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Original Article

Metformin ameliorates insulin resistance, thyroid nodules and thyroid function

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Abstract: Objective: To evaluate the ameliorative impact of metformin on insulin resistance (IR), as well as thyroid nodules (TNs) and function in TN patients with IR. Methods: The clinical data of 128 TN patients with IR admitted to Yantai Laiyang Central Hospital from July 2018 to March 2020 were retrospectively analyzed and categorized into a control group (CNG, n = 64) and a study group (SG, n = 64). Patients in the CNG received standard lifestyle intervention, while those in the SG received standard lifestyle intervention in conjunction with metformin therapy for 1 year of course. Weight-related indicators, IR, thyroid function, TN diameter, and oxidative stress levels were compared between the two groups before and after treatment. Additionally, the safety of metformin was evaluated. Results: Before treatment, no significant differences were observed between the two groups in fasting plasma glucose (FPG), 2-h postprandial glucose (2hPG), glycated hemoglobin (HbA1c), fasting insulin (FINS), homeostatic model assessment of insulin resistance (HOMA-IR), systolic blood pressure (SBP), diastolic blood pressure (DBP), thyroid-stimulating hormone (TSH), malondialdehyde (MDA), TN diameter, and thyroid volume ($P > 0.05$). After treatment, significant statistical differences were observed in the aforementioned indicators between the two groups ($P < 0.05$). After 1 year of treatment, the SG exhibited lower levels of FPG, 2hPG, HbA1c, FINS, HOMA-IR, SBP, DBP, TSH, MDA, TN diameter, and thyroid volume, and showed higher levels of HOMA-β, superoxide dismutase, and glutathione peroxidase levels compared to before treatment ($P < 0.05$). The incidence of adverse reactions in the SG was significantly higher than that in the CNG ($P < 0.05$). Taking metformin and free thyroxine (FT4) were protective factors for TSH ($P < 0.05$). Conclusion: Metformin could significantly improve IR and oxidative stress levels, regulate TSH levels, and shrink TNs in TN patients with IR, with high safety. The administration of metformin and FT4 were identified as protective factors for positive prognosis.

Keywords: Metformin, insulin resistance, oxidative stress, thyroid nodules

Introduction

Insulin resistance (IR) is an independent risk factor in the development and progression of metabolic maladies in individuals with obesity, diabetes, and hypertension. Its main manifestation is hyperinsulinemia. Scientific investigations have illustrated that insulin, akin to thyrotropin, possesses the capacity to induce the proliferations of thyroid cells. Consequently, IR augments thyroid volume and elevates the incidence of thyroid nodules (TNs) [1-3]. Metformin, classified among the biguanides, influences blood glucose levels, orchestrates the modulation of blood lipid levels, and ameliorates IR by restraining excessive renal and hepatic glycogenogenesis, thereby enhancing insulin sensitivity, suppressing lipolysis, and delaying glucose uptake from the gastrointestinal tract [4-6]. A corpus of preceding research endeavors has elucidated [7-9] that metformin therapy for individuals afflicted with IR can reduce TN volume. Nevertheless, the mechanism of action remains unknown, but may be associated with the regulation of thyroid-stimulating hormone (TSH) levels and IR [10]. It merits mention that the incidence of co-morbidity of diabetes and thyroid dysfunction is notably

high. Furthermore, metformin treatment has been reported to reduce the incidence of thyroid diseases in diabetic individuals, and acts a protective factor for the onset of thyroid dysfunction in patients with diabetes [11]. Therefore, this study was conducted to scrutinize the efficacy of metformin in mitigating TNs among patients with IR and to analyze its impact on IR and TNs.

Materials and methods

Clinical data

The study was conducted in accordance with the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Yantai Laiyang Central Hospital. The clinical data of 128 TN patients with IR admitted to Yantai Laiyang Central Hospital from July 2018 to March 2020 were retrospectively analyzed, including 75 males and 53 females, aged 34-72 years, with an average age of (52.1±3.3) years.

Patients who met the following inclusion criteria were included: patients who met the diagnostic criteria of IR in the *World Health Organization classification criteria for glucose metabolic status (1999)* [12], with IR defined as homeostasis model assessment insulin resistance index (HOMA-IR) ≥ 2.5; patients who were diagnosed with TN by fine needle aspiration biopsy and color ultrasound, except for malignant TN and TN > 2 cm in diameter or with eggshell-like calcifications, with normal thyroid function and negative anti-thyroperoxidase antibodies [13]; patients who had not received lipid-lowering treatments or hypoglycemic drugs within 1 month prior to admission; patients who had no chronic microvascular complications.

Patients who met the following exclusion criteria were excluded from the study: those with ketoacidosis, diabetic hyperosmolar syndrome, other severe diabetic conditions, organ function loss, gastrointestinal disease, intractable hypertension, chronic heart failure, rheumatoid arthritis, or malignant tumors; those who received thyroid hormone therapy; those with thyroid volume > 40 mL; those who were allergic to the medication used in this study; those with poor treatment compliance; lactating and pregnant women.

Methodology

Control group (CNG): Patients in the CNG received the following standard lifestyle interventions. Dietary intervention: based on the standard body weight (kg) = height - 105 (cm), the total daily caloric requirements were evaluated according to the individual nutritional demands (20 to 30 kcal per kg body weight), and personalized nutritional prescription was formulated, wherein, the nutritional ratio of each meal was 15% protein, 25% fat, and 60% carbohydrate. The daily fiber intake should not be less than 40 g, and the intake of alcohol and sugary drinks should be limited. Exercise intervention: individualized exercise prescriptions were developed for patients based on the patient's age, sex, weight, and the actual situation. Exercise should be mainly aerobic or endurance exercises such as walking, jogging, tai chi, painting, and calligraphy. Patients were encouraged to adhere to daily exercise, record their body weight once a week, and observe the changes in weight. It was suggested to exercise 3 to 4 times a week for 30 to 60 min each time. Psychological intervention: if patients suffered from inferiority, anxiety, depression, and other psychological problems, they were provided with individualized psychological counseling. Health guidance: during the follow-up period, a health education lecture was held once a week to foster a positive attitude among the patients.

Study group (SG): Patients in the SG received standard lifestyle intervention and metformin. Metformin (Harbin Tongyitang Pharmaceutical Co., Ltd., H20060226; specification: 0.5 g) was administered with an initial dose of 850 mg/day (with dinner). The dose was increased to 1700 mg/day (with breakfast and dinner) 2 weeks later, depending on the tolerance, and maintained until the last enrolled subject completed the 1-year intervention. Changes in blood glucose were monitored during medication. The dose could be continued if the blood glucose was controlled within the normal range, or the dose could be reduced as appropriate if fasting plasma glucose (FPG) was below 3.6 mmol/L.

Outcome measurements

The primary outcome measurements included insulin-related indicators, TN indicators, and thyroid function. The secondary outcome mea-

sures included baseline data, weight-related indicators, oxidative stress response, and adverse reactions.

Baseline data: Baseline data including age, course of disease, systolic blood pressure (SBP), diastolic blood pressure (DBP), TN diameter, smoking, and complications (hyperlipidemia, hypertension, and bronchitis) were observed and compared between the two groups.

Weight-related indicators: The changes in body weight, body mass index (BMI), and waist circumference were observed and compared between the two groups before treatment and after 1 year of treatment.

Insulin-related indicators: Fasting insulin (FINS) levels were measured before treatment and after 1 year of treatment using a microplate reader (HBS-1096B, Nanjing DeTie Experimental Equipment Co., Ltd.), and IR was assessed using a steady-state model. IR score (HOMA-IR) = FINS × FPG/22.5, and basal beta cell function (HOMA-β) = 20 × FINS/(FPG-3.5).

TN indicators: TN diameter and volume were measured utilizing ultrasound examinations before treatment and after 1 year of treatment. Thyroid volume = thickness × width × length/6π.

Oxidative stress response: Fasting venous blood (3 mL) was collected from patients before treatment and after 1 year of treatment. After centrifugation, the serum was collected. Glutathione peroxidase (GSH-PX) and malondialdehyde (MDA) levels were determined utilizing enzyme-linked immunosorbent assay (ELISA) (kits purchased from Shanghai Jichun Industrial Co., Ltd., batch numbers 190114 and 191228). Superoxide dismutase (SOD) was determined utilizing pyrogallol method (kits purchased from Shanghai Yiji Industrial Co., Ltd., batch number 20191012).

Thyroid function: Fasting venous blood (3 mL) was collected from patients before treatment and after 1 year of treatment. After centrifugation, serum was collected, and TSH, free triiodothyronine (FT3), and free thyroxine (FT4) were determined utilizing ELISA.

Adverse reactions: The adverse reactions including hypoglycemia (blood glucose level < 3.9 mmol/L), headaches, gastrointestinal distention, and diarrhea were recorded.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 24.0 statistical software was employed for data analysis. The measurement data were described by mean ± standard deviation (mean ± SD). Independent and paired sample *t*-tests were conducted for comparisons between and within groups, respectively. Counting data were described by percentages and were examined using the chi-square (χ^2) test. Statistical graphs were plotted utilizing the Graphpad prism 7.0 statistical software. $P < 0.05$ was considered a statistically significant difference.

Results

Baseline data

There was no significant difference in baseline data between the two groups ($P > 0.05$) (**Table 1**).

Weight-related indicators

Before treatment, there was no statistical significance in body weight, BMI, and waist circumference between the two groups ($P > 0.05$). After metformin treatment, the body weight, BMI, and waist circumference of the SG were decreased compared with those before treatment ($P < 0.05$), and were significantly lower than those of the CNG ($P < 0.05$). In the CNG, there was no significant difference in body weight, BMI, and waist circumference before and after treatment ($P > 0.05$). The two groups showed statistically significant differences in body weight, BMI and waist circumference after treatment ($P < 0.05$) (**Table 2**).

Insulin-related indicators

Before treatment, there was no significant difference in FINS, HOMA-IR and HOMA-β levels between the two groups ($P > 0.05$). The two groups showed statistically significant differences in FINS, HOMA-IR and HOMA-β after treatment ($P < 0.05$). After metformin treatment, the SG exhibited significant decrease in FINS and HOMA-IR, and showed significant increase in HOMA-β as compared with those before treatment ($P < 0.05$). Furthermore, when compared to the CNG after treatment, these indicators showed statistically significant differences ($P < 0.05$). In the CNG, there was no sig-

Table 1. Comparison of general information (n)/(mean ± SD)

Group	Gender (M/F)	Age (years)	Duration of disease (years)	SBP (mmHg)	DBP (mmHg)	Thyroid nodule diameter (mm)	Smoking	Comorbidities
								Hyperlipidemia/ Hypertension/ Bronchiectasis
Control group (n = 64)	39/25	53.2±3.6	6.25±1.16	143.19±9.85	108.85±8.15	13.45±2.94	9	15/20/9
Study group (n = 64)	36/28	52.0±3.9	6.28±1.37	142.98±10.25	107.76±7.96	13.98±3.02	12	17/23/7
χ^2/t	0.290	1.809	0.134	0.118	0.765	1.006	0.513	0.486
P	0.590	0.073	0.894	0.906	0.445	0.316	0.474	0.784

Note: SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Metformin effectively improves levels of weight-related indicators in TN patients with IR (mean ± SD)

Indicator		Control group (n = 64)	Study group (n = 64)	*t*	*P*
Body weight (kg)	Before treatment	74.39±12.48	73.69±11.59	0.329	0.743
	After 1 year of treatment	72.31±10.04	60.85±11.02	6.150	< 0.001
t		1.0389	6.423		
P		0.301	< 0.001		
BMI (kg/m^2)	Before treatment	26.98±3.25	26.34±3.04	1.151	0.252
	After 1 year of treatment	26.03±3.03	24.46±3.42	2.749	0.007
t		1.710	3.287		
P		0.090	0.001		
Waist circumference (cm)	Before treatment	91.86±1.15	91.77±1.13	0.447	0.656
	After 1 year of treatment	90.13±9.89	86.03±9.77	2.359	0.020
t		1.390	4.669		
P		0.167	< 0.001		

Note: IR: insulin resistance; TN: thyroid nodule; BMI: body mass index.

nificant difference in FINS, HOMA-IR and HOMA-β levels before and after treatment ($P > 0.05$) (**Figure 1**).

Thyroid function

Before treatment, there was no significant difference in FT3, FT4 and TSH levels between the two groups ($P > 0.05$). The two groups exhibited statistically significant difference in TSH before and after treatment ($P < 0.05$). After metformin treatment, the SG showed decreased TSH level compared with that before treatment ($P < 0.05$), and TSH level in the SG was significantly lower than those in the CNG after treatment ($P < 0.05$). There was no statistically significant difference in FT3 and FT4 levels before and after treatment in both groups ($P > 0.05$) (**Figure 2**).

TN indices

Before treatment, the two groups showed no significant difference in TN diameter and thyroid volume ($P > 0.05$). Compared with before treatment, the two groups showed statistically significant difference in TN diameter and thyroid volume after treatment ($P < 0.05$). After metformin treatment, the SG exhibited decreased TN diameter and thyroid volume compared with those before treatment ($P < 0.05$), and TN diameter and thyroid volume in the SG were significantly lower than those in the CNG ($P < 0.05$). There was no significant difference in TN diameter and thyroid volume in the CNG before and after treatment ($P > 0.05$) (**Figure 3**).

Oxidative stress response

Before treatment, the two groups exhibited no statistically significant difference in SOD, GSH-PX, and MDA levels ($P > 0.05$). After metformin treatment, the SG exhibited increased levels of SOD and GSH-PX whereas and decreased level of MDA compared with those before treatment ($P < 0.05$), and when compared to the CNG

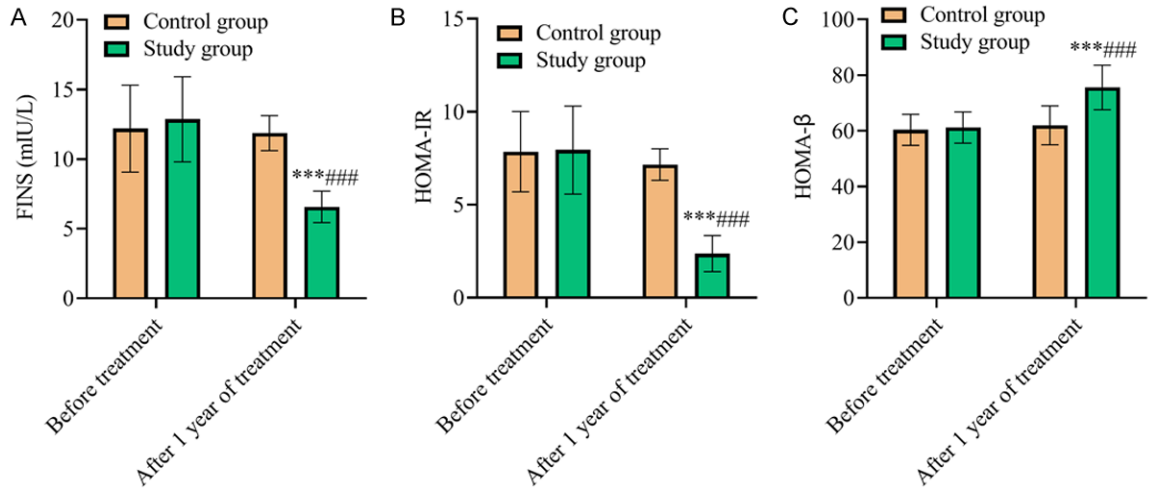

Figure 1. Metformin effectively improved IR in TN patients with IR. A and B: The FINS and HOMA-IR levels were decreased after treatment; C: The HOMA-β level was increased after treatment. Note: compared with before treatment, ***$P < 0.001$; compared with control group, ###$P < 0.001$. IR: insulin resistance; TN: thyroid nodule; FINS: fasting insulin; HOMA-IR: homeostatic model assessment of insulin resistance.

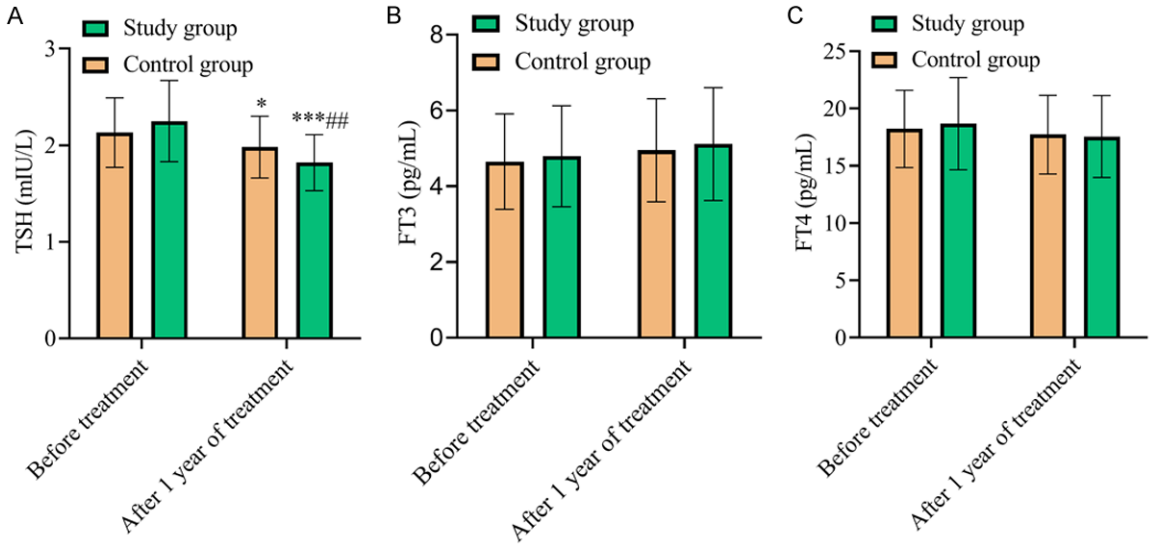

Figure 2. Effect of metformin on thyroid function in TN patients with IR. A: TSH level was significantly reduced after treatment; B and C: FT3 and FT4 exhibited no significant changes. Note: compared with before treatment, *$P < 0.05$, ***$P < 0.001$; compared with control group, ###$P < 0.001$. IR: insulin resistance; TN: thyroid nodule; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine.

after treatment, these indicators showed statistically significant differences ($P < 0.05$). In the CNG, there was no significant difference in SOD, GSH-PX and MDA levels before and after treatment ($P > 0.05$) (**Table 3**).

Adverse effects

The incidence of adverse reactions in the SG was 9.38%. Since patients in CNG were not treated with metformin, no adverse reactions occurred, with an adverse reaction rate of 0%. The incidence of adverse reactions in the SG was significantly higher than that in CNG ($P < 0.05$). Although metformin treatment led to some adverse reactions, they were all well tolerated. In addition, patients benefited largely in body functions from the metformin treatment. It is suggested that metformin had high safety

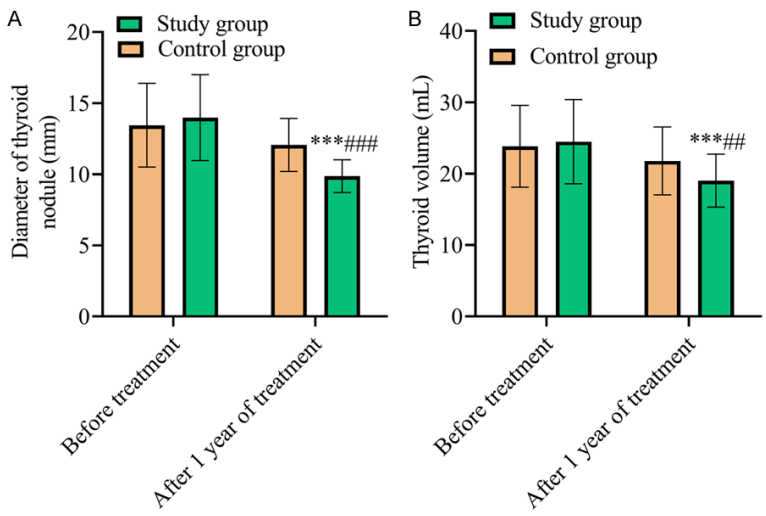

Figure 3. Effect of metformin on TN indicators in TN patients with IR. A: TN diameter was significantly reduced after treatment; B: Thyroid volume was significantly reduced after treatment. Note: compared with before treatment, ***$P < 0.001$; compared with control group, ##$P < 0.01$, ###$P < 0.001$. IR: insulin resistance; TN: thyroid nodule.

and only a few adverse reactions, and no adverse prognosis was observed (**Table 4**).

Analysis of influencing factors affecting prognosis of patients

Utilizing TSH 2.5 mU/L as the cut-off value, the patients were divided into a high TSH group (n = 51) and a low TSH group (n = 77). A multivariate logistic regression analysis was performed using TSH as the dependent variable, and age, sex, metformin usage, course of diabetes, SBP, DBP, TN diameter, smoking, complications, BMI, waist circumference, FINS, HOMA-IR, SOD, MDA, and GSH-PX as independent variables. The results showed that the use of metformin and FT4 were protective factors for TSH ($P < 0.05$) (**Table 5**).

Discussion

IR can trigger diminished glucose utilization and uptake, inducing excessive compensatory insulin secretion to sustain normoglycemia. Unfortunately, this chronic overcompensation further exacerbates pancreatic β-cells [14, 15]. Studies have elucidated the potentiated risks of TN formation and hypertension from IR and hyperinsulinemia [16, 17]. Islet cell dysfunction and IR manifest as hallmarks in patients with type II diabetes mellitus. Consequently, mitigating IR becomes crucial in regulating glucolipid metabolism and enhancing insulin sensitivity.

This research demonstrated that metformin lowered IR and oxidative stress in TN patients with IR. The possible mechanisms can be delineated as follows: (1) Metformin can facilitate anaerobic glycolysis, forestall glucose absorption, and mitigate chronic hyperglycemia-induced β-cell damage, thus restoring β-cell functionality [18]. (2) Metformin may potentiate the affinity and quantity of insulin receptors in peripheral tissues, activate insulin receptor tyrosine kinases, intensify the activity and gene expression of glucose transporter protein type-4, and augment glucose transporter. This, in turn, strengthens glycogen synthesis and glucose oxidation, thus improving β-cell insulin signaling and IR in peripheral tissues [19, 20]. (3) Metformin possesses the capacity to reduce liver gluconeogenesis while elevating glucose utilization and uptake by skeletal muscles and adipose tissues, thereby modulating glucose and lipid metabolism. (4) Patients with hyperinsulinemia and IR are often accompanied by heightened blood pressure, which is associated with increased kidney sodium reabsorption due to IR, heightened activity of sympathetic nervous system, and downregulation of insulin receptors. Metformin can ameliorate IR, thereby aiding blood pressure regulation [21, 22].

TSH enhances protein and nucleic acid synthesis in thyroid follicular epithelial cells, stimulates thyroid cell proliferation, and augments thyroid hormone synthesis and secretion, consequently resulting in TN volume increase. A randomized, double-blind, placebo-controlled clinical trial conducted by Dornelles Severo et al. [23] discerned the capacity of metformin to attenuate TSH levels. A mate-analysis by Wang et al. [24] reported that metformin significantly reduced serum TSH levels, but had no significant effect on serum FT3 and FT4 levels. In congruence with these findings, our study found decreased TSH levels, TN diameter, and

Table 3. Comparison of oxidative stress responses (mean ± SD)

Indicator		Control group (n = 64)	Study group (n = 64)	*t*	*P*
SOD (μ/mL)	Before treatment	196.64±20.15	198.85±19.37	0.633	0.528
	After 1 year of treatment	202.67±23.37	268.89±27.71	14.614	< 0.001
t		1.563	16.573		
P		0.121	< 0.001		
MDA (noml/L)	Before treatment	16.28±2.38	16.38±3.02	0.208	0.836
	After 1 year of treatment	15.95±2.37	6.54±1.15	25.540	< 0.001
t		0.786	24.360		
P		0.433	< 0.001		
GSH-PX (μ/L)	Before treatment	45.19±4.28	46.62±5.08	1.722	0.088
	After 1 year of treatment	46.29±6.78	73.32±8.06	20.531	< 0.001
t		1.098	22.420		
P		0.275	< 0.001		

Note: SOD: superoxide dismutase; MDA: malondialdehyde; GSH-PX: glutathione peroxidase.

Table 4. Comparison of adverse reactions n (%)

Group	Hypoglycemia	Headache	Gastrointestinal distention	Diarrhea	Nausea and vomiting	Total
Control group (n = 64)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Study group (n = 64)	0 (0.00)	1 (1.56)	2 (3.13)	1 (1.56)	2 (3.13)	6 (9.38)
χ^2						48.057
P						< 0.001

Table 5. Logistic regression analysis of factors affecting TSH level

Influencing factor	B	SE	P	OR	*95% CI*
Metformin	-0.309	0.154	0.025	0.819	0.622-0.948
FT4	-0.276	0.127	0.019	0.759	0.601-0.882
Constant	-4.309	3.622	0.188	0.015	

Note: TSH: thyroid-stimulating hormone; FT4: free thyroxine.

thyroid volume in the SG compared to before treatment and the CNG. It is indicated that metformin can promote TN volume reduction and decrease TSH levels. The mechanisms can be explicated thusly: (1) Regulation of leptin expression. Leptin, through JAK-2 signaling pathway, impacts the TSH levels via binding to the corresponding receptors in the hypothalamus and regulating the hypothalamic-pituitary-thyroid axis [25]. In contrast, metformin activates protein kinase, reduces fatty acid oxidation, contributes to catabolism, inhibits anabolic and pro-inflammatory pathways in the adipose tissue. As a result, it improves IR, reduces leptin levels, and inhibits TN growth. (2) Metformin impacts TSH receptor activity and heightens peripheral tissue sensitivity to TSH [26, 27]. (3) Moreover, metformin augments the count of TSH receptors or enhances their affinity of TSH to receptors, thereby diminishing TSH demand. Mi et al. [28] expounded upon metformin's substantial reduction of HOMA-IR in TN patients with IR, thus alleviating IR, diminishing TN size, and lowering serum TSH, FT3, FT4 levels. These findings are consistent with the results of this study, which further demonstrated the clinical efficacy of metformin in TN patients with IR.

In conclusion, metformin emerges as a potent agent in ameliorating IR and oxidative stress levels, modulating TSH levels, and diminishing TNs in patients with TN complicated with IR, with high drug safety. Nevertheless, it's imperative to underscore the limitations of this study, primarily its small sample size, limited range of observational indices, and a short follow-up duration. These limitations may have the potential to introduce bias into the study outcomes. Therefore, the specific mechanism and effect of metformin need to be further studied. No

adverse prognosis was found in the two groups after 1 year of treatment, but due to the retrospective nature of this analysis, no follow-up was conducted on the long-term prognosis of patients, constituting a critical study limitation. Subsequent investigations should address this deficiency by extending the observation period and scrutinizing the factors influencing patient prognoses.

Disclosure of conflict of interest

None.

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References

[1] Khan RMM, Chua ZJY, Tan JC, Yang Y, Liao Z and Zhao Y. From pre-diabetes to diabetes: diagnosis, treatments and translational research. Medicina (Kaunas) 2019; 55: 546.

[2] Huang JX, Liao YF and Li YM. Clinical features and microvascular complications risk factors of early-onset type 2 diabetes mellitus. Curr Med Sci 2019; 39: 754-758.

[3] Zhang J, Jin J, Liu J, He Y, Zhang P, Ye W, Zhu W and Li M. A study of the correlation of insulin resistance and leptin with inflammatory factors and vascular endothelial injury in T2DM patients with CHD. Exp Ther Med 2018; 16: 265-269.

[4] Richter B, Hemmingsen B, Metzendorf MI and Takwoingi Y. Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. Cochrane Database Syst Rev 2018; 10: CD012661.

[5] Grillo CA, Woodruff JL, Macht VA and Reagan LP. Insulin resistance and hippocampal dysfunction: disentangling peripheral and brain causes from consequences. Exp Neurol 2019; 318: 71-77.

[6] Rad SK, Arya A, Karimian H, Madhavan P, Rizwan F, Koshy S and Prabhu G. Mechanism involved in insulin resistance via accumulation of β-amyloid and neurofibrillary tangles: link between type 2 diabetes and Alzheimer's disease. Drug Des Devel Ther 2018; 12: 3999-4021.

[7] Bhansali S, Bhansali A, Dutta P, Walia R and Dhawan V. Metformin upregulates mitophagy in patients with T2DM: a randomized placebo-controlled study. J Cell Mol Med 2020; 24: 2832-2846.

[8] Lentferink YE, Knibbe CAJ and van der Vorst MMJ. Efficacy of metformin treatment with respect to weight reduction in children and adults with obesity: a systematic review. Drugs 2018; 78: 1887-1901.

[9] Madsen KS, Chi Y, Metzendorf MI, Richter B and Hemmingsen B. Metformin for prevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus. Cochrane Database Syst Rev 2019; 12: CD008558.

[10] Derosa G, D'Angelo A, Romano D and Maffioli P. Effects of metformin extended release compared to immediate release formula on glycemic control and glycemic variability in patients with type 2 diabetes. Drug Des Devel Ther 2017; 11: 1481-1488.

[11] Zhu MR and Xu Jin. Analysis of risk factors for co-morbidity of type 2 diabetes mellitus and thyroid dysfunction. Shandong Med J 2021; 61: 63-66.

[12] World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; 1999.

[13] Chinese Medical Association, Journal of the Chinese Medical Association and Chinese Society of General Practice. Guidelines for the primary care of type 2 diabetes mellitus (Practice Edition-2019). Chin J Gen Pract 2019; 18: 810-818.

[14] Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, Craft S, Gandy S, Buettner C, Stoeckel LE, Holtzman DM and Nathan DM. Brain insulin resistance in type 2 diabetes and alzheimer disease: concepts and conundrums. Nat Rev Neurol 2018; 14: 168-181.

[15] Abdul-Ghani MA, Jayyousi A, DeFronzo RA, Asaad N and Al-Suwaidi J. Insulin resistance the link between T2DM and CVD: basic mechanisms and clinical implications. Curr Vasc Pharmacol 2019; 17: 153-163.

[16] Akash MSH, Rehman K and Liaqat A. Tumor necrosis factor-alpha: role in development of insulin resistance and pathogenesis of type 2 diabetes mellitus. J Cell Biochem 2018; 119: 105-110.

[17] Sampath Kumar A, Maiya AG, Shastry BA, Vaishali K, Ravishankar N, Hazari A, Gundmi S and Jadhav R. Exercise and insulin resistance in type 2 diabetes mellitus: a systematic review and meta-analysis. Ann Phys Rehabil Med 2019; 62: 98-103.

[18] Inoue H, Tamaki Y, Kashihara Y, Muraki S, Kakara M, Hirota T and Ieiri I. Efficacy of DPP-4

inhibitors, GLP-1 analogues, and SGLT2 inhibitors as add-ons to metformin monotherapy in T2DM patients: a model-based meta-analysis. Br J Clin Pharmacol 2019; 85: 393-402.
[19] Tong X, Xu J, Lian F, Yu X, Zhao Y, Xu L, Zhang M, Zhao X, Shen J, Wu S, Pang X, Tian J, Zhang C, Zhou Q, Wang L, Pang B, Chen F, Peng Z, Wang J, Zhen Z, Fang C, Li M, Chen L and Zhao L. Structural alteration of gut microbiota during the amelioration of human type 2 diabetes with hyperlipidemia by metformin and a traditional Chinese herbal formula: a multicenter, randomized, open label clinical trial. mBio 2018; 9: e02392-17.
[20] Vallianou NG, Stratigou T and Tsagarakis S. Metformin and gut microbiota: their interactions and their impact on diabetes. Hormones (Athens) 2019; 18: 141-144.
[21] Jia Y, Lao Y, Zhu H, Li N and Leung SW. Is metformin still the most efficacious first-line oral hypoglycaemic drug in treating type 2 diabetes? A network meta-analysis of randomized controlled trials. Obes Rev 2019; 20: 1-12.
[22] Alharbi TJ, Tourkmani AM, Abdelhay O, Alkhashan HI, Al-Asmari AK, Bin Rsheed AM, Abuhaimed SN, Mohammed N, AlRasheed AN and AlHarbi NG. The association of metformin use with vitamin B12 deficiency and peripheral neuropathy in Saudi individuals with type 2 diabetes mellitus. PLoS One 2018; 13: e0204420.
[23] Dornelles Severo M, Stürmer Andrade T, Correa Junior V, Antonio Naujorks A, Gus M and Schaan BD. Metformin effect on TSH in subclinical hypothyroidism: randomized, double-blind, placebo-controlled clinical trial. Endocrine 2018; 59: 66-71.
[24] Wang J, Gao J, Fan Q, Li H and Di Y. The effect of metformin on thyroid-associated serum hormone levels and physiological indexes: a meta-analysis. Curr Pharm Des 2019; 25: 3257-3265.
[25] McCreight LJ, Bailey CJ and Pearson ER. Metformin and the gastrointestinal tract. Diabetologia 2016; 59: 426-435.
[26] Yerevanian A and Soukas AA. Metformin: mechanisms in human obesity and weight loss. Curr Obes Rep 2019; 8: 156-164.
[27] Piskovatska V, Stefanyshyn N, Storey KB, Vaiserman AM and Lushchak O. Metformin as a geroprotector: experimental and clinical evidence. Biogerontology 2019; 20: 33-48.
[28] Mi N and Yang LX. The application effect of metformin in the treatment of thyroid nodule patients with insulin resistance and its effect on serum TSH, FT3 and FT4. Guizhou Med J 2018; 42: 1052-1054.