PRELIMINARY REPORT

Prolonged Exercise Decreases Serum Leptin Concentrations

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Serum leptin and free fatty acid concentrations were determined in two groups of subjects undergoing strenuous exercise: 12 men who fasted overnight and then pedaled a stationary ergometer for 2 hours, and 14 nonfasting ultramarathon runners. Blood samples were collected before exercise, immediately after cessation of exercise, and 6 to 24 hours after the end of the exercise period. Two hours of strenuous pedaling following an overnight fast significantly reduced mean leptin levels by 8.3%; free fatty acids were highly increased and correlated well with the decrease in serum leptin ($r = .737$, $P = .01$). After 6 hours of rest and refeeding, leptin concentrations recovered to preexercise levels and free fatty acid concentrations were decreased to less than preexercise levels. A similar decrease in serum leptin levels (12.3%) occurred in subjects who fasted overnight and then for a period corresponding to the cycle exercise period. The prolonged exercise of an ultramarathon significantly reduced leptin concentrations by 32% in comparison to prerace levels; free fatty acid concentrations were highly increased, but did not correlate with the change in serum leptin concentrations ($r = .366$, $P = .20$). Leptin and free fatty acid concentrations all trended toward prerace levels in blood samples collected 18 to 24 hours after cessation of racing. The results suggest that the negative energy balance of exercise can reduce serum leptin concentrations, but that the significant decrease occurs only at extremes of severity/duration of the exercise-induced negative balance. The possible physiological role of reduced leptin concentrations in response to energy balance and the role of free fatty acids in mediating the response are discussed.

LONG KNOWN TO HAVE an autosomally inherited lesion that resulted in profound obesity and non–insulin-dependent diabetes, the ob/ob mouse was recently shown to have a defect in a 167–amino acid polypeptide produced by fat cells.[1] The protein, termed leptin, is secreted in a 146–amino acid form. Several recent studies have demonstrated that human leptin concentrations in plasma increase with increasing adiposity.[2-4] Studies of mice given injections of leptin have suggested that leptin acts as a hormone to suppress appetite and increase metabolic rate.[5-7] Thus, leptin appears to be involved in the feedback regulation of body (fat) mass.

Recent reports have delineated some of the physiology of leptin.[8] Plasma leptin concentrations were similar to normal levels in individuals with non–insulin-dependent diabetes,[9] human immunodeficiency virus infection,[10] and anorexia nervosa[11] after taking into account the percent body fat of the subjects. Morbidly obese subjects with high plasma concentrations of leptin do not have genetically altered forms of the protein; this finding has been interpreted to suggest that obese humans are resistant to the body mass modulation attributed to leptin.[12-14] A 9-month exercise program reduced leptin concentrations in older women in parallel with reductions in body fat content.[15] A negative energy balance induced by a 7-day dietary restriction reduced plasma leptin concentrations much more than the proportional change in body fat content.[16] Leptin concentrations vary with a diurnal pattern, with higher concentrations at night.[17] Fasting beyond 12 hours disrupts the diurnal pattern and decreases circulating leptin concentrations.[18,19] There is one report concerning the effect of strenuous exercise on serum leptin concentrations: running 20 miles had no detectable effect on serum leptin levels,[20] despite an overnight fast that continued during the exercise period. The purpose of the present study was to determine if a negative energy balance due to physical exertion alters plasma leptin concentrations.

SUBJECTS AND METHODS

Protocol

The study population was composed of two groups. The first group consisted of 12 men (aged 30 ± 2 years; range, 19 to 45) who had fasted overnight and who rode a stationary cycle ergometer in four half-hour segments at approximately 75% peak oxygen uptake, separated by 4-minute rest intervals. Subjects completed the ride with five 1-minute sprints (100% peak oxygen uptake) separated by 3-minute rest periods. Participants were allowed to drink water, and were kept euhydrated by infusion of normal saline during exercise. To determine peak oxygen uptake, subjects cycled at 100, 150, and 200 w for 3 minutes per exercise intensity, followed by 50- or 25-w increments every minute until exhaustion.[21] During the experimental exercise period, every 30 minutes for 5 minutes, expired O_2 and CO_2 were analyzed continuously and averaged over 30-second intervals using an automated open-circuit system for determination of oxygen consumption.[21] At the end of the exercise, participants were given meals (80% carbohydrate, 7% fat, and 13% protein at 0, 2, and 4 hours) and encouraged to eat. A control group (n = 10), which fasted overnight and then for an additional period corresponding to the exercise period, contained four of the original 12 cycle subjects and an additional six men chosen to have an approximately similar adiposity and age as the experimental subjects.

The second group was 14 of 81 runners participating in the Hardrock 100 race, a 101-mile, high-altitude (2,700 to 4,500 m) event held annually in Silverton, CO. The age (mean ± SD) of the ultramarathon runners was 41 ± 13 years (range, 27 to 54); all were males. Participants consumed foodstuffs and drank fluids containing carbohydrates as needed throughout the race. Written informed consent was obtained from all subjects in accordance with protocols approved by the Human Studies Committee of Washington University. Blood specimens

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(yielding serum after centrifugation) were drawn from each participant immediately before the exercise (8:00 AM), at the end of the exercise or race (2:06, 2:08, 2:44, 4:46, 5:21, 6:26, 7:43 AM, 5:00, 7:00, 7:52, 8:40, 10:43, and 11:21 PM for the ultramarathon runners), and either 6 hours (first group) or 18 to 24 hours (second group) after cessation of the exercise.

Analyses

Leptin concentrations were measured with a commercial radioimmunoassay (Linco Research, St Louis, MO) that has been extensively evaluated.[4] The assay, with between-assay and within-assay coefficients of variation of 3.4% to 8.3%, uses a polyclonal antibody made in rabbits to human recombinant leptin. Calibrators and tracer are prepared from human recombinant leptin. Free fatty acids were quantified in serum with a commercial spectrophotometric assay (Wako Chemicals, Richmond, CA) either manually using a Spectronic 1001 spectrophotometer (2-hour exercise group; Spectronic Instruments, Rochester, NY) or by automated analysis on a Hitachi 717 chemistry analyzer (ultramarathon runners; Boehringer Mannheim, Indianapolis, IN). This assay had within assay coefficients of variation of 4% for the manual method and 1.5% for the automated method in the assay linearity range. Body composition was determined by underwater weighing.[22]

Calculations

To determine if there were significant changes in blood variables over time in the ultramarathoners, a one-way repeated-measures ANOVA was performed. To determine if there were significant changes in blood variables over time and/or significant differences between groups of subjects (fasting + 2.5-hour exercise *v* fasting alone), a two-way ANOVA was performed. The Tukey posthoc test was used to further analyze significant main effects. All results are expressed as the mean ± 1 SD.

RESULTS

Two-Hour Exercise

Serum Leptin Concentrations

Serum leptin concentrations in 12 fasting male subjects were 2.85 ± 1.50 μg/L (range, 1.37 to 6.90) before exercise. The subjects had a mean of 15.9% ± 6.7% body fat (range, 3.0% to 28.0%). Leptin concentrations decreased 8.3% compared with paired preexercise values, to 2.61 ± 1.46 μg/L, after 2 hours of strenuous cycling on a stationary ergometer (Fig 1). Serum leptin concentrations returned to near-preexercise levels (3.04 ± 1.59 μg/L) with 6 hours of rest and refeeding. The protocol was repeated with 10 fasting male subjects, omitting the cycling exercise, to determine the effect of the additional period of fasting after an overnight fast on serum leptin concentrations. Serum leptin concentrations decreased from 3.38 ± 1.47 to 2.96 ± 1.19 μg/L (12.3%). Evaluation of the combined data with two-way ANOVA and the Tukey post hoc test found no significant difference between exercise and control groups ($P = .40$), but there was a strongly significant difference between leptin concentrations at the start of the experimental period and its end ($P < .001$) for the combined groups. Thus, 2 hours of strenuous exercise was without discernible effect on circulating leptin concentrations beyond the decrease caused by extended (>12 hours) fasting.

Serum Free Fatty Acids

Free fatty acids before exercise (n = 11) were 520 ± 222 μmol/L (normal, 239 to 843 μmol/L), ranging from 281 to 909 μmol/L. Free fatty acids increased 187% to 1,495 ± 648 μmol/L after 2 hours of cycling. Both the absolute free fatty acid concentration and the increase during the cycling exercise correlated significantly with the change in leptin concentration during the exercise period ($r = .737$, $P = .01$ and $r = .809$, $P = .003$, respectively). Free fatty acid concentrations decreased with 6 hours of rest and refeeding to very low levels (63 ± 24 μmol/L) that were less than preexercise concentrations: the changes in leptin and free fatty acids during the recovery period were not correlated ($r = -.32$, $P = .33$). Free fatty acids measured in 10 subjects who fasted overnight and then fasted an additional period equivalent to the exercise period, without exercise, were similar (363 ± 145 *v* 292 ± 142 μmol/L at start and finish of additional fasting period, respectively). Neither the absolute fatty acid concentration at the end of the fast nor the change in free fatty acids during the recovery period correlated with the change in leptin during the recovery period ($r = .29$, $P = .41$ and $r = .30$, $P = .40$, respectively). Evaluation of data from the exercise and control groups with two-way ANOVA yielded a significant interaction between treatment group and time ($P = .008$), indicating that free fatty acid concentrations increased due to exercise rather than to fasting.

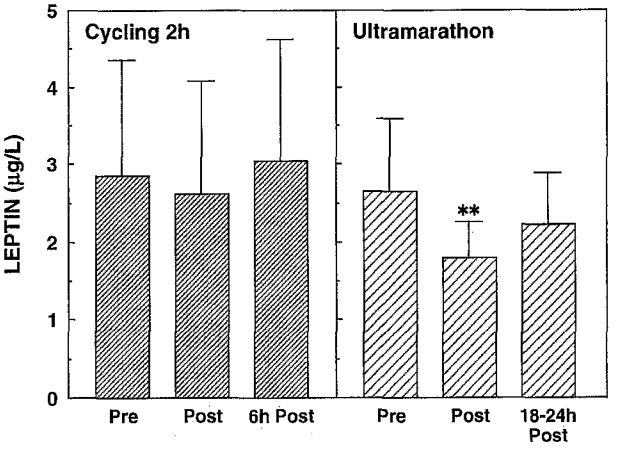

Fig 1. Effect of strenuous exercise on serum leptin concentrations in subjects cycling on an ergometer (n = 12) or in ultramarathon runners (n = 14). Mean ± SD are shown for concentrations in specimens collected immediately before exercise, immediately after completion of exercise, and 6 hours (cycling group) or 18 to 24 hours (ultramarathon group) after completion of exercise. **ANOVA, $P <$ .05 *v* either prerace or 18 to 24 hours postrace.

Oxygen Consumption/Respiratory Exchange Ratio

During the cycle ergometer exercise, the subjects (n = 12) consumed 2.57 ± 0.76 L/min oxygen at a respiratory exchange ratio of 0.92; peak oxygen consumption was 3.52 ± 1.05 L/min.

Sustained Exercise of Ultramarathon

Serum Leptin Concentrations

Serum leptin concentrations in 14 male participants (mean body mass index, 23.9 ± 2.0 kg/m^2) ranged from 1.3 to 4.4 μg/L, with a mean of 2.64 ± 0.94 μg/L in prerace specimens. Ten participants completed the entire 101-mile race; the remaining four participants ran 75, 59, 44, and 19 miles. There was no

clear difference in results for those who completed the race and those who did not, and results for all 14 runners are therefore presented. The mean time spent running was 35.8 ± 11.1 hours. Serum leptin concentrations decreased a mean of 32% to 1.80 ± 0.45 μg/L in comparison to paired prerace values (Fig 1). Leptin concentrations in specimens collected 18 to 24 hours after cessation of racing (2.21 ± 0.68 μg/L) trended toward prerace levels (a 16% decrease, compared with a 32% decrease immediately after cessation of racing). Analysis by one-way ANOVA found significant differences ($P < .05$) between prerace leptin concentrations and both postrace and 18- to 24-hour postrace results.

Serum Free Fatty Acid Concentrations

Free fatty acid concentrations (n = 14) were 320 ± 217 μmol/L (normal, 239 to 843 μmol/L), ranging from 130 to 898 μmol/L in prerace specimens. Postrace free fatty acid concentrations increased 183% to 908 ± 407 μmol/L (range, 261 to 1,796 μmol/L). Neither the absolute postrace value nor the net increase in free fatty acids in postrace specimens compared with prerace specimens correlated significantly with the change in serum leptin as a result of the race ($r = .366$, $P = .20$ and $r = .277$, $P = .34$, respectively). Free fatty acid concentrations 18 to 24 hours postrace were similar to prerace concentrations (270 ± 180 μmol/L). Analysis of the data by one-way ANOVA and Tukey posthoc test found statistically significant differences between prerace and immediately postrace free fatty acid levels ($P < .05$), but prerace and 18- to 24-hour postrace results were statistically similar ($P > .05$). Changes in free fatty acids during the postrace recovery period did not significantly correlate with paired changes in leptin concentrations ($r = .0775$, $P = .79$).

DISCUSSION

Our data show that leptin concentrations decrease in response to the negative energy balance incurred during prolonged strenuous exercise, but that a significant decrease occurs only at extremes of severity/duration of exercise-induced negative energy balance. Rest and refeeding after strenuous exercise caused serum leptin concentrations to trend toward preexercise levels, indicating that exercise (negative energy balance) caused a transient change in leptin secretion/elimination. This finding suggests that leptin might have a role in modulating the acute physiologic response to the negative energy balance of sustained exercise.

Two studies have shown that prolonged (>12 hours) fasting significantly reduces serum leptin concentrations.[18,19] Continuation of an overnight fast was responsible for the modest decrease in leptin concentrations seen in the 2-hour exercise experiment, demonstrating that a 2-hour period of exercise did not significantly alter the leptin response to the negative energy balance of fasting. The significant decrease in leptin after running an ultramarathon cannot be explained solely as an effect of fasting. Although the runners' routine meal schedule was disrupted by the race, all of the runners consumed glucose and other foodstuffs throughout the race, and very small amounts of glucose eliminate the fasting effect on serum leptin concentrations.[18,23] However, it is possible that the energy demands of the ultramarathon combined with decreased caloric intake acted synergistically to reduce leptin concentrations. The known diurnal variation of leptin concentrations[17] also does not explain the decrease in leptin observed in ultramarathon runners; leptin concentrations increase at night in response to the diurnal pattern, and this study observed a day-to-day decrease. Nine of 14 runners finished the race between 11:00 PM and 6:00 AM, which might increase serum leptin concentrations due to the diurnal effect. However, the effects of disruption of the meal schedule or sleep deprivation on diurnal variation, which was determined in normal individuals under normal sleep-wake patterns,[17] have not been established.

These results cannot be explained by potential changes in body fat content that might reduce production of leptin by fat in parallel with a decrease in the body fat that elucidates leptin. First, leptin concentrations returned, or largely returned, to preexercise levels with short periods of rest and refeeding, which did not allow sufficient time to restore the adiposity of the subjects. Second, the magnitude of the decrease in leptin concentrations with exercise was much greater than the possible loss in adipose tissue with exercise. Calculation of fat oxidation (from oxygen consumption data) in the subjects who rode an exercise bike for 2 hours suggests that these subjects metabolized a maximum of 30 g fat. Since the subjects possessed a mean of 11,300 g body fat, the estimated loss of body fat of 0.4% was much less than the transient decrease in leptin in these subjects (8.3%). A calculation of fat oxidation in the ultramarathon runners, assuming that 50% of the overall energy consumed came from fat, suggests that these runners could have metabolized a maximum of approximately 800 g fat. Although percent body fat was not measured in the ultramarathoners, it is not possible that this potential loss constitutes 32% of their average body fat, which would be required if the reductions of serum leptin and body fat content were of similar magnitude.

The exercise-induced negative energy balance experienced during regular training and racing alters body composition; regular exercise increases lean tissue mass and decreases body fat mass. The decrease in serum leptin concentrations induced in the subjects by prolonged exercise is consistent with the suggested relationship between leptin, appetite, and body composition; decreased leptin concentration may increase appetite in the postexercise period. Increased appetite helps subjects maintain body composition in the face of the negative energy balance, and aids recovery by increasing caloric and amino acid support for anabolism.

One means by which the negative energy balance of sustained fasting or exercise could alter circulating leptin concentrations would be to alter (decrease) leptin production. The well-documented decrease in serum leptin concentrations with prolonged fasting, as well as the decrease with prolonged exercise, suggests that a metabolic signal may exist under these conditions that acts to reduce leptin production; one candidate for that signal is free fatty acid concentrations in plasma. A previous study using an adipocyte cell line found that incubation of adipocytes in 25 to 50 μmol/L of a nonmetabolizable fatty acid for 24 hours reduced leptin mRNA levels.[24] It is likely that the exercising subjects in this study had sustained plasma concentrations of free fatty acids much higher than this level, since the mean serum concentrations at the end of both the cycling exercise and the ultramarathon were greater than 10 times this level. The lack of correlation of fatty acid levels with

decreases in leptin concentrations in the ultramarathon runners may reflect the influence of varied meals on free fatty acid levels in these nonfasting subjects. The strong correlation observed for free fatty acid levels with the change in leptin concentrations in 2-hour exercise subjects, who were fasting, is consistent with this rationale, but the lack of correlation in fasting subjects who fasted for the additional period equivalent to the exercise period (but without exercise) with the similar decrease in leptin concentrations suggests that the regulation of leptin and fatty acid responses to exercise has a common basis, rather than a cause-effect relationship. These results support the notion that fatty acid metabolism and leptin homeostasis are related, but do not provide support for the notion that free fatty acids directly modulate serum leptin concentrations, particularly in response to prolonged fasting.

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