Effect of Exercise Training on Serum Leptin Levels in Type 2 Diabetic Patients

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To evaluate the effect of exercise training on serum leptin levels 50 sedentary subjects with type 2 diabetes were enrolled in either 6 weeks of aerobic exercise training with diet therapy (n = 23) or diet therapy alone (n = 27). The training program consisted of walking and cycle ergometer exercise for 1 hour at least 5 times per week, with the intensity of exercise maintained at 50% of maximum oxygen uptake. Serum leptin levels decreased significantly in the exercise training (TR) group (7.2 ± 3.6 to 4.6 ± 2.5 ng/mL, *P* < .05), but not in the sedentary (SED) group (6.9 ± 3.4 to 5.6 ± 2.9 ng/mL). Leptin levels standardized for percentage body fat (dividing serum leptin level by percentage body fat) after treatment were lower in the TR subjects compared with the SED subjects. Body weight and percentage body fat decreased in all patients; however, no significant changes were observed in either group. Fasting concentrations of plasma insulin and cortisol and the urinary excretion of 17-hydroxycorticosteroid (17-OHCS) did not differ between the groups either before or after treatment. Fasting plasma glucose and hemoglobin A_{1c} (HbA_{1c}) improved significantly in both groups, although no significant differences were observed between the groups either before or after treatment. Ventilatory threshold increased significantly in the exercise training subjects. This study demonstrates that exercise training in type 2 diabetic subjects reduces serum leptin levels independent of changes in body fat mass, insulin, or glucocorticoids.

LEPTIN IS REGULATED by body fat mass and recent energy intake. Adipose tissue *ob* messenger RNA expression and circulating leptin levels[1] increase with increasing adiposity. Short-term hypercaloric[2] or hypocaloric[3] feeding without appreciable weight change also alters circulating leptin levels. Energy intake influences circulating leptin levels suggests that energy expenditure, such as exercise, may also affect leptin levels.

Obese persons who incorporate exercise in their weight management program are more likely to comply with their diet and achieve long-term weight reduction than those who do not exercise.[4,5] These investigations suggest that exercise training may alter leptin levels. Exercise is an effective way to enhance insulin sensitivity in type 2 diabetic patients. If exercise training also decreases appetite by altering leptin levels, this is another compelling reason to recommend exercise therapy for all type 2 diabetic patients.

Few studies have investigated the influence of exercise on circulating leptin levels in humans. Acute effects of a single bout of exercise on circulating leptin levels have been studied.[6-10] A single exercise session did not alter leptin levels, unless it provided extreme changes in energy expenditure, such as marathon running.[9] The chronic effects of exercise training on leptin levels are still controversial.[8,11-14] Consequently, we studied the response of serum leptin levels to aerobic exercise training in type 2 diabetic patients.

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SUBJECTS AND METHODS

Previously sedentary type 2 diabetic patients were either enrolled in an exercise training program (TR; 9 men and 14 women) or in a sedentary control group (SED; 11 men and 16 women). All subjects underwent diet therapy. All females were postmenopausal and all were not receiving hormone-replacement therapy. None of the subjects had severe complications of diabetes or an abnormal serum creatinine concentration. Subjects were matched for age (TR 56.0 ± 4.6/SED 57.9 ± 7.6 years), body mass index (26.2 ± 3.5/25.4 ± 3.2 kg/m^2), and fasting concentrations of plasma glucose (10.6 ± 2.8/11.0 ± 3.1 mmol/L), HbA_{1c} (10.0% ± 2.1%/10.1% ± 1.9%), and insulin (41.7 ± 31.0/43.4 ± 28.0 pmol/L). All patients gave informed consent prior to participating in the study, which was approved by an institutional ethics committee.

All participants were hospitalized for 6 weeks. They were placed on a 25- to 27-kcal/kg/d diet (54% to 58% carbohydrate, 22% to 24% protein, 18% to 20% fat) and had 3 meals a day (at 6 AM, noon, and 6 PM). The TR subjects trained at least 5 times per week. Training consisted of walking and cycle ergometer exercise for 1 hour each day. Maximum oxygen uptake ($\dot{V}O_2$max) was measured every 2 weeks. The intensity of the exercise was maintained at 50% of $\dot{V}O_2$max, and was adjusted throughout the training program as aerobic capacity increased.

Serum leptin levels were measured with a radioimmunoassay (Linco Research, St Charles, MO). Plasma insulin concentrations were measured using a microparticle enzyme immunoassay (Dinabot, Abbott Park, IL). Plasma cortisol concentrations were measured using a fluorescence polarization immunoassay (Dinabot). Urinary 17-hydroxycorticosteroid (17-OHCS) excretion were assessed as described previously.[15] Blood samples were obtained at 6 AM after an overnight fast from the antecubital vein with the subject in a supine position. Body composition was measured by dual-energy x-ray absorptiometry, using the Hologic QDR-2000 instrument (Hologic, Waltham, MA).

In the TR subjects, ventilatory threshold and $\dot{V}O_2$max were measured during a standard incremental exercise test performed using a cycle ergometer.[16] Fractions of oxygen and carbon dioxide were analyzed using the Sensormedics MMC metabolic system (Sensormedics, Anaheim, CA).

All data are presented as means ± SD. Comparisons between groups of data were performed using a repeated-measures analysis of variance. Where applicable, post hoc analysis was conducted using the Scheffé procedure. The changes in ventilatory threshold in the TR group were assessed using paired *t* tests. The relation between serum leptin level, which was log-transformed to approximate a normal distribution, and

Table 1. Comparison of Aerobic Exercise Training and Diet Alone on the Physical Characteristics of Type 2 Diabetic Subjects

	TR		SED	
	Before	After	Before	After
Body weight (kg)	60.9 ± 9.8	58.1 ± 7.5	63.0 ± 10.8	60.5 ± 10.5
BMI (kg/m^2)	26.2 ± 3.5	25.2 ± 3.1	25.4 ± 3.2	24.7 ± 3.0
Body fat (%)	32.1 ± 7.4	29.8 ± 7.4	32.2 ± 6.8	30.7 ± 6.7
Ventilatory threshold (mL/kg/min)	14.9 ± 3.3	16.3 ± 3.8*	N/A	N/A

NOTE. Values are means ± SD.
Abbreviations: BMI, body mass index; N/A, not applicable.
*$P < .05$ compared with before treatment.

percentage body fat was evaluated by Pearson's correlation test. Analysis of covariance was used to compare the linear regression lines. *P* values less than .05 were considered statistically significant.

RESULTS

Physical characteristics of the TR and the SED subjects are presented in Table 1. Body weight and body fat were similar in both groups before and after treatment and did not changed with exercise training. Ventilatory threshold increased significantly in the TR subjects ($P < .0001$).

Table 2 shows the average amount of energy expended each week in the TR subjects estimated by oxygen consumption and a frequency of exercise for each week.

Serum leptin levels decreased by 36% in the TR group ($P < .05$), but were not altered significantly in the SED group (Fig 1A). Serum leptin levels standardized for percentage body fat (dividing serum leptin level by percentage body fat) after treatment in the TR group were lower compared with those in SED group ($P < .05$), as shown in Fig 1B.

There was a positive correlation between log-transformed serum leptin levels and percentage body fat before and after treatment in both groups. Comparison of the regression lines before and after treatment showed that serum leptin levels after treatment in the TR group significantly decreased relative to before treatment ($P < .05$), but were not significantly altered in the SED subjects (Fig 2).

To assess insulin and glucocorticoids as mediators of the change in leptin levels with exercise training, we measured fasting plasma insulin and cortisol concentrations and urinary 17-OHCS excretion before and after treatment in both groups (Table 3). Fasting plasma insulin decreased by 21% in the TR subjects and by 11% in the SED subjects, which was not statistically significant. Although plasma cortisol concentrations decreased by 23% in both groups ($P < .05$, respectively), no significant change was observed between the groups either before or after treatment. Urinary 17-OHCS excretion did not decrease significantly in either group.

Table 2. Average Amount of Energy Expenditure in Exercise Training Subjects

	Energy Expenditure (kcal/wk)
1st week	1,632.4 ± 585.1
2nd week	1,732.2 ± 562.9
3rd week	1,834.7 ± 539.6
4th week	1,899.5 ± 607.0
5th week	1,807.1 ± 495.6
6th week	1,828.3 ± 503.3

NOTE. Values are means ± SD.

Fasting plasma glucose and HbA_{1c} improved significantly in both the TR subjects (fasting plasma glucose 10.6 ± 2.8 to 6.8 ± 1.8 mmol/L, $P < .0001$/HbA_{1c} 10.0% ± 2.1% to 8.3% ± 1.6% $P < .05$) and the SED subjects (11.0 ± 3.1 to 7.3 ± 1.6 mmol, $P < 0.0001$/10.1% ± 1.9% to 8.6% ± 1.2%, $P < .05$); however, no significant difference was observed between the groups either before or after treatment.

DISCUSSION

The results of the present study demonstrate that 6 weeks of moderate-intensity aerobic exercise training decreases serum leptin levels in type 2 diabetic subjects. Few studies have investigated whether exercise training influences circulating leptin levels in human. Hickey et al[11] reported that serum leptin levels decreased significantly in females but not males after 12 weeks of aerobic exercise training without alterations in body fat mass. In obese males, Pasman et al[12] found a 23% reduction in plasma leptin levels after 16 months of exercise training (40% to 60% of $\dot{V}O_2$max for 60 minutes, 3 to 4 times per week). In contrast, neither 9 months of exercise (70% of maximal heart rate for 30 minutes to 85% of maximal heart rate for 50 minutes minimum of 3 times per week) in older postmenopausal women[13] nor 20 weeks of endurance training (55% of $\dot{V}O_2$max for 30 minutes to 75% of $\dot{V}O_2$max for 50 minutes, 3 times per week) in 97 sedentary adults[8] had any effect on leptin levels, independent of its effect on adiposity. In these investigations there was no marked differences in the intensity of the aerobic exercise, all exercises were of moderate intensity, and the length of exercise training ranged from 12 weeks to 16 months. It is difficult to conclude that differences in the length of exercise training influenced leptin levels, as both short- and long-term exercise training altered leptin levels. Thus, the effect of exercise training on leptin levels remains controversial.

Only one study has reported the effect of exercise on leptin levels in type 2 diabetic subjects. Ryan and Ehahi[14] showed that circulating leptin levels decreased by 28% after a weight reduction and aerobic exercise program in 4 newly diagnosed type 2 diabetic patients. In their study, the independent effects of weight loss and exercise training could not be ascertained. Our study is the first to examine the effect of exercise training on leptin levels in type 2 diabetic subjects.

The decrease in serum leptin levels in the TR group is larger than that in the SED subjects, and there was no statistical difference in body weight or percentage body fat between the 2

Table 3. Comparison of Aerobic Exercise Training and Diet Alone on Fasting Plasma Insulin and Cortisol Concentrations and Urinary 17-OHCS Excretion in Type 2 Diabetic Subjects

	TR		SED	
	Before	After	Before	After
Fasting plasma insulin (pmol/L)	41.7 ± 31.0	32.8 ± 20.8	43.4 ± 28.0	38.7 ± 26.6
Cortisol (mmol/L)	0.52 ± 0.15	0.40 ± 0.14*	0.51 ± 0.10	0.40 ± 0.10*
Urinary 17-OHCS excretion (mg/m^2/d)	3.6 ± 1.1	3.1 ± 0.9	3.9 ± 1.2	3.4 ± 1.0

NOTE. Values are means ± SD.
*$P < .05$ compared with before treatment.

groups. There was trend toward a greater reduction in body fat mass in the TR subjects compared with the SED group (percentage fat in TR 32.1% ± 7.4% to 29.8% ± 7.4%/in SED 32.2% ± 6.8% to 30.7% ± 6.7%; $P = .12$). Consequently, when serum leptin levels were standardized for percentage body fat after treatment period, those in the TR subjects were also lower than those in the SED group (Fig 1B). The regression lines between serum leptin levels and percentage body fat were significantly different after treatment in the TR subjects.

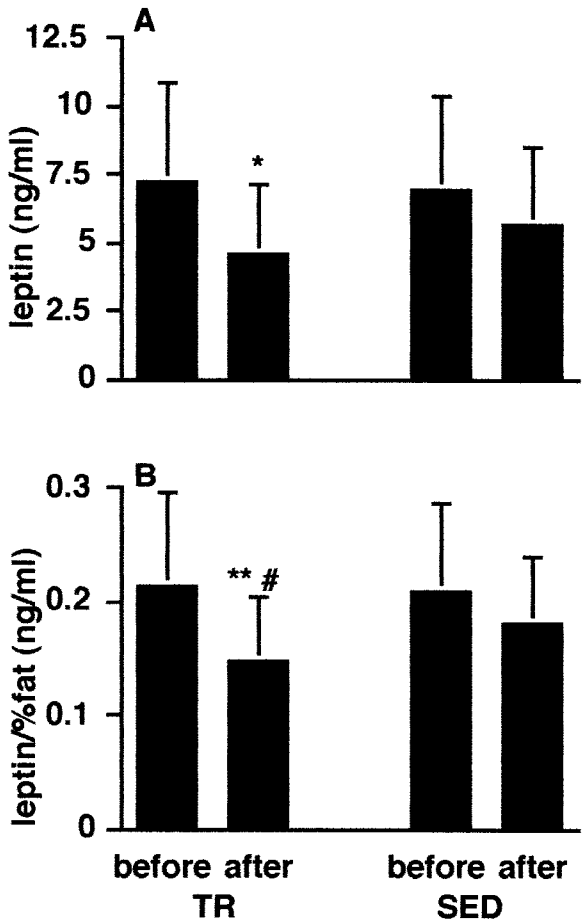

Fig 1. (A) Serum leptin levels before and after treatment, and (B) serum leptin levels standardized for percentage body fat before and after treatment in type 2 diabetic subjects. Mean ± SD. * $P < .05$, ** $P < .01$ *v* before treatment. # $P < .05$ *v* SED.

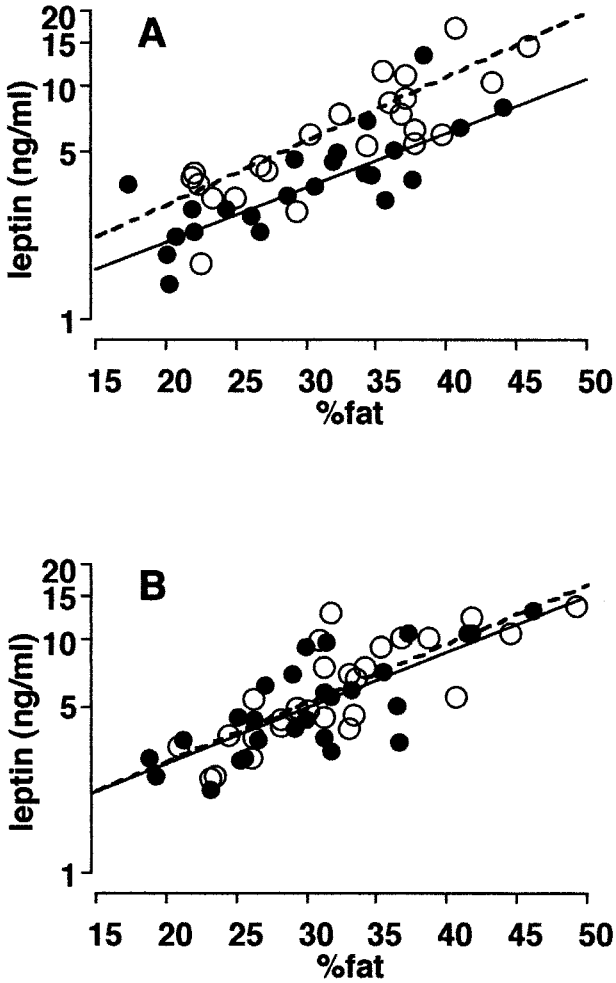

Fig 2. (A) Correlation between serum leptin levels and percentage body fat in exercise training subjects before (○) and after (●) treatment. Serum leptin levels were correlated with percentage of body fat before ($r = .83$; $P < .0001$) and after ($r = .78$; $P < .0001$) treatment. (----) Linear regression line before treatment: log y = 0.028x − 0.097. (—) Linear regression line after treatment: log y = 0.023x − 0.084. (B) Correlation between serum leptin levels and percentage body fat in sedentary subjects before (○) and after (●) treatment. Serum leptin levels were correlated with percentage body fat before ($r = .79$; $P < .0001$) and after ($r = .76$; $P < .0001$) treatment. (----) Linear regression line before treatment: log y = 0.025x − 0.024. (—) Linear regression line for after treatment: log y = 0.024x − 0.032.

The reduction in serum leptin levels with exercise training was independent of changes in body fat.

The mechanisms by which exercise training reduces serum leptin levels are unclear. Both increases and reductions in fat mass (an energy intake and expenditure) have been demonstrated to affect serum leptin level. However, we observed no statistical difference in body weight or percentage body fat between the 2 groups. Furthermore, the reduction in serum leptin levels standardized for percentage body fat was greater in the TR subjects than in the SED group. Differences in a reduction in fat mass between the 2 groups did not affect the change in serum leptin level.

Both in vivo and in vitro studies have shown that glucocorticoids enhance leptin gene transcription and levels of circulating leptin.[17-19] To examine the possibility that glucocorticoids may be affected by exercise training and thus alter serum leptin levels, we measured plasma cortisol concentrations and urinary 17-OHCS excretion before and after treatment. Plasma cortisol and urinary 17-OHCS excretion did not differ between the groups either before or after treatment, and both were unchanged by exercise training. Therefore, the reduction in serum leptin levels by exercise training was not mediated by changes in glucocorticoid concentrations.

Insulin has also been demonstrated to stimulate leptin production.[20] In human studies, circulating leptin levels correlate with basal insulin concentrations.[21] In the present study, baseline plasma leptin levels had a weak correlation with fasting insulin concentrations (data not shown). Fasting plasma insulin decreased by 21% in the TR and by 11% in the SED group after treatment, which was not statistically significant. Hence, plasma insulin dose not appear to mediate the reduction in leptin levels that occurs with exercise training.

Fat cell size has been postulated to be an important regulator of ob mRNA expression. Zachwieja et al[22] demonstrated that both the expression and secretion of leptin were influenced by exercise training in rodents, and that exercised animals had an increased percentage of smaller fat cells. Leptin secretion rates have been noted to be 2 to 3 times greater in subcutaneous fat than in visceral fat tissue in both obese and nonobese humans, which was attributed to a 50% increase in fat cell size.[23] We did not measure regional fat distribution in our study. However, exercise training reduces visceral fat accumulation more than subcutaneous fat accumulation.[24] Therefore, it is difficult to conclude that the exercise-induced reduction in serum leptin level was attributable to differences in fat cell size.

Leptin is bound by plasma proteins.[25] A change in the ratio of bound to unbound leptin might play a role in regulating leptin action. The total mount of leptin may remain stable while exercise training may change the ratio of bound to unbound leptin.

In summary, exercise training reduces serum leptin levels independent of changes in body fat mass, insulin, or glucocorticoids in type 2 diabetic patients.

As there is an increase in leptin levels with increasing adiposity suggesting that obesity is associated with leptin resistance, perhaps reductions in serum leptin levels with exercise training are due to an improvement of leptin resistance, which might make exercise therapy a more effective treatment in patients with type 2 diabetes.

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