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# Compared to moderate-intensity continuous training, short-term high-intensity interval training demonstrates enhanced effects on metabolic flexibility in adult males with obesity

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ABSTRACT

*Background:* The aim of this study was to assess the effects of short-term high-intensity interval training (HIIT) and moderate-intensity continuous aerobic training (MICT) with matched energy expenditure on metabolic flexibility and other metabolic parameters in adult male individuals with obesity.

*Methods:* Twenty male individuals with obesity (age: 21.4 ± 1.5 years; body mass index: 31.0 ± 3.6 kg/m$^2$) were enrolled in this crossover design study. Participants were randomly allocated to an intervention sequence of 1) 3 consecutive days of HIIT (30 min, 6 × 2.5 min bouts at 90 % Peak Oxygen Uptake ($VO_{2peak}$), alternated with 2.5 min active recovery periods [25 % $VO_{2peak}$]), or 2) three consecutive days of MICT (60 min at 50 % $VO_{2peak}$), with a washout period of 1 week. Respiratory quotient (RQ), glucose, C-peptide, insulin, and non-esterified fatty acid (NEFA) levels were measured both during fasting and throughout a 180 min oral glucose tolerance test (OGTT), conducted before and after the intervention. The incremental area under the curve (iAUC) and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) were derived. ΔRQ was defined as the average RQ during the OGTT minus fasting RQ.

*Results:* Short-term exercise intervention significantly reduced HOMA-IR in male individuals with obesity ($P < 0.001$) and concurrently increased the Matsuda index ($P = 0.001$). Both exercise interventions led to a similar decrease in fasting RQ ($P = 0.001$), C-peptide, insulin, and HOMA-IR ($P < 0.001$). Additionally, they reduced the iAUC for glucose and insulin during the OGTT ($P < 0.001$). The ΔRQ and the RQ iAUC in the HIIT group were significantly higher than those in the MICT group, and different exercise intensities exhibited interactive effects within groups for RQ iAUC and ΔRQ ($P = 0.013$ and $P = 0.012$).

*Conclusions:* Two short-term exercise interventions similarly improved glucose tolerance, with HIIT demonstrating a more advantageous effect on metabolic flexibility than MICT in male individuals with obesity.

*Trial registration:* The trial was officially registered at www.chictr.org.cn (ChiCTR2300072884).

## 1. Background

Physiologically, the human body demonstrates substantial adaptability to fluctuating energy sources and demands. This manifests as the capacity to flexibly modulate the ratio of carbohydrates to fat metabolism based on energy substrate availability and requirements. This adaptability is termed metabolic flexibility.[1,2] In metabolically healthy individuals, substrate oxidation can adapt rapidly and comprehensively to changes in dietary intake on, energy requirements, environmental influences, and internal cues.[3] Metabolic inflexibility and impaired metabolic flexibility are primarily characterized by decreased fasting fat oxidation and a diminished ability to increase carbohydrate oxidation in response to food intake.[4] Metabolic inflexibility has been revealed in some populations with chronic diseases, including obesity,[3,5] insulin resistance,[6] and type 2 diabetes.[7,8]

In China, 50.7 % of adults are overweight or obese. The rising male

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predominance in obesity prevalence coincides with testosterone-mediated visceral adiposity and mitochondrial dysfunction that significantly impair metabolic flexibility in this population.[9,10] Overweight and obesity are acknowledged contributors to significant metabolic disorders, including cardiovascular disease and type 2 diabetes.[11] Metabolic flexibility, a critical determinant of energy homeostasis, becomes compromised in obesity, manifesting as impaired substrate switching capacity.[12] This disparity between fat intake and oxidation promotes ectopic lipid accumulation and lipotoxicity,[13,14] characterized by ceramide and diacylglycerol deposition that disrupts insulin signaling, ultimately driving systemic insulin resistance—a hallmark of chronic metabolic diseases.

Exercise is an effective nonpharmacological treatment to enhance metabolic flexibility.[15,16] Eight weeks of moderate-intensity continuous training (MICT) can increase variances in respiratory quotient (RQ) and decrease variances in insulin in individuals with obesity,[17] while short-term high-fat diet challenges reveal enhanced fat oxidation after 10-day MICT protocols.[12] Previous experiments have also validated the beneficial effects of MICT on substrate metabolism in populations with obesity.[12,18,19] In recent years, researchers have discerned that high-intensity interval training (HIIT) can achieve comparable or superior effects to those of MICT in certain aspects, such as improving aerobic capacity, lowering lipids and body weight, and enhancing postprandial insulin sensitivity.[20–22] Additionally, HIIT is recognized for its time-efficiency. Nevertheless, the majority of these studies have not matched the energy expenditure of both forms of exercise; thus, precluding a precise elucidation of metabolic outcomes solely based on exercise intensity. Moreover, long-term studies often accompany changes in body weight and body fat, resulting in research gaps when comparing exercise interventions independent of changes in body composition.

The distinct outcomes of diverse exercise modalities with equal energy expenditure on the short-term enhancement of metabolic flexibility among adults with obesity remain uncertain. Understanding these effects may assist in determining an informed selection of optimal exercise prescriptions to rectify metabolic inflexibility and prevent metabolic pathologies in populations with obesity. Accordingly, our research aimed to investigate the degree of enhancement in metabolic flexibility (assessed via the RQ during fasting and oral glucose tolerance test [OGTT] conditions) following a short-term exercise intervention. Additionally, we sought to discern the differing impacts of MICT and HIIT on metabolic flexibility, as well as related blood metabolic markers (including glucose, insulin, C-peptide, non-esterified fatty acid [NEFA], and insulin sensitivity [HOMA-IR] levels) in adult male patients with obesity. We hypothesized that (1) both short-term MICT and HIIT interventions would enhance metabolic flexibility and blood parameters in adult men with obesity, and (2) HIIT would demonstrate superior effectiveness to MICT in improving metabolic flexibility.

## 2. Materials and methods

### 2.1. Participants

For this study, we recruited 20 sedentary adult male individuals with obesity from the university campus and surrounding communities through online or offline advertising. The eligibility criteria for participation were as follows: male sex; age of 18–40 years; body mass index (BMI) $\geq$ 28 kg/m$^2$ (recommended by Working Group of Obesity, China [WGOC]); body fat percentage of at least 25 %[23]; non-diabetic participants with normal blood glucose (fasting <5.6 mmol/L and 2h postprandial <7.8 mmol/L according to American Diabetes Association [ADA] criteria); non-hypertensive individuals with normal blood pressure (systolic <120 mmHg and diastolic <80 mmHg, per ACC/AHA 2023 guidelines); average daily sedentary time $\geq$8 h or >60 % of the time wearing the accelerometer; and absence of a habitual exercise routine; and no current use of medications or supplements known to affect metabolism (e.g., for weight loss, diabetes, hypertension, lipid disorders, or thyroid function). Individuals who had a weight change exceeding 2 kg within the past 2 months, with diabetes or pre-diabetic conditions, who were taking medications or supplements that affect metabolism, and with physical disabilities or illnesses that affected physical activity were excluded from the study. The participants who completed the initial online assessment proceeded to the laboratory to be screened for physical composition, physical activity levels, blood indicators, medical history, and other relevant parameters.

All eligible participants, who comprehended the experimental procedures and demonstrated willingness to participate, provided written informed consent. This study was approved by the Ethics Committee of Shanghai University of Sports. The trial was officially registered at www.chictr.org.cn (ChiCTR2300072884). This study was conducted in 2023 at Shanghai University of Sport (Shanghai, China).

### 2.2. Study design

The overall experimental design is illustrated in Fig. 1, where participants were required to complete two exercise intervention protocols and undergo metabolic flexibility testing before and after each intervention. The trial implemented a randomized controlled crossover design. After screening for basic conditions and behavioral patterns, participants were randomly assigned to two intervention sequences (HIIT-MICT or MICT-HIIT) via block randomization (block size = 4) generated by Research Randomizer software, with allocation concealment achieved through sequentially numbered sealed opaque envelopes administered by non-study personnel. Each sequence consisted of 3 consecutive days of MICT and HIIT interventions, separated by a standardized 7-day washout period to eliminate carryover effects. Blinded statisticians analyzed outcome data without access to group allocation information.

Prior to the initiation of each exercise group, baseline assessments were performed, including tests for the fasting RQ and an oral glucose tolerance test (OGTT). The post-intervention evaluation comprised assessments conducted on the immediate following day (D1) and 2nd day (D2) after 3 consecutive days of the exercise session. The D1 assessment paralleled the baseline test in terms of content, whereas the D2 assessment gauged the fasting RQ to scrutinize the temporal impact of short-term exercise on resting metabolism.

### 2.3. Body composition

The participants arrived at the laboratory in a fasted condition early in the morning and wearing light clothing. Body composition and weight were assessed using a dual-energy X-ray absorptiometry device (DXA Prodigy; GE Lunar Corp., Madison, WI, USA). Height was measured using a stadiometer (HK6000-ST, Hengkang Jiaye Technology Co., Ltd., Jiangsu Sheng, China). Height and weight were measured with a precision of 0.1 cm and 0.1 kg, respectively. A soft tape measure was employed for the measurement of waist and hip circumferences, and the mean of the two measurements was calculated to the closest 0.5 cm if the difference was $\leq$1 cm.

### 2.4. Cardiorespiratory fitness

Peak oxygen uptake ($VO_{2peak}$) was directly assessed by a graded exercise protocol on a stationary bike (Monark Ergomedic 839E, Varberg, Sweden) while the participants wore a respiratory mask. Gas analysis was conducted using a TrueOne 2400 Metabolic Measurement System (Parvo Medics, Sandy, UT, USA). The exercise test protocol comprised an initial load of 50 W, which increased by 15 W/min while maintaining a cadence of 60 rpm. The participants were encouraged to continue until exhaustion or volitional fatigue. Electrocardiography and heart rate monitoring during testing were performed using a telemetry system (WEP-5200, Nihon Kohden Corp., Tokyo, Japan). The criteria for

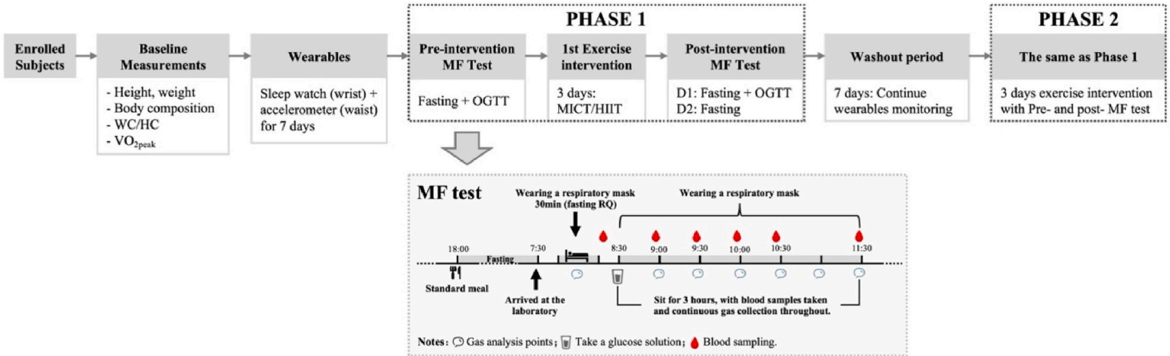


**Fig. 1.** Experimental design diagram Abbreviations: WC, waist circumference; HC, hip circumference; MF, metabolic flexibility; OGTT, oral glucose tolerance test; MICT, moderate-intensity continuous training; HIIT, high-intensity interval training.

determining $VO_{2peak}$ were as follows: 1) $VO_2$ reached a plateau; 2) heart rate reached 95 % of the maximum heart rate (maximum heart rate = 207–0.7 × age); 3) RQ ≥ 1.0; and 4) the participant was requested to stop the experiment based on subjective perception. At least two out of these four conditions should have been met.[24]

### 2.5. Fasting metabolism and OGTT

The fasting and the oral glucose tolerance test conditions, combined with indirect calorimetry, are commonly used to assess metabolic flexibility during the transition from fasted to fed states. Following their arrival at the laboratory at approximately 7:30 the subsequent morning, participants were instructed to sit quietly for 10 min while donning a Polar heart rate monitor. They then wore a TrueOne 2400 mask and rested on the test bed for 30 min in the laboratory, maintained at approximately 25 °C; during this time, they were asked to remain quiet and avoid movement. The fasting blood sample was collected, following which the participants drank the glucose solution. The researchers drew blood at 30, 60, 90, 120, and 180 min. Participants wore masks throughout the entire 3 h OGTT. Gas collection and analysis were conducted using the TrueOne 2400 metabolic analyzer. Key indicators reflecting metabolic flexibility, including RQ, ΔRQ, and area under the incremental RQ curve (RQ iAUC), were measured during the OGTT.

### 2.6. Blood samples

Venous blood was drawn using disposable intravenous indwelling needles (ABLE, 22G Y-type, 0.90 mm × 25 mm) (4 mL per draw) at fasting (0 min) and during OGTT at 30-min intervals (30, 60, 90, 120, and 180 min), with a total sampled volume of 24 mL per subject during each metabolic flexibility test. After 30-min clotting at 22 ± 1 °C, centrifugation was performed at 3000×*g* (Bioridge TDL-8M) for 15 min at 4 °C. Serum aliquots were stored at −80 °C (Haier DW-86L486, dual microprocessor control with ±1 °C stability and 24h temperature monitoring system) with ≤2 freeze-thaw cycles.

Fasting and post-OGTT samples were analyzed for glucose (hexokinase method, Roche Cobas c702), insulin/C-Peptide (ECLIA, Roche Elecsys E601), and NEFAs (enzymatic colorimetry, Wako Diagnostics 999–34691). All assays were conducted at CAP-accredited Qianmai Biomedical Laboratory (Shanghai) following manufacturer protocols, with inter-assay CVs <5 % for all analytes. The iAUC calculations employed trapezoidal method with baseline correction. The insulin sensitivity index (ISI)-Matsuda and insulin resistance index (HOMA-IR) were calculated based on fasting blood glucose, fasting insulin, average 3 h blood glucose concentration, and insulin concentration.[25,26]

### 2.7. Exercise interventions

All exercise training in this study was supervised and conducted at the Fitness Science Museum at the Shanghai University of Sports. The exercise intervention commenced at 19:30 daily. All participants completed 3 consecutive days of HIIT and MICT during the trial period using the stationary bike, selected for its capacity to standardize workload measurement and minimize knee joint stress in obese individuals.[27,28] The exercise protocol referenced the load-intensity scheme in Trombold et al.[29] to match energy expenditure and underwent pre-experimental validation. The HIIT group followed a protocol consisting of alternating intervals: 2.5 min at 25 % $VO_{2peak}$ followed by 2.5 min at 90 % $VO_{2peak}$, repeated for a total duration of 30 min. The MICT group exercised continuously at 50 % $VO_{2peak}$ for 60 min. Exercise intensity (cycle ergometer workload) was calculated based on each participant's measured $VO_{2peak}$ using the standardized formula from the *ACSM's Guidelines for Exercise Testing and Prescription (*10th Edition*)*. During interventions, trained researchers ensured intensity adherence through continuous heart rate monitoring (Polar H10) and real-time workload adjustment (±5W) to maintain target intensity, with all sessions directly supervised.

Given the excess post-exercise oxygen consumption (EPOC) elicited by HIIT, we recruited five male individuals with obesity, each undergoing MICT and HIIT sessions twice in the laboratory. EPOC duration was determined as the time required for oxygen consumption to return to pre-exercise baseline levels and remain stable for at least 10 consecutive minutes, as continuously monitored by gas analyzer (Trueone 2400). The monitoring duration was appropriately extended to ensure consistent total measurement time covering both exercise and recovery periods, ultimately establishing the observation windows: MICT (60-min exercise + 120-min recovery) and HIIT (30-min exercise + 150-min recovery). More concretely, during the HIIT sessions, participants engaged in the prescribed exercise regimen for 30 min, followed by a 150 min sedentary recovery period. In the MICT sessions, participants exercised according to the prescribed regimen for 60 min, followed by a 120 min sedentary recovery period. Throughout the entire experiment, participants' energy expenditure was monitored using the TrueOne gas exchange analyzer to assess whether the total energy expenditure during the "exercise + recovery period" was equivalent between the two modalities.

### 2.8. Metabolic control

The participants were instructed to refrain from engaging in any form of physical exercise outside the experiment. During the week prior to the baseline and washout periods, participants wore motion logger sleep watches (Ambulatory Monitoring, Inc., Ardsley, NY, USA) on their non-dominant wrists to monitor sleep quality. Motionlogger WatchWare 1.94.1.3 software (Amburory Monitoring Inc.) was used with the Sadeh algorithm for scoring.[30] Additionally, participants were requested to wear a compact triaxial accelerometer (ActiGraph wGT3X-BT; Pensacola, FL, USA) to assess their physical activity. The accelerometer was positioned on the hip during hours out of bed, except while bathing or swimming. An accelerometer data analysis was conducted using the ActiLife software (version 6.13.4, ActiGraph). Daily average sedentary and vigorous activity times and percentages were calculated using the Freedson cut-off points.[31] Wearing the accelerometer for a minimum of 10 h per day was determined as valid wear time.[32]

Within 48 h before each assessment, participants were directed to abstain from alcohol and caffeinated beverages, avoid late nights, and manage their diet 24 h before the test by restricting the consumption of high-calorie and high-fat foods. Participants were expected to arrive at the laboratory before 18:00 on the preceding evening. Dinner, a standardized meal contributing to 30 % of the daily energy requirement and comprising 50 % carbohydrate, 30 % protein, and 20 % fat, was completed by 18:30. The participants were advised not to consume more food post-dinner. The period between dinner and sleep was largely sedentary, even though light physical activity was permissible. Normal sleep patterns adhered to certain factors, such as exercise, diet, and sleep, among others, which were meticulously regulated to mitigate their impact on experimental outcomes. Additionally, throughout the exercise intervention period, participants were verbally instructed to maintain their habitual dietary patterns and abstain from alcohol and caffeinated beverages.

### *2.9. Statistical analyses*

ActiLife software processed the accelerometer data. The HOMA-IR was derived based on fasting glucose and insulin levels. For the blood indices of glucose, insulin, C-peptide, and NEFAs during the OGTT, the iAUC was determined with a comparison to fasting levels. In the fasting metabolism tests, the baseline RQ was recorded as $RQ_0$. $\Delta RQ$ was obtained by subtracting $RQ_0$ from the average RQ values at 30, 60, 90, 120, 150, and 180 min during the OGTT, representing the metabolic flexibility of the participants during this test.

The sample size was determined using G*Power 3.1 based on a two-tailed paired *t*-test. Parameters included $\alpha = 0.05$, power $(1-\beta) = 0.8$, and effect size ($f = 0.4$), derived from our pilot study ($n = 10$) showing a mean difference of 1.2 ± 1.8 in insulin sensitivity index (ISI) between HIIT and MICT interventions. A minimum of 14 participants was required to detect this effect. To account for potential attrition (estimated 20 %) and technical failures, 20 participants were enrolled, ensuring adequate statistical power even with a 30 % dropout rate.

Statistical analyses were conducted using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was determined utilizing the Kolmogorov–Smirnov test. Data adhering to the normal distribution were reported as means ± standard deviations; data that did not adhere to the normal distribution were transformed using logarithmic or square root conversions before analysis. Data from the participants were analyzed using a two-way repeated measures analysis of variance. Paired t-tests were employed to test group differences at different time points. A covariance analysis was performed to compare the between-group $\Delta RQ$ (with the remaining RQ as a covariate). All statistics were analyzed using two-tailed tests with significance set at $P < 0.05$.

## 3. Results

### *3.1. Participants*

Initially, 51 volunteers were recruited, 22 of whom were included in the study. Ultimately, only 20 participants (age: 21.4 ± 1.5 years; BMI: 31.0 ± 3.6 kg/m$^2$) successfully completed the study; the two withdrawals were attributed to personal reasons. The participants' characteristics are listed in Table 1.

**Table 1**
Participant characteristics.

| Characteristic | All (n = 20) |
|---|---|
| Age (years) | 21.4 ± 1.5 |
| Height (cm) | 177.6 ± 5.7 |
| Weight (kg) | 99.9 ± 13.7 |
| Body mass index (kg/m$^2$) | 31.0 ± 3.6 |
| Percent body fat (%) | 35.4 ± 4.9 |
| Fat mass (kg) | 34.5 ± 8.4 |
| Fat-free mass (kg) | 62.0 ± 8.1 |
| Waist circumference (cm) | 101.6 ± 7.8 |
| Hip circumference (cm) | 109.3 ± 7.3 |
| Waist-to-hip ratio | 0.93 ± 0.02 |
| Absolute $VO_{2peak}$ (L/min) | 2.74 ± 0.42 |
| Relative $VO_{2peak}$ (mL/kg/min) | 27.84 ± 4.17 |

Data are presented as means ± standard deviations.
$VO_{2peak}$, peak oxygen uptake.

### *3.2. Metabolic control*

Participants in the MICT group exhibited significantly higher energy expenditure during exercise than those in the HIIT group ($P < 0.05$), whereas participants in the HIIT group showed significantly higher energy expenditure during the recovery period than did those in the MICT group ($P < 0.05$). There was no significant difference in total energy expenditure between the two modalities ($P > 0.05$). The energy metabolism results are presented in Supplementary Table 1.

Physical activity and sleep data are presented in Supplementary Table 2, and no significant differences were observed between the baseline and washout periods. Fig. 2 shows the trial flowchart.

### *3.3. Blood samples*

The results of the blood metabolic markers during fasting and OGTT are presented in Table 2. Exercise treatment significantly reduced the glucose iAUC ($P < 0.001$), fasting insulin level ($P < 0.001$), insulin iAUC ($P < 0.001$), fasting C-peptide level ($P < 0.001$), and C-peptide iAUC ($P < 0.001$). Simultaneously, both types of exercise interventions significantly reduced the HOMA-IR ($P < 0.001$) and increased the ISI-Matsuda ($P = 0.001$); the two types of exercises showed an interaction effect on C-peptide iAUC ($P = 0.029$), whereas no group × treatment differences were observed among the other blood indicators. Temporal variations in blood parameters during fasting and the OGTT are presented in Fig. 3; more specific data regarding blood biochemical indicators are presented in Supplementary Table 3.

### *3.4. RQ*

The fasting RQ significantly decreased after both interventions, with no between-group differences. A significant group × treatment effect on the RQ iAUC ($P = 0.012$) and $\Delta RQ$ was observed ($P = 0.013$). Participants in the HIIT group had a significantly lower RQ iAUC (pre: 6.26 ± 5.14 vs. post: 10.12 ± 6.19; $P < 0.05$) and $\Delta RQ$ (pre: 0.038 ± 0.031 vs. post: 0.060 ± 0.037; $P < 0.05$) compared to the corresponding of participants in the MICT group (Table 3). The temporal variations in the RQ during fasting and OGTT are presented in Fig. 3.

On D2, the MICT fasting RQ (0.81 ± 0.05) and HIIT fasting RQ (0.81 ± 0.06) significantly increased compared to the fasting RQ on tD1 ($P < 0.05$), whereas no significant distinction was noted compared to the fasting RQ before exercise.

## 4. Discussion

The main findings of this study were that both MICT and HIIT decreased fasting RQ, indicating improved fasting fat oxidation levels, a key criterion for assessing metabolic flexibility. During the OGTT, both exercise treatments similarly increased insulin sensitivity and reduced

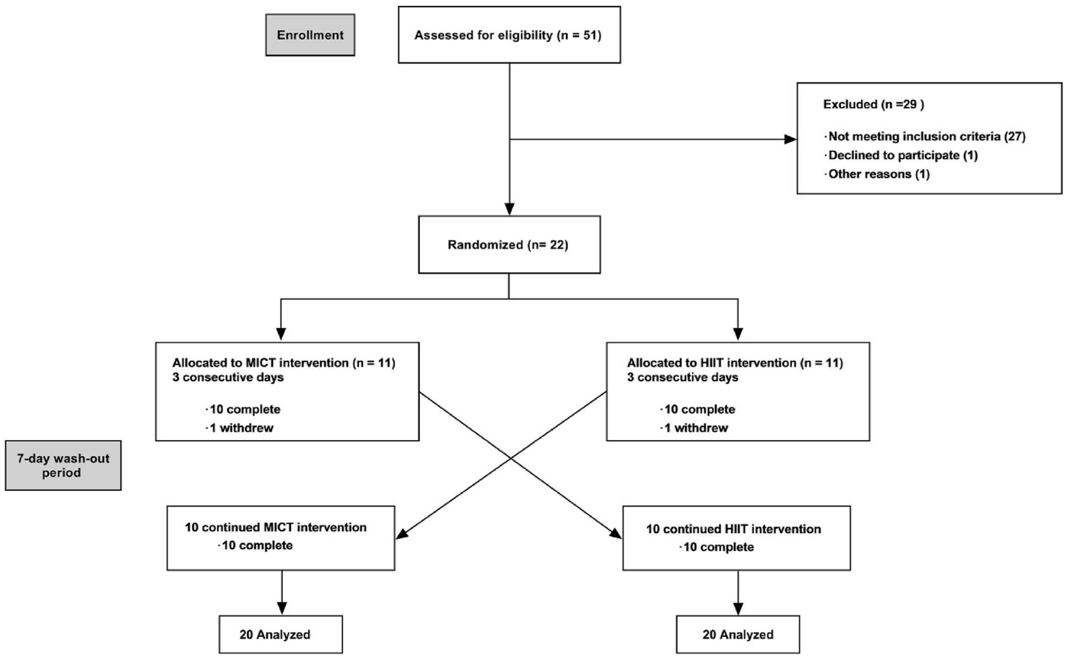


**Fig. 2.** Participant flowchart Abbreviations: HIIT, high-intensity interval training; MICT, moderate-intensity continuous aerobic training.

**Table 2**
Effects of MICT and HIIT on fasting and the OGTT blood biochemical indicators.

| | MICT (n = 20) | | HIIT (n = 20) | | P-value | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | T | G × T |
| HOMA-IR | 4.01 ± 1.61 | 2.80 ± 1.48* | 3.54 ± 1.09 | 2.81 ± 1.20* | 0.001 | 0.384 |
| ISI Matsuda | 2.53 ± 1.00 | 4.19 ± 3.13* | 2.57 ± 0.94 | 3.63 ± 1.92* | 0.001 | 0.436 |
| Glucose (mmol/L) | | | | | | |
| Fasting | 5.01 ± 0.32 | 5.04 ± 0.21 | 5.01 ± 0.25 | 4.95 ± 0.24 | 0.770 | 0.430 |
| Glucose iAUC | 354 ± 151 | 289 ± 88 | 395 ± 118 | 303 ± 101* | <0.001 | 0.527 |
| Insulin (μIU/mL) | | | | | | |
| Fasting | 17.82 ± 6.26 | 12.45 ± 6.22* | 15.93 ± 4.87 | 12.69 ± 5.29* | <0.001 | 0.338 |
| Insulin iAUC | 14035 ± 7256 | 10453 ± 4183* | 13998 ± 6896 | 10558 ± 3824* | <0.001 | 0.926 |
| C-peptide (ng/mL) | | | | | | |
| Fasting | 3.23 ± 0.70 | 2.64 ± 0.50* | 3.05 ± 0.54 | 2.80 ± 0.60* | <0.001 | 0.066 |
| C-peptide iAUC | 949 ± 328 | 842 ± 249 | 1107 ± 326 | 822 ± 193* | <0.001 | 0.029 |
| NEFA (mmol/L) | | | | | | |
| Fasting | 0.48 ± 0.17 | 0.54 ± 0.19 | 0.51 ± 0.19 | 0.52 ± 0.17 | 0.166 | 0.298 |
| NEFA iAUC | −52 ± 23 | −54 ± 26 | −60 ± 26 | −53 ± 24 | 0.513 | 0.220 |

Data are presented as means ± standard deviations.
No significant differences were found between the HIIT and MICT exercise groups. *P < 0.05 compared to MICT. Treatment (T) (Pre vs. Post), and group × treatment (G × T; MICT vs. HIIT).
Abbreviations: HIIT, high-intensity interval training; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; iAUC, incremental area under the curve; ISI, insulin sensitivity index; MICT, moderate-intensity continuous aerobic training; NEFAs, non-esterified fatty acids; OGTT, oral glucose tolerance test.

blood glucose levels. However, compared to those undergoing MICT, male individuals with obesity undergoing HIIT demonstrated a reduction in C-peptide iAUC and an increase in both ΔRQ and RQ iAUC, indicating improved β-cell function and enhanced efficiency in glucose utilization. Overall, these results imply that exercise treatment, regardless of intensity, can improve fasting fat metabolism and glucose tolerance, consistent with previous findings that established the beneficial effects of exercise on metabolic health.[33–36] Moreover, we provide evidence that short-term HIIT can comprehensively improve metabolic flexibility in male individuals with obesity within the physiological framework of fasting and glucose loading, highlighting the importance of exercise intensity for enhancing metabolic flexibility.

The ability to utilize fatty acids during fasting is an important factor for determining metabolic flexibility. We found that both MICT and HIIT significantly lowered the post-exercise RQ in male individuals with obesity. These data imply that exercise, independent of variations in exercise intensity, can enhance fasting fat oxidation in cohorts with obesity, consistent with previous findings.[37,38] These results could be attributed to the ability of exercise to stimulate the effect of catecholamines on β-adrenergic receptors in the adipose tissue, thus, fostering the liberation of NEFA.[39] This aligns with our NEFA data; although there is no significant difference, numerical trends indicate an increase in the NEFA levels following exercise. Simultaneously, the investigation unveiled that a training regimen concurrently upregulated the abundance of positive (CGI-58) and negative (G0S2) regulatory factors governing the activity of adipose triglyceride lipase (ATGL). This augmentation of these protein entities serves to fortify the lipid solubilization regulation within skeletal muscle, thereby fostering a swifter and more precise adjustment of intracellular fatty acid availability.[33] The elevated levels of fatty acids in the blood align with the heightened availability of intracellular fatty acids in tissues, providing a plausible explanation for our findings.

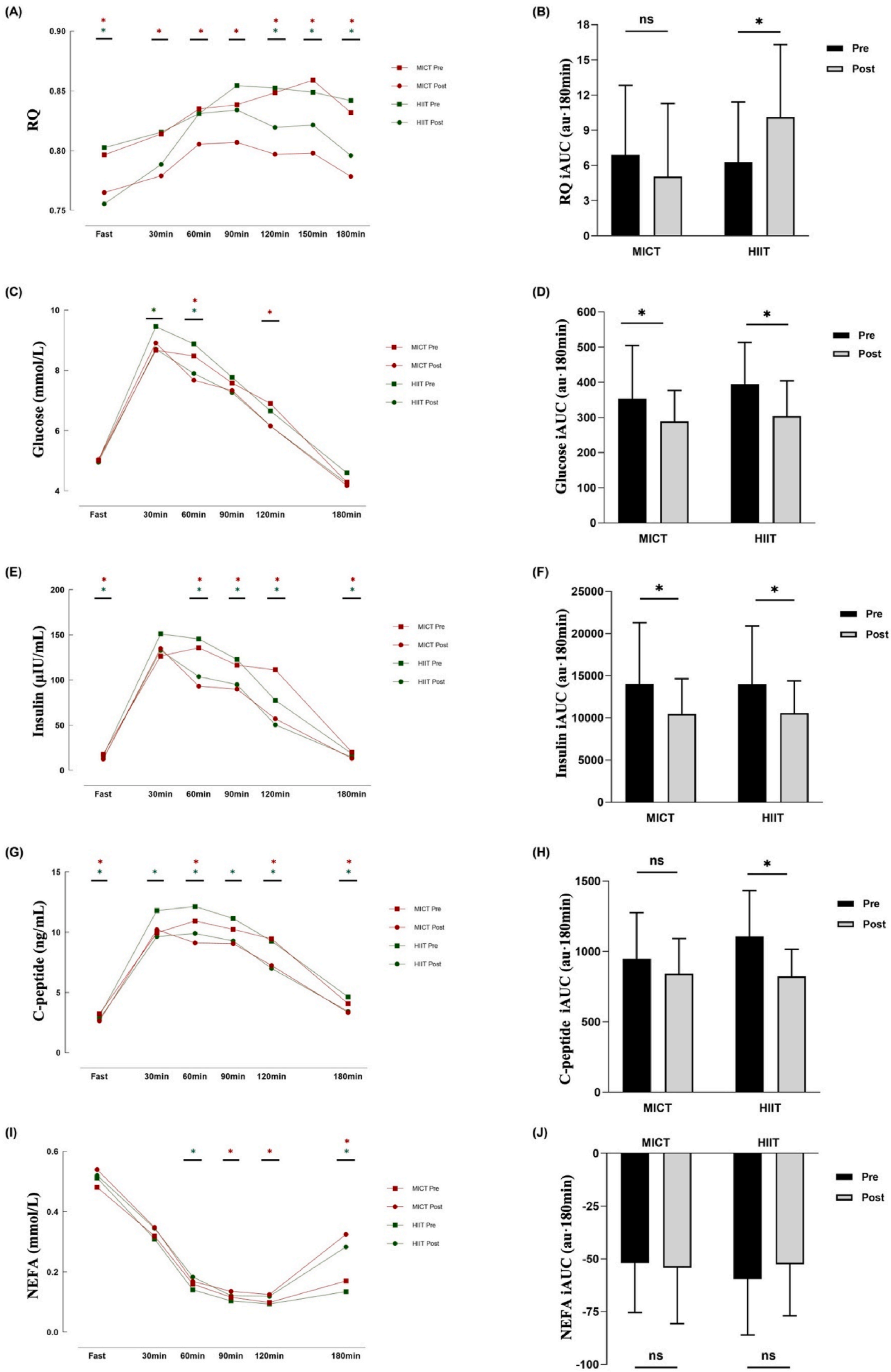


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**Fig. 3.** Influence of MICT and HIIT on metabolic outcomes.
Influence during fasting and the OGTT phase RQ for each group (a), RQ response iAUC (b), blood glucose for each group (c), blood glucose response (iAUC) (d), insulin for each group (e), insulin response (iAUC) (f), C-peptide for each group (g), C-peptide response (iAUC) (h), NEFA for each group (i), and NEFA response (iAUC) (j).
Fasting values correspond to the rest metabolic period, that is, before the OGTT; 30, 60, 90, 120, 150 (only for RQ), 180 min represent the min time after the intake of 75 g of glucose. *within group difference, *$P < 0.05$. MICT group (red) or HIIT group (green). All iAUCs are represented as mean values with standard deviation (SD) (MICT n = 20, HIIT n = 20)
Abbreviations: HIIT, high-intensity interval training; iAUC, incremental area under the curve; MICT, moderate-intensity continuous aerobic training; ns, represents no statistical value; OGTT, oral glucose tolerance test; RQ, respiratory quotient.

Interestingly, we observed a time-dependent effect of short-term exercise on fasting fat oxidation. Our findings indicated no significant difference in the fasting RQ at 36 h post-intervention in comparison to baseline, implying that short-term exercise primarily yields a short-term impact on the reduction of fasting fat oxidation. Exercise-induced changes in fat metabolism may serve to conserve carbohydrates by preserving diminished glycogen stores, redirecting energy utilization toward fat, enhancing peripheral fat oxidation, and attenuating re-esterification rates.[40] Beyond a specific temporal threshold, the replenishment of muscle glycogen depletion incurred by exercise and dissipation of alterations in skeletal muscle metabolism rate and hormone levels may contribute to the restoration of fasted fat oxidation to pre-exercise levels. Schenk et al.[41] suggested that the beneficial influence of exercise on fat metabolism is limited to a 16 h window. Thus, the precise timeframe remains uncertain and requires further experimental validation.

Regarding changes in glucose tolerance during the OGTT, on the one hand, our research found that 3 consecutive days of HIIT and MICT engendered analogous attenuations in the glucose, insulin, and C-peptide AUC during the OGTT in males with obesity. Insulin sensitivity significantly increased to a similar degree in the two groups. It is common to observe an increase in mitochondrial density, skeletal muscle microvascular density, and fat oxidation following training, which is often linked to enhanced insulin sensitivity.[42–45] Additionally, exercise-induced upregulation of nuclear peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) mediates the expression and distribution of glucose transporter 4, thereby enhancing the efficiency of glucose transport[46–48]; on the other hand, a significant intergroup interaction effect was observed for C-peptide iAUC during OGTT. This implies that HIIT elicited a more substantial reduction in C-peptide iAUC, suggesting that alongside the heightened insulin sensitivity and diminished demand, the HIIT intervention elicited a more expedited modulation of C-peptide and insulin secretion by pancreatic β-cells compared to MICT. We conjecture that HIIT potentially augments pancreatic β-cell functionality, thereby enhancing their efficacy or sensitivity in insulin secretion, in alignment with the perspectives articulated by Malin et al.[49]

The variations in RQ, observed during the fasting and postprandial periods (ΔRQ, RQ iAUC), are indicative of the body's capacity to transition between substrate oxidation states and the level of metabolic flexibility. We have discovered noteworthy findings indicating that HIIT significantly increased ΔRQ and RQ iAUC during the OGTT compared to MICT. Incorporating our hematological parameter findings, our findings suggest that alongside equivalent improvements in insulin sensitivity, HIIT demonstrates a superior glucose oxidation rate during OGTT compared to MICT. This implies potentially enhanced insulin utilization within tissues. Consistent with the previously mentioned enhancement of fasting fat oxidation, this suggests that short-term HIIT confers a more comprehensive amelioration of metabolic flexibility in male individuals with obesity within the physiological framework of fasting and glucose loading. In contrast, mitochondrial biogenesis is closely associated with improved metabolic flexibility.[50] Concerning skeletal muscle adaptations, cellular stress and the consequent mitochondrial biogenic signaling are predominantly influenced by the intensity of exercise. Compared to low-intensity exercise, high-intensity exercise elicits an increase in mitochondrial protein synthesis,[51] intimating superior mitochondrial biogenesis when undertaken at high intensities.[15] Investigations have exhibited that one to six bouts of high-intensity intermittent exercise can increase markers of mitochondrial biogenesis, function, and enzymatic activities.[52,53] Conversely, short-term HIIT increases the phosphorylation of AMP-activated protein kinase (AMPK) α1, AMPKα2, and p38 mitogen-activated protein kinase (p38 MAPK) post-exercise, alongside an upregulation in the mRNA expression of PGC-1α at 3 h post-exercise. Consequently, the specific signaling pathways from AMPK and p38 MAPK to PGC-1α may partly elucidate the metabolic adaptations induced by HIIT, including mitochondrial biogenesis and augmented glucose and fatty acid oxidation capacities.[52,54] These alterations provide evidence for the improvement of metabolic flexibility by HIIT. However, our findings do not align with those of Gilbertson et al.,[37] who did not observe any improvement in metabolic flexibility with HIIT. This discrepancy may be attributed to differences in the participants' ages and exercise cycle. Subsequent research should explore how age relates to the impact of exercise on metabolic flexibility in men with obesity.

**Table 3**
Effects of MICT and HIIT on respiratory quotient data during fasting and the OGTT.

| | MICT | | HIIT | | P-value | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | T | G × T |
| Fasting RQ | 0.80 ± 0.04 | 0.77 ± 0.05* | 0.80 ± 0.06 | 0.76 ± 0.06* | 0.001 | 0.469 |
| 30 min | 0.81 ± 0.05 | 0.78 ± 0.03* | 0.82 ± 0.06 | 0.79 ± 0.05 | 0.002 | 0.652 |
| 60 min | 0.84 ± 0.05 | 0.81 ± 0.05* | 0.83 ± 0.05 | 0.83 ± 0.06 | 0.112 | 0.112 |
| 90 min | 0.84 ± 0.05 | 0.81 ± 0.04* | 0.85 ± 0.05 | 0.83 ± 0.07 | 0.023 | 0.610 |
| 120 min | 0.85 ± 0.04 | 0.80 ± 0.05* | 0.85 ± 0.06 | 0.82 ± 0.06* | 0.001 | 0.442 |
| 150 min | 0.86 ± 0.05 | 0.80 ± 0.06* | 0.85 ± 0.06 | 0.82 ± 0.06* | 0.002 | 0.215 |
| 180 min | 0.83 ± 0.03 | 0.78 ± 0.06* | 0.84 ± 0.05 | 0.80 ± 0.05* | <0.001 | 0.718 |
| RQ iAUC | 6.91 ± 5.91 | 5.05 ± 6.24 | 6.26 ± 5.14 | 10.12 ± 6.19* | 0.365 | 0.012 |
| △RQ | 0.041 ± 0.035 | 0.029 ± 0.037 | 0.038 ± 0.031 | 0.060 ± 0.037* | 0.477 | 0.013 |

Data are presented as means ± standard deviations.
ΔRQ represents the postprandial average RQ minus the fasting RQ.
*$P < 0.05$ compared to MICT. Treatment (T) (Pre vs. Post), and group (G) × treatment (G × T; MICT vs. HIIT). ΔRQ outcomes were co-varied for fasting RQ.
Abbreviations: HIIT, high-intensity interval training; iAUC, incremental area under the curve; ISI, insulin sensitivity index; MICT, moderate-intensity continuous aerobic training; OGTT, oral glucose tolerance test; RQ, respiratory quotient.

The innovation of this study lies in: (1) Unlike previous studies focusing solely on acute post-exercise responses (D1), we systematically tracked the sustained effects on Day 2 (D2). The D1 assessment replicated baseline tests, while D2 fasting RQ measurements revealed the time-dependent modulation of resting metabolism by short-term exercise. (2) Our study addresses a gap in literature by demonstrating that HIIT confers more comprehensive improvements in metabolic flexibility than MICT under both fasting and glucose-challenged states in obese males. Additionally, this study had some limitations. First, we employed a 75 g glucose load during the OGTT to simulate postprandial physiology, which failed to reflect real-world circumstances. In daily life, we customarily ingest diverse nutrients rather than solely glucose; however, the use of a single nutrient can eliminate the interference from complex nutritional components and provide a more standardized assessment of glucose metabolism. Future studies should investigate the effects of exercise on lipid metabolism under high-fat conditions. In addition, this investigation has been limited to discussions concerning male individuals. Future research endeavors should endeavor to broaden the scope to encompass a more comprehensive demographic spectrum.

## 5. Conclusion

In conclusion, short-term HIIT or MICT with matched energy expenditure resulted in comparable improvements in fasting fat oxidation and glucose tolerance. However, compared with MICT, short-term HIIT demonstrated a greater improvement in β-cell function and glucose utilization levels after glucose loading, resulting in a superior enhancement in metabolic flexibility. Simultaneously, it possesses a "time-efficiency" advantage. Additionally, changes in fasting oxidation levels induced by short-term exercise returned to pre-exercise levels after 36 h. This study fills a gap in the literature by investigating the acute effects of short-term HIIT on metabolic flexibility, while revealing distinct metabolic impacts of HIIT compared to MICT.

This study offers valuable insights into the selection of exercise regimens for the management of metabolic disorders. Our findings support the idea that short- HIIT can improve metabolic flexibility in male individuals with obesity compared to MICT, suggesting that metabolic flexibility enhancement through exercise may be associated with exercise intensity. These results indicate that HIIT could be preferentially recommended for obese men seeking to enhance their metabolic responsiveness, particularly when time-efficient exercise strategies are needed. In addition, metabolic changes caused by exercise have a time-dependent effect. Consequently, the cultivation of regular long-term exercise habits is crucial to prevent metabolic disease progression. Future studies should investigate whether these acute benefits translate to long-term metabolic improvements, and explore potential differences in the response to different exercise intensities.

## Ethics approval and consent to participate

All eligible participants, who comprehended the experimental procedures and demonstrated willingness to participate, provided written informed consent. This study was approved by the Ethics Committee of Shanghai University of Sports.

## Consent for publication

Not applicable.

## Disclosure statement

The authors report there are no competing interests to declare.

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## Data statement

The data supporting the findings of this study will be made available upon reasonable request from the corresponding author.

## Author contributions

J-ZZ conceived, designed, and directed the study, collected and interpreted the data, and drafted the manuscript; X-ML participated in the study design, supervised the entire study, helped in participant recruitment, and collected the data; S-QL supervised the entire study and facilitated participant recruitment; Z-BC and ZZ developed the study protocol, helped interpret the results, and critically appraised the manuscript. All authors approved the manuscript and the authorship order.

## Author statement

Originality & Concurrent Submission.

We confirm that this manuscript is our original work, has not been published elsewhere, and is not currently under consideration by any other journal.

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## Declaration of competing interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jesf.2025.07.005.

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