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Research article

The Release of Lipolytic Hormones during Various High-Intensity Interval and Moderate-Intensity Continuous Training Regimens and Their Effects on Fat Loss

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Abstract
To investigate the release of lipolytic hormones during various high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), and their effects on fat loss. 39 young women categorized as obese (with a body fat percentage (BFP) ≥30%) were randomly allocated to one of the following groups: all-out sprint interval training (SIT, n =10); supramaximal HIIT ($HIIT_{120}$, 120%$\dot{V}O_{2peak}$, n = 10); HIIT ($HIIT_{90}$, 90%$\dot{V}O_{2peak}$, n = 10), or MICT, (60%$\dot{V}O_{2peak}$, n = 9) for a twelve-week observation period consisting of 3 to 4 exercise sessions per week. Serum epinephrine (EPI) and growth hormone (GH) were measured during the 1st, 20th, and 44th training sessions. Body weight (BW), body mass index (BMI), whole-body fat mass (FM) and BFP were assessed pre- and post-intervention. Following the 1st and 20th sessions, significant increases in EPI ($p < 0.05$) were observed post-exercise in $HIIT_{120}$ and $HIIT_{90}$, but not in SIT and MICT. In the 44th session, the increased EPI was found in SIT, $HIIT_{120}$, and $HIIT_{90}$, but not in MICT ($p < 0.05$). For the GH, a significant increase was observed post-exercise in all groups in the three sessions. The increased EPI and GH returned to baselines 3 hours post-exercise. After the 12-week intervention, significant reductions in FM and BFP were found in all groups, while reductions in BW and BMI were only found in the SIT and HIIT groups. Greater reductions in FM and BFP, in comparison to MICT, were observed in the SIT and HIIT groups ($p < 0.05$). 12-week SIT, $HIIT_{120}$, and $HIIT_{90}$, in comparison to MICT, were more efficacious in fat reduction in obese women, partly benefiting from the greater release of lipolytic hormones during training sessions.

Key words: High-intensity interval training, moderate-intensity continuous training, exercise intensity, lipolytic hormones, obesity.

Introduction

Contemporary research has developed an increased interest in the employment of high-intensity interval training (HIIT) as a time-efficient method for promoting fat loss among individuals identified as obese (Yin et al., 2024; Couvert et al., 2024). Research has demonstrated that, when compared with the same volume of traditional moderate-intensity continuous training (MICT), HIIT can effectively reduce overall body fat (Sultana et al., 2019; Maillard et al., 2016; Wewege et al., 2017) and is even more effective in reducing visceral fat (Maillard et al., 2016; Zhang et al., 2015, 2021).

During HIIT, carbohydrates are the primary fuel source for muscles; fat utilization is minimized because the body relies on carbohydrates as the primary source of energy when exercise intensity exceeds 70% of the maximum oxygen uptake ($\dot{V}O_{2max}$) (Hargreaves and Spriet, 2020). However, the energy expenditure during exercise is not confined to the exercise period alone and occurs throughout the post-exercise period. A meaningful quantity of energy is consumed in the period following physical activity (known as Excess Post-exercise Oxygen Consumption (EPOC) and fat is the primary substrate utilized throughout the EPOC process (Moniz et al., 2020). EPOC is directly correlated with the increased secretion of lipolytic hormone (Borsheim and Bahr, 2003), therefore, HIIT can stimulate the body to release additional lipolytic hormones, enhance EPOC, and consume additional fat which explains one of the mechanisms underlying the fat-reducing effects of HIIT. However, it raises the question of whether the same mechanism applies when comparing HIIT of different intensities with MICT protocols.

Lipolytic hormones, such as epinephrine (EPI) and growth hormone (GH), play a key role in promoting the breakdown of triglycerides by regulating hormone-sensitive lipase and increasing fat consumption (Hansen et al., 2012). Previous studies have demonstrated that the secretion of lipolytic hormones during endurance exercise is directly proportional to exercise intensity (Hansen et al., 2012; Pritzlaff et al., 1999, 2000). Peake et al. (2014) conducted a study comparing HIIT (10 sets of 4-minute exercise at 80%$\dot{V}O_{2max}$ intensity with 2-minute intervals) and MICT (continuous training at 65%$\dot{V}O_{2max}$ with the same energy expenditure). The results made notable observations: firstly, GH levels were significantly elevated after HIIT and MICT sessions, and secondly, the levels of EPI only increased after HIIT (and not MICT). Moreover, GH remained elevated for 2 hours during the recovery period in the HIIT group, compared to only 1 hour in the MICT group (Peake et al., 2014) implying that the secretion of lipolytic hormones is greater and more prolonged with higher-intensity exercise; however, a lack of certainty remains regarding whether this model applies to obese individuals and further study is recommended.

Apart from exercise intensity, the number of intervals and overall duration of exercise are the major influencing factors regarding lipolytic hormones (Jones, 2023; Gallo et al., 2024; Tschakert et al., 2022). Currently, the American College of Sports Medicine (ACSM)

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recommends that adults engage in prolonged exercise for 60 minutes at a moderate intensity, five days per week (ACSM, 2021). Although this recommendation may not be practical or suitable for all individuals (especially those who are inactive or obese). HIIT, which provides short durations of higher-intensity exercise, could be an accessible alternative due to its prominent impact on metabolic responses via exercise intensity (Wewege et al., 2017; Sultana et al., 2019; Sabag et al., 2022a). However, each individual's physiological resilience or exercise durability varies (Jones, 2023; Gallo et al., 2024; Tschakert et al., 2022), making it unclear whether HIIT programs (with different durations and numbers of intervals) have the same effect on lipolytic hormone responses as MICT (with longer durations but the same total exercise volume as HIIT). This uncertainty also extends to whether these effects apply to obese participants.

None of the current literature has established if obese individuals secrete increased amounts of lipolytic hormones during HIIT when compared to MICT; therefore, additional research is required to explore the impact of different intensities of HIIT on the release of lipolytic hormones when compared with MICT. Specifically, it is important to investigate whether the level of lipolytic hormones released during acute exercise is higher in HIIT (compared to MICT) in obese individuals and whether long-term adherence to a HIIT protocol leads to greater fat loss. To establish the role of lipolytic hormones in HIIT-induced fat reduction, this study will compare the release of these hormones following three representative HIIT protocols (90%$\dot{V}O_{2max}$; 120%$\dot{V}O_{2max}$; and all-out sprint intensity) with the levels recorded following MICT. This study hypothesizes that all three HIIT protocols will exhibit a greater fat-reducing effect than MICT which is attributed to the increased elevation of lipolytic hormones associated with acute high-intensity exercise.

Methods

Participants

As outlined by Zhang et al. (2021), the sample size was estimated based on an effect size of 0.5, an α-level of 0.05 ($\eta\rho^2 = 0.20$) and a power of 80%. G*Power was utilized to identify any possible difference in serum EPI between the groups and indicated that 8 participants per group were necessary; therefore, considering a potential dropout rate of 20%, a sample size of 12 participants per group was selected and, in total, sixty obese young women were recruited (see Figure 1). The participants had not experienced significant fluctuations in their body weight (±2kg) in the three months preceding their selection, had not trained, had no history of hormone metabolism, and had no medical reason not to participate in vigorous exercise. Ethical approval was obtained from the Ethical Committee of Hebei Normal University for the Use of Human & Animal Subjects in Research and the study was performed per the Declaration of Helsinki. Each participant completed and signed the consent form and written notice to signify their agreement to participate in this research.

Study design

The subjects were randomly divided into 5 cohorts (consisting of 12 participants per group): sprint interval training group (SIT, 6 seconds of exercise followed by 9 seconds of rest， 40 sets); 120% maximal oxygen uptake ($\dot{V}O_{2max}$) HIIT group ($HIIT_{120}$, 60 seconds of exercise followed by 90 seconds of rest, 16 - 21 sets); 90%$\dot{V}O_{2max}$ HIIT group ($HIIT_{90}$, 240 seconds of exercise followed by 180 seconds of rest, 5 - 7 sets); 60%$\dot{V}O_{2max}$ MICT group (MICT, continuous exercise for 51-61minutes); and the control group without training (CON). The exercise interventions spanned 12 weeks and consisted of 44 sessions (including 3 acute exercise tests): for the first 4 weeks, the participants

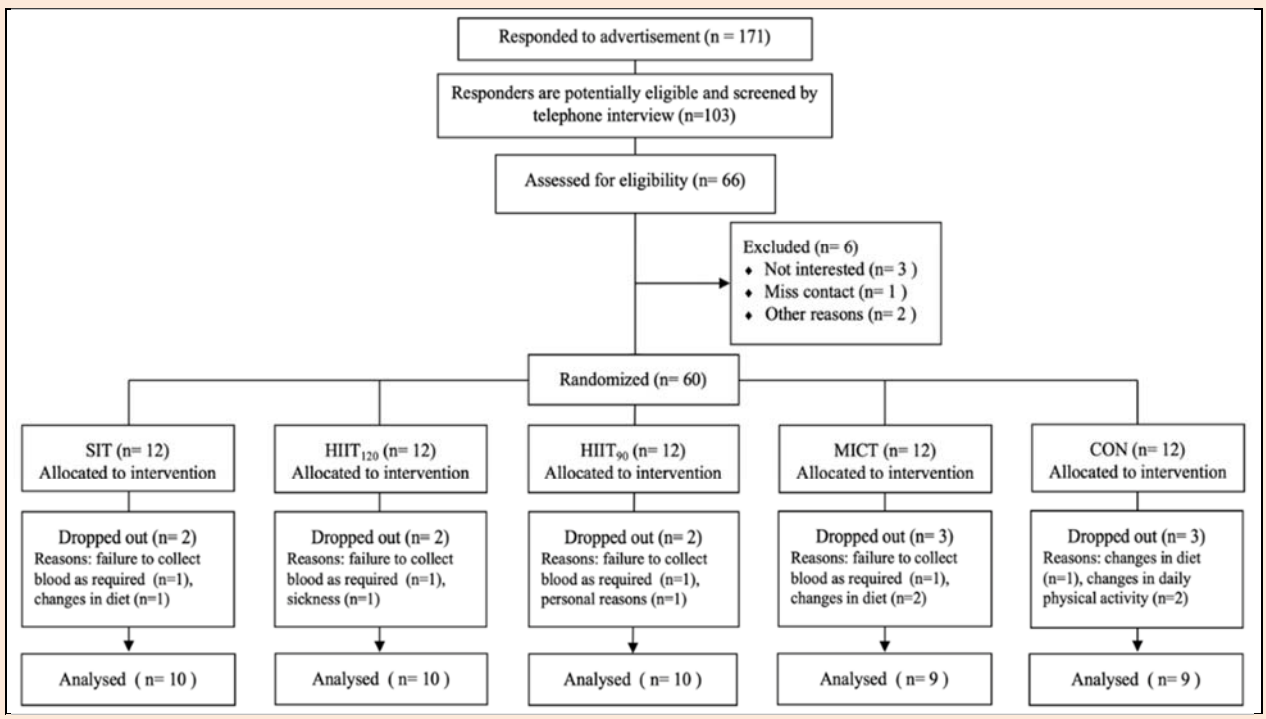

Figure 1. Distribution of study participants.

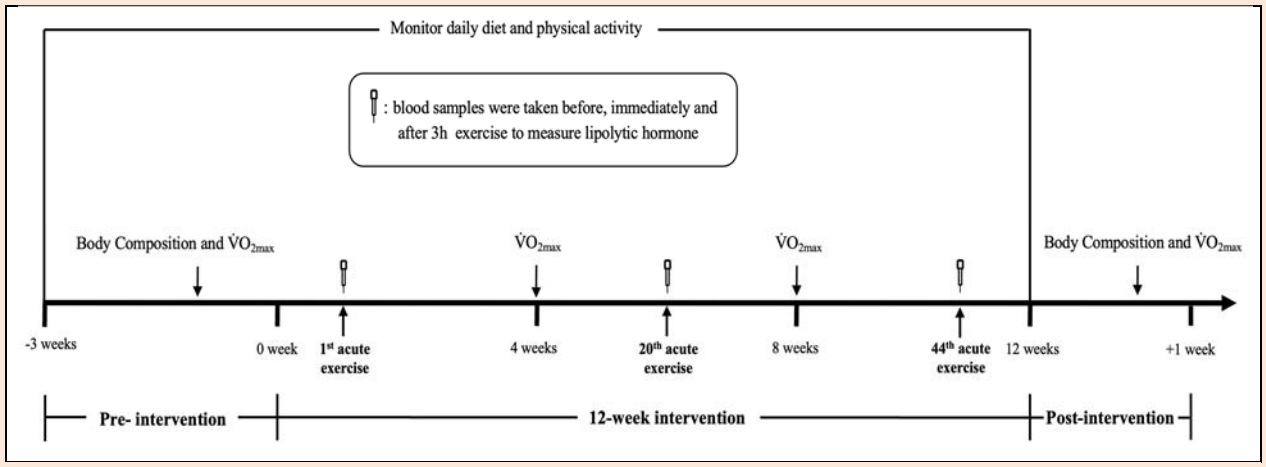

Figure 2. Implementation route of study design.

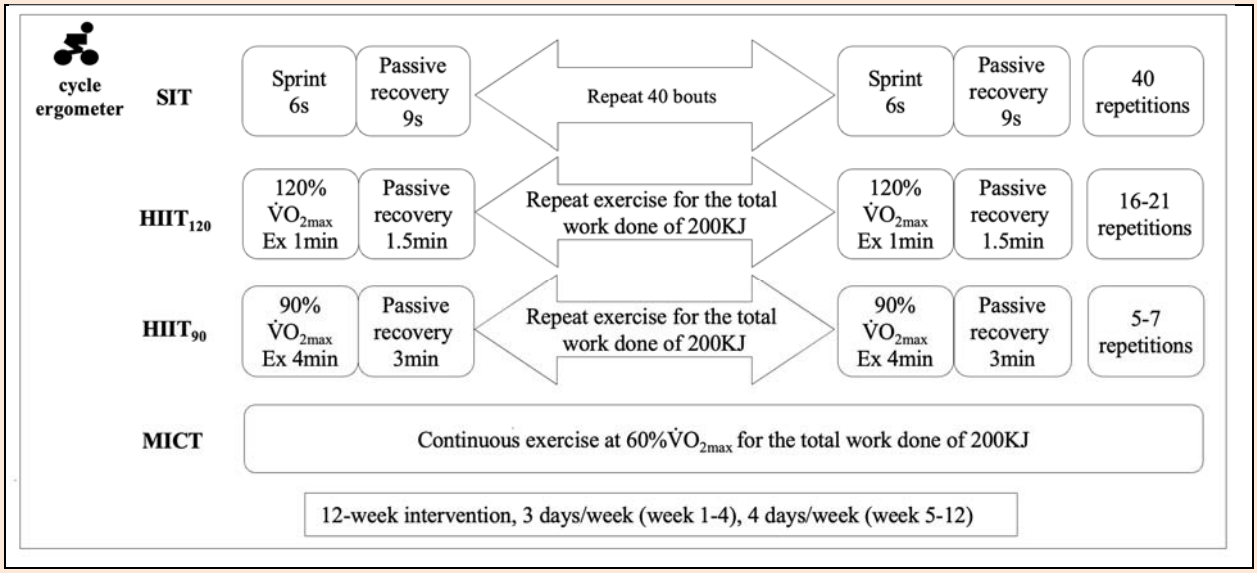

Figure 3. Training protocol of each cohort.

exercised 3 times a week and for each week thereafter, they trained 4 times a week. The acute exercise tests were conducted in the early (1st exercise), middle (20th exercise), and late stages (44th exercise) of the 12-week training period. At the acute exercise tests, median cubital vein blood was collected before, immediately after, and 3 hours after exercise. The EPI and GH levels were tested to observe the effect of HIIT on lipolytic hormones in obese young women. For three weeks before testing, and throughout the 12-week exercise period, the participants' habitual dietary calories and physical activity were monitored and recorded. Additionally, body weight, BMI, body fat, body fat percentage, and body muscle of the participants were tested before and after the 12-week intervention. The technical route of the research scheme is shown in Figure 2.

Training methods

A progressively rising load exhaustion study was conducted via the use of an aerobic cycle ergometer and a gas metabolism analyzer. The exhaustion study began at 50W and increased by 20W every 2 minutes to measure $\dot{V}O_{2max}$ (Quark-PFT-ergo, COSMED, Italy). The assessment concluded when one of the following criteria was met: (i) the increase of exercise intensity resulted in plateaued oxygen uptake; (ii) the respiratory quotient exceeded 1.15; (iii) RPE = 20 or the participant was unable to maintain the required level; or (iv) in the event of significant cardiac or respiratory distress. Following completion of the test, the mean oxygen uptake value in the 30 seconds following load completion was deemed to be the $\dot{V}O_{2max}$. By recording the corresponding value of each intensity power and its average oxygen uptake within the last 30 seconds, a scatter plot was generated to acquire the formula for exercise intensity-oxygen uptake linear regression and calculate the corresponding 120%, 90% and 60%$\dot{V}O_{2max}$ exercise intensity. The exercise duration necessary to accomplish mechanical work to 200KJ was computed via the following formula: Work (KJ) = Work (J) ×1000 = Exercise power (watt) × Exercise time (s) = Exercise power (watt) × Exercise time (min) ×60. The measurement of $\dot{V}O_{2max}$ was conducted at 4-weekly intervals over a period of 12 weeks to modify the intensities of the $HIIT_{120}$, $HIIT_{90}$ and MICT cohorts and ensure the participants maintained the requisite exercise intensity.

Figure 3 contains the training protocols for the high-intensity interval training (HIIT) cohorts (SIT, $HIIT_{120}$ and

$HIIT_{90}$ respectively) alongside those of the MICT group. Those assigned SIT undertook 40 sets of 6-second maximum intensity sprints followed by 9-second intervals on the anaerobic cycle ergometer (Monark 894E, Sweden) for a total exercise duration of four minutes. This period equates to a work/rest recovery period of 6/9 s or a 1 to 1.5 work recovery ratio. The heart rate (HR, Polar, Finland), rating of perceived exertion (RPE, Borg scale 6 - 20, 1973), and peak pedaling power were documented every fifth exercise set. The initial exercise load comprised of 1kg and once the participants had concluded the exercise and their HR and RPE had diminished, the resistance was progressively raised to 3kg. The remaining three cohorts ($HIIT_{120}$, $HIIT_{90,}$ and MICT) utilized the aerobic cycle ergometer (Monark 839E, Sweden) to accomplish mechanical work to 200 KJ. The protocols were as follows: (i) the $HIIT_{120}$ subjects were active for 1 minutes to 120%$\dot{V}O_{2max}$ intensity, rested for 1.5 minutes, and the exercise was repeated 16-21 times to give an exercise component duration of 16-21 minutes which equates to a work/rest recovery period of 1/1.5 minutes or a 1 to 1.5 work recovery ratio; (ii) the $HIIT_{90}$ subjects were active for 4 minutes at an intensity of 90%$\dot{V}O_{2max}$, rested for 3 mins, and the exercise was repeated 5-7 times resulting in a complete exercise duration of approximately 20-28 minutes which equates to a work/rest recovery period of 4/3 minutes or a 1 to 0.75 work recovery ratio; (iii) the MICT subjects exercised continuously for approximately 51-61 minutes at an intensity of 60%$\dot{V}O_{2max}$. For the $HIIT_{120}$ and $HIIT_{90}$ cohorts, assessments of HR and RPE were conducted and documented before and after each session; for the MICT group, the same assessments were conducted at 5-minute intervals.

Physical activity and dietary assessment

Throughout the 3-week pre-intervention and 12-week intervention phases, the participants adopted a daily dietary approach to self-report physical activity and to estimate their habitual energy expenditure. Total energy expenditure, measured in metabolic equivalents (METs)·hr·wk^{-1}, was calculated using reported MET values from the Compendium of Physical Activities combined with the weekly activity recordings. To monitor each participant's caloric intake, their daily diets were meticulously recorded throughout the pre-intervention and intervention periods per the guidelines of the NRISM Sports Nutrition Centre (National Research Institute of Sports Medicine, China) for dietary monitoring. Dietary records and the corresponding energy intake were analyzed using the NRISM dietary and nutritional analysis system (version 3.1) which was specifically designed for Chinese athletes and the general population. Weekly consultations with a dietitian were conducted to ensure consistent adherence to caloric intake guidelines and to mitigate deviations.

Outcome Measures

Lipolysis hormones

Median cubital vein blood was collected before, immediately after, and 3 hours post-exercise (following the earlier (1^{st}), middle (20^{th}), and late exercise sets (44^{th})). The timing of the post-exercise blood samples followed previous research which determined that peak levels of lipolytic hormones (EPI and GH) occur after exercise and that GH levels remain elevated for two hours following the cessation of exercise (Peake et al., 2014); therefore, this research collected blood samples from three hours after exercise to observe the duration of elevated lipolytic hormone levels. The collected data will provide valuable insights regarding the release of lipolytic hormones during high-intensity exercise and the potential fat-reducing effects of HIIT which may be attributed to the cumulative release of lipolytic hormones during extended intensive HIIT sessions.

The median cubital vein blood samples were refrigerated at 4 ℃ for 30 minutes then centrifuged for 15 minutes at a speed of 3000rpm. The resultant supernatant was refrigerated at -80 ℃ and the concentration of lipolytic hormones (EPI and GH) was measured using an enzyme-linked immunosorbent assay. To ensure consistency and accuracy, the same technician performed each test. The EPI testing kits were manufactured by Wuhan Huamei Bioengineering Co., Ltd., and those for GH testing were supplied by RayBiotech (United States). The kits' assay coefficients of variation were 3.48% (EPI), and 4.65% (GH).

Body composition

A bioelectric impedance assessment (Jawon Medical, X-Scan plus II, Korea) was utilized to determine the subjects' body mass (BM), body mass index (BMI), body fat mass (BFM), body fat percentage (BFP), and body muscle mass (BMM). The participants were requested to wear tightly fitting clothes and not wear any metal (such as jewelry and piercings). When assessing body fat, the X-Scan plus II has a retest coefficient of variation of 1.3% and the equation used to compute BMI (kg/m^2) is weight/$(height)^2$.

Statistical analysis

SPSS software (version 26.0) was employed to analyze the data which were given as mean ± standard deviation (SD). The Shapiro-Wilk test of normality was conducted to assess the normal distribution of data. One-way analysis of variance (ANOVA) was employed to analyze the results before the intervention for the individual cohorts. Repeated measures ANOVA was conducted to assess any variances in body composition (5 cohorts*2 time points) and lipolytic hormones (4 cohorts*3 time points) and to examine the interaction, time, and group effects. Upon recognition of a significant interaction, the Newman-Keuls test can be utilized to analyze and compare the simple effect; if the interaction effect is not significant, the main effect is analyzed. The results are considered to be statistically significant if p values of < 0.05 and highly significant if $p < 0.01$.

Results

Participants

To ensure the robustness of its findings, this research adhered rigidly to the pre-determined exercise protocol. Each subject participated in a 12-week exercise intervention which incorporated 3 sessions of acute exercise to assess any fluctuations in the participants' lipolytic hormone levels. A total of 48 participants were deemed to have completed the study (representing an 80% completion rate) and were distributed across the cohorts in the following manner: SIT (n = 10), $HIIT_{120}$ (n = 10), $HIIT_{90}$ (n = 10), MICT (n = 9), and CON (n = 9).

Training monitoring metrics

The parameters noted for each exercise cohort during the intervention (mechanical work, exercise power, exercise time, HR, and RPE) are displayed in Table 1. All exercise groups (except the SIT cohort) achieved mechanical work of 200 KJ which indicates a lower exercise volume in the SIT group. Despite this, the exercise duration remained constant at 4 minutes; however, the resistance on the anaerobic power bike gradually intensified, increasing the work performed by the SIT group, while the other groups maintained a consistent work level with decreasing duration. Throughout the training period, the $HIIT_{120}$, $HIIT_{90}$ and MICT cohorts progressively increased their exercise power resulting in reduced exercise duration over time; however, their work recovery ratios remained static. The HIIT groups displayed a progressive decrease in average HR and an increase in their RPE during training which were not observed in the MICT cohort.

Table 1. The change of work, power, exercise time, HR, and RPE in each group.

	SIT(n = 10)			$HIIT_{120}$（n = 10）			$HIIT_{90}$（n = 10）			MICT（n = 9）		
Week	**1-4**	**5-8**	**9-12**	**1-4**	**5-8**	**9-12**	**1-4**	**5-8**	**9-12**	**1-4**	**5-8**	**9-12**
Work (KJ)	49.1±4.0	57.8 ±3.7**	65.7±5.8**††	200	200	200	200	200	200	200	200	200
Exercise power (Watt)	204.6±16.8	240.8±15.3**	273.6±24.0**††	160.4±18.6	189.5±19.2**	203.8±21.9**††	118.4±11.5	132.6±14.0**	144.4±15.3**††	56.4±10.0	61.0±8.6**	66.6±10.3**†
Exercise time (min)	4	4	4	21.0 ± 2.3	17.7 ± 1.9**	16.6 ± 1.9**††	28.4 ± 2.8	25.5 ± 2.9**	23.3 ± 2.5**††	61.0 ± 11.5	56.0 ± 8.4*	51.4 ± 8.4**††
HR (Beat/min)	175 ± 6	170 ± 5**	166 ± 7**††	170 ± 7	165 ± 5*	163 ± 5*	175 ± 8	174 ± 11	169 ± 7*	137 ± 8	135 ± 7	135 ± 9
RPE	15.7 ± 0.9	16.0 ± 0.6	16.3 ± 1.0*	16.2 ± 1.1	17.2 ± 0.8*	17.1 ± 1.1	15.6 ± 0.8	16.5 ± 1.1	16.7 ± 1.2*	12.2 ± 0.8	12.6 ± 1.9	12.2 ± 0.8

* indicates that compared with 1-4 weeks, $p < 0.05$, ** indicates that compared with 1-4 weeks, $p < 0.01$. † indicates that compared with 5-8 weeks, $p < 0.05$, †† indicates that compared with 5-8 weeks, $p < 0.01$. Work (KJ) = Work (J) * 1000 = Exercise power (watt) * Exercise time (s) = Exercise power (watt) * Exercise time (min) * 60.

Table 2. The Change of Body Composition and $\dot{V}O_{2max}$ Before and After Intervention in Each Group.

	SIT(n = 10)		$HIIT_{120}$ (n = 10)		$HIIT_{90}$(n = 10)		MICT（n = 9）		CON(n = 9)		ANOVA p value
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Group, time, interaction**
Age (yr)	20.6 ± 1.96		19.8 ± 1.9		19.5 ± 1.0		21.0 ± 2.5		21.0 ± 2.1		
Height(cm)	163.2 ± 5.6		163.1 ± 2.7		160.2 ± 7.1		160.0 ± 5.9		161.6 ± 7.3		
Body mass (kg)	71.2 ± 6.5	66.0 ± 6.1**	71.9 ± 9.6	69.5 ± 10.0**	70.3 ± 8.5	65.5 ± 7.0**	66.0 ± 9.6	65.3 ± 8.8	68.7 ± 7.0	69.6 ± 6.4	0.726, 0.000, 0.000
BMI	26.8 ± 2.2	24.8 ± 2.0**	27.1 ± 3.9	26.2 ± 4.0**	27.4 ± 3.1	25.6 ± 3.1**	25.1 ± 2.5	24.8 ± 2.3	26.3 ± 1.8	26.7 ± 1.9	0.657, 0.000, 0.000
Body Fat mass (kg)	23.2 ± 3.5	19.2 ± 3.1**	23.8 ± 5.7	21.7 ± 6.0**	23.8 ± 4.7	19.9 ± 4.3**	20.5 ± 5.1	19.9 ± 4.8**	21.5 ± 2.8	22.3 ± 2.9	0.775, 0.000, 0.000
Body fat percentage	32.5 ± 2.4	29.0 ± 2.7**	32.7 ± 3.4	30.8 ± 4.0**	33.6 ± 3.1	30.1 ± 3.7**	30.7 ± 3.0	30.1 ± 3.0**	31.3 ± 1.8	31.9 ± 2.1	0.767, 0.000, 0.000
Body Muscle mass (kg)	44.0 ± 3.2	43.0 ± 3.5	44.1 ± 3.5	43.8 ± 3.7	42.5 ± 4.0	41.8 ± 3.2	41.7 ± 4.1	41.7 ± 4.8	43.2 ± 4.3	43.3 ± 3.7	0.639, 0.040, 0.277
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	27.8 ± 2.8	33.9 ± 2.5**	27.2 ± 4.2	35.9 ± 5.6**	28.6 ± 2.5	37.1 ± 3.7**	29.0 ± 2.9	34.4 ± 4.2**	30.2 ± 3.1	29.9 ± 2.1	0.474, 0.000, 0.000

** indicates compared with before exercise, $p < 0.01$.

Changes in the levels of lipolysis hormones

The levels of lipolysis hormones (EPI and GH) at baseline (1^{st}), mid (20^{th}), and late (44^{th}) phases of exercise which were measured before, directly following, and 3 hours post-exercise are presented in Figure 4. GH titers were enhanced at all exercise phases by the three forms of HIIT and the MICT, with no variations identified between the groups ($p > 0.05$) (see Figure 4).

In the $HIIT_{120}$ and $HIIT_{90}$ cohorts, elevated levels of EPI were observed in the three training phases directly following exercise when compared to baseline. Within the SIT cohort, that EPI was not elevated (when measured directly following exercise in the early and mid-training assessments); however, they had increased by the latter part of the intervention (12 weeks). No increase in EPI titers was observed following acute MICT in any of the three training periods. Repeated measures of ANOVA demonstrated that in the early training phase, the cohort triad performing HIIT was superior to the group undertaking MICT in achieving higher EPI levels (interaction effect, $p < 0.05$). At the mid-point of training, there was no difference between HIIT and MICT on EPI (interaction effect, $p = 0.112$). However, in the later phase of the exercise program, HIIT was more effective than MICT in increasing EPI concentrations (interaction effect, $p < 0.05$). The EPI differences between the three cohorts performing HIIT before, directly following, and 3 hours

post-exercise failed to reach significance ($p > 0.05$) (see Figure 4).

Changes in body composition and aerobic capacity

The participants within each cohort demonstrated no variations in baseline age, height, BM, BMI, BFM, BFP, BMM and $\dot{V}O_{2max}$ (see Figure 5 and Table 2). In each of the HIIT groups, body weight and BMI were reduced following the 12-week exercise program ($p < 0.01$); no changes were seen in the MICT and CON groups. After the intervention, BFM and BFP were reduced in all intervention cohorts but to a greater extent in the three groups performing HIIT rather than MICT ($p < 0.05$). No differences in BFM and BFP between the HIIT cohorts were observed. The BMM of each group demonstrated no significant change following the 12-week intervention period. Following the training period, $\dot{V}O_{2max}$ was elevated in all the groups: a superior effect was observed among the $HIIT_{120}$ and $HIIT_{90}$ cohorts compared to the MICT group ($p < 0.05$) while the effect of SIT and MICT on $\dot{V}O_{2max}$ was equivalent.

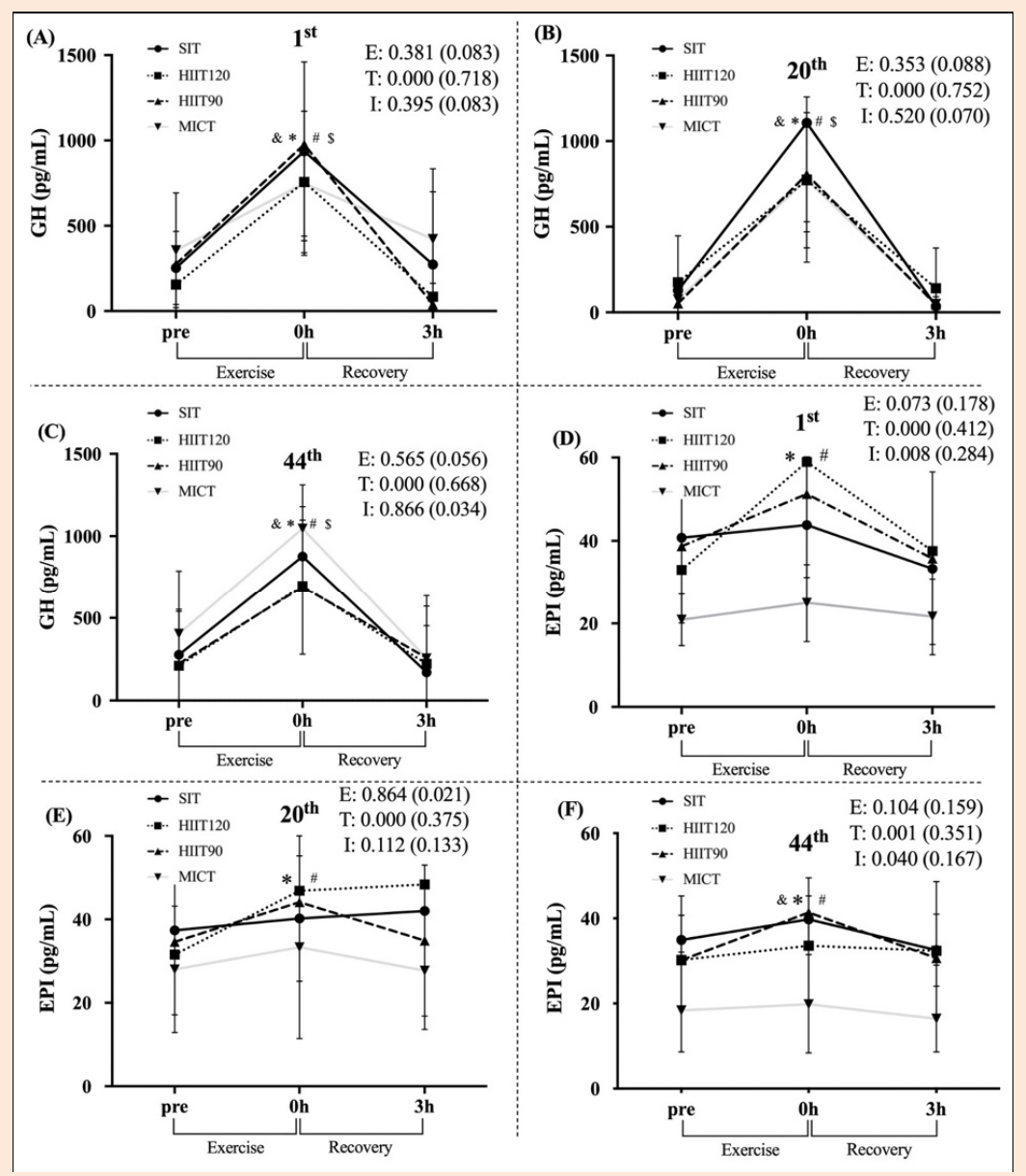

Figure 4. Comparison of GH (A, B, C) and EPI (D, E, F) during three times of acute exercise in 12 weeks of each exercise group. 1^{st}, first acute exercise (A、D); 20^{th}, 20^{th} acute exercise (B、E); 44^{th}, 44^{th} acute exercise (C、F). Pre, before exercise; 0h, immediately after exercise; 3h, 3 hours after exercise. E, group effect (η^2); T, time effects (η^2); I, interaction effects (η^2). * $p < 0.05$, a significant difference compared with pre in the $HIIT_{120}$ group. # $p < 0.05$, a significant difference compared with pre in the $HIIT_{90}$ group. $^{\&}$ $p < 0.05$, a significant difference compared with pre in the SIT group. $^{\$}$ $p < 0.05$, a significant difference compared with pre in the MICT group.

Discussion

This research was designed and implemented to compare the release of lipolytic hormones following high-intensity interval training or continuous exercise and the long-term effects of lipolytic hormones on fat loss. Its results indicate that serum lipolytic hormones (EPI) were elevated in young, obese females in the early and mid-points of the

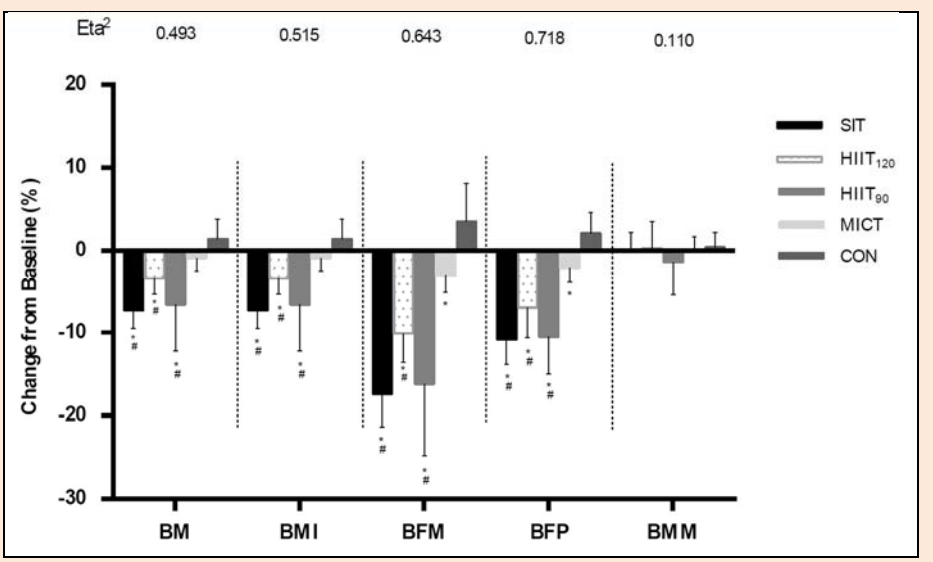

Figure 5. Changes (mean ± SD) in Body mass (BM), Body mass index (BMI), Body fat mass (BFM), Body fat percentage (BFP), and Body muscle mass (BMM) from baseline (0%) to post-training measures among SIT, $HIIT_{120}$, $HIIT_{90}$, MICT, and CON. *Significantly ($p < 0.05$) different from CON; #Significantly ($p < 0.05$) different from MICT. Effect size (Eta^2) for the difference among the five groups is shown on the top of the graph.

training intervention only in the cohorts conducting $HIIT_{120}$ and $HIIT_{90}$; however, in the late training phase, lipolytic hormone releases are consistent across all three of the HIIT cohorts. Of the lipolytic hormones evaluated at the three time points, MICT only induced a rise in GH levels, but this impact was similar to that observed among participants in the three HIIT cohorts. HIIT promotes the release of hormones at a higher rate than MICT which partially explains the efficiency advantage of this form of exercise in reducing fat. However, although the HIIT cohorts demonstrated similar levels of fat reduction, their hormone release levels are different which implies that lipolytic hormones may not be a dominant/unique factor in HIIT-induced fat reduction.

Currently, there is no standardized definition of HIIT protocol (Cao et al., 2021; Maillard et al., 2016; Sabag et al. 2022b; Sultana et al., 2019). Based on exercise intensity and energy systems, HIIT can be classified into the following three forms: HIIT near $\dot{V}O_{2max}$ intensity (usually at 90% $\dot{V}O_{2max}$, primarily utilizing aerobic oxidation for energy supply); HIIT at or above $\dot{V}O_{2max}$ intensity (with anaerobic metabolism contributing to approximately 50% of the total energy supply) (MacInnis et al., 2017; Scott et al., 2024); and repeated intervals of all-out sprints at even higher exercise intensity (where using glycolysis predominates as the main energy source) (Buchheit et al., 2013). Therefore, in this study, four commonly used and classic protocols were employed, with various exercise intensities: SIT, 120$\dot{V}O_{2max}$, 90$\dot{V}O_{2max}$ and 60%$\dot{V}O_{2max}$.

Each participant was closely monitored by the experimenters throughout each training session and strictly followed the exercise protocols. Heart rate and RPE were continuously monitored during exercise to ensure that the exercise intensity for the obese population adhered to the study design and to ensure their safety. In this study, the exercise protocols for each group were set to complete 200KJ of mechanical work based on previous research (Nie et al., 2019). The moderate-intensity MICT group had an exercise duration of approximately 60 minutes to achieve the 200KJ mechanical work, which aligns with the exercise prescription recommendations for the obese population by the ACSM (ACSM, 2021). Although the $HIIT_{120}$ and $HIIT_{90}$, do not adhere to the principle of increasing training load, the total workload had remained at 200KJ, as the same as MICT. The total work was calculated as the average power multiplied by the total time. The average power differed among the two HIIT groups and the MICT group ($HIIT_{120}$ - 48%$\dot{V}O_{2max}$; $HIIT_{90}$ - 51.4%$\dot{V}O_{2max}$; MICT - 60%$\dot{V}O_{2max}$), and the average power can influence acute cardiorespiratory responses (HR and $\dot{V}O_2$) during exercise with fluctuations in average power (Tremblay et al., 2005). Therefore, under the same total work, the HIIT exercise prescriptions in this study, with personalized adjustments on lower average exercise power and exercise duration/number of intervals, and have the time-saving characteristics, may be more suitable for exercise-based weight loss interventions in the obese population.

Effects of MICT and HIIT on lipolytic hormones

The impact of HIIT on fat reduction may be closely related to its influence on lipolytic hormone secretion: exercise acts as a primary trigger for their release which, in turn, induces higher rates of lipid breakdown (Muscella et al., 2020). Previous studies have shown that individuals with obesity and those without obesity have diverse responses to lipolytic hormones following MICT: individuals not classified as obese experience elevated levels of EPI and GH when compared to those classified as obese (Koppo et al., 2010; Weltman et al., 2008), which may be attributed to the lower level of sympathetic excitability of obese people (Hansen et al., 2012). In this study, the EPI of obese young women in the MICT group did not increase following three acute interventions which can be attributed to the fact that the subjects of this study are obese individuals. However, in the $HIIT_{120}$ and $HIIT_{90}$ groups, EPI immediately increased following the 1st and 20th exercises. A study

conducted by Peake et al. (2014) found that HIIT (80%$\dot{V}O_{2max}$) increased EPI release levels whereas they remained unaffected by MICT (65%$\dot{V}O_{2max}$).

Furthermore, in the current study, the SIT program led to an increase in EPI following exercise only during the post-training phase, but not in the pre-and mid-training stages. This increase can be attributed to the fact that the initial resistance during the SIT program started at 1kg and incrementally increased to 3 kg by the later stages, resulting in a lower overall workload in the pre-and mid-training stages. Additionally, the absolute intensity and pure exercise time were relatively low during the pre-and mid-training stages (4 minutes) which may have been insufficient to induce an increase in EPI. This finding may reflect the lower volume of exercise (lower load and/or short exercise time) in the SIT group, as well as the attenuated release of lipolytic hormones seen in obesity (Hansen et al., 2012) and this effect may be mitigated following 12 weeks of fat loss. EPI is secreted by the adrenal medulla which is dominated by the sympathetic nerve; therefore, its secretion will increase alongside any increase in intensity (Verboven et al., 2018). However, its highest intensity was less than the 100%$\dot{V}O_{2max}$ threshold (Pritzlaff et al., 2000) and the EPI release may plateau at the point where the exercise intensity nears or is greater than 100%$\dot{V}O_{2max}$. This factor is crucial when considering the fat reduction noted within the HIIT cohorts in the current study and may explain why the varying intensity of HIIT has equivalent influences on the reduction of fat: HIIT will lead to a higher degree of activation of the sympathetic nervous system (Thompson et al., 2012), which may explain the increase of EPI in obese people in this study.

Obesity inhibits the sympathetic excitation provided by MICT; therefore, this form of exercise does not reach the exercise intensity threshold required to stimulate a significant release of EPI. In this study, the MICT cohort did not exhibit any significant increase in EPI which may also correlate with exercise duration. Several studies have noted the existence of a threshold duration for hormone response to moderate-intensity exercise (Viru, 1992; Tremblay et al., 2005); it is conceivable that the exercise duration adopted by this study failed to attain (or exceed) the required threshold duration resulting in the absence of an increase in EPI levels. Based on the aforementioned observations, it can be inferred that, in contrast with MICT, HIIT can trigger a higher level of EPI liberation into the serum of young females with obesity, and that serum levels are comparable regardless of the HIIT protocol followed.

Epinephrine (EPI) serves as a hormone indicator of training adaptation and can reflect changes in overall workload. In this study, EPI levels did not decrease in each groups during the early, middle, and late stages of training (except for an elevation observed in the SIT group during the late stage) and the exercise power and RPE gradually increased to attain the training principle of relative exercise intensity based on the percentage of $\dot{V}O_{2max}$ (Granata et al., 2018). The purpose of this study was to reduce exercise time to address the issue of time constraints preventing individuals from engaging in physical activity (rather than enhancing exercise performance or outcomes via overload training principles). If future studies are conducted with different goals unrelated to timesaving, attention should be paid to the principle of progressive overload in training load and the potential impact of exercise duration on weight loss and metabolism.

When considering GH of the lipolytic hormone, the increases in GH levels were observed to be comparable across all three forms of HIIT and MICT (in the 1st, 20th, and 44th acute exercise interventions). Sasaki et al. (2014) demonstrated that analogous GH increases were observed following HIIT and MICT in sedentary males before and after a 4-week exercise program which is in alignment with the present data. It could be hypothesized that HIIT and MICT can lead to a comparable rise in circulating GH titers, which is in keeping with the hypothesis that both can promote fat loss as demonstrated. In another study (Peake et al., 2014), elevations in GH levels following HIIT and MICT have been established, although the former leads to a more enduring GH rise. These findings are not in agreement with the currently presented data and may be attributed to the inhibition of GH release during exercise in individuals with obesity (Hansen et al., 2012, Weltman et al., 2008).

In contrast with MICT, HIIT can initiate the release of specific lipolytic hormones such as EPI among young females with obesity; however, when analyzing other lipolytic endocrine components (such as GH), the two exercise paradigms exhibit comparable results. It should be noted that the release of the lipolytic hormone following acute HIIT may not be completely transformed into fatty acid oxidation, and the increase in exercise intensity may weaken the stimulation of EPI on lipolysis (Braun et al., 2018). Additionally, the increased lipolytic hormone may be dispersed rapidly by the body (Thompson et al., 2012). The potential mechanisms of HIIT weight loss correlate with the following factors: HIIT consumes a large amount of glycogen during exercise, fat is fully oxidized in the recovery period and glycogen is synthesized to supplement the carbohydrate consumed during exercise (Kiens and Richter, 1998); HIIT has a greater stimulating effect on muscle; and triglyceride (TG) is redistributed from adipose tissue to muscle (Kuo and Harris, 2016). When considering the adaptive change of adipose tissue following long-term HIIT, this form of exercise can improve the phosphorylation expression of hormone-sensitive lipase (HSL) to improve the lipolysis ability of adipose tissue and promote lipolysis (Liu et al., 2020).

Fat loss effects of MICT and HIIT

Currently, more than 50% of China's adult population is classified as overweight or obese (Pan et al., 2021) which poses a serious risk to their wellbeing and life expectancy. The participants selected for this research were all classified as obese and had a body fat percentage of more than 30%.

The current recommended exercise requirements for the overweight and obese is defined as thirty to sixty minutes of moderate-intensity exercise (i.e., 46-64%$\dot{V}O_{2max}$) at least five days a week or with a higher intensity of exercise (i.e., >64%$\dot{V}O_{2max}$) (ACSM, 2021). However, many people find that the overall volume of exercise proposed is excessive and the duration onerous

which results in a lack of adherence by individuals with obesity (Sharifi et al., 2013).

Previous research has identified that HIIT is an effective approach to weight loss and that a 12-week program of HIIT compared to MICT with identical exercise volume has an equivalent (Zhang et al., 2017) or superior impact on fat loss (Zhang et al., 2021). Additionally, it was noted that HIIT is more efficacious in diminishing visceral fat (Maillard et al., 2016; Zhang et al., 2015; 2021). This research confirms that HIIT promotes fat loss: there was a comparable fat reduction observed across all 3 of the HIIT cohorts. Additionally, the same HIIT cohorts experienced greater fat loss than the MICT group. The changes in body weight and BMI were parallel between the three HIIT groups but there were no changes among the MICT participants which may be due to the small change in fat mass when considering muscle mass, resulting in no difference in total body weight. Contemporary meta-analyses and systematic reviews have additionally supported the impact of HIIT on fat reduction in obesity (Wewege et al., 2017; Sultana et al., 2019; Sabag et al., 2022a). In agreement with other published studies, the current work signifies that HIIT is a superior approach for fat reduction (when compared to MICT) as it exceeds the critical level of 90%$\dot{V}O_{2max}$ (typically over 8 to12 weeks, intervals of 1 to 4 minutes, 1 to 10 repetitions per session, and a work to rest ratio of between 1:1 and 1:2) (Sabag et al. 2022b). Additionally, the contributions of the varying HIIT regimes to fat reduction are comparable. It should be noted that participation in sprint interval training saves time, is more effective at promoting fat loss than MICT, and could provide an alternative exercise program for obese people.

Role of lipolytic hormones in fat loss (during MICT and HIIT)

The process underlying fat reduction following HIIT should be interpreted from the perspectives of both the acute response and adaptation over the long term. In the first instance, the results from repeated measure ANOVA to compare the immediate changes of EPI after three acute exercise sessions showed that both the $HIIT_{120}$ and $HIIT_{90}$ groups exhibited significantly higher EPI changes compared to the MICT group, while there was no difference between the two HIIT groups. Additionally, the SIT group showed significantly higher changes in EPI compared to the MICT group immediately after the 44th exercise session. During the 1st, 20th, and 44th training sessions, both the SIT group and the $HIIT_{120}$ group, as well as the $HIIT_{90}$ group, demonstrated significantly higher levels of post-exercise blood GH compared to pre-exercise levels. During acute exercise, adipose tissue mobilization is regulated by rapid and slow lipolytic hormones, i.e. EPI and GH, respectively (Scott et al., 2024). Lipolytic hormones accelerate lipid mobilization and degradation by attaching to beta-adrenergic receptors, especially within visceral adipose tissue (Hansen et al., 2012; Freda et al., 2008), heightening the activity of hormone-sensitive lipase (HSL) - an essential rate-limiting lipolytic enzyme - and enhancing the decomposition of triglycerides to yield glycerol and fatty acids (Freda et al., 2008; Yang and Mottillo, 2020). Additionally, lipolytic hormones induce the oxidation of lipids which could enhance the rise in EPOC during, and following, exercise. EPOC predominantly derives its energy from the oxidation of lipids (Atakan et al., 2022; Moniz et al., 2020; Islam et al., 2018). Therefore, although the energy source for HIIT in acute exercise is predominantly derived from carbohydrates (Hargreaves and Spriet, 2020), in the present work, the acute HIIT triggered the release of additional lipolytic hormones (EPI and GH) which attached to lipolytic hormone receptors and enhanced HSL function, elevated EPOC, and induced increased lipid degradation and oxidation. Circulation speed is augmented in fat following HIIT (Thompson et al., 2012) which facilitates the delivery of greater titers of lipolytic hormones and further promotes the process.

During long-term adaptation to HIIT, the improved fat reduction is associated with the long-term accumulation of acute HIIT which prompts the body to release additional lipolytic hormones. Furthermore, the continuing fat decrease following HIIT may mitigate against the inactivation of the release of lipolytic hormones seen in individuals with obesity (Hansen et al., 2012). The data acquired by this research indicates that no increase in EPI occurred following exercise in the early and mid-phases of the SIT intervention; however, heightened levels of EPI were observed during the latter stages. These results can be explained by the normalization of lipolytic hormone release following the SIT-induced fat reduction. Liu et al. (2020) used animal models to determine that, following extended periods of HIIT (compared with MICT), lipid cells had a more robust capacity for lipolysis when triggered by the identical lipolytic hormone titer which indicates that high-intensity interval training has a greater effect on heightening the lipolytic hormone sensitivity of adipose tissue. It can be surmised that long-term HIIT can mitigate the inactivation of lipolytic hormone release in individuals with obesity, heighten lipolytic hormone sensitivity and, in the long-term, lead to the release of greater lipolytic hormone concentrations in the course of acute exercise, thus promoting fat reduction.

Study limitations

In this study, there are some confounding factors that should be considered, such as the menstrual cycle of female participants. Although we attempted to record the menstrual cycle of female participants and excluded those using oral contraceptives or individuals with menstrual dysfunction, we were unable to restrict each exercise session to a specific phase of their menstrual cycle, which may have some influence on the results like post-exercise lipolytic hormone response. Moreover, there are limitations in the measurements. One of these limitations concerns the release of the lipolytic hormones following exercise: the time point at which the levels are optimal fluctuates. Due to limitations regarding the frequency of blood drawing, the samples were only collected immediately before exercise, directly following exercise, and three hours after exercise; therefore, the peak levels of the lipolytic hormones may have been missed resulting in an inadequate interpretation of the hormone liberation reactions following exercise. Furthermore, alterations in intra-abdominal visceral fat were not monitored throughout the fat loss.

Subsequent studies could exploit the dose-response association between lipolytic hormone level fluctuations and variations in visceral fat levels to explicate how HIIT can diminish visceral fat levels with greater precision. Finally, the data in the current study pertain only focused on obese young females, as such, generalizability of the data is limited, and further exploration is needed on whether similar patterns exist in other age groups and genders.

Conclusion

When compared with MICT, 12-week SIT, $HIIT_{120}$, and $HIIT_{90}$ interventions were more efficacious in fat reduction in obese women, partly benefiting from the greater release of lipolytic hormones during training sessions

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Key points

- After the 12-week intervention, increased levels of EPI were observed in the acute session following the completion of some varieties of HIIT (excluding MICT).
- Significant increases in GH were observed post-exercise in various HIIT and MICT during the three acute sessions.
- Various HIIT resulted in greater reductions of whole-body fat mass and body fat percentage (when compared to MICT).

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