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ORIGINAL ARTICLE
BODY COMPOSITION, NUTRITION AND SUPPLEMENTATION

Reduced leptin level is independent of fat mass changes and hunger scores from high-intensity intermittent plus strength training

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ABSTRACT

BACKGROUND: This study aimed to analyze the effects of high-intensity intermittent training (HIIT) plus strength training on body composition, hormone related to energy balance (leptin), and hunger scores in physically active non-obese men.
METHODS: Sixteen men were allocated in two different groups, training group (N.=10) performed a combined HIIT (5 km, 1 minute of effort interspersed by 1 minute of rest in passive recovery) followed by strength exercise session (three sets, with load of 8-12 repetition maximum) twice a week, during 8 weeks, while control group (N.=6) did not suffer any intervention. Hunger scores, leptin concentrations and body composition were assessed. Body composition, fasting leptin and hunger score were compared through two-way analysis (group and period) with repeated measures in the second factor while leptin and hunger scores in exercise session pre- and post-8 weeks through two-way analysis (period and time of measurement) with repeated measures in the second factor.
RESULTS: The fasting leptin decreased pre- to post-8week in training group (7.7±4.9 to 2.9±2.1 ng/mL; P=0.012). For leptin response to exercise session there was main effect of training period, with higher values pre- (6.5±3.9 ng/mL) than post-training (2.6±2.1 ng/mL; P<0.001). For hunger scores there was effect of time of measurement (P<0.001), decreasing after breakfast and increasing over the experiment.
CONCLUSIONS: Combined HIIT plus strength training were able to promote alterations in a hormone related to energy balance independent of body composition and hunger index alterations in physically active non-obese men.

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Key words: Leptin - Body composition - High-intensity interval training - Energy metabolism - Exercise.

Physical activity is an important tool for health maintenance, especially to prevent and ameliorate disease conditions. This is possible because physical exercise augments energy expenditure (EE), which is related with a negative energy balance, and decrement in excessive accumulation of abdominal fat inflammation, which are risk factors for cardiometabolic disease.[1] In addition, several hormones and signalling proteins are involved in energy balance control, such as leptin.[2]

Leptin plays a central role in providing information about energy stores,[3] as well as inhibiting orexigenic and activating anorexigenic neurons through suppression of AMP-activated kinase (AMPK), resulting directly in satiety and, thus regulating appetite, increasing

EE [4, 5] and modulating aspects of eating behavior such as meal size, and preferably food reward power.[6]

There is also evidence that appetite control can be improved by an aerobic training program.[7] The increase in hunger scores and decrease in leptin have been shown to be well correlated in the overweight and obese population.[8] It seems that in this population the improvement in appetite control occurs through an increase in satiety [9] and a decrease in leptin levels.[10] The training program should include aerobic and strength exercises for optimal health and fitness benefits,[11] but there is no evidence reporting the effects of an association of high-intensity intermittent training (HIIT) and strength training on these variables.

Aerobic HIIT is characterized by short and repeated bursts separated by recovery periods and it is suggested to be as effective in the improvement of health-related parameters.[12] HIIT exerts superior effects on improving maximum oxygen consumption (VO_{2max}), a strong predictor of mortality,[13] than continuous moderate training.[14, 15] In addition, HIIT as an aerobic training stimulus can be more effective to disrupt energy homeostasis.

Strength training is recognized to enhance muscle strength as well as EE by inducing muscle hypertrophy.[16] Studies have shown that this type of training is important to metabolic and cardiovascular health [17, 18] and can contribute to decreased relative fat mass.[19] Regarding appetite control, the evidences are not abundant as for aerobic training, but strength training seems to induce similar alterations in leptin, although the effect on satiety was not reported.[20] However, it is still not known how combined HIIT plus strength training can induce alterations in energy balance related to appetite control and endocrine regulation.

Thus, the present study aimed to analyze the acute responses and chronic effects of a training program involving combined aerobic (HIIT) plus strength training on body composition, a hormone related to energy balance (leptin), and hunger scores in physically active non-obese men.

Materials and methods

Subjects

The sample consisted of 16 men (6 in the control group and 10 in the training group) aged between 18 and 35 years, considered healthy and physically active through aerobic conditioning determined by VO_{2Peak}. The subjects participated voluntarily in the study after being informed of the procedures, risks, and benefits and signed an informed consent form. This study was approved by the UNESP, Ethics Committee (22793414.7.0000.5402).

Body composition assessment

Total body mass, fat mass, skeletal muscle mass and fat free mass of upper limbs, lower limbs and trunk were measured by octopolar bioelectrical impedance equipment, InBody 720 (Biospace, Seoul, Korea). Frequencies of 1, 5, 50, 250, 500 and 1000 kHz were emitted and received from the counting of the subject in the standing position on the equipment (soles of the feet and heels right and left and palms of the hands and right thumbs and left). The subject followed some recommendations: to wear a minimum clothing, 4 hours of total fasting (food and drinks) as well as do not consume caffeine and do not exercise 12 hours before the assessment. Tests were performed indoors, with controlled temperature between 20 and 25 °C. The methodology of bioelectrical impedance evaluation with octopolar equipment was valid in previous studies.[21-23]

Food control

Concerning the control of eating habits, the volunteers were asked to complete a food recall in which they were required to report the time and details of all food consumed in each meal, accompanied by the amount and method of preparation thereof, for three alternate days after the experimental session, including one weekend day (Saturday or Sunday). Another questionnaire concerning dietary information was completed by volunteers with a food frequency questionnaire. The document presented different types of food and the volunteer reported the frequency and quantity of consumption of each food over a year.

Incremental test

For determination of the aerobic power, the subjects performed a maximal incremental test on the treadmill (Inbramed-ATL, Inbrasport, Porto Alegre, Brazil) until voluntary exhaustion, with the measurement of VO_{2max} (Model Quark PFT Ergo – Cosmed, Rome) ($\dot{V}O_{2max.}$).

Each stage was composed of 2 minutes, the first being performed at a speed of 8 km/h, increasing by 1 km/h at the end of each stage. In addition, heart rate was monitored using a specific monitor (Heart rate monitor Polar S810, GBR) integrated into the gas analysis system. The average of the final 30 seconds was defined as peak oxygen uptake ($\dot{V}O_{2peak}$). The maximal velocity reached in the test was defined as the peak velocity (VO_{2peak}). When the subject was not able to finish the 2-minute stage, the speed was expressed according to the permanence time in the final stage, determined as the following: V_{peak}=velocity of penultimate stage + [(time, in seconds, remained at the final stage multiplied by 1 km/h)/120 s].[24]

Maximum dynamic strength test

Seventy-two hours after the maximum endurance running test, the subjects performed the maximum dynamic strength test through the maximum load that the subject was able to execute in just one repetition maximum (1RM) in the half squat exercise in guided bar (Ipriranga®, Presidente Prudente, Brazil). For this, they followed the recommendations of the American Society of Exercise Physiology.[25]

Before starting the 1RM test, the subjects performed a five minute warm up at 50% of VO_{2peak} and subsequently, two series of eight and three repetitions at 50-80% of 1RM, respectively. To establish the 1RM, single executions were performed with progressively heavier loads to volitional fatigue. The rest interval was three to five minutes and the number of attempts was not more than five. The load lifted highest in the test was regarded as the value of 1RM.

For better control of the 1RM test procedures, each participant had his body position and feet placement in the half-squat exercise recorded and reproduced throughout the study. In addition, a string with adjustable heights was placed behind the participant in order to maintain the bar displacement and knee angle (~90°) constant in each half-squat repetition.

Training protocol - HIIT plus strength training

The subjects performed a warm-up consisting of running at 50% of VO_{2Peak} for 5 minutes at 1% inclination. The HIIT was performed intermittently with subjects running on a treadmill for one minute at 100% of vVO_{2Peak} interspersed by one minute of passive recovery (without exercise) until they had completed 5 km. Strength training was performed with three sets, 10 minutes apart, with a 90-second rest interval between series, with a load of 8-12 RM in the half-squat, bench press, leg extension, leg curl, lat pulldown, dumbbell lateral raise, pushdown, and low pulley. The participants maintained the number of repetitions between 8 and 12RM, and when participants were able to accomplish more than 12RM, the load was adjusted to guarantee the exercise was conducted within the repetition zone.

Acute exercise session assessment

Each subject was submitted to an acute exercise session assessment before and after eight weeks of physical training. These assessments were performed in the morning and consisted of blood samples, standardized breakfast, and a HIIT plus strength exercise session (Figure 1).

Blood samples

Blood samples were collected from an antecubital forearm vein using a needle and vacutainers for determination of total serum to analysis in the following periods: 1) overnight fast (8 to 12 hours); 2) pre-exercise; 3) immediately after exercise (Immediately); 4) 30 minutes after exercise (30 min); 5) 60 minutes after exercise (60 min).

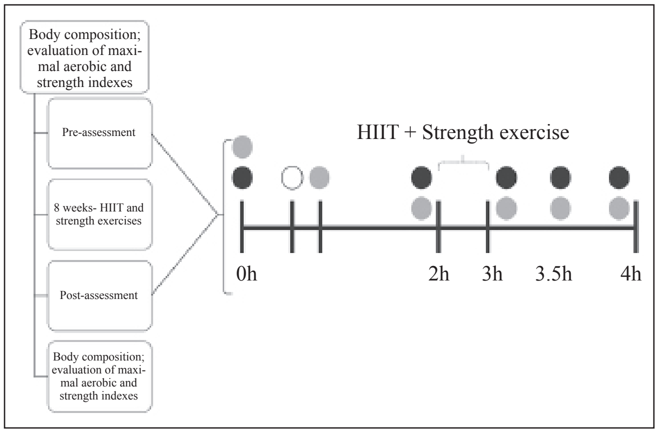

Figure 1.—Experimental design.
Black dot: blood collection; white dots: breakfast; grey dots: hunger score.

Breakfast

After the overnight fasting blood sample collection, a standardized meal was offered, consisting of salt toast, processed UHT cheese ("Ultra High Temperature") and Kefir with yogurt and fruit pulp, food which together adequately provided the macronutrients needed, corresponding to 25% of the estimated daily energy needs for each participant). Moreover, the breakfast constituted easily digestible food and products accessible to the general population. The amount of calories ingested by the subjects was based on the estimated daily energy needs), taking into account the height, and total body mass by adopting a correction factor which adjusted for the caloric value in relation to the physical condition assets.[26]

Hunger scores

Additionally, a scale that measures the degree of participant's hunger was applied at 6 specific times during the experimental session, as follows: 1) before breakfast; 2) immediately after breakfast; 3) pre-exercise session (pre); 4) immediately after exercise session (post-exercise); 5) 30 minutes after exercise session (30-min post-exercise); 6) 60 minutes after exercise session (60-min post-exercise). The perception of hunger was evaluated through a validated Visual Analogue Scale,[20] 10 cm in length, with words at each end, expressing the highest (10 cm) and lowest (0 cm) rating of hunger. Participants were instructed to make a mark on the scale representing their feeling at that moment.

Blood analyses

Approximately 20 mL of blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and anticoagulant gel to separate serum and plasma at 1) overnight fast; 2) pre-exercise; 3) post-exercise; 4) 30-min post-exercise; 5) 60-min post-exercise. The blood was centrifuged at 3000 rpm for 15 minutes at 4 °C. Next, the rates of serum and plasma were stored in Eppendorf type tubes and stored at -20 °C for additional analysis. The concentration of leptin was analyzed through enzymatic assay by ELISA using kits from R&D System (Minneapolis, USA).

Statistical analysis

The data were analyzed using Statistical (version 10.0) and presented as means and standard deviations. For all measured variables, data normality was verified using the Shapiro-Wilk Test. The comparison between control and training groups was done to aerobic fitness, body composition, fast hunger and leptin pre from post the training period due two-way ANOVA (group and training period) with repeated measured in second factor. To compare pre and post training period assessment of the hunger score, and leptin at the different evaluation moments in an exercise session (only to training group) was conduct a two-way ANOVA (training period and time of measurement) with repeated measures in the second factor; when a significant difference was found, Tukey's *post hoc* was utilized. Statistical significance was set at P values <0.05. Standardized effect sizes were also calculated using Cohen's equation (1969) with the following classification values: <0.2 — trivial; >0.2 and <0.6 — small; >0.6 and <1.2 — moderate; >1.2 and <2.0 — large; >2.00 and <4.0 — very large; <4.0 — nearly perfect.[27]

Results

Table I shows body composition, aerobic fitness, leptin and hunger scores values in both control and training group at baseline and post-week 8.

For the comparison between control and training group there was an interaction effect for maximal aerobic speed ($F_{1,14}$=16.87; P=0.001) with increase pre from post-training only to training group (P=0.003; *d*=0.80 [moderate]); and to fasting leptin ($F_{1,14}$=8.09; P=0.012) with decrease pre from post-training (P=0.03; *d*=1.05 [moderate]). There was no effect for fast hunger score, VO_{2peak} and body composition.

For comparison of hunger scores to training group in different moment of measurement and training pe-

TABLE I.—*Characteristic of the sample (N.=16).*

	Control group (N.=6)		Training group (N.=10)	
	Baseline	Post-week 8	Baseline	Post-week 8
Body weight (kg)	78.8±5.1	79.2±4.7	74.7±7.9	75.0±7.9
Muscle mass (kg)	37.5±2.5	37.7±2.5	35.7±4.3	36.0±4.4
Body fat (kg)	13.0±2.2	13.2±2.0	12.0±2.8	12.1±3.4
Body fat (%)	16.4±2.6	16.7±2.3	14.1±3.6	16.2±4.2
Leptin (ng/mL)	6.1±2.5	6.6±3.9	7.7±4.9	2.9±2.1*
Hungry scores (cm)	6.6±2.1	6.1±2.6	6.6±2.6	6.3±3.0
VO_{2peak} (mL/kg/min)	56.3±5.3	54.8±7.0	53.6±5.3	54.5±3.8
vVO_{2peak} (km/h)	15.1±1.3	15.1±1.4	13.9±1.2	14.8±1.1*

VO_2peak: peak oxygen consumption, vVO_2peak.
*P<0.05 was considered as significant from pre to the same group.

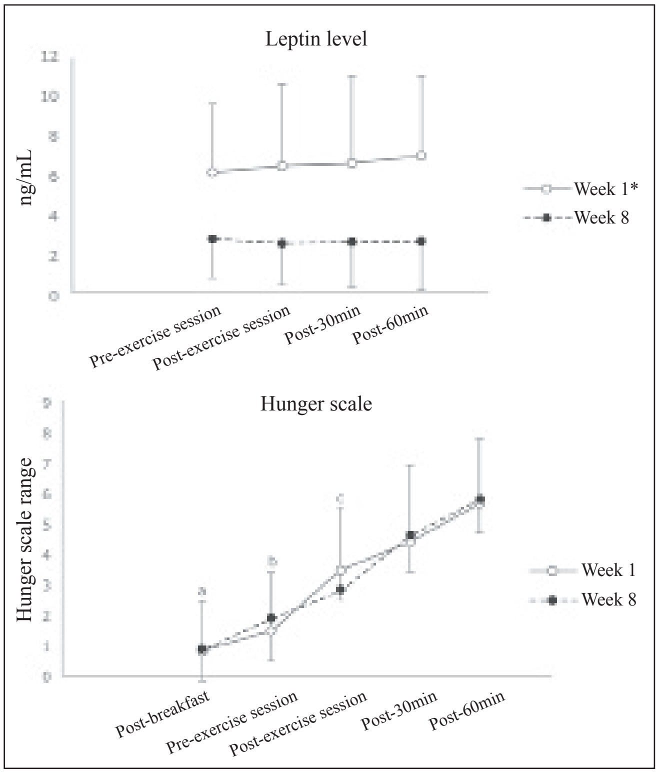

Figure 2.—Comparison of acute exercise session responses.
Values are expressed as mean±standard deviation. Hunger Scores are expressed in centimeters. a: different from immediately post-exercise, post-30 min, and post-60 min; b: different from post-30 min and post-60 min; c: different from post-60 min. Leptin level is expressed in ng/dL.*Different from training period. P value<0.05.

riod there was a main effect of time of measurement ($F_{4,32}$=29.9; P<0.001), with lower values post-breakfast than immediately post-exercise (P=0.001; d=1.10 [moderate]) 30- (P<0.001; d=1.77 [large]; and 60-min post exercise (P<0.001; d=2.60 [very large]); pre-exercise lower than 30- and 60-min post exercise (P<0.001); and immediately post-exercise lower than 60-min post exercise (P<0.001; d=1.05 [moderate]) (Figure 2).

For leptin in exercise session there was a main effect of training period ($F_{1,81}$=9.60; P=0.012), with higher values pre- than post-training (P<0.001; *d*=1.23 [large]).

Discussion

The present study included three measurement stages (overnight fast, postprandial, and post-exercise) which permitted observation of the effects of training on perceptive and metabolic variables associated with hunger, wherein leptin reduction were verified without modifications in hunger scores, fat or fat free mass after 8 weeks of HIIT plus strength training. In addition, a slight increase in aerobic fitness was observed.

The long-term effects of physical training programs on control of appetite is still incipient especially as there are several types of appetite evaluation, for example, via gastrointestinal hormone concentrations,[28] neuropeptides,[29, 30] perception of feelings linked to appetite (*i.e.*, hunger, desire to eat, etc.), and finally through modifications in energy intake (hot and cold meals offered in a laboratory and assessed *via* food diaries).[31] Thus, the high number of different factors makes the definition of a standard response difficult.

The majority of studies has demonstrated that exercise plays a dual-role in increasing orexigenic pathway as well as improving meal induced-satiety, demonstrating anorexigenic action enhancement. Orexigenic pathway is represented by the increase in fasting hunger and improvement in meal induced-satiety through post-meal hunger scores.[32, 33] Thus, the evaluation of appetite control has been assessed in two distinct conditions (fasting and postprandial) and demonstrated improvement in the appetite control system, in overweight and obese people. In the present study there was no effect on fasting hunger or after breakfast and exercise, showing that in normal weight people appetite control remained stable. As proposed by other researchers, this result confirms that normal weight people generally have more accurate appetite control when compared with overweight and obese people.[32, 34]

Although leptin is recognized as an energy sensor according to the amount of adipose tissue and sending afferent signals to the central nervous system (CNS), this adipokine has an important function in energy balance, causing energy conservation and decreased thermogenesis.[35] In this study, leptin concentrations decreased significantly after 8 weeks of HIIT plus strength training without reductions in body fat or changes in hunger scores. Thus, this reduction seems to be related only to an increase in EE.[10] It is important to mention that the increase in resting metabolic rate through exercise could improve appetite control and increase leptin sensitivity, being one of the mechanisms that lead to a reduction in this adipokine. Furthermore, Zacaria *et al.*[36] proposed that high EE inhibits leptin production through norepinephrine action via the β_3-adrenergic receptor and, Gomez-Merino *et al.*[37] considered that the augment in circulating fat-free acid (FFA) could be a possible metabolic signal of leptin level reduction.

Although EE and FFA levels were not measured in this study, the protocol we used is highly intense (see methodology section), which leads to an exacerbated EE, at the same time that HIIT has aerobic characteristics,[38] may be, at least in part, favor an increase in fat oxidation in skeletal muscle. Added to this, strength exercise performed following 5 km-HIIT in the same session could potentiate the EE. Goto *et al.*[17] found that strength exercise performed twice a day for two successive days led to leptin reduction, probably reflecting excessive physical stress and early signs of accumulated fatigue. Although the present study design is different from Goto *et al.*,[17] there is a similarity concerning overload session, resulting in lower long-term leptin concentration. In addition, the decrease in leptin levels could have occurred through an increase in leptin sensitivity, linked to high EE.[39] Therefore, leptin reduction found after 8 weeks of HIIT plus strength training could be explained as a “defence mechanism” to restore the energy deficit since the orexigenic pathway would cease to suffer an inhibition and, thus, stimulate food intake. This condition associated with this argument “defence mechanism” some investigations has been proposed that the decreasing of leptin and other variables as thermogenic adjusts (decreasing resting and no-resting energy expenditure) can occur in order to maintain body weight.[40]

However, we are not able to point if the leptin reduction was resulted of increasing leptin sensibility or it was an attempt to decrease an anorexigenic drive (defence mechanism) in order to avoid weight loss, and this reduction remains elusive and warrants further investigations. Nevertheless, this result is in agreement with other studies that combined aerobic plus strength [41] or plus plyometric training,[42] and also found a decrease in leptin concentration.

Taking into account that HIIT has been considered a potent stimulus to reduction to body fat, and, sometimes, greater than moderate intensity training,[43-45] in the present study, 8 weeks of HIIT plus strength training were not able to reduce fat mass. This can be explained by characteristics of participants, since our sample was composed by physically active men, while the majority of studies was conducted with sedentary people. Thus, the effect that training status on this response exert in the phenomenon deserves future investigations.

Limitations of the study

This study has some limitations. First, EE and concentrations of FFA were not assessed. However, based on published results of a study from our group, we believe that the HIIT protocol generated high EE for session (~523 kcal) and, added to strength training, an exercise session could overcome 800 kcal. Second, hormones more responsive to acute exercise sessions such as acylated ghrelin and peptide YY which were not measured, this could represent a limitation. Third, we did not measure the thermogenic adjustments that would help to contextualize better the effect on body composition.

Conclusions

In summary, HIIT plus strength training was able to modify the concentrations of a hormone related to energy balance (*i.e.*, decreasing leptin), independent of alterations in body composition, hunger score, and satiety. Therefore, it is possible that leptin reduction occurred to maintain body mass, but it would be necessary other measurements as leptin receptor, resting EE and energy intake to verify if there was some compensatory mechanism to prevent changes in body mass.

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