Submitted: 30 August, 2023 Accepted: 13 November, 2023 Published: 29 February, 2024 DOI:10.22514/jomh.2023.124

JOMH
Journal of Men's Health

ORIGINAL RESEARCH

# Effects of short-term high-intensity interval training on growth hormone, cortisol, and leptin levels

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## Abstract

Exercise is known to be very effective for increasing hormone levels; however, the effects of high-intensity interval training (HIIT) protocols are uncertain. The study aimed to examine the effect of HIIT on growth hormone (GH), cortisol, leptin levels and anaerobic capacity (AC) with the intention of contributing to the development of exercise protocols better adapted to athletes' training programs and optimized for hormone regulation. In the study, participants underwent a 2-week HIIT protocol. They were randomly divided into two groups (n = 10 for HIIT and n = 10 for the control) in a double-blind manner. Twenty healthy male athletes aged 18–30 years were subjected to a short-term HIIT exercise on a bicycle ergometer. The blood and Wingate AC data collected in the first and last sessions of the protocol were analyzed, and the GH, cortisol, leptin and AC levels were examined. Based on the results of the analysis, statistically significant differences were observed in GH ($p < 0.05$), cortisol ($p < 0.05$), leptin ($p < 0.001$) and AC ($p < 0.01$) levels between the initial and final tests among the participants. However, while there were no statistically significant differences between the groups in GH ($p = 0.088$), cortisol ($p = 0.905$), leptin ($p = 0.262$) and AC ($p = 0.06$) levels. The study's findings indicate that the short-term HIIT protocol caused significant differences in GH, leptin, cortisol and WAnT AC levels within the group depending on the time main effect. These results suggest that HIIT is efficient for biochemical changes and performance. However, no significant differences were found between the groups. Results suggest that HIIT protocols may elicit different responses between individuals and that time modulates these effects. It is recommended that future studies should be conducted with more participants and longer HIIT protocols.

## Keywords

Anaerobic capacity; Cortisol; Exercise; High-intensity interval training; Hormones; Leptin; Wingate test

## 1. Introduction

The effects of exercise on hormone release have been known for many years, and the effect of exercise on hormone release is an essential part of sports health research and sports physiology [1, 2]. Exercise and physical activity create a state of stress that disrupts the internal hormonal balance [1, 3]. The body's autonomous systems work to maintain consistent conditions despite these stresses [1]. When the inner balance is disturbed, the nervous and endocrine systems are the two most important systems that activate the mechanisms that contribute to the re-establishment of a normal balance [1]. In short, exercise and intense training programs, such as High-Intensity Interval Training (HIIT), initiate a sequence of hormonal adaptation processes that enable the body to cope with the stress of exercise. These adaptation processes can result in an increased or decreased release of specific hormones [1, 3–5]. For instance, high-intensity training can increase the release of cortisol, a stress hormone [4]. Conversely, levels of leptin might decrease in response to exercise [6]. The mechanisms of hormonal changes are highly complex and depend on specific conditions. Therefore, the behavior of hormones can vary according to the duration, intensity and severity of exercise, along with gender, age and fitness level of the individual [1, 3–5]. HIIT is a type of interval training, a cardiovascular exercise strategy alternating short periods of intense anaerobic exercise with less intensive recovery periods until the athlete is too exhausted to continue exercising [4]. Among various training modalities, HIIT is an exercise strategy that induces similar or even superior adaptations compared with traditional moderate-intensity and continuous training according to a range of physiological, performance, and health-related markers [7]. As a popular training strategy today, HIIT is known to improve the physiological, biochemical and performance adaptations of athletes in various disciplines. In fact, HIIT is a versatile method that enhances performance and improves health in elite sports, physical rehabilitation and disease prevention [8, 9]. Furthermore, hormone production is essential for the body's adaptation to HIIT. Secretion rates of some hormones increase during HIIT. The hormonal response is thought to be triggered by metabolic stress caused by HIIT, which affects the integrity of muscles, bones and connective tissues and helps maintain metabolism within the normal range. The level of the hormonal response to HIIT can vary depending on factors such as exercise intensity and duration [9]. HIIT is a short-term training strategy that involves active or passive short recovery periods and bouts of high-intensity exercise [2, 4, 10]. HIIT routines can be easily modified to suit individuals and different sports branches [11, 12]. HIIT requires little or no equipment, saves time and improves an individual's performance within a short time. As such, HIIT is a popular training technique today [13]. HIIT is functional for many individuals thanks to the minimal time commitment it offers [14]. HIIT presents a great advantage in improving athletic performance, and its efficacy has been supported by research studies [15, 16]. The central and peripheral adaptations of HIIT are effective in increasing exercise capacity, for which HIIT has a potent stimulatory role in triggering these adaptations [15, 17]. HIIT sets are relatively short (30–60 s), with rest intervals between sets lasting 15–120 s. The key principles for HIIT involve giving maximal effort and performing to the best of your ability. HIIT is performed at an intensity equal to approximately 90% of maximum oxygen consumption ($VO_{2max}$), usually on a Wingate-style cycle ergometer [15, 18, 19]. There are many studies reporting the differences in intensity, load and rest periods of HIIT. However, the effect of HIIT on physiological and hormonal parameters in healthy individuals has not yet been clarified. In the literature, HIIT is often compared with traditional training methods. In these studies, the effect of HIIT on obesity and diabetes was usually investigated. The main problem of these studies is that they do not address what kind of effect HIIT protocols have on the release of hormones and anaerobic capacity (AC). This study, which evaluates the effects of HIIT on physiological and hormonal levels is crucial for providing important findings that can be added to the literature. Another significant aspect of the present study is the time saving of using HIIT protocols. In adapting to long-term training models, individuals lose time and face a crucial financial burden. HIIT saves time with short training sessions and prevents a lack of time, which is one of the biggest obstacles to physical activity. Because HIIT increases the amount of personal time available that a person has for themselves, these people have more time to socialize when using HIIT. Thus, HIIT protocols also present themselves as a socially impressive element [18, 20, 21]. HIIT training for athletes carries some risks, such as muscle and joint injuries, dehydration, cardiovascular risk, nausea, dizziness, vomiting and fainting. These risks are relatively low in the general population. Overall, HIIT protocols are a safe and effective exercise for healthy adults [8, 21].

The present study aimed to contribute to the development of training protocols that are more suitable for the training programs of athletes and optimized in terms of hormone regulation, by investigating the effects of short-term HIIT on growth hormone (GH), cortisol, leptin levels and AC.

The study hypothesized that HIIT, performed thrice weekly for two weeks would have a greater effect on physical and hormonal parameters than traditional training programmes. This study not only demonstrates the effects of HIIT training on hormone levels and AC, but also provides clinicians, patients and researchers with crucial information to develop more effective and personalized training programmes for hormone regulation in athletes. Such personalized training can support performance enhancement in athletes while optimizing health and fitness. Furthermore, the results of this study could also be useful for managing clinical conditions such as obesity, diabetes or hormone dysregulation.

## 2. Materials and methods

### 2.1 Study design and participants

20 healthy male athletes [22, 23], comprising ten control and ten experimental subjects aged between 18–30 years (control: 21.90 ± 3.98 and experimental: 21.40 ± 2.76), participated voluntarily in the study. The participants demographics are detailed in Table 1. The participant was selected from the Faculty of Sports Sciences with the criteria based on at least 3 years of sports experience, and professional athletes were particularly encouraged to join. The participants have different sports branches. To ensure the homogeneity of the groups, we tried to equally distribute the branches among the groups (control: basketball 5, soccer 3, swimming 1, athletics 1 and experimental: basketball 5, soccer 3, judo 1, gymnastics 1). Participants with similar the body mass index (BMI) characteristics (control 23.20 ± 2.86 and experimental 23.30 ± 2.16) were preferred for the homogeneity of the groups during volunteer selection. The experimental group participated in the short-term HIIT protocol, while the control group only participated in the first and last sessions of the protocol. The hypothesis of the study was that HIIT performed for 2 weeks (3 days a week) would have a greater effect on physical and hormonal parameters than traditional training.

Since the participants were licensed athletes, compliance with the HIIT protocol was high. No supplements were in this study, and no side effects that could threaten the participants' health were reported. The protocol was performed under the supervision of health personnel to ensure safety for possible fainting and nausea due to the high intensity of the training and blood sampling. While there was no negative situation in our study, one participant voluntarily left the study. The study started with 21 participants and was completed with 20 participants. The HIIT protocol was performed in the university biochemistry laboratory.

### 2.2 High-intensity interval training procedure

The study included pre-test and post-test for both the experimental and control groups. The HIIT protocol was modified according to previously published protocols [24, 25] (Fig. 1). Participants were advised to refrain from intense exercise, alcohol and excessive caffeine for 48 hours before all tests and HIIT sessions. Instant communication with participants was facilitated through Zoom and WhatsApp. Additionally,

**TABLE 1. Physical characteristics of the participants.**

| | Groups | | | |
|---|---|---|---|---|
| | Control Group (n = 10) | | Experimental Group (n = 10) | |
| | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI |
| Experience (yr) | 5.60 ± 3.31 | 3.55–7.65 | 7.40 ± 3.37 | 5.31–9.49 |
| Age (yr) | 21.90 ± 3.98 | 19.43–24.37 | 21.40 ± 2.76 | 19.69–23.11 |
| Height (cm) | 179.10 ± 9.96 | 172.93–185.27 | 178.90 ± 8.07 | 173.89–183.90 |
| Body mass (kg) | 75.00 ± 13.74 | 66.49–83.51 | 74.90 ± 9.48 | 69.02–80.78 |
| BMI (kg/m$^2$) | 23.20 ± 2.86 | 21.43–24.97 | 23.30 ± 2.16 | 21.96–24.64 |
| Fat (%) | 13.18 ± 4.98 | 10.09–16.27 | 11.71 ± 3.90 | 9.29–14.13 |
| Fat mass (kg) | 9.91 ± 4.07 | 7.39–12.43 | 8.92 ± 3.56 | 6.71–11.13 |
| FFM (kg) | 64.94 ± 12.44 | 57.23–72.65 | 65.88 ± 7.43 | 61.27–70.49 |
| TBW (kg) | 47.54 ± 9.09 | 41.90–53.18 | 48.24 ± 5.44 | 44.86–51.61 |

*Abbreviations: BMI: Body Mass Index; FFM: Fat-Free Mass; TBW: Total Body Water; 95% CI: 95% Confidence Interval; $\bar{x}$: mean; SD: Standard Deviation.*

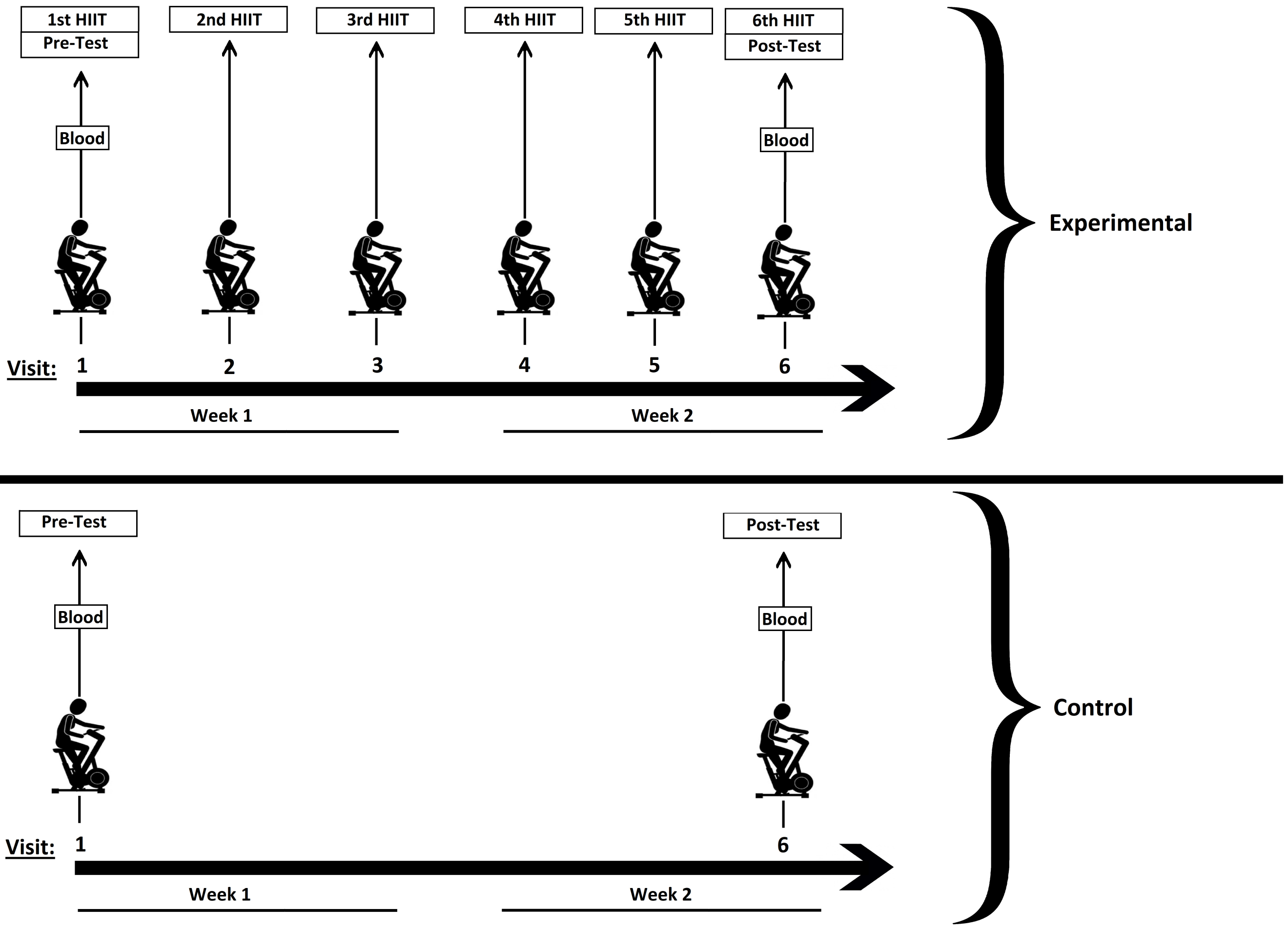


**FIGURE 1. HIIT protocol in experimental and control groups.** HIIT: high-intensity interval training.

all sessions occurred at the same time every day to minimize performance variations due to circadian rhythm. The HIIT protocol was implemented using a Monark bicycle ergometer (Monark Ergomedic 824 E, Varberg, Sweden) and used a computer compatible with this device. In the protocol used the Wingate system, which is commonly used in HIIT training. A preliminary protocol was created for the test, and a load of 75 g per kg of the participant's bodyweight was applied to the protocol. The test protocol was conducted for 2 weeks in total. These 2 weeks encompassed six sessions (three sessions per week) (Fig. 2). There were three sets in each session and in the sets, 30 s of maximum load at full strength intensity and 75 s (50 W) of active rest were applied (Fig. 3). Before and after the sessions, active warm-up and cool-down were performed on the bicycle ergometer for 3 min (at least 60 cadences per minute).

## 2.3 Physical measurements of the volunteers

The bodyweight (±0.1 kg) and body composition of the participants were determined by Bioimpedance Analysis (BIA) using a body analyzer (Tanita BC-418 MA, Tanita Corporation, Tokyo, Japan) on the bare body participants (only light shorts were allowed). Height (±0.1 cm) was measured barefoot with a stadiometer (ADE Telescopic Column Height Rod, GmbH & Co, Hamburg, Germany) that was fixed to the wall.

## 2.4 WAnT anaerobic capacity measurements

The WAnT Anaerobic Capacity values of the participants were determined on a bicycle ergometer in each set (Monark Ergomedic 824 E, Varberg, Sweden).

## 2.5 Blood collection and biochemical analysis

Blood samples were collected for hormonal analyses from the participants after the first and last sessions of the HIIT protocol. Samples were collected at five different time intervals for GH and cortisol hormones (before training, immediately after training, and 15–30–45 min after training). For leptin hormone, samples were collected at three different time intervals (before training, immediately after training and 30 min after training). Participants were not fasting, taking any supplements, and were not on a diet at the time of blood sample collection. At each sample collection point, approximately 10 mL of blood was collected from the participants' forearm vein at the elbow with individual serum collection tubes. Blood sample collection was performed by nurses working at the hospital. Participants did not eat before sample collection and after blood sampling, the participant was offered food. The samples were centrifuged at 3000 rpm for 10 min and stored at −80 °C until the analysis day. At the time of analysis, the samples were transported to the laboratory by cold chain and biochemical analyses were performed. GH and cortisol analyses were performed at University Central Laboratory. These analyses were performed by the ECLIA (Electrochemiluminescence Immunoassay) method with the e-801 modules on the Roche Hitachi Cobas 8000 (Roche/Hitachi MODULAR Analytics Combination Systems, Roche Diagnostics, Indianapolis, IN, USA) device. Leptin levels were determined at University Biochemistry Laboratory. A commercial enzyme-linked immune sorbent assay (ELISA-ELK-Biotech, Wuhan, China, Cat. No: ELK-1160) kit was used for leptin analysis according to the manufacturer's instructions. Leptin levels were determined by an ELISA reader ($\mu$Quant, Bio-Tec, ELx50, Winooski, VT, USA). All biochemical analyses were performed by an expert at the University's central laboratory. The Central Laboratory is registered with External Quality Control Programmes such as UK NEQAS and RİQAS. These control programs perform all tests, and daily control values for each test are recorded (lab code: 580723).

## 2.6 Sample size and blinding

The sample size of the present study was based on the studies of Bonet *et al.* [23] (2020) and Aktas *et al.* [22] (2019). Participants were randomly divided into groups consisting of ten control (five basketball, three soccer, one swimming and one athletics participants) and ten experimental (five basketball, three soccer, one judo and one gymnastics participants) in a double-blind manner (Table 1). Participants were randomly selected into the groups.

## 2.7 Statistical analyses

The data obtained from the study were analyzed with the JASP (Version 0.17.2.1) program. The Shapiro-Wilk test was used to check whether the variables obtained were normally distributed. The test showed that the variables were normally distributed. The normal distribution of the variables was shown with the arithmetic mean and standard deviation as descriptive statistics. As a statistical comparison test, mixed design repeated measures analysis of variance (ANOVA) (Split Plot ANOVA); 5 × 2 groups × 2 time for GH and cortisol/3 × 2 groups × 2 time for AC and leptin) was applied to see the changes in the main effects and interaction data over time. Multiple comparisons were applied to reveal the difference in variables where the main factors or interactions were significant, and the Holm correction method was applied for multiple comparisons. In ANOVA analyses, effect sizes of the group, and group × time effects were calculated using partial eta-square ($\eta^2$), and 0.01–0.06 was considered as a small effect, 0.06–0.14 as a medium effect, and 0.14 and above as a significant effect for classification [26]. A significant result was taken as $p < 0.05$. Confidence intervals are given as 95%.

# 3. Results

The physical characteristics of the groups participating in the study were similar (Table 1); however, there was no statistically significant difference between the experimental and control groups in the participants' sports experience, age, height, weight, BMI, body fat percentage, body fat weight, lean body weight, and total body fluid values ($p > 0.05$).

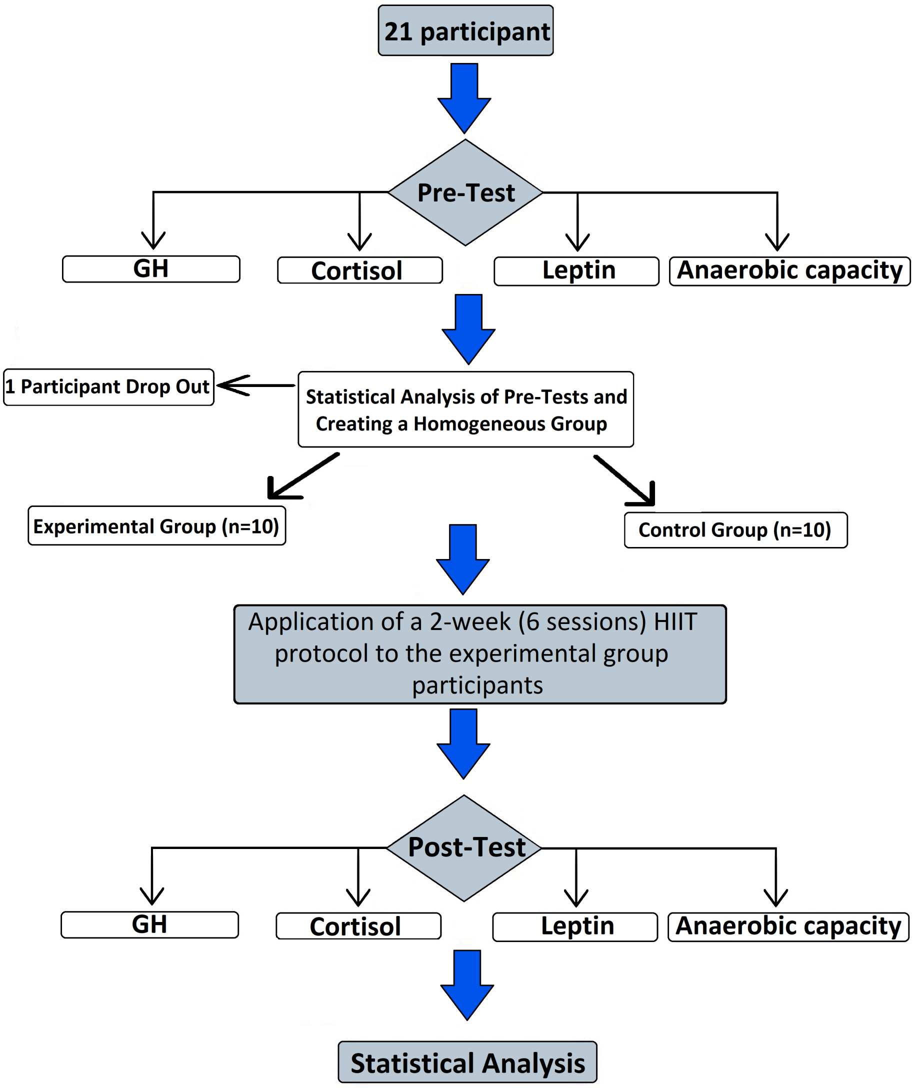


**FIGURE 2. Study design.** HIIT: high-intensity interval training; GH: growth hormone.

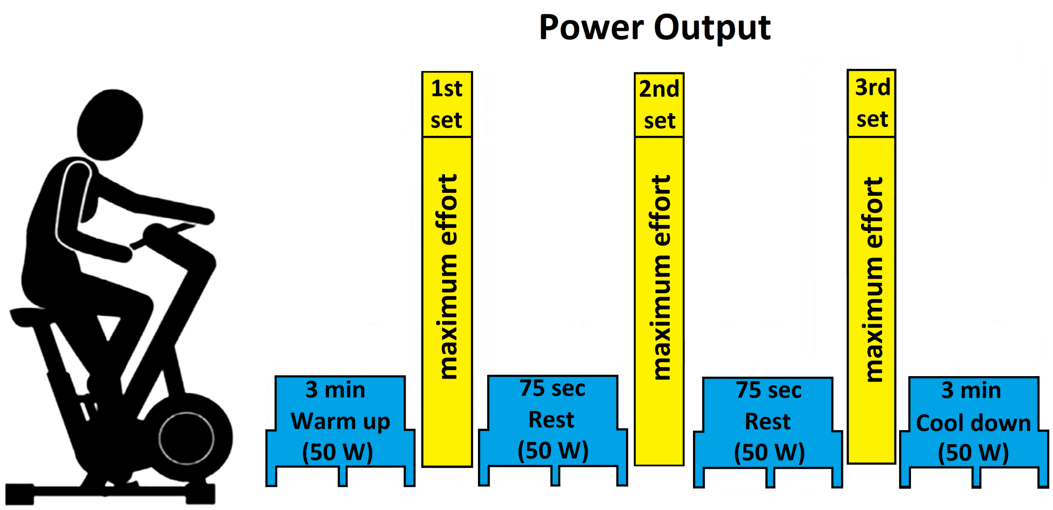


**FIGURE 3. Sets in HIIT sessions.**

### 3.1 Growth hormone

Changes in the levels of GH from acute exercise before and after the 2-week HIIT program are given in Table 2. Changes in GH levels after acute exercise were significant (F2, 36 = 49.21; $p < 0.001$; $\eta^2 = 0.237$). GH level was higher at all measurement times after exercise compared with before exercise ($p < 0.001$). The highest GH level was detected at the third measurement timepoint, which decreased at the following two measurement times ($p < 0.05$). 2-week training program (F1, 18 = 1.86; $p = 0.19$; $\eta^2 = 0.018$) and group factors (F1, 18 = 0.57; $p = 0.46$; $\eta^2 = 0.012$) alone or training-group (F1, 18 = 0.01; $p = 0.98$; $\eta^2 = 0.001$), training-acute exercise (F4, 72 = 1.37; $p = 0.25$; $\eta^2 = 0.005$), acute exercise-group (F4, 72 = 1.903; $p = 0.153$; $\eta^2 = 0.009$), and training-acute exercise-group interactions (F4, 72 = 0.29; $p = 0.088$; $\eta^2 = 0.001$) did not significantly affect GH levels. The changes in GH levels observed in acute exercise were similar in the experimental and control groups before and after HIIT interventions.

### 3.2 Cortisol

The changes in cortisol levels in the control and experimental groups in response to acute exercise before and after the 2-week HIIT program are presented in Table 2. Cortisol hormone levels increased during the recovery period after acute exercise (F4, 72 = 66.427; $p < 0.001$; $\eta^2 = 0.251$). Cortisol levels at all time intervals after exercise were significantly higher than before training ($p < 0.001$). It was also found that the cortisol levels of the participants increased significantly at 15 min ($p < 0.01$) and 30 min after exercise and decreased to pre-exercise levels at 45 min ($p > 0.05$). A decrease in cortisol hormone level was detected during two weeks of HIIT training (F1, 18 = 5.383; $p = 0.032$; $\eta^2 = 0.026$). In contrast, the observed change in cortisol levels after acute exercise was similar during the 2-week HIIT period (F4, 72 = 3.241; $p = 0.051$; $\eta^2 = 0.009$). Cortisol levels were similar in the control and experimental groups (F1, 18 = 0.004; $p = 0.951$; $\eta^2 < 0.001$). Additionally, the increase in cortisol after acute exercise was similar in the control and experimental groups (F4, 72 = 0.883; $p = 0.423$; $\eta^2 = 0.003$). The change in cortisol during the 2-week training period was not different in the control and experimental groups (F1, 18 = 1.841; $p = 0.192$; $\eta^2 = 0.009$). Moreover, the increase in cortisol levels after acute exercise was similar in both the control and experimental groups during the 2-week HIIT period (F4, 72 = 0.098; $p = 0.905$; $\eta^2 < 0.001$).

### 3.3 Leptin

The changes in the responses of leptin levels to acute exercise at the beginning and end of the HIIT program are presented in Table 2. During the 2-week HIIT training program, there was a significant decrease in leptin levels (F1, 18 = 20.463; $p < 0.001$; $\eta^2 = 0.300$). Acute exercise practices (F2, 36 = 0.684; $p = 0.511$; $\eta^2 = 0.005$) and group factors (F1, 18 = 0.576; $p = 0.458$; $\eta^2 = 0.004$) alone or training-group (F1, 18 = 2.666; $p = 0.120$; $\eta^2 = 0.039$), training-acute exercise (F2, 36 = 0.042; $p = 0.907$; $\eta^2 < 0.001$), acute exercise-group (F2, 36 = 1.760; $p = 0.186$; $\eta^2 = 0.013$), and training-acute exercise-group interactions (F2, 36 = 1.385; $p = 0.262$; $\eta^2 = 0.008$) had no significant effect on leptin levels. Acute exercise and group factors did not affect the decrease in leptin levels observed during the 2-week HIIT period.

### 3.4 Anaerobic capacity

The changes in AC in the three Wingate tests performed before and after the 2-week HIIT training program are presented in Table 3. Successive Wingate exercises resulted in a decrease in AC (F2, 36 = 69.829; $p < 0.001$; $\eta^2 = 0.335$). However, after the 2-week HIIT program, there was led to an increase in AC (F1, 18 = 9.049; $p = 0.008$; $\eta^2 = 0.025$). However, AC was similar in the control and experimental groups (F1, 18 = 0.087; $p = 0.771$; $\eta^2 = 0.002$). During the 2-week HIIT period, both the increase in AC and the decrease in AC in the consecutive Wingate test were similar in the control and experimental groups (F2, 36 = 2.894; $p = 0.068$; $\eta^2 = 0.011$).

## 4. Discussion

Our findings suggest that a short-term HIIT protocol leads to significant differences in GH and cortisol levels within the group due to the time main effect. These results indicate that HIIT can influence athletes' hormone regulation and performance and can provide potential benefits when used appropriately. However, due to the fact that the effect of HIIT can vary from person to person under certain conditions, none of the observed differences reached statistical significance between the experimental and control groups in our study. As such, further studies are needed to provide a clear conclusion to this issue.

### 4.1 Effect of short-term HIIT on hormone concentrations (GH, cortisol and leptin)

The effect of short-term HIIT on GH levels is controversial. In general, HIIT is known to increase GH release, but age, stress, fatigue level, inadequate sleep and nutrition, intense and prolonged exercise, and supplement use can decrease GH secretion. According to the results of the study, although the 2-week HIIT program caused a decrease in GH levels in the time main effect, it did not result in a statistically significant difference in GH levels between the groups. Within each group, post-exercise GH values were consistently higher than pre-exercise values at all timepoints. The highest GH level was found 15 min after training, and GH decreased in the following two measurements. Some studies in the literature support the results of the presented research. For instance, in a study involving 24 overweight young women underwent 4 weeks of HIIT, it was reported that HIIT caused a non-significant decrease in GH levels within the groups but no statistically significant change in blood samples taken as pre-test and post-test [27]. It should be noted that the present study was performed with men and that hormone levels in women could be affected differently. On the other hand, some studies [20, 28, 29] have revealed that HIIT affects GH levels and causes statistically significant differences between groups, which contradicts the results of the presented study because there was no statistically significant difference in the study presented.

**TABLE 2. Comparison of the effects of short-term HIIT training on growth hormone, cortisol, and leptin levels.**

| Variable | | Control Group (n = 10) | | | | Experimental Group (n = 10) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre-Training | | Post-Training | | Pre-Training | | Post-Training | |
| | | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI |
| GH (ng/mL) | Rest | 3.09 ± 4.05$^{a}$ | 0.58–5.60 | 3.16 ± 6.90$^{ab}$ | −1.12–7.43 | 5.00 ± 4.94$^{a}$ | 1.94–8.07 | 2.88 ± 4.06$^{a}$ | 0.36–5.39 |
| | | $p = 0.977$ | | | | $p = 0.197$ | | | |
| | Immediately post | 13.67 ± 8.95$^{b}$ | 8.13–19.22 | 12.75 ± 14.57$^{a}$ | 3.72–21.78 | 12.90 ± 8.09$^{b}$ | 7.88–17.91 | 11.33 ± 9.14$^{a}$ | 5.67–16.99 |
| | | $p = 0.003$ | | | | $p < 0.001$ | | | |
| | 15 min. post | 22.43 ± 9.70$^{b}$ | 16.41–28.44 | 17.22 ± 13.37$^{c}$ | 8.94–25.50 | 19.30 ± 8.75$^{ab}$ | 13.88–24.73 | 15.13 ± 9.98$^{b}$ | 8.94–21.32 |
| | | $p = 0.871$ | | | | $p = 0.037$ | | | |
| | 30 min. post | 20.21 ± 8.32$^{ab}$ | 15.05–25.37 | 15.23 ± 13,69$^{a}$ | 6.75–23.72 | 15.48 ± 9.09$^{ab}$ | 9.84–21.11 | 12.09 ± 7.81$^{a}$ | 7.25–16.93 |
| | | $p = 0.010$ | | | | $p = 0.002$ | | | |
| | 45 min. post | 15.23 ± 7.15$^{b}$ | 10.80–19.66 | 11.91 ± 14.11$^{b}$ | 3.17–20.66 | 10.06 ± 7.58$^{ab}$ | 5.37–14.76 | 7.59 ± 5.48$^{ab}$ | 4.19–10.99 |
| | | $p < 0.001$ | | | | $p < 0.001$ | | | |
| Cortisol (µg/dL) | Rest | 11.41 ± 5.79$^{a}$ | 7.82–15.00 | 12.11 ± 4.15$^{ab}$ | 9.54–14.68 | 13.75 ± 5.18$^{a}$ | 10.54–16.96 | 11.89 ± 3.35$^{ab}$ | 9.81–13.96 |
| | | $p = 0.005$ | | | | $p = 0.090$ | | | |
| | Immediately post | 18.43 ± 8.44$^{b}$ | 13.20–23.66 | 18.25 ± 5.09$^{a}$ | 15.10–21.40 | 19.45 ± 6.66$^{b}$ | 15.32–23.58 | 17.29 ± 4.85$^{a}$ | 14.28–20.30 |
| | | $p < 0.001$ | | | | $p = 0.044$ | | | |
| | 15 min. post | 21.38 ± 8.07$^{b}$ | 16.37–26.38 | 20.37 ± 6.04$^{b}$ | 16.63–24.11 | 23.33 ± 4.10$^{bc}$ | 20.79–25.87 | 20.10 ± 4.76$^{b}$ | 17.15–23.05 |
| | | $p = 0.006$ | | | | $p = 0.255$ | | | |
| | 30 min. post | 22.17 ± 7.42$^{b}$ | 17.57–26.76 | 20.77 ± 6.24$^{a}$ | 16.90–24.63 | 23.71 ± 6.00$^{ac}$ | 19.99–27.43 | 19.15 ± 4.26$^{a}$ | 16.51–21.79 |
| | | $p < 0.001$ | | | | $p < 0.001$ | | | |
| | 45 min. post | 22.51 ± 8.34$^{b}$ | 17.34–27.68 | 19.78 ± 6.29$^{c}$ | 15.89–23.68 | 22.84 ± 7.52$^{ab}$ | 18.18–27.50 | 17.08 ± 4.52$^{c}$ | 14.28–19.88 |
| | | $p < 0.001$ | | | | $p < 0.001$ | | | |
| Leptin (µg/dL) | Rest | 3.06 ± 2.19$^{a}$ | 1.96–4.15 | 1.08 ± 0.51$^{a}$ | 1.91–3.59 | 2.01 ± 0.80$^{a}$ | 0.91–3.11 | 1.34 ± 0.49$^{a}$ | 1.97–3.64 |
| | | $p = 0.173$ | | | | $p = 0.140$ | | | |
| | Immediately post | 2.75 ± 1.07$^{a}$ | 2.44–3.81 | 1.18 ± 0.58$^{a}$ | 0.74–1.41 | 2.81 ± 1.43$^{a}$ | 1.49–2.86 | 1.58 ± 0.56$^{a}$ | 1.00–1.67 |
| | | $p = 0.242$ | | | | $p = 0.951$ | | | |
| | 30 min. post | 3.13 ± 1.12$^{a}$ | 0.80–1.56 | 1.19 ± 0.71$^{a}$ | 0.76–1.61 | 2.18 ± 0.93$^{a}$ | 1.20–1.96 | 1.50 ± 0.56$^{a}$ | 1.07–1.92 |
| | | $p = 0.767$ | | | | $p = 0.287$ | | | |

*Notes: There is no difference between groups containing the same letters (a-b-c) in the same column.*
*Abbreviations: GH: Growth Hormone; 95% CI: 95% Confidence Interval; $\bar{x}$: mean; SD: Standard Deviation.*

**TABLE 3. Comparison of the effect of short-term HIIT on anaerobic capacity.**

| Variable | Control Group (n = 10) | | | | Experimental Group (n = 10) | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-Training | | Post-Training | | Pre-Training | | Post-Training | |
| | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI | $\bar{x} \pm$ SD | 95% CI |
| Set 1. | 23.75 ± 4.84$^{a}$ | 21.12–26.39 | 24.21 ± 5.15$^{a}$ | 18.11–22.48 | 23.35 ± 2.84$^{a}$ | 20.71–25.98 | 24.11 ± 1.78$^{a}$ | 17.48–21.86 |
| | $p$ = 0.823 | | | | $p$ = 0.952 | | | |
| Set 2. | 20.30 ± 4.00$^{a}$ | 16.30–20.31 | 20.59 ± 3.89$^{b}$ | 21.65–26.77 | 19.67 ± 2.39$^{b}$ | 13.62–17.63 | 20.86 ± 1.45$^{a}$ | 21.55–26.67 |
| | $p$ = 0.675 | | | | $p$ = 0.842 | | | |
| Set 3. | 18.31 ± 3.33$^{a}$ | 18.64–22.54 | 18.71 ± 2.74$^{b}$ | 17.02–20.40 | 15.62 ± 2.67$^{c}$ | 18.91–22.80 | 20.13 ± 2.34$^{a}$ | 18.43–21.82 |
| | $p$ = 0.062 | | | | $p$ = 0.230 | | | |

*Notes: There is no difference between groups containing the same letters (a-b-c) in the same column.*
*Abbreviations: GH: Growth Hormone; 95% CI: 95% Confidence Interval; $\bar{x}$: mean; SD: Standard Deviation.*

Considering the GH values between groups, studies have reported that HIIT increases GH levels, while others have shown decreases. Considering the studies suggesting that HIIT increases GH levels [20, 28, 29], it is thought that the differences are due to the gender and physical characteristics of the participants. While the present study included young male athletes, the study by Li *et al.* [28] (2022) included 67 adults (male and female) with obesity, and the study by Zhang *et al.* [20] (2020) included 59 female students with obesity. Since HIIT is a high-intensity type of training, it will naturally create different states of exercise stress in these groups. Since it is known that factors such as age, gender, stress level, training type, training intensity, body composition affect GH release, it can be expected that the results of this study may differ from those of the present study. GH release varies depending on age, gender, stress level, training type, training intensity and body composition. Ritsche *et al.* [29] (2014) reported that HIIT reduces GH levels in their study of 19 recreationally active adult men who underwent a 3-week HIIT protocol with 30 s of maximum power loading on a bicycle ergometer, with blood samples being collected every 15 min over 2-hour period. The study concluded that GH levels rise in the first 60 min after which there are regular GH drops for the following 60 min even if HIIT continues. This GH increase and later declines support the theory that changing GH values were due to the time main effect in the study; however, there was no difference between the groups in the present study. In this context, these results contradict the results of the study presented. This discrepancy is due to the differences in the fatigue levels of the volunteers, the number of repetitions in the Wingate sets, and the resting rate during sampling. In the present study, the sets were performed in three repetitions during the training sessions, and the first blood collection was performed immediately after the set. However, Ritsche *et al.* [29] (2014), reported that the number of repetitions varied between 4–6 times, and at the end of the set, 5 min of rest was allowed before blood sampling collection.

Since cortisol is a stress hormone, the stress conditions caused by the intensity, duration, and timing of training can affect cortisol responses in various ways. The literature, does not offer a clear consensus on the effect of HIIT on cortisol levels, with some studies supporting [27, 30] and others opposing [3, 4] the findings of the present study. According to the results of the present study, although 2-week HIIT caused a decrease in cortisol levels in the time main effect within each group, it did not cause a statistically significant difference between the groups. The results of the present study are supported by a study that found HIIT in 30 recreationally active adult men aged 35–40 years had no significant effect on cortisol levels between the experimental and control groups [30]. Similarly, another study on 24 women with obesity showed that 4 weeks (12 sessions with four sets) of HIIT did not cause statistically significant changes in cortisol levels [27]. Conversely, some studies investigating the effect of HIIT on cortisol revealed that HIIT affected cortisol levels and produced statistically significant differences in intergroup levels [3, 4]. Considering the intergroup values in the literature, some studies argue that HIIT increases cortisol levels [4], while others reported that decreased cortisol levels [3]. These results contradict the results of the study because there was no statistically significant difference between the groups in the presented study. For instance, Zar *et al.* [4] (2021) implemented HIIT training in the morning and afternoon in 11 active young men and blood samples were twice taken from the participants, before and after exercise. As a result of the study, it was determined that HIIT performed in the morning caused an increase in cortisol levels, but when performed in the afternoon it did not cause a change in cortisol levels. It is thought that the contradiction between the results of this study and the present study is due to differences in circadian rhythm, training type, duration, and sampling protocol. On the contrary, Irandoust *et al.* [3] (2019) in their study applied 1-week HIIT in 29 women with obesity aged 40–45 years with sleep disorders and concluded that HIIT caused a decrease in cortisol levels. It is possible that the reason for the contradiction between the results of the present study and these results is the preferred volunteer population given that the study was conducted on women with obesity and sleep disorders, therefore, the results obtained might vary in a different population.

In the present study, although 2-week HIIT caused a decrease in leptin levels in the time main effect, it did not cause a statistically significant difference in leptin levels between the groups. The results of the present study are consistent with those of several other studies [31]. For instance, in one study, HIIT was applied for 8 weeks in 18 normal and young men with obesity. According to the results of the study, HIIT did not cause a significant change in leptin levels between the groups [31]. There is no clear result in the literature on the effect of HIIT on leptin levels as some studies have argued that HIIT increases [32] leptin levels, while others showed decreases [6, 33]. In their study, Parastesh and Saremi (2019) applied ten weeks of HIIT training on 42 type-2 diabetic rats and found that HIIT increased leptin levels. Since this was an animal study, it is difficult to extrapolate the finding to humans. In contrast, Maldonado-Martin *et al.* [6] (2021) applied a 16-week HIIT protocol to 77 people (37 women and 40 men) with obesity and hypertension and found that HIIT reduced leptin levels. The results may have been influenced by factors such as gender, duration of HIIT, and the selection of adults with metabolic problems. Similarly, Bonab *et al.* [33] (2020) applied a 12-week (four sessions per week) HIIT program and curcumin supplementation to 30 adult women with obesity in their study, collected blood samples at two time intervals, 48 h before and 48 h after training, and found that leptin levels of women with obesity decreased as a result. Factors such as the sample sizes of the studies, different population characteristics of the participants, different training protocols applied, and different analysis methods used might have influenced the results from the mentioned studies.

### 4.2 Effect of short-term HIIT on anaerobic capacity

In consecutive sets of HIIT implemented for 2 weeks, WAnT AC levels decreased as the main effect of time. However, there was no statistically significant difference in WAnT AC levels between the groups. Some studies [15] are consistent with the results of the present study. For instance, Alsairawan *et al.* [15] (2019) implemented a 2-week (3 days a week for a total of six sessions) Wingate-style HIIT in 23 healthy sedentary university students (nine males and 14 females) aged 18–24 years and examined the effect of HIIT on anaerobic capacity. According to the conclusion of the study, 2-week HIIT did not cause a statistically significant difference in the AC values of the participants. In this study, it was argued that 2 weeks of HIIT protocol was not enough to affect AC. These results suggest that short-term HIIT models might have no or limited effect on AC. There is no clear result in the literature about the effect of HIIT on WAnT AC levels. Some studies in the literature argue that HIIT affects WAnT AC levels [34], which contradicts the results of the presented study. Donie *et al.* [34] (2017) applied a 6-week (three sessions per week) HIIT protocol to 31 athletes (16 males and 15 females) aged 18–20 years in their study and examined the AC. The six-week HIIT application significantly increased the WAnT AC values of the participants. It is thought that different HIIT protocols, different training durations, different sample selections, and different measurement methods led to conflicting results on the effect of HIIT models on WAnT AC. Further studies are needed for more precise results on this subject. In the present study, it was found that 2-week HIIT did not affect WAnT AC levels between the groups, but the effect of HIIT protocols with longer duration might be different. Also, the measurement methods and AC assessment tests used in the study could affect the results.

This study was limited to healthy male athletes aged between 18–30 years with healthy eating habits, without any chronic disease, without any cardiovascular, pulmonary or neuromuscular disease in the past, without any surgery in the lower extremities, without any addictions such as smoking, alcohol, drugs, and who were exercising at least 5 days a week for 2 weeks and 3 days a week. Participants who did not comply with these criteria were excluded from the study.

## 5. Conclusions

According to the findings obtained from this study, no significant difference was found between the groups in the HIIT protocol applied for two weeks. On the other hand, this study shows that the HIIT protocol applied for two weeks caused significant differences in GH, leptin, cortisol and WAnT AC levels within the group due to the main effect of time. These results suggest that time is an effective modulator in high-intensity interval training protocols. The limited sample size of 20 participants represents a major constraint of our study. A larger sample size in future studies may reveal results that will provide the hypothesis of our study. On the other hand, this study was conducted in a short period of 2 weeks. This short period may be insufficient to detect changes in hormone levels. Studies conducted over a longer period may help us better understand the changes over time.

## AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

## AUTHOR CONTRIBUTIONS

NS and MÖ—designed the research study. MÖ—performed the research. MŞ—provided help and advice. NS—analyzed the data. NS, MÖ and MŞ—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Participants were fully informed about the purpose and risks of this study and the testing and training procedures and provided written informed consent. The protocol of this study was approved by the Erciyes University Clinical Research Ethics Committee (ethics code: 2021/200).

## ACKNOWLEDGMENT

We thank the Erciyes University Scientific Research Projects Coordination Unit for their support.

## FUNDING

This research was funded by the Erciyes University Scientific Research Projects Coordination Unit with the project code “TDK-2021-11137”.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**How to cite this article:** Muhammed Öniz, Nazmi Sarıtaş, Meryem Şentürk. Effects of short-term high-intensity interval training on growth hormone, cortisol, and leptin levels. Journal of Men's Health. 2024; 20(2): 51-61. doi: 10.22514/jomh.2023.124.