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Acute effects of high-intensity interval, resistance or combined exercise protocols on testosterone – cortisol responses in inactive overweight individuals


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ABSTRACT
The purpose of this study was to compare the hormonal responses to one session of high-intensity interval training (HIIT, 4×4 min intervals at 85–95% maximum heart rate [HRmax], interspersed with 4 min of recovery at 75–85% HRmax), resistance training (RT at 50-70% of one repetition maximum 12-15 repetitions per set with 60s of recovery) or both (HIIT+RT) exercise protocol in a cohort of physical inactivity, overweight adults (age 18–30 years old). Randomized, parallel-group clinical trial among fifty-one men (23.6±3.5 yr; 83.5±7.8 kg; 28.0±1.9 kg/m2), physical inactivity (i.e., <150 min of moderate-intensity exercise per week for greater than 6 months), with abdominal obesity (waist circumference ≥ 90 cm) or body mass index ≥ 25 and ≤ 30 kg/m² were randomized to the following 4 groups: high-intensity interval training (HIIT, n=14), resistance training (RT, n=12), combined high-intensity interval and resistance training (HIIT+RT, n=13), or non-exercising control (CON, n=12). Cortisol, total- and free-testosterone and total-testosterone/cortisol-ratio (T/C) assessments (all in serum) were determined before (pre) and 1-min post-exercise for each protocol session. Decreases in cortisol levels were −57.08 (95%CI, −75.58 to −38.58; P=0.001; η²=0.61) and −37.65 (95%CI, −54.36 to −20.93; P=0.001; η²=0.51) in the HIIT and control group, respectively. Increases in T/C ratio were 0.022 (95%CI, 0.012 to 0.031; P=0.001; η²=0.49) and 0.015 (95%CI, 0.004 to 0.025; P=0.007; η²=0.29) in the HIIT and control group, respectively. In per-protocol analyses revealed a significant change in cortisol levels [interaction effect F(7.777), η²=0.33] and T/C ratio [interaction effect F(5.298), η²=0.25] between groups over time. Additionally, we showed that in both the intention-to-treat (ITT) and per protocol analyses, HIIT+RT did not change serum cortisol, total or free testosterone. The present data indicate a HIIT reduced cortisol and increased total-testosterone/cortisol-ratio levels significantly in physically inactive adults. Further study is required to determine the biological importance of these changes in hormonal responses in overweight men.
**Abbreviations**

AR  Androgen receptors  
BMI  Body mass index  
HIIT High-intensity interval training  
1RM One repetition maximum  
RPE, Borg scale Rating of perceived exertion  
RT  Resistance training  
SHBG  Sex hormone globulin binding  
TEM  Technical error of measurement  
T/C ratio  Testosterone/cortisol ratio  
WC  Waist circumference
INTRODUCTION

Current lifestyle trends characterized by inactivity and poor dietary habits have led to a dramatic decline in the health of the Latin American population (1). Decreased physical activity and sedentary behavior – combined with poor dietary habits – have been implicated as potential contributing factors in the obesity crisis (2). Overweight and obesity may affect metabolic health with hormonal and sex hormone levels abnormalities (3). In obese men before puberty and at its onset, there has been an increase in free testosterone, in response to the decrease in SHBG that occurs in this condition in order to maintain total testosterone levels (4). Two integral endocrine gland hormones greatly affected by obesity are testosterone and cortisol (3, 5). These hormones have significant effects on both protein metabolism and lipolysis, two metabolic pathways affected by obesity, but also essential in the management and treatment of obesity and cardiovascular comorbidities (5, 6).

Interestingly, physical exercise, particularly physical training is an effective strategy to combat metabolic disorders due to its ability to influence body composition and some biomarkers, such as cholesterol, inflammatory cytokines, and insulin resistance (7, 8). High-intensity exercise (>80% of peak oxygen uptake), has been shown to produce significant increases in circulating hormones (9, 10). Along this line, testosterone and cortisol have been suggested to be important mediators of the exercise-induced hormonal responses, and are considered as useful biomarkers of anabolic and catabolic hormonal control, respectively (11, 12). Furthermore, the testosterone/cortisol ratio (T/C ratio) has been suggested to be an indicator of the anabolic/catabolic status (9). Thereby, either an increase in testosterone, a decrease in cortisol, or a combination of both would indicate the potential overall state of anabolism (13).

However, the relationship between the time course of training adaptations during a training cycle and the parallel time-course of potential changes in resting testosterone, cortisol or T/C ratio is not well established. For example, a 14-day mesocycle with frequent high-intensity interval 5 training (HIIT) sessions induced both endurance adaptions and increases in serum testosterone concentration in thirteen male junior triathletes (15.8±1.8 yr) (14). West et al. (14) showed an acute increase in concentrations of testosterone and cortisol concentrations among twelve healthy young men (21.8±1.2 yr), after several resistance
exercise sessions. In contrast, others have reported significant adaptive responses in professional cyclists to a training program that also induced declining testosterone and increasing cortisol concentrations indicative of an increased catabolic state (15). These discrepancies among studies may occur due to differences in the baseline training status of participants, as well as the training volume/intensity performed.

Obesity induces increased cortisol, while testosterone levels decrease, and several studies have reported that the T/C ratio is significantly correlated with age, body mass index (BMI) and waist circumference (16, 17). In addition, there is considerable information given concerning the endocrine responses to exercise in lean individuals which serves as a point of reference for research using an obese model. Notwithstanding, due to anomalies in the hormonal responses to exercise in obese subjects (18), as well as scarce data on this topic, studies on endocrine responses to exercise in obese individuals are warranted.

Thus, to determine the main factors related to mode of exercise training sessions (i.e., high-intensity interval training - HIIT, resistance training - RT, or combined training – HIIT + RT) that greatly influence acute testosterone and cortisol responses, it is important to create an anabolic hormonal response and optimize the adaptations to training. Although strength and aerobic training intensity volume have a critical influence on the magnitude and/or duration of the acute response of testosterone and cortisol (12), to the best of our knowledge, there are no data available regarding the comparison between HIIT, RT or HIIT + RT on the acute responses of testosterone and cortisol, especially in overweight individuals, who may have risk to altered hormonal responses.

Therefore, the aim of the present study was to compare the acute hormonal responses of cortisol, total- and free-testosterone and total-testosterone/cortisol-ratio at the beginning and after one session of HIIT, RT or both exercise protocol in a cohort of physical inactivity, overweight adults (age 18–30 years old). It was hypothesized that the HIIT + RT protocol (due to the higher intensity) would induce the greatest metabolic perturbations and, therefore, the largest acute responses of cortisol, total- and free-testosterone compared to the RT and the HIIT protocol performed alone.
METHODS

Study design and setting
The present study is a secondary randomized controlled trial (ClinicalTrials.gov ID: NCT02915913; “BrainFit Study”). The study received ethical approval from the Medical Research Ethics Committee of The Universidad Nacional de Colombia (Code N° 018-223-16). Random allocation to treatment is performed at the individual level. Details of sample calculation, randomization, characteristics of participants, design, methods, and measurements of the BrainFit Study had been previously published elsewhere (19), however, the most relevant information is briefly described below.

Participants
Males aged 18–30, physical inactivity (i.e., <150 min of moderate-intensity exercise per week for greater than 6 months, according to an International Physical Activity Questionnaire), with abdominal obesity: waist circumference ≥ 90 cm or excess weight, body mass index ≥ 25 and ≤ 30 kg/m², identified as being willing and with almost immediate availability was enrolled. Inclusion and exclusion criteria are provided in Supplement File Table 1S.

Recruitment
Males who had provided consent-to-contact from a previous study in our laboratory were recruited from different educational institutions (Universidad Nacional, Universidad del Rosario, Universidad Santo Tomás, Universidad Manuela Beltrán and Universidad de la Sabana) in the district of Bogotá, Cundinamarca Department in the Andean region.

Blinding and randomization methods
Randomization into the four study arms was performed by CEMA at the University of Rosario, Bogotá, Colombia, using block randomization with a block size of four. Eligible participants were randomly assigned after completing the baseline measurements to either the control or exercise training groups. The principal investigator coordinated the allocation sequence, and randomization was computer generated. Investigators and statisticians were blinded to treatment allocation throughout the trial protocol. Access to the allocation code was restricted to one study statistician who did not perform the final study analyses. Randomization was conducted independently using sealed opaque envelopes. Moreover,
the importance of maintaining the blinding and allocation concealment was reinforced by regularly scheduled conference calls at the sites and daily meetings with the field investigators.

**Intervention**

Each of the volunteers participated in four randomized trials (HIIT, RT, HIIT + RT and control [no exercise]), and the starting trial was randomized. All the interventions were performed in the same facilities.

1. Non-exercise group (control): During the experimental trial, the control group was in the training facility for the same duration as the other exercise groups, sitting relaxed, and not allowed to consume food.

2. High-intensity interval training (HIIT) group: The HIIT protocol was completed with fast walking or running on a treadmill with the deck inclined (i.e., grade) to reach the desired intensity. All HIIT sessions were preceded with a 5-min warm-up and ended with a 4-min cool down at 65% heart rate reserve until the expenditure of 400 to 500 kcal. The HIIT protocol consisted of four bouts of 4-min intervals at 85–95% HR reserve, interspersed with 4 min of active recovery at 75–85% HR reserve. Participants in the HIIT groups were instructed to reach the target HR for each interval within the first 2 min of the 4-min interval. We calculate the training energy expenditure for participants’ age ranges associated with meeting the consensus public health recommendations from the World Health Organization (20) and the US Department of Health and Human Services (21). During the supervised intervention, we record heart rate (HR) using an HR monitor (Polar Pacer, USA) to ensure compliance with the exercise stimulus at the predetermined target HR zone. In addition, the rating of perceived exertion (RPE, Borg scale) was also measured in each exercise session (15-17 in high intensity and 11-13 to recovery).

3. Resistance training (RT) group: The protocol training phase at 50-70% of one repetition maximum, 1 x 12-15 repetitions, 60 s recovery as many times as needed according to subject weight until the expenditure of 400 to 500 kcal. The five upper body exercises included the bicep screw curl, triceps extension, dumbbell side lateral raise, dumbbell front raise and military press. Three lower body exercises included dumbbell squat, dumbbell front lunge, and dumbbell side lunge. HR and Borg RPE were monitored in each exercise
session. Each session was preceded and followed by a gradual warm-up and cool-down period (both of 10-min duration and consisting of walking and light, static stretching (avoiding muscle pain) in most muscle groups). The cool-down period also includes relaxation and stretching exercises. Each participant's workloads were prescribed on an individual basis using their one repetition maximum results during the initial orientation. Muscular strength was assessed two days before acute intervention using the one repetition maximum (1RM) test, implemented according to similar procedures (22). The 1RM was performed in five upper body exercises included the bicep screw curl, triceps extension, dumbbell side lateral raise, dumbbell front raise, military press and three lower body exercises included dumbbell squat, dumbbell front lunge, and dumbbell side lunge, carried out in the morning between 9 and 11 h, and the highest load of three attempts were reported per exercise.

4. Combined training (HIIT+RT) group: This group was received both the HIIT and RT protocols as described above. Therefore, the energy expenditure associated with the physical training prescribed for the vigorous intensity group was ≈ 400 to 500 kcal/session.

Training intensity and energy expenditure during training

In terms of training intensity, the actual values of intensity were the mean of HR measured in HIIT or combined groups, and the average value of workload and repetitions determined in acute session in RT group, respectively. The exercise training was supervised throughout. Attendance at supervised sessions as well as checks for compliance with target HR and energy expenditure were monitored and recorded by research staff. Energy expenditure was estimated during exercise via indirect calorimetry using a Cosmed K5 portable metabolic system (Rome, Italy) assuming a non-protein respiratory exchange ratio (23).

Blood draws and analysis

Participants arrived at the CEMA laboratory between 6:00 and 9:00 AM, following an overnight fast. Participants were reminded to maintain standardized conditions prior to each assessment point which included arriving in a hydrated state having abstained from caffeine and alcohol consumption for 36 h. In the four groups, blood (10 mL) was drawn by venipuncture at the same time of day (at rest and immediately after intervention). Serum
was then extracted, centrifuged at 3,300 revolutions per minute, aliquoted, and stored at −80°C until subsequent analysis. Concentrations of serum total-free testosterone and cortisol were analyzed by using commercially available electrochemiluminescence immunoassay kits (Roche Diagnostics GmbH, Mannheim, Germany). All analyses were tested in the same assay with standard procedures (Deyi Biomedical Technology Co., Ltd., Beijing, China). Within-assay, the coefficient of variation of standards and samples were: 7% and 6%, and 4%, for total testosterone, free testosterone, and cortisol, respectively.

**Body composition and performance measures**

Body weight (kg) was measured using an electric scale (Model Seca® mBCA 514 Medical Body Composition Analyzer, Hamburg, Germany) with a range of 0 to 200 kg and with an accuracy of within 100 g. Height was measured with a portable stadiometer with a precision of 0.1 mm and a range of 0–2.50 m (Seca® 274, Hamburg, Germany). BMI was calculated by using the formula proposed by Quetelet where BMI = body mass (kg)/height (m2). Body mass index status was evaluated according to the World Health Organization criteria (24).

The waist circumference (WC) (cm) was measured as the narrowest point between the lower costal border and the iliac crest. When this point was not evident, it was measured at the midpoint between the last rib and the iliac crest, using a metal tape measure (Lufkin W606PM®, Parsippany, New Jersey, USA), in accordance with the International Society for the Advancement of Kinanthropometry guidelines (25). The evaluation process was carried out by a team of professionals (2 physical therapy professors) with extensive experience in anthropometric measurement. The technical error of measurement (TEM) values were less than 2% for all anthropometric variables.

Body fat mass was determined with a multifrequency bioelectrical impedance analyser (BIA by a tetrapolar whole body impedance (Model Seca® mBCA 514 Medical Body Composition Analyzer, Hamburg, Germany). A detailed description of the BIA technique can be found in a previous study (26). For the calculation of intra–inter observer TEM, at least 50 subjects needed to be measured (30 men, 20 women, aged 22.3±2.1 yr). The corresponding intra-observer technical error (% reliability) of the measurements was 95%.

**Statistical analysis**
Prior to the planned statistical analyses, a preliminary analysis was conducted (Shapiro–Wilk test) to confirm data distribution normality. A two-way mixed analysis of covariance (ANCOVA) with repeated measures was used to test the main effect (i.e., group effect) and the interaction effect (time and group interaction) with the baseline value as a covariate on the outcome variables (hormonal responses). Cohen's d for effect size was also calculated to determine the magnitude of the group differences. The effect size was classified as small, medium, and medium-to-large effects (<0.20, 0.2–0.6 and 0.6–1.2, respectively), and partial $\eta^2$ was considered small if $\eta^2<0.04$, and large if $\eta^2>0.36$ in interaction effect analysis. Datasets are summarized in the text as mean and standard deviations. All statistical analysis was performed using Statistical Analysis IBM SPSS Statistics version 24.0 (Chicago, IL, USA).

RESULTS
Recruitment began September 1, 2016, and closed on June 30, 2017. The final follow-up visit was in July 2017. Of 70 participants who entered the run-in phase, 56 (80%) were randomized. Reasons for pre-randomization withdrawal included BMI too high, refuse to participate, or a medical condition (Supplement File Figure 1S). Five participants (two from the control group, two from the RT group, and one from the combined group) were excluded from blood samples analyses because serum was technically inadequate for hormonal analysis.

Table 1 shown baseline participant characteristics. No significant intergroup baseline differences were observed.

Acute hormonal response results are displayed in Figure 1 to Figure 4. Decreases in cortisol levels were $-57.08$ (95%CI, $-75.58$ to $-38.58$; $P=0.001$; $\eta^2=0.61$) and $-37.65$ (95%CI, $-54.36$ to $-20.93$; $P=0.001$; $\eta^2=0.51$) in the HIIT and control group, respectively (Figure 1).

Neither exercise protocol significantly increased serum total or free testosterone (Figure 2 and Figure 3, $P>0.05$).
Increases in T/C ratio were 0.022 (95%CI, 0.012 to 0.031; P=0.001; \( \eta^2=0.49 \)) and 0.015 (95%CI, 0.004 to 0.025; P=0.007; \( \eta^2=0.29 \)) in the HIIT and control group, respectively (Figure 4).

In per-protocol analyses, differences between groups over time were noted for: Cortisol (control vs RT, P=0.002), (control vs HIIT+RT, P=0.001), (HIIT vs HIIT+RT, P=0.001), (HIIT vs RT, P=0.002), interaction effect F \( (7.777) \), \( \eta^2=0.33 \); and T/C ratio (control vs RT, P=0.011), (control vs HIIT+RT, P=0.019), (HIIT vs HIIT+RT, P=0.006), (HIIT vs RT, P=0.003), interaction effect F \( (5.298) \), \( \eta^2=0.25 \). (Figure 1 and Figure 4, P>0.05).

**DISCUSSION**

The main findings of the present study were the HIIT protocol induced marked decreases in the serum cortisol concentrations in overweight young male adults, which was not observed following RT and combined protocols. In addition, HIIT protocol induced a significant increase in the T/C ratio in these individuals, which also did not occur following the other protocols.

Previous studies have shown a correlation between the acute response of testosterone to single training sessions and the magnitude of the increase in strength, power and muscle mass resulting from chronic strength training adaptations (27, 28). Besides the important role of testosterone in the synthesis of contractile proteins (29, 30), as well as in the synthesis of neurotransmitters related to strength production (27), the increase in testosterone in response to strength training seems to be related to the magnitude of the increase in the number of androgen receptors (AR) in skeletal muscle human cells (29). Additionally, it has been proposed that sex hormone globulin binding (SHBG) or AR increase after resistance exercise in obese male (5).

Other studies in animal models demonstrated that the amount of ARs plays an important role in the magnitude of muscle adaptations to training (30). Thus, the manipulation of the various factors related to the training session that influence acute hormonal responses, such as volume and intensity, time interval and muscle mass involved can optimize the increase
in testosterone in response to the training session and up-regulate AR expression; thereby, amplifying the anabolic effects (27).

Regarding aerobic training, although the importance of the anabolic hormonal response is not well known; testosterone and cortisol seem to be more responsive to higher intensity exercise (31, 32). Therefore, it is not clear why the HIIT protocol performed in the present study did not elicit a significant elevation in testosterone level. This could be explained by the measurement time; previous studies have been reported peak testosterone concentration between 0 and 10 minutes after exercise (11, 29). Hormonal response to aerobic exercise can be explained in part by increased sympathetic activity during exercise (33) or even by greater vasodilation in the testes stimulated by the increased release of nitric oxide resulting from the exercise (34). Thus, because obesity is also related with damage in endothelial function, it is possible that the mechanism related to vasodilatation in testes may be at least partially impaired and possibly altering the overall endocrine response (7).

On the other hand, some authors have shown that anaerobic exercise (i.e., strength training, anaerobic running or cycling) seems to be a powerful stimulus to increases in testosterone and cortisol levels (26), which can be explained by the marked influence of the anaerobic glycolytic pathway in stimulating acute hormonal increases in response to exercise (35). Therefore, it could be expected that both HIIT and RT sessions used in the present study, in which the glycolytic pathway is strongly activated, have a great potential for stimulating testosterone.

In a study by Lu et al. (35), a correlation between the increase in testosterone and increase in lactate during an incremental protocol was observed in rats. Furthermore, these authors demonstrated in vitro that the direct infusion of lactate in the testes resulted in a dose-dependent increase in testosterone. The AR content was not measured in the present study, and it is possible that the non-significant increase in the circulating total testosterone and decrease in the free testosterone might have had the influence of the increased hormone-receptor interaction. Furthermore, it is well known that the concentrational peak of testosterone occurs around 8 am and decreases to its lowest levels at about 4 pm due to circadian cycle regulation (36). Therefore, the non-significant changes of serum testosterone might have been a result of increased receptor binding and clearance, and not
simply a lack of increased production. Interestingly, Willoughby and Taylor (29) found a positive correlation between the acute response of testosterone and increase number of androgenic receptors after resistance protocol \((r=0.89, p <0.05)\).

However, this hypothesis should be considered with caution and remains speculative. Another explanation for the non-significant changes in testosterone levels is due to overweight/obesity states inducing hormonal abnormalities (3), and specifically, testosterone and cortisol are greatly affected by obesity (37). Future studies comparing testosterone, ARs, SHBG responses to exercise measured during the first-hour post-exercise between healthy-weight and overweight or obese individuals performing the same protocol are needed to determine whether in fact, adiposity may impair increases in testosterone in response to exercise. Finally, while the intensity of the exercise is critical to evoking a hormonal response, so is the dosage of the exercise (i.e., average intensity x total duration), thus perhaps the duration component in our exercise was insufficient. Wilkerson et al. used a short-term continuous exercise protocol, not HIIT, but alluded to this possibility in their work on exercise testosterone responses (38).

With respect to circulating cortisol, a decrease was observed after the HIIT protocol, which disagrees with studies investigating the responses of this hormone after high-intensity exercise bouts. Specifically, investigators observed no changes after moderate interval training of rowing (39), or even acute increases after HIIT at cycle ergometer (40). Cortisol, which is responsible for 95% of glucocorticoid activity, is a catabolic hormone responsible for lipid and protein degradation and subsequent mobilization of energy substrate during exercise (41). Although several studies investigated the cortisol acute responses to aerobic and strength training bouts in healthy-weight individuals (3, 31, 37, 42), there is less information regarding the cortisol responses to HIIT, RT and combined training in overweight male individuals.

Because the role of cortisol in mobilizing energy substrate during exercise, it is not clear why our subjects significantly reduced their cortisol levels following HIIT training, and the same was not observed when observing RT and combined training, even equalizing the total energy expenditure between the exercise sessions. In our study, a decrease in cortisol levels was observed in the control group, which could be attributed to the circadian cycle,
taking into account the peak concentration occurs around 8 am and its progressive decline until reaching the lowest values around 8 pm. (36).

Based on the present results regarding the reduction of cortisol levels after HIIT training (as we implemented), in order to induce a less catabolic response to exercise, HIIT may be suggested as an alternative to improve cardiorespiratory fitness without excessive increases the catabolic state, since it has been shown that continuous aerobic training promotes greater increases in cortisol levels (38). Indeed, some studies have shown that the use of pharmacological cortisol has an inhibitory effect on the steroidogenic process in Leydig cells, via enzymatic inhibition (43) or by eliminating cAMP production (40).

Our findings may suggest that the acute response of cortisol may be favorable in overweight and obese individuals following HIIT or, otherwise suggest that obese individuals have an impaired hypothalamic-pituitary-adrenal axis. The purpose of this study was to investigate whether there are differences in the acute testosterone, cortisol and T/C ratio following HIIT, RT and combined training bouts with similar energy expenditure in overweight individuals. Indeed, our results have shown that HIIT protocol induced a significant increase in the T/C ratio, which appears to be entirely due to the decrease observed in cortisol levels and the maintenance of testosterone levels. One could suggest that this positive change in the T/C ratio is favorable to muscle adaptations induced by HIIT because of a more anabolic environment, but the role of this hormonal ratio as an indicator of muscle adaptations induced by a training is not entirely understood and should be further investigated.

One possible limitation of this study is the absence of continuous aerobic training and combined strength and continuous aerobic training bouts to compare to the exercise protocols investigated. In addition, we investigated the testosterone, cortisol and T/C ratio using specific volume and intensity and our results should not be extrapolated to others exercise protocols.

Moreover, to what extent the acute hormone responses observed in the present study could explain chronic adaptations to training needs to be investigated in a long-term study. On the other hand, this appears to be the first study to compare the acute testosterone and cortisol
responses to HIIT, RT and combined HIIT and RT exercise bouts with similar energy expenditure in overweight individuals with a relatively robust sample size.

In conclusion, the results of this study showed that the HIIT protocol induced a decrease in the cortisol levels as well as an increase in the T/C ratio, whereas non-significant changes were observed following RT or combined HIIT and RT bout. Based on the relationship, suggested in the literature, between acute hormonal responses and chronic neuromuscular adaptations to strength training, this could be positive for the optimization of long-term muscle adaptations. However, the extent of the importance of this response in chronic adaptations to HIIT remains speculative and further studies trying to elucidate hormonal acute responses to HIIT adaptations in overweight individuals are needed to determine whether the meaning of these acute responses.
REFERENCES


Table 1. Baseline participant characteristics by group treatment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=14)</th>
<th>HIIT (n=14)</th>
<th>RT (n=14)</th>
<th>HIIT+RT (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.75 (3.44)</td>
<td>24.50 (3.76)</td>
<td>22.85 (3.16)</td>
<td>22.25 (3.41)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88.64 (8.90)</td>
<td>81.75 (6.78)</td>
<td>83.98 (7.48)</td>
<td>80.65 (6.76)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.75 (0.05)</td>
<td>1.72 (0.05)</td>
<td>1.68 (0.18)</td>
<td>1.69 (0.05)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.75 (2.01)</td>
<td>27.43 (1.74)</td>
<td>27.86 (1.31)</td>
<td>28.18 (1.25)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>97.96 (6.31)</td>
<td>95.36 (4.91)</td>
<td>94.18 (4.68)</td>
<td>96.93 (5.80)</td>
</tr>
<tr>
<td>Body fat percentage, %</td>
<td>28.75 (4.16)</td>
<td>26.21 (4.39)</td>
<td>27.08 (3.78)</td>
<td>28.15 (3.65)</td>
</tr>
<tr>
<td>VO₂max, ml·kg·min⁻¹</td>
<td>41.22 (17.36)</td>
<td>40.61 (16.76)</td>
<td>38.97 (10.55)</td>
<td>37.88 (13.62)</td>
</tr>
<tr>
<td>Exercise test variables, Peak relative VO₂max, ml·kg·min⁻¹</td>
<td>N.A</td>
<td>32.6 (4.2)</td>
<td>29.9 (4.9)</td>
<td>31.6 (3.2)</td>
</tr>
<tr>
<td>EE during training, kcal</td>
<td>N.A</td>
<td>462.69 (74.91)</td>
<td>460.91 (86.77)</td>
<td>461.70 (59.12)</td>
</tr>
<tr>
<td>Total testosterone, ng/ml</td>
<td>4.30 (1.71)</td>
<td>4.44 (1.00)</td>
<td>4.75 (1.96)</td>
<td>4.73 (0.96)</td>
</tr>
<tr>
<td>Free testosterone, pg/ml</td>
<td>12.97 (6.96)</td>
<td>13.67 (3.07)</td>
<td>16.23 (5.20)</td>
<td>16.04 (4.56)</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>120.48 (44.73)</td>
<td>151.14 (27.86)</td>
<td>147.11 (25.97)</td>
<td>147.54 (37.39)</td>
</tr>
<tr>
<td>T/C ratio</td>
<td>0.042 (0.027)</td>
<td>0.031 (0.009)</td>
<td>0.034 (0.015)</td>
<td>0.034 (0.010)</td>
</tr>
</tbody>
</table>

Mean (standard deviation). HIIT, high-intensity interval training; RT, resistant training; BMI, body mass index; EE, energy expenditure; VO₂max, cardiorespiratory fitness; T/C, total-testosterone/cortisol-ratio; NA, not applicable.
Figure 1. Acute effects by group treatment on cortisol responses in inactive overweight individuals

Figure 2. Acute effects by group treatment on free testosterone responses in inactive overweight individuals

Figure 3. Acute effects by group treatment on total testosterone responses in inactive overweight individuals

Figure 4. Acute effects by group treatment on total testosterone/cortisol ratio responses in inactive overweight individuals

Figure Supplemental 1 (FS1). The BrainFit Trial flow diagram

Figure legend. Those whose blood samples (n=5) were technically inadequate and not analyzed. BMI, body mass index.
Highlights

- HIIT reduced cortisol and increased total-testosterone/cortisol-ratio levels significantly in physically inactive adults, which was not observed following RT and combined protocols.
- HIIT protocol induced a significant increase in the T/C ratio in these individuals, which also did not occur following the other protocols.
- HIIT adaptations in overweight individuals are needed to determine whether the meaning of these acute responses.
**Figure 1**

The figure presents cortisol levels pre and post intervention for four groups: CONTROL, HIIT, RT, and HIIT + RT. The top panels display individual cortisol trajectories for each group, with significant decreases noted in the CONTROL and HIIT groups (P=0.001, $\eta^2=0.51$ and $P=0.001$, $\eta^2=0.61$, respectively), while the RT and HIIT + RT groups show no significant change (P=0.679, $\eta^2=0.01$ and $P=0.868$, $\eta^2=0.01$, respectively).

The bottom panel illustrates the between-group differences in cortisol changes, with an interaction effect $F(7,77) = P=0.001$, $\eta^2=0.33$. Post-hoc tests show significant differences between the CONTROL and HIIT groups (P=0.002), between the CONTROL and RT groups (P=0.001), and between the CONTROL and HIIT + RT groups (P=0.001).
Figure 2

CONTROL

HIIT

RT

HIIT + RT

Between-Group difference

Mean [SEM] Free testosterone (pg/mL)

Interaction effect F (0.636)

P=0.591, η²=0.04

Figure 2
Figure 5

ENROLMENT

Indivudual Screening n=70

Assessment of eligibility for study n=60

Excluded:
- BMI too high n=3
- Too active n=7

Eligible and informed consent n=58

Excluded:
- BMI too high n=1
- Medical conditions n=1

Excluded:
- refuse to participate n=2

Randomisation n=56

Allocation

Control n=14
Non-exercise
Discontinued intervention -Missing data for blood sample n=2
Included in analysis n=12

High-intensity interval training n=14
Single Session
Discontinued intervention -none
Included in analysis n=14

Progressive resistance training n=14
Single Session
Discontinued intervention -Missing data for blood sample n=2
Included in analysis n=12

Combined training n=14
Single Session
Discontinued intervention -Missing data for blood sample n=1
Included in analysis n=13

FOLLOW-UP

ANALYSISYS