (22). The results were published from 2016 to 2019, demonstrating that treatment with pioglitazone improves cardiovascular outcomes of non-diabetic, insulin-resistant patients with stroke or TIA (23). In the second cooperative relationship cluster map, the most published author was YONGJUN WANG (10 articles). YJ Wang's study began in 2017, to investigate the impact of insulin resistance on the prognosis of non-diabetic ischemic stroke patients through cross-sectional studies and cohort studies based on the Chinese population (24).

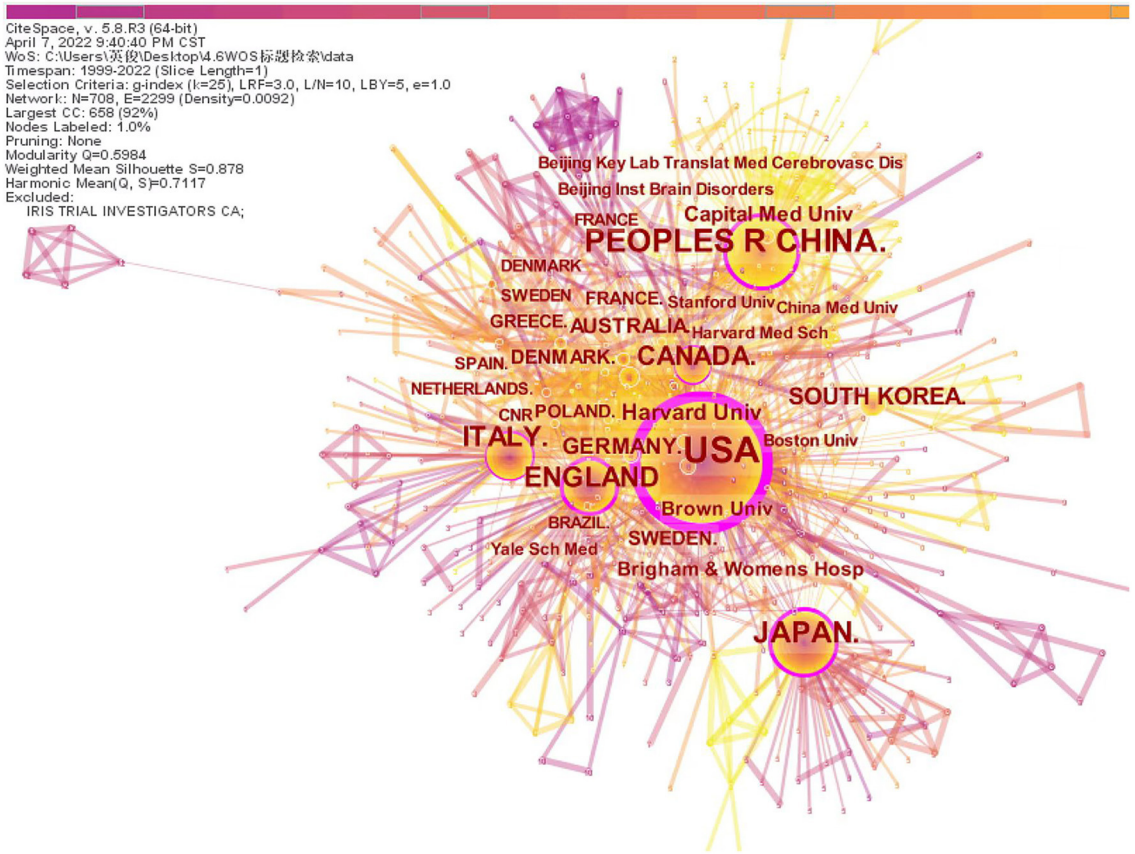

FIGURE 2
The network of countries and institutions.

TABLE 1 Top 10 countries and top five institutes publishing research on insulin resistance and ischemic cerebrovascular disease.

Rank	Country/ region	*N*	Centrality	Institution	*N*	Centrality
1	USA	470	0.39	Harvard University	34	0.09
2	Peoples R China	251	0.11	Capital Med University	32	0.03
3	Japan	139	0.03	Brown University	24	0.06
4	England	109	0.17	Brigham and Womens Hospital	23	0.02
5	Italy	107	0.16	Boston University	16	0.05
6	Canada	84	0.09			
7	South Korea	61	0.00			
8	Germany	51	0.13			
9	Australia	42	0.11			
10	France	40	0.02			

3.4. Co-cited references analysis

Generate co-cited references analysis network map (Figure 4). The results show the number of nodes $N = 1{,}190$, link $E = 3{,}996$, $Q = 0.8795$, and $S = 0.9375$. Each node represents a co-cited reference, the larger the node, the higher the citation frequency, and the links represent the co-citation relationship between different articles. The purple outer circle of the node represents that the references have high betweenness centrality, which is of great research significance in the development process of this field (25).

The top 10 co-cited references ranked according to frequency are listed in Table 3, and they were co-cited more than 200 times. Most of the articles involved are original research papers, laying the knowledge basis for further research on insulin resistance and ischemic cerebrovascular disease. The first high-frequency co-cited reference was the article published in the New Engl J MED by

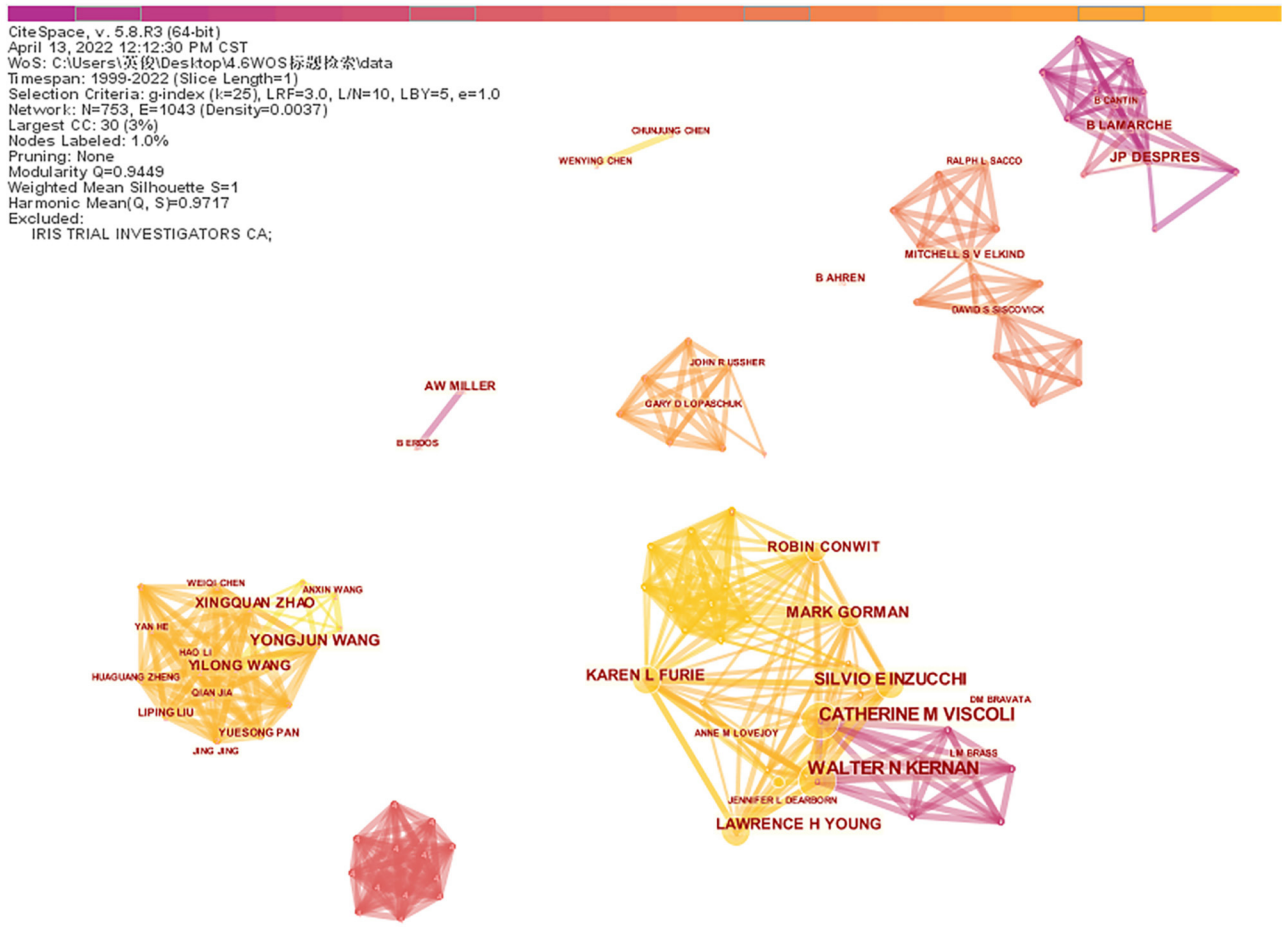

FIGURE 3
The network of co-authors.

TABLE 2 Top 10 authors with the most publications on insulin resistance and ischemic cerebrovascular disease.

Rank	Author	*N*	Year
1	Walter N. Kernan	21	2003
2	Catherine M. Viscoli	18	2002
3	Silvio E. Inzucchi	13	2016
4	Karen L. Furie	12	2016
5	Lawrence H. Young	11	2017
6	Yongjun Wang	10	2017
7	Mark Gorman	10	2016
8	Robin Conwit	8	2017
9	Xingquan Zhao	8	2016
10	Yilong Wang	7	2017

WN Kernan et al. (23), with high centrality, which demonstrated that the application of pioglitazone can benefit patients without diabetes who had insulin resistance along with a recent history of ischemic stroke or TIA through a multicenter, double-blind and randomized controlled trial study of 3,876 patients with ischemic stroke or TIA. The third co-citation article (26), published in 1996, identified fasting insulin concentration as an independent risk factor for ischemic heart disease through a prospective cohort study of Canadian men. The fourth co-cited reference was published on STROKE by Jing et al. (24), which investigated the relationship between insulin resistance and outcome in non-diabetic patients with first-ever acute ischemic stroke through a cross-sectional survey of 1,245 Chinese, and concluded that insulin resistance was associated with an increased risk of death, stroke recurrence, and adverse outcome but not dependence in non-diabetic patients with acute ischemic stroke. The eighth co-citation reference (27) demonstrated that insulin resistance was independently associated with poor functional outcomes in patients with acute ischemic stroke, apart from the risk of short-term stroke recurrence or death.

The top 10 co-cited references ranked according to betweenness centrality are listed in Table 4, serving as a turning point and bridge for the key nodes of this study. The article types mainly include original research papers and reviews. The first co-cited reference (28) with the highest centrality through a cohort study of 1,509 non-diabetic patients in North Manhattan, found that insulin resistance could be used as a predictor of increased risk of incident stroke in non-diabetic individuals. The second co-cited reference (29) used acute ischemic brain injury rat models induced by ligating

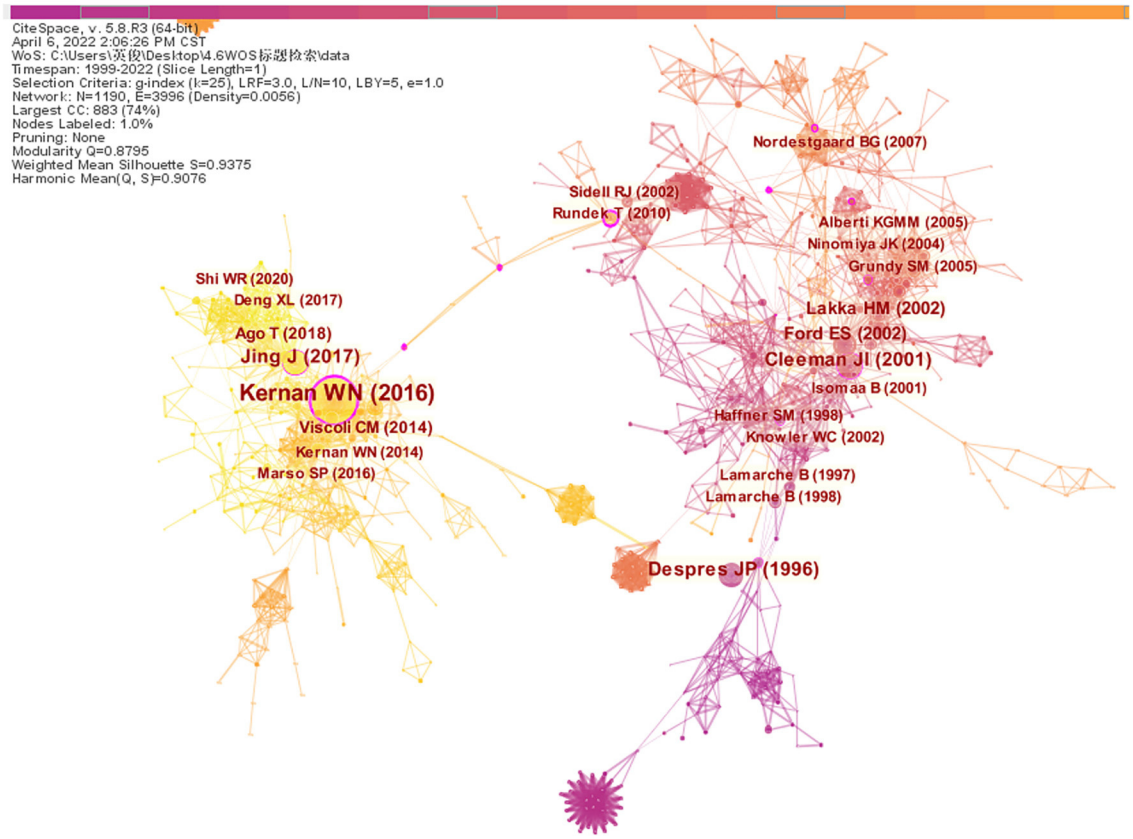

FIGURE 4
The network of co-cited references.

TABLE 3 Top 10 co-cited references with the highest frequency on insulin resistance and ischemic cerebrovascular disease.

Rank	Co-cited reference	Impact factor	Frequency	Centrality
1	Kernan WN, 2016, NEW ENGL J MED, V374, P1321, doi: 10.1056/NEJMoa1506930	91.245	60	0.42
2	Cleeman JI, 2001, JAMA-J AM MED ASSOC, V285, P2486, doi: 10.1001/jama.285.19.2486	56.272	28	0.15
3	Despres JP, 1996, NEW ENGL J MED, V334, P952, doi: 10.1056/NEJM199604113341504	91.245	28	0.00
4	Jing J, 2017, STROKE, V48, P887, doi: 10.1161/STROKEAHA.116.015613	9.910	26	0.13
5	Ford ES, 2002, JAMA-J AM MED ASSOC, V287, P356, doi: 10.1001/jama.287.3.356	7.914	24	0.07
6	Lakka HM, 2002, JAMA-J AM MED ASSOC, V288, P2709, doi: 10.1001/jama.288.21.2709	7.914	21	0.03
7	Viscoli CM, 2014, AM HEART J, V168, P823, doi: 10.1016/j.ahj.2014.07.016	4.749	17	0.01
8	Ago T, 2018, NEUROLOGY, V90, P0, doi: 10.1212/WNL.0000000000005358	9.910	16	0.05
9	Isomaa B, 2001, DIABETES CARE, V24, P683, doi: 10.2337/diacare.24.4.683	19.112	14	0
10	Grundy SM, 2005, CIRCULATION, V112, P2735, doi: 10.1161/CIRCULATIONAHA.105.169404	29.690	14	0.01

TABLE 4 Top 10 co-cited references with the highest centrality on insulin resistance and ischemic cerebrovascular disease.

Rank	Co-cited reference	Impact Factor	Centrality	Frequency
1	Rundek T, 2010, ARCH NEUROL-CHICAGO, V67, P1195, doi: 10.1001/archneurol.2010.235		0.53	14
2	Wang YY, 2011, AM J PHYSIOL-ENDOC M, V300, P0, doi: 10.1152/ajpendo.00301.2010	4.310	0.5	6
3	Kernan WN, 2016, NEW ENGL J MED, V374, P1321, doi: 10.1056/NEJMoa1506930	91.245	0.42	60
4	Nissen SE, 2007, NEW ENGL J MED, V356, P2457, doi: 10.1056/NEJMoa072761	91.245	0.34	7
5	Dormandy JA, 2005, LANCET, V366, P1279, doi: 10.1016/S0140-6736(05)67528-9	79.321	0.29	10
6	Boden-Albala B, 2008, STROKE, V39,P30, doi: 10.1161/STROKEAHA.107.496588	9.910	0.19	8
7	Haffner SM, 1998, NEW ENGL J MED, V339, P229, doi: 10.1056/NEJM199807233390404	91.245	0.16	13
8	Bonora E, 2003, DIABETES CARE, V26, P1251, doi: 10.2337/diacare.26.4.1251	19.112	0.16	8
9	Cleeman JI, 2001, JAMA-J AM MED ASSOC, V285, P2486, doi: 10.1001/jama.285.19.2486	56.272	0.15	28
10	Jing J, 2017, STROKE, V48, P887, doi: 10.1161/STROKEAHA.116.015613	9.910	0.13	26

the right middle cerebral artery and bilateral common carotid arteries, to examine the relationship between adipocytokines and poststroke hyperglycemia. It was found that stroke rats developed glucose intolerance on days 1 and 2 after cerebral ischemic injury, and fasting blood insulin levels and insulin resistance index were higher in stroke rats than in the sham group. Eventually, it proved that sympathetic system excitation after cerebral ischemia and inducing the secretion of proinflammatory cytokines (TNF-α and MCP-1) from adipose tissue may be an underlying mechanism for the disorder of glucose metabolism in rats. The fourth co-cited reference (30) using meta-analysis concluded that rosiglitazone treatment for type 2 diabetes may increase the incidence of myocardial infarction and cardiovascular adverse events. The fifth co-cited reference (31) proved that applying pioglitazone as agonists of peroxisome proliferator-activated receptor gamma (PPAR gamma) can reduce the incidence of macrovascular events in patients with type 2 diabetes. The sixth co-cited reference (32), as part of the North Manhattan study, identified metabolic syndrome as an important risk factor for ischemic stroke through a prospective cohort study. The eighth co-cited reference (33) found an increased risk of progressive carotid atherosclerosis and coronary heart disease in subjects with metabolic syndrome.

3.5. Co-occurring keywords and cluster analysis

Generate co-occurring keywords analysis network map (Figure 5). Results showed that node $N = 729$, link $E = 2805$, $Q = 0.4331$, $S = 0.7318$. A total of 729 keywords were included, with a high degree of correlation between them. Keywords can represent the hotspot and trend of research (34). As shown in Table 5, hotspot keywords are listed based on the frequency of occurrence and mediation centrality (>0.1). Keywords of diseases related to insulin resistance mainly include myocardial ischemia, cardiovascular disease, coronary artery disease, metabolic syndrome, ischemic stroke, diabetes mellitus, hypertension, cerebral ischemia et al. The hotspot keywords of the research direction are association, risk, and prevalence, and the mechanism involved is mainly oxidative stress and inflammation. Among them, adipose tissue, blood pressure, and coronary artery disease have better centrality.

Clustering analysis of co-occurrence keywords was performed, and cluster labels revealed major themes in the research field (Figure 6). We eventually obtained 11 clusters, each with a silhouette value above 0.6, indicating that the clustering results are reliable and meaningful. The cluster label keywords were extracted: #0 oxidative stress, #1 prevalence, #2 adiponectin, #3 low density lipoprotein, #4 therapy, #5 secondary prevention, #6 diabetic cardiomyopathy, #7 physical activity, #8 family history, #9 stroke mortality, #10 thyroid function, and #11 minimal model (Table 6).

3.6. Keywords with citation bursts

The top 20 keywords with the strongest citation burst from 1999 to 2022 are shown in Figure 7. The blue line indicates the time interval, and the red line indicates the period when the keyword burst occurs. "Burst words" refer to words that are frequently cited over a period of time (35). It can predict the migration and changes in research frontiers according to the distribution of keywords with the strongest citation burst. In the previous period, the relationship between glucose metabolism disorders and ischemic cardiomyopathy, hypertension, and coronary artery disease was

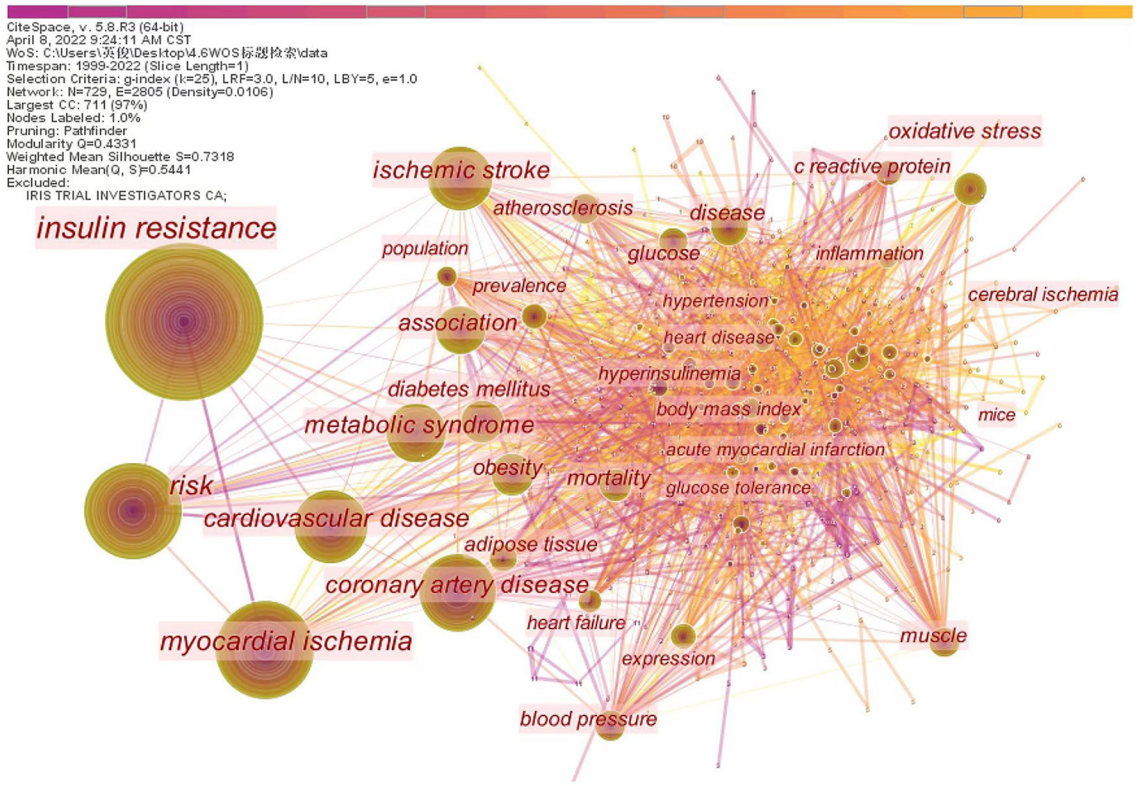

FIGURE 5
The network of co-occurring keywords.

TABLE 5 Top 10 keywords in frequency and top 5 keywords in centrality on insulin resistance and ischemic cerebrovascular disease.

Rank	Frequency	Keywords	Centrality	Keywords
1	930	Insulin resistance	0.11	Coronary artery disease
2	379	Risk	0.11	Disease
3	369	Myocardial ischemia	0.11	Blood pressure
4	251	Cardiovascular disease	0.10	Muscle
5	243	Coronary artery disease	0.10	Adipose tissue
6	211	Ischemic stroke		
7	184	Metabolic syndrome		
8	136	Association		
9	126	Diabetes mellitus		
10	114	Oxidative stress		

mainly studied; from 2000 to 2011, research focused on the mechanisms of intrinsic associations between diseases, including plasminogen activator inhibbitor1, adiponectin, cholesterol, and c reactive protein; in the past 6 years, more attention has been paid to the association between insulin resistance and TIA, ischemia-reperfusion injury and Alzheimer's disease. The pathological mechanisms have focused on oxidative stress and inflammation, and more emphasis is placed on the relationship between insulin resistance and other diseases. The emerging research keywords in recent years represent the current research trends and hotspots in this field.

4. Discussion

4.1. Research progress in insulin resistance and ischemic cerebrovascular disease

Our results show that from 1999 to 2022, a growing number of studies confirm the strong link between insulin resistance and ischemic cerebrovascular disease, indicating that the field has attracted lively discussion and widespread concern in recent years. The United States, China, and Japan are the largest contributors to the number of publications and have played an important

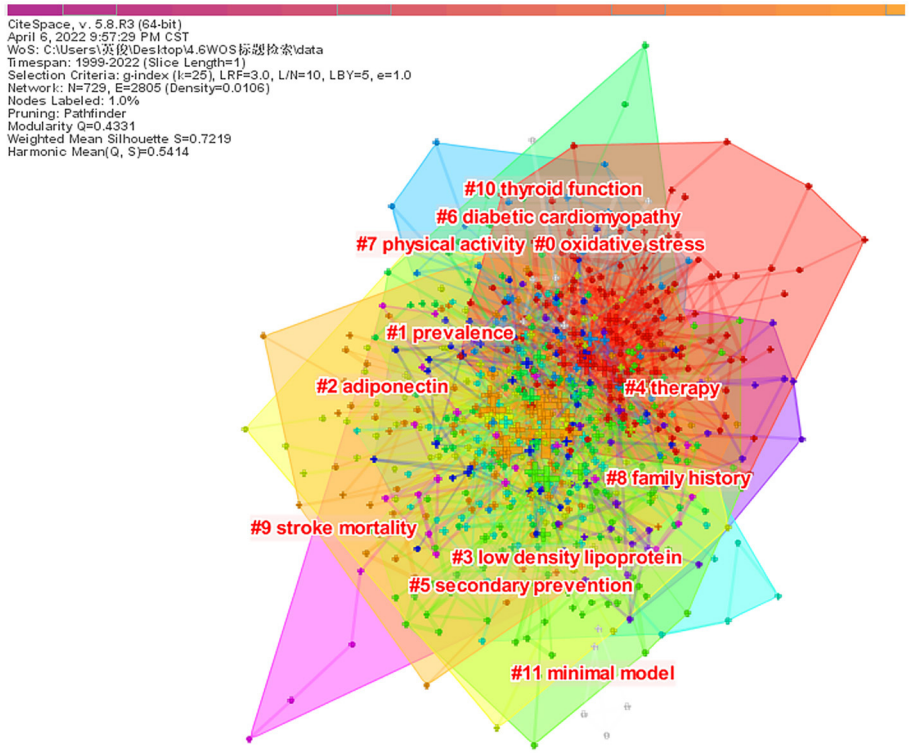

FIGURE 6
Keywords cluster analysis co-occurrence map.

TABLE 6 Keywords cluster analysis.

Cluster-ID	Size	Silhouette	Coverage	Label
0	142	0.616	Oxidative stress; gene expression; cerebral ischemia; nitric oxide synthase; Alzheimer's disease	Oxidative stress
1	111	0.736	Prevalence; hyperinsulinemia; cardiovascular disease; population; risk factor	Prevalence
2	82	0.715	Adiponectin; resistin; ischemic stroke; inflammation; c reactive protein	Adiponectin
3	80	0.787	Low density lipoprotein; cardiovascular risk factor; coronary artery disease; von willebrand factor; risk	Low density lipoprotein
4	78	0.627	Therapy; hyperglycemia; stroke severity; interleukin-1; tissue plasminogen activator	Therapy
5	56	0.799	Secondary prevention; pioglitazone; clinical trial; transient ischemic attack; obesity	Secondary prevention
6	46	0.791	Diabetic cardiomyopathy; heart failure; trimetazidine; fatty acid oxidation; cardiomyopathy	Diabetic cardiomyopathy
7	44	0.774	Physical activity; randomized controlled trial; cardiac rehabilitation; fatty acid; neuroscience	Physical activity
8	31	0.877	Family history; hemostasis; factor alpha; thrombosis; plasminogen activator inhibitor 1 (pai-1)	Family history
9	25	0.899	Stroke mortality; transplantation; renal failure; kidney; cardiovascular disease (CVD)	Stroke mortality
10	7	0.987	Thyroid function; turner syndrome; PKC delta; puberty; genes	Thyroid function

role in the development of the field. However, there are not enough collaborative relationships between authors from different countries and regions, and there is an urgent need to strengthen cooperation between countries, institutions, and authors to conduct relevant research. Major ongoing research trends include three aspects: (1) the association between insulin resistance and ischemic cerebrovascular disease in non-diabetic patients, (2) the intrinsic pathological mechanism between insulin resistance and

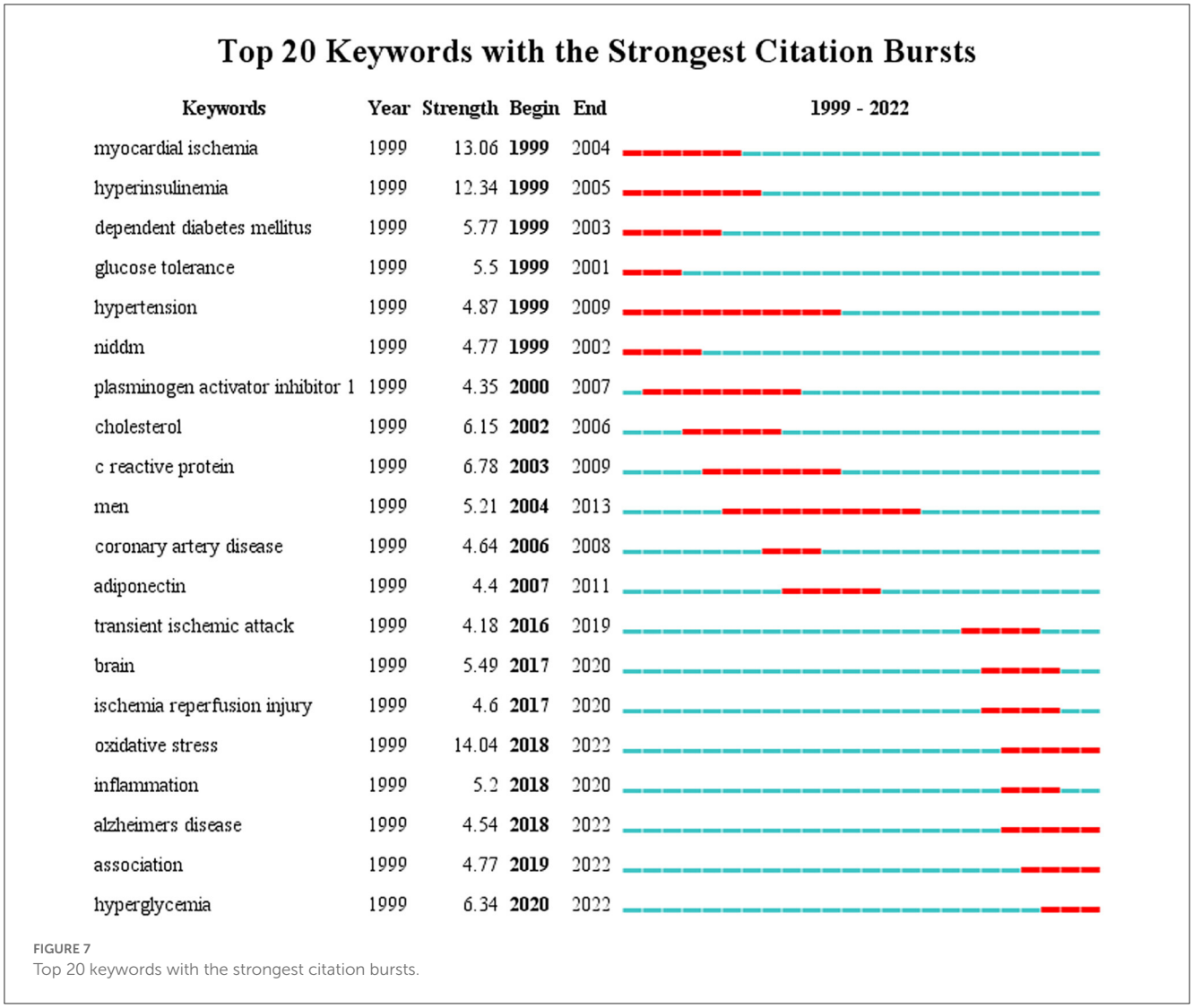

FIGURE 7
Top 20 keywords with the strongest citation bursts.

ischemic cerebrovascular disease, and (3) early intervention of insulin resistance to improve the prognosis of stroke.

4.2. Hot issue of insulin resistance and ischemic cerebrovascular disease

4.2.1. Association

The association between insulin resistance and ischemic cerebrovascular disease has received hot attention in recent years. In clinical studies of insulin resistance, evaluation indexes are more mixed, mostly assessed by Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index (36), or triglyceride-glucose(Ty-G) index (37).

4.2.1.1. Insulin resistance and silent lacunar infarction

Two studies from Korea (38) and Japan (39) evaluated the association between insulin resistance and silent lacunar infarction (SLI), and found that insulin resistance was an independent risk factor for SLI and was positively associated with the incidence and severity of SLI. In elderly patients, impaired insulin sensitivity and decreased muscle strength jointly increased the high risk of SLI.

4.2.1.2. Insulin resistance and cerebral small vessel disease

A Japanese study (40) evaluated the association between insulin resistance and cerebral white matter lesions in non-diabetic patients with ischemic stroke. Insulin resistance was defined as HOMA-IR index $\geq$ 2.5, the degree of periventricular hyperintensity (PVH), as well as deep and subcortical white matter hyperintensity (DSWMH) were measured using brain MRI. The results revealed that insulin resistance was closely related to cerebral white matter lesions in non-diabetic patients with non-cardiac ischemic stroke, with higher HOMA-IR index in patients with heavier PVH and DSWMH. Another study prospectively recruited older, non-diabetic, healthy subjects to assess the association between insulin resistance and the overall cerebral small vessel disease (CSVD) burden (41). The HOMA-IR index $\geq$ 2.80 was defined as Insulin resistance. The results of the study ultimately showed that Insulin resistance, independent of other clinical risk factors, is positively associated with increased severity of overall CSVD burden in a dose-dependent manner.

4.2.1.3. Insulin resistance and post-stroke depression

Two prospective cohort studies in China demonstrated that prediabetes (impaired fasting glucose, impaired glucose tolerance, or HbA1c 5.7–6.4%) was associated with post-stroke depression and could serve as an early predictor (42). In addition, insulin resistance estimated by the HOMA-IR index may have potential clinical significance in identifying stroke patients at risk of developing depression, independent of recognized predictive factors (43).

4.2.1.4. Insulin resistance and risk stratification of ischemic stroke

Triglyceride-glucose index (Ty-G) is considered to be a simple and reliable practical surrogate indicator of insulin resistance. A cross-sectional study from rural areas of northeast China (44) aimed to explore the relationship between Ty-G and ischemic stroke. When Ty-G was divided into quartiles, the risk of ischemic stroke in the top quartile was 1.776 times higher than in the bottom category. Eventually, this finding demonstrates the potential value of Ty-G index in optimizing ischemic stroke risk stratification in the general population. Another retrospective observational multicenter study on the eICU database investigated the prognostic value of the Ty-G index in patients with critically ill stroke (45). The results showed that Ty-G was associated with increased in-hospital mortality in patients with severe ischemic stroke, but not in patients with hemorrhagic stroke. It is therefore concluded that Ty-G may be a potential predictor of hospital and ICU mortality in critically ill stroke patients, especially in ischemic stroke patients. A study in Jiangxi, China, proved that the Ty-G index is potentially useful in the early identification of elderly hypertensive patients at high risk of experiencing a first stroke (46). A recent meta-analysis of Ty-G and stroke risk (47), including 11 cohort studies, found that the risk of ischemic stroke was positively associated with the Ty-G index, and the elevated Ty-G index was an independent risk factor for stroke occurrence, particularly in ischemic stroke.

4.2.1.5. Insulin resistance and the prognosis of ischemic stroke

Two studies from Yongjun Wang's team used the HOMA-IR index and the Ty-G index representing insulin resistance to determine whether it was associated with an adverse clinical outcome of ischemic stroke. One study (48) proved that the Ty-G index was associated with an increased risk of stroke recurrence, all-cause mortality, and neurological deterioration in patients with ischemic stroke. Another explored relationship between insulin resistance and the risk of early neurological deterioration (END) in patients with non-diabetic acute ischemic stroke (49). Ultimately this study provides strong evidence that insulin resistance may be an independent risk factor for END in non-diabetic patients with acute ischemic stroke. However, whether insulin resistance can be used as an independent risk factor for poor prognosis within urgent 3 months remains controversial. Research from Fukuoka, Japan enrolled 4,655 patients with acute ischemic stroke, finding that HOMA-IR was not associated with stroke recurrence or mortality within 3 months of onset (27).

4.2.1.6. Insulin resistance and atherosclerosis

Metabolic syndrome(MetS) is a group of risk factors associated with insulin resistance (50) and is present in about half of the patients with symptomatic intracranial atherosclerotic stenosis (51). The components of MetS interact to affect vascular thickness synergistically and promote the development of subclinical atherosclerosis (52). Atherosclerosis is the main putative mechanism between insulin resistance and ischemic stroke. One study enrolled 1,523 ischemic stroke patients with Ty-G index and carotid artery imaging data (53). Carotid atherosclerosis was measured by common carotid artery intima-media thickness (cIMT). The result demonstrates that a higher Ty-G index was associated with carotid atherosclerosis in patients with ischemic stroke, suggesting that Ty-G could be a promising atherosclerotic marker.

4.2.2. Oxidative stress and inflammation

Since 2018, more attention has been paid to the pathological processes of oxidative stress and inflammatory response between insulin resistance and ischemic cerebrovascular disease.

When insulin binds to insulin receptors on the plasma membrane, the insulin signaling pathway is activated (54), promoting glucose transport and utilization. In the pathological stage of insulin resistance, glucose utilization and uptake by skeletal muscle, adipose tissue, liver, and other organs are weakened, and the liver function of inhibiting gluconeogenesis and promoting glycogen synthesis is decreased, resulting in an increase in peripheral glucose in the blood. Defective inhibition of lipolysis by insulin leads to increased levels of triglycerides (TG), free fatty acids (FFA), and low-density lipoprotein (LDL), and decreased levels of high-density lipoprotein (HDL) (55). Increased level of FFA induce ROS generation through activation of NADPH oxidation and increased ROS induce oxidative stress and ER stress, stimulating adipose tissue to synthesize and secrete a large number of biologically active substances, such as proinflammatory cytokines, acute phase reactants, angiotensin II, leptin, resistin, adiponectin, and plasminogen activator inhibitor-1(PAI-1) (56). Adipose tissue activates the canonical proinflammatory NF-κB pathway, resulting in increased expression of several proinflammatory cytokines, including NLRP3, TNF-α, IL-1β, IL-6, and MCP-1 (57). All the mechanisms contribute to endothelial dysfunction, atherosclerosis, and thrombosis, leading to cardiovascular and cerebrovascular disease.

The brain has been identified as an insulin-sensitive organ with widely distributed insulin receptors (58). Hyperinsulinemia resulting from systemic insulin resistance causes insulin resistance in central neurons (59), which in turn may exacerbate ischemic cerebrovascular damage by downregulating the PI3K-AKT signaling pathway and inhibiting cell survival. Ischemic injury induces cell death through proapoptotic signaling molecules, including forkhead transcription factor (FKHR), GSK-3β, and Bad, which can be prevented by AKT phosphorylation (60). Insulin and insulin-like growth factor 1 (IGF-1) exert neuroprotective effects by activating PI3K-AKT signaling and preventing decreased AKT phosphorylation during ischemia (61). *In vitro* experiments (62) by simulating insulin resistance models of central cortical neurons, found that long-term exposure to insulin increased Akt activation

and severely attenuated this response after subsequent short-term insulin treatment, resulting in reduced neuroprotective effects of insulin and IGF-1. This study demonstrated that central insulin resistance exacerbates cerebral ischemic injury by blunting Akt kinase phosphorylation.

Measures taken against the assembly and activity of the NLRP3 (nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3) inflammasome may be a potential and novel therapy for cerebrovascular ischemic disease concomitant with insulin resistance (63). The NLRP3 inflammasome is a member of the NLR family of innate immune cell sensors. In general, activation of the NLRP3 inflammasome requires two signaling pathways, priming and activating (64). In the priming phase, toll-like receptors (TLRs) recognize a wide variety of danger-associated molecular patterns (65), promote the expression and activation of NLRP1 and NLRP3 inflammasome proteins, and both precursors IL-1β and IL-18 in primary cortical neurons and brain tissue under ischemic conditions. Increased ROS, lysosome rupture, and intracellular potassium efflux activate the NLRP3 inflammasome, promoting the maturation of IL-1β and IL-18 and the release of proinflammatory cytokines (66). Activation of NLRP3 inflammasomes through NF-κB and MAPK signaling pathways to secrete pro-inflammatory cytokines after brain ischemia may be an intrinsic mechanism that enlarges the ischemic damage and causes the disorder of glucose metabolism after stroke (67). The NLRP3 inflammasome may serve as a potential mechanism for the interaction between insulin resistance and ischemic cerebrovascular disease.

4.3. Strengths and limitation

To our knowledge, this is the first study to utilize CiteSpace's co-occurrence and co-citation analysis methods for bibliometric analysis and visual display of insulin resistance and ischemic cerebrovascular disease. However, our study does have limitations. Bibliometric studies rely heavily on databases, while our study did not cover other public and commercial bibliometric databases such as PubMed, Scopus, Medline, and CNKI. Therefore, the data may not be comprehensive. But this was caused by the limitations of the software and databases. For example, PubMed does not provide citation analysis. In addition, WOS has the advantages of wide coverage and strong authority, and it can provide better graphics and more detailed content than Scopus in citation analysis (68). Therefore, even if we only analyzed the literature from WOS, the findings remain reliable. Furthermore, some overlap may occur when analyzing the co-occurrence and clustering of keywords due to the presence of multiple synonyms.

5. Conclusions

The current study does suggest a strong correlation between insulin resistance and ischemic cerebrovascular disease, but whether it can be an independent influence on poor prognosis after stroke remains controversial. In the future, prospective trials with large samples are needed to deeply analyze the role of insulin resistance in the pathogenesis of ischemic cerebrovascular diseases, such as whether there is a correlation between infarction site, infarction size, and insulin resistance after stroke, what kind of people are more prone to glucose metabolism disorders after stroke, what kind of people with post-stroke glucose metabolism disorder are more likely to have poor clinical outcomes. The IRIS trial demonstrated that pioglitazone can improve the clinical outcome of insulin resistance combined with ischemic cerebrovascular disease, but adverse effects such as bone fractures may limit the clinical use of pioglitazone (69). It may be a new research trend to explore drugs that can improve central insulin resistance, alleviate ischemic injury and promote neurological recovery. At present, SGLT2 inhibitors may serve as the first drug that can improve hypothalamic insulin resistance through the blood-brain barrier (70, 71). Inhibition of the NLRP3 inflammasome activation process and thus inhibition of proinflammatory cytokine release may serve as an early modulable target to improve the inflammatory cascade in patients with insulin resistance combined with ischemic cerebrovascular disease. The intrinsic mechanisms underlying the interaction between insulin resistance and ischemic cerebrovascular disease still need further investigation.

Our research results ultimately provide valuable information for potential collaborators and institutions, teasing out the current status, hotspots, and frontiers of insulin resistance and ischemic cerebrovascular disease, which may guide new directions for further research.

Author contributions

XZ and CK designed the study, retrieved the data, performed the statistical analysis, and wrote the first draft. YH made further modifications. XW supervised the whole process and provided modification advice. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Campbell B, Khatri P. Stroke. *Lancet.* (2020) 396:129–42. doi: 10.1016/S0140-6736(20)31179-X

2. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2020 update: a report from the American heart association. *Circulation.* (2020) 141:e139–596. doi: 10.1161/CIR.0000000000000746

3. Feigin VL, Nguyen G, Cercy K, Johnson CO, Alam T, Parmar PG, et al. Global, regional, and country-specific lifetime risks of stroke, 1990 and 2016. *N Engl J Med.* (2018) 379:2429–37. doi: 10.1056/NEJMoa1804492

4. Wang YJ, Li ZX, Gu HQ, Zhai Y, Jiang Y, Zhao XQ, et al. China stroke statistics 2019: a report from the national center for healthcare quality management in neurological diseases, china national clinical research center for neurological diseases, the Chinese stroke association, national center for chronic and non-communicable disease control and prevention, Chinese center for disease control and prevention and institute for global neuroscience and stroke collaborations. *Stroke Vasc Neurol.* (2020) 5:211–39. doi: 10.1136/svn-2020-000457

5. O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the interstroke study): a case-control study. *Lancet.* (2010) 376:112–23. doi: 10.1016/S0140-6736(10)60834-3

6. Lebovitz HE. Insulin resistance: definition and consequences. *Exp Clin Endocrinol Diabetes.* (2001) 109:S135–48. doi: 10.1055/s-2001-18576

7. Domingues N. Insulin resistance as a predictor of cardiovascular diseases. *Rev Port Cardiol.* (2021) 40:545–6. doi: 10.1016/j.repc.2021.06.004

8. Chen W, Wang S, Lv W, Pan Y. Causal associations of insulin resistance with coronary artery disease and ischemic stroke: a Mendelian randomization analysis. *BMJ Open Diabetes Res Care.* (2020) 8:e001217. doi: 10.1136/bmjdrc-2020-001217

9. Chu LM, Liu CC, Yeh CC, Chang YC, Hu CJ, Shih CC, et al. Increased diabetes risk and interaction with social and medical events in patients upon stroke: two nationwide studies. *Atherosclerosis.* (2017) 265:87–92. doi: 10.1016/j.atherosclerosis.2017.08.017

10. Harada S, Fujita WH, Shichi K, Tokuyama S. The development of glucose intolerance after focal cerebral ischemia participates in subsequent neuronal damage. *Brain Res.* (2009) 1279:174–81. doi: 10.1016/j.brainres.2009.05.014

11. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Horwitz RI. Insulin resistance and risk for stroke. *Neurology.* (2002) 59:809–15. doi: 10.1212/WNL.59.6.809

12. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Shulman GI, et al. Impaired insulin sensitivity among nondiabetic patients with a recent tia or ischemic stroke. *Neurology.* (2003) 60:1447–51. doi: 10.1212/01.WNL.0000063318.66140.A3

13. Kleindorfer DO, Towfighi A, Chaturvedi S, Cockroft KM, Gutierrez J, Lombardi-Hill D, et al. 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline from the American heart association/American stroke association. *Stroke.* (2021) 52:e364–467. doi: 10.1161/STR.0000000000000375

14. Chen C, Song M. Visualizing a field of research: a methodology of systematic scientometric reviews. *PLoS ONE.* (2019) 14:e223994. doi: 10.1371/journal.pone.0223994

15. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci U S A.* (2004) 101:5303–10. doi: 10.1073/pnas.0307513100

16. Chen C, Dubin R, Kim MC. Emerging trends and new developments in regenerative medicine: a scientometric update (2000 - 2014). *Expert Opin Biol Ther.* (2014) 14:1295–317. doi: 10.1517/14712598.2014.920813

17. Zhong D, Luo S, Zheng L, Zhang Y, Jin R. Epilepsy occurrence and circadian rhythm: a bibliometrics study and visualization analysis via citespace. *Front Neurol.* (2020) 11:984. doi: 10.3389/fneur.2020.00984

18. Synnestvedt MB, Chen C, Holmes JH. Citespace ii: visualization and knowledge discovery in bibliographic databases. *AMIA Annu Symp Proc.* (2005) 2005:724–8.

19. Kernan WN, Viscoli CM, Inzucchi SE, Brass LM, Bravata DM, Shulman GI, et al. Prevalence of abnormal glucose tolerance following a transient ischemic attack or ischemic stroke. *Arch Intern Med.* (2005) 165:227–33. doi: 10.1001/archinte.165.2.227

20. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Shulman GI, et al. Pioglitazone improves insulin sensitivity among nondiabetic patients with a recent transient ischemic attack or ischemic stroke. *Stroke.* (2003) 34:1431–6. doi: 10.1161/01.STR.0000071108.00234.0E

21. Bravata DM, Wells CK, Kernan WN, Concato J, Brass LM, Gulanski BI. Association between impaired insulin sensitivity and stroke. *Neuroepidemiology.* (2005) 25:69–74. doi: 10.1159/000086286

22. Viscoli CM, Brass LM, Carolei A, Conwit R, Ford GA, Furie KL, et al. Pioglitazone for secondary prevention after ischemic stroke and transient ischemic attack: rationale and design of the insulin resistance intervention after stroke trial. *Am Heart J.* (2014) 168:823–9. doi: 10.1016/j.ahj.2014.07.016

23. Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, et al. Pioglitazone after ischemic stroke or transient ischemic attack. *N Engl J Med.* (2016) 374:1321–31. doi: 10.1056/NEJMoa1506930

24. Jing J, Pan Y, Zhao X, Zheng H, Jia Q, Mi D, et al. Insulin resistance and prognosis of nondiabetic patients with ischemic stroke: the across-china study (abnormal glucose regulation in patients with acute stroke across china). *Stroke.* (2017) 48:887–93. doi: 10.1161/STROKEAHA.116.015613

25. Synnestvedt MB, Chen C, Holmes JH. Visual exploration of landmarks and trends in the medical informatics literature. *AMIA Annu Symp Proc.* (2005) 2005:1129.

26. Després JP, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med.* (1996) 334:952–7. doi: 10.1056/NEJM199604113341504

27. Ago T, Matsuo R, Hata J, Wakisaka Y, Kuroda J, Kitazono T, et al. Insulin resistance and clinical outcomes after acute ischemic stroke. *Neurology.* (2018) 90:e1470–7. doi: 10.1212/WNL.0000000000005358

28. Rundek T, Gardener H, Xu Q, Goldberg RB, Wright CB, Boden-Albala B, et al. Insulin resistance and risk of ischemic stroke among nondiabetic individuals from the northern manhattan study. *Arch Neurol.* (2010) 67:1195–200. doi: 10.1001/archneurol.2010.235

29. Wang YY, Lin SY, Chuang YH, Chen CJ, Tung KC, Sheu WH. Adipose proinflammatory cytokine expression through sympathetic system is associated with hyperglycemia and insulin resistance in a rat ischemic stroke model. *Am J Physiol Endocrinol Metab.* (2011) 300:E155–63. doi: 10.1152/ajpendo.00301.2010

30. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med.* (2007) 356:2457–71. doi: 10.1056/NEJMoa072761

31. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the proactive study (prospective pioglitazone clinical trial in macrovascular events): a randomised controlled trial. *Lancet.* (2005) 366:1279–89. doi: 10.1016/S0140-6736(05)67528-9

32. Boden-Albala B, Sacco RL, Lee HS, Grahame-Clarke C, Rundek T, Elkind MV, et al. Metabolic syndrome and ischemic stroke risk: northern manhattan study. *Stroke.* (2008) 39:30–5. doi: 10.1161/STROKEAHA.107.496588

33. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC, et al. Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: prospective data from the bruneck study. *Diabetes Care.* (2003) 26:1251–7. doi: 10.2337/diacare.26.4.1251

34. Luo H, Cai Z, Huang Y, Song J, Ma Q, Yang X, et al. Study on pain catastrophizing from 2010 to 2020: a bibliometric analysis via citespace. *Front Psychol.* (2021) 12:759347. doi: 10.3389/fpsyg.2021.759347

35. Zhou Q, Kong HB, He BM, Zhou SY. Bibliometric analysis of bronchopulmonary dysplasia in extremely premature infants in the web of science database using citespace software. *Front Pediatr.* (2021) 9:705033. doi: 10.3389/fped.2021.705033

36. Xu J, Viscoli CM, Ford GA, Gorman M, Kernan WN. A diagnostic score for insulin resistance in nondiabetic patients with ischemic stroke or transient ischemic attack. *J Stroke Cerebrovasc Dis.* (2016) 25:1705–12. doi: 10.1016/j.jstrokecerebrovasdis.2016.03.043

37. Yang Y, Ning X, Wang X, Wang J, Zhang Y, Zhong Y, et al. Triglyceride/glucose index is a reliable predictor of insulin resistance in schizophrenia. *Schizophr Res.* (2020) 223:366–7. doi: 10.1016/j.schres.2020.07.005

38. Lee JE, Shin DW, Yun JM, Kim SH, Nam YS, Cho B, et al. Insulin resistance is a risk factor for silent lacunar infarction. *Stroke.* (2016) 47:2938–44. doi: 10.1161/STROKEAHA.116.014097

39. Someya Y, Tamura Y, Kaga H, Sugimoto D, Kadowaki S, Suzuki R, et al. Insulin resistance and muscle weakness are synergistic risk factors for silent lacunar infarcts: the bunkyo health study. *Sci Rep*. (2021) 11:21093. doi: 10.1038/s41598-021-00377-5

40. Katsumata T, Otori T, Nishiyama Y, Okubo S, Nishiyama Y, Nagayama H, et al. Correlation between insulin resistance and white matter lesions among non-diabetic patients with ischemic stroke. *Neurol Res.* (2010) 32:743–7. doi: 10.1179/016164109X12608733393755

41. Yang X, Zhang S, Dong Z, Zi Y, Luo Y, Jin Z, et al. Insulin resistance is a risk factor for overall cerebral small vessel disease burden in old nondiabetic healthy adult population. *Front Aging Neurosci.* (2019) 11:127. doi: 10.3389/fnagi.2019.00127

42. Xiao M, Wang Q, Ren W, Zhang Z, Wu X, Wang Z, et al. Impact of prediabetes on poststroke depression in chinese patients with acute ischemic stroke. *Int J Geriatr Psychiatry.* (2018) 33:956–63. doi: 10.1002/gps.4878

43. Qiu HC, Liu HZ, Li X, Zeng X, Zhao JZ. Insulin resistance as estimated by homeostasis model assessment predicts incident post-stroke depression in chinese subjects from ischemic stroke. *J Affect Disord.* (2018) 231:1–7. doi: 10.1016/j.jad.2018.01.023

44. Shi W, Xing L, Jing L, Tian Y, Yan H, Sun Q, et al. Value of triglyceride-glucose index for the estimation of ischemic stroke risk: insights from a general population. *Nutr Metab Cardiovasc Dis.* (2020) 30:245–53. doi: 10.1016/j.numecd.2019.09.015

45. Zhang B, Liu L, Ruan H, Zhu Q, Yu D, Yang Y, et al. Triglyceride-glucose index linked to hospital mortality in critically ill stroke: an observational multicentre study on eicu database. *Front Med.* (2020) 7:591036. doi: 10.3389/fmed.2020.591036

46. Hu L, Bao H, Huang X, Zhou W, Wang T, Zhu L, et al. Relationship between the triglyceride glucose index and the risk of first stroke in elderly hypertensive patients. *Int J Gen Med.* (2022) 15:1271–9. doi: 10.2147/IJGM.S350474

47. Feng X, Yao Y, Wu L, Cheng C, Tang Q, Xu S. Triglyceride-glucose index and the risk of stroke: a systematic review and dose-response meta-analysis. *Horm Metab Res.* (2022) 54:175–86. doi: 10.1055/a-1766-0202

48. Zhou Y, Pan Y, Yan H, Wang Y, Li Z, Zhao X, et al. Triglyceride glucose index and prognosis of patients with ischemic stroke. *Front Neurol.* (2020) 11:456. doi: 10.3389/fneur.2020.00456

49. Mi D, Wang Y, Wang Y, Liu L. Insulin resistance is an independent risk factor for early neurological deterioration in non-diabetic patients with acute ischemic stroke. *Neurol Sci.* (2020) 41:1467–73. doi: 10.1007/s10072-019-04221-7

50. St-Pierre AC, Cantin B, Mauriège P, Bergeron J, Dagenais GR, Després JP, et al. Insulin resistance syndrome, body mass index and the risk of ischemic heart disease. *CMAJ.* (2005) 172:1301–5. doi: 10.1503/cmaj.1040834

51. Baydar O, Kilic A, Okcuoglu J, Apaydin Z, Can MM. The triglyceride-glucose index, a predictor of insulin resistance, is associated with subclinical atherosclerosis. *Angiology.* (2021) 72:994–1000. doi: 10.1177/00033197211007719

52. Novo S, Peritore A, Guarneri FP, Corrado E, Macaione F, Evola S, et al. Metabolic syndrome (mets) predicts cardio and cerebrovascular events in a twenty years follow-up. a prospective study. *Atherosclerosis.* (2012) 223:468–72. doi: 10.1016/j.atherosclerosis.2012.05.018

53. Miao M, Zhou G, Bao A, Sun Y, Du H, Song L, et al. Triglyceride-glucose index and common carotid artery intima-media thickness in patients with ischemic stroke. *Cardiovasc Diabetol.* (2022) 21:43. doi: 10.1186/s12933-022-01472-1

54. Beale EG. Insulin signaling and insulin resistance. *J Investig Med.* (2013) 61:11–4. doi: 10.2310/JIM.0b013e3182746f95

55. Gaspar RS, Trostchansky A, Paes AM. Potential role of protein disulfide isomerase in metabolic syndrome-derived platelet hyperactivity. *Oxid Med Cell Longev.* (2016) 2016:2423547. doi: 10.1155/2016/2423547

56. Syed IS, Zaman HH, Vethakkan SR, Wan AW. Potential biomarkers of insulin resistance and atherosclerosis in type 2 diabetes mellitus patients with coronary artery disease. *Int J Endocrinol.* (2013) 2013:698567. doi: 10.1155/2013/698567

57. Boden G. Obesity, insulin resistance and free fatty acids. *Curr Opin Endocrinol Diabetes Obes.* (2011) 18:139–43. doi: 10.1097/MED.0b013e3283444b09

58. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Haring HU. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol Rev.* (2016) 96:1169–209. doi: 10.1152/physrev.00032.2015

59. Bruce KD, Hanson MA. The developmental origins, mechanisms, and implications of metabolic syndrome. *J Nutr.* (2010) 140:648–52. doi: 10.3945/jn.109.111179

60. Kawano T, Fukunaga K, Takeuchi Y, Morioka M, Yano S, Hamada J, et al. Neuroprotective effect of sodium orthovanadate on delayed neuronal death after transient forebrain ischemia in gerbil hippocampus. *J Cereb Blood Flow Metab.* (2001) 21:1268–80. doi: 10.1097/00004647-200111000-00003

61. Zhao H, Sapolsky RM, Steinberg GK. Phosphoinositide-3-kinase/akt survival signal pathways are implicated in neuronal survival after stroke. *Mol Neurobiol.* (2006) 34:249–70. doi: 10.1385/MN:34:3:249

62. Kim B, Sullivan KA, Backus C, Feldman EL. Cortical neurons develop insulin resistance and blunted akt signaling: a potential mechanism contributing to enhanced ischemic injury in diabetes. *Antioxid Redox Signal.* (2011) 14:1829–39. doi: 10.1089/ars.2010.3816

63. Hong P, Gu RN, Li FX, Xiong XX, Liang WB, You ZJ, et al. Nlrp3 inflammasome as a potential treatment in ischemic stroke concomitant with diabetes. *J Neuroinflammation.* (2019) 16:121. doi: 10.1186/s12974-019-1498-0

64. Kelley N, Jeltema D, Duan Y, He Y. The nlrp3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci.* (2019) 20:3328. doi: 10.3390/ijms20133328

65. García-Culebras A, Durán-Laforet V, Peña-Martínez C, Moraga A, Ballesteros I, Cuartero MI, et al. Role of tlr4 (toll-like receptor 4) in n1/n2 neutrophil programming after stroke. *Stroke.* (2019) 50:2922–32. doi: 10.1161/STROKEAHA.119.025085

66. Seoane PI, Lee B, Hoyle C, Yu S, Lopez-Castejon G, Lowe M, et al. The nlrp3-inflammasome as a sensor of organelle dysfunction. *J Cell Biol.* (2020) 219:e202006194. doi: 10.1083/jcb.202006194

67. Fann DY, Lim YA, Cheng YL, Lok KZ, Chunduri P, Baik SH, et al. Evidence that nf-κb and mapk signaling promotes nlrp inflammasome activation in neurons following ischemic stroke. *Mol Neurobiol.* (2018) 55:1082–96. doi: 10.1007/s12035-017-0394-9

68. Falagas ME, Pitsouni EI, Malietzis GA, Pappas G. Comparison of pubmed, scopus, web of science, and google scholar: strengths and weaknesses. *FASEB J.* (2008) 22:338–42. doi: 10.1096/fj.07-9492LSF

69. Viscoli CM, Kent DM, Conwit R, Dearborn JL, Furie KL, Gorman M, et al. Scoring system to optimize pioglitazone therapy after stroke based on fracture risk. *Stroke*. (2018). doi: 10.1161/STROKEAHA.118.022745

70. Pawlos A, Broncel M, Wozniak E, Gorzelak-Pabiś P. Neuroprotective effect of sglt2 inhibitors. *Molecules*. (2021) 26:7213. doi: 10.3390/molecules26237213

71. Kullmann S, Hummel J, Wagner R, Dannecker C, Vosseler A, Fritsche L, et al. Empagliflozin improves insulin sensitivity of the hypothalamus in humans with prediabetes: a randomized, double-blind, placebo-controlled, phase 2 trial. *Diabetes Care.* (2022) 45:398–406. doi: 10.2337/dc21-1136

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Investigating the association between fasting insulin, erythrocytosis and HbA1c through Mendelian randomization and observational analyses

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Background: Insulin resistance (IR) with associated compensatory hyperinsulinemia (HI) are early abnormalities in the etiology of prediabetes (preT2D) and type 2 diabetes (T2D). IR and HI also associate with increased erythrocytosis. Hemoglobin A1c (HbA1c) is commonly used to diagnose and monitor preT2D and T2D, but can be influenced by erythrocytosis independent of glycemia.

Methods: We undertook bidirectional Mendelian randomization (MR) in individuals of European ancestry to investigate potential causal associations between increased fasting insulin adjusted for BMI (FI), erythrocytosis and its non-glycemic impact on HbA1c. We investigated the association between the triglyceride-glucose index (TGI), a surrogate measure of IR and HI, and glycation gap (difference between measured HbA1c and predicted HbA1c derived from linear regression of fasting glucose) in people with normoglycemia and preT2D.

Results: Inverse variance weighted MR (IVWMR) suggested that increased FI increases hemoglobin (Hb, b=0.54 ± 0.09, p=2.7 x 10^{-10}), red cell count (RCC, b=0.54 ± 0.12, p=5.38x10^{-6}) and reticulocyte (RETIC, b=0.70 ± 0.15, p=2.18x10^{-6}). Multivariable MR indicated that increased FI did not impact HbA1c (b=0.23 ± 0.16, p=0.162) but reduced HbA1c after adjustment for T2D (b=0.31 ± 0.13, p=0.016). Increased Hb (b=0.03 ± 0.01, p=0.02), RCC (b=0.02 ± 0.01, p=0.04) and RETIC (b=0.03 ± 0.01, p=0.002) might modestly increase FI. In the observational cohort, increased TGI associated with decreased glycation gap, (i.e., measured HbA1c was lower than expected based on fasting glucose, (b=-0.09 ± 0.009, p<0.0001)) in people with preT2D but not in those with normoglycemia (b=0.02 ± 0.007, p<0.0001).

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Conclusions: MR suggests increased FI increases erythrocytosis and might potentially decrease HbA1c by non-glycemic effects. Increased TGI, a surrogate measure of increased FI, associates with lower-than-expected HbA1c in people with preT2D. These findings merit confirmatory studies to evaluate their clinical significance.

KEYWORDS

insulin resistance, hyperinsulinemia, type 2 diabetes (T2D), hemoglobin A1c, erythrocytosis

Introduction

The type 2 diabetes (T2D) pandemic is a major public health challenge, affecting more than 420 million people worldwide (1, 2). Insulin resistance (IR) and associated compensatory hyperinsulinemia (HI) are early abnormalities in the pathogenesis of prediabetes (preT2D) and Type 2 diabetes (T2D) (3). Although reduced insulin action (IR) is implicated in hyperglycemia, some aspects of insulin signaling pathways are preserved in states of IR. Consequently, some manifestations associated with IR are due to HI (4, 5). Close surveillance and timely intervention in people with IR and HI can potentially prevent T2D and remit/improve glycemia in those who develop T2D (6–8).

Increasingly, hemoglobin A1c (HbA1c) has replaced fasting glucose and/or the 75 g oral glucose tolerance test to diagnose preT2D, T2D and T2D remission. HbA1c is also used to set glycemic targets for people with diabetes (9–11). Advantages to using HbA1c compared to fasting glucose include convenience and use of an assay that is standardized, stable and reproducible with limited intraindividual variability. Further, it provides an average measure of glycemia in the prior 2 to 3 months (1, 12) (1). However, altered red cell lifespan and erythrocytosis can affect HbA1c measurement by non-glycemic pathways. This has implications in patients with red cell disorders and hemoglobinopathies (1, 13). In people without T2D, including those with preT2D, non-glycemic parameters are a major predictor of HbA1c. Higher hemoglobin (Hb) associates with lower HbA1c (14, 15). Observational studies have also reported higher Hb and red cell count with increased IR and HI (16–18). Whether this association is causal is not established nor is it known if it impacts HbA1c measurement through non-glycemic pathways. HI can potentially increase cell proliferation and thus plausibly mediate the increased erythrocytosis seen in people with IR and HI (19).

Mendelian randomization (MR) can be used to infer potential causal associations between an exposure and an outcome by assessing the effects of genetic variants robustly associated with the exposure in one population on the outcome of interest in a separate cohort (2 sample MR) (20). We undertook bidirectional MR to investigate potential causal associations between fasting insulin adjusted for BMI (FI) and measures of erythrocytosis (hemoglobin, measured as g/L of blood, red cell count and reticulocyte count) in people of European ancestry. We used summary statistics from the largest genome wide association studies (GWAS) to date in this population. We undertook multivariable MR to assess the non-glycemic effects of FI on HbA1c by adjusting for elevated fasting glucose (FG) and type 2 diabetes (T2D). We also explored the association between the triglyceride-glucose index (21), a surrogate measure of IR and HI and glycation gap (difference between measured HbA1c and predicted HbA1c from fasted glucose measurement), in a cohort of Canadian adults with normoglycemia and preT2D.

Methods

Cohorts

Demographic details of the cohorts used for MR analyses have been included in Table 1 (22–28). GWAS summary statistics for FI, HbA1c and FG were derived from GWAS undertaken by MAGIC (22). Summary statistics for T2D were derived from DIAGRAM/GERA/UK Biobank consortia (26). All other summary statistics were from the UK Biobank (24, 25, 27).

Overlap between exposure and outcome cohorts

There is no reported overlap between the cohorts.

Primary MR analyses

For our primary analysis, we undertook bidirectional inverse variance weighted (IVW) MR with FI as exposure (Supplementary File 2) and Hb, red cell count (RCC), reticulocyte count (RETIC) as outcomes. A p value of <0.05 was considered significant for primary and secondary analyses. We followed the recently published STROBE-MR reporting guidelines (Checklist in Supplementary File 1) (29). As we used publicly available summary statistics from GWAS, we did not seek institutional approval. Informed consent was obtained from the investigators from each participant in the original study.

TABLE 1 Cohort details for Mendelian Randomization (MR) analyses.

Trait	Population cohort	Mean Age	% Female	Sample size	Cases	Controls	PMID
Fasting Insulin (FI)	MAGIC	50.7	51.2	151,013	Not applicable	Not applicable	34059833
Type 2 Diabetes (T2D)	DIAGRAM/GERA/UK Biobank	54.1/63.3/56.9*	50.1/59.0/54.2*	655,666	61,714	593,952	30054458
Fasting Glucose (FG)	MAGIC	50.9	47.7	133,010	Not applicable	Not applicable	22885924
Hemoglobin (Hb)	UK Biobank	56.7	54.9	563,946	Not applicable	Not applicable	32888493
Red Cell Count (RCC)	UK Biobank	56.7	54.9	545,203	Not applicable	Not applicable	32888493
Reticulocytes (RETIC) ***	UK Biobank	56.7	54.9	408,112	Not applicable	Not applicable	32888494
HbA1c**	MAGIC	52.3	57.9	146,806	Not applicable	Not applicable	34059833
HbA1c**/***	UK Biobank	56.7	54.9	389,889	Not applicable	Not applicable	34017140

*Study-specific characteristics were not available for all UK Biobank data and was extrapolated from data available.
[a]Output from MRC IEU GWAS pipeline analysis using Phesant derived variables from UK Biobank, version 2: https://doi.org/10.5523/bris.pnoat8cxo0u52p6ynfaekeigi.
**To minimize overlap, bidirectional MR analyses with FI was undertaken with HbA1c measure in the UK Biobank, but for WHR adjusted for BMI analyses HbA1c was assessed in MAGIC.
***Estimated from available UK Biobank data (PMID 32888493) as data not available.

Secondary analyses

As we found a potential causal association between FI and erythrocytosis, we undertook univariable MR to investigate the association between FI and HbA1c followed by multivariable MR adjusted for FG, T2D or Hb. Adjustment for FG and T2D was not undertaken in combination due to concerns about collinearity (30). Adjustment for T2D and Hb in combination was not undertaken as the F-statistic was <10, indicative of a weak instrument.

MR assumptions: MR is based on three assumptions. First, the instrument is robustly associated with the exposure. Therefore, we only used SNPs that were genome-wide significantly associated for all the instruments (20). Second, that the instrument does not influence the outcome *via* another pathway other than the outcome i.e., no horizontal pleiotropy (20). Finally, the instrument is not influenced by any confounders (20). For univariable MR, we used inverse weighted MR (IVWMR) and additional sensitivity analyses including MR-Egger, weighted median, weighted mode and leave-one-out analyses.

IVWMR was performed by undertaking meta-analysis of the individual Wald ratio for each SNP in the instrument. By permitting a non-zero intercept, MR-Egger relaxes the assumption of no horizontal pleiotropy and returns an unbiased causal estimate, in the case of horizontal pleiotropy, providing that the horizontal pleiotropic effects are not correlated with the SNP-exposure effects (InSIDE assumption) (20, 31). The median effect of all SNPs in the instrument was used for analysis using weighted median MR, which permits SNPs with a greater effect on the association to be evaluated by weighting the contribution of each SNP by the inverse variance of its association with the outcome: this is robust even if only 50% of the SNPs satisfy all three MR assumptions (32). Finally, SNPs were clustered into groups based on similarity of causal effects for weighted mode MR, with the cluster with the largest number of SNPs deriving the causal effect estimate (33). Cochrane's Q test was used to assess heterogeneity, while leave-one-out analyses were conducted to assess if any MR estimate was biased by a single SNP potentially with horizontal pleiotropic effect (20) and the F statistic was calculated to assess the strength of the instrument exposure (20, 34, 35).

Univariable MR was conducted using the "TwoSampleMR" package in R (R studio® v1.3.1073 and R® v4.0.3). Linkage disequilibrium (LD) pruning was used to select a proxy (r^2>0.8) if a SNP was not directly matched from the 1000 Genomes project (Version 0.5.6, Released 2021-03-35). The "ggplot2" and "metaphor" packages in R were used to create plots. We undertook inverse variance weighted multivariable MR (IVW Multivariable MR) to assess the effect of FI on HbA1c after adjustment for FG and T2D and Hb (36). Multivariable MR was conducted using both the "TwoSampleMR", "Multivariable MR" and "RMultivariable MR" packages in R (R studio® v1.3.1073 and R® v4.0.3), where the latter two packages assessed heterogeneity *via* Cochrane's Q test and strength of the instrument *via* F statistics (34, 36). Plots were generated using "plotobject".

Observational study

Demographic details for this study have been included in Table 2. We received institutional approval from University Health Network (UHN) research ethics board for the observational study. As we analyzed anonymized data, we did not obtain consent from individual patients. We undertook analyses in a cohort of patients without T2D (n=7600 of whom 1096, i.e., 14.4%, had pre-T2D), who attended one of UHN's, an academic health center in Toronto, Canada, outpatient clinics between 2006 and 2022. We excluded patients who had attended diabetes clinics in the prior 2 years, those with fasting glucose ≥ 7mmol/L, HbA1c ≥ 6.5%, age >65 years or <18 years or Hb outside the sex-specific normal range. We did not undertake analyses in patients with diabetes as we did not have access to their medical records and could not ascertain the type of diabetes or their medications (e.g., insulin and sodium glucose co-transporter 1 inhibitors), which can impact both glycemia and erythrocytosis (19, 37)

Using R studio® v1.3.1073 and R® v4.0.3, predicted HbA1c was assessed based on regression analysis of fasting glucose adjusted for age and sex. To estimate the potential non-glycemic contribution to HbA1c, we assessed the glycation gap which is defined as the difference between measured HbA1c and predicted HbA1c (based on fasting glucose). We then assessed the association between triglyceride-glucose index (21), a surrogate measure of HI and IR, and the glycation gap. Triglyceride-glucose index (21) was calculated as ln [fasting triglyceride (mg/dL) × fasting plasma glucose (mg/dL)/2]. Correction factors of 88.57 and 18 were used to convert triglycerides to mg/L and fasting glucose to mg/dL, respectively.

The rms and lattice packages were used to fit a regression model and for estimation. The beta coefficient, standard error, y-intercept and p-value were analyzed in order to determine if there was an association between triglyceride-glucose index and glycation gap for all participants, those with pre-T2D (HbA1c 6-6.4% and fasting glucose 6-6.9 mmol/l) and those with normoglycemia (specified as HbA1c < 6% and fasting glucose <6 mmol/l). A p-value of < 0.05 was specified as being significant. The R^2 and adjusted R^2 were also analyzed to determine the fit of the model. An ANOVA table further analyzed if there was a linear relationship present.

Several diagnostic plots were created to test the presence of linearity and evaluate the fit of our model. A Normal Q-Q plot was created to test if the data had a normal distribution. If the data points fell onto a reasonably straight line, this would indicate a well fit model. Using the xyplot function, two plots were then created, both examining the residuals (residuals versus fitted values and residuals versus triglyceride-glucose index). If the plots produced a straight line, this would indicate a linear relationship. If the line was curved, this would indicate nonlinearity and splines would be required to analyze the cubic model.

Finally, the model was assessed for overfitting *via* validation. A set of random numbers was generated and the 0.632 Bootstrap method was used (38). The 0.632 Bootstrap method was chosen as to reduce bias by using correction factors. The R^2 and mean squared error (MSE) were analyzed. An overfit model would produce a significantly different R^2 and a higher MSE. Further, an optimism greater than 0.1 would suggest overfitting as well as a slope with shrinkage. A decrease in g-index would also be suggestive of overfitting. It should be noted that MSE and g-index may be difficult to interpret as they vary based on sample size and range of data. The same analyses were undertaken for triglyceride-glucose index on Hb.

Results

Primary analyses

Univariable MR analyses of FI and erythrocytosis (Hb, RCC, RETIC)

Univariable inverse variance weighted MR suggests increased FI increases Hb (b=0.54 ± 0.09, $p=2.7x10^{-10}$, RCC (b=0.54 ± 0.012, $p=5.38x10^{-6}$) and RETIC (b=0.70 ± 0.15, $p=2.18x10^{-6}$), with concordant results with MR-Egger, weighted median, weighted mode and simple mode MR analyses (Table 3; Figures 1, 2; Supplementary File 5).

Inverse variance weighted MR suggests increased Hb (b=0.03 ± 0.01, p=0.02), RCC (b=0.02 ± 0.01, p=0.04) and RETIC (b=0.03 ± 0.01, p=0.002) might modestly increase FI, but MR-Egger, weighted median, weighted mode and simple mode MR analyses did not find evidence for potential causal association (Supplementary Files 3, 5).

Secondary analyses

Univariable and multivariable MR analyses of FI as exposure (adjusted for FG, T2D and Hb) and HbA1c as outcome

Univariable inverse variance weighted MR suggests that increased FI does not significantly increase HbA1c (b=0.23 ± 0.16, p=0.16) (Table 4; Figure 3; Supplementary File 5).

Multivariable MR suggests FI decreases HbA1c after adjusting for T2D (b=-0.30 ± 0.13, p=0.02. After adjusting for Hb (b=0.36, $p=9.14x10^{-4}$), FI increases HbA1c, but the F-statistic of <10 precludes definitive conclusion. There was no significant effect of FI on HbA1c after adjusting for FG alone (b=-0.221 ± 0.13, p=0.096) (Table 4; Supplementary File 5).

Exploratory analyses

MR Analyses exploring association between Hb and HbA1c

MR suggests a bidirectional relationship between Hb and HbA1c. Univariable inverse variance weighted MR suggests increased Hb decreases HbA1c (b=-0.105, $p=1.17x10^{-13}$) concordant with MR-Egger, weighted median and mode but not simple mode analyses (Supplementary Files 4, 5). Reverse inverse variance weighted MR suggests increased HbA1c decreases Hb (b=-0.867, $p=6.02x10^{-7}$) concordant with MR-Egger, weighted median and simple mode but not weighted mode analyses (Supplementary Files 4, 5).

Observational study

Cohort details and descriptive statistics regarding the 7600 participants can be found in Tables 2, 5. Consistent with the MR analyses, increased TGI was associated with increased Hb (b=1.88 ± 0.19, p<0.001) (Figure 4A). Linear regression analysis yielded this equation for predicted HbA1c derived from fasting glucose and adjusted for age and sex: predicted HbA1c=4.163+0.172*(fasting glucose). In the cohort overall (the majority of whom had normoglycemia), (b=0.073 ± 0.008, p<0.0001) and in people with normoglycemia (b=0.023 ± 0.007, p<0.0001), increased triglyceride-glucose index was associated with an increase in glycation gap. However, among people with pre-T2D, increased triglyceride-glucose index was associated with a decreased glycation gap i.e., measured HbA1c was lower than that predicted by fasting glucose (b=-0.087 ± 0.009, p<0.0001) (Figures 4B–D).

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TABLE 2 Baseline characteristics for participants of observational study.

Characteristic	Healthy Participants, N = 6,504*	Participants with Pre-T2D, N = 1,096**	p-value[2]
Sex			0.076
Female	3,147 (48%)	562 (51%)	
Male	3,357 (52%)	534 (49%)	
Age (years)	50 (41, 58)	56 (49, 61)	<0.001
HbA1c (%)	5.40 (5.20, 5.60)	6.10 (6.00, 6.20)	<0.001
Fasting Glucose (mmol/L)	5.10 (4.70, 5.50)	5.70 (5.20, 6.20)	<0.001
Triglyceride (mmol/L)	1.16 (0.85, 1.68)	1.33 (0.99, 1.92)	<0.001
HDL Cholesterol (mmol/L)	1.29 (1.07, 1.57)	1.19 (1.01, 1.43)	<0.001
Hemoglobin (g/L)	145 (136, 154)	144 (134, 153)	<0.001
Triglyceride Glucose Index	8.46 (8.12, 8.85)	8.71 (8.36, 9.08)	<0.001
Predicted A1c, adjusted for age, sex (%)	5.04 (4.97, 5.11)	5.14 (5.06, 5.23)	<0.001
Glycation Gap***	0.36 (0.12, 0.57)	1.01 (0.89, 1.13)	<0.001

*Median (IQR); n (%).
**Wilcoxon rank sum test; Pearson's Chi-squared test.
***Actual HbA1c – Predicted HbA1c.

TABLE 3 Univariable MR analyses of fasting insulin (FI) as exposure and hemoglobin (Hb), red cell count (RCC) and reticulocyte count (RETIC) as outcomes.

Method	β	Standard Error	P	Egger-Intercept	p_{Egger}	Cochrane's Q	Q df	p_Q	I^2	F
Univariable MR Analysis — Exposure: FI (49 SNPs, single nucleotide polymorphisms), Outcome: Hb										
MR Egger	0.678	0.231	0.005	-0.002	0.534	857.625	47	$1.95x10^{-149}$	94.520	19.221
Weighted median	0.506	0.047	$6.22x10^{-27}$							19.221
Inverse variance weighted	0.544	0.086	$2.70x10^{-10}$			864.803	48	$2.80x10^{-150}$	94.450	19.221
Simple mode	0.624	0.090	$9.43x10^{-9}$							19.221
Weighted mode	0.575	0.063	$4.54x10^{-12}$							19.221
Univariable MR Analysis — Exposure: FI (49 SNPs), Outcome: RCC										
MR Egger	1.070	0.307	0.001	-0.009	0.068	1520.582	47	$8.23x10^{-288}$	96.909	19.221
Weighted median	0.377	0.055	$7.66x10^{-12}$							19.221
Inverse variance weighted	0.539	0.118	$5.38x10^{-6}$			1633.210	48	0	97.061	19.221
Simple mode	0.401	0.134	0.004							19.221
Weighted mode	0.443	0.098	$3.85x10^{-5}$							19.221
Univariable MR Analysis — Exposure: FI (49 SNPs), Outcome: RETIC										
MR Egger	-0.026	0.379	0.946	0.012	0.045	1744.044	47	0	97.305	19.221
Weighted median	0.605	0.061	$6.49x10^{-23}$							19.221
Inverse variance weighted	0.698	0.147	$2.18x10^{-6}$			1902.129	48	0	97.477	19.221
Simple mode	0.819	0.116	$6.18x10^{-9}$							19.221
Weighted mode	0.684	0.076	$7.48x10^{-12}$							19.221

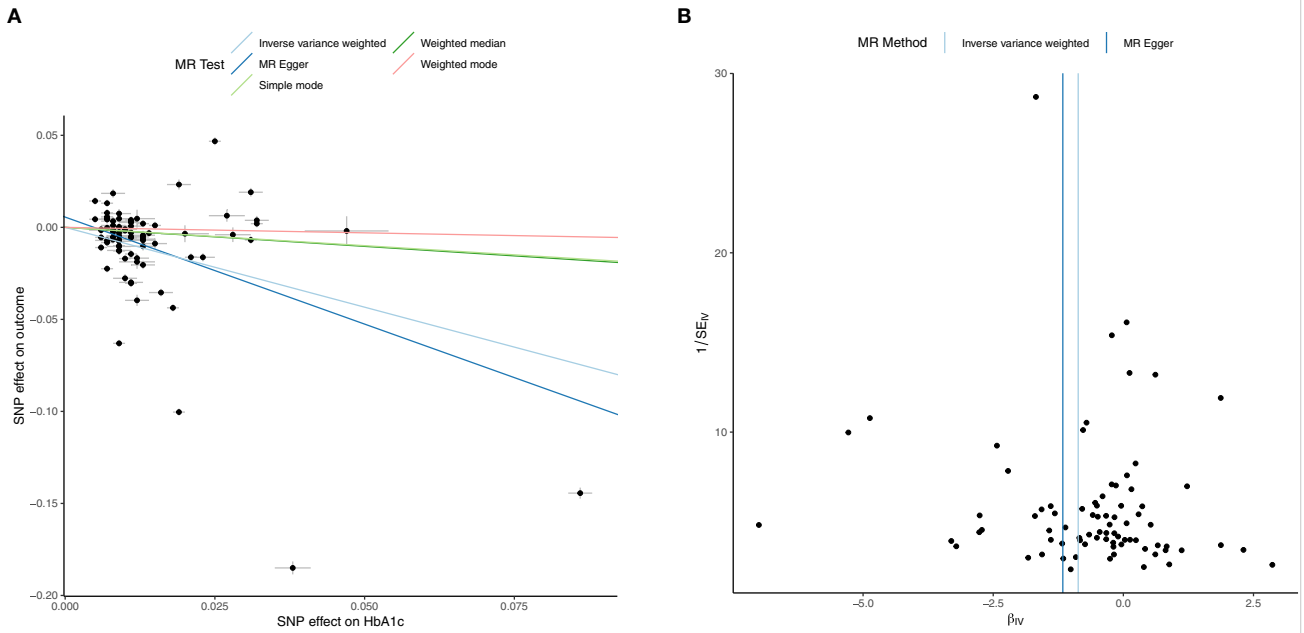

FIGURE 1
Univariable Mendelian Randomization (MR) Analysis — Exposure: fasting insulin (FI), Outcome: hemoglobin (Hb)— **(A)** Scatter plot showing the single nucleotide polymorphisms (SNPs) associated with FI against SNPs associated with Hb (vertical and horizontal black lines around points show 95% confidence intervals (CI) for five different Mendelian Randomization (MR) association tests **(B)** Funnel plot of the effect size against the inverse of the standard error for FI against Hb.

For all analyses, the R^2 adjusted and unadjusted were almost identical and the regression p-values were <0.005. Normal Q-Q plots for all data were suggestive of a normal distribution and good fit. Plots for residuals versus triglyceride-glucose index and residuals versus fitted values suggested linearity and that likely all relationships were accounted for in the model. Using the 0.632 Bootstrap method, validation was carried out and resulted in corrected R^2 and corrected slopes that were relatively similar to the original values. The R optimism was found to be 0.0116, which was less than the cut-off of 0.1. The MSE increased by 1.57% and the g-index decreased by 3.97%. These results indicate that the model was not overfit.

Discussion

Epidemiological data suggests that IR and HI are associated with increased erythrocytosis (16–18), which may plausibly be secondary to HI mediated erythrocytosis (19). Our MR analyses suggests that a causal association between HI and increased erythrocytosis. MR further suggests that increased FI after adjustment for T2D reduces HbA1c. MR also indicates a bidirectional inverse relationship between Hb and HbA1c. Collectively, this data suggest that HI mediated erythrocytosis might potentially lower HbA1c by non-glycemic effects with the transition from normoglycemia to T2D. Our observational data was concordant with the MR analyses. It showed that increased triglyceride-glucose index was associated with higher Hb. Further, increased triglyceride-glucose index associated with lower-than-expected HbA1c based on fasting glycemia. These findings await confirmation and assessment of clinical significance in well-designed prospective studies across the glycemic spectrum from normoglycemia to preT2D and T2D.

Increased FI is a recognized compensatory feature of IR. Some features of IR and HI such as increased hepatic glucose production are likely a consequence of reduced insulin action, while others such as hepatic steatosis and dyslipidemia are likely due to increased insulin action *via* signaling pathways that are not perturbed in IR (4, 5). *In vitro* studies suggest that insulin can increase erythrocytosis (19). This suggests that increased insulin action i.e., HI likely underpins the increased erythrocytosis seen with IR and HI. Further studies are needed to confirm these findings and explore underlying mechanisms and signaling pathways.

The potential non-glycemic impacts of increased fasting insulin on HbA1c might lead to lower-than-expected HbA1c based on glycemia and thus have implications for people with IR and HI during screening for preT2D and T2D. HbA1c is increasingly used to diagnose these conditions, in lieu of fasting glucose/oral glucose tolerance test measures, and to set glycemic targets for treatment (1, 9–12). Interestingly, observational data indicates that ~40% of people with T2D diagnosed based on more than one measure of elevated fasting glucose and/or post OGTT glucose, have HbA1c below the diabetes threshold (39). The potential non-glycemic effects of increased FI on HbA1c may also be particularly

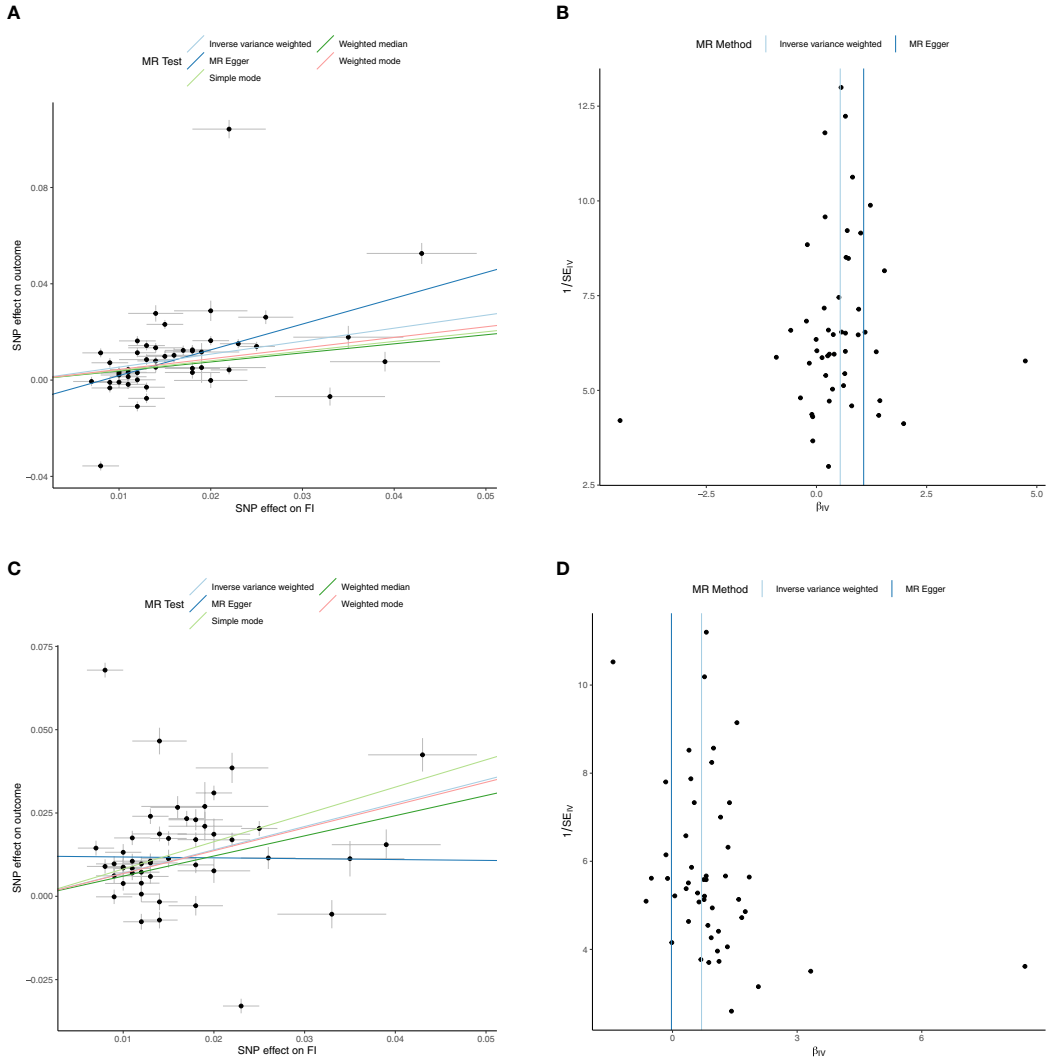

FIGURE 2
Univariable Mendelian Randomization (MR) Analysis — Exposure: fasting insulin (FI), Outcome: red cell count (RCC) and reticulocyte count (RETIC)— **(A)** Scatter plot showing the single nucleotide polymorphisms (SNPs) associated with FI against SNPs associated with RCC (vertical and horizontal black lines around points show 95% confidence intervals (CI) for five different Mendelian Randomization (MR) association tests **(B)** Funnel plot of the effect size against the inverse of the standard error for each SNP for FI against RCC **(C)** Scatter plot showing the single nucleotide polymorphisms (SNPs) associated with FI against SNPs associated with RETIC (vertical and horizontal black lines around points show 95% confidence intervals (CI) for five different Mendelian Randomization (MR) association tests **(D)** Funnel plot of the effect size against the inverse of the standard error for each SNP for FI against RETIC.

TABLE 4 Univariable MR analyses of fasting insulin (FI) and glycated hemoglobin (HbA1c) as outcome followed by multivariable MR adjusted for elevated fasting glucose (FG), type 2 diabetes (T2D) and increased hemoglobin (Hb).

Method	β	Standard Error	P	Egger-Intercept	p_{Egger}	Cochrane's Q	Q df	p_Q	I^2	F
Univariable MR Analysis — Exposure: FI (49 SNPs, single nucleotide polymorphisms), Outcome: HbA1c										
MR Egger	0.226	0.436	0.608	$2.70x10^{-5}$	0.997	2610.360	47	0	98.199	19.221
Weighted median	0.348	0.055	$1.72x10^{-10}$							19.221
Inverse variance weighted	0.227	0.162	0.162			2610.361	48	0	98.161	19.221
Simple mode	0.689	0.097	$5.57x10^{-9}$							19.221

(Continued)

TABLE 4 Continued

Method	β	Standard Error	P	Egger-Intercept	p_{Egger}	Cochrane's Q	Q df	p_Q	I^2	F
Weighted mode	0.432	0.118	$6.42x10^{-4}$							19.221
Multivariable MR Analysis — Exposure: FI adjusted FG (30 SNPs), Outcome: HbA1c										
Inverse Variance Weighted	-0.221	0.133	0.096			1111.614	82	$3.42x10^{-180}$	92.623	20.75
Multivariable MR Analysis — Exposure: FI adjusted T2D (21 SNPs), Outcome: HbA1c										
Inverse Variance Weighted	-0.305	0.126	0.016			1211.384	112	$8.18x10^{-184}$	90.754	14.524
Multivariable MR Analysis — Exposure: FI adjusted Hb (16 SNPs), Outcome: HbA1c										
Inverse Variance Weighted	0.363	0.110	$9.14x10^{-4}$			8336.312	403	0	95.166	4.547

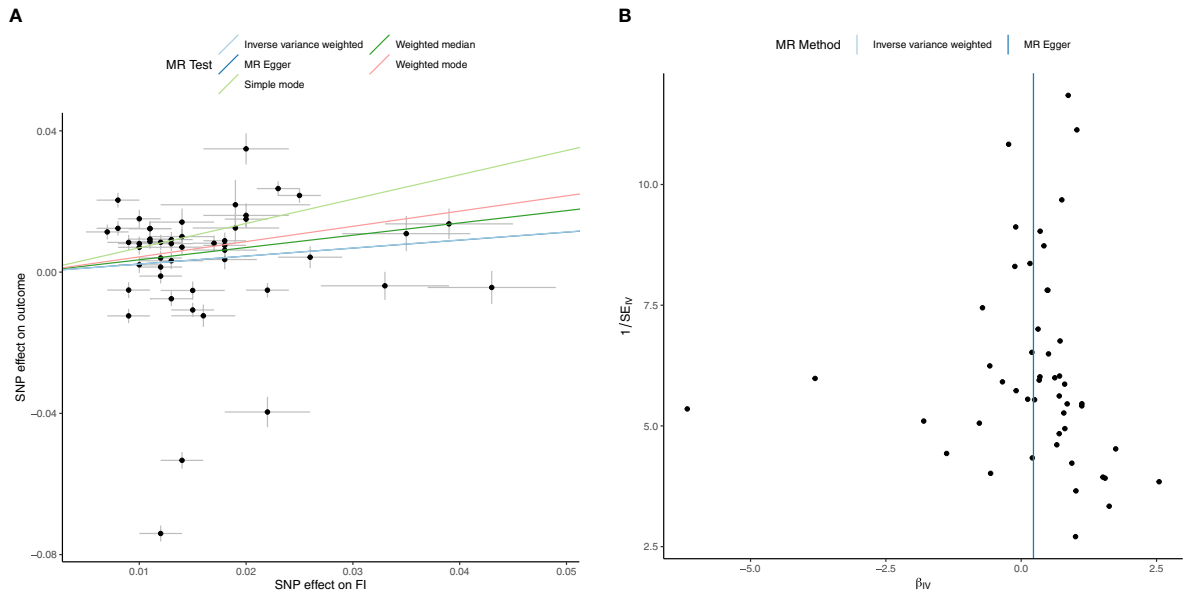

FIGURE 3
Univariable Mendelian Randomization (MR) Analysis — Exposure: fasting insulin (FI), Outcome: HbA1c— **(A)** Scatter plot showing the single nucleotide polymorphisms (SNPs) associated with FI against SNPs associated with HbA1c (vertical and horizontal black lines around points show 95% confidence intervals (CI) for five different Mendelian Randomization (MR) association tests **(B)** Funnel plot of the effect size against the inverse of the standard error for FI against HbA1c.

TABLE 5 Linear regression for Triglyceride Glucose Index (exposure) on glycation gap* (outcome).

Population (n)	Beta	Standard error	y-intercept	p-value
Pre-T2D (1096)	-0.087	0.009	1.77	<0.0001
Healthy (6504)	0.023	0.007	0.14	<0.0001
HbA1c < 5% (681)	0.071	0.01	0.41	<0.0001
HbA1c 5 – 5.4% (2948)	0.047	0.005	0.6	<0.0001
HbA1c 5.5 – 5.9% (2875)	0.054	0.006	1.06	<0.0001

*Calculated as actual HbA1c – predicted HbA1c using model adjusted for age, sex (21).

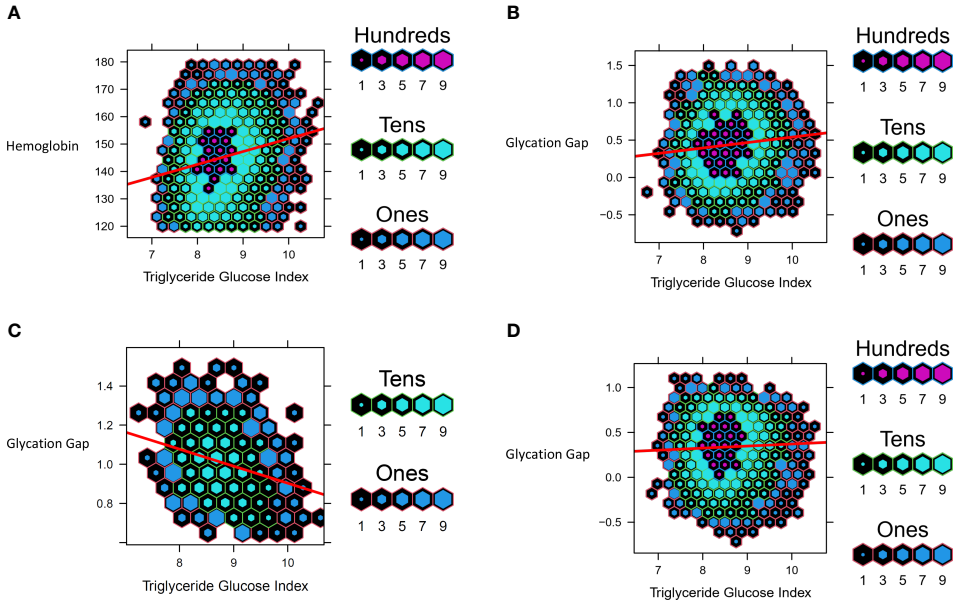

FIGURE 4
Data from UHN cohort. Hexbin plots represent number of study participants with the observed and calculated values, where the color and size of each individual hexagon correlates to the number of participants with the corresponding values. The red line represents the regression line for each cohort. **(A)** Association between Triglyceride Glucose Index and Hemoglobin **(B)** Association between Triglyceride Glucose Index and Glycation Gap in all participants **(C)** Association between Triglyceride Glucose Index and Glycation Gap in with pre-T2D **(D)** Association between Triglyceride Glucose Index and Glycation Gap in those with normoglycemia.

pertinent for weight loss induced T2D diabetes remission. HbA1c is the recommended glycemic parameter to define remission in a patient population with high prevalence of IR and HI (11).

The strengths of this study include MR analyses with the largest sample sizes in populations of European ancestry and likely minimal/no overlap between participants in the exposure and outcome cohorts. Our study has several limitations. The findings may not apply to other ethnic groups given that we used populations with European ancestry only. This may especially be a concern in populations with higher prevalence of hemoglobinopathies and red cell disorders (13, 40–43). Additionally, analyses were not stratified by sex which is a major determinant of body composition, IR and HI (44). For our observational data we did not have access to individual level data including medications and comorbidities. Due to these limitations, we also excluded patients with biochemical evidence of T2D as we could not reliably ascertain the type of diabetes and account for the potential impact of medications which might impact red cell parameters. We derived predicted HbA1c from fasting glucose and did not account for post-prandial readings which is a major limitation. Finally, we did not have measures of FI in the observational cohort and therefore used surrogate measures of IR and HI in our analyses.

In conclusion, our data suggests that increased FI, a feature of IR, may increase erythrocytosis and might potentially lower HbA1c independent of glycemia. As these findings might have implications for the diagnoses and management of preT2D and T2D, it merits well designed prospective confirmatory studies across the glycemic spectrum to confirm these findings and assess whether these effects are clinically relevant.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by University Health Network. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SD, AP, AN, and RK designed the study. All authors analyzed the data. AN, RK, and SD wrote the manuscript and all authors read and edited the manuscript. SD is the guarantor of this work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1146099/full#supplementary-material

References

1. Selvin E. Hemoglobin a 1c-using epidemiology to guide medical practice: Kelly West award lecture 2020. *Diabetes Care* (2021) 44(10):2197–204. doi: 10.2337/dci21-0035

2. WHO Global Report. *Global report on diabetes* (2016). Available at: http://www.who.int/about/licensing/copyright_form/index.html%0Ahttp://www.who.int/about/licensing/copyright_form/index.html%0Ahttp://www.who.int/about/licensing/copyright_form/index.html%0Ahttps://apps.who.int/iris/handle/10665/204871%0Ahttp://www.who.int.

3. Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: An analysis from the Whitehall II study. *Lancet* (2009) 373:2215–21. doi: 10.1016/S0140-6736(09)60619-X

4. Brown MS, Goldstein JL. Selective versus total insulin resistance: A pathogenic paradox. *Cell Metab* (2008) 7:95–6. doi: 10.1016/j.cmet.2007.12.009

5. Semple RK, Sleigh A, Murgatroyd PR, Adams CA, Bluck L, Jackson S, et al. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *J Clin Invest* (2009) 119:315–22. doi: 10.1172/JCI37432

6. Aminian A, Vidal J, Salminen P, Still CD, Hanipah ZN, Sharma G, et al. Late relapse of diabetes after bariatric surgery: Not rare, but not a failure. *Diabetes Care* (2020) 43:534–40. doi: 10.2337/dc19-1057

7. Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. *Lancet Diabetes Endocrinol* (2019) 7:344–55. doi: 10.1016/S2213-8587(19)30068-3

8. Nathan DM, Barrett-Connor E, Crandall JP, Edelstein SL, Goldberg RB, Horton ES, et al. Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications: the DPP outcomes study. *Lancet Diabetes Endocrinol* (2015) 3:866. doi: 10.1016/S2213-8587(15)00291-0

9. Diabetes Canada Clinical Practice Guidelines Expert C, Berard LD, Siemens R, Pharm B, Woo V. Monitoring Glycemic Control. *Can J Diabetes* (2018) 42 (Suppl 1): S47–53. doi: 10.1016/j.jcjd.2017.10.007.

10. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. Management of hyperglycaemia in type 2 diabetes, 2018. a consensus report by the American diabetes association (ADA) and the European association for the study of diabetes (EASD). *Diabetologia* (2018) 61:2461–98. doi: 10.1007/s00125-018-4729-5

11. Riddle MC, Cefalu WT, Evans PH, Gerstein HC, Nauck MA, Oh WK, et al. Consensus report: Definition and interpretation of remission in type 2 diabetes. *Diabetes Care* (2021) 44:2438–44. doi: 10.2337/dci21-0034

12. Reed J, Bain S, Kanamarlapudi V. A review of current trends with type 2 diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. *Diabetes Metab Syndr Obes* (2021), 3567–602. doi: 10.2147/DMSO.S319895

13. Lacy ME, Wellenius GA, Sumner AE, Correa A, Carnethon MR, Liem RI, et al. Association of sickle cell trait with hemoglobin A1c in African americans. *JAMA* (2017) 317:507–15. doi: 10.1001/jama.2016.21035

14. Fizelova M, Stančáková A, Lorenzo C, Haffner SM, Cederberg H, Kuusisto J, et al. Glycated hemoglobin levels are mostly dependent on nonglycemic parameters in 9398 Finnish men without diabetes. *J Clin Endocrinol Metab* (2015) 100:1989–96. doi: 10.1210/jc.2014-4121

15. Jansen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BHR, Snieder H. Determinants of HbA1c in nondiabetic Dutch adults: Genetic loci and clinical and lifestyle parameters, and their interactions in the lifelines cohort study. *J Intern Med* (2013) 273:283–93. doi: 10.1111/joim.12010

16. Barazzoni R, Cappellari GG, Semolic A, Chendi E, Ius M, Situlin R, et al. The association between hematological parameters and insulin resistance is modified by body mass index – results from the north-East Italy MoMa population study. *PloS One* (2014) 9:e101590. doi: 10.1371/journal.pone.0101590

17. Woo M, Hawkins M. Beyond erythropoiesis: Emerging metabolic roles of erythropoietin. *Diabetes* (2014) 63:2229–31. doi: 10.2337/db14-0566

18. Barbieri M, Ragno E, Benvenuti E, Zito GA, Corsi A, Ferrucci L, et al. New aspects of the insulin resistance syndrome: Impact on haematological parameters. *Diabetologia* (2001) 44(10):1232–7. doi: 10.1007/s001250100634

19. Ratajczak J, Zhang Q, Pertusini E, Wojczyk BS, Wasik MA, Ratajczak MZ. The role of insulin (INS) and insulin-like growth factor-I (IGF-I) in regulating human erythropoiesis. studies in vitro under serum-free conditions–comparison to other cytokines and growth factors. *Leukemia* (1998) 12:371–81. doi: 10.1038/sj.leu.2400927

20. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-base platform supports systematic causal inference across the human phenome. *eLife* (2018) 7:e34408. doi: 10.7554/eLife.34408.001

21. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord* (2008) 6:299–304. doi: 10.1089/met.2008.0034

22. Chen J, Spracklen CN, Marenne G, Varshney A, Corbin LJ, Luan J, et al. The trans-ancestral genomic architecture of glycemic traits. *Nat Genet* (2021) 53:840–60. doi: 10.1038/s41588-021-00852-9

23. Vuckovic D, Bao EL, Akbari P, Lareau CA, Mousas A, Jiang T, et al. The polygenic and monogenic basis of blood traits and diseases. *Cell* (2020) 182:1214–1231.e11. doi: 10.1016/j.cell.2020.08.008

24. Chen MH, Raffield LM, Mousas A, Sakaue S, Huffman JE, Moscati A, et al. Trans-ethnic and ancestry-specific blood-cell genetics in 746,667 individuals from 5 global populations. *Cell* (2020) 182:1198–1213.e14. doi: 10.1016/j.cell.2020.06.045

25. Mbatchou J, Barnard L, Backman J, Marcketta A, Kosmicki JA, Ziyatdinov A, et al. Computationally efficient whole-genome regression for quantitative and binary traits. *Nat Genet* (2021) 53:1097–103. doi: 10.1038/s41588-021-00870-7

26. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun* (2018) 9. doi: 10.1038/s41467-018-04951-w

27. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, et al. Large-Scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet* (2012) 44:991–1005. doi: 10.1038/ng.2385

28. Pulit SL, Stoneman C, Morris AP, Wood AR, Glastonbury CA, Tyrrell J, et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum Mol Genet* (2019) 28:166–74. doi: 10.1093/hmg/ddy327

29. Skrivankova VW, Richmond RC, Woolf BAR, Davies NM, Swanson SA, Vanderweele TJ, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomisation (STROBE-MR): explanation and elaboration. *BMJ* (2021) 375. doi: 10.1136/bmj.n2233

30. Nguyen A, Khafagy R, Gao Y, Meerasa A, Roshandel D, Anvari M, et al. Association between obesity and chronic kidney disease: multivariable mendelian randomization analysis and observational data from a bariatric surgery cohort. *Diabetes* (2023). doi: 10.2337/figshare.21913053

31. Bowden J, Smith GD, Burgess S. Mendelian randomization with invalid instruments: Effect estimation and bias detection through egger regression. *Int J Epidemiol* (2015) 44:512–25. doi: 10.1093/ije/dyv080

32. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* (2016) 40:304–14. doi: 10.1002/gepi.21965

33. Hartwig FP, Smith GD, Bowden J. Robust inference in summary data mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol* (2017) 46:1985–98. doi: 10.1093/ije/dyx102

34. Sanderson E, Spiller W, Bowden J. Testing and correcting for weak and pleiotropic instruments in two-sample multivariable mendelian randomization. *Stat Med* (2021) 40:5434–52. doi: 10.1002/sim.9133

35. Sadreev II, Elsworth BL, Mitchell RE, Paternoster L, Sanderson E, Davies NM, et al. Navigating sample overlap, winner's curse and weak instrument bias in mendelian randomization studies using the UK biobank. *medRxiv* (2021). doi: 10.1101/2021.06.28.21259622v1

36. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol* (2019) 48:713–27. doi: 10.1093/ije/dyy262

37. Inzucchi SE, Zinman B, Fitchett D, Wanner C, Ferrannini E, Schumacher M, et al. How does empagliflozin reduce cardiovascular mortality? insights from a mediation analysis of the EMPA-REG OUTCOME trial. *Diabetes Care* (2018) 41:356–63. doi: 10.2337/dc17-1096

38. Efron B, Tibshirani R. Improvements on cross-validation: The .632+ bootstrap method. *J Am Stat Assoc* (1997) 92:548. doi: 10.2307/2965703

39. Griffin SJ, Borch-Johnsen K, Davies MJ, Khunti K, Rutten GE, Sandbæk A, et al. Effect of early intensive multifactorial therapy on 5-year cardiovascular outcomes in individuals with type 2 diabetes detected by screening (ADDITION-europe): A cluster-randomised trial. *Lancet* (2011) 378:156–67. doi: 10.1016/S0140-6736(11)60698-3

40. Sarnowski C, Leong A, Raffield LM, Wu P, de Vries PS, DiCorpo D, et al. Impact of rare and common genetic variants on diabetes diagnosis by hemoglobin A1c in multi-ancestry cohorts: The trans-omics for precision medicine program. *Am J Hum Genet* (2019) 105:706–18. doi: 10.1016/j.ajhg.2019.08.010

41. Chai JF, Kao SL, Wang C, Jun-Yu Lim V, Khor IW, Dou J, et al. Genome-wide association for HbA1c in Malay identified deletion on SLC4A1 that influences HbA1c independent of glycemia. *J Clin Endocrinol Metab* (2020) 105:3854–64. doi: 10.1210/clinem/dgaa658

42. Wu W-C, Lacy ME, Correa A, Carnethon M, Reiner AP, Eaton CB, et al. Association between hemoglobin A1c and glycemia in African americans with and without sickle cell trait and whites, results from CARDIA and the Jackson heart study. *J Diabetes Treat* (2018) 3.

43. Paterson AD. HbA1c for type 2 diabetes diagnosis in africans and African americans: Personalized medicine NOW! *PloS Med* (2017) 14:e1002384. doi: 10.1371/journal.pmed.1002384

44. Lagou V, Mägi R, Hottenga JJ, Grallert H, Perry JRB, Bouatia-Naji N, et al. Sex-dimorphic genetic effects and novel loci for fasting glucose and insulin variability. *Nat Commun* (2021) 12:1–18. doi: 10.1038/s41467-020-19366-9

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Insulin amyloidosis: A case report

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Insulin amyloidosis is a rare form of localized amyloidosis due to insulin aggregation into subcutaneous amyloid fibrils. We describe the case of a 55years old male with insulin-requiring type 1 diabetes presenting with two non-inflammatory intra-dermal nodules associated with local lymph node enlargement. Diagnosis was confirmed by Congo red coloration of the amyloid deposit and insulin protein identification on mass spectrometry. Insulin amyloidosis is a potential complication of repeated subcutaneous insulin injections. The main risk factor is the intrinsic characteristic of the insulin used. Insulin amyloidosis leads to systemic metabolic consequences such as chronic hyperglycemia or unpredictable hypoglycemia, as well as unesthetic cutaneous lumps or abscesses. Standard-of-care is yet to be defined but mainly rely on therapeutical education of insulin injections, while surgical excision is reported to improve glycemic control in some patients.

KEYWORDS

amyloidosis, insulin, type 1 diabetes, hyperglycemia, case report

Highlights

- Insulin amyloidosis (AIns) is a localized form of amyloidosis appearing in some insulin-requiring diabetic patients.
- AIns manifests as painless, non-inflammatory subcutaneous lumps, which may be overlooked as lipohypertrophy.
- In some cases, a granulomatous inflammation around the amyloid deposits can be evidenced on a 18-FDG-PET scan.
- The biopsy typically reveals amyloid deposits further characterized by immunohistochemistry, mass spectroscopy, or sequencing.
- In addition to esthetic discomfort or local infection (abscesses), AIns can lead to chronic hyperglycemia and unpredictable hypoglycemia.
- First line of care consists in patient education to avoid subsequent insulin injections in the same, or affected locations, while surgical removal has been shown to improve glycemic control in some situations.

Introduction

Amyloidosis is a heterogenous group of diseases defined by systemic or localized amyloid fibril deposits in tissues and extracellular spaces of organs (1). Amyloid fibril is an insoluble structure resulting of a protein's abnormal folding and aggregation in cross β-sheets, characterized by a green or yellow birefringence in polarization microscopy after Congo red staining. This criteria is key to retain the diagnosis of amyloidosis. In 2022, up to 42 different

forms of amyloidosis were reported in the international classification established by the International Society of Amyloidosis (ISA) based on the protein contained in the amyloid deposits. Addition of a new protein to the classification implies that its whole sequence has been characterized and published (1).

Insulin's amylogenic potential was first demonstrated in 1983 by Störkel et al. who reported the presence of amyloid deposits on a systematic skin biopsy performed in a patient treated with daily injections of porcine insulin (2). Dische confirmed the observation 5 years later and identified insulin as the component of the amyloid substance in a patient by using protein sequencing (3). Insulin amyloidosis is now recognized as a local form of amyloidosis of iatrogenic origin, abbreviated to AIns amyloidosis in the international nomenclature (1). Insulin amyloidosis is a rare, unrecognized and probably under-diagnosed condition due to its mostly asymptomatic nature (4). However, it may have clinical significance in some situations. We report the case of a 55 years old patient with insulin amyloidosis of the left arm in the context of persistent chronic hyperglycemia.

Case description

A 55-year-old man initially presented to oncology and internal medicine consultation for several subcutaneous masses that progressively appeared on his left arm during spring 2021. Employed in construction and green spaces, his main medical history was type 1 diabetes treated by basal-bolus insulin therapy (64 IU/day at the time of the medical record), hypercholesterolemia treated with rosuvastatin, and active alcohol and tobacco consumption. He also received aspirin 160 mg daily in a primary prevention of cardiovascular events.

The patient's type 1 diabetes was diagnosed at the age of 26 and was characterized by anti-GAD antibody positivity, poor glycemic control (8.6% of glycated hemoglobin in early 2022, 7,8-8,8% during the last 10 years) and an absence of ocular, renal, neurological or cardiovascular complication detected at the term of the last check-up in 2022. Regarding his history of diabetes treatment, he had been following a basal-bolus insulin therapy since the time of diagnosis, comprising insulin glargine as a long-acting insulin, and various types of rapid-acting insulin (human insulin initially for 16 years, insulin asparte for 10 years then insulin lispro subsequently for the last 3 years).

The initial clinical examination revealed two non-inflammatory mobile, firm but painless, subcutaneous nodules measuring 2 and 5 cm on the outer and inner sides of the left forearm (Figure 1A). Two lymph node enlargements were palpable on the elbow and the left armpit, both mobile and painless. Patient's general condition was normal, and there was no fever or other symptoms. There was no history of recent travel and neither infectious nor animal contact.

Biologically, there was no inflammatory syndrome, no renal failure, no elevation of troponin nor NT-proBNP, no abnormal blood count, no disturbance of hemostasis or transaminases, but isolated cholestasis with gamma-GT at 145 IU/l (3-ULN). There was no proteinuria, plasma protein electrophoresis showed normal levels of gamma globulins at 8.9 g/l with polyclonal shape, serum and urine free light chains and angiotensin converting enzyme were normal. Immunophenotyping of circulating lymphocytes only revealed an increase in the CD4/CD8 ratio with no argument for a lymphoid hemopathy. Broad infectious tests ruled out an infection.

A soft tissue ultrasound (Figure 1B) confirmed the two sub-cutaneous lesions of the left forearm, the main one measuring 4 cm in its longitudinal axe, associated with four enlarged lymph nodes located around elbow and armpit. An MRI of the left upper limb revealed the two sub-cutaneous lesions characterized by T2 hyposignal and enhancement upon gadolinium (Figure 1C). A 18-FDG-PET-CT-scan showed an isolated hypermetabolism (Figure 1D) colocalizing with the two subcutaneous masses and the enlarged local lymph nodes. The surgical biopsy of one lesion revealed granulomatous remodeling in contact with amyloid deposits (Figures 2A,B), displaying red-green birefringence under polarized light after Congo red staining (Figure 2C). Bacteriological and mycobacteriological cultures of the surgical specimen were both negative. Proteomic analyses using mass spectrometry confirmed the insulin origin of the amyloid deposits leading to the diagnosis of insulin amyloidosis (Figure 2D). Upon further medical history taking, the patient revealed using these two locations (among others) as injections sites for insulin.

Discussion

Insulin amyloidosis is a rare and probably under-diagnosed condition due to its mostly asymptomatic nature (4). However, it may have clinical significance in some situations, like in our patient, with persistent chronic hyperglycemia. Indeed, AIns is a rare complication of insulin therapy in diabetic patients, mainly type 1 diabetes (66% of reported cases, like our patient), with a history of diabetes ranging from 7 to 60 years (4). Insulin is a 51-amino-acid polypeptide made of one α-chain and one β-chain linked by disulfide bridges. Although the α-chain also exhibits a propensity to aggregate, the β insulin chain is the one chain mostly involved in β cross-sheet assembly, under the influence of several factors specific to the therapeutic formulation of insulin: pH of the solution (an acidic pH promotes aggregation), high insulin concentration, elevated shear forces exerted in insulin pens or pumps, adjuvant molecules used in the formulations (e.g., silicone oil), and even the type of the insulin itself, as human insulin appear to more likely aggregate into sheets than insulin of porcine origin (4–7). Regarding analogs, they all have been reported susceptible to form amyloid fibrils, especially insulin lispro according to the work by Woods et al. (6) These five factors combined with the wide use of insulin pens and pumps since the late 1980s may partly explain the increasing number of reported cases (4). Another explanation could be a closer monitoring of insulin injection sites in diabetic patients and thus more frequent diagnoses of AIns amyloidosis (7). In the medical case we herein report, the patient had been treated with pens of rapid-acting human insulin for approximately 16 years upon diagnosis of type 1 diabetes before being treated with insulin analogs (insulin asparte first, then insulin lispro) for another 13 years, along with insulin glargine for the whole duration of the disease. Insulin amyloidosis subcutaneous nodules appeared 29 years after the disease's onset, at a time when insulin lispro was being used as the rapid-acting insulin.

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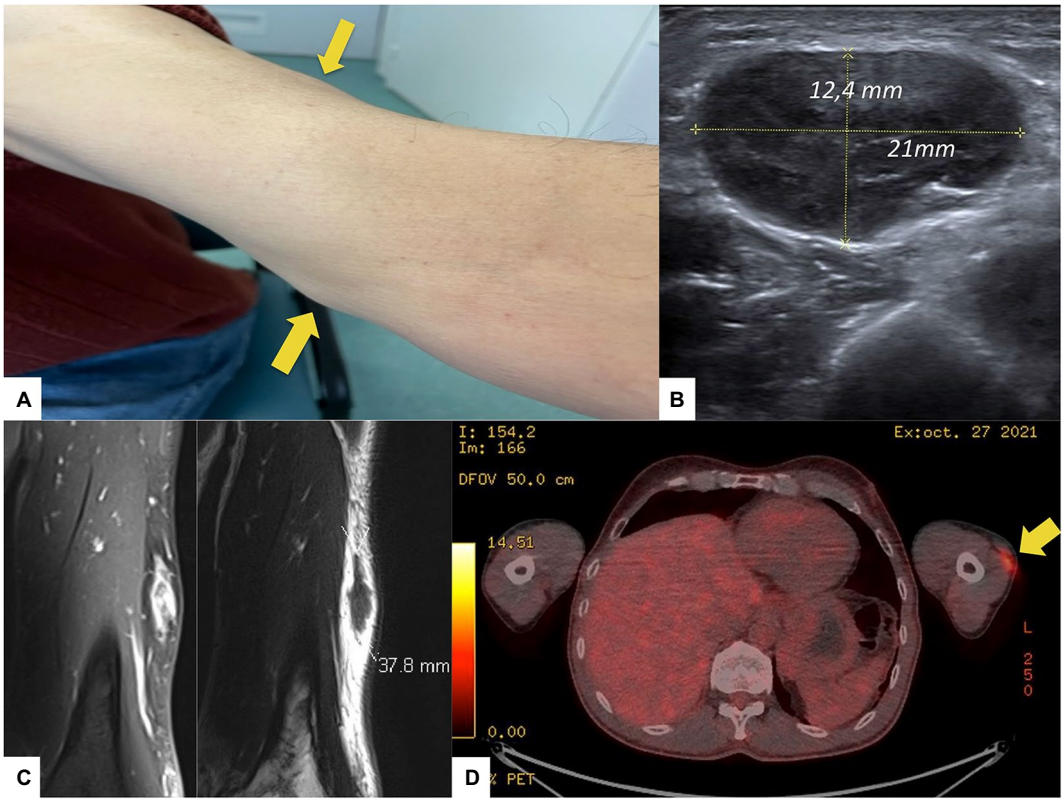
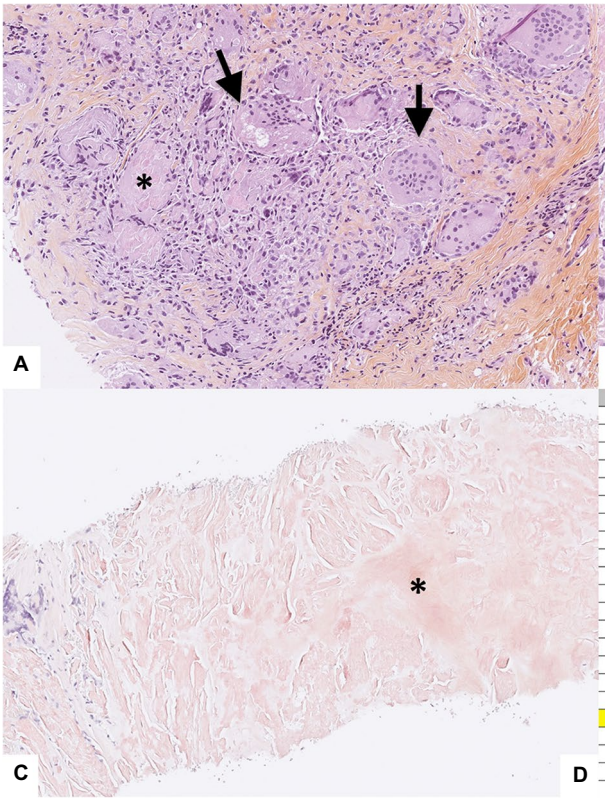


FIGURE 1
Clinical and paraclinical features of the patient. **(A)** Presence of two subcutaneous nodules on the left arm (arrows), **(B)** Soft tissue ultrasound of the left arm evidencing and measuring one subcutaneous mass, **(C)** MRI frontal slices of the left upper limb (T1 with gadolinium injection on the left, and T2 on the right), and **(D)** 18-FDG-PET-CT scan showing a 18-FDG uptake by the nodule (arrow) on the left arm.

Classified as a local form of amyloidosis, AIns amyloidosis mainly manifests as firm subcutaneous nodules or masses at one or more insulin injection sites (5). Most of reported cases describe a local impact ranging from simple esthetic discomfort to infectious complications and abscesses (3). Association with lymph node enlargement at lymphatic drainage sites, as described in our case, is however uncommon: only one other case reported amyloid deposits associated with a regional adenopathy (5). Nevertheless, AIns amyloidosis can lead to more systemic symptoms, as it is often associated with metabolic complications such as hypoglycemia, or more frequently, chronic hyperglycemia. In a recent literature review, Nilsson described the difficult control of diabetes in patients with AIns amyloidosis, with a mean glycated hemoglobin of 9.7% (4). Indeed, diabetic patients with amyloid nodules prefer injecting insulin in these amyloid sites because so injections are less painful, while it is now clear that insulin absorption is much lower at these sites, around 34% of the usual absorption (6, 7). Several hypotheses have been raised: mechanical obstruction to injected insulin, or local enzymatic degradation or conversion of injected insulin into monomers by the amyloid fibrils (4). On the other hand, hypoglycemia is also frequently observed in these patients, possibly due to an unpredictable release of the insulin accumulated at the injection site(s) (4).

Like for other forms of amyloidosis, histological diagnosis of AIns amyloidosis relies on the proof of amyloid deposition using Congo red staining and characterization of the amyloid deposit using immunochemistry, mass spectrometry, amino acid analysis or sequencing (4). However, as AIns amyloidosis is a rare and poorly known condition manifesting with non-specific clinical and biological features, clinicians should rule out differential diagnoses, such as multiple myeloma, systemic amyloidosis, and other malignancies. Indeed, the occurrence of a subcutaneous mass in insulin-requiring diabetic patients is a frequent issue affecting 27 to 64.4% of these patients (8). Lipohypertrophy is the main differential diagnosis, manifesting as painless subcutaneous masses at the insulin injection site(s). In this context, the biopsy is discriminating, as lipohypertrophy is related to adipocyte hypertrophy and not insulin amyloid deposit with granulomatous reaction (5). Nonetheless, to avoid systematic skin biopsy, some authors emphasize the interest of imaging to differentiate insulin amyloidosis from lipohypertrophy, the latter distinguishing on MRI by a fat-like signal, while in our patient AIns amyloidosis displayed a gadolinium-enhanced T2 hypointense lesion (7). However, MRI characteristics of insulin amyloidosis remains scarcely known. In the work by Nagase *et al*, insulin amyloidosis seems to induce T1 hyposignal, although no details were given about T2 features and gadolinium enhancement in this report (9). In addition, we report an 18-FDG uptake by insulin amyloidosis nodules. This is interesting because amyloid deposits are mostly acellular, being composed of aggregates of misfolded proteins that are likely to induce granulomatous inflammatory reaction to resorb it. Another work by Albert *et al* reported the presence of a granulomatous reaction around the subcutaneous amyloid deposit



accession	description	MS/MS
CO6A3_HUMAN	Collagen alpha-3(VI) chain	68
CO6A1_HUMAN	Collagen alpha-1(VI) chain	26
FINC_HUMAN	Fibronectin	26
ALBU_HUMAN	Albumin	22
COCA1_HUMAN	Collagen alpha-1(XII) chain	21
APOA4_HUMAN	Apolipoprotein A-IV	19
TENA_HUMAN	Tenascin	19
APOE_HUMAN	Apolipoprotein E	18
APOA1_HUMAN	Apolipoprotein A-I	14
CO6A2_HUMAN	Collagen alpha-2(VI) chain	13
IGG1_HUMAN	Immunoglobulin gamma-1 heavy chain	12
VTNC_HUMAN	Vitronectin	11
VIME_HUMAN	Vimentin	10
ACTB_HUMAN	Actin, cytoplasmic 1	9
FBN1_HUMAN	Fibrillin-1	9
HBB_HUMAN	Hemoglobin subunit beta	9
PEDF_HUMAN	Pigment epithelium-derived factor	9
INS_HUMAN	Insulin	8
COEA1_HUMAN	Collagen alpha-1(XIV) chain	7
IGHG3_HUMAN	Immunoglobulin heavy constant gamma 3	7
IGHG4_HUMAN	Immunoglobulin heavy constant gamma 4	7
SAMP_HUMAN	Serum amyloid P-component	4

FIGURE 2
Histological analyses of the biopsy of one subcutaneous nodule. **(A)** HES section at low magnification (x10) evidencing granulomas with giant cells (arrows) phagocytizing the amorphous substance (*), **(B)** HES section at higher magnification (x40), **(C)** Section at low magnification (x4) stained with Congo Red evidencing the amyloid deposit (*), and **(D)** Detailed results of the mass spectrometry evidencing the insulin protein highlighted in yellow, along with the serum amyloid P-component in red (of note, immunoglobulin heavy gamma chains were of polyclonal origin).

in the skin of a 59-year old patient with severe insulin resistance (10). We believe that 18-FDG uptake is mostly due to the granulomatous inflammation, as observed in histological sections of patient's lesions, possibly combined with high local insulin concentrations resulting in increased glucose uptake by immune and stromal cells.

Apart from lipohypertrophy, it is important to bear in mind the possibility of other causes of subcutaneous mass in patients with diabetes, such as skin cancer, lymphoma, or local infection (*staphylococcus* sp., *streptococcus sp., mycobacterium sp., fungi*). Insulin resistance syndrome characterized by insufficient effect of subcutaneous insulin compared to intravenous in diabetic patient must also be considered (11). Of note, Soudan *et al* reported a high dermal insulin concentration in these patients, but Congo red staining seeking amyloid deposit was not performed suggesting that a fraction of these patients might rather have had AIns amyloidosis (11). Other important differential diagnoses include more common forms of amyloidosis such as AL amyloidosis, AA amyloidosis or ATTR amyloidosis. In our case, the positive diagnosis of AIns amyloidosis was made after the surgical biopsy showed a typical birefringence with Congo red staining, along with the evidence of insulin deposition on mass spectrometry. Regarding differential diagnoses, clinical, biological, radiological and histopathological findings were inconsistent with lipohypertrophy (the biopsy showed no adipocyte hypertrophy), other forms of amyloidosis (no light chain elevation, no monoclonal spike, no past or present chronic inflammation, or other amyloidogenic protein on mass spectrometry), malignancy (no other hypermetabolic location on 18-FDG PET-CT) or infection (blood, bacteriological and mycobacteriological cultures of the biopsy were negative).

Finally, as many rare presentations of common diseases, management of AIns amyloidosis is not standardized. Therapeutic education on insulin injections seems a legitimate first-line treatment so that insulin injections can be carried out in other sites and preferentially in unaffected areas, at a lower dosage and under close blood sugar control to avoid hypoglycemia. This strategy may be sufficient to improve glycaemia in most patients, as elicited in a case reported by Nagase et al. where the switch of insulin injections sites alone could reduce insulin dosage by 47% in a patient (9, 11). Surgical removal can be another treatment option, although its indications remain to be better defined. In some cases, it may improve glycemic control (12). Thus, severe and/or refractory insulin resistance despite therapeutic education to insulin injections could be potential indications for surgical removal of the nodules.

In conclusion, AIns amyloidosis remains an incompletely understood complication of insulin treatment in patients living with diabetes due to the low number of reported cases. Its real incidence and prevalence in insulin-requiring diabetic subjects is probably underestimated and under-diagnosed. This case description aims at providing physicians with a better understanding of this disease to potentially lead to a better recognition and treatment.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

AD was responsible for formulating writing ideas and writing the manuscript. AD, MT, MC, CH, and ER were responsible for collecting information. ER was responsible for revising the manuscript and guiding the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Buxbaum JN, Dispenzieri A, Eisenberg DS, Fändrich M, Merlini G, Saraiva MJM, et al. Amyloid nomenclature 2022: update, novel proteins, and recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. *Amyloid*. (2022) 29:213–9. doi: 10.1080/13506129.2022.2147636

2. Störkel S, Schneider HM, Müntefering H, Kashiwagi S. Iatrogenic, insulin-dependent, local amyloidosis. *Lab Investig*. (1983) 48:108–11.

3. Dische FE, Wernstedt C, Westermark GT, Westermark P, Pepys MB, Rennie JA, et al. Insulin as an amyloid-fibril protein at sites of repeated insulin injections in a diabetic patient. *Diabetologia*. (1988) 31:158–61. doi: 10.1007/BF00276849

4. Nilsson MR. Insulin amyloid at injection sites of patients with diabetes. *Amyloid*. (2016) 23:139–47. doi: 10.1080/13506129.2016.1179183

5. Nagase T, Katsura Y, Iwaki Y, Nemoto K, Sekine H, Miwa K, et al. The insulin ball. *Lancet*. (2009) 373:184. doi: 10.1016/S0140-6736(09)60041-6

6. Woods RJ, Alarcón J, McVey E, Pettis RJ. Intrinsic fibrillation of fast-acting insulin analogs. *J Diabetes Sci Technol*. (2012) 6:265–76. doi: 10.1177/193229681200600209

7. Surmacz-Chwedoruk W, Nieznańska H, Wójcik S, Dzwolak W. Cross-seeding of fibrils from two types of insulin induces new amyloid strains. *Biochemistry*. (2012) 51:9460–9. doi: 10.1021/bi301144d

8. Blanco M, Hernández MT, Strauss KW, Amaya M. Prevalence and risk factors of lipohypertrophy in insulin-injecting patients with diabetes. *Diabetes Metab*. (2013) 39:445–53. doi: 10.1016/j.diabet.2013.05.006

9. Nagase T, Iwaya K, Kogure K, Zako T, Misumi Y, Kikuchi M, et al. Insulin-derived amyloidosis without a palpable mass at the insulin injection site: a report of two cases. *J Diabetes Investig*. (2020) 11:1002–5. doi: 10.1111/jdi.13199

10. Albert SG, Obadiah J, Parseghian SA, Yadira Hurley M, Mooradian AD. Severe insulin resistance associated with subcutaneous amyloid deposition. *Diabetes Res Clin Pract*. (2007) 75:374–6. doi: 10.1016/j.diabres.2006.07.013

11. Soudan B, Girardot C, Fermon C, Verlet E, Pattou F, Vantyghem M. Extreme subcutaneous insulin resistance: a misunderstood syndrome. *Diabetes Metab*. (2003) 29:539–46. doi: 10.1016/S1262-3636(07)70069-1

12. Shiba M, Kitazawa T. Progressive insulin-derived amyloidosis in a patient with type 2 diabetes. *Case Reports Plastic Surg Hand Surg*. (2016) 3:73–6. doi: 10.1080/23320885.2016.1247650

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Predictive model of diabetes mellitus in patients with idiopathic inflammatory myopathies

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Objectives: Cardiovascular diseases are the common cause of death in patients with idiopathic inflammatory myopathies (IIMs). Diabetes mellitus was associated with higher cardiovascular mortality, but few studies focused on the risk of diabetes mellitus in IIMs patients. Our study is aimed at developing a predictive model of diabetes mellitus in IIMs patients.

Methods: A total of 354 patients were included in this study, of whom 35 (9.9%) were diagnosed as new-onset diabetes mellitus. The predictive nomogram was drawn based on the features selected by least absolute shrinkage and selection operator (LASSO) regression, univariate logistic regression, multivariable logistic regression, and clinical relationship. The discriminative capacity of the nomogram was assessed by C-index, calibration plot, and clinical usefulness. The predictive model was verified by the bootstrapping validation.

Results: The nomogram mainly included predictors such as age, gender, hypertension, uric acid, and serum creatinine. This predictive model demonstrated good discrimination and calibration in primary cohort (C-index=0.762, 95% CI: 0.677-0.847) and validation cohort (C-index=0.725). Decision curve analysis indicated that this predictive model was clinically useful.

Conclusions: Clinicians can assess the risk of diabetes mellitus in IIMs patients by using this prediction model, and preventive measures should be taken early for high-risk patients, ultimately reducing the adverse cardiovascular prognosis.

KEYWORDS

diabetes mellitus, idiopathic inflammatory myopathies, nomogram, predictive model, cardiovascular diseases

1 Introduction

Idiopathic inflammatory myopathies (IIMs) are a group of rare autoimmune diseases characterized by chronic inflammatory infiltration of the skeletal muscle and proximal muscle weakness, which affect approximately 14.0 to 17.4 per 100,000 person-years (1). Multiple organs are involved in patients with IIMs, including skin, lungs, heart, and gastrointestinal tract, etc. Although the overall survival rate of patients with IIMs has improved, cardiac involvement remains a poor prognostic factor that as a cause of death has been reported in 10-20% of IIMs patients (2–4). Numerous studies demonstrated that the risk of cardiac involvement in patients with IIMs was higher than that in the general population, which was the same as other connective tissue diseases (CTD) (5, 6). Moreover, diabetes mellitus, a well-known traditional risk factor for cardiovascular events, was associated with higher cardiovascular mortality in patients with CTD, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (7, 8). However, the underlying mechanisms and pathogenesis of diabetes mellitus in these diseases remained unknown. Limited evidence revealed that inflammation, insulin resistance and pancreatic β cell dysfunction may play an important role in the process (8–10). More recent studies showed that diabetes mellitus in IIMs patients was not uncommon, as it occurred in about 4.2% to 29% of patients, whose prevalence was also higher than that in age- and sex-matched healthy controls (11–13). Despite the prevalence of diabetes mellitus in IIMs patients was high, it was often ignored the presence and onset of diabetes mellitus by clinicians. Of note, Yu et al. pointed out that diabetes mellitus was positively associated with mortality in patients with polymyositis and dermatomyositis (HR=2.57, 95%CI: 1.38-4.80, $P< 0.0001$) (9). In addition, patients with diabetes mellitus not only reduce the quality of life but also may lead to serious complications that increase medical expenses (14, 15). Therefore, it was crucial to predict the risk of developing diabetes mellitus in IIMs patients and then take preventive measures early for high-risk cases, and ultimately reduce the adverse cardiovascular prognosis.

To data, some models and clinical nomograms have been developed to predict the incidence of diabetes mellitus, but most of them are focused on the general population and rarely involved in subjects with autoimmune diseases. As far as we know, no study has been conducted to predict the incidence of diabetes mellitus in IIMs patients. Hence, the purpose of the current study is to establish an effective prediction model for diabetes mellitus based on demographic and clinical features of IIMs patients.

2 Materials and methods

2.1 Study population and follow-up evaluation

We conducted a retrospective cohort study including patients with IIMs who underwent a regular follow-up at the Third People's Hospital of Chengdu between January 2010 and December 2020. The diagnosis of IIMs was determined by experienced clinicians according to the criteria of Bohan and Peter (16). All participants received periodical follow-ups and clinical examinations (at least once every three months) during the study period. The follow-up time was defined as the time from the onset of IIM to the date of diagnosis of diabetes mellitus or the last visit, whichever occurred first. Patients were not included if they had previous history of type 1 or type 2 diabetes, malignant tumor, infectious diseases, hyperthyroidism, congenital heart disease, myocardial infarction, heart failure, chronic obstructive pulmonary disease, severe hepatic and renal insufficiency, and overlap syndrome at baseline. Furthermore, subjects with irregular follow-up or incomplete data were also excluded from the study. Finally, this study included a total of 354 patients who met the above-mentioned criteria. This retrospective study was approved by the Ethic Committee of the Third People's Hospital of Chengdu and performed in accordance with the Declaration of Helsinki (2019 S-20). All patients signed written informed consent.

Information, such as demographic characteristics, clinical manifestations, and drug administration, on each patient was collected through face-to-face interviews by trained physicians. After a 12-h fasting period, venous blood was collected in the morning from all subjects, and laboratory parameters were determined by using standard clinical laboratory techniques.

2.2 Diabetes mellitus assessment

During the study period, a fasting plasma glucose (FPG) ≥7.0 mmol/L, and/or self-reported diabetes can be considered as new-onset diabetes (17). Plasma glucose levels were measured on YSI glucose analyzer 2700 by the glucose oxidase method. Patients were checked at the time of diagnosis of diabetes mellitus or the last visit, whichever came first.

2.3 Data collection

The patients' data were recorded in electronic medical records system during routine clinical follow-up. Demographics, clinical manifestations, laboratory parameters, and drug administration were systematically extracted from electronic medical records. Data were collected by two trained graduate students and checked by an experienced clinician.

2.4 Definition

In the current study, the diagnosis of overlap syndrome was based on the American College of Rheumatology (ACR) criteria for SLE (18), RA (19) and systemic sclerosis (20). Smoking was defined as having at least one cigarette per day and persisting for more than one year (21). Hypertension was defined as systolic blood pressure (SBP) ≥140mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg, and/or the use of antihypertensive medication (22). The following laboratory parameters were assessed: total protein (TP, normal range: 60-83 g/L), albumin (ALB, normal range: 35-55 g/L), urea (normal range: 3.38-8.57 mmol/L), serum creatinine (Scr, normal range: 53-140 μmol/L), serum uric acid (UA, normal

range: 240-490 μmol/L), triglyceride (TG, normal range: 0.29-1.83 mmol/L), total cholesterol (TC, normal range: 2.8-5.7 mmol/L), high-density lipoprotein-cholesterol (HDL-C, normal range: >0.9 mmol/L), low-density lipoprotein-cholesterol (LDL-C, normal range: <4.0 mmol/L), C-relative protein (CRP, normal range: <10 mg/L), and erythrocyte sedimentation rate (ESR, normal range: <40 mm/h).

2.5 Statistical analysis

All data mainly including baseline characteristics, clinical manifestations, laboratory parameters, and drug administration were expressed as count (%) or mean ± SD. All statistical analyses were conducted by using the R software (Version 4.1.0; https://www.R-project.org). A *P*-value of less than 0.1 (two-tailed) was considered statistically significant.

The least absolute shrinkage and selection operator (LASSO) regression was a punitive regression method, which estimated the regression coefficient by maximizing the logarithmic likelihood function and limited the sum of the absolute values of the regression coefficients (23). And the LASSO regression removed unnecessary covariates, which was applied to the reduction of high dimensional data. In this study, we first selected the most important variables related to diabetes mellitus in patients with IIM by using the LASSO regression model. Then, the features selected by the LASSO regression were used for univariate logistic regression and multivariable logistic regression analysis. The features were considered as odds ratio (OR) having 95% confidence interval (CI) and as *P*-value. The development of predictive model for diabetes mellitus in IIMs patients was based on the results of the LASSO regression, multivariable logistic regression, and clinical relationship.

The nomogram was utilized to show the risk prediction model of new-onset diabetes mellitus in patients with IIMs. The prediction model was validated from three aspects: discrimination ability, calibration ability, and clinical usefulness. Harrell's C-index was used to evaluate the predictive accuracy of the nomogram (24). The C-index can range from 0.5 to 1.0. The C-index of 0.5, which represented random chance and this model had no predictive value, and the C-index of 1.0, which indicated exactly the same and this model had perfect discrimination. In general, C-index >0.7 was considered to have better discrimination ability. The goodness of fit was assessed using a calibration curve, and the area under the curve (AUC) of receiver operating characteristic (ROC) was similar to the C-index. Decision curve analysis (DCA) was also conducted to evaluate the clinical usefulness of the prediction model by quantifying the net benefits for a range of threshold probabilities in the whole cohort (25). This nomogram was further validated by bootstrapping (1000 bootstrap replicates) to calculate the relatively correctional C-index.

3 Results

3.1 Patients' characteristics

A total of 354 IIMs patients met the inclusion criteria for this study, with the mean age of 48 years (range: 18-82 years), of whom 67.2% were female. This cohort consisted of 95 patients with polymyositis, 247 patients with dermatomyositis, and 12 patients with inclusion body myositis. The median follow-up time in the current study was 6 months (ranging from 1 to 120 months). There were 35 out of 354 patients (19 female and 16 male) who developed diabetes mellitus, with the mean age of 55 years (range: 20-76 years). The mean of baseline FPG of the included subjects was 4.72 ± 0.77 mmol/L, and there was no statistical difference in FPG between patients with or without diabetes mellitus (4.87 ± 0.73 mmol/L vs. 4.70 ± 0.78 mmol/L). The detailed characteristics of patients with IIMs were presented in Table 1.

3.2 Features selection

The LASSO regression model was applied to select the most optimal predictive features. In this study, there were 35 variables for LASSO logistic regression analysis, and 7 of them had nonzero coefficients (Figure 1). These 7 features included age, gender, hypertension, UA, Scr, ESR, and Raynaud's phenomenon. Then, these 7 features were analyzed by univariate logistic regression and multivariable logistic regression, and the results pointed out that age, gender, hypertension, UA, and Scr were statistically significant between the two groups. Based on the results of LASSO regression analysis and logistic regression analysis, and clinical correlation, we selected age, gender, hypertension, UA, and Scr as predictors of diabetes mellitus in IIMs patients (Tables 2, 3).

3.3 Development of an individualized prediction model

As shown in Figure 2, the nomogram was drawn to provide a quantitative and convenient tool to predict the risk of diabetes mellitus in IIMs patients by using age, gender, hypertension, UA and Scr. The point of each predictor can be determined by drawing a vertical line to the point axis, and the total points can be calculated by summing point of each related factor in the nomogram. The higher the total points, the higher the risk of developing diabetes mellitus in patients with IIMs.

3.4 Validation of the nomogram of diabetes mellitus in IIMs patients

The calibration curve of the prediction diabetes mellitus nomogram in these patients presented good calibration (Figure 3). The C-index of this predictive nomogram was 0.762 (95% CI: 0.677-0.847) for the primary cohort, which was confirmed to be 0.725 by bootstrapping validation. The AUC value of this prediction model was 0.754 (95% CI: 0.665-0.843). DCA was used to evaluate the clinical usefulness of the predictive nomogram. From the perspective of the decision curve (Figure 4), if the threshold probability of a patient and a doctor was >2% and <68%, respectively, using this nomogram to predict the risk of diabetes mellitus in IIMs patients would achieve a favorable net

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TABLE 1 Clinical and demographic characteristics of IIMs patients.

Characteristics	Diabetes mellitus (n=35)	Non-diabetes mellitus (n=319)	Total (n=354)
Age (mean ± SD) (years)	55 ± 14	48 ± 13	48 ± 14
Gender (n,%)			
Female	19 (54.3)	219 (68.7)	238 (67.2)
Male	16 (45.7)	100 (31.3)	116 (32.8)
FPG (mean ± SD) (mmol/L)	4.96 ± 0.71	4.71 ± 0.78	4.73 ± 0.77
Follow-up (n,%) (months)			
<6	15 (42.9)	149 (46.7)	164 (46.3)
≥6	20 (57.1)	170 (53.3)	190 (53.7)
Smoking (n,%)	3 (8.6)	23 (7.2)	26 (7.3)
Hypertension (n,%)	10 (28.6)	40 (12.5)	50 (14.1)
Dysphagia (n,%)	10 (28.6)	78 (24.5)	88 (24.9)
Myalgia (n,%)	14 (40.0)	159 (49.8)	173 (48.9)
Arthralgia (n,%)	10 (28.6)	133 (41.7)	143 (40.4)
Rash (n,%)	25 (71.4)	216 (67.7)	241 (68.1)
Lung involvement (n,%)	13 (37.1)	114 (35.7)	127 (35.9)
Gottron's sign (n,%)	8 (22.9)	88 (27.6)	96 (27.1)
Raynaud's phenomenon (n,%)	0 (0.0)	33 (10.3)	33 (9.3)
ESR positive (n,%)	21 (60.0)	232 (72.7)	253 (71.5)
CRP positive (n,%)	21 (60.0)	179 (56.1)	200 (56.5)
TP (n,%)			
Below normal	11 (31.4)	94 (29.5)	105 (29.7)
Normal	24 (68.6)	222 (69.6)	246 (69.5)
Above normal	0 (0.0)	3 (0.9)	3 (0.8)
ALB (n,%)			
Below normal	18 (51.4)	151 (47.3)	169 (47.7)
Normal	17 (48.6)	168 (52.7)	185 (52.3)
Urea (n,%)			
Below normal	4 (11.4)	40 (12.5)	44 (12.4)
Normal	30 (85.7)	256 (80.3)	286 (80.8)
Above normal	1 (2.9)	23 (7.2)	24 (6.8)
Scr (n,%)			
Below normal	21 (60.0)	157 (49.2)	178 (50.3)
Normal	14 (40.0)	162 (50.8)	176 (49.7)
UA (n,%)			
Below normal	19 (54.3)	98 (30.7)	117 (33.1)
Normal	16 (45.7)	221 (69.3)	237 (66.9)
TG (n,%)			

(Continued)

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TABLE 1 Continued

Characteristics	Diabetes mellitus (n=35)	Non-diabetes mellitus (n=319)	Total (n=354)
Normal	17 (48.6)	160 (50.2)	177 (50.0)
Above normal	18 (51.4)	159 (49.8)	177 (50.0)
TC (n,%)			
Below normal	2 (5.7)	21 (6.6)	23 (6.5)
Normal	27 (77.2)	246 (77.1)	273 (77.1)
Above normal	6 (17.1)	52 (16.3)	58 (16.4)
HDL-C (n,%)			
Below normal	10 (28.6)	79 (24.8)	89 (25.1)
Normal	25 (71.4)	240 (75.2)	265 (74.9)
LDL-C (n,%)			
Normal	32 (91.4)	298 (93.4)	330 (93.2)
Above normal	3 (8.6)	21 (6.6)	24 (6.8)
Antinuclear antibody positive (n,%)	21 (60.0)	214 (67.1)	235 (66.4)
Anti SSA antibody positive (n,%)	4 (11.4)	43 (13.5)	47 (13.3)
Anti SSB antibody postive (n,%)	0 (0.0)	16 (5.0)	16 (4.5)
Anti-SCL-70 antibody postive (n,%)	0 (0.0)	5 (1.6)	5 (1.4)
Anti-Jo1 antibody postive (n,%)	1 (2.9)	23 (7.2)	24 (6.8)
Use of GC (n,%)	15 (42.9)	137 (42.9)	152 (42.9)
Use of MTX (n,%)	3 (8.6)	34 (10.7)	37 (10.5)
Use of CTX (n,%)	1 (2.9)	8 (2.5)	9 (2.5)
Use of HCQ (n,%)	1 (2.9)	23 (7.2)	24 (6.8)
Use of AZA (n,%)	1 (2.9)	6 (1.9)	7 (2.0)
Use of TII (n,%)	0 (0.0)	9 (2.8)	9 (2.5)
Use of TGP (n,%)	2 (5.7)	7 (2.2)	9 (2.5)

FPG, fasting plasma glucose; ESR, erythrocyte sedimentation rate; CRP, C-relative protein; TP, total protein; ALB, albumin; Scr, serum creatinine; UA, uric acid; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; GC, glucocorticoid; MTX, methotrexate; CTX, cyclophosphamide; HCQ, hydroxychloroquine; AZA, azathioprine; TII, tripterygium wilfordii; TGP, total glucosides of paeony.

benefit than the scheme. What's more, the net benefit was comparable with several overlaps, on the basis of the predictive nomogram of diabetes mellitus in this range. In short, these results all suggested this model had good discrimination.

4 Discussion

Cardiac involvement in patients with IIMs was mostly atypical and seldom attracted the attention of clinicians. However, increasing amounts of data demonstrated that cardiovascular events were the common cause of death in IIMs. Early identification and control of cardiovascular risk factors can reduce mortality and improve the quality of life in IIMs patients. Diabetes mellitus was an indispensable risk factor for cardiovascular diseases, so it was greatly significant to identify the risk of diabetes mellitus in patients with IIMs. As a tool to evaluate the risk and prognosis of disease, nomogram has been paid more attention and applied in medical research and clinical practice. To the best of our knowledge, our study was the first to provide a relatively accurate prediction model of diabetes mellitus in patients with IIMs based on generally available clinical features. Nomogram illustrated that older age, male, hypertension, low levels of UA and Scr were more likely to develop diabetes mellitus in patients with IIMs. The C-index of the constructed nomogram and the internal validation was up to 0.762 and 0.725, respectively, which demonstrated that this predictive model had adequate discrimination and calibration. Furthermore, the decision curve analysis indicated this nomogram also had good clinical usefulness. Therefore, we think that clinicians can assess the risk of diabetes

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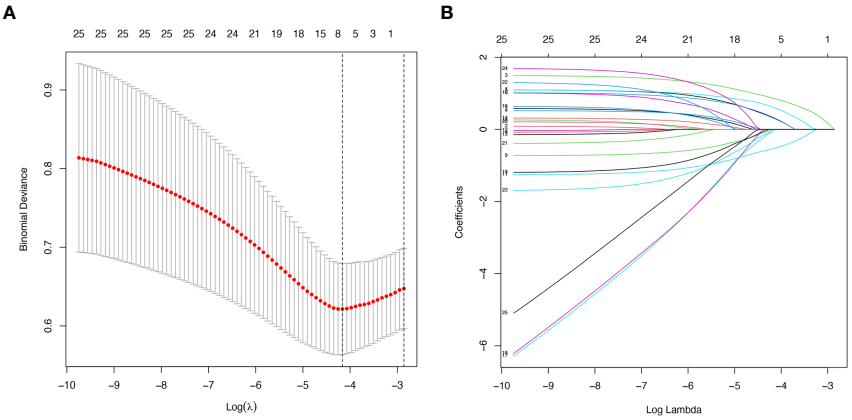

FIGURE 1
Demographic and clinical features selection using the LASSO regression model. **(A)** Optimal tuning parameter (λ) selection in the LASSO regression model used ten-fold cross-validation via minimum criteria. The binomial deviance curve was plotted versus log (λ). Dotted vertical lines were drawn at the optimal values by using the minimum criteria and 1 standard error (SE) of the minimum criteria (the 1-SE criteria). **(B)** LASSO coefficient profiles of the 35 features. A coefficient profile plot was produced against the log (λ) sequence. Vertical line was drawn at the value selected using ten-fold cross-validation, where optimal lambda resulted in 7 features with nonzero coefficients.

TABLE 2 Univariate logistic regression for features of diabetes mellitus.

variable	Odds ratio (95%CI)	*P*-value
Age, years		
≥60	1.5280(1.1220-2.0820)	0.0070
45-59	1.5330(0.9760-2.4080)	0.0640
18-44	1.0000(Ref.)	—
Hypertension		
Yes	3.8670(1.4640-10.2150)	0.0060
No	1.0000(Ref.)	—
Gender		
Male	2.6750(1.1890-6.0150)	0.0170
Female	1.0000(Ref.)	—
Scr		
Below normal	2.4910(1.0970-5.6530)	0.0290
Normal	1.0000(Ref.)	—
UA		
Below normal	2.7990(1.2830-6.1090)	0.0100
Normal	1.0000(Ref.)	—
Raynaud's phenomenon		
Yes	0.3320(0.0420-2.6100)	0.2950
No	1.0000(Ref.)	—
ESR		
Below normal	1.9600(0.8870-4.3350)	0.0960
Normal	1.0000(Ref.)	—

Scr, serum creatinine; UA, uric acid; ESR, erythrocyte sedimentation rate.

mellitus in IIMs patients by referring to this prediction model. For high-risk patients, preventive measures should be taken as soon as possible to reduce or delay the occurrence of diabetes mellitus.

The findings of our study were consistent with those of previous studies in other CTD or general population. For example, age and gender were considered to be important risk factors for diabetes mellitus, and the risk of diabetes mellitus increased with age. Similarly, a cross-sectional study demonstrated that the prevalence of diabetes mellitus increased with age, and subjects aged 60 years or older and aged 44-59 years had 2.35-fold and 2.77-fold increased risk of diabetes mellitus compared to subjects younger than 44 years, respectively (26). It may be contributed to the gradual decline of pancreatic β cell function with aging, eventually resulting in relative or absolute lack of insulin and altered glucose metabolism (27). It was worth noting that the epigenetic changes caused by aging may also affect pancreatic islets gene expression and insulin secretion. Age-related changes in pancreatic islets DNA methylation also can increase insulin resistance, impaired pancreatic β cell function, and induce diabetes mellitus (28). In addition, the prevalence of IIMs patients in women was higher than that in men, and the latter had a higher risk of developing diabetes mellitus than females that was also similar to the general population (29, 30). A study on the complications of polymyositis revealed that the prevalence of diabetes mellitus had a difference in men and women (26.1% vs 13.5%), but it was not statistically significant (P=0.201) (13). The relationship between gender and diabetes mellitus may be associated with sex steroids (31). The levels of testosterone in men decreased with the increase of age, and lower levels of serum testosterone in men were often correlated with insulin resistance, obesity and metabolic compromise (32, 33). It has been confirmed that low levels of serum testosterone in men may be applied to predict the development of type 2 diabetes (34). Additionally, there were great differences in pancreatic islets DNA methylation between genders, and extensive DNA methylation often occurred

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TABLE 3 Multivariable logistic regression model for features of diabetes mellitus.

Intercept and variable	Prediction model		
	β	Odds ratio (95%CI)	*P*-value
Intercept	-4.3077	0.0134 (0.0041-0.0386)	<0.0001
Age, years			
≥60	0.9137	2.4936(0.9027-7.1909)	0.0811
45-59	0.7651	2.1492(0.8413-5.7979)	0.1157
18-44	—	1.0000(Ref.)	—
Hypertension			
Yes	1.2247	3.4033(1.2833-8.8116)	0.0119
No	—	1.0000(Ref.)	—
Gender			
Male	1.0126	2.7529(1.2436-6.1956)	0.0129
Female	—	1.0000(Ref.)	—
Scr			
Below normal	0.8587	2.3602(1.0669-5.3914)	0.0365
Normal	—	1.0000(Ref.)	—
UA			
Below normal	0.9381	2.5550(1.1942-5.5531)	0.0160
Normal	—	1.0000(Ref.)	—

Scr, serum creatinine; UA, uric acid.

in women that can lead to increased insulin secretion, reducing the risk of diabetes mellitus (28). Hypertension was not uncommon in patients with IIMs, and the reported prevalence of hypertension in IIMs patients varied from 38.7 to 71% based on different patient selection and different definitions of disease used in the studies (12, 13). Patients with hypertension probably tended to have concomitant metabolic disorders, and researchers had identified that patients with hypertension were nearly 2.5 times more likely to develop diabetes mellitus than healthy subjects (35). In patients with hypertension, there was a 9% elevation in the risk of diabetes mellitus for each 10 mmHg increased in systolic blood pressure (36). Hypertension and diabetes mellitus often coexist and interact with each other, and their pathogenesis may be related to inflammation. Proinflammatory cytokines such as tumor-necrosis factor α (TNF-α) and interleukin-6 (IL-6) not only involved in the pathogenesis of hypertension, but also interfered with the insulin signaling pathways which were associated with insulin resistance and diabetes mellitus (37, 38). UA was mostly produced by the liver and excreted by the kidneys, and UA played a pivotal role in the antioxidant defense system in humans (39). Notably, Pitocco et al. pointed out that there was a negative correlation between UA and glycemia (r=-0.28, P=0.027), which was consistent with the result of current study (39). However, the precise mechanism of hypouricemia on the development of diabetes mellitus in IIMs patients was still poorly determined. Limited evidence demonstrated that the relationship between UA and glycemia may be contributed to the excessive production of NO caused by oxidative stress, and NO can restrict the production of UA by inhibiting the activity of endothelial xanthine oxidase activity (39, 40). Furthermore, oxidative stress often presents in the early stage of diabetes mellitus (39). To date, the study focusing on low Scr and diabetes mellitus in autoimmune diseases were rare. However, a large prospective study demonstrated that low Scr was associated with an increased risk of diabetes mellitus in the general population (41). Creatinine was a metabolite of muscle creatine and its

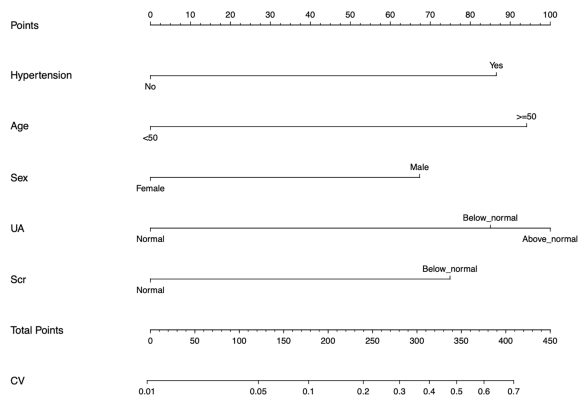

FIGURE 2
Developed the predictive nomogram of diabetes mellitus in IIMs patients. The predictive nomogram was developed in the cohort, with the age, gender, hypertension, uric acid (UA), and serum creatinine (Scr) incorporated.

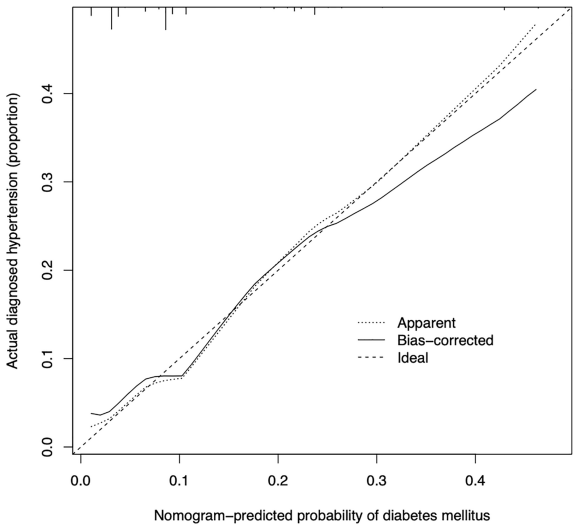

FIGURE 3
Calibration curves of the predictive nomogram of diabetes mellitus in the cohort. The x-axis represents the predicted risk of diabetes mellitus. The y-axis represents the probability of the actual diagnosed diabetes mellitus. The diagonal dotted line represents a perfect prediction by an ideal model. The solid line represents the performance of the nomogram, of which a closer fit to the diagonal dotted line represents a better prediction.

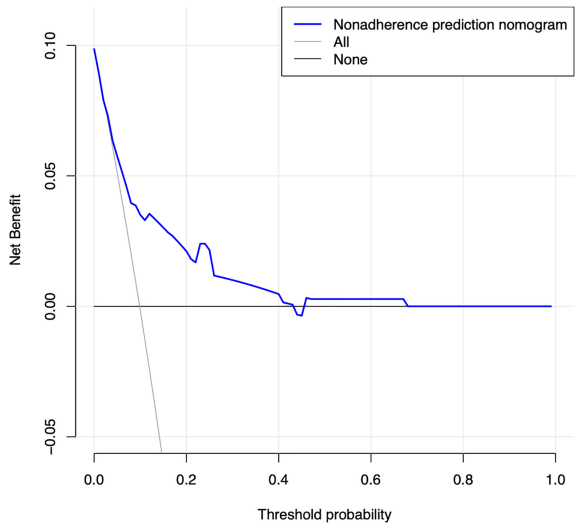

FIGURE 4
Decision curve analysis for the predictive nomogram of diabetes mellitus. The y-axis measures the net benefit. The dotted line represents the risk of predictive nomogram. The thin solid line represents the assumption that all patients have diabetes mellitus. Thin thick solid line represents the assumption that no patients have diabetes mellitus. The decision curve demonstrates that if the threshold probability of a patient and a doctor is >2% and <68%, respectively, using this nomogram to predict diabetes mellitus of IIMs adds more benefit than the scheme.

concentration in serum was proportional to muscle mass, muscle mass was negatively related to insulin resistance and diabetes (42). Thus, it was speculated that low serum creatinine may be a predictor of diabetes mellitus (43). It was worth mentioning that whether the clinical manifestations and the treatment of IIMs were associated with the development of diabetes mellitus was still unknown. However, a study presented that hydroxychloroquine (HCQ), methotrexate (MTX) and tumor-necrosis factor inhibitors (TNFi) were related to decreased risk of incident diabetes mellitus in RA patients, and glucocorticoid (GC) was associated with increased risk of diabetes mellitus in a dose-dependent manner (44). The mechanism underlying the diabetes risk reduction with HCQ, MTX and TNFi may be attributed to the reduction of inflammatory, the improvement of glucose metabolism and pancreatic β cell function (44). Glucocorticoid increased glucose by augmenting hepatic gluconeogenesis, inhibiting glucose uptake in adipose tissue, and antagonizing insulin-mediated glucose disposal in a dose-dependent manner (45, 46). Therefore, drugs should be reasonably selected to reduce the risk of diabetes in the treatment of autoimmune diseases.

There are currently no studies on diabetes risk prediction models in connective tissue diseases; But in recent years, multivariate risk scores have been developed to predict the risk of diabetes in healthy individuals and most risk scores contain typical diabetes risk factors such as age, gender, degree of obesity, family history of diabetes and blood pressure status (47–50). In this study, a prediction model of diabetes prevalence in IIMs was developed, and the important characteristics derived included age, gender, hypertension, UA, and Scr, which were somewhat coincident and similar to the results of previous studies (48). Due to different research factors and statistical methods, different prediction models may also get different prediction findings. Previous studies mostly used univariate or multivariate analysis to obtain the results, which may be prone to multicollinearity problems. LASSO regression analysis can better resolves confound from multicollinearity (51). It builds a model with better accuracy and stability by reducing the complexity of the model. After filtering out meaningful variables, univariate and multivariate logistic regression analyses were used again to make the results more reliable and stable. The constructed model was also verified for its discrimination, calibration and clinical applicability, thus we can establish a model with better validity and applicability.

There are some limitations to this study that are worth mentioning. First, our study is a retrospective study in which individuals with incomplete data are exclude, which may lead to selection bias. Second, although this study includes a wide range of potential predictors, there are other factors that cannot be acquired, such as drinking status, family history of diabetes, and the dosage of drugs, etc, which may lead to the limited prediction power of the model. Third, the sample size of this cohort is relatively small and it is not representative of all Chinese patients with IIMs. Nonetheless, as far as we know, this is the largest prediction model focused on diabetes mellitus in autoimmune diseases. Fourth, the prediction model is not validated externally. Therefore, more prospective studies are needed to further confirm the current results and make it universally applicable.

In conclusion, we have established a relatively accurate and simple nomogram based on five risk factors, namely age, male, hypertension, hypouricemia, and low serum creatinine, to predict

the risk of developing diabetes mellitus in IIMs patients. Clinicians and patients can take more necessary measures early to reduce the incidence of diabetes mellitus for high-risk patients.

Data availability statement

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

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethic Committee of the Third People's Hospital of Chengdu (2019 S-20) on 20 March 2021. The patients/participants provided their written informed consent to participate in this study.

Author contributions

QN and LQ performed the study design and wrote the manuscript. QN, WY and QL performed data collection and analysis. LQ and TY conducted validation and formal analysis. HW and JW substantially revised, commented on and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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. Furst DE, Amato AA, Iorga SR, Gajria K, Fernandes AW. Epidemiology of adult idiopathic inflammatory myopathies in a U.S. managed care plan. *Muscle Nerve* (2012) 45:676–83. doi: 10.1002/mus.23302

2. Sultan SM, Ioannou Y, Moss K, Isenberg DA. Outcome in patients with idiopathic inflammatory myositis: morbidity and mortality. *Rheumatology* (2002) 41:22–6. doi: 10.1093/rheumatology/41.1.22

3. Schiopu E, Phillips K, MacDonald PM, Crofford LJ, Somers EC. Predictors of survival in a cohort of patients with polymyositis and dermatomyositis: effect of corticosteroids, methotrexate and azathioprine. *Arthritis Res Ther* (2012) 14:R22. doi: 10.1186/ar3704

4. Liu XH, Feng XJ, Shi JY, Jia FW, Liu YX, Zhu YL, et al. The quest for diagnostic approaches of cardiac involvement in polymyositis and dermatomyositis. *Ann Palliat Med* (2020) 9:2256–70. doi: 10.21037/apm-19-650

5. Gupta R, Wayangankar SA, Targoff IN, Hennebry TA. Clinical cardiac involvement in idiopathic inflammatory myopathies: a systematic review. *Int J Cardiol* (2011) 148:261–70. doi: 10.1016/j.ijcard.2010.08.013

6. Opinc AH, Makowski MA, Łukasik ZM, Makowska JS. Cardiovascular complications in patients with idiopathic inflammatory myopathies: does heart matter in idiopathic inflammatory myopathies? *Heart Fail Rev* (2021) 26:111–25. doi: 10.1007/s10741-019-09909-8

7. Skielta M, Söderström L, Rantapää-Dahlqvist S, Jonsson SW, Mooe T. Trends in mortality, co-morbidity and treatment after acute myocardial infarction in patients with rheumatoid arthritis 1998-2013. *Eur Heart J Acute Cardiovasc Care* (2020) 9:931–8. doi: 10.1177/2048872619896069

8. Zeng YJ, Zeng FQ, Dai L, Yang C, Lin BZ, Zheng DH, et al. Characteristics and risk factors for hyperglycemia in Chinese female patients with systemic lupus erythematosus. *Lupus* (2010) 19:1344–50. doi: 10.1177/0961203310375439

9. Yu KH, Wu YJ, Kuo CF, See LC, Shen YM, Chang HC, et al. Survival analysis of patients with dermatomyositis and polymyositis: analysis of 192 Chinese cases. *Clin Rheumatol* (2011) 30:1595–601. doi: 10.1007/s10067-011-1840-0

10. Danve AS, Kulkarni S. Do tumor necrosis factor (TNF) inhibitors improve the glycemic control in patients with rheumatoid arthritis and concomitant diabetes mellitus? *Am J Ther* (2017) 24:e347–50. doi: 10.1097/mjt.0000000000000297

11. Pi H, Zhou H, Jin H, Ning Y, Wang Y. Abnormal glucose metabolism in rheumatoid arthritis. *BioMed Res Int* (2017) 2017:9670434. doi: 10.1155/2017/9670434

12. Narayanaswamy AS, Akhtar M, Kumar N, Lazar AI. Polymyositis–a review and follow up study of 24 cases. *J Assoc Physicians India* (1993) 41:354–6.

13. Diederichsen LP, Diederichsen AC, Simonsen JA, Junker P, Søndergaard K, Lundberg IE, et al. Traditional cardiovascular risk factors and coronary artery calcification in adults with polymyositis and dermatomyositis: a Danish multicenter study. *Arthritis Care Res (Hoboken)* (2015) 67:848–54. doi: 10.1002/acr.22520

14. Souza FH, Levy-Neto M, Shinjo SK. Prevalence of clinical and laboratory manifestations and comorbidities in polymyositis according to gender. *Rev Bras Reumatol* (2011) 51:428–83.

15. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of diabetes 2016. *J Diabetes Res* (2016) 2016:6989453. doi: 10.1155/2016

16. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* (1975) 292:344–7. doi: 10.1056/nejm197502132920706

17. Inzucchi SE. Clinical practice. diagnosis of diabetes. *N Engl J Med* (2012) 367:542–50. doi: 10.1056/NEJMcp1103643

18. Hochberg MC. Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* (1997) 40:1725. doi: 10.1002/art.1780400928

19. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American college of Rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis* (2010) 69:1580–8. doi: 10.1136/ard.2010.138461

20. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 Classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis* (2013) 72:1747–55. doi: 10.1136/annrheumdis-2013-204424

21. Wang Y, Wu H, Liu Q, Wang C, Fu L, Wang H, et al. Association of CHRNA5-A3-B4 variation with esophageal squamous cell carcinoma risk and smoking behaviors in a Chinese population. *PloS One* (2013) 8:e67664. doi: 10.1371/journal.pone.0067664

22. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International society of hypertension global hypertension practice guidelines. *Hypertension* (2020) 75:1334–57. doi: 10.1097/hjh.0000000000002453

23. Sauerbrei W, Royston P, Binder H. Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat Med* (2007) 26:5512–28. doi: 10.1002/sim.3148

24. Harrell FEJr., Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA* (1982) 247:2543–6.

25. Vickers AJ, Cronin AM, Elkin EB, Gonen M. Extensions to decision curve analysis, a novel method for evaluating diagnostic tests, prediction models and molecular markers. *BMC Med Inform Decis Mak* (2008) 8:53. doi: 10.1186/1472-6947-8-53

26. Wang R, Zhang P, Li Z, Lv X, Cai H, Gao C, et al. The prevalence of pre-diabetes and diabetes and their associated factors in northeast China: a cross-sectional study. *Sci Rep* (2019) 9:2513. doi: 10.1038/s41598-019-39221-2

27. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. *Diabetes* (2003) 52:1738–48. doi: 10.2337/diabetes.52.7.1738

28. Davegårdh C, García-Calzón S, Bacos K, Ling C. DNA Methylation in the pathogenesis of type 2 diabetes in humans. *Mol Metab* (2018) 14:12–25. doi: 10.1016/j.molmet.2018.01.022

29. Limaye VS, Blumbergs P, Roberts-Thomson PJ. Idiopathic inflammatory myopathies. *Intern Med J* (2009) 39:179–90. doi: 10.1159/000212374

30. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, et al. Prevalence of diabetes among men and women in China. *N Engl J Med* (2010) 362:1090–101. doi: 10.1056/nejmoa0908292

31. Allan CA. Sex steroids and glucose metabolism. *Asian J Androl* (2014) 16:232–8. doi: 10.4103/1008-682x.122589

32. Jones TH. Effects of testosterone on type 2 diabetes and components of the metabolic syndrome. *J Diabetes* (2010) 2:146–56. doi: 10.1111/j.1753-0407.2010.00085.x

33. Zeng QS, Xu CL, Liu ZY, Wang HQ, Yang B, Xu WD, et al. Relationship between serum sex hormones levels and degree of benign prostate hyperplasia in Chinese aging men. *Asian J Androl* (2012) 14:773–7. doi: 10.1038/aja.2012.32

34. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL, Rancho Bernardo S. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the rancho Bernardo study. *Diabetes Care* (2002) 25:55–60. doi: 10.2337/diacare.25.1.55

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

36. Li X, Wang J, Shen X, An Y, Gong Q, Li H, et al. Higher blood pressure predicts diabetes and enhances long-term risk of cardiovascular disease events in individuals with impaired glucose tolerance: Twenty-three-year follow-up of the daqing diabetes prevention study. *J Diabetes* (2019) 11:593–8. doi: 10.1111/1753-0407.12887

37. Bautista LE, Vera LM, Arenas IA, Gamarra G. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-alpha) and essential hypertension. *J Hum Hypertens* (2005) 19:149–54. doi: 10.1038/sj.jhh.1001785

38. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* (2006) 17:4–12.

39. Pitocco D, Di Stasio E, Romitelli F, Zaccardi F, Tavazzi B, Manto A, et al. Hypouricemia linked to an overproduction of nitric oxide is an early marker of oxidative stress in female subjects with type 1 diabetes. *Diabetes Metab Res Rev* (2008) 24:318–23. doi: 10.1002/dmrr.814

40. Cote CG, Yu FS, Zulueta JJ, Vosatka RJ, Hassoun PM. Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide. *Am J Physiol* (1996) 271:L869. doi: 10.1152/ajplung.1996.271.5.l869

41. Bao X, Gu Y, Zhang Q, Liu L, Meng G, Wu H, et al. Low serum creatinine predicts risk for type 2 diabetes. *Diabetes Metab Res Rev* (2018) 34:e3011. doi: 10.1002/dmrr.3011

42. Park J, Mehrotra R, Rhee CM, Molnar MZ, Lukowsky LR, Patel SS, et al. Serum creatinine level, a surrogate of muscle mass, predicts mortality in peritoneal dialysis patients. *Nephrol Dial Transplant* (2013) 28:2146–55. doi: 10.1093/ndt/gft213

43. Hjelmesaeth J, Røislien J, Nordstrand N, Hofsø D, Hager H, Hartmann A. Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study. *BMC Endocr Disord* (2010) 10:6. doi: 10.1186/1472-6823-10-6

44. Xie W, Yang X, Ji L, Zhang Z. Incident diabetes associated with hydroxychloroquine, methotrexate, biologics and glucocorticoids in rheumatoid arthritis: A systematic review and meta-analysis. *Semin Arthritis Rheum* (2020) 50:598–607. doi: 10.1016/j.semarthrit.2020.04.005

45. Movahedi M, Beauchamp ME, Abrahamowicz M, Ray DW, Michaud K, Pedro S, et al. Risk of incident diabetes mellitus associated with the dosage and duration of oral glucocorticoid therapy in patients with rheumatoid arthritis. *Arthritis Rheumatol* (2016) 68:1089–98. doi: 10.1002/art.39537

46. Do TTH, Marie G, Héloïse D, Guillaume D, Marthe M, Bruno F, et al. Glucocorticoid-induced insulin resistance is related to macrophage visceral adipose tissue infiltration. *J Steroid Biochem Mol Biol* (2019) 185:150–62. doi: 10.1016/j.jsbmb.2018.08.010

47. Allaoui G, Rylander C, Averina M, Wilsgaard T, Fuskevåg OM, Berg V. Longitudinal changes in blood biomarkers and their ability to predict type 2 diabetes mellitus-the tromsø study. *Endocrinol Diabetes Metab* (2022) 5:e00325. doi: 10.1002/edm2.325

48. Arellano-Campos O, Gómez-Velasco DV, Bello-Chavolla OY, Cruz-Bautista I, Melgarejo-Hernandez MA, Muñoz-Hernandez L, et al. Development and validation of a predictive model for incident type 2 diabetes in middle-aged Mexican adults: the metabolic syndrome cohort. *BMC Endocr Disord* (2019) 19:41. doi: 10.1186/s12902-019-0361-8

49. Schmid R, Vollenweider P, Bastardot F, Vaucher J, Waeber G, Marques-Vidal P. Current genetic data do not improve the prediction of type 2 diabetes mellitus: the CoLaus study. *J Clin Endocrinol Metab* (2012) 97:E1338–41. doi: 10.1210/jc.2011-3412

50. Raynor LA, Pankow JS, Duncan BB, Schmidt MI, Hoogeveen RC, Pereira MA, et al. Novel risk factors and the prediction of type 2 diabetes in the atherosclerosis risk in communities (ARIC) study. *Diabetes Care* (2013) 36:70–6. doi: 10.2337/dc12-0609

51. Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc Ser B Methodol* (1996) 58:267–88. doi: 10.1111/j.2517-6161.1996.tb02080.x

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The effects of sucrose and arsenic on muscular insulin signaling pathways differ between the gastrocnemius and quadriceps muscles

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Introduction: Insulin resistance in muscle can originate from a sedentary lifestyle, hypercaloric diets, or exposure to endocrine-disrupting pollutants such as arsenic. In skeletal muscle, insulin stimulates glucose uptake by translocating GLUT4 to the sarcolemma. This study aimed to evaluate the alterations induced by sucrose and arsenic exposure in vivo on the pathways involved in insulinstimulated GLUT4 translocation in the quadriceps and gastrocnemius muscles.

Methods: Male Wistar rats were treated with 20% sucrose (S), 50 ppm sodium arsenite (A), or both (A+S) in drinking water for 8 weeks. We conducted an intraperitoneal insulin tolerance (ITT) test on the seventh week of treatment. The quadriceps and gastrocnemius muscles were obtained after overnight fasting or 30 min after intraperitoneal insulin injection. We assessed changes in GLUT4 translocation to the sarcolemma by cell fractionation and abundance of the proteins involved in GLUT4 translocation by Western blot.

Results: Male rats consuming S and A+S gained more weight than control and Atreated animals. Rats consuming S, A, and A+S developed insulin resistance assessed through ITT. Neither treatments nor insulin stimulation in the quadriceps produced changes in GLUT4 levels in the sarcolemma and Akt phosphorylation. Conversely, A and A+S decreased protein expression of Tether containing UBX domain for GLUT4 (TUG), and A alone increased calpain-10 expression. All treatments reduced this muscle's protein levels of VAMP2. Conversely, S and A treatment increased basal GLUT4 levels in the sarcolemma of the gastrocnemius, while all treatments inhibited insulin-induced GLUT4 translocation. These effects correlated with lower basal levels of TUG and impaired insulin-stimulated TUG proteolysis. Moreover, animals treated with S had reduced calpain-10 protein levels in this muscle, while A and A+S inhibited insulin-induced Akt phosphorylation.

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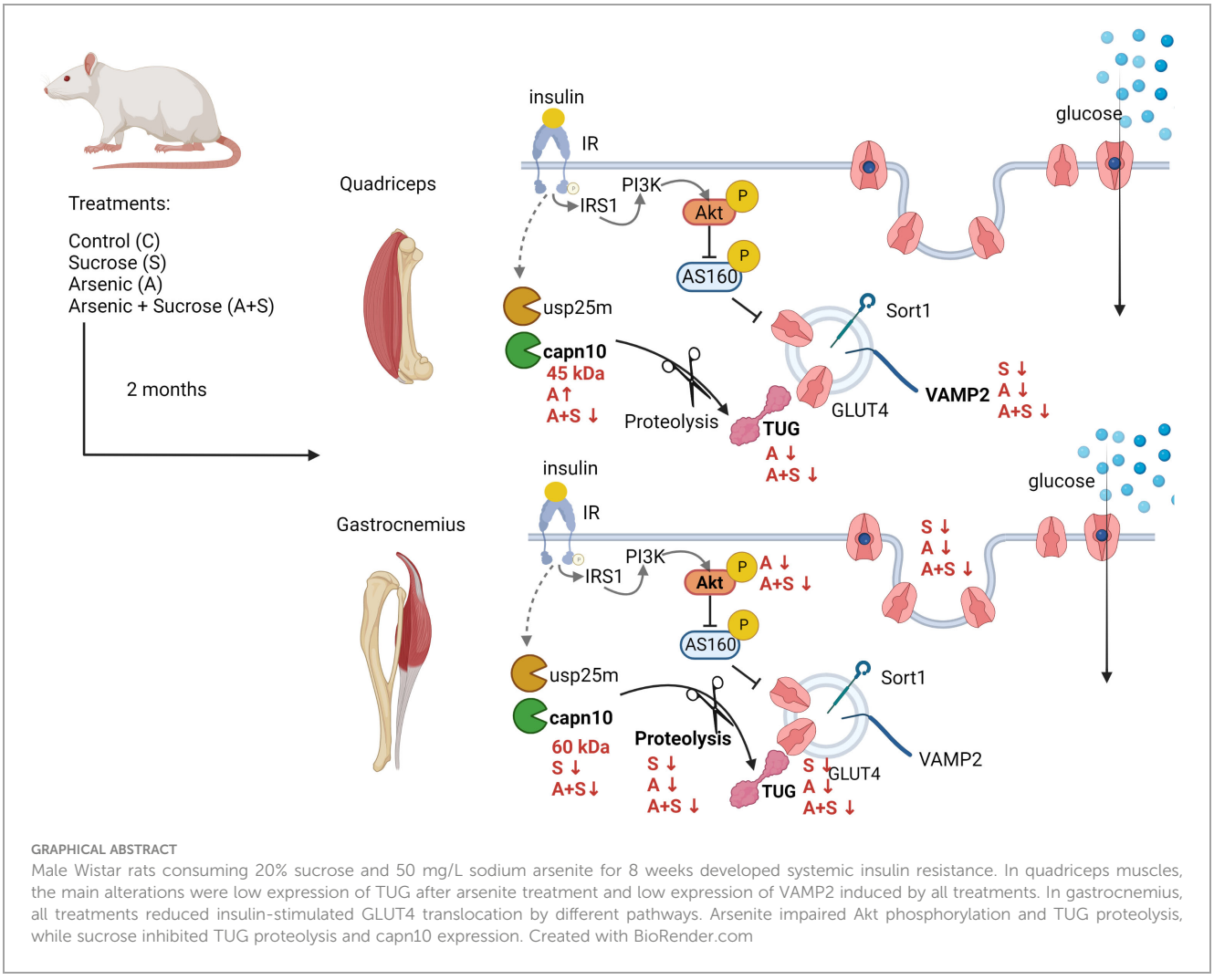
Conclusion: Arsenic and sucrose induce systemic insulin resistance due to defects in GLUT4 translocation induced by insulin. These defects depend on which muscle is being analyzed, in the quadriceps there were defects in GLUT4 retention and docking while in the gastrocnemius the Akt pathway was impacted by arsenic and the proteolytic pathway was impaired by arsenic and sucrose.

KEYWORDS

insulin resistance, skeletal muscle, GLUT4, arsenic, metabolic syndrome



GRAPHICAL ABSTRACT
Male Wistar rats consuming 20% sucrose and 50 mg/L sodium arsenite for 8 weeks developed systemic insulin resistance. In quadriceps muscles, the main alterations were low expression of TUG after arsenite treatment and low expression of VAMP2 induced by all treatments. In gastrocnemius, all treatments reduced insulin-stimulated GLUT4 translocation by different pathways. Arsenite impaired Akt phosphorylation and TUG proteolysis, while sucrose inhibited TUG proteolysis and capn10 expression. Created with BioRender.com

Introduction

Metabolic syndrome (MS) is a condition characterized by at least three of the following signs: insulin resistance, central obesity, impaired fasting glucose, hypertension, and dyslipidemia. It increases the risk of developing type 2 diabetes (T2D), non-alcoholic fatty liver disease, cardiovascular diseases, and some forms of cancer (1). Classical risk factors for this condition include a sedentary lifestyle and consuming hypercaloric diets often including sweetened drinks (1). In addition, there is increasing evidence that exposure to endocrine-disrupting chemicals, such as arsenic, raises the risk of developing the signs of MS (2). However, the possible interaction between diet and arsenic exposure on the development of the signs of MS is poorly characterized.

Arsenic is an environmental pollutant distributed worldwide that can be produced by natural and anthropogenic sources (3).

Exposure to arsenic through drinking water is associated with a higher risk of developing T2D and MS (4–8). Moreover, arsenic exposure induces systemic and muscle insulin resistance *in vivo* and *in vitro*, resulting in structural damage of muscle and loss of lean body mass (9–12). Specifically, arsenic inhibits insulin-stimulated glucose uptake (ISGU) by decreasing insulin-induced translocation of the glucose transporter-4 (GLUT4) to the plasma membrane in muscle fibers and adipocytes *in vitro* (12–15). Nevertheless, most of these studies focused on phosphorylated levels of Akt, whose alterations are known to have minimal impact on insulin-stimulated GLUT4 translocation in skeletal muscle (16, 17). At the same time, arsenic's effects on other insulin signaling steps remain largely understudied.

Skeletal muscle is a central tissue coordinating the body's energy balance; in the presence of insulin, it is responsible for up to 80% of whole-body glucose disposal (18). Moreover, muscle insulin resistance results in poor glycemic control and is a primary factor in determining the development of T2D (19). Mechanistically, activation of insulin receptor (IR) induces its transphosphorylation at several tyrosine residues, which in turn triggers different signaling pathways controlling ISGU, glycogen synthesis, lipid accretion, protein synthesis, and muscle growth (18). Regarding ISGU, IR activation promotes GLUT4 mobilization from the intracellular GLUT4 storage vesicles (GSV) to the sarcolemma and T-tubules (18) by activating several pathways. These include 1) the recruitment and activation of the phosphatidyl inositol 3 kinase (PI3K), inducing the synthesis of phosphatidyl inositol 3,4,5 triphosphate (PIP3), and activating protein kinase Akt (18). Active Akt phosphorylates and inhibits the proteins TBC1D1 and TBC1D4 (also known as AS160) in muscle, which are negative regulators of GLUT4 translocation (18). 2) The proteolytic pathway, involving the activation of the proteases usp25m and calpain-10 (capn10), which cleave the Tether containing UBX domain for GLUT4 (TUG) protein, releasing the GSVs from their perinuclear location (20, 21). The coordination between these pathways is necessary to effectively translocate GLUT4 to the sarcolemma and induce ISGU (22, 23). However, there is evidence that alterations of the IRS/PI3K/Akt/TBC1D1/4 axis are not a principal cause of muscle insulin resistance, and recent works focus on pathways independent of this axis (17, 21).

Skeletal muscle is a highly heterogeneous tissue of different proportions of oxidative and glycolytic myofibers, satellite cells, connective tissue, vascular cells, and neuron axons. The interactions between these components determine each muscle bed's mechanical and metabolic properties, resulting in different responses to pathophysiological conditions, including insulin resistance (24–26). Notably, a specific muscle bed's response during pathological conditions does not depend only on fiber composition, and muscles with similar fiber compositions differ in their response to environmental cues (25). Thus, comparing the defects in insulin signaling between multiple muscle groups is needed to determine how each muscle responds to environmental cues.

Previously, we reported that a model of MS in Wistar rats consuming 20% sucrose through drinking water for 2 months developed central obesity, insulin resistance, cardiac arrhythmias, hypertriglyceridemia, and alterations in pancreatic beta-cells (27–30). Thus, we aimed to evaluate the alterations induced by sucrose and arsenic consumption *in vivo* on the canonical and proteolytic pathways that control insulin-stimulated GLUT4 translocation in the quadriceps and gastrocnemius muscles.

Materials and methods

Animals and treatments

All animal protocols involved in this study were approved by the Animal Care Committee of the Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM; CICUAL MHU189-22). Animal care was performed according to the International Guiding Principles for Biomedical Research Involving Animals, Council for International Organizations of Medical Sciences, 2010. The animals used for this work are part of a more extensive study aimed at identifying the mechanisms induced by arsenic and sucrose that contribute to the development of MS. Most of their tissues and organs were collected for several analyses.

A total of 104 young male Wistar rats (250-280 g, approximately 8 weeks of age, 26 rats for each condition) were obtained from the local animal facility of IFC, UNAM. The animals were housed in a cycle of 12 hours of light and 12 hours of darkness, at 20-23°C and 40% relative humidity. The rats were randomly assigned to each of the experimental conditions: control (C), maintained with tap water; sucrose (S), treated with 20% sucrose in drinking water; arsenic (A), treated with 50 ppm of sodium arsenite in drinking water; arsenic + sucrose (A + S), treated with 20% sucrose and 50 ppm of sodium arsenite in drinking water. Although this arsenite dose is considerably higher than the environmentally relevant concentrations for human populations, it is important to note that rats are resistant to the toxic effects of arsenic (3). Thus, we established the arsenite dose based on previous works showing that this concentration promotes proatherogenic dyslipidemias and hypertension in rats (31, 32). We also calculated the arsenic intake by measuring the water consumption of the animals (Supplementary Figure 1A). In our model, the rats treated with A and A+S consumed 4.19 ± 0.69 and 5.03 ± 0.99 mg of arsenic/kg of body weight/day (Supplementary Figures 1B, C), which is close to the non-observed adverse effect level calculated for orally ingested trivalent arsenic in rats (5 mg/kg/day) and is similar to the doses that induce insulin resistance and non-alcoholic fatty liver disease in rat models (33–36).

All animals were fed *ad libitum* with a standard chow diet for rats (Lab Diet 5001), as previously reported (37). We replaced drinking water with different treatments three times per week to prevent the growth of microorganisms and prevent arsenite oxidation. The treatments lasted for 8 weeks. All measurements and experiments were performed in the animals after fasting for 13 hours (8:00 PM to 9:00 AM). During this period, the treatments were replaced by plain water.

For determinations done during fasting (six animals for each condition), rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) prior to the dissection of

tissues. For determinations after insulin stimulation (20 animals for each condition, 10 stimulated with vehicle and 10 stimulated with insulin), fasted animals were given an intraperitoneal injection with 0.2 UI/kg of human insulin (Humulin®, Eli Lilly and Co., México) or an equivalent volume of sterile water (vehicle, less than 0.2 mL per rat). The animals were anesthetized after 30 min of stimulation. The quadriceps and the lateral head of the gastrocnemius muscles were excised, weighted, and quickly frozen in dry ice. Tissues were stored at -70°C until needed.

Somatometric and biochemical parameters

We monitored the body weight of the animals every week and before sacrifice. The animal's length was measured from the tip of the nose to the anus, and this data was used along with the final body weight to calculate the body mass index (BMI) (37). Blood fasting glucose concentration was measured by drawing blood from the tail vein before anesthetizing the animals and evaluated with a hand glucometer (Accu-Check, Hoffman La Roche, Basel, Switzerland). After sacrifice, the blood was drawn from the inferior cava vein into heparinized tubes, and plasma was isolated by centrifuging blood samples at 2000 rpm for 15s min at 4°C. Triglyceride levels were assessed using colorimetric glycerol phosphate oxidase and phenol 4-aminoantipyrene methods, using a Randox RX Imola, according to the manufacturer's protocol. Insulin levels in plasma were assessed with an Ultrasensitive rat insulin ELISA system according to the manufacturer's instructions (10-1137-10; Mercodia Uppsala, Sweden).

Insulin tolerance test

We performed an ITT one week before the end of the treatment, as previously described (37). Briefly, 12 h-fasted rats received an intraperitoneal injection with 0.2 IU/kg of Humulin® (Eli Lilly and Co., México). Blood samples were drawn from the tail vein immediately before the injection (time 0) and 15, 30, 60, 90, and 120 min after injection. Glucose concentrations were measured with a hand glucometer (Accu-Check, Hoffman La Roche, Basel, Switzerland). Animals that suffered from distress or that were not properly injected were eliminated from the analysis.

Preparation of whole muscle lysates

Approximately 500 mg of muscle tissue were minced while still frozen and thawed in RIPA buffer (140 mM NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, and 0.1% SDS) freshly supplemented with cOmplete™, Mini Protease Inhibitor Cocktail (Roche) and 1 mM of sodium fluoride. We sonicated samples three times for 15 seconds each. Then, we centrifuged the samples at 10,000 X g for 18 min at 4°C. Supernatants were transferred to new tubes and the protein concentration was quantitated using the DC™ protein assay kit (Bio-Rad Laboratories, Hercules, CA US). The lysates were stored at -70°C until needed.

GLUT4 translocation to sarcolemma and T-tubules

Sarcolemma fractions were obtained as described (38–40). Briefly, the lateral gastrocnemius and quadriceps muscles from insulin-stimulated rats were excised, and 0.5 mg of muscle was minced and quickly placed on 5 mL of ice-cold homogenization buffer (20 mM HEPES pH 7.4, 1 mM EDTA, 250 mM sucrose) freshly supplemented with protease inhibitors. Tissues were homogenized with a PRO250 tissue homogenizer (PRO Scientific, Monroe, CT USA) with three bursts of 10 seconds each. Homogenates were centrifuged in a benchtop centrifuge at 2000 X g for 10 minutes. The supernatant was transferred to new tubes and centrifuged in a JA-20.1 rotor at 9000 X g for 20 min. Then, the supernatant was centrifuged again in a 50Ti rotor at 180,000 X g for 90 min. All centrifugation steps were performed at 4°C. The pellet was resuspended in RIPA buffer and quantitated as described for whole muscle lysates.

Quantitative immunoblot

Equal amounts of protein were mixed with 2X Laemli buffer and heated for 5 min at 85°C. Samples were loaded onto 12% SDS-PAGE and transferred at 15 V for 1 hour in a semi-dry chamber (Bio-Rad Laboratories, Hercules, CA US) to Immobilon-P, PVDF membranes (Millipore, MA, USA). Membranes were blocked for 1 hour at room temperature (RT) with tris-buffered saline + 0.1% Tween-20 (TBS-T) and 4% Blotto non-fat dry milk (Santa Cruz Biotechnology, Dallas, TX USA). Primary antibodies were incubated in TBS-T + 4% milk at 4°C overnight, according to the conditions described in Supplementary Table 1. The membranes were washed three times with TBS-T and incubated with the corresponding secondary antibody in TBS-T + 4% milk for 1 h at room temperature (horseradish peroxidase (HRP) goat anti-mouse dilution factor 1:4000, cat: sc-2005 Santa Cruz Biotechnology; and Peroxidase IgG Fraction Monoclonal Mouse Anti-Rabbit IgG, light chain specific, dilution factor 1:6000, cat: 211-032-171 Jackson Immunoresearch). To establish quantitative detection systems for each protein, we performed curves loading different amounts of total muscle lysate from a control rat. Then, the linearity of the detection system for each antibody was established (Supplementary Figure 2) (41). For each protein, the best running conditions were chosen based on these curves and are summarized in Supplementary Table 1. The blots were developed with ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences, Chicago, IL USA) in a C-Digit scanner (LI-COR Biosciences, NE USA). We used the optical density of the entire lane in the gels stained with Coomassie brilliant blue (CBB) as a loading control, as previously described (20). The immunoblots were analyzed using the Image Studio Lite ver. 5.2 Software (LI-

COR Biosciences, NE USA). The results are presented as fold changes relative to the protein abundance observed in control animals.

Cell culture and transfections

COS7 cells were obtained from the American Type Culture Collection (ATCC) and cultured as previously described (42). pcDNA3.1 plasmids containing CAPN10 isoforms a and c were gifted by Dr. Yasuko Ono (42). pTT3 plasmid containing SORT1-bio-His was a gift from Gavin Wright (Addgene plasmid # 52024). Cells were seeded in 3.5 cm dishes and transfected with 1.5 µg of the corresponding plasmid using the TransIT-X2 transfection system (Myrus Bio). Cells were collected 24 h after transfection and processed for Western blot as previously described (42).

Statistical analysis and image assembly

All the experiments and determinations were done in at least four animals for each condition. Data analysis was done with GraphPad Prism 8.0 software. For all the experiments, we performed two-way ANOVA with Tukey's *post hoc* test, with differences at $p<0.05$ considered statistically significant. Graphs present the mean ± standard error of the mean (SEM), and each circle denotes the individual animals used. The final figures were assembled using Adobe Photoshop 2022.

Results

Sucrose and arsenite induce metabolic syndrome signs

Rats treated for 8 weeks with sucrose (S) and arsenite + sucrose (A+S) gained more body weight compared with control (C) and arsenite (A)-treated rats (Figure 1A). Likewise, the body mass index (BMI) of animals in the 8th week of treatment with S and A+S was significantly higher than C and A-treated rats (Figure 1B). Thus, in our model, arsenic intake does not affect body weight gain induced by sucrose ingestion.

Neither S, A, nor A+S treatments altered fasting glucose blood levels compared with the control (Figure 1C). Sucrose consumption increased triacylglyceride plasma levels, but this effect was prevented in animals consuming A+S (Figure 1D). During the ITT, rats treated with S and A+S had statistically significantly higher blood glucose levels at 15 minutes. At the same time, S, A, and A+S displayed higher levels at 30 and 60 min of the ITT ($p<0.05$), displaying an increased area under the curve (AUC; C vs S

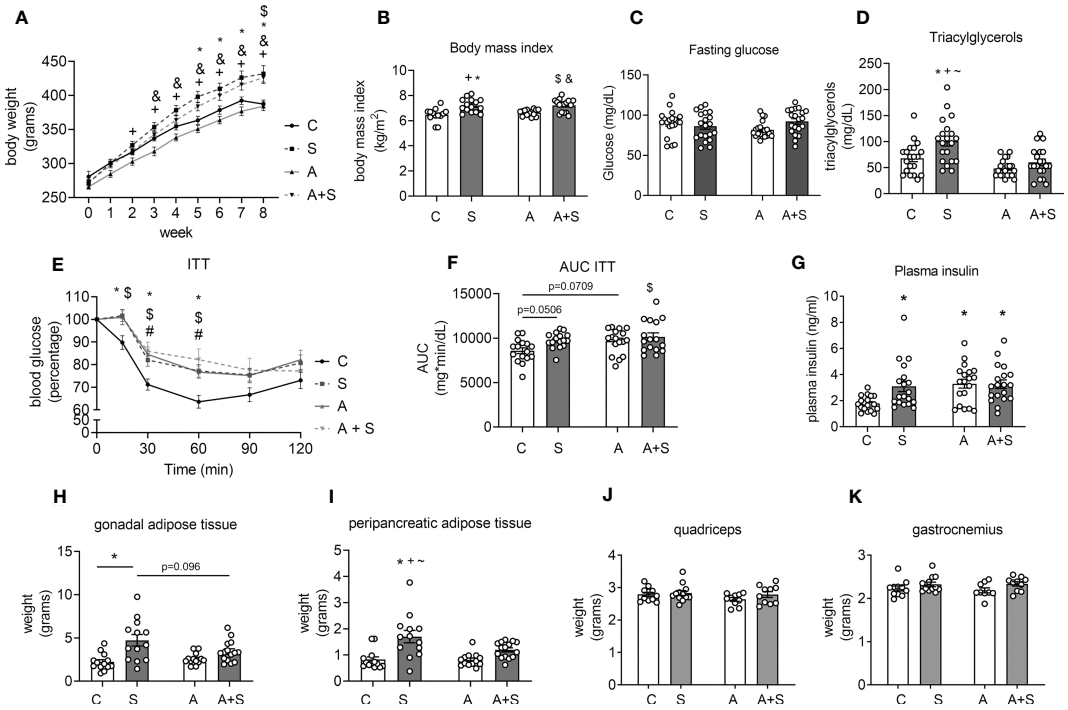


FIGURE 1
Characterization of the effects of sucrose and arsenic intake on parameters related to metabolic syndrome. **(A)** Weekly changes in body weight during the treatment of 8 weeks (n= 14 C, 15 S, 16 A, 17 A+S). **(B)** Body mass index at the end of treatment (n= 14 C, 15 S, 16 A, 17 A+S). **(C)** Blood fasting glucose (n= 20 animals in each condition). **(D)** Plasma triacylglyceride levels (n=20 animals in each condition). **(E)** Insulin tolerance test (ITT) was performed in the 7th week of treatment (n= 16 C, 16 S, 17 A, 17 A+S). **(F)** Area under the curve (AUC) calculated from the ITT. **(G)** Plasma insulin levels in fasted animals (C= 19 in each condition). **(H–K)** Gonadal and peripancreatic adipose tissue weight (n= 13 C, 13 S, 13 A, 15 A+S). **(I, J)** Quadriceps and gastrocnemius weight (n= 11 C, 12 S, 10 A, 11 A+S). The graphs present the mean ± S.E.M., and individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's *post hoc* test, and statistically significant differences were considered when $p<0.05$. *S vs C, $^{+}$S vs A, $^{\#}$A vs C, $^{\$}$C vs A+S, $^{\&}$A vs A+S, $^{\sim}$S vs A+S.

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p=0.0506, C vs A p=0.0709 and C vs A+S p<0.05), indicating that all treatments induced insulin resistance (Figures 1E, F). Consistent with the presence of insulin resistance observed after the ITT, all treatments increased plasma insulin levels compared with the control (Figure 1G). The evidence shows that sucrose induces at least three of the MS signs (obesity, hypertriglyceridemia, and insulin resistance), while arsenic only favored the development of insulin resistance and prevented the hypertriglyceridemia induced by sucrose without altering body weight gain.

Interestingly, the weight and the percentage of body weight of the peripancreatic and gonadal adipose tissues increased in S-treated animals but not in A+S, compared with C and A (Figures 1G, H, Supplementary Figure 3). The weight and the percentage of body weight of the quadriceps and gastrocnemius muscles was not different between the groups (Figures 1I, J, Supplementary Figure 3).

Arsenic and sucrose altered GLUT4 trafficking to the sarcolemma

Since glycolytic muscles, such as quadriceps and gastrocnemius are more susceptible to metabolic dysfunction induced by high-sucrose diets (43), and these muscles are among the largest muscle groups in rats, we studied how insulin signaling is affected by the treatments in these muscles. We obtained sarcolemma fractions from the quadriceps and gastrocnemius muscles 30 min after intraperitoneal injection with 0.2 IU/kg insulin to evaluate the abundances of GLUT4 and GLUT1 (the glucose transporter constitutively present at the sarcolemma).

Neither insulin stimulation nor treatments altered GLUT4 and GLUT1 abundances in sarcolemma fractions from the quadriceps (Figures 2A–C), indicating that this muscle group has low insulin sensitivity, at least under our conditions. In contrast, insulin stimulation increased GLUT4 abundance in the sarcolemma from the gastrocnemius in control animals (Figures 2D, E). Moreover, S and A induced a statistically significant increase in basal GLUT4 levels, while S, A, and A+S blunted insulin-stimulated GLUT4 translocation (Figures 2D, E), correlating with the results from the ITT.

As expected, GLUT1 levels in sarcolemma fractions did not change due to insulin and treatments in gastrocnemius (Figure 2F). These results indicate that S, A, and A+S specifically affect GLUT4 trafficking induced by insulin while impairing basal GLUT4 intracellular retention in gastrocnemius.

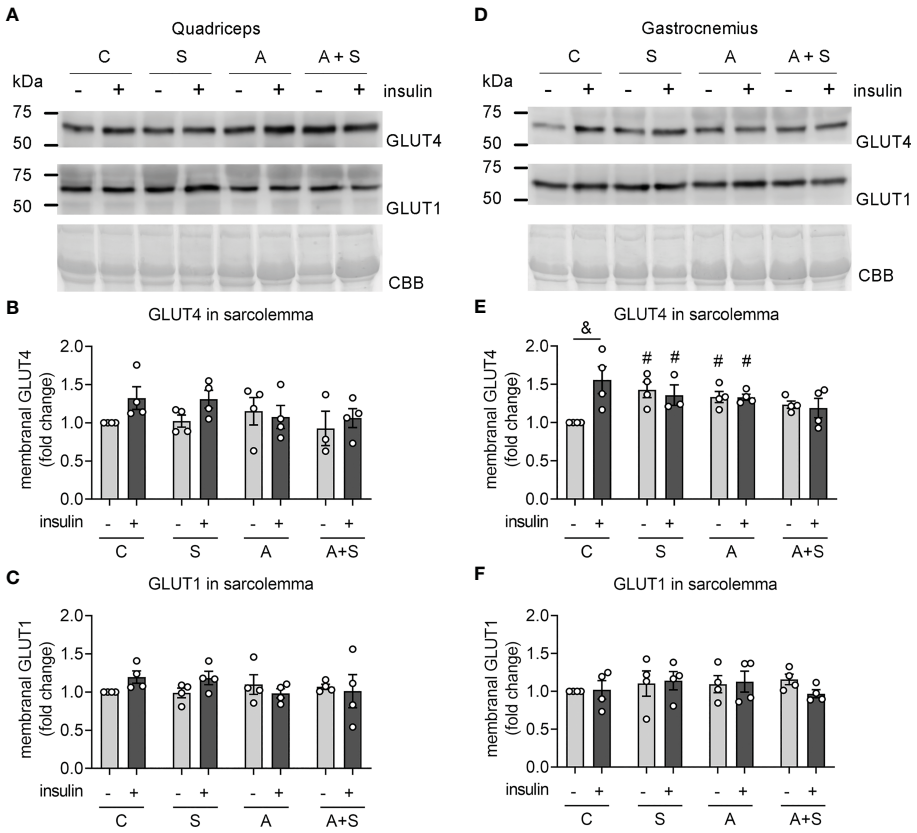

FIGURE 2
Effects of sucrose and arsenic intake on GLUT4 abundance in the sarcolemma. We determined the protein abundances of GLUT4 and GLUT1 in sarcolemma fractions 30 min after stimulation with an intraperitoneal injection of 0.2 IU/kg of insulin. **(A–C)** Results in quadriceps. **(D–F)** Results in gastrocnemius. **(A, D)** Representative Western blot. **(B, E)** Quantitation of GLUT4 levels in the sarcolemma. **(C, F)** GLUT1 was used as a negative control of a protein constitutively expressed at the sarcolemma. The graphs present the mean ± S.E.M. of four animals for each condition. Individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's *post hoc* test, and statistically significant differences were considered when p<0.05. $^{\&}$Effect of insulin. $^{\#}$Effect of treatment, compared with Control without insulin.

Arsenite and sucrose impair the expression of GSV markers

Defects in insulin-stimulated GLUT4 translocation during insulin resistance can be due to impaired sorting of GLUT4 into the GSVs and the downregulation of regulatory proteins of GSV trafficking, such as VAMP2 and sortilin (44–47). Thus, we tested whether the effects observed in GLUT4 abundance in the sarcolemma could be related to changes in total GLUT4, VAMP2, and sortilin levels. The total abundance of GLUT4 was unchanged under all conditions in both muscles, demonstrating that the alterations observed in GLUT4 abundance in the sarcolemma were due to impaired signaling and trafficking, rather than changes in GLUT4 expression (Figures 3B, F). Conversely, protein levels of VAMP2 (a member of the v-SNARE family of proteins, which is important for GSV docking and fusion with the plasma membrane (18)) decreased in the quadriceps muscles from animals treated with S, A, and A+S, while there were no effects in the gastrocnemius (Figures 3C, G). Sortilin is an important protein for GLUT4 recruitment into the GSVs (46). Nevertheless, we did not find differences in the levels of this protein in both muscles (Figures 3D, H).

Intriguingly, the band detected by the sortilin antibody in both muscles was around 60 kDa, which is lower than the expected molecular weight of 92 kDa. We validated that this signal was, in fact, sortilin by exogenously expressing human sortilin in COS7 cells and comparing the signal with those observed in rat muscles (Supplementary Figure 4). As expected, COS7 cells transfected with the plasmid containing human sortilin expressed two bands of approximately 100 and 90 kDa, corresponding to pro-sortilin and its mature form. In contrast, both muscles expressed only the 60 kDa form of insulin at levels compared with the exogenously expressed sortilin in COS7 cells, thereby demonstrating that this signal is, in fact, an isoform of sortilin. Nevertheless, the entries for the rat, mouse, and human sortilin gene (accession numbers: 83576, 20661, and 6272, respectively) show only one isoform (100 kDa) in rats, two isoforms (91 and 87 kDa) in mice, and two isoforms (88 and 77 kDa) in humans. Thus, the structure of this muscle isoform in rats remains to be determined.

Arsenite, but not sucrose, inhibits Akt phosphorylation

Since no defects in GSV markers in the gastrocnemius could explain the alterations observed in GLUT4 trafficking, we next tested whether the treatments affected the abundance of Akt and insulin-stimulated Akt phosphorylation at serine residue 473, which is a marker of fully active Akt (48).

Neither treatment altered the abundance of total Akt protein in the quadriceps nor the gastrocnemius muscles (Figures 4A–D). Comparable to the changes in GLUT4 abundance in the sarcolemma, insulin injection induced Akt phosphorylation in the gastrocnemius but not the quadriceps from control animals (Figures 4E–H). Interestingly, insulin-induced Akt phosphorylation did not change in the gastrocnemius from S-treated animals (Figures 4G, H), while arsenite blunted insulin-stimulated pAkt levels independently of sucrose intake (Figures 4G, H). No changes in pAkt levels were observed in the quadriceps after any treatment (Figures 4E, F).

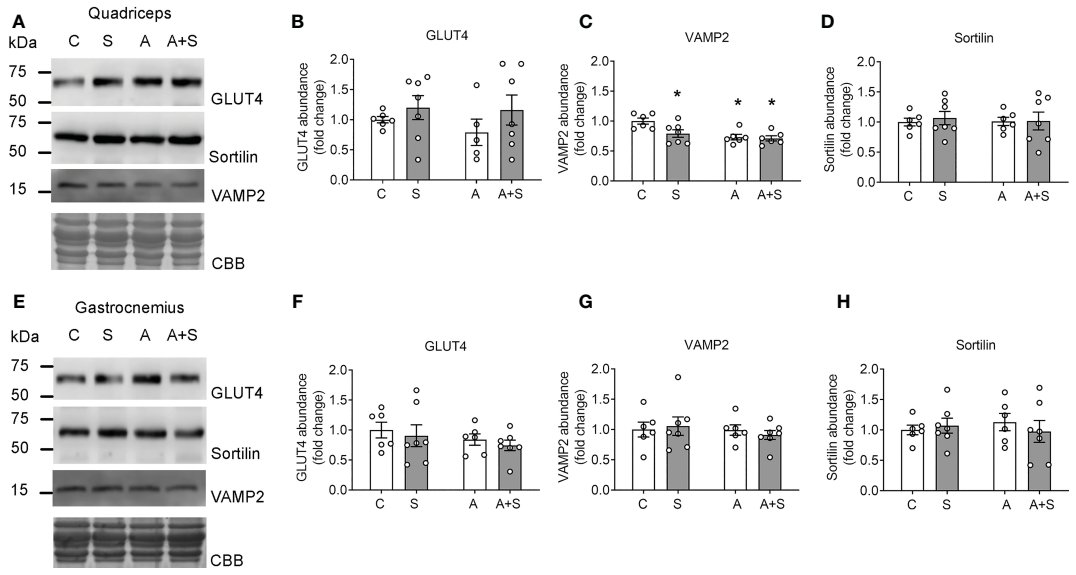

FIGURE 3
Sucrose and arsenic impair GSV markers in the quadriceps but not in the gastrocnemius. Total muscle lysates were prepared from animals after overnight fasting. **(A–D)** Results in quadriceps. **(E–H)** Results in gastrocnemius. **(A, E)** Representative Western blot. **(B, F)** Quantitation of total GLUT4 levels. **(C, G)** VAMP2 quantitation. **(D, H)** Sortilin quantitation. The graphs present the mean ± S.E.M. of six animals for each condition. Individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's *post hoc* test, and statistically significant differences were considered when $p<0.05$. *vs Control animals.

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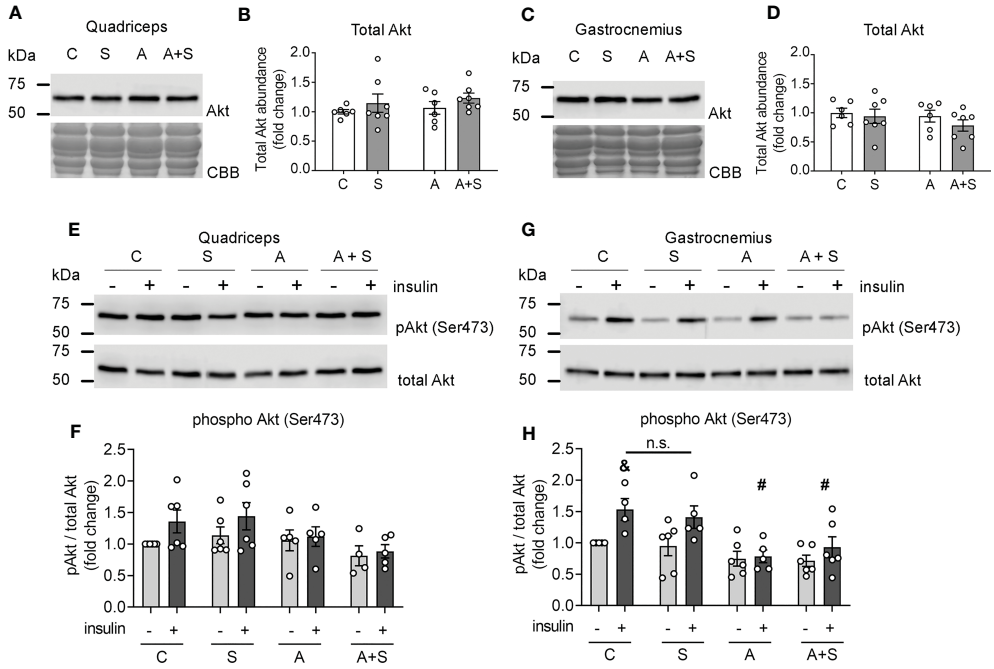

FIGURE 4
Arsenite impairs Akt phosphorylation induced by insulin. **(A–D)** Total lysates from fasted rats were used for total Akt determination in quadriceps **(A, B)** and gastrocnemius **(C, D)** muscles. **(E–H)** Lysates from rats stimulated with vehicle or insulin were used to evaluate phosphor Akt (Ser473) levels in quadriceps **(E, F)** and gastrocnemius **(G, H)**. The graphs present the mean ± S.E.M. of six animals in each condition. Individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's *post hoc* test, and statistically significant differences were considered when $p<0.05$. [&]Insulin vs the same treatment with vehicle. [#]vs Control with insulin. n.s. stands for non-significant.

Sucrose and arsenite intake have different effects on capn10 and TUG proteolysis

Insulin promotes TUG proteolysis and dissociation of TUG-GLUT4 complexes, releasing the GSVs (23). Moreover, TUG's N- and C-terminal fragments promote GSV trafficking and expression of thermogenic genes, respectively (21, 23). Thus, we evaluated the possible alterations in the two proteases that perform TUG proteolysis [capn10 and usp25m (20, 21)], as well as changes in the abundance of intact TUG and its proteolytic C-terminal fragments..

We did not find changes in the abundance of the protease usp25m under any conditions in either the quadriceps or the gastrocnemius (Figures 5A, B, D, E). In both muscles, capn10 was detected as three main putative isoforms (60, 50, and 45 kDa; the 60 kDa isoforms being the most abundant in both muscles) and unidentified high molecular weight bands, as described previously (42, 49). In the quadriceps, the treatments altered neither the main isoform of 60 kDa nor the 50 kDa isoform. However, A-treated animals displayed higher levels of capn10 45 kDa isoform compared with the control, and this increase was prevented in A+S (Figure 5C). In contrast, in the gastrocnemius muscle from S and A+S-treated animals, the abundance of capn10 60 kDa isoform was significantly reduced compared with the control animals, without changes in the 50 and 45 kDa isoforms (Figure 5F). Of note, by comparing the rat capn10 isoforms present in these muscles with exogenously expressed human CAPN10a and CAPN10c isoforms in COS7 cells, it can be reasonably concluded that the predominant capn10 isoform present in muscle (60 kDa isoform) corresponds to isoform c (capn10c). In comparison, the capn10 bands at 50 and 45 kDa could be proteolytic fragments or unknown splicing isoforms (Supplementary Figure 5).

Next, we measured the changes in the abundance of intact TUG after 30 min of insulin stimulation, as a marker of TUG proteolysis (21, 23). Concordantly with the lack of response to insulin stimulation in the quadriceps at the levels of GLUT4 translocation and Akt phosphorylation, the levels of intact TUG were not altered after insulin stimulation in the control animals (Figures 6A, B). Nevertheless, the arsenic treatment decreased basal and insulin-stimulated TUG abundance, regardless of sucrose intake (Figures 6A, B). In contrast, TUG abundance in the gastrocnemius muscle from control animals was significantly reduced after insulin stimulation (Figures 6E, F), which is consistent with the loss of intact TUG due to insulin-stimulated proteolysis. In this muscle, S, A, and A+S reduced the basal abundance of TUG and completely abolished insulin-stimulated TUG proteolysis (Figures 6E, F). To further characterize the deregulation of TUG proteolysis, we evaluated the abundance of the proteolytic fragments of this protein. In our model, we found two main fragments containing the C-terminal domain of TUG of 42 and 37 kDa (Figures 6A, B). In the quadriceps, the 42 kDa fragment was induced in animals exposed to arsenite and stimulated with insulin (Figures 6A, C), while the fragment of 37 kDa was reduced in animals exposed to sucrose during fasting, but this was reversed after insulin stimulation (Figures 6A, D). In the gastrocnemius, insulin increased the abundance of both fragments in control animals (Figures 6E, G, H). Interestingly, the levels of the

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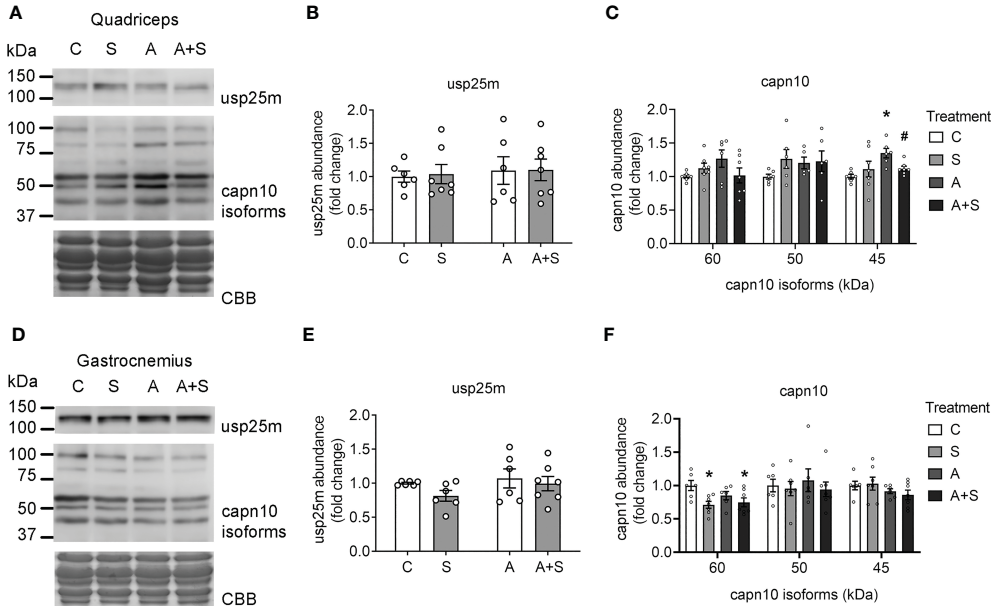

FIGURE 5
Sucrose and arsenic intake affect CAPN10 abundance. Total lysates from fasted rats were used to quantitate the levels of Usp25m and capn10 proteases in quadriceps **(A–C)** and gastrocnemius **(D–F)**. The graphs present the mean ± S.E.M. of six animals in each condition. Individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's *post hoc* test, and statistically significant differences were considered when $p<0.05$. * vs control, # vs A.

42 kDa fragment increased in S and A animals but not in A+S animals, independently of the stimulation with insulin (Figures 6E, G). This effect was not observed in the abundance of the fragment of 37 kDa in arsenite-treated animals, but this fragment was increased in S-exposed animals during fasting but not after insulin stimulation (Figures 6E, H).

Discussion

Skeletal muscle plays a pivotal role in controlling whole-body glucose homeostasis, and muscle insulin resistance is a major factor in the pathophysiology of MS and T2D (18). In the present study, we compared the effects of sucrose and arsenic intake for two months in developing the signs of MS, along with the alterations in muscle insulin signaling pathways that control GLUT4 translocation to the sarcolemma.

Our results showed that both sucrose and arsenite induced whole-body insulin resistance, even when arsenite did not modify body weight and prevented hypertriglyceridemia induced by sucrose. Interestingly, sucrose and arsenite had no additive effects on insulin resistance. Based on the calculations of the arsenic intake in our model (4.19 ± 0.69 and 5.03 ± 0.99 mg/kg/day), we can compare our data with previous reports of insulin resistance and hyperinsulinemia induced by intraoral administration of arsenite in male rats (2.5-5 mg/kg/day) (34, 36). Thus, it is appropriate to state that arsenic exposure in the range of 2.5-5 mg/kg/day induces insulin resistance without the development of obesity, at least in rat models.

On the other hand, arsenite did not affect the increase in body weight induced by sucrose intake, but it prevented adipose tissue hypertrophy of the two adipose tissue depots that we analyzed. These discrepancies could be related to arsenic-induced lipodystrophy, resulting in the lower ability of adipose tissue to handle an excess of calories and favoring ectopic lipid deposition in other tissues (2, 34, 50). Thus, our results indicate that arsenite affects the development of MS traits induced by sugary drinks in different ways.

Skeletal muscle accounts for up to 80% of the total glucose disposal during insulin stimulation, and defective GLUT4 trafficking in skeletal muscle is a major factor in developing MS and T2D (18). Since all treatments reduced insulin-induced glucose disposal during the ITT, we tested whether arsenite and sucrose affect some of the pathways involved in GLUT4 trafficking to the sarcolemma.

In the quadriceps, insulin stimulation (0.2 IU/kg) did not increase GLUT4 abundance in the sarcolemma, nor promote Akt phosphorylation and TUG proteolysis. Moreover, neither arsenite nor sucrose altered the levels of GLUT4 in the sarcolemma and phospho-Akt in this muscle group. Nevertheless, all treatments diminished VAMP2 levels, and arsenite and sucrose + arsenite diminished the basal levels of TUG, but this latter effect was probably due to enhanced TUG proteolysis and lower gene expression, respectively. Although low TUG levels result in impaired intracellular retention of GLUT4 and high levels of GLUT4 in the sarcolemma during fasting (23), we did not find changes in GLUT4 levels in the sarcolemma in the quadriceps from arsenic-treated rats.

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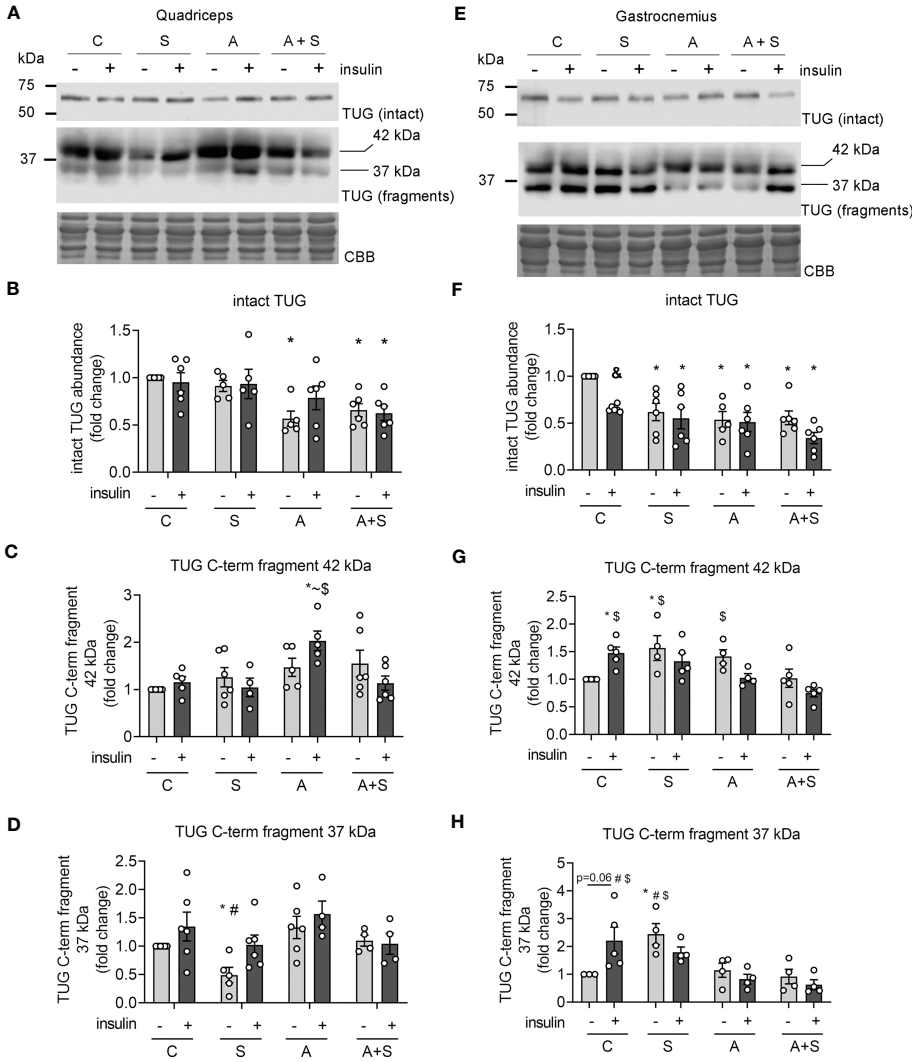

FIGURE 6
Effects of arsenic and sucrose intake on TUG proteolysis. Lysates from rats stimulated with vehicle or insulin were used to evaluate the abundance of intact TUG and its C-terminal fragments in quadriceps **(A–D)** and gastrocnemius **(E–H)**. The graphs present the mean ± S.E.M. of six animals in each condition. Individual animals are expressed as white circles. All data were analyzed by two-way ANOVA with Tukey's post hoc test, and statistically significant differences were considered when $p<0.05$. * vs control without insulin, ~ vs S insulin, # vs A, $ vs A+S insulin, & Insulin vs the same treatment with vehicle.

Although the lack of insulin response in quadriceps might seem paradoxical, most studies on GLUT4 translocation in muscle use high insulin doses (about 2 IU/kg), which are 10 times higher than the insulin doses used for the treatment of T2D patients and the dose used for this study (0.2 IU/kg) (2, 21, 51). Moreover, different muscle groups have distinct insulin sensitivity, especially at submaximal concentrations (52). Therefore, we propose that the quadriceps muscle has low insulin sensitivity in rats, and alterations related to GSV trafficking include reduced VAMP2 after sucrose and arsenite treatment and decreased TUG abundance after arsenic treatment.

In contrast, insulin induced a ~1.5-fold increase in GLUT4 levels in the sarcolemma from gastrocnemius, concordant with the expected increase of membranal GLUT4 described in skeletal muscle (18). Likewise, insulin stimulated Akt phosphorylation and TUG proteolysis in this muscle. In parallel with systemic insulin resistance, all treatments blunted insulin-induced GLUT4 recruitment to the sarcolemma in the gastrocnemius. Interestingly, only arsenite treatment impaired Akt phosphorylation, independently of sucrose intake, while all treatments dulled TUG proteolysis induced by insulin in the gastrocnemius. It is of note that the levels of the proteolytic fragments of TUG were differentially deregulated by treatments: sucrose increased the abundance of both fragments, arsenite only increased the fragment of 42 kDa, and arsenite + sucrose did not change the abundance of any fragment; thus, we hypothesize that sucrose and arsenite accelerate basal proteolysis of TUG, while TUG gene expression is impaired in animals consuming both factors. Whether this increase in basal TUG proteolysis is carried out by capn10 and usp25m or by other proteases that cleave this protein [such as calpain-1 and the ubiquitin-proteasome system (20, 23)] remains to be explored.

The results in the gastrocnemius muscle suggest that sucrose and arsenic intake impair GLUT4 trafficking in gastrocnemius through different pathways: while sucrose deregulates the proteolytic pathway, arsenic affects both proteolytic and PI3K-Akt pathways. However, Akt levels must be reduced by more than 90% to effectively impair GLUT4 translocation in skeletal muscle (16), and arsenic reduced Akt phosphorylation by ~60%. Also, reduced p-Akt levels due to hypercaloric diets do not necessarily correlate with reduced phosphorylation of its target proteins (TBC1D1 and TBC1D4) and GLUT4 translocation (17). Thus, we can hypothesize that impaired TUG proteolysis exerts a higher contribution to blunted GLUT4 translocation after arsenic and sucrose exposure.

Consistent with previous reports (10, 13, 17, 21), our data suggest that the proteolytic pathway is more sensitive to environmental cues than the PI3K-Akt pathway, and arsenic has a high impact on Akt phosphorylation, probably by inhibiting PDK-1 (14). Notably, we found higher levels of GLUT4 in the sarcolemma during fasting in the gastrocnemius from sucrose and arsenite-treated animals. As previously stated, low levels of TUG in skeletal muscle result in the defective retention of GLUT4 in intracellular compartments (23). Therefore, the high fasting levels of GLUT4 at the sarcolemma could be due to the enhanced basal TUG proteolysis in animals consuming sucrose and arsenite separately and the low expression of TUG in animals consuming both factors. We hypothesize that the impaired intracellular retention of GLUT4 in this muscle could contribute to maintaining normal blood glucose levels, even in insulin-resistant states.

Although the deregulation of the proteolytic pathway is an important factor contributing to insulin resistance in muscle, the precise upstream mechanisms that regulate this pathway under physiological conditions remain largely unknown. Interestingly, high-fat dietary intake reduces the protein levels of the protease usp25m in the quadriceps of mice (23). Nevertheless, we did not find changes in the levels of this protease after sucrose and arsenic exposure in rats in any of the muscles analyzed. We found that capn10 isoforms were differentially deregulated in the quadriceps and gastrocnemius muscles. Whether these discrepancies could be due to species-specific mechanisms or the differences in the source of calory surplus (fat vs sucrose) remains to be determined in future studies. Nevertheless, neither change in capn10 isoforms, nor usp25m levels fully explain why all treatments inhibited TUG proteolysis. Further research is needed to understand the precise mechanisms that activate these proteases in skeletal muscle and how environmental and dietary factors affect their activity.

Conclusion

Our results indicate that arsenic and sucrose induce systemic insulin resistance related to defects in the translocation of GLUT4 to the sarcolemma in skeletal muscle. Importantly, sucrose and arsenic do not have additive effects in developing insulin resistance. The defects in the pathways involved in this process depend on the muscle analyzed and the environmental factors. In the quadriceps, the main alterations were related to markers of GSV intracellular retention and docking. At the same time, in the gastrocnemius, sucrose altered the proteolytic pathway and arsenic impaired Akt phosphorylation and TUG proteolysis.

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 Animal Care Committee of the Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM; CICUAL MHU189-22).

Author contributions

PP: conceptualization, experimental design, investigation, data curation, draft writing and editing, visualization. MV: experimental design, investigation, data curation, manuscript review and editing. AS: experimental design, manuscript review and editing, funding acquisition. POW: conceptualization, experimental design, manuscript review and editing, funding acquisition. MH: conceptualization, experimental design, manuscript review and editing, funding acquisition, project management. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

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References

1. Hiriart M, Velasco M, Larqué C, Diaz-Garcia CM. Metabolic syndrome and ionic channels in pancreatic beta cells. *Vit Hormones* (2014) 95:87–114. doi: 10.1016/B978-0-12-800174-5.00004-1

2. Pánico P, Velasco M, Salazar AM, Picones A, Ortiz-Huidobro RI, Guerrero-Palomo G, et al. Is arsenic exposure a risk factor for metabolic syndrome? a review of the potential mechanisms. *Front Endocrinol (Lausanne)* (2022) 13:878280. doi: 10.3389/fendo.2022.878280

3. ATSDR. Toxicological profile for arsenic. *US Departament Health Hum Serv* (2013) 2013:24. doi: 10.1155/2013/286524

4. Ochoa-Martínez ÁC, Ruiz-Vera T, Almendarez-Reyna CI, Zarazúa S, Carrizales-Yáñez L, Pérez-Maldonado IN. Impact of arsenic exposure on clinical biomarkers indicative of cardiovascular disease risk in Mexican women. *Ecotoxicol Environ Saf* (2019) 169:678–86. doi: 10.1016/j.ecoenv.2018.11.088

5. Sung T, Huang J, Guo H. Association between arsenic exposure and Diabetes: a meta-analysis. *BioMed Res Int* (2015) 2015:1–10. doi: 10.1155/2015/368087

6. Spratlen MJ, Grau-Perez M, Best LG, Yracheta J, Lazo M, Vaidya D, et al. The association of arsenic exposure and arsenic metabolism with the metabolic syndrome and its individual components: prospective evidence from the strong heart family study. *Am J Epidemiol* (2018) 187:1598–612. doi: 10.1093/aje/kwy048

7. Kazemifar AM, Shafikhani AA, Mozhdehipanah H, Khamesi S, Arami M. Evaluation of different types of arsenic methylation and its relationship with metabolic syndrome in an area chronically exposed to arsenic. *Environ Anal Health Toxicol* (2020) 35:e2020006. doi: 10.5620/eaht.e2020006

8. Wang S, Chang F, Liou S, Wang H, Li W, Hsieh DPH. Inorganic arsenic exposure and its relation to metabolic syndrome in an industrial area of Taiwan. *Environ Int* (2007) 33:805–11. doi: 10.1016/j.envint.2007.03.004

9. Ambrosio F, Brown E, Stolz D, Ferrari R, Goodpaster B, Deasy B, et al. Arsenic induces sustained impairment of skeletal muscle and muscle progenitor cell ultrastructure and bioenergetics. *Free Radic Biol Med* (2014) 74:64–73. doi: 10.1016/j.freeradbiomed.2014.06.012

10. Yang L, Qiu T, Yao X, Jiang L, Wei S, Pei P, et al. Taurine protects against arsenic trioxide-induced insulin resistance *via* ROS- autophagy pathway in skeletal muscle. *Int J Biochem Cell Biol* (2019) 112:50–60. doi: 10.1016/j.biocel.2019.05.001

11. Mondal V, Hosen Z, Hossen F, Siddique AE, Tony SR, Islam Z, et al. Arsenic exposure-related hyperglycemia is linked to insulin resistance with concomitant reduction of skeletal muscle mass. *Environ Int* (2020) 143:105890. doi: 10.1016/j.envint.2020.105890

12. Padmaja Divya S, Pratheeshkumar P, Son Y-O, Vinod Roy R, Andrew Hitron J, Kim D, et al. Arsenic induces insulin resistance in mouse adipocytes and myotubes *Via* oxidative stress-regulated mitochondrial Sirt3-FOXO3a signaling pathway. *Toxicol Sci* (2015) 146:290–300. doi: 10.1093/toxsci/kfv089

13. Walton FS, Harmon AW, Paul DS, Drobná Z, Patel YM, Styblo M. Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. *Toxicol Appl Pharmacol* (2004) 198:424–33. doi: 10.1016/j.taap.2003.10.026

14. Paul DS, Harmon AW, Devesa V, Thomas DJ, Stýblo M. Molecular mechanisms of the diabetogenic effects of arsenic inhibition of insulin signaling by arsenite and methylarsonous acid. *Environ Health Perspect* (2007) 115:734–42. doi: 10.1289/ehp.9867

15. Xue P, Hou Y, Zhang Q, Woods CG, Yarborough K, Liu H, et al. Prolonged inorganic arsenite exposure suppresses insulin-stimulated AKT S473 phosphorylation and glucose uptake in 3T3-L1 adipocytes: involvement of the adaptive antioxidant response. *Biochem Biophys Res Commun* (2011) 407:360–5. doi: 10.1016/j.bbrc.2011.03.024

16. Jaiswal N, Gavin MG, Quinn WJ, Luongo TS, Gelfer RG, Baur JA, et al. The role of skeletal muscle akt in the regulation of muscle mass and glucose homeostasis. *Mol Metab* (2019) 28:1–13. doi: 10.1016/j.molmet.2019.08.001

17. Hoehn KL, Hohnen-Behrens C, Cederberg A, Wu LE, Turner N, Yuasa T, et al. IRS1-independent defects define major nodes of insulin resistance. *Cell Metab* (2008) 7:421–33. doi: 10.1016/j.cmet.2008.04.005

18. Sylow L, Tokarz VL, Richter EA, Klip A. The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. *Cell Metab* (2021) 33:758–80. doi: 10.1016/j.cmet.2021.03.020

19. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* (2009) 32(Suppl 2):S157–63. doi: 10.2337/dc09-s302

20. Pánico P, Hiriart M, Ostrosky-Wegman P, Salazar AM. TUG is a calpain-10 substrate involved in the translocation of GLUT4 in adipocytes. *J Mol Endocrinol* (2020) 65:45–57. doi: 10.1530/JME-19-0253

21. Habtemichael EN, Li DT, Alcázar-Román A, Westergaard XO, Li M, Petersen MC, et al. Usp25m protease regulates ubiquitin-like processing of TUG proteins to control GLUT4 glucose transporter translocation in adipocytes. *J Biol Chem* (2018) 293:10466–86. doi: 10.1074/jbc.RA118.003021

22. Sylow L, Kleinert M, Pehmøller C, Prats C, Chiu TT, Klip A, et al. Akt and Rac1 signaling are jointly required for insulin-stimulated glucose uptake in skeletal muscle and downregulated in insulin resistance. *Cell Signal* (2014) 26:323–31. doi: 10.1016/j.cellsig.2013.11.007

23. Habtemichael EN, Li DT, Camporez JP, Westergaard XO, Sales CI, Liu X, et al. Insulin-stimulated endoproteolytic TUG cleavage links energy expenditure with glucose uptake. *Nat Metab* (2021) 3:378–93. doi: 10.1038/s42255-021-00359-x

24. de Wendt C, Espelage L, Eickelschulte S, Springer C, Toska L, Scheel A, et al. Contraction-mediated glucose transport in skeletal muscle is regulated by a framework of AMPK, TBC1D1/4, and Rac1. *Diabetes* (2021) 70:2796–809. doi: 10.2337/db21-0587

25. Umek N, Horvat S, Cvetko E. Skeletal muscle and fiber type-specific intramyocellular lipid accumulation in obese mice. *Bosn J Basic Med Sci* (2021) 21:729–37. doi: 10.17305/bjbms.2021.5876

26. James DE, Burleigh KM, Storlien LH, Bennett SP, Kraegen EW. Heterogeneity of insulin action in muscle: influence of blood flow. *Am J Physiol-Endocrinol Metab* (1986) 251:E422–30. doi: 10.1152/ajpendo.1986.251.4.E422

27. Velasco M, Larqué C, Gutiérrez-Reyes G, Arredondo R, Sanchez-Soto C, Hiriart M. Metabolic syndrome induces changes in KATP-channels and calcium currents in pancreatic β-cells. *Islets* (2012) 4:302–11. doi: 10.4161/isl.21374

28. Larqué C, Velasco M, Navarro-Tableros V, Duhne M, Aguirre J, Gutiérrez-Reyes G, et al. Early endocrine and molecular changes in metabolic syndrome models. *IUBMB Life* (2011) 63:831–9. doi: 10.1002/iub.544

29. Albarado-Ibañez A, Avelino-Cruz JE, Velasco M, Torres-Jácome J, Hiriart M. Metabolic syndrome remodels electrical activity of the sinoatrial node and produces arrhythmias in rats. *PloS One* (2013) 8:e76534. doi: 10.1371/journal.pone.0076534

30. Cruz-cruz I, Bernate-obando G, Larqué C, Escalona R, Pinto-almazán R, Velasco M. Early effects of metabolic syndrome on ATP-Sensitive potassium channels from rat pancreatic beta cells. *Metabolites* (2022) 12:1–12. doi: 10.3390/metabo12040365

31. Afolabi OK, Wusu AD, Ogunrinola OO, Abam EO, Babayemi DO, Dosumu OA, et al. Arsenic-induced dyslipidemia in male albino rats: comparison between trivalent and pentavalent inorganic arsenic in drinking water. *BMC Pharmacol Toxicol* (2015) 16:15. doi: 10.1186/s40360-015-0015-z

32. Yang H-T, Chou H-J, Han B-C, Huang S-Y. Lifelong inorganic arsenic compounds consumption affected blood pressure in rats. *Food Chem Toxicol* (2007) 45:2479–87. doi: 10.1016/j.fct.2007.05.024

33. Holson JF, Stump DG, Clevidence KJ, Knapp JF, Farr CH. Evaluation of the prenatal developmental toxicity of orally administered arsenic trioxide in rats. *Food Chem Toxicol* (2000) 38:459–66. doi: 10.1016/S0278-6915(00)00015-6

34. Jia X, Qiu T, Yao X, Jiang L, Wang N, Wei S, et al. Arsenic induces hepatic insulin resistance via mtROS-NLRP3 inflammasome pathway. *J Hazard Mater* (2020) 399:123034. doi: 10.1016/j.jhazmat.2020.123034

35. Wei S, Qiu T, Yao X, Wang N, Jiang L, Jia X, et al. Arsenic induces pancreatic dysfunction and ferroptosis via mitochondrial ROS-autophagy-lysosomal pathway. *J Hazard Mater* (2020) 384:121390. doi: 10.1016/J.JHAZMAT.2019.121390

36. Izquierdo-Vega JA, Soto CA, Sanchez-Peña LC, De Vizcaya-Ruiz A, Del Razo LM. Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite. *Toxicol Lett* (2006) 160:135–42. doi: 10.1016/j.toxlet.2005.06.018

37. Velasco M, Ortiz-Huidobro RI, Larqué C, Sánchez-Zamora YI, Romo-Yáñez J, Hiriart M. Sexual dimorphism in insulin resistance in a metabolic syndrome rat model. *Endocr Connect* (2020) 9:890–902. doi: 10.1530/EC-20-0288

38. Zhang J, Oh E, Merz KE, Aslamy A, Veluthakal R, Salunkhe VA, et al. DOC2B promotes insulin sensitivity in mice via a novel KLC1-dependent mechanism in skeletal muscle. *Diabetologia* (2019) 62:845–59. doi: 10.1007/s00125-019-4824-2

39. Merz KE, Tunduguru R, Ahn M, Salunkhe VA, Veluthakal R, Hwang J, et al. Changes in skeletal muscle PAK1 levels regulate tissue crosstalk to impact whole body glucose homeostasis. *Front Endocrinol (Lausanne)* (2022) 13:821849. doi: 10.3389/fendo.2022.821849

40. Zhou M, Sevilla L, Vallega G, Chen P, Palacin M, Zorzano A, et al. Insulin-dependent protein trafficking in skeletal muscle cells. *Am J Physiol Endocrinol Metab* (1998) 275(2):E187-96. doi: 10.1152/ajpendo.1998.275.2.E187

41. McDonough AA, Veiras LC, Minas JN, Ralph DL. Considerations when quantitating protein abundance by immunoblot. *Am J Physiology-Cell Physiol* (2014) 308:C426–33. doi: 10.1152/ajpcell.00400.2014

42. Ono Y, Doi N, Shindo M, Pánico P, Salazar AM. Cryptic splicing events result in unexpected protein products from calpain-10 (CAPN10) cDNA. *Biochim Biophys Acta (BBA) - Mol Cell Res* (2022) 1869:119188. doi: 10.1016/j.bbamcr.2021.119188

43. Warren BE, Lou P-H, Lucchinetti E, Zhang L, Clanachan AS, Affolter A, et al. Early mitochondrial dysfunction in glycolytic muscle, but not oxidative muscle, of the fructose-fed insulin-resistant rat. *Am J Physiol Endocrinol Metab* (2014) 306:658–67. doi: 10.1152/ajpendo.00511.2013.-Although

44. Maier VH, Melvin DR, Lister CA, Chapman H, Gould GW, Murphy GJ. *V-and t-SNARE protein expression in models of insulin resistance normalization of glycemia by rosiglitazone treatment corrects overexpression of cellubrevin, vesicle-associated membrane protein-2, and syntaxin 4 in skeletal muscle of zucker diabetic fatty rats* (2000). Available at: http://diabetesjournals.org/diabetes/article-pdf/49/4/618/365088/10871200.pdf.

45. Livingstone R, Bryant NJ, Boyle JG, Petrie JR, Gould GW. Diabetes is accompanied by changes in the levels of proteins involved in endosomal GLUT4 trafficking in obese human skeletal muscle. *Endocrinol Diabetes Metab* (2022) 5:e361. doi: 10.1002/edm2.361

46. Tsuchiya Y, Hatakeyama H, Emoto N, Wagatsuma F, Matsushita S, Kanzaki M. Palmitate-induced down-regulation of sortilin and impaired GLUT4 trafficking in C2C12 myotubes. *J Biol Chem* (2010) 285:34371–81. doi: 10.1074/jbc.M110.128520

47. Bogan JS. Regulation of glucose transporter translocation in health and diabetes. *Annu Rev Biochem* (2012) 81:507–32. doi: 10.1146/annurev-biochem-060109-094246

48. Sugiyama MG, Fairn GD, Antonescu CN. Akt-ing up just about everywhere: compartment-specific akt activation and function in receptor tyrosine kinase signaling. *Front Cell Dev Biol* (2019) 7:70. doi: 10.3389/fcell.2019.00070

49. Ma H, Fukiage C, Kim YH, Duncan MK, a. RN, Shih M, et al. Characterization and expression of calpain 10: a novel ubiquitous calpain with nuclear localization. *J Biol Chem* (2001) 276:28525–31. doi: 10.1074/jbc.M100603200

50. Ceja-Galicia ZA, Daniel A, Salazar AM, Pánico P, Ostrosky-Wegman P, Díaz-Villaseñor A. Effects of arsenic on adipocyte metabolism: is arsenic an obesogen? *Mol Cell Endocrinol* (2017) 452:25–32. doi: 10.1016/j.mce.2017.05.008

51. Chun J, Strong J, Urquhart S. Insulin initiation and titration in patients with type 2 diabetes. *Diabetes Spectr* (2019) 32:104–11. doi: 10.2337/ds18-0005

52. James DE, Jenkins AB, Kraegen EW. Heterogeneity of insulin action in individual muscles in vivo: euglycemic clamp studies in rats. *Am J Physiol-Endocrinol Metab* (1985) 248:E567–74. doi: 10.1152/ajpendo.1985.248.5.E567

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Association of physical activity and sitting with metabolic syndrome and hyperglycemic clamp parameters in adolescents – BRAMS pediatric study

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Introduction: Obesity and metabolic syndrome (MetS) have immediate and long-term consequences on adolescent health and well-being. Among the available treatments for MetS in adolescents, behavioral interventions such as increasing physical activity (PA) are preferred. This study aimed to investigate the association of PA and sitting time with MetS and a complete set of metabolic health parameters.

Methods: Data from the Pediatric Brazilian Metabolic Syndrome Study (BRAMS-P), a cross-sectional multicenter study conducted using a convenience sample of 448 Brazilian adolescents (10y–19y), were used. Sociodemographic and lifestyle information were collected using a standardized questionnaire. Daily PA and sitting time were estimated from the International PA Questionnaire. Anthropometric parameters, body composition, and blood pressure were measured by trained researchers. Blood lipids, uric acid, hepatic enzymes, creatinine, glycated hemoglobin, glucose, and insulin were measured in fasting blood samples, and the Homeostasis Model Assessment for Insulin Resistance was calculated. A subsample of 57 adolescents underwent the hyperglycemic clamp protocol.

Results: The odds for metabolic syndrome were higher among adolescents who spent >8h sitting (OR (95%CI)=2.11 (1.02 – 4.38)), but not in those classified as active (OR (95%CI)=0.98 (0.42 – 2.26)). Adolescents who spent more time sitting had higher BMI, waist circumference, sagittal abdominal diameter, neck circumference, percentage of body fat, and worse blood lipid profile. The insulin sensitivity index was moderately and positively correlated with moderate-to-high PA in minutes per day (rho=0.29; p=0.047).

Conclusion: Time spent sitting was associated with worse metabolic parameters and must be restricted in favor of adolescent health. Regular PA is associated with improved insulin sensitivity and may be encouraged not only in adolescents with obesity or metabolic disorders but also to prevent adverse metabolic outcomes in normal-weight adolescents.

KEYWORDS

physical activity, international physical activity questionnaire, sitting, metabolic syndrome, adolescents, hyperglycemic clamp

1 Introduction

Adolescence is a critical period in human development given the physiological, sociological, psychological, and reproductive maturation that occurs during this stage of life (1). The prevalence of obesity in children and adolescents between 5 and 19 years of age has almost doubled during the last 20 years, reaching 18.4% globally (2), raising concerns about its immediate and long-term consequences on adolescents' health and well-being (3).

Adolescents with obesity have higher risk of anxiety and depression (4), polycystic ovary syndrome (5), insulin resistance, hypertension and dyslipidemia, many of which share components with the so-called metabolic syndrome (MetS) (6). In addition, when the onset of these metabolic disorders occurs during childhood or adolescence, there is an increased risk for diabetes, cardiovascular diseases, and some types of cancer before the age of 45 years, posing a huge burden upon health systems around the globe (7).

As defined by the International Diabetes federation (IDF), MetS is a cluster of interrelated risk factors for cardiovascular disease and type 2 diabetes, including abdominal obesity, high cholesterol levels, hypertension, and impaired insulin sensitivity, which are defined by anthropometric, blood pressure, and blood biomarkers specific cut-off values depending on adolescent's age range (8). Along with obesity, the prevalence of MetS is increasing, reaching approximately 35.5 million adolescents worldwide (9).

Among the available treatment options for MetS in children and adolescents, behavioral interventions such as improving dietary quality and adequate physical activity (PA) are prioritized over drugs and surgical therapy (10). In this sense, studies have shown that greater amounts of moderate-to-high intensity PA, objectively measured (accelerometer) and self-reported, are associated with a lower risk for MetS and other cardiometabolic health outcomes in adolescents (11–13). For sedentary behavior, on the other hand, while screen time is a well-known risk factor for MetS, in children and adolescents, as reviewed elsewhere (14), there are conflicting results and recommendations on sitting time (11, 12).

Moreover, most studies have investigated basic outcomes in relation to PA and sedentary behavior, such as body mass index and metabolic syndrome components (11, 14); however, few studies have investigated a complete set of metabolic health biomarkers, which compromises physical activity and sedentary behavior (12). To our knowledge, only one study has assessed the relationship between physical activity and direct measures of insulin sensitivity and beta-cell function using the hyperglycemic-clamp protocol in adolescents (15), and no study has investigated these outcomes in relation to sedentary behaviors. Thus, the present study aimed to investigate the association of moderate-to-high-level physical activity and sitting time with MetS and a complete set of metabolic health outcomes, including the investigation of hyperglycemic clamp parameters in a subsample.

2 Materials and methods

2.1 Study design

The present study used data from the Pediatric Brazilian Metabolic Syndrome Study (BRAMS-P), a cross-sectional study conducted on a convenience sample of adolescents between 2011 and 2013, which took place in health centers, ambulatories, public schools, and public universities across three Brazilian cities: Campinas, Itu, and Sao Paulo.

Individuals between 10 and 19 years of age were invited to participate and had a body mass index above the 5th percentile, according to the Centers for Disease Control and Prevention growth chart for age and sex (16). Individuals were excluded at the time of data and sample collection, if they were pregnant, or presented with liver disease, nephropathy, hypothyroidism, hyperthyroidism, diabetes mellitus, genetic syndrome diagnosis, and delayed neuropsychomotor development, as well as those who were using either systemic corticosteroids or drugs with hypoglycemic properties.

For the present study, further exclusions were made if individuals had incomplete data to diagnose metabolic syndrome (missing values for any of the following: plasma high-density lipoprotein cholesterol [HDL-c] concentration, fasting glucose, blood pressure, and waist circumference) or did not complete the International Physical Activity Questionnaire (IPAQ).

All participants and their legal guardians were informed of the study protocol, and those who agreed to participate signed an informed consent form. The study protocol was approved by the Committee for Research Ethics of the School of Medical Sciences of UNICAMP (protocol n. 900/2010, CAAE: 0696.0.146.146-10) and

is in accordance with the Brazilian law and the ethical principles of Helsinki Declaration.

2.2 Clinical evaluation

Data on demographic (age and sex) and socioeconomic (chief-or-the-family educational level, and Brazilian economic classification table) characteristics, as well as on family health history (hypertension, obesity, dyslipidemia, cardiovascular disease and diabetes), smoking habits, alcohol intake, other illicit drug use, supplement use, medicine use, and sleeping habits were collected by trained interviewers using a standardized questionnaire.

Sexual maturity was rated according to Tanner scale (17), which was presented to participants in a reserved room by trained researchers and self-declared, and pubertal development was determined as pre-pubertal (Tanner I), pubertal (Tanner II-IV) and post-pubertal (Tanner V). Further information on BRAMS-p self-assessment method can be found elsewhere (18). Blood pressure was measured using a mercury-based sphygmomanometer with auscultatory approach, following National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents recommendations (19).

Additionally, Campinas and Itu centers used a IPAQ-short form applied by trained interviewers adapted to the Brazilian population (20), from which the time spent on moderate and intense PA as well as the time spent on sitting position per day were calculated.

Adolescents were classified as having metabolic syndrome following the IDF criteria (8).

2.3 Anthropometric measurements and body composition parameters

Adolescents were asked to wear light clothing and no shoes during all the anthropometric and body composition evaluation. Body weight was measured using a digital scale with capacity for 150 Kg and precision of 0,1 Kg, and height was measured with adolescents standing in an orthostatic position against a wall, using a fixed stadiometer with capacity for 220 cm and precision of 0,1 cm. Body mass index (BMI) was, then, calculated as body weight, in Kg, divided by squared height, in cm. BMI was transformed into z-score using the LMS parameters from the World Health Organization (WHO) BMI-for-age growth chart for boys and girls, and classified as overweight and obesity according to the WHO cut-off points (21).

Waist circumference was measured by trained researchers positioning the tape at the midpoint between the last rib and the iliac crest. Hip circumference was measured positioning the tape at the biggest circumference between the waist and knees while adolescents were at the stand position with feet 30 cm apart (22). The sagittal abdominal diameter was measured using the Holtain-Kahn Abdominal Caliper (Holtain Ltd, Crymych, United Kingdom), at the umbilicus level after a normal exhalation while the subjects were in a supine position with their knees slightly bent on a firm examination table (22). The neck circumference was measured positioning the tape at the midpoint of the neck length (23).

Percentage body fat was estimated using tetrapolar bioimpedance (Biodynamics, model 310, Shoreline, Washington, USA) validated for epidemiological studies (24).

2.4 Biochemical markers

Blood samples were collected after a 12-hour overnight fasting, and centrifuged for plasma storage at 80°C. Plasma samples were transported to the UNICAMP Clinical Hospital laboratory, where creatinine, glucose, total cholesterol, HDL-c, low-density lipoprotein cholesterol (LDL-c), triglycerides, uric acid, gamma-glutamil transferase (gamma-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glycated hemoglobin were measured using standard protocols (25). Insulin plasma levels were measured by enzyme-linked immunosorbent assay kit (EZHI-14K; Millipore; St. Louis, Missouri, USA) at the Laboratory of Diabetes and Metabolism Investigations (LIMED).

The Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was calculated as the product of the fasting plasma insulin level (in milliunits per liter) and the fasting plasma glucose level (in millimoles per liter), divided by 22.5 (26).

2.5 Metabolic syndrome criteria

Metabolic syndrome was defined according to the International Diabetes Federation criteria (27). For adolescents aging 10 to 16 years, the MetS was established whenever high waist circumference was present (> 90^{th} percentile) along with at least two of the following components: high blood pressure (systolic or diastolic blood pressure > 95^{th} percentile); low HDL-c (≤ 40 mg/dL); and high fasting glucose (>100 mg/dL). For adolescents aging more than 16 years, MetS was established when three or more of the following components were present: high waist circumference (≥94 cm for men, and ≥80 cm for women); high blood pressure (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥ 85 mmHg); low HDL-c (≤ 40 mg/dL for men, and ≤ 50 mg/dL for women); and high fasting glucose (> 100 mg/dL).

2.6 Hyperglycemic clamp protocol

Participants underwent a 2-hour hyperglycemic clamp (with blood glucose acutely raised and maintained at approximately 225 mg/dL; to convert to millimoles per liter, multiply by 0.0555) according to the protocol previously described by Arslanian (28).The insulin sensitivity index (ISI) was calculated as the mean exogenous glucose infusion rate from 60 to 120 minutes of the clamp protocol, adjusted for urinary glucose excretion (subtraction), divided by the mean insulin concentration of the period, and it was then corrected for lean body mass (29). The Disposition Index (DI), which represents the beta-cell function

relative to insulin sensitivity, was calculated as the product of ISI vs. the area under the curve of the first phase of the insulin secretory rate (30).

2.7 Statistical analysis

Continuous variables were tested for normality using the Kolmogorov-Smirnov test and, as the vast majority did not have satisfactory adhesion to normal distribution, results are presented as median (min-max). Categorical variables are presented in absolute and relative frequency.

To compare means between adolescents with and without the metabolic syndrome, the Mann-Whitney test was applied. The chi-squared test was applied to compare frequencies between different metabolic syndrome status.

To check for the correlation between time spent on moderate to high intensity PA, as well as time spent sitting per day, and metabolic parameters the Spearman's coefficient was used, adjusted for confounding variables. To estimate the odds for metabolic syndrome in adolescents that referred more than 60 minutes per day of moderate to high intensity PA, as well as those that referred more than 8 hours per day of sitting, a multiple logistic regression was used, adjusted for confounding variables.

The confounding factors used were: age (years), sex (dichotomous), smoking status (yes/no to "have you smoked 100 cigarettes or more during your whole life?"), alcohol intake (yes/no to "Have you drink one dose or more of alcoholic beverage the past month?"), puberal status, medicine use (yes/no), sleep (in hours, for the correlation coefficient test, and > 8 hours/night in the logistic regression). Time spent sitting and time spent on moderate to high intensity PA were also added as confounding factors of each other's exposure.

All analysis were conducted using Stata SE software, version 17.0 (StataCorp LLC, Texas, EUA).

3 Results

After applying the exclusion criteria, the final sample of the present study comprised 448 adolescents and a subsample of 57 individuals who participated in the hyperglycemic clamp protocol (Figure 1).

There was a balance between males and females, with the majority of the sample classified as pubertal, with a median age of 14 years (10 to 19 years), median time spent in moderate-to-high level PA of 24 min per day (varying from 0 to 509 min), and median time spent sitting of 7 h per day (varying from 0.1 to 18 h) (Table 1).

Comparisons between adolescents with (n=38) and without (n=410) metabolic syndrome showed that those with metabolic syndrome were more frequently male and referred to smoking habits and medication use more frequently than those without metabolic syndrome (Table 1). Among adolescents with at least one of metabolic syndrome components, 2% had high plasma glucose (n=9), 13% had high blood pressure (n=58), 42% had high waist circumference (n=189), and 47% had low HDL-c (n=212) (Supplemental Figure S1 shows a Vann's diagram for intersection

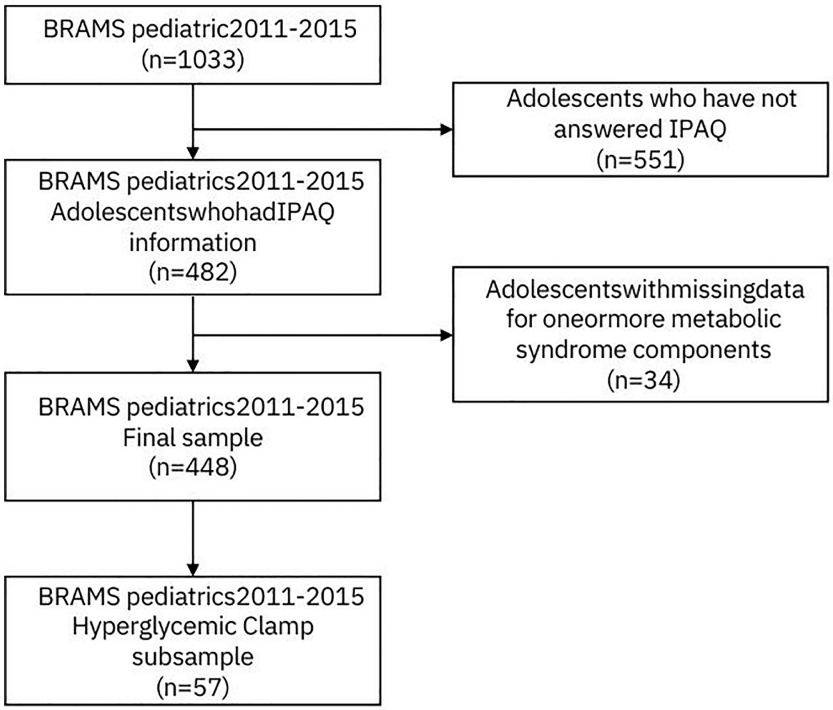

FIGURE 1
Flowchart on inclusion and exclusion criteria, BRAMS pediatrics, 2011-2015. IPAQ, International Physical Activity Questionnaire.

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TABLE 1 Sociodemographic and lifestyle characteristics of the total sample, and across metabolic syndrome status, BRAMS pediatrics, 2011-2015.

Characteristics	Total sample (n=448) N (%)/median (min – max)	Metabolic syndrome		P value
		No (n=408) N (%)/median (min – max)	Yes (n=40) N (%)/median (min – max)	
Sex				0.028
Female	199 (44)	233 (57)	16 (40)	
Male	249 (56)	175 (43)	24 (60)	
Age (years)	14 (10 – 19)	14 (10 – 19)	16 (10 – 19)	0.022
Puberal status				0.460
Prepuberal	31 (7)	27 (7)	4 (11)	
Puberal	310 (69)	285 (70)	25 (63)	
Post-puberal	106 (24)	95 (23)	11 (26)	
Nutritional status*				<0.001
Underweight	3 (1)	3 (1)	0 (0)	
Normal weight	165 (37)	165 (40)	0 (0)	
Overweight	108 (24)	107 (26)	1 (3)	
Obesity	172 (38)	133 (33)	39 (97)	
Smoking status				0.008
≥ 1 cigarette per month	2 (1)	0 (0)	2 (5)	
< 1 cigarette per month	446 (99)	408 (100)	38 (95)	
Alcohol use				0.265
≥ 1 dose per month	41 (9)	39 (10)	2 (5)	
< 1 dose per month	407 (91)	369 (90)	38 (95)	
Sleep				0.268
Sufficient (> 8 hours/day)	288 (65)	265 (65)	23 (59)	
Insufficient (< 8 hours/day)	157 (35)	141 (35)	16 (41)	
Medicine use				0.001
No	433 (98)	399 (99)	34 (87)	
Yes	10 (2)	5 (1)	5 (13)	
Moderate-to-high level physical activity (min/day)	24 (0 – 509)	24 (0 – 508)	36 (0 – 411)	0.350
Time spent sitting (hours/day)	7.0 (0.1 – 18.0)	7.0 (0.1 – 18.0)	8.1 (2.3 – 18)	0.070

Continuous variables are presented as median (min-max), and categorical variables are presented in absolute (relative) frequency. To compare means between groups, the Mann-Whitney test was applied, and, to compare frequencies, the chi-squared test was used, or *Fisher's exact test. P values<0.05 were considered statistically significant.

between metabolic syndrome components in the total sample). Adolescents with metabolic syndrome were older, had worse anthropometric parameters, higher systolic and diastolic blood pressure, higher plasma cholesterol, triglyceride, uric acid, gamma-GT, ALT, insulin, and HOMA-IR, and lower HDL-c levels than those who did not (Tables 1, 2). Additionally, in the subsample that undertook the hyperglycemic clamp protocol, adolescents with metabolic syndrome had a lower glucose infusion rate, ISI, and DI than those without metabolic syndrome (Table 3).

The odds for metabolic syndrome were higher among adolescents who spent more than 8 hours per day sitting, but not in those who spent more than 60 minutes a day of moderate-to-high PA (Table 4).

Adolescents who spent more time sitting had higher BMI, waist circumference, sagittal abdominal diameter, neck circumference, percentage body fat, plasma LDL-c, and triglycerides as well as lower HDL-c, while none of these correlations were found for time spent in moderate-to-high PA (Table 5). Among the investigated parameters from the hyperglycemic clamp protocol, ISI had a moderate and

TABLE 2 Anthropometric parameters, biochemical indicators, and blood pressure of the total sample, and across metabolic syndrome status, BRAMS pediatrics, 2011-2015.

Characteristics	Total sample (n=448) Median (min-max)	Metabolic syndrome		P value
		No (n=410) Median (min-max)	Yes (n=38) Median (min-max)	
Anthropometry and body composition				
BMI (z-score)	1.5 (-3.0 – 4.5)	1.4 (-3 - 4)	3 (1.7 - 4.5)	<0.001
Waist circumference (cm)	83 (49 – 139)	80 (49 - 136)	104 (86 - 139)	<0.001
Waist-to-hip ratio	0.85 (0.52 – 0.68)	0.8 (0.5 - 1.9)	0.9 (0.8 - 1.1)	<0.001
Sagittal abdominal diameter (cm)	17.5 (10.5 – 33.9)	17 (11 - 29)	23 (17 - 34)	<0.001
Neck circumference (cm)	33.5 (25.5 – 46.0)	33 (26 - 45)	39 (32 - 46)	<0.001
Body fat (%)	28.5 (4.9 – 67.8)	28 (5 - 68)	37 (19 - 49)	<0.001
Biochemical indicators				
Total cholesterol (mg/dL)	157 (67 – 286)	156 (90 - 286)	169 (67 - 234)	0.001
HDL-c (mg/dL)	46 (24 – 101)	47 (24 - 101)	36 (27 - 56)	<0.001
LDL-c (mg/dL)	92 (26 – 223)	91 (37 - 223)	95 (26 - 168)	0.054
Triglycerides (mg/dL)	73 (12 – 358)	71 (12 - 233)	156 (45 - 358)	<0.001
Uric acid (mg/dL)	4.7 (0.9 – 10.0)	4.6 (0.9 - 10)	6.2 (3.8 - 9.1)	<0.001
Gamma-GT (U/L)	17 (4 – 131)	17 (4 - 131)	22 (10 - 50)	<0.001
AST (U/L)	20 (9 – 61)	20 (9 - 61)	20 (15 - 34)	0.644
ALT (U/L)	15 (5 – 151)	15 (5 - 151)	19 (9 - 68)	<0.001
HbA1c (%)	5.4 (3.4 – 6.5)	5.4 (3.4 - 6.5)	5.4 (4 - 6.1)	0.318
Glucose (mg/dl)	81 (46 – 110)	81 (46 - 110)	83 (56 - 102)	0.151
Insulin (mU/L)	12.3 (1.4 – 64.7)	12 (1 - 65)	23 (3 - 57)	<0.001
HOMA-IR	2.4 (0.3 – 14.2)	2.2 (0.3 - 12.6)	4.3 (0.6 - 14.2)	<0.001
Blood pressure				
Systolic (mmHg)	110 (75 – 170)	110 (75 - 150)	124 (90 - 170)	<0.001
Diastolic (mmHg)	70 (50 – 110)	70 (50 - 100)	80 (50 - 110)	<0.001

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMI, Body mass index; Gamma-GT, gamma-glutamil transferase; HDL-c, high density lipoprotein cholesterol; HOMA-IR, homeostasis assessment model for insulin resistance; LDL-c, Low density lipoprotein cholesterol. Continuous variables are presented in median (min –max). Means comparison were conducted using the Mann-Whitney's test. P values <0.05 were considered statistically significant.

positive correlation with moderate-to-high PA, in minutes per day, as shown in Figure 2. For the subsample that participated in the hyperglycemic clamp protocol, adolescents who had more moderate-to-high PA daily had lower BMI (rho=-0.31; p=0.031), higher plasma HDL-c (rho=0.35; p=0.016), lower plasma triglyceride levels (rho=-0.32; p=0.027), and lower plasma insulin levels (rho=-0.30; p=0.038), whereas the time spent sitting had no statistically significant correlation with any of the metabolic parameters.

4 Discussion

The present study showed that adolescents who spent more time sitting had higher odds for MetS, higher BMI, waist circumference, sagittal abdominal diameter, neck circumference, percentage of body fat, plasma LDL-c, and triglycerides, as well as lower HDL-c. For the subsample from the hyperglycemic clamp protocol, on the other hand, those who spent a greater amount of time on moderate-to-high-level PA had higher insulin sensitivity, as measured by the ISI.

The results regarding the relationship between sitting time and the odds of MetS and its components are controversial. Bae et al., for instance, found in a representative sample of Korean adolescents (12y – 18y), that for each additional hour in daily sitting time, the odds of having at least one MetS component increased by 2% (31). Similarly, Sisson et al. found that daily sitting time was correlated with HOMA-IR in a representative sample of adolescents in the United States (32). Yin and colleagues, on the other hand, besides

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TABLE 3 Characterization of the hyperglycemic clamp subsample, and across metabolic syndrome status, BRAMS pediatrics, 2011-2015.

Characteristics	Total subsample (n=57) N (%)/median (min – max)	Metabolic syndrome		P value
		Total subsample (n=57) N (%)/median (min – max)	Yes (n=10) N (%)/median (min – max)	
Sex				0.730
Female	28 (49)	24 (51)	4 (40)	
Male	29 (51)	23 (49)	6 (60)	
Age (years)	14 (10 – 18)	14 (10 – 18)	14.5 (11 – 18)	0.505
Puberal status				0.112
Prepuberal	1 (2)	0 (0)	1 (10)	
Puberal	30 (52)	24 (51)	6 (60)	
Post-puberal	26 (46)	23 (49)	3 (30)	
Nutritional status*				0.036
Underweight	7 (12)	7 (15)	0 (0)	
Normal weight	14 (25)	14 (30)	0 (0)	
Overweight	36 (63)	26 (55)	10 (100)	
Obesity				
Alcohol use				0.574
≥ 1 dose per month	5 (9)	5 (11)	0 (0)	
< 1 dose per month	52 (91)	42 (89)	10 (100)	
Sleep				0.041
Sufficient (> 8 hours/day)	29 (51)	27 (57)	2 (20)	
Insufficient (< 8 hours/day)	28 (49)	20 (43)	8 (80)	
Medicine use				1.000
No	53 (96)	43 (96)	10 (100)	
Yes	2 (4)	2 (4)	0 (0)	
Moderate-to-high level physical activity (min/day)	26 (0 – 304)	29 (0 – 304)	6 (0 – 61)	0.051
Time spent sitting (hours/day)	8 (0 – 16)	8 (0 – 16)	9 (5 – 14)	0.204
Glucose infusion rate (mg)	7 (2 – 18)	7 (3 – 18)	5 (2 – 12)	0.019
Insulin sensitivity index	0.05 (0.01 – 0.19)	0.05 (0.01 – 0.19)	0.02 (0.01 – 0.11)	0.011
Disposition index	515 (42 – 2298)	570 (42 – 2298)	255 (54 – 934)	0.034

Continuous variables are presented as median (min-max), and categorical variables are presented in absolute (relative) frequency. To compare means between groups, the Mann-Whitney test was applied, and, to compare frequencies, the Fisher's exact test was used. P values<0.05 were considered statistically significant.

reporting a relation between sitting time and higher odds for abdominal obesity in a school-based sample of Chinese children and adolescents (6y – 14y), found no association between sitting time and the odds for MetS (33). Similarly, Oliveira and colleagues found that sitting time was not associated with obesity indicators or blood pressure in a Brazilian sample of 6264 adolescents (14y – 19y) (34).

A plausible theory for these controversial results lies in the different activities that compose the amount of sitting time in each study. Sitting time can be subdivided into screen time (TV, computer, and video game), educational activities (homework, classrooms, reading), and others (12). Of these subcategories, strong evidence points to screen time as an important risk factor for MetS in adolescents (14), whereas there is no evidence of harm related to other kinds of sitting activities.

Evidences are, on the other hand, concordant about the effect of moderate-to-high level PA on metabolic health (11–13). The results presented here are in accordance with the work published by Lee

TABLE 4 Odds ratio for metabolic sydrome across physical activity and sitting categories in adolescents (n=448), BRAMS pediatrics, 2011-2015.

PA and sitting categories	Metabolic syndrome	
	OR (IC 95%)	Adjusted OR (IC 95%)
≥ 60 min/day of moderate-to-high PA (n=109)	1,20 (0,58 – 2,49)	0,98 (0,42 – 2,26)
≥ 8hours/day sitting (n=191)	1,93 (1,01 – 3,73)	2,11 (1,02 – 4,38)

PA, Physical activity. Odds ratio for metabolic syndrome estimated from a multiple logistic regression, ajdusted for age (years), sex (dichotomous), smoking status (yes/no), alcohol intake (yes/no), puberal status, medicine use (yes/no), and sufficient sleep (yes/no). Time spent sitting and time spent on moderate to high intensity physical activity were treated as confounding factors of each other's exposure. Odds ratio with 95% confidence intervals that do not contain the number 1 were considered statistically significant.

et al., who used the hyperglycemic clamp protocol to check for improvements in insulin sensitivity after aerobic and resistance exercise interventions in 43 adolescent boys (12y 0 18y) and showed that increasing moderate-to-high level PA is effective in reducing abdominal adiposity, hepatic lipid accumulation, and, therefore, insulin sensitivity (15). Similarly, in the present study, adolescents that referred more time in moderate-to-high level PA had lower BMI, lower plasma triglyceride levels, and higher insulin sensitivity.

There is biological plausibility for these results. Among other beneficial effects, moderate-to-high levels of PA increase energy

TABLE 5 Correlation between time spent on physical activity and sitting, and metabolic parameters in adolescents, BRAMS pediatrics, 2011-2015.

Metabolic parameters	Sitting (hours/day)	Moderate-to-hight level Physical activity (min/day)
	Rho	Rho
Anthropometry and body composition		
BMI (z-score)	0,15*	-0,04
Waist circumference (cm)	0,16*	-0,01
Waist-to-hip ratio	0,09	-0,03
Sagittal abdominal diameter (cm)	0,12*	-0,03
Neck circumference (cm)	0,11*	|<0,01|
Body fat (%)	0,13*	-0,07
Biochemical indicators		
Total cholesterol (mg/dL)	0,07	|<0,01|
HDL-c (mg/dL)	-0,10*	0,03
LDL-c (mg/dL)	0,12*	-0,02
Triglycerides (mg/dL)	0,10*	0.02
Uric acid (mg/dL)	0,06	0,03
Gamma-GT (U/L)	0,05	-0,09
AST (U/L)	-0,06	0,03
ALT (U/L)	0,01	-0,06
HbA1c (%)	-0,01	0,05
Glucose (mg/dl)	0,09	0,18*
Insulin (mU/L)	0,06	-0,05
HOMA-IR	0,06	-0,01
Blood pressure		
Sistolic (mmHg)	0,05	0,01
Diastolic (mmHg)	0,03	-0,02

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMI, Body mass index; Gamma-GT, gamma-glutamil transferase; HDL-c, high density lipoprotein cholesterol; HOMA-IR, homeostasis assessment model for insulin resistance; LDL-c, Low density lipoprotein cholesterol. Correlation was estimated by Spearman's coefficient, adjusted for age (years), sex (dichotomous), smoking status (yes/no), alcohol intake (yes/no), puberal status, medicine use (yes/no), and sleep (hours/night). Time spent sitting and time spent on moderate to high intensity physical activity were treated as confounding factors of each other's exposure. *P values <0.05 were considered statistically significant.

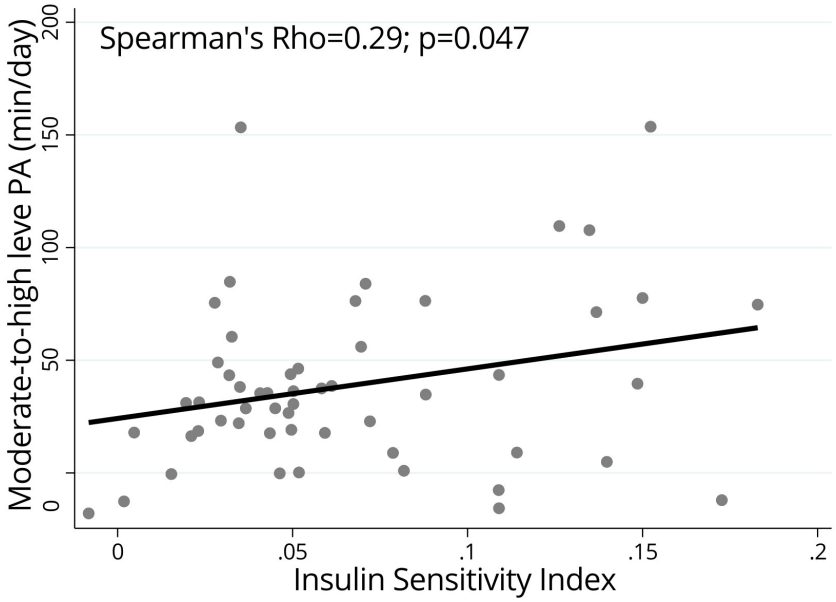

FIGURE 2
Correlation between the hyperglycemic clamp Insulin Sensitivity Index and time spent in moderate-to-high level physical activity (min/day) fitted values in adolescents (n=57), BRAMS pediatric, 2011-2015. PA, physical activity; Rho - Spearman's correlation coefficient adjusted for age, sex, smoking status, alcohol intake, puberal status, medicine use, sleep, and time spent sitting.

expenditure by triggering fatty acid and carbohydrate uptake and oxidation in skeletal muscles, as well as by increasing mitochondrial biogenesis (35). In addition, regular physical exercise decreases systemic low-grade inflammation and modulates the gut microbiome favoring lipid and glucose metabolism, short-chain fatty acids uptake, and secretion of gut hormones with insulin sensitizing effects (36). Ultimately, PA improves insulin sensitivity, as body adiposity, inflammation and disruptive glucose metabolism are critical nodes of insulin resistance pathophysiology (37).

The apparent inconsistency between the results found in total sample compared to that found in the subsample that participated in the hyperglycemic clamp protocol are, in fact, mostly due to the higher sensitivity of hyperglycemic clamp protocol to capture insulin resistance and beta-cell function compared to fasting plasma insulin and glucose levels. Moreover, effects of sitting time were not detected in the aforementioned subsample, and this was probably due to small sample size and, accordingly, lower statistical power.

Some limitations of the present study must be acknowledged. First, even though IPAQ-short form has been previously validated for the Brazilian population (20), self-reported physical activity may be subject to memory bias. Considering that the hyperglycemic clamp protocol is a direct measurement of insulin sensitivity and secretion, an objective measurement of physical activity and sedentary behavior, such as information collected with accelerometers, would improve the precision of the correlation estimation between the two variables. Another limitation is related to the cross-sectional design of the present study, which precludes causal inference and raises concerns about possible reverse causation effects that confuse the results. Reverse causation is a common issue in cross-sectional studies with PA because, on one hand, individuals with overweight, obesity, or metabolic disorders are more likely to have just initiated regular exercise, underestimating the beneficial effects of PA, and, on the other hand, individuals with more serious health issues may be more likely to become inactive, overestimating the protective effects of PA (38).

The present study had several strengths. First, the detailed assessment of metabolic parameters in a large sample of adolescents is rarely found in the literature and allows further investigations on the association of lifestyle and sensitive markers of metabolic disorders, which was extended to a subsample of individuals who participated in the hyperglycemic clamp protocol, a gold standard for insulin secretion evaluation, and a direct measurement of insulin sensitivity (28). Statistical correction for pubertal status and sleep was an important asset, as these factors are well-known confounders, as shown by previous studies with the BRAMS-P dataset using the hyperglycemic clamp protocol (30, 39). Another advantage of the present study was to use the time spent sitting and time spent on moderate-to high-intensity physical activity as confounding factors of each other's exposure, which favors the interpretation of the results.

In conclusion, independently of the time invested in moderate-to-high-level PA daily, the time spent sitting must be restricted in favor of adolescents' metabolic health. While our study point to an increase in MetS odds in adolescents that spend more than 8-hours sitting, further studies are needed to investigate the optimum recommendations for sitting and resting time in children and adolescents, standardizing this cut-off point across countries and investigating if the type of activity carried out during this sitting time (e.g.: studying, reading, watching TV) have different impacts in human health. Efforts to fight sedentary behavior in children and adolescents are urgent, especially considering that in 2018, 37% of adolescents globally were sedentary (more than three hours of sitting daily outside school) (40), and this prevalence has rapidly increased, according to recent studies (41), caused by the COVID-

19 pandemic. In addition, the World Health Organization recommendations on regular PA are reinforced here to improve insulin sensitivity not only in adolescents with obesity or metabolic disorders but also to prevent adverse metabolic outcomes in normal-weight adolescents (42).

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 Committee for Research Ethics of the School of Medical Sciences of UNICAMP (protocol n. 900/2010, CAAE: 0696.0.146.146-10). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

TS, MN and BG contributed to the conception and design of the study. MZ, MA, AR, AV, and BG contributed with data collection. TS and MN conducted statistical analysis and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1191935/full#supplementary-material

References

1. Norris SA, Frongillo EA, Black MM, Dong Y, Fall C, Lampl M, et al. Nutrition in adolescent growth and development. *Lancet* (2022) 399(10320):172–84. doi: 10.1016/S0140-6736(21)01590-7

2. UNICEF. *The state of the world's children 2019. children, food and nutrition: growing well in a changing world*. New York, Ny (2019).

3. Jebeile H, Kelly AS, O'Malley G, Baur LA. Obesity in children and adolescents: epidemiology, causes, assessment, and management. *Lancet Diabetes Endocrinol* (2022) 10(5):351–65. doi: 10.1016/S2213-8587(22)00047-X

4. Mannan M, Mamun A, Doi S, Clavarino A. Prospective associations between depression and obesity for adolescent males and females- a systematic review and meta-analysis of longitudinal studies. *PloS One* (2016) 11(6):e0157240. doi: 10.1371/journal.pone.0157240

5. Vilmann LS, Thisted E, Baker JL, Holm JC. Development of obesity and polycystic ovary syndrome in adolescents. *Horm Res Paediatr* (2012) 78(5–6):269–78. doi: 10.1159/000345310

6. Brandão AP, Brandão AA, Berenson GS, Fuster V. Síndrome metabólica em crianças e adolescentes. *Arq Bras Cardiol* (2005) 85(2):79–81. doi: 10.1590/S0066-782X2005001500001

7. Horesh A, Tsur AM, Bardugo A, Twig G. Adolescent and childhood obesity and excess morbidity and mortality in young adulthood–a systematic review. *Curr Obes Rep* (2021) 10(3):301–10. doi: 10.1007/s13679-021-00439-9

8. Zimmet P, Alberti GB, Kaufman F, Tajima N, Silink M, Arslanian S, et al.. The IDF consensus definition of the metabolic syndrome in children and adolescents. *Pediatr Diabetes* (2007) 8(5):299–306. doi: 10.1111/j.1399-5448.2007.00271.x

9. Noubiap JJ, Nansseu JR, Lontchi-Yimagou E, Nkeck JR, Nyaga UF, Ngouo AT, et al. Global, regional, and country estimates of metabolic syndrome burden in children and adolescents in 2020: a systematic review and modelling analysis. *Lancet Child Adolesc Health* (2022) 6(3):158–70. doi: 10.1016/S2352-4642(21)00374-6

10. Fornari E, Maffeis C. Treatment of metabolic syndrome in children. *Front Endocrinol* (2019) 10. doi: 10.3389/fendo.2019.00702

11. Skrede T, Steene-Johannessen J, Anderssen SA, Resaland GK, Ekelund U. The prospective association between objectively measured sedentary time, moderate-to-vigorous physical activity and cardiometabolic risk factors in youth: a systematic review and meta-analysis. *Obes Rev* (2019) 20(1):55–74. doi: 10.1111/obr.12758

12. Chaput JP, Willumsen J, Bull F, Chou R, Ekelund U, Firth J, et al. WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5–17 years: summary of the evidence. *Int J Behav Nutr Phys Activity* (2020) 17. doi: 10.1186/s12966-020-01037-z

13. Leister KR, Cilhoroz BT, Rosenberg J, Brown EC, Kim JY. Metabolic syndrome: operational definitions and aerobic and resistance training benefits on physical and metabolic health in children and adolescents. *Diabetes Metab Syndrome: Clin Res Rev* (2022) 16(6):102530. doi: 10.1016/j.dsx.2022.102530

14. Wu J, Zhang H, Yang L, Shao J, Chen D, Cui N, et al. Sedentary time and the risk of metabolic syndrome: a systematic review and dose–response meta-analysis. *Obes Rev* (2022) 23. doi: 10.1111/obr.13510

15. Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys. *Diabetes* (2012) 61(11):2787–95. doi: 10.2337/db12-0214

16. Kuczmarski RJNational Center for Health Statistics (U.S.) and National Health and Nutrition Examination Survey (U.S.). *2000 CDC growth charts for the united States: methods and development*. Dept. of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics, USA (2002). p. 190.

17. Tanner JM. *Growth at adolescence. 2nd ed*. Oxford: Blackwell Scientific Publications (1962).

18. da Silva C de C, Vasques ACJ, Zambon MP, Camilo DF, De Bernardi Rodrigues AM, Antonio MÂRGM, et al. Sagittal abdominal diameter resembles waist circumference as a surrogate marker of insulin resistance in adolescents–Brazilian metabolic syndrome study. *Pediatr Diabetes* (2018) 19(5):882–91. doi: 10.1111/pedi.12664

19. Falkner B, Daniels SR, Flynn JT, Gidding S, Green LA, Ingelfinger JR, et al. *National high blood pressure education program working group on high blood pressure in children and adolescents chair members national institutes of health staff staff ACKNOWLEDGMENTS*. Available at: http://publications.aap.org/pediatrics/article-pdf/114/Supplement_2/iv/1001963/iv.pdf.

20. Pinto Guedes D, Correa Lopes C, Elisabete Ribeiro Pinto Guedes J. Reprodutibilidade e validade do questionário internacional de atividade fisica em adolescentes ARTIGO ORIGINAL. *Rev Bras Med Esporte* (2005) 11:151–58. doi: 10.1590/S1517-86922005000200011

21. de Onis M. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* (2007) 85(09):660–7. doi: 10.2471/BLT.07.043497

22. da Silva C de C, Vasques ACJ, Zambon MP, Camilo DF, De Bernardi Rodrigues AM, Antonio MÂRGM, et al. Sagittal abdominal diameter resembles waist circumference as a surrogate marker of insulin resistance in adolescents-Brazilian metabolic syndrome study. *Pediatr Diabetes* (2018) 19(5):882–91. doi: 10.1111/pedi.12664

23. Stabe C, Vasques ACJ, Lima MMO, Tambascia MA, Pareja JC, Yamanaka A, et al. Neck circumference as a simple tool for identifying the metabolic syndrome and insulin resistance: results from the Brazilian metabolic syndrome study. *Clin Endocrinol (Oxf)* (2013) 78(6):874–81. doi: 10.1111/j.1365-2265.2012.04487.x

24. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol* (1986) 60(4):1327–32. doi: 10.1152/jappl.1986.60.4.1327

25. HC-UNICAMP. *UNICAMP clinical hospital laboratory protocols* (2023). Available at: https://hc.unicamp.br/especialidades/patologia-clinica/.

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

27. Alberti SG, Zimmet P, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The IDF consensus definition of the metabolic syndrome in children and adolescents. *Pediatr Diabetes* (2007) 8:299–306. doi: 10.1016/S0140-6736(07)60958-1

28. Arslanian SA. Clamp techniques in paediatrics: what have we learned? *Horm Res Paediatr* (2005) 64(Suppl. 3):16–24. doi: 10.1159/000089313

29. Camilo DF, Vasques ACJ, Hayashi K, Tura A, da Silva C de C, Zambon MP, et al. Adiposity and family history of type 2 diabetes in an admixed population of adolescents: associations with insulin sensitivity, beta-cell function, and hepatic insulin extraction in BRAMS study. *Diabetes Res Clin Pract* (2018) 137:72–82. doi: 10.1016/j.diabres.2017.12.013

30. de Cassia da Silva C, Zambon MP, Vasques ACJ, Camilo DF, de Góes Monteiro Antonio MÂR, Geloneze B. The threshold value for identifying insulin resistance (HOMA-IR) in an admixed adolescent population: a hyperglycemic clamp validated study. *Arch Endocrinol Metab* (2023) 67(1):119–25. doi: 10.20945/2359-3997000000533

31. Bae KN, Kim HR, Rhie YJ, Lee KH, Nam HK. Daily sitting time associated with the risk of metabolic syndrome in Korean adolescents. *J Pediatr Endocrinol Metab* (2018) 31(1):63–9. doi: 10.1515/jpem-2017-0110

32. Sisson SB, Shay CM, Camhi SM, Short KR, Whited T. Sitting and cardiometabolic risk factors in U. *S. adolescents. J Allied Health* (2013) 42(4):236–42.

33. Yin N, Yu X, Wang F, Yu Y, Wen J, Guo D, et al. Self-reported sedentary behavior and metabolic syndrome among children aged 6–14 years in Beijing, China. *Nutrients* (2022) 14(9):1869. doi: 10.3390/nu14091869

34. Oliveira LMFTd, Ritti-Dias RM, Farah BQ, Christofaro DGD, Barros MVGd, Diniz PRB, et al. Does the type of sedentary behaviors influence blood pressurein adolescents boys and girls? a cross-sectional study. *Cien Saude Colet* (2018) 23(8):2575–85. doi: 10.1590/1413-81232018238.23612016

35. Kränkel N, Bahls M, Van Craenenbroeck EM, Adams V, Serratosa L, Solberg EE, et al. Exercise training to reduce cardiovascular risk in patients with metabolic syndrome and type 2 diabetes mellitus: how does it work? *Eur J Prev Cardiol* (2019) 26(7):701–8. doi: 10.1177/2047487318805158

36. Zhang L, Liu Y, Sun Y, Zhang X. Combined physical exercise and diet: regulation of gut microbiota to prevent and treat of metabolic disease: a review. *Nutrients* (2022) 14(22):4774. doi: 10.3390/nu14224774

37. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* (2016) 126(1):12–22. doi: 10.1172/JCI77812

38. Lee DH, Rezende LFM, Ferrari G, Aune D, Keum N, Tabung FK, et al. Physical activity and all-cause and cause-specific mortality: assessing the impact of reverse causation and measurement error in two large prospective cohorts. *Eur J Epidemiol.* (2021) 36(3):275–85. doi: 10.1007/s10654-020-00707-3

39. De Bernardi Rodrigues AM, da Silva C de C, Vasques ACJ, Camilo DF, Barreiro F, Cassani RSL, et al. Association of sleep deprivation with reduction in insulin sensitivity as assessed by the hyperglycemic clamp technique in adolescents. *JAMA Pediatr* (2016) 170(5):487. doi: 10.1001/jamapediatrics.2015.4365

40. Pechtl SML, Kim LP, Jacobsen KH. Physical inactivity and sedentariness: languorous behavior among adolescents in 80 countries. *J Adolesc Health* (2022) 70 (6):950–60. doi: 10.1016/j.jadohealth.2021.12.017

41. Bozzola E, Barni S, Ficari A, Villani A. Physical activity in the COVID-19 era and its impact on adolescents' well-being. *Int J Environ Res Public Health* (2023) 20 (4):3275. doi: 10.20945/2359-3997000000533

42. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World health organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med* (2020) 54(24):1451–62. doi: 10.1136/bjsports-2020-102955

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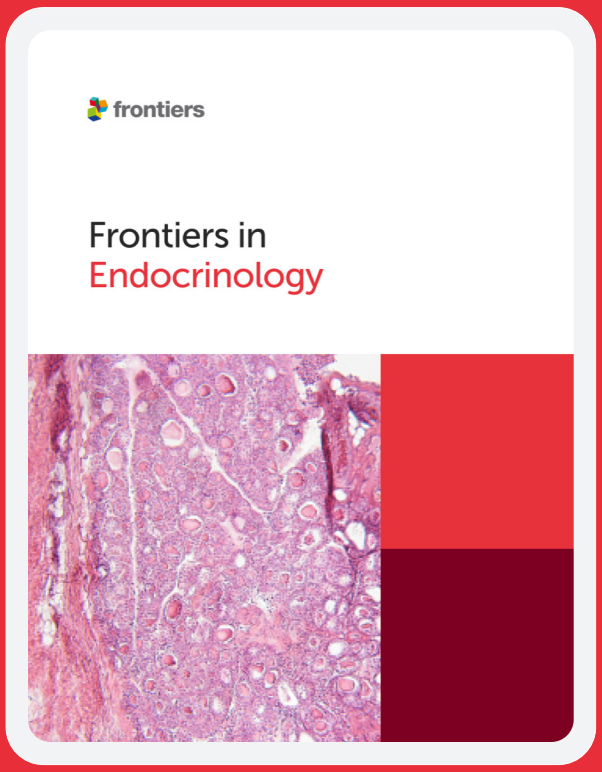

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