Acute exercise effect on postabsorptive serum leptin

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Fisher, Jonathan S., Rachael E. Van Pelt, Oren Zinder, Michael Landt, and Wendy M. Kohrt. Acute exercise effect on postabsorptive serum leptin. J Appl Physiol 91: 680–686, 2001.—We postulated that high circulating cortisol levels during intense exercise would lead to increased serum leptin concentrations. Young, lean men ate a small meal and then exercised on a cycle ergometer for 41 min or rested on a control day. Serum leptin concentration was 10% greater during exercise than in the control condition (P < 0.05). Directly after exercise, serum leptin dropped to ~10% less than the control level (P < 0.05) but had recovered to the nonexercised level after ~2 h of recovery. Rapid exercise effects on circulating leptin were related to changes in hemoconcentration rather than changes in leptin mass. When serum leptin was normalized to serum protein, leptin increased by 10% in the exercise condition compared with control by the end of recovery (P < 0.05). Although exercise increased serum cortisol concentration threefold, there was no relation between differences in cortisol and exercise vs. control differences in normalized leptin. The increased leptin mass after exercise may have been related to greater plasma glucose concentration during recovery after exercise compared with the control condition.

LEPTIN, a hormone secreted by adipocytes, may contribute to long-term control of energy balance and body composition by interaction with receptors in the hypothalamus (31). For example, mice and children who cannot express leptin lack feedback control of their energy intake and are massively obese (22). Circulating leptin level in humans follows a diurnal pattern, with zenith and nadir at about midnight and shortly after the morning breakfast, respectively (30, 32). Despite early reports that feeding did not affect circulating leptin concentrations (3), further research has now shown a positive relation between food intake or hyperinsulinemia on blood leptin concentration (28, 30). Additionally, there may be an interactive effect of cortisol and insulin on blood leptin levels. For example, Laferrière et al. (17) studied the effects of normal vs. elevated glucocorticoid levels (via placebo or dexamethasone) on blood leptin under fed and fasting conditions.

Leptin concentrations decreased as is typical during fasting (16, 28) by ~40% over the 9-h period in both placebo and dexamethasone conditions. Feeding alone prevented the fasting-related decline in circulating leptin, as has been well demonstrated by others (28, 30). However, dexamethasone combined with meals increased plasma leptin by twofold over feeding alone. Thus it appears that insulin and/or carbohydrate flux into adipocytes is responsible for regulation of leptin after meals, and glucocorticoids may act synergistically with insulin to increase circulating leptin.

Although several investigators have not found an acute effect of exercise on circulating leptin (6, 9, 16, 26), others have reported that moderate-intensity exercise was associated with a decline in circulating leptin (5, 6, 15, 32). Among the important variables that may have contributed to the heterogeneity in results were differences in accounting for hemoconcentration and control of fed and fasting states. Because of the purported effects of glucocorticoids on leptin, we hypothesized that high-intensity exercise would lead to an increase, rather than a decrease, in blood leptin levels. We designed our experiment to avoid the countervailing effect of fasting on leptin levels that might mask a potential exercise effect. We postulated that, in fed subjects, the large increase in circulating cortisol provoked by high-intensity exercise would stimulate an increase in serum leptin concentrations. This hypothesis was tested in a group of sedentary men who were similar in terms of adiposity and leptin levels, and measures were made to adjust for the changes in hemoconcentration that occur with exercise.

MATERIALS AND METHODS

Experimental subjects. Young, lean, sedentary, nonsmoking male subjects (n = 8; for subject characteristics, see Table 1) were recruited. All subjects signed informed consent forms. All procedures were approved by the Washington University Institutional Review Board.

Descriptive data. Each subject was scanned by dual-energy X-ray absorptiometry (QDR-1000w; Hologic; enhanced whole body analysis software, version 5.4) for determination of body composition. Maximal aerobic power (V̇O2 max) was

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>29.2±1.7</td>
</tr>
<tr>
<td>Mass, kg</td>
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<tr>
<td>Height, cm</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.9±0.4</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>8.1±1.5</td>
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<tr>
<td>Lean mass, kg</td>
<td>61.3±2.1</td>
</tr>
<tr>
<td>Fat content, %</td>
<td>11.5±1.9</td>
</tr>
<tr>
<td>VO₂max, l/min</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>VO₂max, ml·kg⁻¹·min⁻¹</td>
<td>43.6±2.4</td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>181±5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 men. BMI, body mass index; VO₂max, maximal aerobic power; HR, heart rate.

measured by cycle ergometry (10) by using a Max-1 online CO₂ and O₂ monitoring system (Physio-Dyne Instrument, Quogue, NY).

Test sessions. On two separate occasions, 1–2 wk apart, each subject reported to the laboratory after an overnight fast at 8:00 AM. A catheter was inserted into an antecubital vein and perfused with 0.9% saline to maintain patency. The subject then consumed a bagel (~53 g carbohydrate, ~11 g protein, ~2 g fat) and 355 ml of orange juice (37 g carbohydrate) within 15 min (total energy intake was ~425 kcal). One hour after the subject began consumption of the meal, exercise commenced. For the control trial, the overnight fast and the morning meal were replicated, but the subject did not exercise.

Exercise. Subjects began cycling with 5 min of warm-up at 50% of the power output equivalent to the power output at VO₂max. Subjects then cycled at the power output equivalent to 85% VO₂max for 5 min (i.e., one intense exercise period) followed by a 3-min recovery at 50% of VO₂max. Subjects completed four intense periods followed by recovery cycling. The final period of recovery lasted 7 min, for a total of 41 min of exercise. All subjects were able to complete the first hard exercise period at 85% of VO₂max. Thereafter, work rates were adjusted downward when leg fatigue limited performance. The average work rates for exercise and recovery stages were 76 ± 2 and 43 ± 2% of VO₂max, respectively. Total energy expenditure during the exercise period was estimated to be 475 ± 35 kcal (1), approximately isocaloric with the energy content of the preexercise meal.

Blood sampling. Blood samples were taken before the meal (~60 min), before exercise (0 min), after each hard cycling work rate (10, 18, 26, and 34 min), after exercise (41 min), and during recovery (60, 90, 120, and 240 min). Blood samples were taken during the control trial at the same time points. An aliquot of plasma was placed on ice for immediate determination of plasma glucose. Additional serum and plasma samples were stored at −80°C until analysis of other metabolites and hormones.

Serum leptin (19) and insulin (23) were determined by radioimmunoassay. Serum cortisol was determined with an Abbot Laboratories Tdx analyzer, using reagents provided by the manufacturer. Plasma glucose was measured by the glucose oxidase method (Beckmann Glucose Analyzer 2). Serum free fatty acids (FFA) were determined with an assay kit (Wako Chemicals, Dallas, TX). Plasma epinephrine and norepinephrine were determined by radioenzymatic assay (29). Serum protein concentration was assayed with the biuret method (4).

Serum leptin concentration was expressed as a percentage of the level at time 0. To account for exercise-related changes in hemoconcentration, serum leptin concentrations were also normalized to serum protein content. We made the assumption that rapid changes in blood volume would be mirrored by changes in serum protein concentration and that rapid changes in serum leptin should parallel changes in serum protein.

For statistical comparisons of hormone and metabolite concentrations between control and exercise trials, data were averaged over three periods: exercise (10–41 min), early recovery from exercise (60–120 min), and late recovery from exercise (180 and 240 min). Data were analyzed by paired t-tests (α = 0.05).

Relations between leptin and the following measures were determined by regression analysis: 1) the ratio of final (240 min) normalized leptin in the exercise condition to that in the control condition, and 2) the ratio of the metabolite or hormone area under the curve (AUC) for the exercise condition to that in the control condition. Hierarchical regression analysis was determined by entering AUC factors in two sets: 1) glucose, insulin, and glucose × insulin, and 2) cortisol and insulin × cortisol. Significance testing for the sets (2) was determined with the level for significance set at P < 0.05.

RESULTS

Serum leptin concentration (uncorrected for hemoconcentration) increased by ~10% (P < 0.05) compared with control immediately after the start of exercise and dropped to ~10% lower (P < 0.05) than control directly after exercise (Fig. 1A). Serum leptin then recovered to control level by 240 min. When serum leptins were normalized to serum protein content, it was apparent that the rapid, exercise-related changes in serum leptin were secondary to changes in blood volume and not to changes in leptin mass (Fig. 1B). After normalization to serum protein concentrations, there were no differences between exercise and control leptin concentrations during or immediately after exercise. However, from 90 min onward, there was a linear increase in normalized leptin in the exercise condition, such that it was 10% higher (P < 0.05) in the exercise than in the control condition by the end of the trial. The higher normalized leptin toward the end of the trial in the exercise compared with the control condition does not appear to be a random occurrence, because normalized leptin increased linearly in the exercise condition (r = 0.4, P < 0.05), whereas there was no relation between time and normalized leptin in the control condition (r = 0.006, P > 0.97).

Serum cortisol rose threefold during exercise and remained elevated compared with control (P < 0.05) for the duration of the trial (Fig. 2A). Plasma epinephrine and norepinephrine (Fig. 2, B and C) were elevated during exercise (P < 0.05) but dropped quickly after exercise and were not different during recovery from exercise (Fig. 2B).

Serum insulin (Fig. 3A) increased after the meal but was suppressed during exercise compared with control (P < 0.05). After exercise, insulin rebounded to control level. This rebound was concomitant with a postexercise rise in plasma glucose (Fig. 3B) compared with control (P < 0.05). Serum FFA were not different between control and exercise conditions until there

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was a small rise during late recovery from exercise ($P < 0.05$, Fig. 4).

Relations between final, normalized leptin and AUC measures are shown in Fig. 5. Relations were not statistically significant. However, when entered into hierarchical regression analysis as sets, nutritive factors (glucose, insulin, and glucose × insulin) explained 86% of the variance in final leptin concentrations ($P < 0.05$; Table 2). Inclusion of hormonal factors (cortisol, cortisol × insulin) in the regression model increased the amount of variance explained to 98%, a statistically significant ($P < 0.05$) increment of 12% over the variance explained by nutritive factors alone.

DISCUSSION

Previously, other investigators have studied the effects of continuous, moderate-intensity exercise on circulating leptin concentrations (3, 5, 6, 9, 15, 26). They have found either no exercise-related change in circulating leptin (3, 6, 9, 26) or an exercise-related decrease in blood leptin (5, 15, 18). To our knowledge, ours is the first study to examine the effects of intense, intermittent exercise on circulating leptin levels during exercise and during recovery. We used a small meal to halt the well-established fasting-related decline in circulating leptin (17, 28, 30) and hypothesized that an increase in blood cortisol induced by high-intensity exercise would lead to an increase in circulating leptin. This hypothesis was based on the finding by Laferrière and colleagues (17) of a synergistic effect of feeding and cortisol on serum leptin concentration.
We found an increase in serum leptin at the start of exercise and a decrease at the end that were not apparent after leptin concentration was normalized to serum protein concentration. Therefore, it appears that acute exercise-related changes in leptin are secondary to fluctuations in blood volume. In light of these findings, all subsequent analyses of the leptin data took into account changes in hemoconcentration.

Serum leptin recovered to control level by 180 min (139 min after the end of exercise). When normalized to serum protein, there was an increase in serum leptin.

Fig. 3. Serum insulin (A) and plasma glucose (B) changes during and after exercise. *Difference between exercise and control means for the time period denoted by the associated line, $P < 0.05$.

Fig. 4. No increase in serum free fatty acids during exercise. *Difference between exercise and control means for the time period denoted by the associated line, $P < 0.05$.

Fig. 5. Relations between leptin and glucose (A), insulin (B), cortisol (C), and free fatty acids (FFA; D). Correlations are between leptin and hormone or metabolite areas under the curve (AUC). y-Axis: ratio of final (240-min time point) normalized exercise leptin to final normalized control leptin, expressed as a percent. x-Axis: ratio of hormone or metabolite AUC for the exercise trial vs. the AUC for the control trial, expressed as a percent. None of the correlations was statistically significant.
after exercise compared with the control condition. Our finding of an increase in circulating leptin after high-intensity exercise is in contrast to the 30% decline in leptin observed by Duclos et al. (5) in fed subjects 2 h after moderate-intensity exercise. A recent study found that circulating leptin decreased two- to threefold more after 3 h of moderate cycling exercise in fasted subjects than in a fasted control group that rested (15). However, fed subjects in the same study who completed a 42-km run did not have a decrease in blood leptin during the run. Cortisol levels were not elevated during 3 h of cycling, but they doubled during the marathon run. The investigators suggested that elevated cortisol during the long run may have prevented a decline in leptin.

Most studies have found no acute effect of exercise on circulating leptin. Hickey et al. (9) found no change in blood leptin, even after correction for hemococoncentration, during a 2-h run by fasted, well-trained young men. Similarly, circulating leptins were not altered by 10–12 min of cycling followed by a VO2 max test (3). Racette et al. (26) observed no exercise effect on arterial plasma leptin, venous plasma leptin, or leptin production when subjects cycled for 60 min at 50% of VO2 max. Furthermore, plasma leptin was not acutely decreased by running at 70% VO2 max, long enough to burn 800 or 1,500 kcal (6). Fasting leptin concentration was decreased by ~20% 2 days after the running exercise for both levels of energy expenditure (6). We did not measure plasma leptin levels 48 h after the exercise in the present study and cannot rule out the possibility that the leptin levels of our subjects would have displayed a delayed decline if the energy expenditure of exercise had not been compensated by increased caloric intake (11).

The evolution of opinion on the acute effects of insulin on circulating leptin may aid interpretation of the above exercise studies. Early investigations (3) found no effect of meals on circulating leptin. However, once the appropriate fasting controls were added (17, 28), it became clear that insulin and meals had a profound, acute effect on circulating leptin: low physiological levels of insulin prevented the fasting-related decline in leptin, whereas higher insulin doses caused an increase in circulating leptin. Keeping in mind that blood leptin normally declines during fasting (17, 28), a finding that exercise maintains circulating leptin concentrations implies that exercise opposes the normal, fasting-related leptin decrease. To our knowledge, only one study examining the effects of exercise on leptin has included both a control (resting only) and an exercise trial performed by the same subjects. In that study (16), serum leptin declined by ~3 ng/ml in fasted subjects over 30 min of cycling exercise at 80% of VO2 max followed by 80 min of rest; a similar decline occurred during the rest-only trial.

What exercise-related factor may have prevented the fasting-related decline in circulating leptin in some studies (3, 9, 15, 26) and led to an increase in leptin normalized to serum protein in fed subjects in the present study? We hypothesized that exercise-induced increases in cortisol would lead to a rise in circulating leptin because administration of glucocorticoids has been found to increase circulating leptin in humans (17, 20). We found only a weak relation between cortisol and differences in protein-normalized leptin, but nutritive factors (glucose, insulin, and the glucose × insulin interaction) were highly related to differences in leptin. Insulin has previously been shown to be a secretagogue for leptin in humans (28). There is also evidence that increased glucose flux in adipocytes may have an independent effect on leptin production. For example, one study reported that blocking glucose uptake or metabolism, but not insulin signaling pathways, prevented insulin-stimulated leptin secretion in cultured adipocytes (24). It has been suggested that a glucose-derived component of the hexosamine biosynthetic pathway (e.g., the principle product UDP-N-acetylglucosamine) mediates glucose effects on leptin expression (33). For example, leptin protein and mRNA have been shown to increase in adipocytes and even skeletal muscle (33) after hyperinsulinemic perfusion of rats with UDP-N-acetylglucosamine precursors, and overexpression of glutamine-fructose-6-phosphate amidotransferase, the gateway enzyme for the hexosamine biosynthetic pathway, also increased leptin mRNA in adipocytes of mice (21).

Hilton and Loucks (11) examined sedentary and exercising women for whom daily energy expenditure was matched with daily energy intake (~1,400 kcal/day expended by walking was replaced by an equivalent dietary intake in the exercising women), and they concluded that exercise stress without decreased energy availability did not affect circulating leptin levels. When daily energy expenditure exceeded energy intake (accomplished by caloric restriction in the sedentary women or exercise without dietary compensation for energy expenditure in trained women), circulating leptin levels plunged ~70% in the sedentary women but only ~50% in trained women. Hilton and Loucks ascribed the different effects to preferential oxidation of fat and thus carbohydrate-sparing in trained women, and they suggested that the higher carbohydrate availability in trained women blunted the effects of negative energy balance on circulating leptin levels. However, in the present study, absolute carbohydrate

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### Table 2. Regression analysis of factors associated with leptin

<table>
<thead>
<tr>
<th>Factor</th>
<th>$R^2$</th>
<th>Increment in $R^2$</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>$R^2 = 0.86$</td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose × insulin</td>
<td>$R^2 = 0.98$</td>
<td>IR$^2 = 0.12$</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Hormonal together with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nutritive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol × insulin</td>
<td>$R^2 = 0.98$</td>
<td>IR$^2 = 0.12$</td>
<td>P &lt; 0.05</td>
</tr>
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$R^2$, fraction of leptin variance shared by the factors included in the regression analysis. IR$^2$, increment in $R^2$ (or additional shared fraction of variance) after inclusion of the second set of factors in the regression analysis.
availability would no doubt have been reduced in the exercise condition compared with the control condition. On the other hand, the higher glucose and insulin concentrations after exercise could have increased glucose flux in leptin-producing cells. This could be especially important in skeletal muscle, which characteristics increases glucose uptake (independently of and additive to the effects of insulin) after contractile activity (12) and has been reported to express leptin in response to glucose uptake (33).

Serum FFA were not elevated during high-intensity exercise in this study, perhaps due to a lingering inhibitory effect of postprandial hyperinsulinemia on lipolysis (14). Moderate-intensity exercise (~60% of VO2 max) in fasted subjects characteristically increases whole body mobilization of FFA after 30–60 min of exercise (13, 27). However, carbohydrate feeding before exercise was reported to suppress exercise-related stimulation of lipolysis (13, 14). Moreover, fatty acid mobilization may not have time to increase during high-intensity exercise (~85% of VO2 max) that cannot be maintained for prolonged periods (27). Regulation of lipolysis by exercise is potentially relevant to regulation of circulating leptin levels, because lipolysis and leptin production appear to be inversely controlled. For example, Duclos et al. (5) reported that exercise-related increases in FFA and glycerol concentrations after exercise could have increased glucose uptake (independently of and additive to the effects of insulin) after contractile activity (12) and has been reported to express leptin in response to glucose uptake (33).

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The relative concentrations of the hormones and metabolites that seem to upregulate (cortisol, insulin, glucose) or downregulate (epinephrine) plasma leptin levels change rapidly during and after exercise, depending on exercise intensity, exercise duration, and the fitness level of the subject. How these hormones may interact to prevent the decline in leptin under some conditions and not others remains to be shown.

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