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Insulin resistance and serum metabolomics improvement in subjects with impaired glucose tolerance treated with *Hibiscus esculentus* L.

Smanjenje insulinske rezistencije i poboljšanje metabolomičkih osobina seruma kod osoba sa smanjenom tolerancijom na glukozu, tretiranih biljkom *Hibiscus esculentus* L.

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Abstract

Background/Aim. Prediabetes (PD) refers to the condition in which the blood sugar level is higher than normal but has not reached the diagnostic criteria for diabetes mellitus (DM) yet. Impaired glucose tolerance (IGT) is a major prediabetic symptom since most patients with type 2 DM have progressed from the previous PD phase. The aim of the study was to observe the changes in serum metabolomics in patients with IGT treated with *Hibiscus esculentus* L. (*H. esculentus*) combined with the change of lifestyle. **Methods.** Sixty patients with IGT were divided into two groups. In one group, the subjects made a lifestyle change (LC group, simple diet control), and the other group of subjects made a lifestyle change combined with *H. esculentus* (LCH group) treatment with daily consumption of 20 g of dried *H. esculentus* fruit tea. The aim was to compare the blood glucose, homeostasis model assessment-estimated insulin resistance (HOMA-IR) index, and serum metabolomics after a 60-day clinical observation period. **Results.** There was no statistical significance in the glucose level between the two groups by the end of the observation period. The HOMA-IR index in the LCH group was lower than in the LC group (1.7 ± 1.1 vs. 2.4 ± 1.2, $p = 0.030$). Serum metabolomics revealed that the levels of d-galactose, d-glucose, turanose, and uric acid in the LCH group were significantly lower than those in the LC group (16.7 ± 3.9 mmol/L vs. 21.2 ±2.9 mmol/L, 101.5 ± 40.2 mmol/L vs. 132.9 ± 36.7 mmol/L, 1.8 ± 1.6 mmol/L vs. 3.76 ± 2.46 mmol/L, 44.56 ± 15.7 μmol/L vs. 67.8 ± 23.5 μmol /L, respectively). The levels of lactic acid and conjugated linoleic acid in the LCH group were significantly higher than those in the LC group (3.3 ± 0.5 mmol/L vs. 2.3 ± 0.8 mmol/L, 6.9 ± 6.1 mmol/L vs. 2.1 ± 1.2 mmol/L, respectively). **Conclusion.** *H. esculentus*, combined with a change of lifestyle, can reduce insulin resistance and the levels of multiple monosaccharides and blood uric acid in IGT patients. Regulation of the metabolism of lactic acid and conjugated linoleic acid may be the potential mechanism of how *H. esculentus* reduces insulin resistance.

Key words:
glucose intolerance; insulin resistance; life style; metabolomics; plants, medicinal; prediabetic state; serum.

Apstrakt

Uvod/Cilj. Predijabetes je stanje u kome je nivo šećera u krvi viši od normalnog, ali još nije dostigao dijagnostičke kriterijume za dijabetes melitus (DM). Smanjena tolerancija na glukozu (STG) je glavni predijabetesni simptom a kod većine obolelih od DM tipa 2 bolest se razvila kao posledica progresije predijabetesa. Cilj rada bio je da se odrede promene metabolomičkih osobina seruma kod osoba sa STG, koje su konzumirale čaj od biljke *Hibiskus esculentus* L. (*H. esculentus*), u kombinaciji sa promenom načina života. **Metode.** Ukupno 60 ispitanika sa STG podeljeno je na dve grupe. Jedna grupa ispitanika promenila je način života (jednostavna kontrola ishrane) – KI grupa, a u drugoj grupi bili su ispitanici koji su, pored toga što su promenili način života, konzumirali i čaj od biljke *H. esculentus* (na dnevnom nivou 20 grama sušenog voćnog čaja) – KIH grupa. Cilj je bio da se uporede vrednosti glukoze u krvi, insulina, indeksa rezistencije na insulin (*homeostasis model assessment of insulin resistance* – HOMA-IR) i metabolomičkih osobina seruma između te dve grupe ispitanika nakon 60 dana kliničke opservacije. **Rezultati.** Nije bilo statistički značajne razlike u nivou glukoze u krvi između dve grupe po završetku 60-dnevnog perioda opservacije. Indeks HOMA-IR u grupi KIH bio je niži nego u KI grupi (1,7 ± 1,1 vs. 2,4 ± 1,2, $p = 0,030$). Rezultati ispitivanja metabolomičkih osobina seruma otkrili su da su nivoi d-galaktoze, d-glukoze, turanoze i mokraćne kiseline u KIH grupi bili značajno niži u poređenju sa KI grupom (16,7 ± 3,9 mmol/L vs. 21,2 ± 2,9 mmol/L, 101,5 ± 40,2 mmol/L vs. 132,9 ± 36,7 mmol/L, 1,8 ± 1,6 mmol/L vs. 3,76 ± 2,46

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mmol/L, 44,56 ± 15,7 μmol/L vs. 67,8 ± 23,5 μmol/L, redom). Nivoi mlečne kiseline i konjugovane linoleične kiseline u KIH grupi bili su značajno viši nego kod ispitanika iz KI grupe (3,3 ± 0,5 mmol/L vs. 2,3 ± 0,8 mmol/L, 6,9 ± 6,1 mmol/L vs. 2,1 ± 1,2 mmol/L, redom). **Zaključak**. Unos biljke *H. esculentus* u kombinaciji sa promenama načina života može umanjiti insulinsku rezistenciju i smanjiti nivo većeg broja monosaharida i mokraćne kiseline u krvi osoba sa STG. Regulisanje metabolizma mlečne kiseline i konjugovane linoleične kiseline može biti potencijalni mehanizam koji objašnjava kako *H. esculentus* smanjuje insulinsku rezistenciju.

Ključne reči:
glukoza, netolerancija; insulin, rezistencija; način života; metabolomika; biljke, lekovite; predijabetesno stanje; serum.

Introduction

The analysis report of the global diabetes mellitus (DM) prevalence survey by The International Diabetes Federation (IDF) showed that the number of global diabetic patients reached 415 million in 2015, and by 2040, the global number of diabetic adults is expected to reach 629 million [1]. Prediabetes (PD) refers to the state in which the blood sugar level is higher than normal but has not reached the diagnostic criteria for DM yet. Most patients with type 2 DM (T2DM) progress from the PD condition. Impaired glucose tolerance (IGT) is a major prediabetic symptom, and IDF reported that the global prevalence of IGT in 2017 reached 374 million [1]; in 2013, the survey in China also showed that the prevalence of IGT was as high as 15.5% [2]. Surveys have shown that 70% of people with PD can progress to T2DM [3]. Therefore, how to prevent or delay the progress of PD into DM is the focus of DM prevention. Appropriate lifestyle interventions can delay the progression of PD to D [4–6]. Diet regulation is an important way of changing lifestyle in the population with PD [7]. *Hibiscus esculentus* L. (*H. esculentus)* is a herbaceous plant also known as *Abelmoschus esculentus* L. of the Malvaceae family. Recent studies have shown that *H. esculentus* can help lower blood sugar and blood lipids and may become an early dietary choice for DM [8]. It has also been found that *H. esculentus* and its extracts can regulate dipeptidyl peptidase-4 (DPP-4), protect the islet β cells, and improve insulin sensitivity [9, 10]. However, there are few clinical reports on *H. esculentus* regulating glucose metabolism in IGT patients, and the mechanism of *H. esculentus* regulating blood glucose still needs to be studied and clarified. Through comprehensive, real-time, and systematic contour analysis of metabolites, metabolomics research has made significant breakthroughs in recent years in screening DM biomarkers, analyzing metabolic pathways, and studying molecular pathways for pharmacological effects [11]. In this study, high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry analysis were applied to compare the changes in the glucose metabolism and serum metabolomics of IGT patients after simple lifestyle control or combined with *H. esculentus* tea consumption, aiming to explore the possible regulation pathways of blood glucose metabolism by *H. esculentus.*

Methods

General information

Inclusion criteria: a total of 60 IGT patients that were admitted to our hospital from May to November 2019 and met the inclusion criteria were enrolled and divided into the LC group (subjects made a lifestyle change) and LCH group (subjects made a lifestyle change combined with *H. esculentus*) according to the random number expression method, with 30 cases in each group; the diagnosis was in compliance with the 1999 glucose tolerance abnormality diagnostic criteria (fasting blood glucose < 7 mmol/L, blood glucose at 2-hr glucose load ≥ 7.8 mmol/L, < 11.1 mmol/L) by World Health Organization (WHO) [12]; the patients were all < 75 years old, including 32 males and 28 females; disease duration 0–12 months.

Exclusion criteria: patients with confirmed DM [type 1 DM (T1DM), T2DM, or special types of DM)], severe cardiac insufficiency (New York Heart Association cardiac function class Ⅲ or higher), myocardial infarction, chronic liver disease or severe liver dysfunction (glutamyl aminotransferase ≥ 3 times of the normal upper limit), malignant tumors, tuberculosis, recent administration of nephrotoxic drugs, or cognitive abnormalities and estimated glomerular filtration rate (eGFR) < 60 mL/min. Estimated GFR complies with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation:

The equation for females – $144 \times [\text{serum creatinine (sCR) } /0.7)]^{-0.329} \times 0.993^{\text{age}}$ (if sCR ≤ 0.7 mg/dL) or $144 \times (\text{sCR } /0.7)^{-1.209} \times 0.993^{\text{age}}$ (if sCR > 0.7 mg/dL).

The equation for males – $141 \times (\text{sCR } /0.9)^{-0.411} \times 0.993^{\text{age}}$ (if sCR ≤ 0.7 mg/dL) or $141 \times (\text{sCR } /0.9)^{-1.209} \times 0.993^{\text{age}}$ (if sCR > 0.7 mg/dL).

This study was conducted in accordance with the declaration of Helsinki, following the approval from the Ethics Committee of the Shanghai University of Traditional Chinese Medicine (Ethical Committee Approval number PTEC-R-2018-53-1, from October 25, 2018). Written informed consent was obtained from all participants.

There was no statistically significant difference in the basic information, such as age, gender, and course of the disease, between the two groups (Table 1, $p > 0.05$).

The following steps were used for preparing fruit tea of dried *H. esculentus*: fresh *H. esculentus* (cultivated and harvested by Nanjing Youhewo Agricultural Co., Ltd.) was removed from the pedicel, entirely cleaned, and then the middle part was cut with a diameter of 15–20 mm into 5-mm thin slices with sterilized stainless steel knives, followed by 240 min of 60 °C air drying with the speed of 1 m/s (the vacuum drying oven: Type zKn4025, Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd.). The quality inspection was performed by the Comprehen-

Table 1

Comparison of clinical data between groups

Parameter	LC	LCH	F/χ^2	*p*-value
Age (years)	41.4 ± 5.9	40.8 ± 5.4	0.331	0.668
Male/female (n)	14/16	18/12	1.071	0.301
Course of disease (months)	7.3 ± 1.9	7.3 ± 1.6	2.4	0.943
Fasting blood glucose (mmol/L)	5.5 ± 1.0	5.6 ± 0.9	0.226	0.877
2-hr postprandial blood glucose (mmol/L)	8.9 ± 1.4	8.61.5	1.092	0.293
Glycated hemoglobin (%)	6.9 ±1.8	6.9 ± 1.8	0.082	0.987
Fasting insulin (mmol/L)	10.4 ± 6.0	10.5 ± 5.8	0.032	0.962
2-hr postprandial insulin (mmol/L)	64.5 ± 22.3	65.0 ± 23.2	0.022	0.626
ALT (U/L)	24.3 ±10.9	24.470 ± 10.2	0.153	0.961
Creatinine (μmol/L)	63.8 ± 18.4	64.5 ± 17.9	0.075	0.876

ALT – alanine transaminase; LC – lifestyle change group; LCH – lifestyle change combined with *H. esculentus* treatment group; Results are given as mean ± standard deviation; F/χ^2 test is performed between the LC and LCH group.

sive Testing and Inspection Center of Gaochun Dist., Nanjing, which obtained the following results: no metal or non-metallic foreign matter remaining, moisture content 3.4%, and lead/hexachlorocyclohexane/dichlorodiphenyltrichloroethaneno detection. Twenty g of the material was then seal-packed in polyethylene bags sterilized with an ozone concentration of ≥ 20 mg x m^{-3} for 30 min [13].

Groups and treatments

After 2 weeks of diet and exercise education for all the study subjects to cultivate their self-monitoring blood glucose habits to prevent the occurrence of hypoglycemia events or other adverse events, the study subjects started a 60-day clinical observation. The LC group continued changing their lifestyle, while the LCH group was given 20 grams of *H. esculentus* dried fruit tea daily (divided into 3 servings, brewed with 200 mL of warm water, to be consumed after breakfast, lunch, and dinner with chewed pulp) in addition to the lifestyle change. During that period, the study subjects were required to self-monitor the finger-terminal blood glucose before meals and at 9 pm twice a week.

Observation indices

After 60 days of observation, the serum fasting and 2-hr postprandial glucose, insulin, glycated hemoglobin, and lipids were compared between the two groups. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) index (fasting blood glucose × fasting insulin level/22.5) was calculated and compared, together with comparing the differences in the serum metabolomics between the two groups.

Specimen collection and processing

All the study subjects were fasting for more than 10 hrs before the blood sampling from the medial cubital vein in the morning. Then, 1.5 mL of the blood was collected into one coagulation tube with inert separation gel, one ethylenediamine tetraacetic acid-anticoagulation tube, and one heparin-anticoagulation tube, respectively. After standing for 30 min, the blood was centrifuged (3,500 rpm/15 min), and the supernatant serum and plasma were collected and stored frozen at -70 °C. The enzymatic method was used to detect the blood glucose concentration, alanine transaminase (ALT), and sCR (Beckman Coulter Au Chemistry Systems, Suzhou, China); HPLC was used to detect the glycated hemoglobin (Arkray, Koka-shi, Japan); direct chemiluminescence was used to detect the serum insulin level (Simens Healthineers, Wuxi, China). Processing of metabolomics specimens: 400 μL of ice-cold methanol solution and 10 μL of internal standard (0.3 mg/mL, 2-chlorophenylalanine and undecanoic acid) were added to the 100 μL of the serum and mixed thoroughly, followed by 15 min centrifugation at 12, 000 rpm at 4 °C. Then, 200 μL of concentrated and dried supernatant was transferred into an injection vial; 30 μL of methoxypyridine solution (20 mg/mL) was added, shaken vigorously, and incubated at 37 °C for 1.5 hrs. Next, 30 μL of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), containing 1% chlorotrimethylsilane (TMCS) was added, incubated at 70 °C for 1 h, and then put to sit at room temperature. Reagents such as methanol were purchased from Sinopharm Group Co Ltd, Shanghai, China [14, 15].

Analysis of gas chromatography-mass spectrometry

Spectrometry was performed on a gas chromatography-mass spectrometer (Agilent, 7890A-7000, Palo Alto, California, US) and a vacuum concentrator (Labogene, MaxiVac Alpha, Copenhagen, Denmark). Chromatographic conditions were column HP-5 (30 m × 0.25 mm × 0.25 μm) and helium as the carrier gas. The heating processes were as follows: initial 80 °C for 2 min, increasing to 320 °C with the increasing speed of 10 °C/min, maintaining for 6 min. The inlet temperature was 280 °C. Mass spectrometry conditions were ionization mode EI, ion source temperature 230 °C, and temperature of the ion transmission line 150 °C. Scan mode was full scan, 50 ~ 600 m/z (mass-to-charge ratio).

Processing of metabolomics data

The experimental data were input into various (X) forms of chromatography-mass spectrometry (XCMS) data analysis platforms for extraction, peak alignment, retention time correction, etc., to finally obtain the original data. The

area of the original data was then normalized using Excel. The normalized data were then input into SIMCA-P14 software for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) for observing the natural distribution between samples and visually observing the possible existence of differences. The differential metabolites between the two groups were searched by the OPLS-DA method, and the variable importance in the projection (VIP) value (threshold value > 1) of the OPLS model was combined with the *p*-value (threshold value = 0.05) of the *t*-test to find the most differentially expressed metabolites. The identification of such metabolites used the National Institute of Standards and Technology (NIST) database.

Statistical analysis

SPSS 21.0 statistical software was used for processing. The measurement data were expressed as mean ± standard deviation (SD). The changes in each observation index before and after treatment were tested by the *t*-test. All the statistical results were tested by the two-sided test, with $p < 0.05$ being considered statistically significant.

Results

Observation indices

In the LCH group, the fasting insulin level after treatment was 7.4 ± 4.2 mmol/L, which decreased significantly compared with that in the LC group (9.8 ± 4.4 mmol/L, $p = 0.035$). There were no significant differences in the fasting blood glucose, 2-hr postprandial blood glucose, blood lipids, and glycated hemoglobin between the two groups. In the LCH group, the HOMA-IR index after treatment was 1.7 ± 1.1, lower than that in the LC group (2.4 ± 1.2, $p = 0.030$) (Table 2).

Analysis of gas chromatography-mass spectrometry metabolomics

PCA analysis obtained the spatial distribution of serum in the two groups (Figure 1A). The samples in the LC and LCH groups did not show significant separation, and most of the serum samples in the two groups were distributed within the 95% confidence interval. To further verify the separation

Table 2

Changes in serum metabolomics in the two groups after the 60-day clinical observation period

Parameter	LC		LCH		*p*-value	
	before	after	before	after	before	after
Fasting blood glucose (mmol/L)	5.6 ± 1.0	5.1 ± 0.7	5.6 ± 0.9	5.4 ± 0.8	0.877	0.125
2-h postprandial blood glucose (mmol/L)	8.9 ± 1.4	8.7 ± 1.2	8.6 ± 1.5	8.4 ± 1.3	0.293	0.261
Glycated hemoglobin (%)	6.9 ± 1.8	6.6 ± 1.4	6.9 ± 1.8	6.6 ± 1.4	0.987	0.989
Fasting insulin (mmol/L)	10.5 ± 6.0	7.4 ± 4.2	10.5 ± 5.8	9.8 ± 4.4	0.962	0.035*
2-hr postprandial insulin (mmol/L)	64.4 ± 22.3	58.7 ± 17.6	65.0 ± 23.2	60.1 ± 15.4	0.926	0.738
HOMA-IR	2.6 ± 1.7	2.4 ± 1.2	2.6 ± 1.6	1.7 ± 1.1△	0.976	0.030*
TC (mmol/L)	4.6 ± 1.6	4.3 ± 1.6	4.6 ± 1.6	4.2 ± 1.4	0.939	0.836
TG (mmol/L)	2.4 ± 1.0	1.9 ± 0.8	2.4 ± 1.1	1.9 ± 0.8	0.907	0.861
LDL (mmol/L)	3.1 ± 1.1	2.3 ± 1.0	3.0 ± 1.1	2.2 ± 1.0	0.867	0.723
HDL (mmol/L)	1.8 ± 1.3	1.9 ± 1.2	1.8 ± 1.3	1.9 ± 1.1	0.935	0.995
ALT (U/L)	24.3 ± 10.9	24.3 ± 12.7	24.5 ± 10.2	23.1 ± 11.0	0.961	0.697
Creatinine (μmol/L)	63.8 ± 18.4	62.4 ± 20.3	64.5 ± 17.9	62.9 ± 20.5	0.876	0.930
Uric acid (μmol /L)	201.1 ± 105.9	194.3 ± 104.4	198.6 ± 103.3	191.6 ± 102.2	0.929	0.922

LC – lifestyle change group; LCH – lifestyle change combined with *H. esculentus* treatment group; HOMA-IR – homeostasis model assessment-estimated insulin resistance index; TC – total cholesterol; TG – triglycerides; LDL – low-density lipoprotein; HDL – high-density lipoprotein; ALT – alanine transaminase; Results are given as mean ± standard deviation; △ *p* < 0.05 – comparison with the data before treatment; **p* < 0.05 – comparison with the LC group.

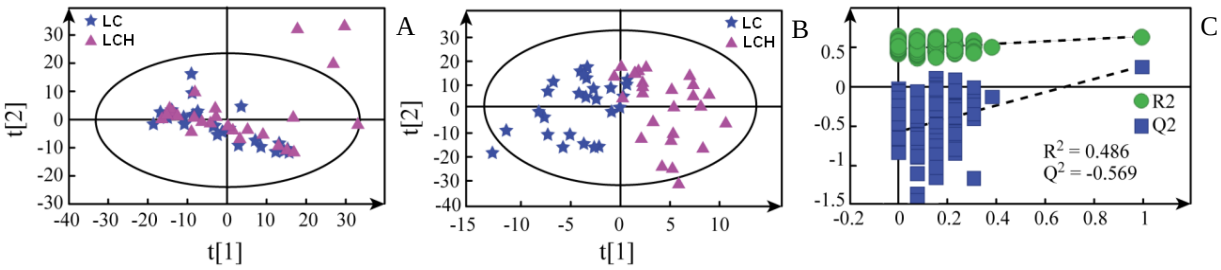

Fig. 1 – A) Principal component analysis between groups; B) Orthogonal partial least squares discriminant analysis between groups; C) Replacement verification model.
LC – lifestyle change group; LCH – lifestyle change combined with *H. esculentus* treatment group

Table 3

Differential serum metabolomics between the two groups

Metabolites	LC	LCH	VIP	*p*-value
d-galactose (mmoI/L)	21.2 ± 2.9	16.7 ± 3.9	1.57	0.004
d-glucose (mmoI/L)	132.9 ± 36.7	101.5 ± 40.2	1.03	0.003
Turanose (mmoI/L)	3.7 ± 2.4	1.8 ± 1.6	1.07	0.002
Lactic acid (mmoI/L)	2.3 ± 0.8	3.3 ± 0.53	1.15	0.006
Uric acid (μmoI /L)	67.8 ± 23.5	44.5 ± 15.7	1.17	0.008
CLA (mmol/L)	2.1 ± 1.2	6.9 ± 6.1	1.08	0.0003

LC – lifestyle change group; LCH – lifestyle change combined with *H. esculentus* treatment group; VIP – variable importance in the projection; CLA – conjugated linoleic acid.
Results are given as mean ± standard deviation.

between the two groups, OPLS-DA analysis was performed on the two groups' sera (Figure 1B). The results showed that the samples of the two groups significantly separated, with interpretability $R^2X = 0.895$, $R^2Y = 0.738$, and predictability $Q^2 = 0.651$, indicating that the fit and prediction ability of the OPLS-DA data model can better explain the differences between the two groups. The replacement test was used to verify the model, and after 200 times of replacement tests, the intercept was $R^2 = 0.486$ and $Q^2 = -0.569$ (Figure 1C), indicating that the model had good reliability.

The VIP value of differential metabolites can reflect the importance of each variable in establishing the model. In this study, VIP ≥ 1 and a two-sided *t*-test ($p < 0.05$) were used to screen and identify the major differential metabolites in the two groups. The results showed that the plasma lactate level in the LCH group was 3.3 ± 0.5 mmol/L, higher than that in the LC group (2.3 ± 0.8 mmol/L, $p = 0.006$). In the LCH group, the levels of serum uric acid, plasma d-galactose, d-glucose, and turanose were 44.5 ± 15.7 μmol/L, 16.7 ± 3.9 mmol/L, 101.5 ± 40.2 mmol/L, 1.8 ± 1.6 mmol/L, respectively, all significantly lower than those in the LC group (67.8 ± 23.5 μmol/L, 21.2 ± 2.9 mmol/L, 132.9 ± 36.7 mmol/L, 3.7 ± 2.4 mmol/L, respectively, with $p = 0.008$, 0.004, 0.003, 0.002, respectively). The plasma-conjugated linoleic acid (CLA) level in the LCH group was 6.9 ± 6.1 mmol/L, which was significantly higher than that in the LC group (2.1 ± 1.2 mmol/L, $p = 0.003$) (Table 3).

Discussion

The purpose of this study was to compare whether *H. esculentus* combined with lifestyle change can improve glucose metabolism abnormalities in IGT patients more efficiently than just simple lifestyle changes and analyze possible metabolic pathways of such regulatory effects. The results showed that the fasting insulin level and HOMA-IR of patients in the LCH group were significantly lower than those in the LC group, suggesting that the patients had reduced insulin resistance. Insulin resistance is the basic pathological mechanism throughout the development of T2DM, which also causes other metabolic disorders, such as islet β-cell secretion defects, insulin resistance in peripheral tissues, increased liver glucose output, lipid metabolism disorders, weakened insulinotropic effect, and hyperglycemia. Insulin resistance also participates in the whole occurrence and development process of T2DM and its complications. Because of that, early improvement of insulin resistance may help delay the onset and progression to T2DM [16]. Our results suggest that *H. esculentus* combined with lifestyle change can significantly improve insulin resistance in IGT patients and may help delay their transition to type T2DM. The hypoglycemic effect of *H. esculentus* has been observed in several animal studies. For instance, Khatun et al. [17] reported that *H. esculentus* extract can reduce blood glucose by reducing glucose intestinal absorption in rats. Previous studies reported that *H. esculentus* extract significantly reduced the fasting blood glucose and glycated hemoglobin, and improved the liver function damage in diabetic rats, the super-dose of which is not toxic to rats [18]. Certain *in vitro* tests have confirmed and explored the specific molecular mechanism of *H. esculentus* in improving insulin resistance. Other studies have confirmed that *H. esculentus* polysaccharides can improve insulin sensitivity in mice, and the effect may depend on the regulation of the liver X receptor and the expressions of peroxisome proliferators-activated receptor (PPAR) and its target genes [19, 20]. Our results show that *H. esculentus* combined with lifestyle significantly improves insulin resistance in IGT patients more than lifestyle management alone and validate previous scholars' conclusions that *H. esculentus* can improve insulin resistance at a clinical level. Since IGT patients had no significant changes in clinical DM when their plasma glucose changed, our study results did not observe significant reductions in the levels of glucose and glycated hemoglobin in IGT patients before and after treatment. However, *H. esculentus* can improve insulin resistance in patients, implying it assists in long-term glucose metabolism regulation and DM prevention in IGT patients. Thereby prolonging the observation period may provide more clinical evidence on the regulation of glucose metabolism by *H. esculentus.*

We analyzed the differential metabolites between the two groups through metabolomics to explore possible metabolic pathways involved in regulating glucose metabolism and insulin action. The results showed that the plasma d-galactose, d-glucose, and turanose were significantly lower in the LCH group than in the LC group, suggesting that monosaccharide molecules in the LCH group were significantly reduced. These monosaccharide molecules can be interchanged with glucose in peripheral blood, which is an important factor leading to elevated plasma glucose and insulin

resistance. Our results show that the monosaccharide molecular metabolites of the patients in the LCH group are significantly reduced, which provides theoretical support for our hypothesis of the clinical application of *H. esculentus* to regulate blood sugar and suggests that the monosaccharide metabolism pathway may be the main metabolic pathway involved in the improvement of insulin resistance by *H. esculentus*.

Our metabolomics analysis also showed that the blood lactic acid level in the LCH group was significantly higher than that in the LC group, suggesting that the lactic acid metabolic pathway may be involved in the metabolic mechanism of *H. esculentus* regulating insulin action. The role of circulating lactic acid in glucose metabolism is mainly manifested in regulating liver glucose output. Lactic acid is the final product of glycolysis and is also the main raw material of liver gluconeogenesis, which can increase liver glucose output and maintain fasting blood glucose stability. In the state of insulin resistance, the inhibitory effect of insulin on the activation pathway of liver gluconeogenesis key enzymes weakens, leading to the abnormal activation of the gluconeogenesis pathway in liver cells; therefore, the liver glucose output continues to be activated when the blood glucose increases, resulting in blood glucose elevation. The lactic acid in the liver is taken up by hepatocytes through the lactic acid cycle, causing lactic acid to participate in liver gluconeogenesis and blood sugar increase. The increase in the lactic acid level by the hypoglycemic agent metformin may be related to inhibiting the uptake and utilization of lactic acid by liver cells, thereby reducing the liver gluconeogenesis precursors and inhibiting the liver glucose output [21, 22]. Our study observed that the insulin resistance in the LCH group improved while the lactate level increased, suggesting that *H. esculentus* may be involved in regulating the lactic acid cycle and liver glucose output, thereby reducing insulin resistance. Nevertheless, the specific mechanism of *H. esculentus* regulating gluconeogenesis and lactic acid metabolism still needs further studies. There was no statistical significance in liver enzymes and serum creatinine levels between the two groups, suggesting that the increased lactic acid level in the LCH group did not cause liver or kidney function damage. However, the effect of *H. esculentus* on lactic acid metabolism needs to be observed for a longer period of time for further evaluation.

Our study found that the blood uric acid level in the LCH group was significantly lower than that in the LC group, suggesting that inhibition of uric acid overproduction may be involved in the improvement of insulin resistance metabolic pathways by *H. esculentus*. Recent studies have confirmed that elevated uric acid is directly involved in the development of lipid metabolism disorders and DM, as well as their complications [23]. Studies have found that prediabetic patients with hyperuricemia have higher insulin levels, suggesting that increased blood uric acid worsens insulin resistance and accelerates the progression of type T2DM [24]. In addition, persistent hyperuricemia can aggravate islet β-cell damage [25]. Zhu et al. [26] and other studies have found that hyperuricemia inhibits the activities of insulin signaling pathway protein kinase B (PKB) and insulin receptor substrate (IRS) and increases insulin resistance through oxidative stress, thus leading to insulin resistance in mice. Our results support the findings of other scholars. However, the molecular mechanism of *H. esculentus'* regulation of the uric acid metabolic pathway needs further studies. We have used mass spectrometry to detect the main components of dried *H. esculentus* fruit tea, which showed polysaccharides and flavonoids, etc. We also used the water extraction method to extract the *H. esculentus* polysaccharides and applied them in high-fat diet-induced insulin-resistant mice, and found that they can reduce pyruvate-induced blood sugar increase and decrease the expression of the key enzymes of hepatic gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate (G6P)], suggesting that *H. esculentus* may have a regulatory effect on hepatic gluconeogenesis. The main components of *H. esculentus* are quercetin and isoquercetin. Our *in vitro* experiments have confirmed that they can reduce the expression of PEPCK and G6P in hepatocytes and activate adenosine 5-monophosphate-activated protein kinase (AMPK) and its phosphorylation simultaneously, which have similar effects to metformin. This study is a clinical observation of *H. esculentus* on glucose metabolism in the IGT population. The results suggest that *H. esculentus* can improve insulin resistance and increase blood lactate levels, which supports the results of *in vitro* studies and suggests that *H. esculentus* may be involved in regulating hepatic gluconeogenesis, thereby reducing hepatic glucose output and reducing blood sugar [27].

Metabolomics analysis also showed that the CLA level in the LCH group was significantly higher than that in the LC group. CLA is an isomer of linoleic acid and is widely found in ruminant meat and dairy products [28, 29]. Recent studies have found that it has the effects of improving immunity, confronting cancer, atherosclerosis, and oxidation, reducing blood sugar and lipids, and improving insulin sensitivity [30, 31]. Its effect on glycolipid metabolism may be related to activating PPARα, down-regulating CD36, and promoting acetyl-CoA carboxylase (ACC) phosphorylation [32, 33]. Studies have shown that CLA can act as a ligand of the nuclear transcription factor PPAR-γ to increase the expression of adiponectin mRNA, play a role in glucose metabolism organs such as the liver and skeletal muscles, and improve insulin resistance [34]. Recent studies have confirmed that CLA promotes white adipocyte differentiation by activating PPAR-γ, which increases insulin-sensitive small adipocytes, thereby increasing insulin sensitivity [34]. Our research shows that *H. esculentus* combined with lifestyle change increases the CLA level, which is consistent with previous results on CLA regulating glucose metabolism; therefore, this study can provide a clinical basis for *H. esculentus* in ameliorating insulin resistance and preventing the progression of IGT.

Conclusion

This study found that *H. esculentus* combined with lifestyle change can improve insulin resistance in patients with glucose tolerance. Metabolomics analysis showed that the

contents of various monosaccharides such as galactose and serum uric acid decreased, while the levels of serum lactic acid and CLA increased in the LCH group, suggesting the involvement of the two elements in *H. esculentus*' improving insulin sensitivity in IGT patients. In addition, our findings imply *H. esculentus* regulating glucose metabolism may be related to inhibiting the liver glucose output by reducing liver lactic acid circulation. However, this study has a small number of study subjects and a short observation cycle. In the future, more prediabetic patients need to be included for long-term clinical observations to verify the mechanism of *H. esculentus* in improving early-stage abnormal glucose metabolism. In addition, metabolomics analysis only suggested the effects of *H. esculentus* on glucose metabolism-related products, more in-depth research is needed in the future to explore the molecular mechanism of *H. esculentus* in improving early-stage metabolic abnormalities and insulin resistance so as to provide a more theoretical basis and research directions for *H. esculentus*' antidiabetic effects.

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Conflicts of interest

The authors declare no conflict of interest.

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