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Effects of High-Intensity Exercise on Leptin and Testosterone Concentrations in Well-Trained Males

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Objective: A number of investigations have examined the effect of exercise on leptin concentrations, because leptin is associated with obesity, satiety, and reproductive function. High-intensity exercise is known to increase testosterone, an inhibitor of leptin. The objective of the study was to determine whether the leptin responses to a progressive, intermittent exercise protocol were related to serum testosterone concentrations. Most previous studies have examined leptin responses to low or moderately high exercise intensities. A second objective was to determine whether leptin responses were different than previous experiments using intermittent moderate and high-intensity exercise.

Methods: Well-trained runners completed strenuous intermittent exercise consisting of treadmill running at 60, 75, 90, and 100% $VO_{2\,max}$ and a subsequent resting control trial was also conducted.

Results: There were significant increases in mean serum levels of leptin and testosterone with both quickly returning to baseline during recovery, but no relationship between the two hormones was found. After examining individual data for both hormones, it was discovered that subjects could be classified as leptin responders or nonresponders, whereas testosterone increased in all subjects. Responders had elevated serum leptin levels at baseline and exhibited increases after high-intensity exercise, whereas nonresponders did not show changes in leptin during exercise.

Conclusions: Data suggest testosterone levels do not acutely affect leptin responses to exercise or 1-h of recovery. Moreover, varied leptin responses to intense exercise in comparable well-trained runners was observed and was associated with baseline leptin concentrations.

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Introduction

Leptin, a hormone found primarily in adipose tissue, is a 16 kDa protein with a helical structure similar to cytokines *(1,2)* that plays an important role in the regulation of body weight and energy homeostasis *(3,4)*. Neurons in the arcuate, ventromedial, and dorsomedial hypothalamic nuclei that are sensitive to leptin express neuropeptides/neurotransmitters that are associated with central regulation of energy balance *(5)*. Leptin is regulated by the status of fat storage, with larger adipocytes containing more leptin than smaller ones in the same individual *(6)*. Nutrition-related control of leptin has been suggested to be partially regulated by insulin. It has been demonstrated that leptin mRNA expression increases after elevation of insulin in response to feeding *(7)* and a decline in serum leptin levels follows reduction in insulin during fasting *(8)*.

The discovery of leptin *(2)* has led to numerous experiments to better understand its function and a portion of those studies have focused on leptin and exercise. A major reason for this is that exercise reduces obesity, thus if leptin levels are affected, this may provide some explanation of how exercise affects obesity. Moreover, an array of hormones are affected by exercise that have been shown alone to alter leptin concentrations *(9)*.

We have recently reviewed the extensive literature on leptin and exercise *(9)* noting that studies of short-term exercise (<60 min) and circulating leptin have reported no change, increases, or reductions in leptin concentrations, with factors such as hemoconcentration and diurnal decline accounting for the latter two *(10–12)*. We have reported relatively stable leptin levels in response to exercise in postmenopausal women *(12)*, but have noted variability among subjects.

Gender influences leptin concentrations *(13)* with higher levels in females than males *(14,15)*. Higher leptin levels in females have been attributed to a number of factors includ-

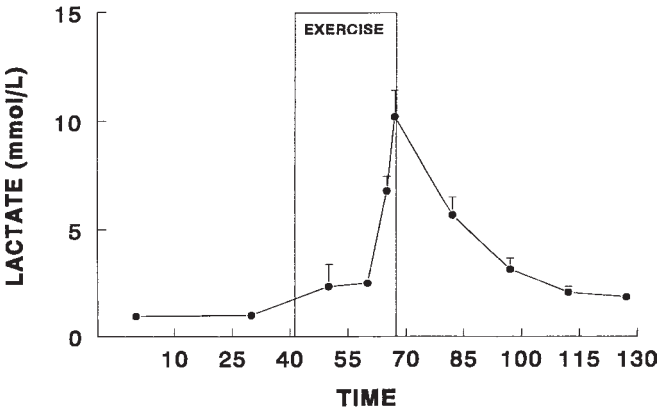

Fig. 1. Plasma lactate concentrations before exercise, after each exercise intensity, and for 1 h of recovery. Data depict means ± SE for $n = 7$. Each concentration was compared with the –10 min resting concentration, $*p < 0.05$; $**p < 0.01$.

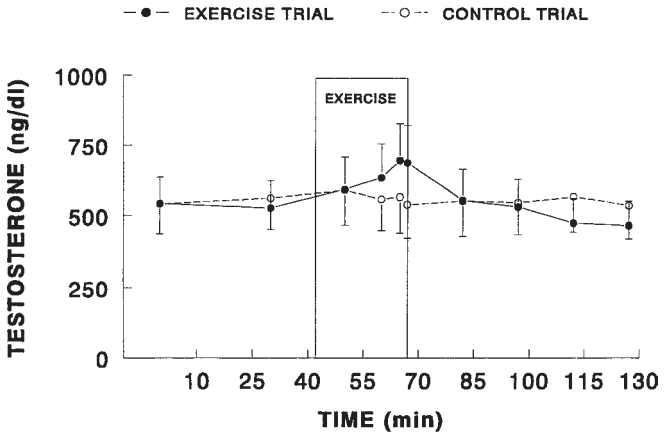

Fig. 2. Serum testosterone concentrations for the entire group ($n = 7$) before exercise, after each exercise intensity, and for 1 h of recovery. Data depict means ± SE for exercise (●) and control ( ) trials, $n = 7$. There was a significant ($p < 0.01$) time effect and a significant ($p < 0.01$) time ↔ trial interaction.

ing (a) stimulation by estrogens, (b) inhibition by androgens, and (c) fat-depot differences in leptin expression *(16,17)*.

It is known that testosterone concentrations rise in response to exercise only at higher exercise intensities *(18)* and that training may increase those responses *(19)*. We have recently reported glucoregulatory responses to exercise in a group of well-trained males *(20)*. We have extended these studies to determine the leptin responses to a progressive, intermittent exercise protocol and ascertained whether any relationship exists between testosterone and serum leptin. Most previous studies have examined leptin responses to low or moderately high exercise intensities *(9)*. A second objective was to determine whether leptin responses using an intermittent moderate and high-intensity exercise protocol were different than responses reported in previous experiments.

Results

Plasma volume was not reduced more than 9.9% between any two time points during exercise and only 2.2% during recovery. Plasma lactate levels (Fig. 1) rose significantly ($p < 0.01$) during exercise demonstrating the rigorousness of the protocol.

Testosterone: There was a significant time effect ($p < 0.01$) and time ↔ trial interaction ($p < 0.01$) for testosterone. Thus, the exercise trial produced significantly different testosterone responses compared with the control trial. Testosterone concentrations (Fig. 2) rose from 526 ± 97.54 ng/dL, prior to exercise, to peak at 695 ± 132.12 ng/dL after 5 min at 90% $VO_{2\,max}$ and remained elevated after 100% $VO_{2\,max}$. Individual testosterone data revealed testosterone increases in all subjects during the exercise period. A correlation matrix demonstrated that there was no relationship between leptin and testosterone during or following exercise (data not shown).

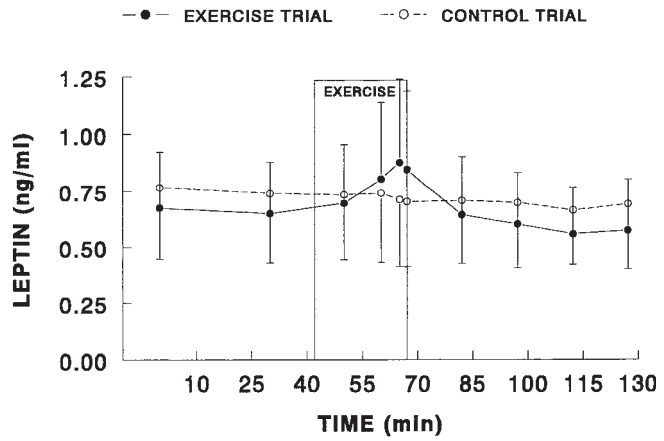

Fig. 3. Serum leptin concentrations for the entire group ($n = 7$) before exercise, after each exercise intensity, and for 1 h of recovery. Data depict means ± SE for exercise (●) and control ( ) trials. There was a significant ($p < 0.01$) time effect and a significant ($p < 0.01$) time ↔ trial interaction.

Leptin: There was a significant time effect ($p < 0.01$) and time ↔ trial interaction ($p < 0.01$) for leptin. Thus, the exercise trial produced significantly different leptin concentrations compared with the control trial. Leptin concentrations for the entire group (Fig. 3) rose from 0.67 ± 0.25 ng/mL at rest to peak at 0.87 ± 0.36 ng/mL after 5 min at 90% of $VO_{2\,max}$ then declined to resting values during recovery. Individual leptin data revealed different responses among subjects with some showing an increase ($n = 3$) and others ($n = 4$) showing no response during the exercise trial. We calculated the mean ± SE concentrations for the three responders and four nonresponders (Fig. 4). Data clearly show different resting leptin concentrations between the two groups with peak values of 1.7 ± 0.55 ng/mL for responders and no change for nonresponders. Using independent *t*-tests we found a significantly higher ($p < 0.05$)

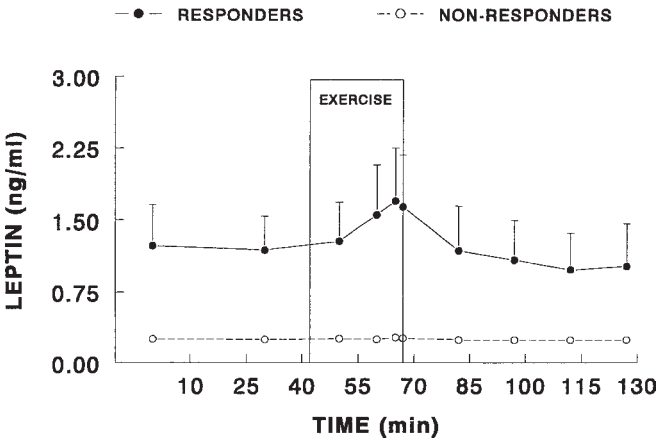

Fig. 4. Serum leptin concentrations for leptin responders (●), $n = 3$, and leptin nonresponders ( ), $n = 4$, before exercise, after each exercise intensity, and for 1 h of recovery. The control trial is not shown for clarity. Data depict means ± SE.

Table 1
Endocrine and Descriptive Data
for Leptin Responders ($n = 3$) and Nonresponders ($n = 4$)

	Nonresponders	Responders
Age (y)	28.3 (3.0)	29.3 (3.4)
Weight (kg)	73.3 (5.7)	73.5 (1.3)
Body fat (%)	9.6 (2.0)[a]	13.06 (1.3)
$VO_{2\ max}$ (mL/kg/min)	61.8 (2.7)	59.9 (2.4)
Resting glucose (mg/dL)	88.3 (3.8)	83.3 (8.7)
Resting insulin (μIU/mL)	4.8 (0.9)	4.6 (1.1)
Resting leptin (ng/mL)	1.19 (0.35)[a]	0.25 (0.01)
Resting testosterone (ng/mL)	670.7 (89.8)	564.5 (36.3)
Resting cortisol (μg/dL)	19.4 (3.3)	16.1 (3.2)
Percent of plasma volume shift after run at 75% $VO_{2\ max}$	−2.9 (0.5)	−4.62 (3.0)
Percent of plasma volume shift after run at 90% $VO_{2\ max}$	−2.1 (1.3)	−8.79 (3.3)

[a]Significantly different ($p < 0.05$) than responders. Values are presented as means ± SE.

leptin concentration at all 10 time points for the responders versus the nonresponders. There was no difference ($p > 0.05$) in testosterone responses between these two subgroups at any exercise or recovery time point. We further examined age, weight, body fat, $VO_{2\ max}$, glucose, insulin, and cortisol concentrations in the responders and nonresponders to determine whether these factors could explain the different leptin levels (Table 1). Among these factors the only one (other than resting leptin levels) that was significantly different ($p < 0.05$) between responders and nonresponders, was percentage body fat, although BMI and percentage body fat in both groups was in the normal range.

Discussion

Previous investigations have demonstrated that androgens have a suppressive effect on leptin *(17,21)*. We have demonstrated that a progressive intermittent, intense exercise protocol elicits increases in leptin and testosterone concentrations, with both quickly returning to baseline values during recovery. Individual data revealed similar testosterone responses for all subjects, whereas leptin increased during exercise in some subjects (responders), but did not change in others (nonresponders). There was no relationship between testosterone and leptin concentrations during exercise or recovery. These data suggest testosterone does not acutely affect leptin levels during exercise or 1 h of recovery and that there may be individual differences in leptin responses to intense exercise.

Previous studies have shown that testosterone levels increase from resistance exercise *(22,23)* and running exercise *(24,25)* of sufficient volume and that exercise intensity increases this response *(26)*. The testosterone increases in the present study are likely due to the intensity of the exercise protocol. We *(18)* and others *(27)* have postulated possible mechanisms for the increases in testosterone, including hemoconcentration, reduced metabolic clearance, and increased lactate concentrations.

Previous studies investigating effects of exercise on leptin have mostly reported no change or a slight reduction in leptin concentrations *(9)*. There are a few investigations, however, that have documented leptin increases from running *(11,28)*; however, leptin increases were attributed to plasma volume shifts and thus hemoconcentration. Therefore, the findings of the present study are novel in this regard. In the present study, some individuals showed dramatic leptin increases whereas others did not, suggesting that there are leptin responders and nonresponders. The observed increases in leptin in these individuals were in response to a rigorous progressive bout of intermittent running exercise, which was different from protocols used in previous studies.

Although plasma volume shifts appeared slightly greater in the responders after running at 75% and 90% of $VO_{2\ max}$ (Table 1), these differences (approx 1.5–6.0% greater plasma volume shift for responders) are much less than the leptin differences (approx 300% greater leptin for responders) and thus cannot account for the different leptin responses. Insulin and glucose concentrations in response to exercise were not different between responders and nonresponders and appear not to explain the variation. Moreover, resting cortisol, which has been shown to promote leptin production *(9)*, was not significantly different between responders and nonresponders.

Percentage body fat was significantly greater in the responders compared with the nonresponders and may have played a role in the variable leptin responses. Moreover, independent *t*-tests revealed significantly higher leptin concentrations at all 10 time points for the responders versus the

nonresponders. If a greater amount of fat tissue for leptin expression is present, it could contribute to an increase in the detectable plasma peptide pool. Another interesting difference was found regarding resting leptin levels in these otherwise comparable individuals who were all well trained and had low body fat. Those individuals with higher baseline leptin levels were those that showed the dramatic increases in leptin during the exercise trial. This is not unlike other physiological situations in which baseline leptin concentrations frequently predict the changes that occur following some intervention, be it exercise or weight loss or some physiological state that may alter serum leptin concentrations *(29,30)*. Clearly, further studies are needed to determine why one group of individuals so closely matched to another group is markedly different in baseline leptin concentrations. Future studies with a larger number of subjects will be required to answer this question.

In conclusion, we have demonstrated that testosterone levels do not acutely affect leptin responses to exercise or during one hour of recovery. Data show that leptin increases can occur even in the presence of elevated testosterone. Moreover, there may be different leptin responses to intense exercise in well-trained runners. Data suggest for individuals with elevated serum leptin levels at baseline that an intense bout of short-term interval exercise can increase serum leptin levels. For individuals with lower baseline concentrations, there will be no change in serum leptin levels with the same exercise. Future studies are needed to elucidate the cause of these observations and determine whether degree of adiposity is a primary factor affecting different leptin responses to exercise.

Materials and Methods

Subjects

Seven male subjects were recruited from the university community and provided written consent for participation in the study. A description of the subjects and exercise protocol that was conducted is described in our earlier report *(20)*. In brief, the subjects' mean (±SEM) age, weight, height, percentage fat, and $VO_{2\ max}$ were 28.71 ± 2.91 y, 73.39 ± 4.14 kg, 179.80 ± 2.53 cm, 11.08 ± 1.01%, and 61.01 ± 2.37 mL/kg/min, respectively. Subjects that participated had no history of cardiovascular or metabolic diseases, were between the ages of 18 and 39 yr, were following a normal dietary regimen, and were not taking any medications. All had previous running experience and a well-trained aerobic fitness level ($VO_{2\ max}$ > 52.0 mL/kg/min). The study was approved by the Southeastern Louisiana University Institutional Review Board.

Preliminary Trial

Subjects completed a preliminary trial to determine standard fitness measures that included body composition (skinfold measures) and cardiorespiratory fitness ($VO_{2\ max}$). Subjects completed a graded exercise test to exhaustion at a constant grade. The treadmill speeds (at a 4% treadmill grade) that corresponded with 60, 75, 90, and 100% $VO_{2\ max}$ were calculated from a regression equation generated from the relationship between VO_2 and treadmill speed in the preliminary trial.

Exercise and Control Trials

Subjects were refrained from exercise and alcohol 24 h before testing and reported for the exercise trial at 07:45 following an overnight fast. An intravenous catheter (Travenol, 22 gauge, 32 mm) was inserted into an antecubital vein and a normal saline lock was attached. At 08:30, 40 min prior to exercise (–40) and at 09:00, 10 min prior to exercise (–10), resting blood samples were collected from the catheter. Subjects then completed an intermittent treadmill exercise protocol at four speeds predicted to elicit a specific percentage of $VO_{2\ max}$: 60% $VO_{2\ max}$ for 10 min, 75% $VO_{2\ max}$ for 10 min, 90% $VO_{2\ max}$ for 5 min, and 100% $VO_{2\ max}$ for 2 min. After each workload was completed at the prescribed intensity and duration, treadmill speed was reduced to a walking speed (for 3.5–4 min) to allow a blood sample to be collected. Gas samples were collected continuously and confirmed that the actual VO_2 corresponded with the predicted VO_2 for each workload.

In addition to blood samples collected from the intravenous catheter after each workload (60, 75, 90, 100% $VO_{2\ max}$), samples were also collected every 15 min during a 1-h recovery (R15, R30, R45, and R60). Sera from blood samples were stored at –20°C until assayed. One month later, subjects reported to the lab at 07:45 following an overnight fast for a control trial. Blood samples were drawn (in the seated position) at the identical times of the exercise trial, but exercise was excluded in this trial.

Analyses

Hemoconcentration was calculated *(31)* from hematocrit (microhematocrit method) and hemoglobin levels (Sigma Chemical, St. Louis, MO). Plasma lactate was determined in duplicate using an enzymatic, colorimetric method (cat. no. 735-10, Sigma Chemical). Plasma glucose was determined in duplicate using an enzymatic (hexokinase), colorimetric method (cat. no. 115-A, Sigma Chemical). Serum samples were assayed in duplicate for leptin using a sensitive (0.5 ng/mL) and highly specific IRMA assay (Diagnostic Systems Laboratories, Webster, TX). Serum samples were assayed for testosterone, insulin, and cortisol by a chemiluminescent enzymatic immunoassay (Immulite, Diagnostic Products Corporation, Los Angeles, CA). The interassay coefficients of variation for leptin, testosterone, insulin, and cortisol were 8.8%, 7.6%, 10.6%, and 5.6%, respectively. Intraassay coefficients of variation for leptin, testosterone, insulin, and cortisol were < 5.0%. The sensitivity of the assay for testosterone, insulin, and cortisol was 20 ng/mL, 1 μg/dL, and 2 μIU/mL, respectively.

Statistics

Two different statistical methods were used. A 2 ↔ 10 (trial ↔ time point) repeated measures ANOVA was used to examine hormone changes over time and hormone concentrations between trials. Independent *t*-tests were used to compare leptin responders and nonresponders. To examine the relationship between leptin and testosterone concentrations, we used Pearson correlation coefficients. Data are expressed as means ± SE. All comparisons were considered statistically significant at $p < 0.05$.

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