ABSTRACT

Objective: This study aimed to analyze the immediate effects of concurrent training on leptin and cortisol levels in overweighted young adults. Methods: This study used a quasi-experimental methodology. We included 20 volunteers of both sexes, randomly divided into a training competitor group (TCG n = 10) and a control group (C n = 10). Blood collection was performed in individuals at rest after fasting for 12 hours. The leptin and cortisol levels were analyzed by radioimmunoassay and chemiluminescence immunoassay before and immediately after training. Two-way ANOVA was used for statistical analysis with a significance level of p < 0.05. Results: In the analysis of leptin levels, there was significant difference between groups (TCG x C) in the pre intervention (p = 0.02) and post intervention (p = 0.01). In the intra groups, no significant changes were found, and in the analysis of cortisol levels between groups (TGC x C), a significant difference in the pre intervention (p = 0.01) and post intervention (p = 0.01) was observed; however, in the intra groups there were no significant changes. Conclusion: A single concurrent training session is not sufficient to promote acute changes in the leptin and cortisol levels of the volunteer overweighted young adults in this study.

Keywords: obesity, cycling, resistance training.

INTRODUCTION

Over the 1990 decade, the percentage of individuals with overweight and obesity has significantly increased\(^1\), and nowadays is considered a global epidemics\(^2\). Although the scientific community does not know if overweight and obesity are multifactorial phenomena, it acknowledges the need to explore many ways of increasing the daily energetic cost in order to control and reduce the prevalence of these diseases\(^3\).

The recent advances in the endocrinology and metabolism field show that, contrary to what was believed some years ago, the adipocyte is not a cell of energy storage, but it also synthesizes and releases many substances, including leptin\(^3\), which derives from the Greek leptis, which means thin\(^4\), and was discovered in 1994. Leptin is a peptide hormone composed of 167 amino acids and is mainly produced in the white adipose tissue, being greater in the subcutaneous fat when compared with the visceral adipose tissue\(^5\), but is different between the adipocytes. Its release occurs during the peak of the night and the first hours of the morning, and its plasma half-life is of 30 minutes\(^5\).

Leptin concentration is partially related to the size of the mass of the adipose tissue in the body\(^6\). It is known as the “satiety hormone” for having influence on the appetite reduction process\(^7\). Its secretion may be influenced by many physiological mechanisms, such as fasting, the glucocorticoids, the sympathetic activity, physical exercise and alterations in the body weight and energetic balance, which can drastically alter the quantity of leptin intrinsically associated with fat mass\(^8\) fall in its production, and/or its activity over the receptors of the hypothalamus could distort the positive energetic balance, creating the obesity scenario\(^9\).

Many studies show that the leptin plasma levels are not altered according to the acute aerobic exercise in athletes or non athletes. Ribeiroet al.\(^1\) report that it has been common to communication that exercise, either acute or chronic, causes decrease of leptin concentration. Kraemer et al.\(^1\), in a revision study, suggest that there are many studies on the effect of acute exercise on leptin, and that the majority did not presented any alteration or decrease in the leptin concentration; however, the studies are very contradictory due to the acute or chronic plasma leptin aerobic physical activity.

The study by Weltman et al.\(^1\) reported that 30 minutes of exercise above or below the lactate threshold, are able to lead to acceleration of metabolism rate, but the exercise intensity may have not been sufficient to alter the leptin concentrations in young men during exercise or recovery periods (3.5 hours post-years), compared with control values. In this study, the exercise intensity was not a factor which affected the leptin responses in young men. Tuominen et al.\(^1\) found 34% of reduction in the leptin concentrations in the plasma 44 hours after two hours of exercise at 75% of \(\dot{V}O_{2\text{max}}\). In the study by Olive and Miller\(^1\), the leptin plasma concentrations were analyzed in the 24 and 48 hours after one hour of moderate exercise periods (~ 900kcal spent) and after intense exercise of short duration (~ 200kcal spent), where decrease of 18% and 40%, respectively after the long duration and moderate intensity activity was observed. None alteration has occurred after the leptin activity concentration of short duration and high intensity.

Cortisol is the main glucocorticoid produced by the adrenal and its secretion is related to many factors, such as: rhythm of the circadian cycle\(^14\), stress, hypoglycemia and some amino acids\(^18\). The cortisol circulating concentration is at maximal values at the first morning hours, immediately before waking up, and progressively decreases completely during the day, presenting very low levels at night\(^19,20\). Exercise and training process are classified as stress.
to the human body\textsuperscript{11} and powerful stimulators of fast secretion\textsuperscript{12}.

During acute exercise, in a test of maximum oxygen consumption ($\dot{V}O_{2max}$), the level of blood cortisol increases according to the exercise intensity, once the work load is above the critical threshold (50-60\% $\dot{V}O_{2max}$)\textsuperscript{21,22}. Therefore, in the same absolute intensity of exercise, cortisol may present low response from the practice of an exercise training program. In the submaximal exercise, the cortisol response is more variable and influenced by many external factors\textsuperscript{19}.

If the intensity is below the critical threshold, then the cortisol levels may not increase above the rest levels, or may become in fact reduced\textsuperscript{23}. However, if it is above the critical threshold intensity, then the cortisol levels will initially increase and subsequently enter a plateau (steady-state exercise)\textsuperscript{24}, since while cortisol increases during exercise, at the end of it many alterations and possible effects of this hormone occur during the early recovery\textsuperscript{25}.

The correlation between cortisol and the weight loss process is explained by the effects of the intermediary metabolism\textsuperscript{26}, which among the main functions we can mention the maintenance of glycemia and consequent lipolysis stimulation.

Considering hence that one of the stimulating factors of leptin secretion is the increased cortisol level, the aim of this study was to analyze the acute effect of concurrent training on the leptin and cortisol serum levels in overweight young adults.

**METHODOLOGY**

**Study outlining**

This study is quasi-experimental for trying to establish cause/ effect correlations\textsuperscript{27}.

**Sample**

The non-probabilistic sample was limited to 20 individuals sorted in two groups: concurrent training – TCG (10), and control group – C (10), age between 20 and 35 years, of both sexes, who exercised in the Westfit health club, located in the Realengo neighborhood, in Rio de Janeiro city. The subjects were randomly selected and volunteers, following these inclusion criteria: non-athletes, practitioners of indoor cycling and bodybuilding, with a minimum of six months of activity and weekly frequency of three times, who were with body mass index (BMI) between 25.0 and 29.9, characterizing overweight.

The selected subjects were told about the eating routine and training protocol, signed the Free and Clarified Consent Form and the PAR-Q – Physical Activity Readiness Questionnaire application.

This research is according to the Ethical Guidelines for Biomedical Research Involving Humans according to the principles established in the Declaration of Helsinki (1996). The research Project was approved in Rio de Janeiro, Brazil, by the Ethics in Research Committee of the Castelo Branco University under the protocol number 0157/2008.

**DATA COLLECTION**

**Measurement protocol**

During the weeks which preceded the day of the test, the anthropometric measures, one repetition maximum (1RM) test and submaximal oxygen consumption ($\dot{V}O_{2submaximal}$) were performed with the subjects of the sample groups.

The collection of the anthropometric measures of total body mass, fat mass, lean mass, height and BMI was performed according to the protocol by the International Society for the Advancement of Kinanthropometry (ISAK)\textsuperscript{28}, in a room specific to functional evaluation in the Westfit health club premises with the instruments: a Filizola\textsuperscript{®} digital scale with anthropometer previously calibrated, a Sanny\textsuperscript{®} metal measuring tape and a calculator.

The 1RM test\textsuperscript{29} was performed only in the C, to measure the intensity of each exercise which was part of the strength training protocol. The exercises were performed in Biotech\textsuperscript{®} machines and the total used weight (long bar, short bar and free weights) was previously checked on a Filizola\textsuperscript{®} digital scale.

Two weeks after the 1RM test, the measure of the $\dot{V}O_{2submaximal}$ for cycle ergometer expressed in kg.min (ml)\textsuperscript{−1} was done only in the C group, to determine the individual intensity to be used in the indoor cycling class. The test followed the Balke/ACSM protocol (1980), which allows suitable physiological adaptation, as well as guarantees the cardiac safety of the individuals. The test was performed in an Inbrasport\textsuperscript{®} cycle ergometer previously calibrated.

One week after the $\dot{V}O_{2submaximal}$ test, the subjects of the TCG and C group were referred to the blood collection. Both groups received an eating routine of 1,200kcal which was prescribed by nutritionist Fernanda Albuquerque de Andrade, CRN 2003100304, to be followed on the day prior to the test in order to avoid possible increase in fat intake.

**Protocol for concurrent training**

The exercise sequence for the TCG consisted of two exercise modalities, one aerobic (indoor cycling) and one strength (bodybuilding). The concurrent training occurred in the following order: indoor cycling practice followed by bodybuilding practice, with no intervals between them, in a single session with approximate duration of two hours.

**Protocol for indoor cycling**

The cycling classes were performed in a separate room, specific to this practice, with temperature of 17ºC in Tomawank bicycles, model XL. Duration of the indoor cycling classes was of 50 minutes with no interruption, with cadence set at 80rpm, according to the bpm of the music corresponding to the necessary rpm, since, according to Mello\textsuperscript{30}, the rpm has straight relation with the music bpm. The class protocol followed the specifications of the sports continuous training\textsuperscript{11} with intensity at 65\% of $\dot{V}O_{2submaximal}$ for all subjects in the TCG. The OMNI RES scale (subjective perception scale for resistance exercise) for cycling was used for intensity maintenance for the whole time of the exercise\textsuperscript{12}.

**Protocol of strength training**

The strength training session was performed immediately after the indoor cycling session in bodybuilding machines named Biotech\textsuperscript{®}. The program consisted of three bouts, 65\% of 1RM load for each of the proposed exercises, with 20 repetitions and interval of one to two minutes between sets for recovery of the worked muscle group\textsuperscript{31}, since, according to Kawakami et al\textsuperscript{33}, the isometric muscular activities repeated at high intensity may produce decrease in strength with time. The strength training protocol (ST) was composed of seven exercises for up-
per and lower limbs, in the following order: supported row, leg press 45°, bench press (long bar), knee extension (extensor chair), straight bar curl (short bar), knee flexion (flexor chair) and triceps extension (high pulley). The session had approximate duration of one hour and 10 minutes.

**Protocol for blood collection**

The volunteers were asked to arrive at the collection place on the set day at eight o'clock in the morning, on 12-hour absolute fast to better evaluate the basal serum leptin and cortisol levels of the subjects of TCG and C groups at absolute fast. The 20 blood samples of leptin and cortisol of both groups were collected by the Sérgio Franco Medicina Diagnóstica Laboratory, Rio de Janeiro, in an itinerant laboratory in the Westfit health club. Immediately after the end of the training session, the collection of more than 20 blood samples of leptin and cortisol was performed in both groups at the same conditions and places described above. The method used by the laboratory for the analysis of the leptin samples was the radioimmunoassay, where the reference values are: up to 18.5 ng/mL in women and up to 9.2 ng/mL in men. The method used by the laboratory for the cortisol samples analysis was the immunoenzimatic assay by chemiluminescence, which has reference values of 5.0 to 25.0 mcg/dL in the seven to nine o'clock in the morning period.

**STATISTICAL ANALYSIS**

All statistical procedures were processed in the Statistical Package for the Social Sciences program (SPSS 14.0, Chicago, USA). The sample characterization of the population was investigated for applying the described techniques. In order to obtain a description of the collected data, mean measure was used, which is a central tendency measure, and standard deviation for the dispersion measures. Inferential analysis of the study used two-way ANOVA in order to determine differences between the groups at different moments. The sample homogeneity and the results were analyzed by the Shapiro Wilk test, and for sample normality the Tukey post moments. The sample homogeneity and the results were analyzed by the Shapiro Wilk test, and for sample normality the Tukey post hoc test was used. The significativ level adopted was of \( p < 0.05 \).

**RESULTS**

Table 1 presents the descriptive results of the anthropometric variables of the TCG and C groups, as well as the normality analysis of the sample through the Shapiro-Wilk test (SW). Each group was composed of 10 subjects.

It can be analyzed that all groups presented normal distribution or all the analyzed variables. Kurtosis was also performed to guarantee homogeneity of the sample. BMI between 26.84 and 27.5 was observed, which lets classify them as with overweight. Table 2 presents the descriptive results of the serum level of the training competitor group (TCG) and control group (C) at the pre and post-intervention moments. Normality was also verified through the SW test.

When the mean values of the first and second leptin collection, it was observed that the TCG group presented values of 14.53 ng/mL ± 6.79 and 13.09 ng/mL ± 8.46, and was within the reference mean thresholds expected for plasma leptin (until 13.85 ng/mL ± 6.57 for women and men), while the C group which obtained values of 27.09 ng/mL ± 10.70 and 21.49 ng/mL ± 9.44, these were much higher than the mean reference thresholds.

Table 3 presents the descriptive results of the cortisol serum level in two moments (pre and post-intervention).

The descriptive analysis of the plasma cortisol samples of the TCG and C groups demonstrated that the values obtained in the first and second collections of both groups were within the mean values of 15.0 ± 14.14 mcg/dL for the seven to nine o’clock time.
Table 4 presents the leptin statistical values for the TCG and C groups. The data presented significant difference when the groups were compared to the pre and post-intervention moments.

Intergroup significant difference has been observed (TCG x C) in the pre-intervention (p = 0.02) and post-intervention moments (p = 0.01). In the intragroup analysis significant alterations have not been observed.

Table 5 presents the cortisol statistical values for the TCG and C groups. In the intergroup analysis (TCG x C), significant difference was observed at the pre-intervention (p = 0.01) and post-intervention moments (p = 0.01). In the intragroup analysis significant alterations have not been observed.

**DISCUSSION**

According to the revision study by Kraemer et al., many studies which have investigated the effects of the acute exercise on leptin showed reduction or no alteration in the concentrations. Steinberg et al and Jen et al. stated that the articles published have demonstrated that the leptin plasma levels are reduced after physical training in animals.

In the study by Estadella et al., the found results verified that endurance training in rats would avoid increase of leptin plasma levels induced by a hyperlipidic diet, and that decrease in the leptin levels was also followed by lower body weight and adipose mass gain and lower levels of serum triglycerides and insulin.

Kraemer et al. state that many studies say that the leptin plasma levels are not altered due to acute aerobic exercise in athletes or non-athletes. However, the decrease of these levels may be connected to the time of leptin collection after the exercise session, while leptin is directly or indirectly related to the control of the energy balance in the long run. Besides being an important lipostate (measurer of the lipid buildup of the body), leptin increases lipolysis and lipidic oxidation.

The research by Rosa et al. investigated the effects of the con-current training on the leptin concentrations in young adults in overweight state. Their results pointed that there was reduction of the variable levels. Such decrease in the leptin concentrations do not seem to depend on the performance order of the concurrent training, since in the studies by Rosa et al., the used protocols caused reduction in the concentrations of this hormone regardless of the performance order.

In the present study, leptin presented significant difference (p = 0.02) when the pre-moments of the groups were compared, and in mean values, the C group was higher (27.09 ± 10.70). The two-way ANOVA results pointed the existence of statistically significant difference (p = 0.01) in the comparison of the pre moment of the C group with the post moment of the TCG group.

In the study by Bouassida et al., the leptin behavior was evaluated in trained and untrained individuals after exercise in cycle ergometer. The protocol consisted of two exercise periods: 45 min and 85 min. The leptin samples were collected before, during, at the end, after two hours and 24 hours of recovery from each protocol. It was verified that leptin did not significantly alter during both protocols for both groups, but was lower (p < 0.05) in all samples in the trained group when compared with the untrained group.

In our research, the leptin mean values demonstrated slight decrease in both groups (TCG: 14.53 ± 6.79 to 13.09 ± 8.46; C: 27.09 ± 10.70 to 21.49 ± 9.44) after the used training protocol. Such findings corroborate Essig et al., who state that the exercise effects with period equal or longer than 60 minutes usually do not reveal any decrease or slight decrease in the leptin levels post-exercise, and Ribeiro et al., mentioning that the report that acute or chronic physical exercise has been commonly causing decrease in the leptin concentrations.

However, according to Fisher et al., the studies suggest that exercise practiced in an acute manner (< 60 min) does not present acute effect on the leptin production, regardless of the exercise intensity in healthy men and women and that the reduction found may be attributed to the circadian rhythm or hemo concetration. Hulver and Houward also questioned whether exercise per se causes this reduction or if body fat decrease, the typical chronic response to physical training or the energetic imbalance created would not be responsible for this decrease.

Essig et al., in their study, also found low leptin concentration in trained men after 48 hours of exercise, but not immediately after exercise and 24 hours after it. It was two separated years of treadmill running – 800 kcal and 1,500 kcal. The authors speculate that the alterations in the leptin stimulus, as well as the alterations in the inhibitors, may have affected the leptin concentrations.

Longer exercise duration has also been widely discussed. The study performed by Keller et al. tried to observe the leptin behavior after three hours of exercise in cycle ergometer. It was observed that the leptin concentration decreased after this period.

In the study by Landt et al., reduction of 8% in the fasting leptin was observed followed by two hours of exercise in cycle ergometer at 75% VO2max and ended with five chunks of one-minute sprints. The same authors observed in another study that the leptin concentration before and after exercise of a group of ultramarathon runners presented decrease of 32% after mean of 35 hours of continuous exercise, which, according to the authors,
suggests that the most remarkable decrease occurred due to the energy imbalance and that leptin may serve as an important sign of this imbalance, but only in extreme situations.

Despite the found results, some studies did not observe any alteration in the leptina concentration, such as the study by Racette et al.62, who measured the difference of arteriovenousleptin concentration after 60 minutes of exercise on ergometric bicycle and a study by Nindlet al.68, where exercise with weights was applied in physically active individuals and the decrease of the leptin levels occurred after only nine hours of post-exercise. In the present study there was no significant difference in the comparison of the leptin concentration in the pre and post in the two groups (TCG – p = 0.98; C – p = 0.51). According to the obtained results, it can be observed that there was no significant difference in the analyzed variable after the intervention protocol.

The study by Cruz et al.49 did not demonstrate significant alterations in the leptin concentrations after a protocol of concurrent training with characteristics of modality, intensity and duration similar to the ones used in the present study, in which significant difference was not observed in the leptin levels of the participants either.

Due to its nature of fast response to many kinds of stress, which usually occurs within some minutes, cortisol points strength exercises as powerful promoters to reach fast increase of its concentrations in the blood stream20, also due to its characteristic to promote adaptative changes in the body. However, some studies35-65-67 did not find differences in plasma cortisol after an acute strength training session. The study by Kraemer et al.62 tried to analyze the cortisol acute response after eight bouts of 10RM in leg press with interval times of one minute and three minutes between bouts, and recorded that, with one- minute interval, there was significant increase compared with the three minutes for the same exercise protocol. In the present study, the bodybuilding protocol followed an interval time of one to two maximal minutes for three bouts of 20 repetitions, according to the ACSM guidelines23, differently from the study by Kraemer et al.62, which could have been another factor which led to cortisol increase besides the number of bouts and repetitions performed.

Foschini and Prestes53 mentioned studies which used number of bouts and repetitions similar to the ones used by Kraemer et al.62 and which presented increase in the plasma cortisol concentration.

However, some studies55-66-67 did not find differences in plasma cortisol levels after exercise. Grandys et al.59, for example, analyzed the plasma cortisol between other hormones before and after five weeks of endurance training in cycle ergometer – in the continuous method (2x/week at 90% VO2max) and intermittent method (2x/week at 50% VO2max) – on Mondays and Thursdays, and at the end no alterations in the plasma cortisol were found.

The results presented in the current research demonstrate that a single session of the concurrent training protocol used in the present study was not able to generate the necessary stress to increase the plasma cortisol secretion in sufficient quantity to promote increase of the serum leptin levels in overweight young adults.

CONCLUSIONS

The results presented in the present research demonstrate that a single session of the concurrent training program used in this study was not able to generate the necessary stress to increase the plasma cortisol secretion in sufficient quantity to promote increase in the serum leptin levels in the evaluated individuals.