

Pharmacological and metabolic effects of Bifidobacterium triple viable capsules and Five-animal Play in patients with impaired glucose tolerance

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Abstract: Background: Impaired glucose tolerance (IGT) is a reversible pre-diabetic condition characterized by early disruptions in insulin resistance and glucose control. Wingless-type MMTV integration site family member 5a/ secreted frizzled-related protein 5 (Wnt5a/Sfrp5) signaling and GLP-1 modulation have been identified as possible treatment approaches. **Object:** The effects of low-intensity aerobic activity, Huatuo Five-animal Play exercise and bifidobacterium triple viable capsules on glucose–lipid metabolism and GLP-1/Wnt5a/Sfrp5 signaling were assessed in this randomized, parallel-group clinical trial involving ninety persons with IGT. **Methods:** The designated intervention was given to participants for four weeks. Before and during therapy, measurements were taken of fasting blood glucose (FBG), 2 h post-load glucose (2hFBG), glycosylated hemoglobin (HbA1c), fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), serum lipids and circulating GLP-1, Wnt5a and Sfrp5. **Results:** All treatments improved lipid indices, characterized by reductions in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), along with an increase in high-density lipoprotein cholesterol (HDL-C) and significantly lowered FBG, 2hFBG, HbA1c, fasting insulin and HOMA-IR ($P<0.05$). Compared with aerobic exercise, Five-animal Play generated higher decreases in FBG, 2hFBG and HOMA-IR and a more significant decline in Wnt5a ($P<0.05$). Outcomes in the Five-animal Play group were comparable to the probiotic group. There were no negative responses or toxicities to the kidneys or liver. **Conclusion:** Huatuo Five-animal Play may offer a safe, mechanism-based non-pharmacological approach for early metabolic intervention in pre-diabetes.

Keywords: Five-animal Play exercise; Glucose metabolism; Impaired glucose tolerance; Traditional Chinese medicine exercise therapy

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INTRODUCTION

Mild hyperglycemia, insulin resistance and early abnormalities in glucose–lipid metabolism are the hallmarks of IGT, a condition that lies between normal glucose homeostasis and overt type 2 diabetes mellitus (T2DM). Almost all patients who develop T2DM pass through a pre-diabetic stage such as IGT, and prospective studies have demonstrated that timely intervention during this window can delay or prevent progression to diabetes and its microvascular and macrovascular complications (Carrasco-Zanini *et al.*, 2022; Chen *et al.*, 2021; Lian *et al.*, 2022). Conventional pharmacotherapy is effective but may be restricted by cost, tolerability and long-term adherence, whereas lifestyle-based therapies such as diet modification and structured physical exercise are being regarded as crucial components of comprehensive pre-diabetes management. Accumulating evidence has pointed out the critical role of the gut microbiota (GM)–host axis in energy homeostasis, low-grade inflammation and insulin signaling,

therefore linking intestinal dysbiosis to IGT and T2DM (Scheithauer *et al.*, 2020; Wu *et al.*, 2023; Liu *et al.*, 2024).

Probiotics primarily affect glucose metabolism through metabolites obtained from the microbiota, especially short-chain fatty acids (SCFAs) produced by bacterial fermentation of dietary fiber. Bifidobacterium and other beneficial genera increase the synthesis of acetate, propionate and butyrate, which activate intestinal enteroendocrine L cells' free fatty acid receptors (FFAR2/FFAR3) and increase the secretion of glucagon-like peptide-1 (GLP-1). GLP-1 increases insulin sensitivity, delays stomach emptying, inhibits glucagon release and improves glucose-dependent insulin secretion. Furthermore, by restricting endotoxin transfer and bolstering gut barrier integrity, SCFAs lower systemic inflammation. When taken as a whole, these endocrine and anti-inflammatory benefits offer a molecular foundation for probiotics' ability to enhance glucose tolerance in pre-diabetic situations. Bacterial fermentation of food substrates generates SCFAs, which influence insulin

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sensitivity, inflammatory pathways and epithelial integrity (Mukhopadhyaya and Louis, 2025; Facchin *et al.*, 2024). Decreases in beneficial genera such as *Lactobacillus* and *Bifidobacterium* have been associated with disturbed glucose and lipid metabolism and increased metabolic inflammation (Razmpoosh *et al.*, 2019; Kim, 2023; Ney *et al.*, 2023). Probiotic preparations of bifidobacteria can partially reconstitute microbial balance, stimulate SCFA production and improve glycaemic control; thus, these represent a promising supplementary therapy in pre-diabetes and T2DM. Recent metabolomics and microbiota-focused studies further demonstrate that probiotic- and TCM-based interventions regulate glucose metabolism through microbiota-derived metabolites and endocrine signaling networks, including SCFA-mediated GLP-1 secretion and inflammatory pathway modulation (Li *et al.*, 2022; Lang *et al.*, 2023; Su *et al.*, 2023; Cheng *et al.*, 2024). These findings support a systems-level view of microbiota–endocrine interactions in metabolic regulation.

At the molecular level, GLP-1 and the Wnt5a/Sfrp5 signaling pathway have emerged as critical nodes that integrate gut-derived signals with insulin secretion and tissue insulin sensitivity. In response to nutrients and microbial metabolites, intestinal L cells secrete GLP-1, which decreases blood sugar through increasing glucose-dependent insulin secretion, suppressing glucagon release, delaying stomach emptying and regulating appetite (Fujiwara *et al.*, 2019; Kyriachenko *et al.*, 2019). Wnt5a, a non-canonical Wnt ligand, promotes pro-inflammatory signaling and has been implicated in obesity-associated insulin resistance, while secreted frizzled-related protein 5 (Sfrp5) is an endogenous antagonist of Wnt5a capable of counteracting metabolic inflammation and improving insulin sensitivity (Cisneros *et al.*, 2020; Rohm *et al.*, 2022; Xu *et al.*, 2023). Clinical evidence suggests that GLP-1 receptor agonists may improve circulating Wnt5a and Sfrp5 levels in T2DM patients, suggesting functional crosstalk between these pathways (Xu *et al.*, 2023).

For people with or at risk for diabetes, regular aerobic and mind-body exercise is a proven non-pharmacological intervention that improves body composition, insulin sensitivity, cardiovascular risk and cardiorespiratory fitness (Evans *et al.*, 2019; Wang *et al.*, 2023; Vazquez-Guajardo *et al.*, 2024). Exercise has been demonstrated to modify the makeup of the GM, increase the production of SCFA and change the cytokine and myokine profiles that converge on similar pathways as probiotic therapy, in addition to macroscopic metabolic effects (Cataldi *et al.*, 2022; Chen *et al.*, 2024). Huatuo Five-animal Play is a traditional form of Chinese mind-body workout regimen that consists of slow and synchronized movements with controlled breathing and mental focus. It mimics the breathing and movement patterns of five animals, namely tigers, deer, bears, apes and birds. Five-animal Play has been documented to favourably influence body

composition and metabolic status in middle-aged and older adults and improve blood pressure, lipid profiles, glycaemic indices and physical function in a range of chronic conditions (Yu, 2008; Guo *et al.*, 2018; Wang *et al.*, 2019; Zou *et al.*, 2019). Nevertheless, little is known about the molecular underpinnings, especially with regard to its possible influence on GLP-1/Wnt5a/Sfrp5 signaling in pre-diabetic conditions. Few clinical trials have, to date, explicitly compared a probiotic pharmaceutical preparation with various types of exercise, including conventional mind-body exercise, in patients with IGT while concurrently evaluating clinical effectiveness, GLP-1/Wnt5a/Sfrp5 signaling and glucose-lipid metabolism. Traditional treatments have multi-target regulatory effects on metabolic, inflammatory and endocrine pathways, such as insulin signaling, lipid metabolism and immune modulation, according to recent network pharmacology and TCM mechanistic studies (Cheng *et al.*, 2024; Li *et al.*, 2024; Wang *et al.*, 2025; Bin *et al.*, 2025; Xing *et al.*, 2024). The evaluation of Huatuo Five-animal Play as an integrative therapeutic operating across interconnected metabolic networks is supported mechanistically by these systems-based findings. Understanding if Huatuo Five-animal Play produces metabolic effects through mechanisms similar to or separate from those of a *Bifidobacterium*-based probiotic may assist in presenting this traditional exercise as a rational, mechanism-based supplement to pharmaceutical or microbiota-targeted treatments.

Therefore, the present randomized clinical trial aimed to investigate the effects of *Bifidobacterium* triple viable capsules, low-intensity aerobic exercise and Huatuo Five-animal Play exercise on glucose metabolism, lipid profiles and blood GLP-1, Wnt5a and Sfrp5 levels in individuals with IGT. Our hypothesis was that Huatuo Five-animal Play, at least partially through up-regulation of GLP-1 and Sfrp5 and down-regulation of Wnt5a, would improve insulin resistance and glucose tolerance to a degree comparable to probiotic therapy and superior to traditional aerobic exercise. This would provide a mechanistic basis for its use as a non-pharmacological therapeutic modality in IGT.

The non-canonical Wnt5a/Sfrp5 signaling axis is important for insulin resistance and metabolic inflammation at the molecular level. Wnt5a is a pro-inflammatory ligand that promotes adipose inflammation and hinders insulin signaling by activating β -catenin-independent pathways, such as JNK- and Ca²⁺-dependent signaling. Type 2 diabetes, obesity and decreased glucose tolerance have all been linked to elevated Wnt5a expression. By aggressively binding Wnt ligands through its cysteine-rich domain, secreted frizzled-related protein 5 (Sfrp5) opposes Wnt5a, reducing inflammatory signaling and enhancing insulin sensitivity. This axis is a viable target for early intervention in IGT because an imbalance favoring increased Wnt5a

and decreased Sfrp5 contributes to persistent low-grade inflammation and glucose dysregulation. As a mind-body workout that incorporates controlled breathing and movement, Huatuo Five-animal Play may increase insulin sensitivity, lessen inflammation brought on by stress and improve autonomic balance. These actions offer a biological foundation for the possible regulation of endocrine and inflammatory pathways associated with poor glucose tolerance.

MATERIALS AND METHODS

Source of patients

All subjects in this study were acquired from the physical examination department and massage department of the First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine, a total of 90 cases. Patients were assigned to three groups (group A, B and C) in a randomized manner, with 30 cases in each group. Randomization was performed using a computer-generated random number sequence created by an independent statistician who was not involved in participant recruitment or intervention delivery. Eligible participants were enrolled by clinical investigators at the study site. Group allocation was implemented by a separate research coordinator according to the randomization sequence, assigning participants to the bifidobacterium triple viable capsule group (Group A), low-intensity aerobic exercise group (Group B), and Huatuo Five-animal Play exercise group (Group C). This study was not prospectively registered.

Diagnostic criteria

IGT diagnostic criteria (Yameny, 2024): (1) $6.1 \text{ mmol/L} \leq$ fasting plasma glucose (FPG) $< 7.0 \text{ mmol/L}$; (2) $7.8 \text{ mmol/L} \leq 2\text{h}$ after glucose load, venous blood glucose $< 11.1 \text{ mmol/L}$. Those who meet the above two items can be diagnosed as IGT.

Inclusion criteria

(1) Patients diagnosed with IGT; (2) Patients' ages ranged between 18 and 70 years; (3) patients had no serious liver and kidney function damage, history of diabetes and complications; (4) Patients had no serious cardiovascular and cerebrovascular diseases, severe spinal-related diseases and mental diseases; (5) the Patient had not taken hypoglycemic and lipid-lowering drugs; (6) Patients can voluntarily and actively cooperate with exercise and moderate-intensity exercise (60%-70% maximum heart rate) can be performed after exercise risk assessment; (7) Patients voluntarily signed informed consent.

Exclusion criteria

(1) Patients who took antibiotics, steroids, gastrointestinal motility drugs, microecological preparations, yogurt and other preparations affecting intestinal flora in the past month; patients with a history of gastrointestinal diseases such as diarrhea and dysentery; or patients with a history

of gastrointestinal surgery; (2) The patient has a disease that seriously threatens his life or affects his voluntary movement; (3) Patients with severe hypertension ($\geq 160/100 \text{ mmHg}$), severe liver and kidney dysfunction ($\text{ALT} > 100 \text{ U/L}$ or $\text{AST} > 100 \text{ U/L}$ or total bilirubin $> 34.2 \text{ umol/L}$); (4) Pregnant and lactating women; (5) Poor compliance, failure to follow the doctor's advice to take medicine or exercise on time, affecting the evaluation of curative effect and failure to complete the treatment; (6) Patients with recurrent peptic ulcer, allergic constitution and mental illness; (7) Patients with elevated blood glucose caused by diabetes, liver disease, drugs and other factors; (8) Patients who are participating in clinical studies of other drugs.

Withdrawal criteria

Cases that fail to complete this clinical study should be considered as shedding cases: (1) Subjects who have not been followed up regularly and have incomplete clinical data; (2) Subjects who had serious adverse reactions, complications, or other special circumstances during the trial and should not continue to participate; (3) Patients who were not satisfied with the curative effect and were required to withdraw automatically; (4) Poor compliance of the subjects, failure to use drugs or exercise as required, or serious adverse events during the test; (5) Although the test can be completed, the amount of medication or exercise during the test is not up to standard, self-adjustment of drugs or exercise; (6) The reasons for shedding cases need to be recorded in detail and the results of the last efficacy test indicators should be turned into the final results for statistical analysis and the clinical medical records should be retained for reference.

Intervention methods

There was no significant difference in daily diet and physical activity among all groups. The diet was dominated by cereals such as wheat and rice, vegetables and a small amount of meat. Participants were advised to stick to their regular diet and lifestyle, avoid mental stress, abstain from probiotics and yogurt and avoid moderate-to-intense exercise and other fitness regimens during the study.

Based on the original lifestyle, group A was given Bifidobacterium triple viable capsules orally (Specification: 210 mg/capsule, Batch No.: 0472016111, Shanghai Xinyi Pharmaceutical Co., Ltd.), 3 capsules/time, twice a day for 4 weeks.

Based on the original lifestyle, group B adopted low intensity cycling exercise. According to the Karvonen formula, the effective heart rate for low-intensity exercise is $(220 - \text{age} - \text{rest heart rate}) \times 60\% + \text{rest heart rate}$. Exercise at an effective heart rate for 30 minutes a day, 6 times a week for 4 weeks. Exercises such as warm-up and stretching are not included in the time and the duration of the effective heart rate is taken as the criterion.

Based on their original lifestyle, the subjects in group C practiced Five-animal Play under the guidance of professional doctors. The practice site is the massage department of the First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine. Practice Five-animal Play three times a day, about 30 minutes each time, 6 days a week for 4 weeks, supervised by the same group of professional doctors and exercise guidance. If you can complete the standard exercise action by yourself through the assessment, the patient is instructed to practice at home and regularly play the amount of exercise on the online WeChat group. All subjects were tested before and after treatment.

Observation indicators

General indicators include age (years), gender, course of disease (years), weight (kg), waist circumference (cm), hip circumference (cm), body mass index (BMI) and waist-to-hip ratio (WHR). Body weight, waist circumference, hip circumference, BMI and waist-to-hip ratio were measured 1 day before and 1 month after the experiment. $BMI = \text{weight (kg)} / \text{square of height (m}^2\text{)}$. $WHR = \text{waist circumference} / \text{hip circumference}$.

Efficacy observation indicators: FPG, 2 h postprandial blood glucose (2hPPG) and HbA1c in serum were detected by an automatic biochemical analyzer (Mindray BS-280, China).

Related protein indicators: ELISA was used to detect the levels of GLP-1, Wnt5a and Sfrp5 in serum before and after treatment. Human GLP-1 ELISA kit from Shanghai Sangon Biotech (China), Item No. D711371. Human Wnt5a ELISA kit from Shanghai Fuyu Biotechnology Co., LTD. (Item No. FY-04136H2). Human Sfrp5 ELISA Kit from Wuhan Fenn Biotechnology Co., LTD. (Item No. EH1598). Using commercially available enzyme-linked immunosorbent assay (ELISA) kits, serum levels of glucagon-like peptide-1 (GLP-1), wingless-type MMTV integration site family member 5a (Wnt5a), secreted frizzled-related protein 5 (Sfrp5) and fasting insulin were measured in accordance with the manufacturer's instructions. A human GLP-1 ELISA kit (Shanghai Sangon Biotech Co., Ltd., China; Cat. No. D711371; assay range: 0.5–20 pmol/L; limit of detection [LOD]: 0.1 pmol/L; intra-assay coefficient of variation [CV] < 8%; inter-assay CV < 10%) was utilized to quantify GLP-1. A human Wnt5a ELISA kit (Shanghai Fuyu Biotechnology Co., Ltd., China; Cat. No. FY-04136H2; assay range: 0.1–10 ng/mL; LOD: 0.05 ng/mL; intra-assay CV < 8%; inter-assay CV < 10%) was used to measure the levels of Wnt5a. A human Sfrp5 ELISA kit (Wuhan Fenn Biotechnology Co., Ltd., China; Cat. No. EH1598; assay range: 1–50 ng/mL; LOD: 0.3 ng/mL; intra-assay CV < 8%; inter-assay CV < 10%) was used to assess Sfrp5 quantities. A human insulin ELISA kit (Solarbio Science and Technology Co., Ltd., China; Cat. No. SEKH-0219-96T; assay range: 2–200

pmol/L; LOD: 1.0 pmol/L; intra-assay CV < 10%; inter-assay CV < 12%) was used to measure fasting insulin. Every serum sample was examined twice and statistical analysis was performed using the mean value. To reduce batch effects, samples from various research groups were dispersed at random among assay plates. Laboratory staff who were blind to group assignment carried out all measurements.

GM-related indicators: 5 ml of venous blood was taken in the early morning of the day before the start of the experiment and the early morning of the next day, after one month. The blood samples were placed at $22 \pm 2^\circ\text{C}$ for 30 min and the serum was taken after 15 min at a speed of 3000 r/min and the serum was stored in a -70°C refrigerator for testing. Fasting serum insulin (FINS) was detected by ELISA and insulin resistance index (HOMA-IR) and insulin sensitivity index (HOMA-ISI) were calculated. ELISA kit from China Solarbio Technology Co., LTD. (Item No. SEKH-0219-96T). $HOMA-IR = (\text{FBG} \times \text{fasting insulin}) / 22.5$. $HOMA-ISI = 1 / (\text{FBG} \times \text{fasting insulin})$. Insulin concentrations while fasting were given in pmol/L. Fasting insulin levels were converted to $\mu\text{U/mL}$ using the usual conversion factor ($1 \mu\text{U/mL} = 6.945 \text{ pmol/L}$) in order to calculate the homeostasis model assessment of insulin resistance (HOMA-IR). Consequently, the following formula was used to determine HOMA-IR: $HOMA-IR = [\text{FBG (mmol/L)} \times \text{fasting insulin } (\mu\text{U/mL})] / 22.5$. For instance, a participant's HOMA-IR value would be 0.69 if their fasting plasma glucose was 6.0 mmol/L and their fasting insulin was 18.0 pmol/L (or 2.59 $\mu\text{U/mL}$).

LM-related indexes: Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were detected by an automatic biochemical analyzer (Mindray BS-280, China).

Safety observation indicators: include (1) General physical examination, including heart rate and blood pressure in resting state and exercise state; (2) Blood, urine and stool routine examination; (3) Liver and kidney function indicators: alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (Scr), serum cystatin C (CysC) and endogenous creatinine clearance rate. In order to facilitate clinical calculation, this study used the endogenous creatinine clearance rate to reflect renal damage. All safety observation indices were detected once before treatment and after treatment.

Follow-up

Weekly offline outpatient follow-up and daily online follow-up modes were adopted. Group B and Group C WeChat groups were formed to avoid privacy leakage using numbered notes. The daily exercise card is for special reasons, such as failing to exercise and private chat

supervision is to ensure that each group of people exercises 6 times a week exercise frequency. An offline outpatient follow-up was conducted once a week to exercise and guide the action details to ensure the quality of exercise and even to complete the record.

Throughout the study, adherence to the intervention was tracked. Weekly outpatient follow-up and participant self-report were used to measure medication adherence for the Bifidobacterium group. Compliance was defined as taking at least 90% of the prescribed capsules. During outpatient follow-up, supervised attendance was noted for the low-intensity aerobic exercise group and heart rate was tracked to make sure the goal heart rate zone—which was determined using the Karvonen formula—was reached. The Huatuo Five-animal Play group self-reported the completion of home-based sessions through daily exercise check-ins in the WeChat group and supervised practice attendance was tracked. The percentage of prescribed sessions completed over the course of the four-week intervention period was used to calculate adherence.

Efficacy determination

(1) *Clinical cure*: clinical symptoms disappeared and all the observation indexes returned to normal. (2) *Markedly effective*: clinical symptoms disappeared; BMI and WHR decreased by more than 1/10; serum insulin, blood glucose and 1/2 HOMA-IR were normal, HbA1c did not decrease obviously or showed a downward trend. (3) *Effective*: clinical symptoms improved compared with before treatment; BMI and WHR decreased $\leq 1/10$ compared with those before treatment. Serum insulin, blood glucose, HOMA-IR and HbA1c decreased to an insufficiently marked effective standard. (4) *Ineffective*: clinical symptoms were not improved or deteriorated; the observation indexes did not change or increase.

Statistical analysis

SPSS 22.0 statistical software was used for data analysis. The data of dichotomous variables were expressed by frequency and the chi-square test was used. The data that did not conform to the normal distribution were expressed as [M (Q1, Q3)] and the non-parametric test was used. $P < 0.05$ was considered statistically significant. Lipid parameters are provided as TC, TG, HDL-C and LDL-C (mmol/L) and statistical significance is defined as $P < 0.05$. All continuous variables are expressed in SI units.

RESULTS

Participant flow and trial completion

90 individuals with impaired glucose tolerance were evaluated for eligibility and randomly assigned in a 1:1:1 ratio to three groups: the Huatuo Five-animal Play exercise group (Group C, $n = 30$), the low-intensity aerobic exercise group (Group B, $n = 30$) and the Bifidobacterium triple viable capsule group (Group A, $n = 30$). Following

randomization, there were no withdrawals or exclusions and every randomly assigned participant finished the 4-week intervention. As a result, the final statistical analysis included every participant in each group ($n = 30$ per group).

Intervention adherence

All three interventions were generally well-adhered to. The mean medication adherence rate in the bifidobacterium group (Group A) was 94.6% and none of the subjects reported taking less medicine than the predetermined compliance level. Heart rate monitoring verified that the target heart rate zone was reached during more than 85% of recorded sessions in the low-intensity aerobic exercise group (Group B), where participants attended 92.1% of supervised exercise sessions. Over the course of the intervention, self-reported completion of recommended home-based sessions averaged 90.8% in the Huatuo Five-animal Play group (Group C), while supervised session attendance was 93.4%. Overall adherence did not differ significantly between the three groups.

Comparison of baseline data of three groups of patients

Post-intervention, Group A experienced a significant drop in body weight and BMI ($P < 0.05$), yet no substantial changes were observed in waist circumference, hip circumference and the WHR ($P > 0.05$). Similarly, Group B saw significant reductions in weight, BMI and waist circumference ($P < 0.05$), with no notable alterations in hip circumference and WHR ($P > 0.05$). Group C also demonstrated significant decreases in weight and BMI after treatment ($P < 0.05$), without any significant variations in waist circumference, hip circumference and WHR ($P > 0.05$). When contrasted with Group A, Group C exhibited a markedly lower weight following Five-animal Play therapy ($P < 0.05$). Moreover, group C's weight and BMI post-treatment were substantially lower than those of Group B ($P < 0.05$). For further insights, refer to Table 1.

The clinical efficacy evaluation of three groups of patients

The curative effect of the three groups before and after treatment was observed. The total effective rate of treatment was Group A $>$ Group C $>$ Group B. The cure rate of Group C was the highest and the cure rate of Group B was the lowest. However, there was no significant difference in the total effective rate between the three groups ($P > 0.05$). As shown in table 2.

GLP-1, Sfrp5 and Wnt5a protein expression in the three groups

Based on the post-treatment assessments, Group C showed a marked increase in the quantities of GLP-1 and Sfrp5 ($P < 0.05$) and a notable reduction in Wnt5a ($P < 0.05$) while both Group A and Group B had shown similar increases in the amounts of GLP-1 and Sfrp5, in comparison to Group C; however, Wnt5a concentrations were not significantly different in any of the three groups ($P > 0.05$). Comparative

analysis showed no significant disparities in protein index levels between Group C and Group A after treatment ($P>0.05$). However, Group C's Wnt5a levels were considerably lower than those of Group B ($P<0.05$), while GLP-1 and Sfrp5 levels were not statistically different ($P>0.05$). For detailed data, consult table 3.

Analysis of GM parameters across the three groups

Post-intervention, there was a significant reduction in FBG, 2hFBG, HbA1c, fasting insulin and HOMA-IR in all three groups compared to pre-intervention levels ($P<0.05$).

When comparing Group C's GM markers post-intervention to those of Group A, no significant differences were found ($P>0.05$). However, Group C exhibited obviously lower FBG, 2hFBG and HOMA-IR compared to Group B post-intervention ($P<0.05$). The reductions in HbA1c and fasting insulin in Group C were not obviously different from those observed in Group B ($P>0.05$). Refer to table 4 for a detailed comparison.

Correlation analysis between molecular markers and glucose metabolism

To further study potential processes, correlation analyses were done between changes (Δ = post-treatment – pre-treatment) in GLP-1, Wnt5a and Sfrp5 and changes in glycaemic markers (FBG, 2hFBG, fasting insulin and HOMA-IR).

- Significant relationships were observed:
- Δ GLP-1 was inversely associated with Δ FBG ($r = -0.34$, $P < 0.01$) and Δ HOMA-IR ($r = -0.39$, $P < 0.01$), indicating that larger increases in GLP-1 were associated with greater improvements in insulin sensitivity.
- There was a positive relation between Δ Wnt5a and Δ HOMA-IR ($r = 0.41$, $P < 0.01$), suggesting that a decrease in Wnt5a was associated with a decrease in insulin resistance.
- Δ Sfrp5 negatively linked with Δ HOMA-IR ($r = -0.36$, $P < 0.01$) and Δ FBG ($r = -0.28$, $P < 0.05$).

These data support a mechanistic connection between manipulation of the GLP-1/Wnt5a/Sfrp5 axis and benefits in glucose metabolism.

Analysis of LM parameters across the three groups

Post-intervention, Groups A and C experienced a significant surge in TC, triglycerides (TG) and LDL-C levels ($P < 0.01$), along with a marked decline in HDL-C levels ($P<0.05$). Group B also showed significant increases in TC and TG levels ($P<0.01$) and a decrease in HDL-C levels ($P<0.05$), yet there was no notable change in LDL-C levels ($P>0.05$).

The TC levels in Group C post-intervention were considerably higher than those in Group A ($P<0.05$), with no significant variations in other lipid parameters ($P>0.05$). Similarly, HDL-C levels in Group C were obviously lower than in Group B post-intervention ($P<0.05$), while other

indices remained unaffected ($P>0.05$). For further details, refer to table 5.

Safety evaluation of three groups of patients after treatment

No adverse reactions and liver and kidney function damage occurred in the three groups.

DISCUSSION

This randomized clinical research of patients with IGT, we compared the metabolic and mechanistic effects of three interventions: Bifidobacterium triple viable capsules, low-intensity aerobic activity and Huatuo Five-animal Play mind–body exercise. After four weeks of treatment, all three strategies considerably reduced FBG, 2-hour post-load glucose, HbA1c, fasting insulin and HOMA-IR, indicating improved glycaemic management and insulin resistance. This molecular paradigm is supported by our results of decreased circulating Wnt5a and elevated Sfrp5 after intervention, indicating that improvements in glucose metabolism may be mediated, at least partially, by suppression of Wnt5a-driven inflammatory signaling. Furthermore, a growing body of research points to a functional crosstalk between GLP-1 and Wnt5a/Sfrp5 signaling, whereby GLP-1's insulin-sensitizing and anti-inflammatory actions may indirectly decrease Wnt5a activity while promoting Sfrp5-associated anti-inflammatory control. Serum GLP-1 and Sfrp5 levels increased significantly, while Wnt5a levels fell or tended to decrease, indicating a positive regulation of pathways implicated in metabolic inflammation and insulin sensitivity. Huatuo Five-animal Play has shown a reduction in Wnt5a that is more pronounced than traditional aerobic exercise, as well as greater reductions in body weight, BMI, FBG, 2-hour FBG and homeostasis model assessment of insulin resistance (HOMA-IR). It also resulted in metabolic improvements comparable to those of the probiotic used in this study. This indicates that Huatuo Five-animal Play may be a non-pharmacological method for targeting glucagon-like peptide 1 (GLP-1), Wnt5a and secreted frizzled-related protein 5 (Sfrp5) signaling in patients with IGT.

Huatuo Five-animal Play is associated with the modulation of GLP-1/Wnt5a/Sfrp5 signaling, according to favorably published evidence, which points to the interconnectivity of the gut microbiome. from the gut to the gut to body fat to body fat inflammation and insulin resistance (Fujiwara, et al. 2019; Cisneros et al., 2020; Wu et al., 2023; Liu et al., 2024). Based on the results of our studies, there were significant increases in GLP-1 and Sfrp5 levels in all three groups treated, indicating an increase of enteroendocrine activity and a decrease in pro-inflammatory signals from the Wnt5a pathway. The decreased levels of Wnt5a seen in the Wuqinqi treatment group when compared with the other groups indicate that Huatuo Five-animal Play likely provides benefits to insulin sensitivity and decreases inflammation, via the non-canonical Wnt pathway.

Table 1: Baseline characteristics and anthropometric changes before and after treatment.

Index	Time	Group A	Group B	Group C
		(n=30)	(n=30)	(n=30)
Gender (male / female)		16/14	15/15	14/16
Age [years, M (Q1, Q3)]		46.00 (36.75, 51.00)	54.00 (41.75, 62.25)	47.50 (38.50, 56.25)
Course of disease [month, M (Q1, Q3)]		9.50 (5.00, 13.25)	9.00 (5.00, 11.50)	9.00 (6.00, 13.00)
Body weight [kg, M (Q1, Q3)]	Pretreatment	72.00 (67.88, 76.88)	69.75 (63.38, 76.00)	73.75 (64.38, 78.00)
	After 1 month of treatment	71.50 (67.88, 76.25)*	69.75 (63.38, 75.00)*	73.75 (64.13, 76.75)* ^{ab}
BMI [Kg/m ² , M (Q1, Q3)]	Pretreatment	25.97 (24.76, 26.62)	25.25(24.34, 26.05)	26.03 (24.92, 27.07)
	After 1 month of treatment	25.93 (24.76, 26.58)*	25.09 (24.33, 25.62)*	25.69 (24.92, 26.70)* ^b
Waistline [cm, M (Q1, Q3)]	Pretreatment	88.50 (85.00, 93.12)	88.00 (83.88, 90.50)	89.50 (86.00, 95.25)
	After 1 month of treatment	88.50 (85.00, 93.00)	87.50 (83.75, 90.50)*	90.00 (87.50, 94.25)
Hipline [cm, M (Q1, Q3)]	Pretreatment	98.00 (94.00, 102.25)	100.50 (94.00, 103.00)	99.00 (96.00, 103.25)
	After 1 month of treatment	98.00 (94.00, 102.25)	100.00 (94.00, 102.00)*	99.00 (96.00, 103.00)
WHR [M (Q1, Q3)]	Pretreatment	0.89 (0.86, 0.93)	0.89 (0.87, 0.93)	0.91 (0.88, 0.92)
	After 1 month of treatment	0.89 (0.87, 0.93)	0.89(0.86, 0.92)	0.91(0.87, 0.91)

Note: Data are presented as M (Q1, Q3). Compared with pretreatment, * $P < 0.05$, ** $P < 0.01$; Compared with Group A, ^a $P < 0.05$; Compared with Group B, ^b $P < 0.05$. (TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol).

Table 2: Comparison of curative effect between the three groups (n).

Group/Curative effect	Cure	Markedly effective	Effective	Ineffective	χ^2	P
Group A (n=30)	3	18	6	3	1.976	0.474
Group B (n=30)	2	17	4	7		
Group C (n=30)	6	12	6	6		

Note: Total effective rate = cure + markedly effective + effective; P-value from chi-square test.

Table 3: Serum GLP-1, Wnt5a, and Sfrp5 levels before and after treatment.

Index	Time	Group A (n=30)	Group B (n=30)	Group C (n=30)
GLP-1 (pmol/L)	Pretreatment	6.91 (6.18, 8.38)	6.52 (6.08, 6.95)	6.81 (6.51, 7.02)
	After 1 month of treatment	9.68 (8.26, 10.30)*	9.86 (9.01, 10.73)*	9.88 (9.02, 10.73)*
Wnt5a (ng/mL)	Pretreatment	1.51 (1.20, 2.28)	1.68 (1.20, 2.46)	1.46 (1.14, 2.33)
	After 1 month of treatment	1.37 (1.05, 1.83)	1.54 (1.08, 1.98)	1.19 (0.78, 1.71)* ^b
Sfrp5 (ng/mL)	Pretreatment	29.34 (26.79, 30.85)	29.76 (26.75, 31.86)	28.72 (26.94, 32.22)
	After 1 month of treatment	37.23 (35.12, 42.67)*	38.82 (34.05, 46.80)*	38.82 (34.06, 46.80)*

Note: Compared with the test results before treatment, * $P < 0.05$, ** $P < 0.01$; Comparison with group A, ^a $P < 0.05$; Comparison with group B, ^b $P < 0.05$. (GLP-1: glucagon-like peptide-1, Wnt5a: wingless-type MMTV integration site family member 5a, Sfrp5: secreted frizzled-related protein 5).

Table 4: Changes in glucose metabolism indexes before and after treatment.

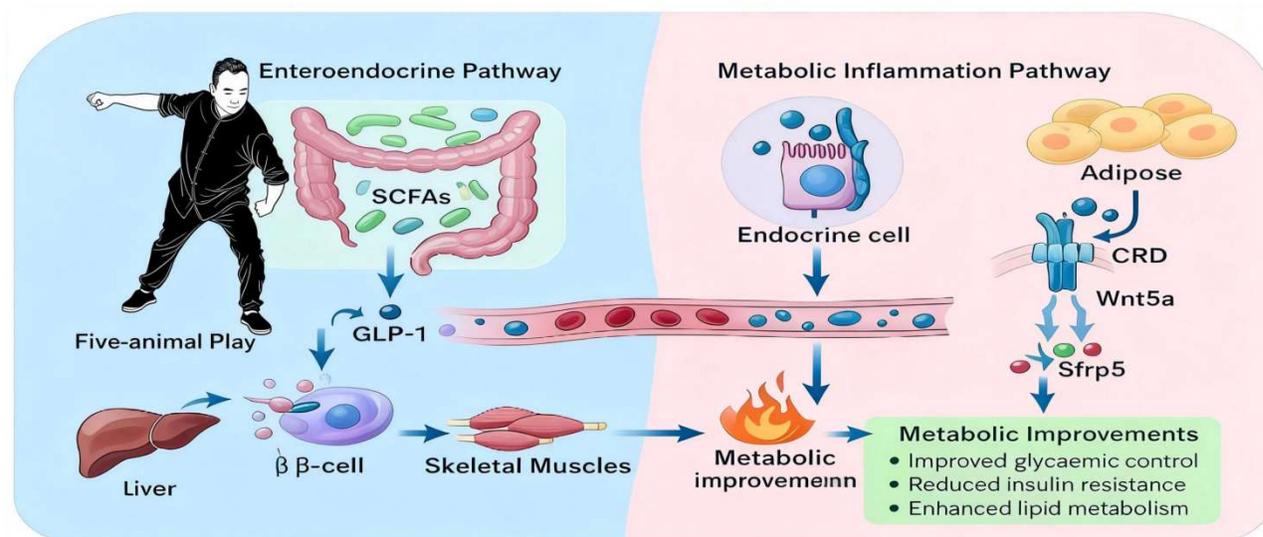
Index	Time	Group A (n=30)	Group B (n=30)	Group C (n=30)
FBG (mmol/l)	Pretreatment	6.48 (6.35, 6.62)	6.39 (6.31, 6.62)	6.43 (6.29, 6.66)
	After 1 month of treatment	5.95 (5.80, 6.23)*	6.32 (6.14, 6.39)*	6.03 (5.80, 6.16) ^{ab}
2hFBG (mmol/l)	Pretreatment	9.50 (8.90, 9.75)	9.50 (9.10, 9.63)	9.50 (8.90, 9.70)
	After 1 month of treatment	9.15 (8.58, 9.41)*	9.20 (8.48, 9.43)*	8.70 (8.08, 9.23) ^{ab}
HbA1c (mmol/L)	Pretreatment	6.30 (6.20, 6.42)	6.30 (6.19, 6.37)	6.37 (6.13, 6.51)
	After 1 month of treatment	6.23 (6.14, 6.34)*	6.26 (6.20, 6.32)*	6.33 (6.10, 6.45)*
Fasting insulin (pmol/L)	Pretreatment	17.09 (15.79, 18.72)	17.20 (15.48, 18.18)	17.25 (15.64, 18.80)
	After 1 month of treatment	16.35 (14.50, 17.49)*	16.48 (15.18, 17.58)*	16.42 (14.77, 17.85)*
HOMA-IR	Pretreatment	4.91 (4.45, 5.34)	4.87 (4.50, 5.31)	4.92 (4.40, 5.30)
	After 1 month of treatment	4.29 (3.90, 4.56)*	4.58 (4.28, 4.80)*	4.35 (3.96, 4.56) ^{ab}

Note: Compared with the test results before treatment, *P<0.05, **P<0.01; Comparison with group A, ^aP<0.05; Comparison with group B, ^bP<0.05. (HOMA-IR is the insulin resistance index; HbA1c is glycosylated hemoglobin; FBG is fasting blood glucose).

Table 5: Changes of lipid metabolism indexes in the three groups before and after treatment [M (Q1, Q3)]

Index	Time	Group A (n=30)	Group B (n=30)	Group C (n=30)
TC (mmol/L)	Pretreatment	5.42 (5.24, 5.62)	5.35 (5.26, 5.46)	5.30 (5.24, 5.51)
	After 1 month of treatment	5.35 (5.15, 5.52)**	5.18 (5.11, 5.33)**	5.12 (5.05, 5.28) ^{ab}
TG (mmol/L)	Pretreatment	1.96 (1.85, 2.16)	1.98 (1.87, 2.06)	1.96 (1.84, 2.06)
	After 1 month of treatment	1.88 (1.82, 2.00)**	1.84 (1.75, 2.02)**	1.90 (1.75, 2.00)**
HDL-C (mmol/L)	Pretreatment	1.36 (1.25, 1.50)	1.34 (1.23, 1.45)	1.31 (1.20, 1.37)
	After 1 month of treatment	1.48 (1.33, 1.54)*	1.42 (1.35, 1.57)*	1.35 (1.28, 1.46) ^{ab}
LDL-C (mmol/L)	Pretreatment	3.21 (3.01, 3.46)	3.28 (2.96, 3.50)	3.19 (2.97, 3.42)
	After 1 month of treatment	3.12 (2.88, 3.29)**	3.10 (2.89, 3.17)	2.96 (2.74, 3.13)**

Note: Compared with the test results before treatment, *P<0.05, **P<0.01; Comparison with group A, ^aP<0.05; Comparison with group B, ^bP<0.05. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

**Fig. 1:** Proposed mechanism of Five-animal Play intervention in IGT.

Note: SCFAs: short-chain fatty acids; GLP-1: glucagon-like peptide-1; Sfrp5: secreted frizzled-related protein 5; CRD: cysteine-rich domain; Wnt5a: wingless-type MMTV integration site family member 5a. The mechanism of Five-animal Play intervention on IGT.

This provides insight into the future use of Huatuo Five-animal Play as a therapeutic approach, since increased Wnt5a levels and decreased Sfrp5 levels have both been associated with obesity and related diseases, including adipose tissue inflammation and transition to T2DM (Rohm *et al.* 2022; Xu *et al.* 2023).

Five-animal Play may also promote autonomic regulation and lessen stress-related neuroendocrine activity as a mind-body intervention that emphasizes deep breathing and mental attention. This could indirectly lower metabolic inflammation and improve insulin sensitivity. There are many ways in which the use of Five-animal Play may impact GLP-1, Wnt5a and Sfrp5. Mind-body qigong has been shown to support microcirculation and metabolic function, minimize neuroendocrine activity associated with stress and improve autonomic balance (Guo, *et al.* 2018; Wang, *et al.* 2019; Zou, *et al.* 2019). Regular Five-animal Play practice has also been shown to positively impact intestinal microecology and SCFA production, which can activate FFAR2/3 on intestinal L cells to promote GLP-1 secretion (Kyriachenko *et al.*, 2019; Yu, 2008; Zou *et al.*, 2019). A hypothesis in which gut-derived signals and adipokine-mediated Wnt regulation simultaneously contribute to better insulin signaling is consistent with the observed rise in GLP-1 in our Five-animal Play group, along with increased Sfrp5 and decreased Wnt5a. Our results provide indirect support for this mechanistic framework, even though we did not directly measure the composition of the GM or the amounts of SCFA.

This domain is highly homologous to the CRD of the Wnt transmembrane receptor coiled-coil protein, which can competitively bind to the extracellular ligand Wnt5a protein of the Wnt signaling pathway, thereby inhibiting the activity of the Wnt signaling pathway. When Sfrp5 is up-regulated and Wnt5a is down-regulated, it can promote islet cell secretion, produce insulin and reduce insulin resistance to improve abnormal GM (Fig. 1).

Correlation between molecular changes and metabolic improvement

Correlation analyses further substantiated the mechanistic importance of these pathways. Reductions in HOMA-IR and fasting glucose were linked to increases in GLP-1 and Sfrp5, whereas improvements in insulin resistance were linked to decreases in Wnt5a. These data demonstrate that modification of the GLP-1/Wnt5a/Sfrp5 axis contributed directly to the metabolic improvements observed. The significant reduction in Wnt5a and increases in GLP-1 and Sfrp5 observed in the Five-animal Play group suggest that this exercise acts on the same mechanistic pathways targeted by modern therapeutic agents and microbiota-modulating probiotics.

Comparison with probiotic therapy and conventional aerobic exercise

Bifidobacterium triple viable capsules are a widely used

probiotic product in clinical practice. Consistent with recent results in T2DM, we found that probiotic administration significantly reduced glycaemic indices and HOMA-IR and elevated GLP-1 and Sfrp5 levels (Razmpoosh *et al.*, 2019; Kyriachenko *et al.*, 2019; Liu *et al.*, 2024). Huatuo Five-animal Play may produce equivalent metabolic effects by non-pharmacological manipulation of common signaling pathways, as evidenced by the Five-animal Play group's improvement in most glucose metabolism metrics being comparable to that of the probiotic group. It is important from a translational perspective because when someone correctly instructs and supervises mind-body exercises, these types of exercises have low direct costs, few contraindications and high acceptance; moreover, mind-body exercises appear to have greater effects than low-intensity aerobic exercise on weight, BMI, FBG levels, 2-hour FBG, homeostasis model assessment of insulin resistance (HOMA-IR), as well as a significantly greater decrease in Wnt5a levels while showing similar increases in GLP-1 and Sfrp5 as those found in low-intensity aerobic exercise. The integrated nature of Five-animal Play is a combination of controlled breathing, coordinated movement and mental focus, which may create additional adaptations in autonomic, endocrine or inflammatory activity, over and above those achieved via traditional aerobic exercise of the same intensity (Evans *et al.*, 2019; Wang *et al.*, 2023). Additionally, Five-animal Play also includes variations in postures and utilizes multi-joint, whole body exercises that elicit greater activation of muscle which, in turn, increases glucose uptake and storage in the skeletal muscle, thus improving the effectiveness of insulin on these tissues. Our data support the hypothesis that not all forms of exercise are physiologically comparable and that traditional mind-body practices may offer specific advantages in pre-diabetic patients.

Effects on lipid metabolism and overall metabolic risk

After four weeks of intervention, Five-animal Play, aerobic exercise and probiotic therapy all produced moderate but substantial improvements in lipid metabolism in our group, as seen by decreases in TC, TG and LDL-C and elevations in HDL-C. These modifications, albeit statistically slight, may lead to a more favourable cardiovascular risk profile, particularly when paired with improved glycaemic management and lower HOMA-IR. The lipid-modifying effects of probiotics and exercise are well documented and are thought to involve alterations in bile acid metabolism, SCFA production, hepatic lipid synthesis and clearance and changes in adipokine and cytokine secretion (Razmpoosh *et al.*, 2019; Cataldi *et al.*, 2022; Chen *et al.*, 2024; Facchin *et al.*, 2024). Five-animal Play, by integrating physical activity with breathing and relaxation techniques, may additionally reduce sympathetic overactivity and chronic stress, thereby indirectly benefiting lipid metabolism.

Safety and clinical implications

There were no significant side effects or clinically significant changes in liver and kidney function and all

three procedures were well tolerated. This is particularly encouraging in a pre-diabetic population, in whom long-term safety and acceptance of preventive therapies are critical. The absence of side effects, coupled with improvements in glycaemic and lipid indices, shows that Huatuo Five-animal Play could be safely adopted as an addition to regular lifestyle guidance and, where appropriate, microbiota-targeted or pharmaceutical therapy in individuals with IGT. For contexts where access to pharmaceuticals is restricted or patients are unwilling to commence pharmacological therapy, Five-animal Play may offer a culturally acceptable, low-cost strategy to postpone or prevent progression to T2DM. For contexts where access to pharmaceuticals is restricted or patients are unwilling to commence pharmacological therapy, Five-animal Play may offer a culturally acceptable, low-cost strategy to postpone or prevent progression to T2DM.

Limitations and future directions

This study has a number of shortcomings. First, our ability to identify between-group differences in some characteristics and evaluate the long-term stability of the observed effects may have been hampered by the small sample size and short intervention time (4 weeks). Longer-term research is required to demonstrate the stability and permanence of these effects, even though significant metabolic and molecular alterations were seen after 4 weeks. This duration probably reflects early adaptive responses. Second, even though we measured important molecular markers (GLP-1, Wnt5a and Sfrp5), we did not directly assess the composition of the GM, SCFA concentrations, or other inflammatory mediators, which would have given us a clearer understanding of the mechanisms behind the microbiota–GLP-1–Wnt axis. Also, there is limited causal inference about microbiota-mediated mechanisms because gut microbiome composition and microbial metabolite profiles were not explicitly examined, despite the assessment of important endocrine and inflammatory indicators. Third, despite frequent supervision and follow-up, inter-individual variations in adherence, baseline physical activity and lifestyle factors could not be completely controlled. Fourth, the results may not be applicable to different demographics or healthcare environments because the study was carried out at a single facility. Larger, multi-center cohorts and longer follow-up times should be included in future research to verify the sustainability of metabolic improvements and assess clinically significant outcomes including the development of microvascular and macrovascular problems and the progression to type 2 diabetes. Integration of high-throughput microbiome profiling, SCFA measurements and broader panels of inflammatory and metabolic indicators would help explain the specific mechanisms by which Five-animal Play and probiotic therapy affect GLP-1/Wnt5a/Sfrp5 signaling and insulin resistance. Additionally, researching combination tactics (e.g., probiotic + Five-animal Play) may indicate possible

additive or synergistic benefits on metabolic control in pre-diabetic situations.

CONCLUSION

Huatuo Five-animal Play exercise treatment significantly improved glucose metabolism, insulin resistance and lipid profiles in patients with IGT, with therapeutic results comparable to those of Bifidobacterium triple viable capsules and superior to traditional aerobic exercise. These advantages were supported by positive regulation of GLP-1, Wnt5a and Sfrp5, suggesting that Five-animal Play may work through mechanisms relevant to recognized metabolic and inflammatory pathways. Given its safety, accessibility and mechanistic plausibility, Five-animal Play may serve as a viable non-pharmacological supplement targeting the GLP-1/Wnt5a/Sfrp5 axis in the therapy of pre-diabetes. Larger and longer-term investigations integrating GM analysis are necessary to corroborate these findings.

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Authors' contributions

Yu Yang and Xiangyong Gan contributed equally to this work and share co-first authorship. Yu Yang was responsible for study conceptualization and design and drafted the original manuscript. Xiangyong Gan contributed to participant recruitment, intervention coordination, data collection and drafting of the original manuscript. Qilin Xiao, Yingye Liang, and Yuhang Zeng assisted with participant recruitment, intervention coordination and data collection. Zhenghong Ye, Rundong Lin, and Tianxia Diao performed laboratory assays and data analysis. Zhiming Ye and Yi Liang conducted statistical analysis and data interpretation. Weibo Hong was responsible for project administration and resource management. Yufeng He contributed to study conceptualization and design, manuscript review and editing, supervision and served as the corresponding author. All authors reviewed and approved the final manuscript.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The research received ethical approval from the Guangxi University of Traditional Chinese Medicine's Institutional Review Board (Ethics No: 2022-041-02) and accepted its supervision. All subjects voluntarily signed the informed consent form.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

- Carrasco-Zanini J, Pietzner M, Lindbohm JV, Wheeler E, Oerton E and Kerrison N (2022). Proteomic signatures for identification of impaired glucose tolerance. *Nat. Med.*, **28**(11): 2293-2300.
- Cataldi S, Bonavolontà V, Poli L, Clemente FM, De Candia M, Carvutto R and Fischetti F (2022). The relationship between physical activity, physical exercise and human gut microbiota in healthy and unhealthy subjects: A systematic review. *Biology (Basel)*, **11**(3): 479.
- Chen Y, Yang K, Xu M, Zhang Y, Weng X and Luo J (2024). Dietary patterns, gut microbiota and sports performance in athletes: A narrative review. *Nutrients*, **16**(11): 1634.
- Chen Y, Zhang P, Wang J, Gong Q, An Y and Qian X (2021). Associations of progression to diabetes and regression to normal glucose tolerance with development of cardiovascular and microvascular disease among people with impaired glucose tolerance: A secondary analysis of the 30-year Da Qing diabetes prevention outcome study. *Diabetologia*, **64**(6): 1279-1287.
- Cheng M, Li T, Hu E, Yan Q, Li H and Wang Y (2024). A novel strategy of integrating network pharmacology and transcriptome reveals antiapoptotic mechanisms of Buyang Huanwu decoction in treating intracerebral hemorrhage. *J. Ethnopharmacol.*, **319**: 117123.
- Cisneros E, Di Marco F, Rueda-Carrasco J, Lillo C, Pereyra G and Martín-Bermejo MJ (2020). Sfrp1 deficiency makes retinal photoreceptors prone to degeneration. *Sci. Rep.*, **10**(1): 5115.
- Evans PL, McMillin SL, Weyrauch LA and Witczak CA (2019). Regulation of skeletal muscle glucose transport and glucose metabolism by exercise training. *Nutrients*, **11**(10): 2432.
- Facchin S, Bertin L, Bonazzi E, Lorenzon G, De Barba C and Barberio B (2024). Short-chain fatty acids and human health: From metabolic pathways to current therapeutic implications. *Life (Basel)*, **14**(5): 559.
- Fujiwara Y, Eguchi S, Murayama H, Takahashi Y, Toda M, Imai K and Tsuda K (2019). Relationship between diet/exercise and pharmacotherapy to enhance the GLP-1 levels in type 2 diabetes. *Endocrinol. Diabetes Metab.*, **2**(3): e00068.
- Guo Y, Xu M, Wei Z, Hu Q, Chen Y and Wei Y (2018). Beneficial effects of qigong Five-animal Play in the improvement of health condition, prevention and treatment of chronic diseases: Evidence from a systematic review. *Evid. Based Complement. Alternat. Med.*, **2018**: 3235950.
- Kim CH (2023). Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cell Mol. Immunol.*, **20**(4): 341-350.
- Kyriachenko Y, Falalyeyeva T, Korotkyi O, Molochek N and Kobylak N (2019). Crosstalk between gut microbiota and antidiabetic drug action. *World J. Diabetes*, **10**(3): 154-164.
- Lang J, Li L, Quan Y, Tan R, Zhao J and Li M (2023). LC-MS-based metabolomics reveals the mechanism of the anti-gouty arthritis effect of Wuwei Shexiang pill. *Front. Pharmacol.*, **14**: 1213602.
- Lian K, Feng H, Liu S, Wang K, Liu Q and Deng L (2022). Insulin quantification towards early diagnosis of prediabetes/diabetes. *Biosens. Bioelectron.*, **203**: 114029.
- Li H, Tan Y, Cheng X, Zhang Z, Huang J and Hui S (2022). Untargeted metabolomics analysis of the hippocampus and cerebral cortex identified the neuroprotective mechanisms of Bushen Tiansui formula in an A β 25-35-induced rat model of Alzheimer's disease. *Front. Pharmacol.*, **13**: 990307.
- Liu Y, Chang J and Bai LD (2024). Intestinal flora: A new perspective on type 2 diabetes. *World J. Clin. Cases*, **12**(11): 1996-2007.
- Mukhopadhyaya I and Louis P (2025). Gut microbiota-derived short-chain fatty acids and their role in human health and disease. *Nat. Rev. Microbiol.*, **23**(10): 635-651.
- Ney LM, Wipplinger M, Grossmann M, Engert N and Mosig AS (2023). Short-chain fatty acids: Key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biol.*, **13**(3): 230014.
- Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M and Yousefinejad A (2019). The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo-controlled trial. *Diabetes Metab. Syndr. Obes.*, **12**: 175-182.
- Rohm TV, Meier DT, Olefsky JM and Donath MY (2022). Inflammation in obesity, diabetes and related disorders. *Immunity*, **55**(1): 31-55.
- Scheithauer TP, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, Van Raalte DH and Herrema H (2020). Gut microbiota as a trigger for metabolic inflammation in obesity and type 2 diabetes. *Front. Immunol.*, **11**: 571731.
- Su M, Tang T, Tang W, Long Y, Wang L and Liu M (2023). Astragalus improves intestinal barrier function and

- immunity by acting on intestinal microbiota to treat T2DM: A research review. *Front. Immunol.*, **14**: 1243834.
- Vazquez-Guajardo M, Rivas D and Duque G (2024). Exercise as a therapeutic tool in age-related frailty and cardiovascular disease: Challenges and strategies. *Can. J. Cardiol.*, **40**(8): 1458-1467.
- Wang K, Liu S, Kong Z, Zhang Y and Liu J (2019). Mind-body exercise (Five-animal Play) for patients with chronic obstructive pulmonary disease: A systematic review and meta-analysis of randomized controlled trials. *Int. J. Environ. Res. Public Health.*, **16**(1): 72.
- Wu J, Yang K, Fan H, Wei M and Xiong Q (2023). Targeting the gut microbiota and its metabolites for type 2 diabetes mellitus. *Front. Endocrinol. (Lausanne).*, **14**: 1114424.
- Xu W, Sang YQ, Liu XK, Geng HF, Wang B and Shi L (2023). Effect of glucagon-like peptide-1 receptor agonist on insulin secretion index and serum Wnt5a protein in patients with new-onset type 2 diabetes mellitus. *J. Diabetes Metab. Disord.*, **22**(1): 539-545.
- Yu DH (2008). Changes of fat metabolism in middle-aged and elderly people before and after exercise of Health Qigong and Five-animal Play for 6 months. *Chin. J. Sports Med.*, **27**(5): 610-611.
- Zou L, Zhang Y, Sasaki JE, Yeung AS, Yang L and Loprinzi PD (2019). Five-animal Play qigong as an alternative exercise for improving risk factors associated with metabolic syndrome: A meta-analysis of randomized controlled trials. *Int. J. Environ. Res. Public Health.*, **16**(8): 1396.