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ORIGINAL

# Effects of breaking up prolonged sitting *via* exercise snacks intervention on the body composition and plasma metabolomics of sedentary obese adults: a randomized controlled trial

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**Abstract.** Obesity resulting from long-term sedentary a significant threat to human health. This study explores the effects of exercise snack intervention on body composition and plasma metabolomics in sedentary obese adults. Participants in the snack group were subjected to 4 days of sprint exercises by stair-climbing per week for 12 weeks. Systemic and regional fat mass, epicardial adipose tissue (EAT), abdominal visceral (AVFA) and subcutaneous (ASFA) fat area and plasma metabolomics data were measured before and after intervention. A higher improvement of EAT, AVFA and ASFA in the snack group compared to that in the control group, with a significant interaction effect ($p < 0.05$). The key differential metabolites between the two groups include isoleucine, glycine and serine. The proposed exercise snack effectively reduced the amount of AVFA and EAT. The change in body composition may be associated with the altered pathways of isoleucine, glycine, and serine metabolism.

*Key words*: Exercise snacks, Obese adults, Plasma metabolomics

## Introduction

Obesity resulting from long-term sedentary lifestyles poses a significant threat to human health and has impacted over 10% of the global population, making it a considerable health concern in various countries worldwide [1]. The ectopic accumulation of abdominal visceral fat and epicardial fat further elevates the risk of sequential metabolic complications, including hypertension, diabetes, fatty liver, and cardiovascular disease [2, 3]. As a non-drug intervention therapy, exercise can effectively mitigate obesity and regulate metabolic processes by enhancing energy expenditure and stimulating the release of lipolytic hormones [4, 5].

Increasing evidence suggests that high-intensity exercise can reduce visceral fat, thus effectively enhancing health and improving body metabolism [6, 7]. Low-volume Sprint Interval Training (SIT) involving repeated short-term (10–30 sec) "all-out" exercise followed by a brief recovery period is often considered the most time-efficient alternative to traditional endurance exercise [8, 9]. Nevertheless, traditional SIT typically requires specialised exercise equipment, such as an anaerobic ergometer, and specific exercise areas, making it impractical for many sedentary office workers with limited time. Moreover, exercising once a day may not be sufficient to counteract prolonged sitting habits [10].

Recently, "exercise snacks" have emerged as a new intervention, defined as short, vigorous bouts of exercise (lasting no more than 1 min) performed intermittently throughout the day [11]. Previous research indicates that a 4-min stair climbing is an effective exercise snack to improve postprandial insulin and total non-esterified

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fatty acid levels in obese adults on the same day [12]. Stair sprinting protocol can be seamlessly integrated into daily routines, offering a convenient approach to mitigate long-term sedentary behaviour. Despite its promising outcome, the long-term efficacy of "sports snacks" for fat reduction remains ambiguous.

In view of this, it is imperative to develop more convenient and practical exercise prescriptions specifically for obese people who frequently lead an unhealthy, sedentary lifestyle. By employing the concept of metabonomics, this study investigated the effect of a 12-week exercise snacks intervention on the body composition of sedentary obese adults. The findings of this study will not only address the pressing need for effective interventions but also provide the theoretical basis for relevant researchers exploring the implementation of exercise snacks as a viable therapy.

## Method

### *Participants*

A total of 27 sedentary adults with a daily energy expenditure of less than 4 metabolic equivalents (METs) (calculated using a continuous physical activity questionnaire (IPAQ)) and a BMI of higher than 25 kg/m$^2$ participated voluntarily in this study from May to September 2022. The recruitment process involved an intra-school campaign and personalised interviews to ascertain the eligibility of potential participants. The exclusion criteria include those who practice drinking alcohol over 100 mL a week, consume diet supplements and/or drugs, suffer from a long-term health disorder, and experience difficulties engaging in regular physical activities. In addition, the Consolidated Standards of Reporting Trials (CONSORT) workflow diagram (Supplementary Fig. 1) were adopted to track the clinical phase throughout the study. All participants received detailed information about the study objectives, the physical, imaging, and biochemical procedures, as well as the stair sprint exercise. Prior to commencing the study, they were provided with informed consent forms to fill out as a formal agreement to participate in this research and given the opportunity to withdraw at any time if they experience physical or psychological discomfort. Strict confidential measures were also enforced throughout the study period. Furthermore, this study was endorsed by the Ocean University of China Ethics Board (Number: OUC-HM-2021023; Data:15/10/2021). The clinical registration of this experiment was registered on the ISRCTN (Registration number: ISRCTN85480568; Date: 20/04/2024). All experimental procedures were performed following the principles outlined in the Declaration of Helsinki.

### *Study Design*

This study was designed as a single-blind randomised controlled trial. Using the coin flip method, participants who met the inclusion criteria were randomly assigned to the snack group, which engaged in stair sprint exercise, or the control group. The supervisory personnel remained unaware of the group assignments throughout the study. Fig. 1 depicts the overall experimental intervention process. In order to monitor habitual energy intake and expenditure separately, each participant was instructed to report their daily dietary intake and physical activities 3 weeks prior to the intervention until its completion. Besides, their systemic and regional fat mass, epicardial adipose tissue (EAT), abdominal visceral (AVFA) and subcutaneous (ASFA) fat area, plasma metabolomics data, and maximum oxygen uptake were measured one week prior to intervention. Additionally, participants in the snack group were subjected to 4 days of sprint exercises by stair-climbing per week for 12 consecutive weeks. In contrast, those in the control group were excluded from any exercises. The above parameters were measured again one week after the intervention was completed. All participants on the training day studied in the teaching building during the daytime, and the researchers monitored each exercise of the participants to ensure their safety.

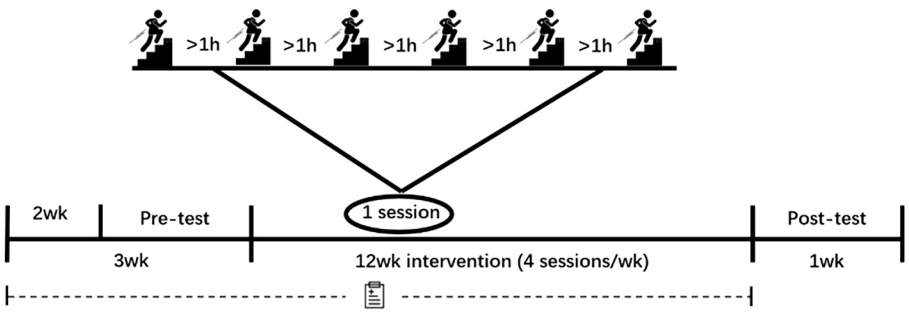


**Fig. 1** Experimental intervention flowchart

### *Calorie Expenditure and Intake*

The daily energy consumption of participants was assessed through a 24-hour dietary recall method. A special questionnaire, developed in accordance with China's Sports Nutrition Centre of the National Research Institute of Sports Medicine (NRISM) guidelines, was utilised to record the caloric intake (foods and beverages) estimation consumed within the previous day (from midnight to midnight). A dietician guided the participants to provide detailed information on the type of food, diet size, and preparation method. Subsequently, the NRISM dietary and nutritional analysis system (version 3.1) was employed to assess the measured energy intake. Participants were offered dietary suggestions whenever deviations from the recommended daily caloric intake were identified.

Daily physical activities, excluding cycle ergometer training and sedentary activities, were evaluated through a 24-hour activity recall approach. Participants were instructed to recall and report the physical activities they performed from midnight-to-midnight on the previous day. A structured, self-reported instrument was used to record details regarding the type and intensity of the physical activities that they engaged in, such as leisure cycling and brisk walking. The duration of each activity was recorded in intervals of at least 5 min. Then, the data were converted into estimates of energy expenditure using standard MET codes from the 2011 Compendium of Physical Activity [13].

### *Body Composition Test*

Body fat mass measurement and blood sample collection were conducted on the same day, with the AVFA and maximal oxygen uptake ($VO_{2max}$) determined on the following day. Pre- and post-intervention assessments were performed using a similar approach to avoid the menstrual phases among female participants. For the body composition and blood tests, the participants arrived at the laboratory at 8:00 am after fasting at least 8 hours and abstained from performing any intense exercise for 48 hours. A dual-energy X-ray absorptiometry (DEXA, Discovery Wi, USA) was employed to analyse body mass, body fat percentage, and fat mass of the whole body, trunk, android, and gynoid regions. A trained technologist assisted in adjusting the regional demarcations according to the previously adopted guidelines [14].

A multidetector computed tomography (CT) scanner (Somaton Sensation 64, Siemens, Germany) fixed at an acquisition protocol of 120 kVp and 150 mA was applied for assessing the EAT and cross-sectional AVFA and ASFA. For the scanning process, participants laid on their backs with their arms naturally positioned at their sides. Scanned images (5 mm width) at the umbilical level (near the L4–L5 intervertebral space) were then captured for analysis. The built-in volume calculation software of the CT scanner was applied to determine the number of voxels in the entire data set of both AVFA and ASFA, with a CT number range from –190 to –30 HU for visceral and subcutaneous fat. Single-slice scans for the AVFA and ASFA showed a high correlation with the corresponding volumetric reconstructions at the umbilicus ($r \geq 0.85$) [15]. A method similar to a previous study [16] was also employed for the EAT volume evaluation. This reference method manually traces the contour of the pericardium using a cursor pointer to reconstruct 1-mm thick axial slices. Pericardium contour was monitored at specific intervals, starting from the lower visible level of the pulmonary artery bifurcation until the top visible level of the pulmonary valve (PV), and then every 20 mm from there until the first slice where the diaphragm becomes visible, followed by every 10 mm until the last slice of the visible pericardium.

The same technicians were in charge of conducting the DEXA and CT analyses throughout the study, and they were not provided with information regarding the participants and group categories. Note that the intra-observer coefficient of variation (CV) for the fat variable determination was ≤4.6% and ≤4.1% for DEXA and CT, respectively.

### *Maximal Oxygen Uptake Test*

The participants' $VO_{2max}$ was assessed through a graded cycling exercise protocol commencing at 60 W and a pedal frequency of 60 revolutions per minute (RPM). For women, the power output was raised by 20 watts every 2 min and 40 watts every 2 min for men until exhaustion. During the test, the $VO_2$ was measured using a gas metabolism analyser (Quark-PFT, COSMED, Italy), while the heart rate (HR) level was determined using an HR monitor (H12, Polar, Finland). The $VO_{2max}$ and $HR_{max}$ values are the average values of the last 30 seconds of the last stage.

### *Non-targeted Metabolomics*

Participants were seated for the blood test before drawing 5 mL of venous blood from the antecubital vein *via* venipuncture. The collected blood samples were transferred into a heparin anticoagulant tube, followed by centrifugation at 2,000 *g* for 10 min at 4°C. Subsequently, the upper plasma was extracted and placed in a –80°C refrigerator for storage. Supplementary Table 1 provides a more precise description of the experimental and analytical methods for the subsequent liquid phase non-targeted metabolomics.

### *Interventions*

The intervention period was performed four times a week for 12 consecutive weeks in a fixed main teaching building, where participants typically worked, studied, read, and participated in the exercise. To monitor the number of steps and HR during each exercise session, participants wore exercise wristbands (Watch S3, Xiao mi, China). Additionally, the rate of perceived exertion (RPE) (category-ratio with a 0–10 scale) of participants was continuously monitored to ensure safety during each exercise session. For the snack group, participants were instructed to walk to a nearby stairwell adjacent to the laboratory. They were then asked to ascend four flights of stairs without skipping steps (60 steps) as fast and as safely as possible. Exercise snacks were conducted 6 times a day, with intervals of more than 1 hour between them. As sprinting up the stairs may not have been feasible for obese participants, they were allowed to climb the stairs at a self-selected challenging pace.

### *Data Analysis*

G*power software (University of Trier, Germany, version 3.1.9.7) was utilised to determine the sample size previously by calculating power. Besides, the Shapiro-Wilk normality test was applied to evaluate the normal distribution of the variables, while two-way repeated ANOVA measures was used to estimate the differences in body composition parameters across two time points and two groups. In instances of a significant interaction, *post-hoc* analysis for ANOVA was performed to identify the simple main effects using the Bonferroni test. The $p < 0.05$ value represents a statistical significance. Data were presented as means ± standard deviation (SD).

**Table 1** Physical characteristics and exercise intensity of the participants

| | Control group | Snack group |
|---|---|---|
| Gender | Male 6; Female 7 | Male 7; Female 7 |
| Age (y) | 21.08 ± 1.32 | 22.14 ± 1.88 |
| Height (cm) | 167.38 ± 8.71 | 167.07 ± 9.09 |
| Weight (kg) | 75.07 ± 11.50 | 78.82 ± 15.27 |
| Mean HR (b/min) | | 117.7 ± 20.5 |
| Mean RPE | | 5.1 ± 2.1 |
| Exercise steps | | 60 |
| Duration (s) | | 22.7 ± 9.8 |

Note: HR = Heart Rate; RPE = Rate of Perceived Exertion.

## Results

### *Participants*

The exercise information and physical properties of the participants are shown in Table 1. Accordingly, all participants completed each intervention and test without any adverse events. The sample size was determined using G*power with a *post-hoc* assumption power (1-β err prob) of 0.97. Based on the results, the age, height, and weight were indifferent across the five groups ($p > 0.05$).

### *Calorie Expenditure and Intake*

The calorie intake and energy expenditure of the participants 3 weeks before and during the 12-week intervention are shown in Table 2. The findings highlighted a statistically insignificant difference between the groups before and after the intervention ($p > 0.05$).

### *Maximum Oxygen Uptake*

The $VO_{2max}$ revealed an insignificant difference among the groups before intervention ($p > 0.05$). Comparatively, the $VO_{2max}$ in the snack group was increased significantly after the intervention (33.14 ± 4.97 to 37.61 ± 3.87, $p < 0.05$). Nevertheless, the measured $VO_{2max}$ in the control group was insignificantly different (31.67 ± 5.09 to 32.06 ± 4.95, $p > 0.05$).

### *Change in Body Composition Before and After Intervention*

The difference in body composition before and after intervention in the snack and control groups is presented in Table 3. The baseline data indicated a statistically insignificant difference among the groups before intervention ($p > 0.05$). However, the weight, fat percentage, total fat mass, android, and trunk area fat mass in the snack group substantially improved after 12 weeks of intervention ($p < 0.05$). Furthermore, the repeated measures ANOVA results suggest that the snack group achieved a higher improvement than that in the control

**Table 2** Diet and physical activity

| | Control group | | Snack group | |
|---|---|---|---|---|
| | Pre | 12-wk | Pre | 12-wk |
| Estimated daily energy intake (kJ/d) | 8,184.62 ± 632.22 | 8,234.15 ± 556.76 | 7,999.29 ± 699.46 | 8,072.29 ± 590.68 |
| Estimated daily energy expenditure (kJ/d) | 1,926.23 ± 273.78 | 1,983.62 ± 267.71 | 2,046.57 ± 281.99 | 2,037.43 ± 226.41 |

group, with a significant interaction effect ($p < 0.05$) except for the fat content in the gynoid area ($p > 0.05$).

Fig. 2 depicts the changes in EAT, ASFA, and AVFA in the snack and control groups before and after intervention. While no statistically significant difference in the baseline data among the two groups was observed before intervention ($p > 0.05$), the snack group recorded a considerable enhancement for all three indicators after intervention ($p < 0.05$). Additionally, the repeated measures ANOVA outcomes showed a higher improvement in the snack group compared to that in the control group, with a significant interaction effect ($p < 0.05$).

### *Non-targeted Metabolomics*

The score plot of the Partial Least Squares Discriminant Analysis (PLS-DA) model between snack and control groups is illustrated in Fig. 3, which indicates a clear and accurate dispersion degree of the two data sets. In addition, Fig. 4 shows the heatmap of metabolites in the offspring of the two intervention groups. The analysis identified 723 metabolites in the liquid phase of the non-targeted metabolomics. The key differential metabolites between the two groups include asparagine, carnitine, creatine, isoleucine, glucose, glutamate, glycine, serine, leucine, and proline, where the main enriched pathways are "valine, leucine, and isoleucine biosynthesis" and

**Table 3** Changes in body composition before and after intervention

| | Control group | | Snack group | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Weight | 75.07 ± 11.50 | 75.34 ± 11.91 | 76.81 ± 14.76 | 74.29 ± 14.55 |
| Fat% | 41.97 ± 4.82 | 42.17 ± 4.95 | 41.71 ± 4.71 | 39.59 ± 4.35* |
| Total Fat mass | 31.75 ± 6.59 | 31.82 ± 6.31 | 32.49 ± 4.79 | 30.04 ± 4.45* |
| Android area | 2.53 ± 0.53 | 2.55 ± 0.49 | 2.76 ± 0.78 | 2.54 ± 0.69* |
| Gynoid area | 5.85 ± 0.69 | 5.84 ± 0.77 | 6.10 ± 1.07 | 5.69 ± 1.03 |
| Trunk area | 17.28 ± 2.72 | 17.24 ± 2.73 | 17.68 ± 2.23 | 16.26 ± 1.73* |

Note: Fat% = Body fat percentage. * indicates a significant difference compared to pre-intervention.

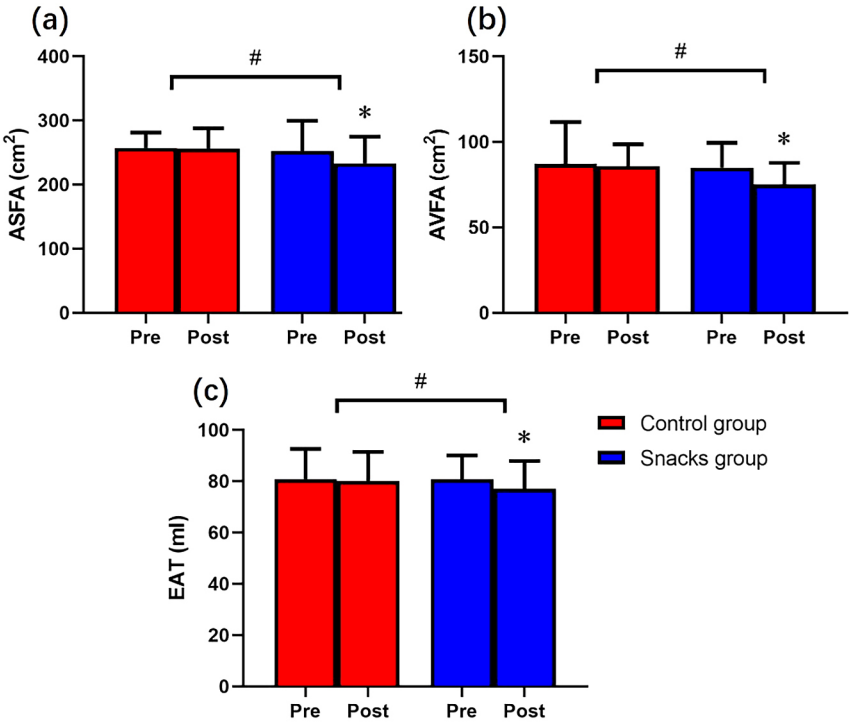


**Fig. 2** Changes of adipose tissue variables in the two groups before and after intervention

Note: * indicates a statistically significant difference compared to before intervention; # indicates a statistically significant difference compared to the control group.

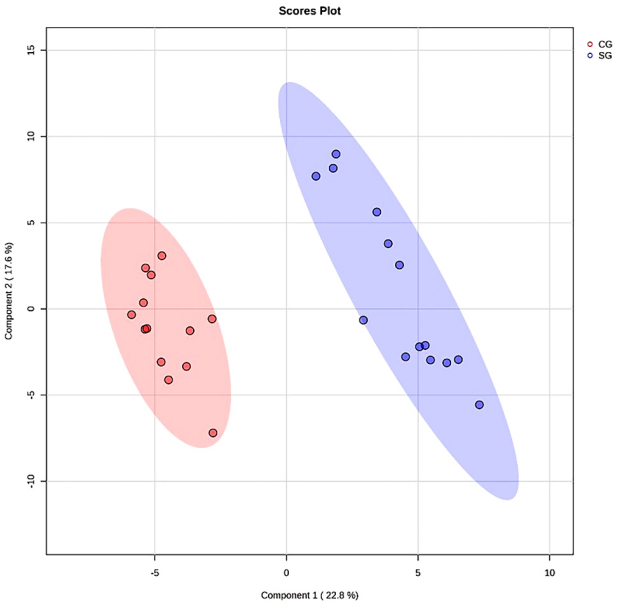


**Fig. 3** The Partial Least Squares Discriminant Analysis of the Control and Snack groups after intervention

Note: The red circle represents the control group; The blue circle represents the snack group.

"glycine, serine, and threonine metabolism." Supplementary Figs. 2, 3 and Graphical Abstract provide more detailed information.

Apart from that, Table 4 shows the correlation between plasma differential metabolites and adipose tissue variables. A significant positive correlation was recorded between glutamine, glycine, pyruvate, and serine and AVFA ($p < 0.05$). In contrast, glucose and leucine showed a significantly negative correlation with AVFA ($p < 0.05$). Besides, 2-oxoisocaproate and glucose achieved a significantly positive correlation with ASFA ($p < 0.05$). Meanwhile, glycine showed a significantly negative correlation with ASFA ($p < 0.05$). It was also found that asparagine and glucose recorded a significantly positive correlation with EAT ($p < 0.05$) compared to the significantly negative correlation between glutamine and pyruvate.

## Discussion

According to the non-target metabonomics analysis *via* Liquid Chromatography-mass Spectrometry (LCMS),

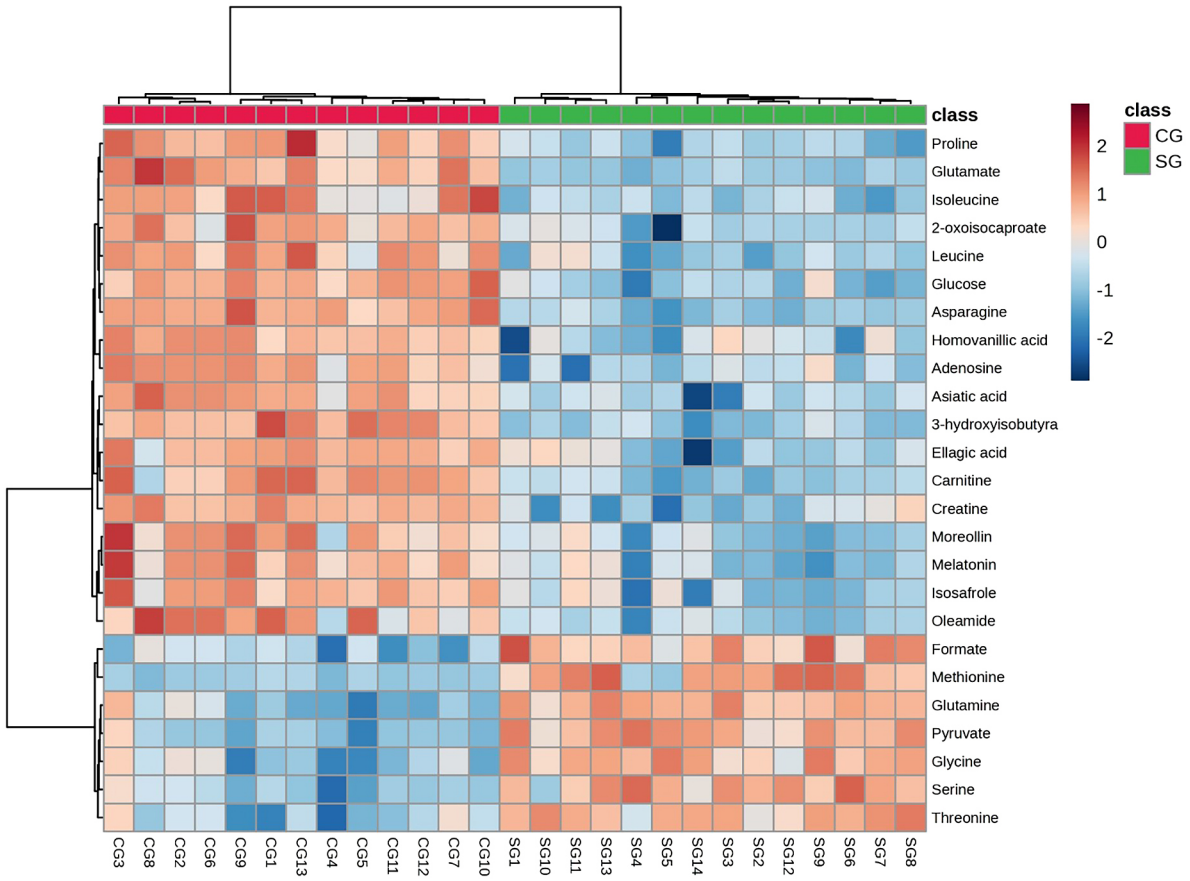


**Fig. 4** The metabolite heatmap chart of the Control and Snack groups after intervention

Note: The left side represents the metabolite heatmap of each sample in the control group; The right side represents the metabolite heatmap of each sample in the snack group.

**Table 4** Correlations coefficients between metabolites and adipose tissue value

| AVFA | *r* | 95%CI | *p* |
|---|---|---|---|
| Glucose | 0.41 | 0.03 to 0.68 | 0.035 |
| Glutamine | –0.49 | –0.73 to –0.13 | 0.010 |
| Glycine | –0.54 | –0.77 to –0.21 | 0.001 |
| Leucine | 0.45 | 0.08 to 0.71 | 0.019 |
| Pyruvate | –0.48 | –0.73 to –0.13 | 0.011 |
| Serine | –0.52 | –0.75 to –0.18 | 0.005 |
| ASFA | | | |
| 2-oxoisocaproate | 0.49 | 0.13 to 0.73 | 0.005 |
| Glucose | 0.34 | 0.04 to 0.61 | 0.047 |
| Glycine | –0.40 | –0.68 to –0.02 | 0.020 |
| EAT | | | |
| Asparagine | 0.44 | 0.07 to 0.70 | 0.022 |
| Glucose | 0.43 | 0.06 to 0.70 | 0.026 |
| Glutamine | –0.40 | –0.68 to –0.03 | 0.037 |
| Pyruvate | –0.39 | –0.68 to –0.02 | 0.039 |

Note: AVFA = Abdominal Visceral Fat Areas; ASFA = Abdominal Subcutaneous Fat Areas; EAT = Epicardial Adipose Tissue.

this study explored the influence of exercise snacks on the body composition of obese adults. The findings revealed that adopting exercise snacks as an alternative therapy to break a sedentary lifestyle effectively enhanced the AVFA and EAT in obese adults. The AVFA level observed were primarily associated with leucine and serine, while the EAT level were essentially linked to asparagine. These findings suggest that the implementation of exercise snacks may alter the plasma metabolite level by minimising the adipose tissue content.

The exercise protocol applied in this study involved supervised exercise snacks and was adopted from a previous study [12]. The exercise protocol also exhibited similar intensity to those described in past studies [11, 17]. The average HR during exercise sessions in this study was 117.7 ± 20.5 beats/min, slightly higher than that reported in the previous study. The difference in HR could be attributed to the longer duration of the stair-climbing and exercise implemented in this study. Given the brief duration of each exercise session (approximately 22 sec), most traditional indicators, such as the maximum HR percentage or $VO_{2max}$, may not accurately reflect the physiological demands imposed on the heart or skeletal muscle. Thus, this study also assessed the average RPE after exercise, which was 5.1 ± 2.1. This method was found to be more suitable for characterising the nature of sports snacks.

Epicardial fat, which originates from the splanchnopleuric mesoderm, lacks a clear separation from the myocardium by any fascia and shares microcirculation from the coronary arteries with the myocardium [18, 19]. Compared to other fat deposits, epicardial fat exhibits a smaller cell size, distinct fatty acid composition, and higher fatty acid but lower glucose metabolism [20]. Similar to excessive accumulation of AVFA, epicardial fat can easily contribute to the development and progression of chronic metabolic diseases [21]. The findings of this study demonstrate that exercise snacks effectively enhance ectopic fat deposition in obese adults, minimizing both AVFA and EAT. This improvement may be attributed to the increased oxygen consumption resulting from frequent snacks and exercise [22], as well as the secretion of lipolytic hormone after exercise [23]. Furthermore, the results in past acute studies supported the beneficial effects of exercise snacks on glucose and lipid metabolism in obese individuals, thus corroborating this research outcome [12].

On the contrary, the exercise snacks intervention exerted a substantial impact on plasma metabolites. Valine, isoleucine, and leucine, which are crucial components of branched-chain amino acids (BCAA), were found to have reduced the leucine and isoleucine levels in the plasma of obese adults after exercise. Moreover, the leucine level exhibited a significantly positive correlation with AVFA. A recent study revealed a significant correlation between leucine and isoleucine levels with

AVFA, indicating the potential involvement of BCAA in visceral fat deposition over time [24]. This supports the results of this study. The possible weight loss effect of leucine is mediated by changes in energy efficiency caused by increased energy expenditure. For example, supplementing with leucine increases energy expenditure in mice with high fat diet [25, 26] and genetically obese mice [27]. A study has shown that the disruption of the BCATM gene leads to an increase in plasma BCAA levels and an increase in oxygen consumption. Therefore, the weight and fat of $BCATM^{-/-}$ mice decreased. Furthermore, glycine demonstrated a significant negative correlation with AVFA in this study. This may be related to the antioxidant function of glycine, which is a tripeptide glutathione (GSH; composed of glutamate, cysteine, and glycine) and is the most abundant antioxidant in cells [28]. If any of the three amino acids of glutathione are restricted, the synthesis of glutathione will not be ideal, which may lead to decreased insulin sensitivity [29]. Another study revealed that glycine treatment in sucrose-fed rats led to a reduction in intra-abdominal fat and non-esterified fatty acids, along with an increase in liver mitochondrial respiration [30]. The results suggest a potential association between glycine and the effects on fatty acid oxidation, circulating non-esterified fatty acids, and, ultimately, intra-abdominal fat accumulation, which aligns with the findings of this study. Additionally, Newgard *et al.* recorded an elevated level of arginine, BCAA, glutamate/glutamine, aspartate/asparagine, phenylalanine, and tyrosine in obese individuals [31]. Other researchers have also noted a positive link between glutamate, BCAA, tyrosine, and visceral adipose tissue area [32]. Nevertheless, the correlation between aspartic acid and epicardial fat has not been evaluated in past studies. Based on the results of this study, aspartic acid was found to significantly reduce after intervention, along with a positive correlation with epicardial fat. The changes in these amino acids may not be unique to snack sports. In Duft *et al.*'s study, aerobic combined with resistance training was used for a 12 week exercise intervention, three times a week. Compared to the control group, pyruvate, glutamate, and glucose also showed significant differences [33]. In summary, exercise snacks may impact the ectopic deposition of fat in the body by modulating the levels of body metabolites.

Regardless of the remarkable results, there are several constraints in this study. Firstly, the study included a relatively small sample size, consisting of only 27 individuals, which may not be sufficiently large for robust statistical analysis. Secondly, this study exclusively focused on evaluating obese adults, all of whom were college students, thus limiting the generalizability of the results to other populations. Additionally, this study solely assessed the metabolome in plasma without examining the proteome and transcriptome, thereby restricting the comprehensive interpretation of the results. Considering this, future studies should expand the sample size and include a more diverse participant pool. Besides, employing multi-sample and multi-omics approaches, such as evaluating participants' intestinal flora or plasma proteome, could offer more in-depth insights into the effects of exercise snacks on the body composition and metabolism in obese adults.

## Conclusion

This study conducted a single-blind randomised controlled trial to investigate the effects of exercise snacks on breaking prolonged sitting of obese adults to improve body composition and plasma metabonomics of sedentary obese adults. The findings demonstrate that the proposed exercise snack protocol effectively reduced the amount of AVFA and EAT. The change in body composition and plasma metabonomics may be associated with the altered pathways of valine, leucine, isoleucine, glycine, and serine metabolism.

## Graphical Abstract

Differential metabolites and pathway enrichment after 12 weeks of exercise snack.

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Not Applicable.

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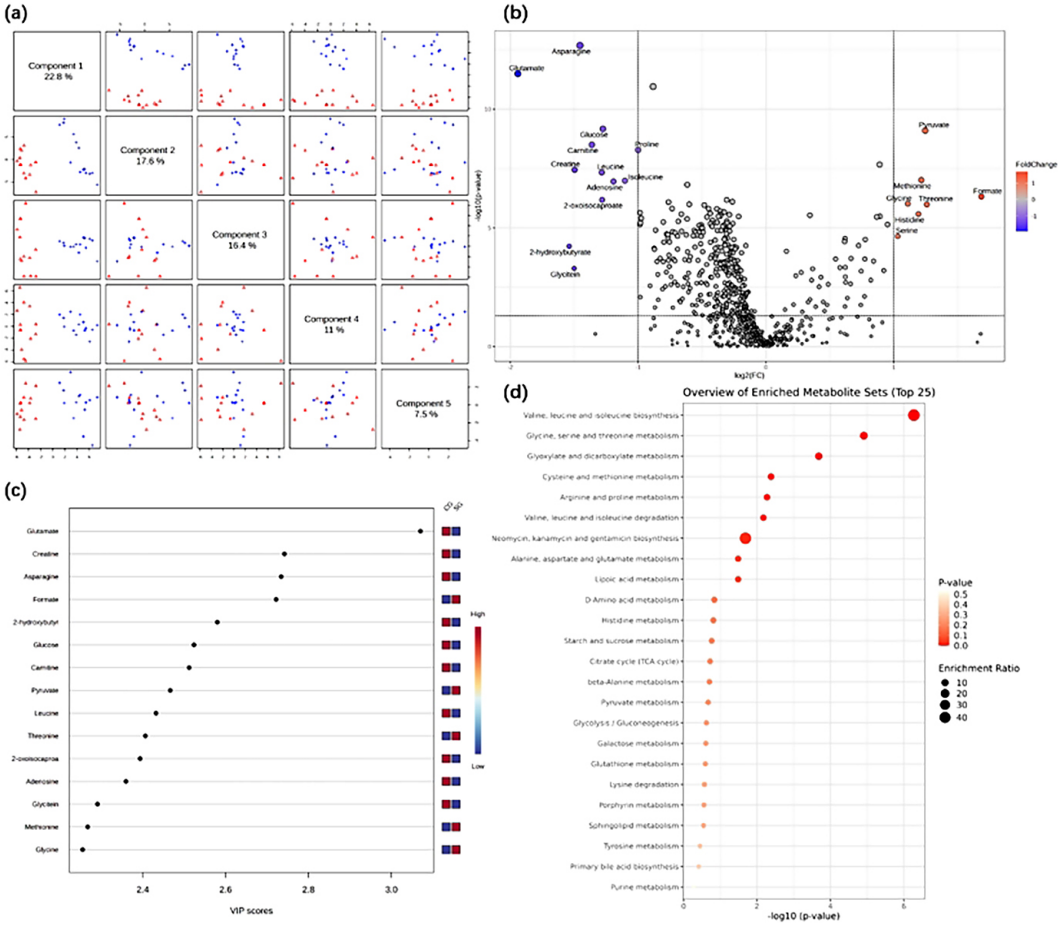
## Author Contributions

J.Z. and X.G. wrote the main manuscript text. D.Z. prepared Figures and Tables. J.Z. and X.G. analyzed the data. C.J. and W.Y. drafted and reviewed the manuscript. Informed consent was obtained from all participants included in the study.

## Competing Interests

The authors declare no competing interests.




**Graphical Abstract**

## Data Availability

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

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