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The effect of short-term exercise on plasma leptin levels in patients with anorexia nervosa

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Abstract

Plasma leptin concentrations are markedly reduced in malnourished patients with anorexia nervosa (AN). Whether the long-term underweight and low-fat stores affect the leptin response to exercise remains unknown. We investigated the effect of 45-minute cycle ergometer exercise (2 W kg^{-1} of lean body mass [LBM]) on plasma leptin, norepinephrine (NE), glycerol, and insulin levels in 10 patients with AN and in 15 healthy age-matched women (C). Plasma leptin levels immediately and 90 minutes after the exercise bout were significantly reduced compared with basal leptin levels in both AN and C groups ($P < .05$). Compared with the control trial, leptin levels were significantly lower immediately and 90 minutes after exercise in the AN group ($P < .05$) but not in the C group. Basal and exercise-induced plasma glycerol and NE levels did not differ significantly between the groups. Basal and exercise-induced plasma insulin levels were significantly lower in the AN group compared with the C group ($P < .05$). In conclusion, we demonstrated that a single bout of low-intensity exercise significantly reduces plasma leptin levels in patients with AN. In healthy women, exercise had no effect on lowering leptin concentrations beyond the diurnal decrease that occurs in the absence of exercise. Neither NE nor insulin are responsible for the different response of leptin to exercise in AN.

1. Introduction

Leptin is a hormone with multiple functions, including predominantly long-term regulation of body weight, energy balance, and body temperature [1,2]. It is produced predominantly by adipocytes and causes satiety by regulating hypothalamic neurotransmission and energy expenditure [3]. However, large variations in plasma leptin levels have been noted for a given level of body fat [4-6], suggesting that other factors, such as catecholamines, insulin, free fatty acids, food intake, gender, and sex, may be involved in the regulation of leptin levels. Recent data have provided evidence on the regulatory role of leptin in energy intake and energy expenditure in humans [7,8]. Thus, exercise with concomitant alterations in energy balance may influence leptin metabolism. However, in humans, the effect of exercise on leptin is not defined. Either a reduction [9-11] or no change [12-16] in leptin levels have been reported after a single bout of exercise.

Exercise alters concentrations of certain hormones that may alter leptin concentrations, including insulin, cortisol, catecholamines, and growth hormone [17-19]. Among these, lipolytic norepinephrine (NE) and antilipolytic insulin play an important counteracting role in the regulation of leptin production. As the sympathetic nervous system (SNS) was established to have a tonic inhibitory action on leptin synthesis [20], adrenergic regulation may contribute to the rapid decline in circulating leptin that occurs when the SNS is activated, such as during fasting or exercise [20-22]. On the contrary, it has been demonstrated that leptin expression occurs after elevation of insulin in response to feeding [23], and leptin levels decline after reduction in insulin during fasting [4]. This decline of leptin during fasting should be reversed by insulin administration [24].

Anorexia nervosa (AN) is a psychiatric disorder characterized by intensive fear of gaining weight leading to deliberate food intake reduction and life-threatening weight

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and fat loss in affected patients [25]. As expected, severe malnutrition in AN is associated with altered glucose and lipid metabolism and multiple endocrine perturbations. Some of these abnormalities may be linked to alterations in adipocytokine production [26]. Several studies have previously described markedly reduced basal plasma leptin levels in malnourished and underweight anorectic patients [27-29]. However, responses of plasma leptin to a single bout of exercise in patients with AN have not been explored.

We hypothesized that in underweight patients with AN who have markedly low plasma leptin [29] and insulin levels (Dostalova et al, 2006, in press) and increased exercise-induced adipose tissue NE and glycerol levels [19], the production of leptin during a single bout of low-intensity exercise could be altered and could contribute to extreme sensitivity of these patients to energy imbalance and consequently to weight loss (ie, relapse). Therefore, we decided to investigate the effect of a single bout of exercise on plasma leptin, NE, insulin, and glycerol levels in patients with AN. To account for a diurnal variation of leptin and for the effect of 12 hours' fasting of the subjects, a control trial consisting of the same blood-sampling protocol without exercise was conducted the next day after exercise.

2. Subjects and methods

2.1. Study subjects

Ten women with a restrictive type of AN (age, 22.1 ± 1.0 years; body mass index [BMI], 15.7 ± 0.47 kg m^{-2}; percent body fat, 7.1% ± 0.88%) and 15 healthy age-matched women (control [C]; age, 21.3 ± 0.9 years; BMI, 21.2 ± 0.42 kg m^{-2}; percent body fat, 24.3% ± 0.79%) were enrolled in this study. All subjects included in the study were nonsmokers, had no allergies, and had been free of medications for at least 3 weeks before the study. Professional athletes were not included in the study. The healthy control subjects had no history of obesity or malnutrition, hypertension, gastrointestinal diseases, eating disorders, or other psychiatric disorders, and had normal physical examination and electrocardiogram (ECG) results. Blood tests confirmed normal blood count and liver and renal functions. Patients with AN were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (American Psychiatric Association, 1994) after detailed medical and psychiatric evaluation. All patients with a restrictive type of AN were examined after 2 weeks of hospitalization in the Department of Psychiatry and were clinically stable and in relatively good health except for their eating disorder. All healthy women were studied on the 7th to 10th day of their menstrual cycle, whereas all patients with AN had amenorrhea. All participants provided written informed consent before participating in the study, which was approved by the Human Ethic Review Committee, Institute of Endocrinology, Prague, Czech Republic, and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

2.2. Experimental procedures

Upon enrollment in the study, subjects were asked to fast (from 8:00 PM) and drink only water the night before the study and not to use stimulant drugs, such as caffeine, nicotine, or alcohol, for 2 days before the experiment. The week before the study, the maximum oxygen uptake was determined in a treadmill test using standard procedures (Bruce protocol). All subjects were admitted to the Institute of Endocrinology at 7:00 AM in the following 2 days. After a short medical examination (blood pressure, heart, and respiratory rate measurement, ECG), percent body fat was estimated by anthropometric measurement and bioimpedancy (TANITA, Tokyo, Japan). All subjects were then placed in supine position on a comfortable bed in a room kept at 23°C to 25°C. An intravenous catheter was placed in the antecubital vein, and a normal saline lock was attached. Blood collection started at least 45 minutes after catheter insertion and bed rest to reach a steady-state condition. The first blood samples were collected at 8:00 AM after 12 hours of fasting (resting levels), then immediately after the exercise bout (at 10:00 AM), and finally, 90 minutes after the end of the exercise bout (at 11:30 AM). Similarly, the next day blood samples were taken at 8:00, 10:00, and 11:30 AM without the exercise stimulation. All blood samples were taken when subjects were in a supine position. For each blood draw, the first 3 mL of blood (with saline from the catheter lock) preceding a 20-mL draw was withdrawn into a discard tube. After each draw, the catheter was flushed with physiological saline (3 mL) to maintain patency. On the first day, subjects underwent 45 minutes of physical exercise on an electromagnetically braked bicycle ergometer (Cateye, Japan) at a power output of 2 W kg^{-1} of lean body mass (LBM). After finishing an exercise bout, all subjects assumed a resting supine position on a comfortable bed for 90 minutes. The metabolic rate during exercise was measured as oxygen consumption with a modified paramagnetic oxygen analyzer (Spirolyt, Junkalor, Dessau, Germany). ECG, heart rate, and blood pressure were monitored with an Eagle 3000 cardiomonitor (Marquette, Milwaukee, WI). Oxygen consumption, heart rate, and blood pressure during the 45-minute exercise were measured every 5 minutes. Blood samples for leptin assay were collected into chilled polypropylene tubes containing Na_2EDTA and antilysin. Plasma was immediately separated from whole blood by centrifugation at 3000 rpm for 20 minutes at 4°C, and stored at −20°C until assay.

2.3. Analytical procedures

Plasma leptin concentrations were determined by a commercial radioimmunoassay (Linco Research, St Charles, MO). The detection limit of the assay was 0.05 ng mL^{-1}, with intra-assay coefficients of variation (CVs) of 5.25% and 5.97%, and interassay CVs of 8.9% and 8.67% for low (0.44 ng mL^{-1}) and high (4.24 ng mL^{-1}) leptin levels, respectively.

Table 1
Characteristics of the study subjects

	C (n = 15)	AN (n = 10)
Age (y)	21.3 ± 0.9	22.1 ± 1.0
Height (cm)	171.9 ± 1.86	170.8 ± 1.11
Weight (kg)	62.2 ± 1.54	45.8 ± 1.89*
Lean body mass (kg)	39.1 ± 0.76	37.8 ± 1.01
BMI (kg m^{-2})	21.2 ± 0.42	15.7 ± 0.47*
Body fat (%)	24.3 ± 0.79	7.1 ± 0.88*
Leptin (ng mL^{-1})[a]	7.4 ± 0.81	1.5 ± 0.32*
NE (pg mL^{-1})[a]	151.6 ± 18.26	177.1 ± 22.37
Glycerol (μmol L^{-1})[a]	135.8 ± 17.41	139.9 ± 19.08
Insulin (pmol L^{-1})[a]	28.3 ± 4.53	14.2 ± 3.67*
Glucose (mmol L^{-1})[a]	4.7 ± 0.08	4.1 ± 0.11

Values are expressed as mean ± SEM. n indicates number of probands.
[a] Blood samples were taken at 8.00 AM (exercise trial).
* $P < .05$ vs control subjects.

Plasma NE was assayed by high-performance liquid chromatography, using electrochemical detection after purification on alumina [30]. Plasma glycerol was measured by radiometric kit (Randox Laboratories, GY105, Montpellier, France). Plasma insulin was measured by a commercial radioimmunoassay (Immunotech, Prague, Czech Republic). Sensitivity was 3.7 pmol L^{-1} and the intra- and interassay variability was 3.4% and 4.3%, respectively. Plasma glucose levels were measured in a Cobas Integra 400 plus system (Roche Diagnostics, Mannheim, Germany). All samples were run 4 times (2 assays in duplicate).

2.4. Data analysis

All data are presented as mean ± SEM. Data were analyzed by 2-way analysis of variance (ANOVA) with factors AN and time, and using Bonferroni intervals. Tukey interaction between factors was incorporated into the statistical model. Statistical differences between groups were analyzed by the Mann-Whitney rank sum test. Statistical differences between the phases of the experiment were analyzed by Wilcoxon paired test. A P value equal to or less than .05 denoted statistical significance. All statistics were performed using Statgraphics Plus 3.3 (Manugistics, Rockville, MA).

Table 2
Circulatory response of the study subjects

	C (n = 15)	AN (n = 10)
Heart beat (beats min^{-1})		
Rest	83 ± 2.5	71 ± 2.9*
Exercise	107 ± 3.1†	102 ± 1.9†
Systolic blood pressure (mmHg)		
Rest	110 ± 3.3	90 ± 3.3*
Exercise	125 ± 2.1†	103 ± 2.8*,†
Diastolic blood pressure (mmHg)		
Rest	74 ± 2.8	59 ± 2.9*
Exercise	77 ± 3.5	63 ± 3.3*
$\dot{V}O_2$ (mL kg^{-1} min^{-1})		
Rest	3.1 ± 0.6	2.9 ± 0.7
Exercise	9.2 ± 0.4†	7.4 ± 0.5*,†
$\dot{V}O_2$max (mL kg^{-1} min^{-1})	36.5 ± 2.4	24.3 ± 3.5*
$\dot{V}O_2$max (L min^{-1})	2.3 ± 0.15	1.1 ± 0.17*

Values are expressed as mean ± SEM. Exercise results are maximal values attained during the investigation.
* $P < .05$ vs control subjects.
† $P < .05$ vs resting values.

3. Results

The baseline characteristics and the circulatory response of the study subjects on exercise stimulation are shown in Tables 1 and 2, respectively. Patients with AN were extremely malnourished as evidenced by severely decreased BMI and body fat content relative to the C group (Table 1).

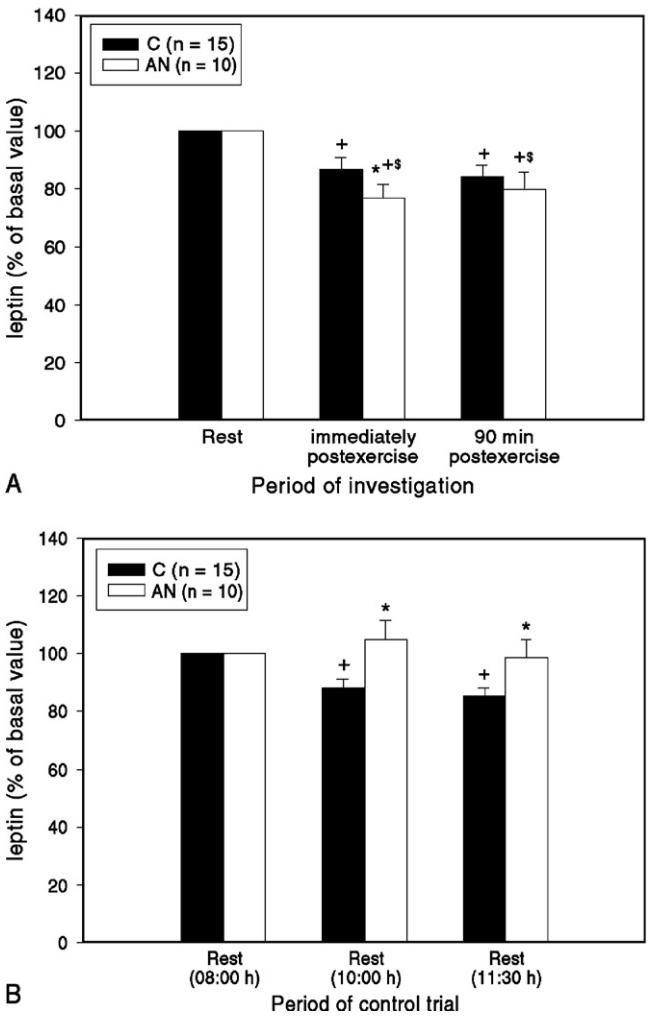

Fig. 1. A, Plasma leptin levels (percent of basal value) under the effect of a single bout of exercise (2 W kg^{-1} of LBM, 45 minutes) in patients with AN (n = 10) and in healthy women (C, n = 15). Plasma leptin levels were measured at rest (8:00 AM), immediately after the end of the exercise bout (10:00 AM) and after 90 minutes of recovery (11:30 AM). Values are expressed as mean ± SEM. $^{+}P < .05$ vs basal levels; $^{*}P < .05$ vs C group; $^{\$}P < .05$ vs control trial levels. B, Plasma leptin levels (percent of basal value) during the control trial in patients with AN (n = 10) and in healthy women (C, n = 15). Plasma leptin levels were measured at 8:00, at 10:00, and at 11:30 AM. The same probands as in the exercise trial participated in the control trial on the following day after exercise. Values are expressed as mean ± SEM. $^{+}P < .05$ vs basal levels; $^{*}P < .05$ vs C group.

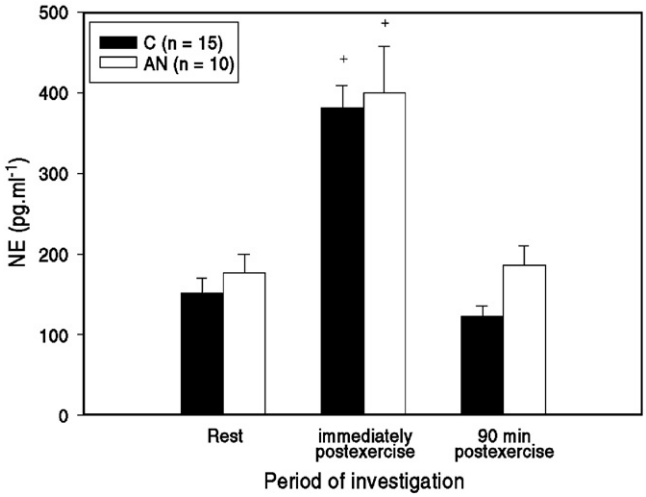

Fig. 2. The effect of a single bout of exercise (2 W kg^{-1} of LBM, 45 minutes) on plasma NE (picograms per milliliter) levels in patients with AN (n = 10) and in healthy women (C, n = 15). Plasma NE levels were measured at rest (8:00 AM), immediately after the end of the exercise bout (10:00 AM) and after 90 minutes of recovery (11:30 AM). Values are expressed as mean ± SEM. $^{+}P < .05$ vs basal levels.

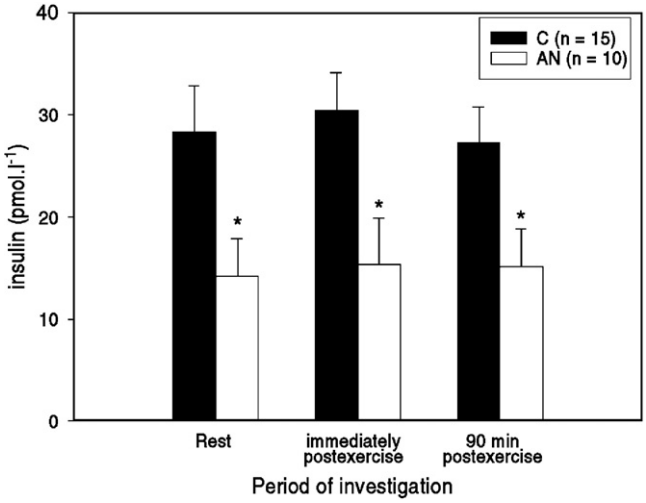

Fig. 4. The effect of a single bout of exercise (2 W kg^{-1} of LBM, 45 minutes) on plasma insulin (picomoles per liter) levels in patients with AN (n = 10) and in healthy women (C, n = 15). Plasma insulin levels were measured at rest (8:00 AM), immediately after the end of the exercise bout (10:00 AM), and after 90 minutes of recovery (11:30 AM). Values are expressed as mean ± SEM. $*P < .05$ vs C group.

Baseline plasma insulin levels were significantly reduced in the AN group compared with the C group, whereas fasting plasma glucose did not significantly differ between the groups studied (Table 1). The oxygen consumption under basal conditions was comparable in the groups studied. However, the maximum oxygen consumption ($\dot{V}O_2max$) as well as the highest value of oxygen consumption reached during exercise was significantly lower in patients with AN (Table 2). The resting heart rate and systolic and diastolic blood pressure were markedly lower in patients with AN than in the C group (Table 2). Exercise stimulation led to significant increases in heart beat and systolic blood pressure, but not in diastolic blood pressure in both groups when compared with resting values (Table 2).

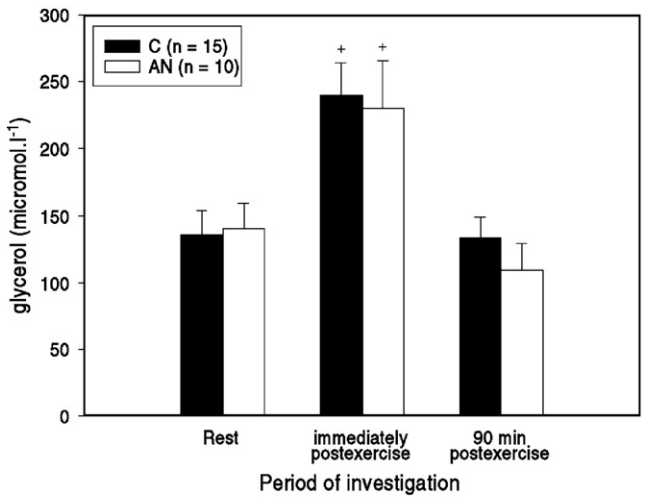

Fig. 3. The effect of a single bout of exercise (2 W kg^{-1} of LBM, 45 minutes) on plasma glycerol (micromoles per liter) levels in patients with AN (n = 10) and in healthy women (C, n = 15). Plasma glycerol levels were measured at rest (8:00 AM), immediately after the end of the exercise bout (10:00 AM), and after 90 minutes of recovery (11:30 AM). Values are expressed as mean ± SEM. $^{+}P < .05$ vs basal levels.

3.1. Basal and exercise-induced plasma leptin concentrations

Basal plasma leptin levels were markedly lower in the AN group than in the C group (Table 1). Plasma leptin levels measured immediately after the exercise bout and after 90 minutes of recovery significantly decreased in both groups compared with basal levels (Fig. 1A).

3.2. Comparison of exercise-induced and control trial leptin levels

For the C group, leptin levels declined to the same extent during control and exercise trials. However, in the AN group, leptin concentrations declined only during the exercise trial (Fig. 1A, B).

3.3. Basal and exercise-induced plasma NE, glycerol, and insulin concentrations

Basal and exercise-induced plasma NE (Fig. 2) and glycerol (Fig. 3) levels were not significantly different between the groups. Plasma NE as well as glycerol levels significantly increased during exercise and decreased to the levels conformable to those observed under basal conditions 90 minutes after the end of an exercise bout (Figs. 2 and 3). Basal and exercise-induced plasma insulin levels were significantly lower in patients with AN compared with controls (Fig. 4). In both groups, insulin levels were not significantly changed immediately after and 90 minutes after exercise compared with basal levels (Fig. 4).

3.4. Relationship of plasma leptin with other parameters

Plasma leptin levels correlated positively with BMI (R = 0.68, P = .03) and negatively with plasma glycerol levels (R = −0.82, P = .04) in the AN group, but not in the C group. We did not confirm significant correlation between baseline, exercise-stimulated, and postexercise leptin levels, respectively, and percent body fat, plasma NE, insulin, and glucose levels in any group studied.

4. Discussion

The most important finding of this study is that a single bout of moderate-intensity exercise significantly reduces plasma leptin levels in underweight patients with a restrictive type of AN, but not in healthy normal-weight women in comparison with leptin levels found in these subjects during the control trial. Furthermore, basal and exercise-induced plasma NE as well as glycerol levels were not significantly different in patients with AN compared with the C group. Basal and exercise-induced plasma insulin levels were significantly reduced in patients with AN compared with the C group. In both groups, NE as well as glycerol levels were significantly increased and plasma insulin levels were not changed immediately after exercise compared with basal levels.

Studies that have investigated effects of short-term exercise on leptin have shown either reductions [9,10,31] or no change [12] in leptin concentrations. In the study of Jurimae and Jurimae [10], relatively short-term (30 minutes) intense exercise significantly decreased plasma leptin levels, and in the study of Elias et al [9], short-term exhaustive exercise in male volunteers led to decline in plasma leptin levels. However, a control trial was not conducted in these studies to determine whether diurnal changes accounted for observed reductions. In the study of Kraemer et al [31], 30 minutes of exercise at 80% of $\dot{V}O_2$max was associated with reduced leptin concentrations in postmenopausal women, but the reductions were due to the circadian rhythms of leptin as determined from control trial samples from the same subjects. Thus, reported reductions in leptin levels during a single bout of exercise could be attributed to circadian rhythms. The absence of any reduction in leptin reported in short-term exercise study may be due to the limited energy expenditure of these exercise bouts [18,32], or the protocol of these studies excluded prolonged postexercise blood sampling. Previous studies that used aerobic running exercise in lean healthy men have reported delayed leptin reduction 24 and/or 48 hours after exercise but not immediately after the exercise bout [33,34].

The positive attribute of our study is that results found under the effect of exercise were compared with those obtained during control trial in the same subjects on the following day to control for diurnal changes and/or the effect of prolonged overnight fasting, previously underlined to be important for the validity of obtained data [14]. It is well established that circulating leptin levels in humans follow a diurnal pattern, with zenith and nadir at about midnight and shortly after the morning breakfast, respectively [33,35]. The reduction in plasma leptin levels that we observed during exercise in the C group appears to be a continuation of the natural late-night/early-morning decline in plasma leptin levels [35], as both exercise and control trial leptin concentrations declined in nearly the same manner. Another possibility could be that the observed decrease of plasma leptin levels in the C group is due to prolonged overnight fasting in both exercise and control trials, with exercise of low-energy expenditure having no effect on leptin levels [32]. Moreover, it is also possible that if our subjects had exercised for a longer period and/or we had measured plasma leptin levels for a longer time, an exercise-induced reduction in leptin might have taken place in healthy women. Patients with AN could have altered timing of the leptin regulation in acute energy disbalance.

We did not find any reduction in plasma leptin levels during the control trial in patients with AN. Balligand et al [36] previously demonstrated that AN is associated with the complete lack of diurnal leptin pulsation, and short-term refeeding is not sufficient to restore this abnormality. We suppose that patients with AN are adapted to a state of chronic starvation with markedly low leptin and insulin levels and prolonged fasting is not sufficient to further decrease plasma leptin levels in these patients. However, this observation does not explain the rapid decrease of plasma leptin levels after a single bout of exercise that we observed in patients with AN.

Leptin responds to the difference between intake and expenditure in healthy women [32]. It was previously underlined that the threshold for energy deficit (short-term exercise energy expenditure considerably exceeds energy intake) must achieve the leptin levels being suppressed by exercise. Subjects in our study underwent the exercise trial of intensity about 25% $\dot{V}O_2$max after 12 hours of overnight fasting. Compared with other studies, the intensity of exercise in our study is low, but the intensity and duration of exercise in our study had been limited by the physical condition of extremely malnourished patients with AN. Fitting the intensity of the exercise, we further had to bear in mind the aim of this study—to investigate the leptin response to exercise of intensity that could be reached in normal daily life to test the possible hypersensitivity of AN to energy expenditure. However, the rapid decrease of leptin during low-intensity exercise does not support our original hypothesis of leptin's role in the tendency to weight reduction after low-intensity energy expenditure in patients with AN. The sensitive response of leptin to exercise in these patients is probably a part of adaptive mechanisms leading to conservation of energy and to increased feelings of hunger after energy expenditure. Because the previous diet, meal schedule, and physical activity of patients with AN had been controlled during the 2 weeks of hospitalization, the results of our study could not be influenced by

preexercise trial fasting and/or extreme physical activity of patients with AN. However, we could not guarantee the unitary daily energy intake/energy expenditure in patients with AN. The daily energy value had been chosen individually with respect to the prehospitalization energy intake and psychological attitude to food intake of the patient. However, we guarantee that all patients with AN included in the study consumed their diet as oral meals 6 times a day within the usual 12-hour span and adhered to sedentary behavior for 2 weeks before the exercise trial.

To the best of our knowledge, there are no published data on the influence of a single bout of exercise on plasma leptin levels in patients with AN. Previously published studies showed that leptin decrease under the effect of exercise is independent of weight reduction [37,38]. These studies included a training program with diet therapy, or only diet therapy lasting for 6 weeks [37], or a very low energy diet and training program for 4 months [38]. However, patients with AN are in a state of chronic malnutrition persisting for several years associated with chronic metabolic and endocrine adaptation to this starvation status. Rapid exercise-induced leptin decline in patients with AN could be a part of chronic adaptation to energy deficit in these patients, with many factors influencing this abnormally sensitive and prompt response to energy expenditure. This response of leptin to exercise mirrors the prompt leptin increase in patients with AN, not seen in healthy women (Dostalova et al, unpublished results), after consumption of a standardized mixed meal (2451 kJ, 32.6 g of fat, 17.6 g of protein, 50.0 g of carbohydrate).

Exercise has been shown to alter concentrations of certain hormones and neurotransmitters that may influence leptin concentrations [14,18,19,39]. Among these, catecholamines have been shown to inhibit leptin production as well as plasma leptin levels [40,41], whereas insulin has been shown to stimulate leptin [24]. Although we had initially hypothesized that the activity of the SNS and insulin could be important in the regulation of leptin during short-term exercise, the finding of different exercise-induced leptin response in AN, yet no difference in NE and insulin responses to exercise between groups, indicates that something other than NE (SNS activity) and insulin was responsible for the different leptin responses. However, we could not exclude the possibility that chronically low insulin levels that we found in patients with AN affect glucose uptake in adipocytes and, thus, production of leptin in adipose cells [42]. Kolaczynski et al [43] concluded that insulin does not stimulate short-term leptin production, but they demonstrated a long-term effect of insulin on leptin production both in vivo and in vitro.

Although we failed to find differences in NE and glycerol responses to exercise between AN and the C group, our group has previously found that AN is associated with in vivo increased basal as well as exercise-stimulated SNS activity, especially increased NE levels, and unchanged basal but significantly increased exercise-stimulated lipolysis in the subcutaneous abdominal adipose tissue [19]. Regulation of lipolysis by exercise is potentially relevant to regulation of circulating leptin levels because lipolysis and leptin production during exercise have been found to be inversely controlled [44]. Thus, we could not exclude the influence of excited activity of the SNS and of increased lipolysis in the abdominal adipose tissue of patients with AN during a short-term exercise [19] on exercise leptin production in these patients.

In summary, we demonstrated that patients with AN who have significantly decreased fasting plasma leptin levels show a significant fall in leptin levels immediately after and 90 minutes after a single bout of exercise. On the other hand, the decrease in plasma leptin observed during both exercise and control trials in healthy women could be explained by prolonged overnight fasting and/or circadian variation of leptin lacking in AN. Neither NE nor insulin are the salient factors responsible for the different response of leptin to exercise in AN. Further investigation is needed to classify the particular role of altered regulation mechanisms on plasma leptin in the state of acute energy disbalance in patients with AN.

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