Concurrent strength and aerobic exercise reduce plasma leptin and cortisol levels.

Ejercicios aeróbicos y de fuerza concurrentes reducen los niveles plamaticos de leptina y cortisol.

Treinamento concorrente de força e aeróbico reduz os níveis plasmáticos de leptina e cortisol.

[Research Article]

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Abstract

To analyze the correlation between leptin and cortisol after a concurrent training (CT) session. Thirty male volunteers (age: 27.7 ± 5.1 years, BMI: 27.08 ± 1.42 kg/m²) were randomly assigned to an experimental group (EG: n=15) and a control group (CG:...

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Baseline leptin and cortisol levels were determined by collecting blood samples. EG was subjected to CT (indoor cycling followed by strength training), whereas CG performed no exercise. After CT, blood samples were collected in EG and CG to determine the hormone variables. EG showed a significant decrease (p<0.05) intra and intergroup in leptin and cortisol levels. Only the EG showed a significant correlation (r=0.756; p<0.05) between leptin and cortisol levels. CT session is linked to in decreased leptin and cortisol levels, exhibiting a positive correlation between them.

**Keywords:** adipokines, health promotion, hormones, physical exercise, overweight.

**Introduction**

Obesity is strongly linked to an increased risk of developing metabolic syndrome (Loprinzi & Cardinal, 2012), musculoskeletal disorders, psychosocial problems (Brennan et al., 2009), and even COVID-19 (Pérez et al., 2021). In the last few years,
adipose tissue has emerged as an endocrine organ, owing to its ability to secrete and produce bioactive substances known as adipokines, including leptin (Rosa et al., 2010; Coles, 2016).

Leptin, identified as an anti-obesity hormone (Haleem et al., 2015), is primarily secreted by white adipose tissue but also produced in small amounts by the stomach, mammary glands, brown adipose tissue, and placenta. It is involved in regulating body weight, appetite, and energy expenditure (Negrão & Licínio, 2000; Licínio, 1998; Rosa et al., 2022). However, several data (e.g., Conde et al., 2010) have reported an association between high leptin levels and the development of inflammatory and autoimmune conditions. Myokines have emerged as a class of molecular mediators responsible for fat browning. Adipokines influence and modulate their activity, further confirming the intricate crosstalk between skeletal muscle and adipose tissue. This interaction is crucial in regulating thermogenesis and energy expenditure (Rodriguez et al., 2016).

Leptin mutates the expression and activity of multiple hypothalamic peptides through specific receptors (Negrão & Licínio, 2000), with five distinct isoforms: the long receptor (Ob-Rb), short receptors (Ob-Ra, Ob-Rc, and Ob-Rd), and the soluble receptor (Ob-Re). The Rb receptor isoform is expressed in some areas of the brain and responds to central leptin actions. It belongs to the family of class 1 cytokine receptors, which contain an extracellular ligand binding domain, a transmembrane spanning domain, and a cytoplasmic signaling domain. Dysfunctions in this receptor can lead to obesity and leptin resistance, increasing blood leptin levels (Wunderlich et al., 2013). In turn, high leptin levels in the blood facilitate the secretion of pro-inflammatory cytokines, resulting in the risk of developing inflammatory diseases (Likuni et al., 2008). Leptin levels can be influenced by various factors, such as fasting, sympathetic activity, insulin, body weight turns, glucocorticoids, and physical exercise (Rosa et al., 2012).

Likewise, cortisol is an essential body glucocorticoid that play a critical role in maintaining homeostasis. However, high levels may produce deleterious effects, such as a raised risk of cardiovascular and metabolic disorders, as well as immune, cognitive, and emotional diseases (Dedovic & Duchesne, 2012). Moreover, cortisol secretion levels are considered a trustworthy and accurate marker of physiological stress responses (Metzenthin et al., 2009).
The relationship between leptin and cortisol has been described as leptin levels are influenced by glucocorticoids, including cortisol, which stimulate leptin gene transcription and production (Negrão & Licínio, 2000; Licínio, 1998). Nevertheless, despite the relationship between these hormones, there is no consensus regarding their response to physical exercise.

Rosa et al. (2010) reported a significant reduction in leptin levels after a single session of concurrent training (CT). However, exercise intensity seems to have a substantial effect on these results, although no significant alterations in leptin levels were observed after a single moderate-intensity CT session compared to a high-intensity session (Rosa et al., 2009). A significant decrease in cortisol levels was observed after different CT protocols (Rosa et al., 2011). Cadore et al. (2010) found no changes in cortisol levels after an 8-week CT intervention.

Prior research has examined the impacts of aerobic or strength exercise on cortisol (Metzenthin et al., 2009) and leptin levels (Jürimäe, 2005). Combining aerobic and strength exercises into a single training session, or CT, is a commonly employed regimen in exercise training (Rosa et al., 2012).

Due to conflicting data on plasma leptin and cortisol levels following CT, as a limited understanding of their correlation post-physical exercise, additional research is necessary to gain clarity and insight into this matter.

Therefore, is study aimed to investigate the correlation between a single CT session and serum leptin and cortisol levels in overweight adults.

Methods

Sample

Cross-section study with 30 male volunteers (27.7 ± 5.1 years old; BMI 27.08 ± 1.42 kg/m²), physically active following the American College of Sports Medicine (ACSM) standards (ACSM, 2013), randomly divided into two groups: experimental group (EG, N = 15) and control group (CG, N = 15).

The volunteers engaged in regular physical exercise three times a week for a minimum of six months and presented no apparent risk factors following the criteria of risk stratification of the American Heart Association (ACSM, 2013).
This research attended the guidelines for research involving human beings, according to the Declaration of Helsinki (WMA, 2008). The Human Research Ethics Committee of Castelo Branco University (UCB/RJ) approved the project by protocol n°: 0-2008-01-89.

**Data Collection**

Data collection occurred in three moments: an initial moment (M1) to explain the research procedures, followed by another moment for conducting the tests: anthropometric evaluation and strength test (M2) and concurrent training session (M3).

During M2, anthropometric measurements and one repetition maximum (1RM) test were conducted; blood samples were collected, and concurrent training was carried out at M3, as described below.

![Figure 1: study design.](image)

**Anthropometric measurements**

At moment 2, the volunteers realized an anthropometric evaluation, composed of body weight and height, to calculate their body mass index (BMI) by dividing body weight in kg by the square of the height in meters (kg/m²).

An analogic scale was used to evaluate body weight and height, with a 150-kg capacity and 100g accuracy (Filizola, São Paulo, Brazil). The measurements attended the standards of the *International Society for the Advancement of Kinanthropometry – ISAK* (Marfell-Jones et al., 2006).

**One repetition maximum test (1RM)**

The one repetition maximum (1RM) test (Baechle & Earle, 2000) was performed at the moment 2 to establish and control the intensity in the exercises: supported rowing, leg press 45°, straight bench press, knee extensor, elbow extensor, knee flexor, and elbow flexor. The warm-up consisted of 15 repetitions with 50% overload, estimated according to the training weight. Three minutes later, the first of the
three attempts at each movement was carried out. The initial overload was estimated according to the overload used in the volunteers’ training sessions.

The tests stopped when voluntary concentric failure occurred at 1RM. The movement execution speed was 2 seconds for each phase (concentric / eccentric) without any interval allowed in between these. If the overload for 1RM was not reached after 3 attempts, the test was cancelled and performed on a non-consecutive day. The intervals between attempts for each exercise were set at 5 minutes to recover.

**Leptin and cortisol blood sample**

Serum blood samples were the taken early in the morning (6:30 a.m.) and immediately after the CT session (at 8:30 a.m.) to determine baseline and post-CT session leptin and cortisol levels. Participants were required to fast for 12 hours and sleep for a minimum of eight hours, prior to blood sample. None of the participants performed no physical exercise in the day before this session to avoid any kind of interference on blood variables (Rosa et al., 2012).

Blood was collected using mineral-free needles (25 × 0.7 mm), mineral-free vacuum tubes, and latex-free gloves (Lengruber, Rio de Janeiro, Brazil). Blood samples were collected at the data collection site by qualified nurses from Sérgio Franco Laboratory, Brazil, directly after CT (for the EG) or equivalent control time (for the CG).

ELISA human commercial kit (Linco Research, St. Charles, MI) was used to determine blood leptin and cortisol levels (radioimmunoassay for leptin and chemiluminescent immunoenzymatically assay for cortisol). All procedures were performed according to the manufacturer’s instructions. The intra-assay coefficient of variation for leptin and cortisol measurements was < 10%.

After the analyses, blood samples were discarded according to ANVISA guidelines (National Health Surveillance Agency, Law RDC 306/12/ 2004).

**Breakfast**

The subjects were provided with a breakfast meal that consisted of the following items: 200 mL of fat-free yogurt, 2 slices of light whole-wheat bread, 30g of fresh white cheese, 10g of butter, and 1 medium-sized banana, following nutrition instructions.

**Concurrent strength and aerobic exercises**
Forty minutes after breakfast, the participants in the experimental group (EG) underwent a CT session that included aerobic exercise: a 40-minute continuous indoor cycling class, intensity varying from 5 to 7 on the OMNI scale of perceived exertion for cycling (Robertson, 2004).

The resistance training session consisted of 3 sets per exercise performed until exhaustion. The intensity of the resistance training session was 85% of 1RM for the following exercises with a 2-3-minute interval between sets: supported rowing, leg press 45°, straight bench press, knee extensor, elbow extensor, knee flexor, and elbow flexor.

CG did not engage in any exercise and did not consume any food between breakfast and the final blood collection.

**Statistical analysis**

Statistical analysis was performed using the *Statistical Package for the Social Sciences* software (SPSS, Chicago, IL). Descriptive statistics were performed to determine the mean and standard deviation. The Shapiro-Wilk (SW) test was used to verify the data normality. A paired Student’s t-test was used to analyze intragroup differences, while an independent student's t-test was used to analyze intergroup differences. Pearson’s correlation assessed the association between leptin and cortisol levels. In addition, the effect size (d) was calculated to verify the magnitude of the study findings. The significant value adopted was 95% (p-value ≤ 0.05).

**Results**

Table 1 presents the anthropometric data, which were normally distributed. All participants were classified as overweight (BMI of 25.0 to 29.9) according to the World Health Organization criteria (WHO, 2021).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Age [years]</th>
<th>Body Mass [kg]</th>
<th>Height [m]</th>
<th>BMI [kg/m²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>15</td>
<td>27.9 ± 4.97</td>
<td>76.9 ± 17.9</td>
<td>1.67 ± 0.14</td>
<td>26.84 ± 1.51</td>
</tr>
<tr>
<td>SW (p-value)</td>
<td>0.84</td>
<td>0.13</td>
<td>0.57</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>15</td>
<td>27.5 ± 5.48</td>
<td>80.1 ± 14.6</td>
<td>1.70 ± 0.12</td>
<td>27.5 ± 5.48</td>
</tr>
<tr>
<td>SW (p-value)</td>
<td>0.47</td>
<td>0.39</td>
<td>0.51</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EG, experimental group; CG, control group; SW: Shapiro-Wilk normality test.
Figure 2 illustrates the pre-and post-intervention leptin levels, while Figure 3 displays the pre-and post-intervention cortisol levels. In EG, a significant 20% reduction in leptin levels was observed after the CT session. Similarly, the CG experienced a 19% decrease in leptin levels. Regarding cortisol levels, the EG showed a significant 38% reduction in serum levels, whereas the CG exhibited a higher reduction of 49%.

The effect size in the EG was strong for leptin and cortisol ($d_{\text{leptin}} = 0.96 > 0.82$; $d_{\text{cortisol}} = 2.11 > 0.82$) and indicates an improvement in results (i.e., reduction in leptin and cortisol levels following CT) in a substantial number of these participants. In the CG, the effect size was moderate for leptin and strong for cortisol ($d_{\text{leptin}} = 0.63 < 0.82$; $d_{\text{cortisol}} = 3.24 > 0.82$).

The effect size in the EG ($d_{\text{leptin}} = 0.96 > 0.82$; $d_{\text{cortisol}} = 2.63 > 0.82$) indicates an improvement in results (i.e., reduction in leptin and cortisol levels following CT) in a substantial number of these participants.

*Figure 2. Leptin serum levels in control subjects before sham exercise (CG pre) and after sham exercise (CG Post) and in subjects before CT (EG Pre) and after CT (EG Post). ***, $p \leq 0.001$, intragroup difference; ###, $p \leq 0.001$, intergroup difference relative to the same sampling time.*
Figure 3. Cortisol serum levels in control subjects before sham exercise (CG pre) and after sham exercise (CG Post) and in subjects before CT (EG Pre) and after CT (EG Post). ***, p ≤ 0.001, intragroup difference; ###, p ≤ 0.001, intergroup difference relative to the same sampling time.

Figure 4 presents the correlation between leptin and cortisol levels in both groups. A significant correlation was observed in EG only in the post-test between leptin and cortisol.
Figure 4. Correlation analysis between leptin and cortisol levels per group. Pearson’s r statistic and the p-value are in the bottom right corner.

Discussion

The major findings of this study were the significant reductions in leptin and cortisol levels after a single CT session and the significant correlation between these blood variables at the -post-CT session on EG.

Although the study participants were active and engaged in regular physical exercise, all of them were classified as overweight according to the WHO (26). This was also observed in a study by Rosa et al. (2010), who investigated the effects of a similar CT session to that applied here on the leptin levels of overweight adults, reporting a significant decline in leptin content after the intervention.

According to a review by Hulver & Houmard (2003), studies that investigated exercise sessions lasting one hour or more, such as the present one, are more representative regarding changes in serum leptin concentrations. These results suggest that exercise-induced reductions in leptin could potentially be influenced by changes in nutrient availability or nutrient flux at the adipocyte level, which are the main producers and secretors of leptin.

The impacts of physical exercise on leptin levels appear to be dependent on both the intensity and duration of the exercise. In this respect, Rosa et al. (2009) found that a similar training session to that used here yielded the same results in terms of its effect on leptin concentration, whereas high-intensity exercise promoted an apparent negative energy balance, decreasing leptin levels.

Jürimäe (2005) also reported a significant reduction in leptin levels, as observed in the present study, where leptin concentrations declined after the concurrent training intervention. However, Rosa et al. (2011) analyzed the influence of different concurrent training sequences on leptin concentrations and found a significant reduction in leptin levels after the session in which aerobic exercise was performed before strength exercise, and vice versa. These findings also appear in the present study, where a reduction in leptin concentration was observed.

Research by Conde et al. (2010) found an association between high leptin levels and the development of inflammatory and auto-immune processes. According to Likuni et al. (2008), excessive leptin production contributes to pro-inflammatory cytokine
secretion. Sansoni et al. (2016) compared trained runners and controls with a low physical activity profile before and after a 65 km mountain ultra-marathon. The authors observed that only glucagon and leptin displayed differences between trained runners and controls at rest among the metabolic hormones assessed. Specifically, they noted that leptin levels decreased in runners following the mountain ultramarathons while all other variables increased. In addition, increased leptin levels cause oxidative stress in endothelial cells with a vascular calcifying effect, promoting platelet aggregation and increasing atherogenesis and cardiovascular disease risk (Wunderlich et al., 2013; Nakata et al., 1999; Matsubara et al., 2002). The significant decrease in leptin levels in this study was positive in terms of reducing the risk of inflammatory, auto-immune, and cardiovascular diseases.

Furthermore, a notable decrease in cortisol levels was observed following the concurrent training session. The effects of concurrent training and strength training were reported, respectively, by Rosa et al. (2011; 2016) and Kraemer (1998). In addition, Rosa et al. (2012) also examined the acute effect of concurrent training on cortisol levels and investigated whether the exercise sequence could influence the concentrations of this hormone. The results demonstrated that the concurrent training protocol used led to a significant reduction in cortisol levels, regardless of the exercise sequence.

It is interesting to note that the response of cortisol to exercise can vary, and an increase in cortisol levels was observed such by Izquierdo et al. (2009), who used strength training, and França (2006), who examined hormonal response after a marathon.

The decline in cortisol concentration found in the present study was beneficial to overall health since cortisol is the main stress hormone, and high levels can be detrimental, increasing the risk of cardiovascular and metabolic disorders, as well as immune, cognitive, and emotional diseases (Dedovic & Duchesne, 2012). With respect to the pre-and post-intervention correlation between leptin and cortisol in both groups, a significant medium to high correlation was found only in the EG.

Leptin levels can be influenced by glucocorticoids, including cortisol, which stimulates the transcription of the leptin gene, leading to an increase in leptin production (Negrão & Licinio, 2000). This could explain the correlation observed between the
variables. These data corroborate research by Licínio (1998), who demonstrated a correlation between leptin and cortisol concentrations.

Lombardi et al. (2012) suggested a possible relationship between bone and energy metabolism. The authors examined professional cyclists in the Giro d’Italia 2011 stage race, evaluating the markers of bone and energy metabolism (bone alkaline phosphatase, tartrate-resistant acid phosphatase 5b, total and undercarboxylated osteocalcin, leptin, and adiponectin) and the hormones (cortisol and testosterone). The results showed an increase in levels of the undercarboxylated form of osteocalcin, which was indirectly related to improved energy expenditure through adipokine modifications, as well as a decrease in leptin and reduced adiponectin, with means high energy consumption and optimization of energy expenditure.

The circadian rhythm and individual variability may have interfered with the results of this study due to the emotional and biological effects that subjects were exposed to. Indeed, these factors can be regarded as limitations of the study and may help explain some of the baseline differences observed. The levels of various hormones, including leptin and cortisol, exhibit circadian variation and fluctuations. In some cases, these variations are attributed to pulses from the regulatory endocrine axis, while in others, they are influenced by changes in humoral stimuli caused by individual environmental or behavioral factors (Dedovic & Duchesne, 2012).

In conclusion, the present study revealed that a single concurrent training session significantly decreased blood levels of leptin and cortisol. Furthermore, a significant correlation between these variables after intervention was observed only in the EG after CT session.

The study highlights the need for additional research to explore the relationship between leptin, cortisol, and other hormones related to adipose tissue and stress. Future studies can investigate the long-term effects of concurrent training on hormonal regulation and its impact on various health outcomes.

**Practical applications:**

Overall, the practical applications of this study suggest that incorporating concurrent training into exercise routines can have positive effects on hormonal regulation, weight management, and stress reduction. This study’s findings have practical applications in several areas, such as:
1. Exercise prescription: CT can be recommended for individuals looking to reduce leptin and cortisol levels. It could be used for promoting hormonal changes associated with adipose tissue and stress.

2. Weight management: As leptin is a hormone involved in regulating appetite and energy expenditure, the reduction in leptin levels observed after CT suggests that this exercise modality may benefit weight management.

3. Stress reduction: The significant reduction in cortisol levels observed after CT indicates that this exercise approach may have stress-reducing effects.

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Conflict of interest and disclosures

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