Acute and chronic effects of exercise on leptin levels in humans

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The large number of studies on the genetic and nongenetic determinants of obesity (2, 4), relatively little is known about the genes likely involved in the regulation of body weight over an extended period of time.

A major breakthrough came with the cloning of the ob (obese) gene (29) and its receptor (27). The ob gene codes for a protein of 146 amino acids, known as leptin, secreted by the white adipose tissue. Soon after the discovery of the ob gene, three studies have shown that administration of leptin to the obese (ob/ob) mouse was associated with a reduction of adiposity, a decrease in serum glucose and insulin levels, and an increase in metabolic rate and locomotor activity (5, 10, 19). These studies suggest that leptin plays a central role in the regulation of food intake and energy balance in mice.

No mutations in the ob gene have been identified in humans so far, but ob gene expression and serum leptin levels were found to be highly correlated with percentage of body fat and to decline after weight loss (6, 17). Large variations in serum leptin concentrations have been noted for a given level of body fat (6), suggesting that other factors may be involved in the regulation of leptin levels.

Among these factors, insulin, corticosteroids, free fatty acids, and food intake have been implicated (7, 9, 18, 21, 23), but little is known about the role of exercise. Exercise represents the most variable fraction of energy expenditure in humans. Considering the role of leptin on energy expenditure in the obese mouse and the role of exercise in maintenance of diet-induced weight loss, exercise could be an important determinant of leptin levels in human. Very few studies have investigated the effects of exercise and endurance training on leptin levels. One study performed in animals has shown that acute exercise in rats was associated with a 30% reduction of the expression of the ob gene in the adipose tissue (30). In humans, only two studies have been reported. In one study, leptin concentrations were measured in 13 lean (average percent body fat of 9.7%) long-distance male runners before and after completion of a 20-mile treadmill run at 70% of maximal oxygen uptake (VO2max) (12). Pre- and postexercise leptin concentrations were 2.19 ± 0.32 and 2.14 ± 0.35 (SE) ng/ml, respectively. These results

Obesity is associated with several morbid conditions such as non-insulin-dependent diabetes mellitus, dyslipidemias, cardiovascular disease, and some forms of cancer and therefore represents a major health problem. Although excess caloric intake and/or reduced energy expenditure from physical activity may be responsible for the increased prevalence of obesity (16, 24), genetic factors appear to be important determinants of the susceptibility to become obese (2). Despite
suggest that, in trained individuals (average V\textsubscript{O}\textsubscript{2max} of 62.9 ± 2.2 ml·min\(^{-1}·kg\(^{-1}\)), acute exercise has no effects on circulating leptin levels (12). In another study, of sedentary postmenopausal women aged 60–72 yr, leptin levels were evaluated in response to exercise training consisting of a 2-mo flexibility exercise program followed by a 9-mo exercise program that included walking, jogging, and stair climbing (13). Significant reductions in serum leptin levels were observed after exercise training, but these were explained by changes in fat mass (13). The present study was undertaken to assess both the acute effects of exercise and chronic effects of endurance training on plasma leptin levels in sedentary adult men and women.

MATERIAL AND METHODS

Population. Subjects of the HERITAGE Family Study were used for the purpose of this study. The specific aims, design, and methodology of the study have been described in detail elsewhere (3). Briefly, Caucasian and black families were recruited, tested, exercise trained in the laboratory with a rigorously controlled standardized training program for 20 wk, and retested. All family members were sedentary, defined at baseline as having had no regular physical activity over the previous 6 mo. A total of 97 subjects (51 men and 46 women), ranging in age from 17 to 40 yr [24.5 ± 5.8 (SD) yr] were selected from the database for this study. They were selected according to the following criteria: only unrelated subjects from the offspring generation; only Caucasians; and only subjects for whom blood samples were available at all time points and with no hemolysis. Subjects of the offspring generation were selected to maximize the response to training, which tends to decrease as individuals get older.

Training protocol. Each subject was trained on a computer-controlled cycle ergometer three times a week for 20 wk by using the same standardized protocol. The intensity and duration of the training program were adjusted every 2 wk. The subjects worked at an intensity corresponding to 55% of V\textsubscript{O}\textsubscript{2max} for 30 min per session at the beginning, increasing progressively toward an intensity of 75% of V\textsubscript{O}\textsubscript{2max} for 50 min during the last 6 wk of the training protocol. Training intensities were adjusted individually by a computer system that recorded all training data and automatically adjusted the power output of the cycle ergometer to keep the heart rate response of the subject within 5 beats of the programmed heart rate at all times during all training sessions.

Concomitant variables. Body composition and fasting levels of glucose and insulin were measured before and after training. Fat mass, fat-free mass, and percent body fat were determined from body density measurements obtained by underwater weighing. Plasma glucose concentrations were determined enzymatically, whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation. Fasting insulin and the insulin-to-glucose ratio were used as indexes of insulin sensitivity.

Exercise tests. Three exercise tests, conducted on a stationary cycle ergometer (Ergo-Metrics 800S, Sensor Medics), were administered to the subjects before training and at the end of the 20-wk training program: a maximal exercise test, a submaximal exercise test, and a submaximal/maximal exercise test. The data of the present study were derived from the submaximal/maximal exercise test. During that test, subjects exercised for 10–12 min at an absolute power output of 50 W (50W) and progressed to a maximal level of exertion. For this test, a venous catheter was inserted in the left arm to obtain blood samples at rest before the test (Rest), at 50W, and immediately on completion of the test (Max). Subjects were asked to refrain from eating for at least 2 h before the test. This test was repeated after the endurance training program and was conducted at the same time of the day as the pretraining test. The blood samples were centrifuged after the test and stored at −80°C until determination of leptin levels. Plasma total proteins were also measured to account for the effects of hemocenteration during the test.

Plasma leptin determination. Leptin levels were measured by radioimmunoassay (Linco, St. Charles, MO). The assay detects human leptin with a sensitivity (lowest detectable level) of 0.5 ng/ml in plasma. The intra-assay coefficient of variation was 7%.

Statistical analyses. The acute and chronic effects of exercise on leptin levels were assessed separately in men and women by using a repeated-measures analysis of variance for one factor, the time effect. For acute effects, the time has three levels, i.e., Rest, 50W, and Max, whereas for chronic effects the time has two levels, i.e., pre- and posttraining. All the analyses were performed by using SAS software (version 6.08) for personal computer.

RESULTS

The effects of the endurance training program on body composition, fasting glucose and insulin, and V\textsubscript{O}\textsubscript{2max} are presented in Table 1 for men and women separately. The endurance training resulted in significant (P < 0.001) improvements of V\textsubscript{O}\textsubscript{2max}, which increased from 3.3 to 3.8 l/min in men (15.7%) and from 2.1 to 2.5 l/min in women (18.8%). The endurance training resulted in significant (P < 0.0001) decreases in fat mass and percent body fat in men but not in women. Significant increases in fat-free mass were also observed in both men and women. No significant changes in indicators of glucose and insulin metabolism were noted after exercise training.

The average plasma leptin levels measured at Rest before endurance training were 4.6 ± 4.4 ng/ml in men and 11.9 ± 8.5 ng/ml in women. As a group, the subjects of the present study were not obese, with average body fat mass, kg 17.1

| Table 1. Effects of a 20-wk endurance training program on body composition, fasting glucose and insulin, and V\textsubscript{O}\textsubscript{2max} |
|---|---|---|---|
| Variable | Men (n = 51) | Women (n = 46) |
| | Pretraining | Posttraining | Pretraining | Posttraining |
| BMI, kg/m² | 25.5 ± 5.0 | 25.4 ± 5.1 | 23.0 ± 3.5 | 23.1 ± 3.6 |
| Fat-free mass, kg | 64.1 ± 7.8 | 64.8 ± 7.8t | 46.1 ± 5.1 | 46.9 ± 4.9t |
| Fat mass, kg | 17.1 ± 11.1 | 16.2 ± 11.0t | 16.6 ± 7.6 | 16.4 ± 7.7 |
| Percent body fat | 21.0 ± 8.1 | 19.7 ± 8.1t | 25.5 ± 7.8 | 25.0 ± 7.8 |
| Insulin, pmol/l | 67.7 ± 39.4 | 65.1 ± 37.5 | 55.5 ± 22.5 | 57.1 ± 25.3 |
| Glucose, mmol/l | 4.9 ± 0.4 | 5.0 ± 0.4t | 4.7 ± 0.4 | 4.7 ± 0.3 |
| Insulin-to-glucose ratio | 13.6 ± 7.6 | 12.7 ± 6.9 | 11.7 ± 4.5 | 12.1 ± 5.2 |
| V\textsubscript{O}\textsubscript{2max}, l/min | 3.3 ± 0.5 | 3.8 ± 0.5t | 2.1 ± 0.3 | 2.5 ± 0.4t |

Values are means ± SD. n = No. of subjects; V\textsubscript{O}\textsubscript{2max}, maximal O\textsubscript{2} uptake; BMI, body mass index. Effects of endurance training are tested by an analysis of variance (ANOVA) for repeated measures.

*P < 0.05, †P < 0.01, ‡P < 0.0001.
LEPTIN LEVELS AND EXERCISE

Mass index values of 25 and 23 in men and women, respectively, and a mean percent body fat of 21% in men compared with 26% in women. Plasma leptin levels were strongly correlated with percent body fat in both men \((r = 0.83, P < 0.0001)\) and women \((r = 0.74, P < 0.0001)\), as illustrated in Fig. 1. Leptin levels were also significantly correlated (results not shown) with fasting insulin \((r = 0.54, P < 0.0001\) for men; \(r = 0.43, P < 0.01\) for women).

The acute and chronic effects of exercise on leptin levels are summarized in Table 2. On average, the pretraining leptin levels remained unchanged during the exercise test \((P\) values adjusted for hemoconcentration), with values from 4.6 to 4.7 ng/ml in men \((P = 0.78)\) and from 11.9 to 11.7 ng/ml in women \((P = 0.45)\). The same trend was observed posttraining, with no significant effects in men \((P = 0.61)\) and women \((P = 0.77)\).

The effects of exercise training on leptin levels are also summarized in Table 2. After endurance training, men exhibited significant reductions in leptin levels measured either at Rest \((P = 0.004)\), 50W \((P = 0.009)\), or at Max \((P = 0.044)\), with average training-induced changes \((\Delta)\) in leptin levels of \(-0.67\), \(-0.59\), and \(-0.45\) ng/ml, respectively. In women, the chronic effects of exercise training on leptin levels were not significant, except for an increase \((11.7\) vs. \(13.0\) ng/ml; \(P = 0.03)\) noted at Max. To determine whether the effects of exercise training were attributable to changes in fat mass, the analyses were repeated by adding this covariate in the model. As indicated by the \(P\) values given in Table 2, the effects of training on leptin were no longer significant after changes in fat mass are accounted for.

Figure 2 presents the individual pre- and posttraining plasma leptin values measured at Rest in men (Fig. 2A) and women (Fig. 2B) separately. There were considerable interindividual differences in the leptin response to exercise training, some individuals showing increases and others showing decreases or no changes after endurance training.

**DISCUSSION**

Administration of leptin to the ob/ob mouse reveals that leptin influences oxygen consumption, locomotor activity, and heat production \((10, 19)\), suggesting that this hormone plays an important role in the regulation of energy expenditure besides its effect on the control of food intake. The present study was undertaken to study the leptin response to the acute (single bout of exercise) and chronic (20 wk of exercise training) effects of exercise in men and women.

Our results indicate that leptin levels are not altered during exercise and that endurance training is associated with a reduction in circulating levels of leptin in men but not in women. Men exhibited a significant \((P < 0.00001)\) reduction of 6% in percent body fat in response to training compared with a nonsignificant reduction of 2% in women. After changes in fat mass in the analyses are accounted for, the effects of training were no longer significant, suggesting that there are no chronic effects of exercise on leptin levels, independent of fat mass changes. However, this adjustment appears to have different effects in men and women. As indicated in Table 2, adjustment for fat mass resulted in an increase of the \(P\) values for chronic effects in men, whereas in

**Table 2. Acute and chronic effects of exercise on plasma leptin levels**

<table>
<thead>
<tr>
<th></th>
<th>Leptin Levels, ng/ml</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Rest</td>
<td>50W</td>
</tr>
<tr>
<td>Men</td>
<td>51</td>
<td>4.6±4.4</td>
<td>4.7±4.4</td>
</tr>
<tr>
<td>Pretraining</td>
<td></td>
<td>3.9±4.2</td>
<td>4.1±4.4</td>
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<tr>
<td>Posttraining</td>
<td></td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>P value for chronic effects Without adjustment</td>
<td></td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Adjusted for fat mass changes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>46</td>
<td>11.9±8.5</td>
<td>11.7±7.6</td>
</tr>
<tr>
<td>Pretraining</td>
<td></td>
<td>12.4±8.1</td>
<td>12.8±8.1</td>
</tr>
<tr>
<td>Posttraining</td>
<td></td>
<td>0.43</td>
<td>0.06</td>
</tr>
<tr>
<td>P value for chronic effects Without adjustment</td>
<td></td>
<td>0.22</td>
<td>0.03</td>
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<td>Adjusted for fat mass changes</td>
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Values are means ± SD. n, No. of subjects; Rest, before test; 50W, 10- to 12-min submaximal/maximal exercise at 50-W absolute power output; Max, after test. \(P\) values are derived from an ANOVA for repeated measures. For acute effects, \(P\) values are adjusted for hemoconcentration.
women the opposite effect was observed. This observation suggests that the leptin response to exercise is gender specific. To further investigate this issue, we computed correlations between leptin levels measured at Rest before training and their changes after endurance training with changes in body composition. No association was observed between pretraining leptin levels and changes in body composition, but changes in leptin levels were found to be correlated with changes in body composition in women ($r = 0.34 \leq r \leq 0.37; P < 0.01$) but not in men. Because the leptin response to endurance training was negatively correlated with the pretraining leptin levels ($r = -0.33$ and $r = -0.32$ in men and women, respectively), we computed correlations between changes in leptin and body composition after accounting for the effects of pretraining leptin levels. The same trend was observed, with no association in men, whereas the association observed in women became more significant ($0.39 \leq r \leq 0.49; P < 0.005$). These associations will need to be investigated further with a larger sample size, but they are in agreement with studies that have shown that there are gender differences in leptin levels independent of body composition (11, 22).

One factor that could contribute to this gender difference in leptin levels and their response to training is the sex hormones. The leptin levels of pre- and postmenopausal women after adjustment for fat mass were compared in two different studies, one reporting no significant difference (11), whereas another reported significantly higher levels in premenopausal women (22). In postmenopausal women, fat mass-adjusted leptin levels were reported to be unaltered by hormone replacement therapy (11, 13). These studies suggest that after differences in body fat mass are accounted for, leptin levels in women are independent of reproductive status (pre- vs. postmenopausal women) and exogenous sex hormones (hormone replacement therapy).

The absence of significant fat loss in women after the endurance training program could be explained by modifications of their dietary intake. Although energy intake was not assessed in the present study, cholesterol and fat intake were monitored with the EPAT (Eating Pattern Assessment Tool) questionnaire (20). The Eating Pattern Assessment Tool questionnaire is a simple tool used to score the frequency of ingestion of high-fat high-cholesterol food groups. On the basis of this questionnaire, no significant changes were noted in the intake of fat and cholesterol over the 20-wk of endurance training among participants of the HERITAGE study (A. Walker et al., unpublished observations).

Studies in animals suggest that insulin could play a role in the modulation of ob gene expression (7, 23). Recent in vivo (clamp studies) and in vitro (adipocytes from adipose tissue biopsies) studies have shown that there are no acute effects of insulin on leptin secretion (8, 15, 28). A number of studies have shown that exercise training is associated with improvements in glucose tolerance and insulin sensitivity (1). Results of the present study based on fasting insulin or insulin-to-glucose ratio suggest that insulin sensitivity was not altered by endurance training, which could perhaps explain the absence of chronic effects of regular exercise on leptin levels.

Another important finding of this study is the presence of large interindividual differences in the leptin response to exercise. Despite moderate changes in average leptin values, some individuals exhibited a large increase or decrease in leptin levels, whereas others showed no change. A few factors have to be considered in the interpretation of these results. The first is that exercise training might produce alterations in the production and/or clearance of leptin that could not be reflected by a single plasma measurement of leptin levels at a given point in time. Furthermore, there is now evidence that leptin circulates in either a free form (presumably the bioactive form) or is bound to leptin-binding proteins and that the ratio of these two forms varies between lean and obese individuals (26). Furthermore, the same study has shown that in lean subjects with 21% or less body fat, which is a level similar to the men in the present study, the majority of
leptin (60–98%) circulated in the bound form and that fasting had no effects on bound leptin (26). This observation could explain the absence of an exercise training effect on total leptin as measured in the present study.

Second, leptin levels used for this report were not measured in a fasting state. This could partly explain the variation observed in the leptin response. However, this is not likely to be a major factor, because results from other studies suggest that leptin levels do not rise postprandially (8, 25). Moreover, leptin levels also exhibit diurnal variation, with a rise during the overnight fast (25) and a fall in the morning if fasting is prolonged (14). In the present study, blood samples for leptin assays were taken at the same time of the day before and after training, and diurnal variation could not be responsible for the interindividual differences observed in the leptin response to exercise training.

In summary, results of this study suggest that there are no acute or chronic effects of exercise on leptin levels in humans, independent of changes in fat mass. As a matter of fact, most of the variation in leptin levels associated with acute exercise or with endurance training appeared to be within the confidence intervals of the leptin assay, although one cannot rule out a minor contribution from other sources.

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