Effect of long-term changes in diet and exercise on plasma leptin concentrations[1–3]

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ABSTRACT

Background: Although it is known that plasma leptin concentrations correlate with the amount of adipose tissue in the body, little information is available on the long-term effects on leptin concentrations of changes in diet and exercise.

Objective: We wanted to examine whether changes in dietary energy sources and exercise-mediated energy expenditure, alone or in combination, affect plasma leptin concentrations.

Design: In a randomized, 2 × 2 factorial trial, 186 men with metabolic syndrome were divided into 4 groups: diet, exercise, a combination of diet and exercise, and control. Data on dietary intake, physical fitness, and demographics were collected and plasma leptin concentrations were measured before and after a 1-y intervention period.

Results: Plasma leptin concentrations, body mass index, and fat mass decreased in association with long-term reductions in food intake as well as increased physical activity. By adjusting for either body mass index or fat mass, we observed a highly significant reduction in plasma leptin concentration after both the diet and the exercise interventions. There was no interaction between the interventions, suggesting a direct and additive effect of changes in diet and physical activity on plasma leptin concentrations.

Conclusion: Long-term changes in lifestyle consisting of decreased intake of dietary fat and increased physical activity reduced plasma leptin concentrations in humans beyond the reduction expected as a result of changes in fat mass. *Am J Clin Nutr* 2001;73:240–5.

KEY WORDS Leptin, diet, exercise, metabolic syndrome, men, weight loss, lifestyle

INTRODUCTION

Robust biological mechanisms that resist changes in body fat content are responsible for the weight regain that usually follows weight loss, provided that food is available (1, 2). Several hormones play important roles in keeping body weight stable (2). Leptin is one of the newly discovered hormones that may be of marked importance in the regulation of body fat (3). This 16-kDa peptide is expressed and secreted in proportion to adipocyte size and number and circulates in plasma in a concentration highly correlated with body fat mass (3, 4–7). Administration of recombinant leptin to mice with mutations in the leptin gene indicated that leptin participates in the regulation of food intake and energy expenditure (8–10). However, because there are large variations in leptin concentrations among individuals with similar body compositions, it is likely that factors other than adipose mass influence plasma leptin concentrations (5, 6, 11–13). Potential modifiers of leptin concentrations are energy-yielding nutrients such as fatty acids, carbohydrates, proteins, and alcohol. Most studies published so far indicate that fasting and refeeding may change plasma leptin concentrations, whereas little is known about the effect of specific nutrients in humans (14).

Physical activity is important for long-term regulation of body weight, partly because it increases the resting metabolic rate (15, 16). Weight reduction after physical exercise is correlated with reductions in plasma leptin concentrations in obese middle-aged women (17). However, results regarding the effects of exercise on plasma leptin concentrations, independent of fat mass, are conflicting (18–20).

The aim of the present study was to examine whether improvement in the cardiovascular disease risk factor profile induced by changes in lifestyle among sedentary individuals (21–23) has any effects on plasma leptin concentrations. We measured plasma leptin concentrations in men with moderately elevated blood pressure and lipid concentrations who were assigned to the single or combined intervention of physical training and diet for 1 y.

SUBJECTS AND METHODS

Study population and design

Samples and data were retrieved from the Oslo Diet and Exercise Study, a randomized, 2 × 2 factorial intervention trial (21, 22). The 2 interventions were physical exercise and dietary

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change, alone or in combination, lasting 1 y. The experimental design, recruitment of participants, and laboratory procedures are described in detail elsewhere (21, 22). The ethical principles of the Helsinki Declaration were followed and the trial was approved by the local ethical committee.

The trial included 186 men aged ($\bar{x}$ ± SD) 44.9 ± 2.5 y with mildly elevated diastolic blood pressure of 87.9 ± 8.1 mm Hg, plasma HDL-cholesterol concentration of 1.01 ± 0.17 mmol/L, triacylglycerol concentration of 2.28 ± 1.13 mmol/L, total cholesterol concentration of 6.3 ± 0.8 mmol/L, and body mass index (BMI; in kg/m^2) of 28.6 ± 3.4 (21). The participants were randomly allocated to the diet group (n = 44), the exercise group (n = 48), the combined diet and exercise group (n = 57), or the control group (n = 37).

Dietary counseling was provided to the participants in the diet group and the combined diet and exercise group at the start of the study and then after 3 and 9 mo. The advice was individually tailored according to dietary habits and risk factor profile. Increased consumption of fish and fish products, vegetables, and fiber-rich products containing complex carbohydrates and reduced intake of saturated fat and cholesterol were recommended.

The exercise program entailed supervised endurance exercise, such as aerobics, circuit training, and fast walking and jogging, 3 times/wk. Each workout lasted 60 min. The exercise group and the combined diet and exercise group were not separated during training. Attendance at each workout was recorded, as was the physical activity some participants did at home. This corresponded to an average of 1.8 h/wk throughout the year. Furthermore, all participants were interviewed at the end of the trial about changes in physical activity habits. The diet group and the control group did not change their physical activity habits during the 1-y period.

Laboratory procedures

Blood samples were collected between 0800 and 1000 after the subjects had fasted overnight and abstained from smoking and after they were recumbent for 10 min. Furthermore, the participants were told to abstain from vigorous exercise for 4 d before blood sampling. Cardiovascular disease risk factors were assessed in each participant before and after the 1-y intervention by means of a standard clinical examination performed by a cardiologist.

Euglobulin clot lysis time was measured in fresh plasma. All other indexes were measured in batches at the end of the trial from samples that had been stored at −70°C (20). Insulin was measured by radioimmunoassay (Linco Research, St Charles, MO) (24). Intraassay CVs were estimated to be 8% for measurements in the range of 0–144 pmol, 7% for measurements between 144 and 576 pmol, and 9% for measurements >576 pmol. Interassay CVs were 11%, 14%, and 12% at low, medium, and high (>996 pmol) concentrations, respectively. Other components (glucose, lipids, and factor VII) were quantified by standard methods (21, 22). Plasma leptin was measured by competitive radioimmunoassay (Linco Research) with use of [^{125}I]leptin as a tracer (25). The intraassay CV was 5.5% and the interassay CV was 3.8%.

Blood pressure was measured by using automatic equipment (Vita-Stat blood pressure monitor; VitaStat Medical Services Inc, Bellevue, WA) after the subjects had rested supine for 10 min. Aerobic capacity was estimated directly by using a modified Balke test protocol (26). Body weights were measured by using a Lindel balance scale (Samhald, Klippan, Sweden) while the participants wore only underclothes. Heights were measured at the same time while subjects were not wearing shoes. Percentage body fat was measured by using a body-composition analyzer (Futurex-5000; Futurex Inc, Gaithersburg, MD) based on near-infrared interactance (27). The height, weight, frame size, and activity level of each person were entered into the body-composition analyzer. Subjects were seated with their right forearm supported on a table and optical density levels were measured at the arterial midline of the right biceps. Output, which was programmed by the manufacturer using their standard equation, was recorded as percentage body fat. Intrasubject variation was 1–2%. By analyzing the change in percentage body fat resulting from the intervention, we minimized the overestimation of body fat in lean subjects and underestimation in obese subjects. Maximal oxygen uptake ($\dot{V}O_2$max) was measured during a treadmill test in which expired air was analyzed by using an MMC Horizon System (SensorMedics, Yorba Linda, CA; 21).

Dietary assessment was accomplished by using an optical-mark-readable food-frequency questionnaire that has been extensively validated (28, 29). All participants completed the food-frequency questionnaire both before and after the intervention. On the basis of the given frequencies of use and the self-estimated portion sizes (according to given alternatives in household measures), daily intakes of energy and nutrients were calculated by using a food database and software system at the Institute for Nutrition Research, University of Oslo. Cod liver oil was included in the nutrient calculations. To assess compliance with diet, plasma concentrations of fatty acids were also measured at the start and end of the trial. Smoking habits were estimated by measurement of serum thiocyanate concentrations and by self-report.

Statistics

At baseline, the mean plasma leptin concentration was lower in smokers (8.2 ± 4.0 mg/L, n = 63) than in nonsmokers (10.3 ± 7.4 mg/L, n = 122) (P = 0.048). Because neither self-reported smoking status nor plasma thiocyanate concentrations changed significantly during the course of intervention, and because the smokers were randomly distributed in all 4 intervention groups, smokers were not treated differently from nonsmokers within the same intervention group.

Because leptin values were skewed to the left, the values were logarithmically transformed. Fat mass (defined as body weight multiplied by percentage body fat) was skewed to the right and thus transformed values were also used for this variable. Response variables (leptin and fat mass) were defined as the differences between log values at baseline and after 1 y of intervention. Analysis of leptin response adjusted for BMI or fat mass was done by the following per subject calculation:

$$\text{Log}[(\text{leptin/fat mass})_{1\text{ y}}/(\text{leptin/fat mass})_{\text{baseline}}] \qquad (1)$$

Each of the response variables was then used as a dependent variable in a multiple regression model with the design variables of diet (yes or no), exercise (yes or no), and their cross product to test for interaction as regressors. First, a pretest of interaction was performed and if not significant, the interaction term was removed from the model. The model was then rerun with only the 2 main effects to be tested. Spearman's rank-order correlation coefficients were used to assess correlations with plasma leptin concentrations.

The data are presented as means ± SDs. P values <0.05 were considered statistically significant. Data were analyzed with use

TABLE 1
Plasma leptin concentrations, body mass index, and body fat mass at baseline and the change in these variables after a 1-y intervention[1]

Intervention	Leptin		BMI		Fat mass		Fat mass	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
	μg/L		*kg/m²*		*%*		*kg*	
Group data								
Control (n = 37)	12.0 ± 10.1	0.5 ± 4.6	28.8 ± 3.4	0.3 ± 0.8	24.5 ± 3.0	−0.1 ± 1.6	22.5 ± 4.8	0.05 ± 1.6
Diet (n = 44)	8.7 ± 4.3	−0.7 ± 3.0[2]	27.8 ± 3.5	−1.3 ± 1.5[3]	24.5 ± 3.4	−1.4 ± 2.2[3]	22.7 ± 4.9	−1.2 ± 2.0[3]
Exercise (n = 48)	9.8 ± 4.9	−0.4 ± 2.3	28.2 ± 3.3	−0.3 ± 1.3	24.9 ± 3.3	−1.0 ± 2.5[2]	22.5 ± 5.2	−0.9 ± 2.4[2]
Diet and exercise (n = 57)	9.1 ± 6.2	−2.2 ± 2.4[4]	26.2 ± 2.6	−1.8 ± 1.3[4]	24.1 ± 3.7	−2.4 ± 2.5[4]	20.5 ± 5.1	−1.1 ± 1.8[3]
Pooled data[5]								
Dietary[6] (n = 101)	8.9 ± 5.5	−1.7 ± 3.0[4]	28.6 ± 3.1	−1.8 ± 1.4[4]	24.3 ± 3.6	−2.0 ± 2.4[4]	22.1 ± 4.5	−1.8 ± 2.1[4]
Exercise[7] (n = 105)	9.4 ± 5.6	−1.4 ± 2.5[3]	28.3 ± 3.3	−1.1 ± 1.5[3]	24.5 ± 3.5	−1.8 ± 2.6[3]	22.0 ± 4.6	−1.6 ± 2.3[3]

[1] $\bar{x}$ ± SD.
[2] $P < 0.05$.
[3] $P < 0.01$
[4] $P < 0.001$.
[5] No significant effects were found with either nondietary or nonexercise intervention.
[6] Diet group plus diet and exercise group.
[7] Exercise group plus diet and exercise group.

of the computer program SIGMASTAT (version 2.0; Jandel Scientific Software, Erkarthm, Germany) and JMP STATISTIC for Apple Macintosh (version 3.2; SAS Institute Inc, Cary, NC).

RESULTS

Effect of intervention on plasma leptin concentration

After dietary intervention (n = 101: diet group and combined diet and exercise group), plasma leptin concentrations were reduced (**Table 1**). The change in plasma leptin concentration after dietary intervention was significantly greater than that after 1 y of nondietary intervention (ie, in the control group and the exercise group; $P < 0.001$). Likewise, the exercise intervention (n = 105: exercise group and combined diet and exercise group) reduced plasma leptin concentrations and the change in plasma leptin concentration after exercise intervention was significantly different from that after nonexercise intervention (P = 0.002). Logarithmic transformation of the plasma leptin concentration did not alter the conclusions for either intervention (dietary compared with nondietary or exercise compared with nonexercise).

BMI was lower after dietary intervention than after nondietary intervention ($P < 0.001$) and after exercise intervention than after nonexercise intervention (P = 0.005). Fat mass was also lower after dietary intervention ($P < 0.001$, calculated as percentage fat mass and kg body wt) and after exercise intervention [P = 0.002 (%) and P = 0.003 (kg)].

Change in the plasma leptin concentration correlated with change in BMI after both types of interventions (r = 0.397 for dietary intervention and r = 0.485 for exercise intervention, $P <$ 0.001 for both). Interestingly, change in the plasma leptin concentration correlated with change in percentage body fat after exercise intervention (r = 0.229, P = 0.02) but not after dietary intervention (r = 0.156). After adjustment for BMI and fat mass, either as percentage fat or in kg body wt, plasma leptin concentrations were reduced after both dietary intervention ($P < 0.001$ for all adjustments) and exercise intervention ($P < 0.001$ for BMI and fat mass in kg; P = 0.002 for percentage fat).

There were no significant interactions between the dietary and exercise interventions when calculated as simple responses on a logarithmic scale for leptin (P = 0.32) and fat mass (P = 0.89) or when calculated as responses adjusted for BMI (P = 0.32), percentage fat (P = 0.37), and fat mass (P = 0.35). This finding indicates that both types of interventions had independent effects on plasma leptin concentrations.

Dietary intake

Baseline values for intakes of energy and nutrients and changes after 1 y of intervention are shown in **Table 2**. Dietary advice promoted reduced intakes of total energy, protein, fat (including most types of fatty acids), cholesterol, alcohol, and sugar in the diet group and the combined diet and exercise group. The reported reductions in intake of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in the diet group were not significantly different from those in the combined diet and exercise group. The intake of 20–22-carbon n−3 fatty acids increased in both dietary intervention groups. The only significant change in dietary intake in the exercise group was a lower intake of fat as a percentage of total energy intake; intakes of other nutrients remained unchanged in the exercise and control groups during the 1-y intervention period.

Correlation between leptin and dietary intake

Neither baseline intake nor change in intakes of total energy, protein, carbohydrates, and cholesterol correlated with the plasma leptin concentration or with change in the plasma leptin concentration in any intervention group. At baseline, the plasma leptin concentration correlated positively with the intake of vitamin A (r = 0.152, P = 0.04) and negatively with the intake of alcohol (r = −0.155, P = 0.04) for all subjects (n = 180). The reduced intake of fat and fatty acids, including saturated, monounsaturated, and polyunsaturated fatty acids, correlated positively with change in the plasma leptin concentration in the diet group (data not shown).

Reductions in intakes of total energy and dietary fat correlated with change in the plasma leptin concentration after both dietary intervention and nonexercise intervention. Reductions in intakes

TABLE 2
Intakes of energy and nutrients[1]

	Control group (n = 36)		Diet group (n = 43)		Exercise group (n = 47)		Diet and exercise group (n = 54)	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Energy (MJ)	10.1 ± 2.7	−0.3 ± 2.7	11.1 ± 3.5	−2.3 ± 2.6[2]	10.6 ± 2.6	−0.5 ± 2.8	10.2 ± 2.6	−1.6 ± 2.9[2]
Protein (g)	99.0 ± 26.6	−2.1 ± 28.6	106.5 ± 40.3	−13.4 ± 29.9[3]	100.0 ± 18.7	−4.2 ± 25.6	100.2 ± 26.9	−7.7 ± 25.6[4]
Fat								
(g)	90.1 ± 25.7	−3.2 ± 30.2	102.0 ± 37.8	−32.5 ± 31.4[2]	88.8 ± 26.9	−7.4 ± 33.8	90.4 ± 28.1	−26.5 ± 28.9[2]
(% of energy)	33.4 ± 5.6	−0.7 ± 4.8	33.7 ± 4.7	−5.1 ± 5.4[2]	31.0 ± 4.5	−1.6 ± 4.8[4]	32.3 ± 5.2	−5.3 ± 5.4[2]
Carbohydrate (g)	270.3 ± 86.5	−6.8 ± 76.8	297.9 ± 92.2	−42.3 ± 71.6[2]	301.2 ± 82.5	−11.1 ± 78.3	284.3 ± 78.4	−24.6 ± 95.4
Sugar (g)	39.1 ± 28.9	−6.4 ± 29.0	41.9 ± 25.1	−16.8 ± 22.9[2]	44.5 ± 32.6	−5.3 ± 27.5	47.8 ± 34.1	−19.6 ± 33.8[2]
Alcohol (g)	11.8 ± 19.5	−0.6 ± 3.7	11.5 ± 9.4	−2.5 ± 5.3[3]	12.4 ± 17.7	2.3 ± 13.3	12.3 ± 15.2	−2.8 ± 11.0
Cholesterol (mg)	439 ± 131	−15 ± 160	462 ± 192	−121 ± 149[2]	414 ± 117	−25 ± 115	412 ± 118	−94 ± 130[2]
Vitamin A (mg)	1.8 ± 1.4	0.2 ± 1.6	1.8 ± 1.6	−0.4 ± 1.4	1.9 ± 1.27	−0.1 ± 1.1	1.8 ± 1.2	−0.2 ± 1.3
Vitamin D (μg)	6.2 ± 3.8	−0.2 ± 3.5	6.4 ± 4.5	−0.1 ± 3.6	7.0 ± 5.1	0.2 ± 4.4	6.7 ± 5.8	0.8 ± 6.3
Vitamin C (mg)	123 ± 70	−16 ± 51	155 ± 121	−9 ± 98	134 ± 77	−1.0 ± 82	109 ± 48	16 ± 85
Fatty acids								
Saturated (g)	34.2 ± 9.8	−0.9 ± 12.4	38.5 ± 14.7	−13.6 ± 12.6[2]	34.6 ± 13.2	−3.0 ± 12.9	34.8 ± 11.7	−12.1 ± 10.7[2]
Monosaturated (g)	33.1 ± 9.9	−1.6 ± 11.2	37.2 ± 13.6	−12.3 ± 11.3[2]	31.9 ± 9.2	−2.9 ± 12.3	33.0 ± 10.5	−10.2 ± 11.3[2]
Polyunsaturated (g)	16.0 ± 5.8	−0.5 ± 5.7	18.8 ± 8.0	−4.9 ± 6.6[4]	15.6 ± 5.6	−1.2 ± 7.7	15.9 ± 5.8	−2.9 ± 6.9[3]
18:2n−6	11.9 ± 4.7	−0.4 ± 4.4	14.1 ± 5.9	−4.0 ± 5.0[2]	11.4 ± 4.9	−1.0 ± 6.1	11.9 ± 4.9	−2.6 ± 5.8[3]
18:3n−3	1.6 ± 0.7	−0.1 ± 0.6	1.9 ± 0.8	−0.5 ± 0.7[2]	1.5 ± 0.6	−0.1 ± 0.8	1.6 ± 0.7	−0.3 ± 0.7[3]
20:4n−6	0.21 ± 0.07	0.00 ± 0.09	0.25 ± 0.14	−0.07 ± 0.11[2]	0.19 ± 0.05	−0.02 ± 0.06	0.20 ± 0.06	−0.04 ± 0.07[2]
20:5n−3	0.24 ± 0.27	−0.01 ± 0.19	0.22 ± 0.20	0.08 ± 0.22[4]	0.26 ± 0.25	0.03 ± 0.20	0.24 ± 0.24	0.11 ± 0.29[3]
22:5n−3	0.05 ± 0.03	0.00 ± 0.02	0.05 ± 0.04	0.00 ± 0.03	0.05 ± 0.03	0.00 ± 0.03	0.05 ± 0.04	0.01 ± 0.04
22:6n−3	0.45 ± 0.38	−0.02 ± 0.27	0.44 ± 0.33	0.11 ± 0.31[4]	0.49 ± 0.38	0.04 ± 0.33	0.44 ± 0.38	0.19 ± 0.47[3]
20–22:n−3	0.73 ± 0.68	−0.03 ± 0.48	0.70 ± 0.56	0.19 ± 0.55[4]	0.80 ± 0.66	0.07 ± 0.56	0.73 ± 0.65	0.30 ± 0.79[3]

[1] $\bar{x}$ ± SD.
[2] $P \leq 0.001$.
[3] $P \leq 0.01$.
[4] $P \leq 0.05$.

of total energy and carbohydrate, but not change in fat intake, correlated with change in the plasma leptin concentration after exercise intervention (**Table 3**).

Correlation between leptin and cardiovascular disease risk factors

We found a strong correlation at baseline between the plasma concentrations of leptin and insulin and between the plasma concentrations of leptin and glucose (both fasting and after a standard 75-g oral glucose load). No significant correlation was observed between plasma concentrations of HDL_2, HDL_3, LDL cholesterol, or lipoprotein(a) and leptin, either at baseline or after the intervention (data not shown).

Analysis by 2 × 2 factorial design showed a significant correlation between changes in the plasma concentrations of leptin and insulin (fasting) in all groups (**Table 4**). After exercise intervention, changes in the plasma insulin concentration (after a glucose load) and in cholesterol failed to correlate with change in the plasma leptin concentration, in contrast with the finding in the other groups. Change in the plasma concentration of glucose (fasting), the plasma concentration of apolipoprotein A-I, and $\dot{V}O_2$max correlated with change in the plasma leptin concentration only after exercise intervention. After both dietary and exercise intervention, change in blood pressure (systolic and diastolic) correlated with change in the plasma leptin concentration.

TABLE 3
Correlation between change in nutrient intake and change in the plasma leptin concentration after 1 y of intervention[1]

	Intervention			
	Dietary (n = 97)	Nondietary (n = 83)	Exercise (n = 101)	Nonexercise (n = 79)
Energy (MJ)	0.239[2]	0.150	0.207[2]	0.264[2]
Fat				
(g)	0.222[2]	0.156	0.153	0.375[3]
(% of energy)	0.080	0.096	0.030	0.379[3]
Carbohydrate (g)	0.194	0.152	0.240[2]	0.139
Sugar (g)	0.123	0.099	0.099	0.255[2]

[1] Analyses were based on factorial design. Dietary intervention = diet group plus diet and exercise group; exercise intervention = exercise group plus diet and exercise group.
[2] $P \leq 0.05$.
[3] $P \leq 0.001$.

DISCUSSION

This is the first study to investigate the effect of long-term changes in lifestyle components such as diet and exercise, and in particular the combination of the 2, on plasma leptin concentrations. Both interventions had a strong effect, reducing the plasma leptin concentration beyond the effect expected as a result of changes in body weight and fat mass.

After dietary intervention, leptin, BMI, and fat mass were reduced, and change in the plasma leptin concentration correlated with change in BMI. However, change in the plasma leptin concentration failed to correlate with change in percentage body

TABLE 4
Correlation between change in hemostatic variables and plasma lipid concentrations and change in the plasma leptin concentration[1]

	Intervention			
	Dietary (n = 100)	Nondietary (n = 85)	Exercise (n = 104)	Nonexercise (n = 81)
Insulin (pmol/L)				
BL	0.383[2]	0.287[2]	0.384[2]	0.325[3]
AL	0.284[3]	0.324[3]	0.186	0.453[2]
Glucose (mmol/L)				
BL	0.159	0.116	0.271[3]	0.095
AL	0.237[4]	0.164	0.384[2]	0.232[4]
Total cholesterol (mmol/L)	0.221[4]	0.270[3]	0.165	0.417[2]
Apolipoprotein A-I (g/L)	0.172	0.146	0.258[3]	0.151
Apolipoprotein B (g/L)	−0.255[3]	−0.308[3]	−0.277[3]	−0.379[2]
Factor VII (%)	0.144	0.181	−0.038	0.480[2]
Systolic blood pressure (mm Hg)	0.255[3]	−0.050	0.254[3]	0.177
Diastolic blood pressure (mm Hg)	0.256[3]	0.119	0.288[3]	0.262[4]
$\dot{V}O_2$max (mL/kg·min)	−0.109	−0.210	−0.371[2]	0.105

[1] Correlation coeffiecients were calculated on the basis of changes after 1 y of intervention. Analyses were based on factorial design. Dietary intervention-diet group plus diet and exercise group; exercise intervention-exercise group plus diet and exercise group; BL, before load, AL, after load; $\dot{V}$O2 max, maximal oxygen uptake.
[2] $P \leq 0.001$.
[3] $P \leq 0.01$.
[4] $P \leq 0.05$.

fat resulting from dietary intervention, suggesting a direct effect of dietary fat on the plasma leptin concentration. Several explanations are possible for this effect of dietary fat. We assumed that there was no time bias in reported dietary intake because there was a 1-y period between each report; thus, participants were unlikely to have remembered the details of their first report when completing the food-frequency questionnaire the second time. During this study period, however, the participants might have been influenced by being participants in the study and may have underestimated their dietary intakes at the end of the intervention. The mechanism by which the accumulation of triacylglycerol influences the expression of leptin in adipose tissue is not known. In humans and in rodents, concentrations of circulating leptin are reduced after fasting and increased after overfeeding (30). During relatively short-term fasting and refeeding experiments, Kolaczynski et al (30) found that BMI values were not markedly altered, suggesting that the leptin concentration may be regulated by factors other than body fat. Leptin concentrations do not change after normal meal consumption (6), but dietary changes over a period of 1 y may modify plasma leptin concentrations and energy balance.

Reductions in intakes of fat and fatty acids in the combined diet and exercise group were similar to those in the diet group (Table 2); however, no correlation was found between change in the plasma leptin concentration and fat intake (as shown by the nondietary and exercise interventions; Table 3). This may suggest that increased physical activity overrides the correlation between the plasma leptin concentration and dietary fat intake. Physical activity may reduce leptin messenger RNA expression in rats (31) and lower the abdominal tissue leptin production rate in humans (32). In the present study, we observed a reduction in plasma leptin concentrations, BMI, and fat mass after exercise intervention; however, the effect on leptin concentration was also strongly significant after adjustment for either BMI or fat mass.

Lifestyle changes such as those in this randomized trial were reported previously to improve carbohydrate metabolism, reduce insulin resistance (33), and reduce blood pressure in persons with hypertension (23). Considine (34) hypothesized that changes in energy intake or expenditure may be detected by the adipocyte and thus influence synthesis of leptin via insulin, corticoids, and epinephrine. A training program that improves insulin sensitivity could alter leptin concentrations independently of adipose tissue mass (34). Exercise is often added to energy restriction in the treatment of obesity and it also has preventive effects on the development of diabetes (35, 36). The combined diet and exercise intervention reduced insulin resistance in our patients with metabolic syndrome (33) and we observed a strong correlation between change in the plasma leptin concentration and changes in insulin and glucose. Pasman and Saris (19) studied the effect of long-term exercise training on leptin concentration and concluded that regular exercise allows "resetting" of the leptin concentration so that a lower concentration can be maintained at a certain body fat content. In a study of well-trained runners, short-term exercise was found to have no detectable effect on serum leptin concentrations (18). Physical activity is known to affect sympathetic nerve signals (37). An increase in sympathetic nerve activity promotes down-regulation of plasma leptin concentrations (38), but the dynamics between changes in energy metabolism and leptin is not understood. We conclude that long-term diet and exercise interventions may have direct effects on the plasma leptin concentration beyond the effect expected due to changes in fat mass.

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