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Plasma leptin in moderately obese men: independent effects of weight loss and aerobic exercise

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Thong, Farah S. L., Robert Hudson, Robert Ross, Ian Janssen, and Terry E. Graham. Plasma leptin in moderately obese men: independent effects of weight loss and aerobic exercise. *Am J Physiol Endocrinol Metab* 279: E307–E313, 2000.—The independent effects of weight loss and exercise on plasma leptin and total (AT), subcutaneous (SAT), and visceral (VAT) adipose tissue were investigated in 52 obese men. Subjects were randomly assigned to four 12-wk protocols: *1*) control (C, $n = 8$), *2*) diet-induced weight loss (DWL, $n = 14$), *3*) exercise-induced weight loss (EWL, $n = 14$), and *4*) exercise with weight maintenance (EWS, $n = 16$). Plasma leptin was unchanged in C (from 7.8 ± 1.3 to 7.7 ± 1.0 ng/ml). Equivalent weight loss (7.5 kg) decreased leptin significantly but similarly (DWL, from 8.5 ± 1.0 to 4.8 ± 0.6 ng/ml; EWL, from 10.1 ± 1.0 to 5.0 ± 0.6 ng/ml). Exercise in the absence of weight loss did not alter leptin levels (from 10.1 ± 1.3 to 9.2 ± 1.2 ng/ml). Changes in leptin correlated with changes in AT and SAT (both $P < 0.05$) but not in VAT. We conclude that reduction in adipose tissue after weight loss results in a collateral decrease in circulating leptin, and exercise, independent of its effects on weight loss, has no profound influence on leptin secretion.

ob gene; caloric restriction; adipose tissue; regional fat distribution; body composition; weight control

LEPTIN IS SECRETED IN A pulsatile manner (25, 29) by adipose tissue in proportion to its size, providing a feedback signal in the regulation of body weight homeostasis (5, 17, 21, 31). Exogenous administration of leptin to obese and lean rodents induces weight loss through suppression of appetite, reduction of adipose tissue mass, and increased thermogenesis (9, 21). Despite intensive research, the pathophysiological role of leptin in human obesity remains to be delineated. Paradoxically, *ob* gene expression and leptin levels in obese humans reflect total adiposity, suggesting that human obesity is not associated with leptin deficiency, but likely with insensitivity to endogenous leptin, possibly because of an inability of leptin to cross the blood-brain barrier and/or a defect in leptin receptor signaling (2, 27). In addition to fat mass, insulin (14, 28), glucocorticoids (6, 13), and sex hormones (3) are factors thought to be important in the regulation of leptin expression and secretion. Regional fat differences may contribute to this control mechanism, because subcutaneous adipocytes express and secrete more leptin compared with visceral adipocytes, possibly because of their intrinsic properties or distinct regional regulation (18, 19).

In humans, leptin levels decrease in response to weight loss (4, 5, 30). Similarly, short-term fasting decreases and overfeeding increases leptin levels, but these changes are disproportionate to changes in adiposity (1, 7, 15). Because changes in energy intake can up- or downregulate leptin expression, alterations in energy expenditure via exercise might also influence leptin levels. Plasma leptin levels are unaltered by an acute bout of exercise (10, 16, 22). Plasma leptin decreased after a 20-wk endurance training program, but this was attributed to a loss of fat mass (22). In contrast, plasma leptin was decreased after an ultramarathon race (16) and after a 12-wk endurance training program in females, but not in males (11). In obese men, the number of training hours per week correlated significantly with changes found in plasma leptin, which led the authors to conclude that exercise training, independent of body fat, decreased circulating leptin levels (20). However, the possibility cannot be excluded that the reduction of leptin is secondary to changes in energy balance rather than to exercise itself.

The direct impact of exercise on *ob* gene expression and leptin secretion, independent of its effects on energy balance, has not been explored in humans. Therefore, the purpose of the present study was to examine the independent effects of diet and exercise on plasma leptin levels in moderately obese men in the presence or absence of weight loss. Because *ob* gene expression and leptin secretion by regional fat depot are distinct,

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we also investigated the response of plasma leptin to weight loss and exercise in relation to alterations in subcutaneous and visceral adipose tissue.

MATERIALS AND METHODS

Subjects. Fifty-two sedentary males with upper body obesity participated in this study [age, 44.4 ± 1.8 yr; body wt, 98.4 ± 1.2 kg; body mass index (BMI), 31.4 ± 0.3 kg/m^2; waist-to-hip ratio (WHR), 1.00 ± 0.01]. The subjects' characteristics before the study are presented in Table 1. All subjects underwent a preparticipation medical exam, including screening for normal glucose tolerance and plasma lipid profile. The protocol for this study was approved by the Queen's University Ethics Committee, and informed written consent was obtained from each subject.

Experimental protocol. A detailed description of this study is described elsewhere (23). Briefly, the subjects were randomly assigned to one of the four following conditions for 12 wk: *1*) control (C), *2*) diet-induced weight loss (DWL), *3*) exercise-weight stable (EWS), and *4*) exercise-induced weight loss (EWL). All subjects followed a weight maintenance diet for a 4- to 5-wk baseline period. All diets consisted of ~55–60% carbohydrate, 15–20% protein and 20–25% fat. To ensure accuracy of the prescribed energy requirement, body weight was monitored during this period. The C group was asked to maintain body weight by consuming a weight maintenance diet during the 12-wk period. The weight loss program was designed to achieve 0.6 kg weight loss/wk. The subjects in the DWL group were asked to reduce the weight maintenance diet by 700 kcal/day. The EWL group was required to consume a weight maintenance diet of 700 kcal/day in conjunction with daily exercise sessions. Thus the negative energy balance in the EWL group was designed to match that of the DWL group. To maintain body weight in the EWS group, energy expended during the daily exercise sessions (~700 kcal/day) was matched by a concomitant increase in caloric intake. Subjects were required for the duration of the study to keep detailed daily food records, which were analyzed and reviewed by a dietitian. Body weight was used as a means to verify adherence to the respective treatments by all subjects. In *weeks 6* and *7*, when doubly labeled water measurements were made, diet records were analyzed with the Food Processor software (ESHA Research, Salem, OR). Detailed descriptions of the method and results are presented in detail elsewhere (23).

Maximal aerobic capacity. Maximal O_2 uptake ($\dot{V}O_{2\,max}$) was assessed with a graded test on a motorized treadmill. Depending on the subject's capabilities, the initial workload was set at a constant speed of 4.8 to 6.9 km/h, and the grade was set at 0%. After the initial 2 min, the grade was increased by 2% every 2 min for 4 min and then by 1% every 2 min thereafter until volitional exhaustion. $\dot{V}O_2$ was measured with a SensorMedics 2900 metabolic cart (SensorMedics, Yorba Linda, CA). In all subjects, $\dot{V}O_{2\,max}$ was measured before treatment and at *weeks 4* and *8* (EWL and EWS only) after treatment.

Exercise protocol. The exercise program consisted of supervised brisk walking or jogging on a motorized treadmill daily for 12 wk. The duration of the exercise session was determined by the time required to expend 700 kcal. The subjects exercised at an intensity that corresponded to no more than 75% of their respective $\dot{V}O_{2\,max}$ (~80% maximal heart rate). Energy expenditure was determined by heart rate and $\dot{V}O_2$ data obtained during the pretreatment exercise test and was adjusted from the results of the subsequent tests performed at *weeks 4* and *8*. Heart rate was measured every 5 min during each exercise session with an automated heart rate monitor (Polar Oy, Finland).

Magnetic resonance imaging. The detailed procedure for obtaining whole body MRI data is described elsewhere (24). Briefly, the data were obtained with a General Electric 1.5-tesla magnet by use of T1-weighted spin echo pulse sequences. The MRI data were analyzed with specifically designed computer software (Tomovision). The results presented are converted from volume (liters) to mass (kg) by multiplying the volumes by the assumed constant density for fat (0.92 kg/l), fat-free skeletal muscle (1.04 kg/l), and fat free lean tissue density (1.08 kg/l). Detailed MRI results are presented elsewhere (23).

Blood sampling. Blood samples were obtained from all subjects at 0800 after an overnight fast before and after treatment. All subjects were asked to consume a weight maintenance diet for 3 days and to avoid strenuous exercise for 4 days. Posttreatment blood samples from subjects in the exercise groups were obtained 4 days after their last exercise sessions.

Blood analysis. Fasting plasma leptin was assayed by means of a ^{125}I RIA kit for human leptin (Linco Research, St. Charles, MO). The minimal detectable limit of the leptin assay kit is 0.5 ng/ml. Plasma leptin concentrations were determined as the average of duplicate determinations. To minimize the effects of assay variability, samples from each subject were analyzed with the same assay. The intra- and interassay coefficients of variation were 5 and 4%, respectively.

Table 1. *Descriptive characteristics*

	Control (n = 8)	DWL (n = 14)	EWS (n = 14)	EWL (n = 16)
Anthropometric				
Age, yr	46.0 ± 3.8	42.6 ± 2.6	44.7 ± 2.0	45.0 ± 1.9
Weight, kg	96.7 ± 3.2	96.1 ± 2.4	97.9 ± 2.4	101.5 ± 2.0
BMI, kg/m^2	30.7 ± 0.6	30.7 ± 0.5	31.3 ± 0.6	32.3 ± 0.5
Waist circumference, cm	108.7 ± 1.7	109.1 ± 1.5	110.0 ± 1.8	112.0 ± 1.2
WHR	1.00 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	1.01 ± 0.01
$\dot{V}O_{2\,max}$, ml · min^{-1} · kg^{-1}	38.0 ± 2.3	37.7 ± 1.1	38.0 ± 1.7	37.6 ± 1.9
MRI, kg				
Total AT	30.5 ± 1.6	28.4 ± 1.3	30.6 ± 1.8	33.1 ± 1.4
Subcutaneous AT	22.3 ± 1.4	21.8 ± 1.3	23.4 ± 1.4	24.9 ± 1.3
Visceral AT	4.1 ± 0.6	3.2 ± 1.0	3.4 ± 0.3	3.9 ± 0.2

Values are means ± SE. DWL, diet-induced weight loss; EWS, exercise-weight stable; EWL, exercise-induced weight loss; BMI, body mass index; WHR, waist-to-hip ratio; $\dot{V}O_{2\,max}$, maximal O_2 uptake; AT, adipose tissue.

Table 2. *Absolute and relative changes in body composition and $\dot{V}O_{2max}$ in response to diet and exercise*

	Control (n = 8)		DWL (n = 14)		EWS (n = 14)		EWL (n = 16)	
	Abs	%	Abs	%	Abs	%	Abs	%
Weight, kg	0.1 ± 0.3	0.1 ± 0.3	−7.4 ± 0.2*,†	−7.7 ± 0.3*,†	−0.6 ± 0.2‡,§	−0.6 ± 0.2‡,§	−7.6 ± 0.1*,†	−7.5 ± 0.2*,†
BMI, kg/m^2	−0.03 ± 0.1	−0.2 ± 0.4	−2.4 ± 0.1*,†	−7.7 ± 0.3*,†	−0.3 ± 0.1‡,§	−0.9 ± 0.3‡,§	−2.4 ± 0.1*,†	−7.5 ± 0.3*,†
WHR	−0.001 ± 0.01	−0.1 ± 0.5	−0.04 ± 0.01*,†	−4.1 ± 0.5*,†	−0.01 ± 0.01‡	−1.3 ± 0.5‡	−0.03 ± 0.01*	−2.7 ± 0.5*
$\dot{V}O_{2max}$, ml · kg^{-1} · min^{-1}	0.07 ± 1.3	1.2 ± 3.3	0.8 ± 0.9†,§	1.9 ± 2.6†,§	7.3 ± 1.5*,‡	19.6 ± 3.9*,‡	8.8 ± 0.9*,‡	24.5 ± 2.7*,‡

Values are means ± SE. Abs, absolute; %, relative. $P < 0.05$: *vs. Control, †vs. EWS, ‡vs. DWL, §vs. EWL.

Statistical analysis. Statistical analyses were performed with the use of an SAS statistical package (Cary). Plasma leptin concentrations were log-transformed to normalize the distribution. Within-group differences were made with a one-way ANOVA; comparison between groups was assessed by two-way repeated-measures ANOVA. Where applicable, a Tukey's post hoc comparison was made. Regression analyses, Pearson product-moment correlation, and partial correlation coefficients were used to evaluate associations among different variables. Statistical significance was accepted at $P < 0.05$. All data presented are means ± SE, unless otherwise indicated.

RESULTS

The descriptive characteristics of the 52 men who participated in this study are presented in Table 1. All 52 men were similar in all anthropometric measures, maximal aerobic capacity, adipose tissue, and skeletal muscle mass. There were no significant differences detected in baseline characteristics between groups.

Weight loss and anthropometric variables. Body weight was maintained in both C and EWS groups (Table 2). Mean weight loss in DWL and EWL were 7.4 ± 0.2 and 7.6 ± 0.1 kg, respectively, which represents a decrease of 7.7 ± 0.3 and 7.5 ± 0.3% of their initial respective weights and was not significantly different between groups. Similarly, BMI was maintained in both C and EWS groups. As shown in Table 2, mean BMI decreased by 2.4 ± 0.1 kg/m^2 (−7.7 ± 0.3%) in DWL and 2.4 ± 0.1 kg/m^2 (−7.5 ± 0.3%) in EWL.

Exercise training. Adherence to the exercise training program was similar between EWS (98.0 ± 0.6%) and EWL (98.4 ± 0.3%). The average duration (EWS, 63.3 ± 2.0 min vs. EWL, 60.4 ± 2.5 min) and intensity (EWS, 77.0 ± 1.2% vs. EWL, 77.0 ± 1.0% $\dot{V}O_{2\,max}$) of the exercise sessions were not significantly different between these groups. Similarly, energy expended during the exercise sessions was similar between EWS (692 ± 3 kcal) and EWL (698 ± 3 kcal). The exercise protocol resulted in significant but similar improvement in $\dot{V}O_{2\,max}$ in the subjects in the two exercise groups (Table 2). The improvements in $\dot{V}O_{2\,max}$ in EWS (19.6 ± 3.9%) and in EWL (24.5 ± 2.7%) were significantly different compared with C and DWL (Table 2). $\dot{V}O_{2\,max}$ remained unchanged in both C and DWL.

Plasma leptin. As shown in Fig. 1, baseline leptin concentrations were similar between the groups. Plasma leptin remained unchanged in C. A significant but similar decrease in plasma leptin was observed after weight loss in DWL (from 8.5 ± 1.0 to 4.8 ± 0.6 ng/ml) and in EWL (from 10.1 ± 1.0 to 5.0 ± 0.6 ng/ml). The reductions in plasma leptin after weight loss (DWL, 42 ± 3%; EWL, 51 ± 3%) were significantly different compared with C and EWS. Conversely, exercise without any weight loss (EWS) had no effect on plasma leptin levels (before, 10.1 ± 1.3 ng/ml; after, 9.2 ± 1.2 ng/ml), and this did not significantly differ from C (Fig. 1). There were wide interindividual differences in leptin response to exercise in the absence of weight loss, with the majority of individuals showing no change, whereas several individuals showed increases and others decreases in plasma leptin in response to exercise.

Adipose tissues. The absolute and relative changes in adipose tissue are presented in Table 3. There were no significant changes in adipose tissue mass observed in C. The principal effect of weight loss was reduction in adipose tissue in DWL and EWL, with a greater reduction observed in EWL than in DWL ($P < 0.05$). This difference remained significant despite controlling for pretreatment values. Exercise without weight loss (EWS) resulted in a significant decrease in adipose tissue mass, but this reduction was not significantly different compared with C. Weight loss, regardless of the manner in which it was achieved, resulted in similar but significant reduction in total subcutaneous (SAT) and visceral (VAT) adipose tissue. These reductions were significantly greater compared with C and EWS. VAT was significantly reduced in EWS, and this reduction was significantly greater compared with C

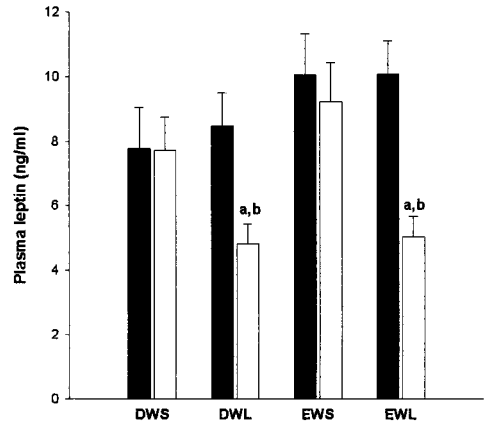

Fig. 1. Baseline (filled bars) and posttreatment (open bars) plasma leptin concentrations in control [diet-weight stable (DWS)], diet-weight loss (DWL), exercise-weight stable (EWS), and exercise-weight loss (EWL). a, $P < 0.05$ vs. pretreatment (within group); b, $P < 0.05$ vs. posttreatment DWS and post-EWS (between groups).

Table 3. *Absolute and relative changes in total AT, SAT, and VAT in response to diet and exercise*

	Control (n = 8)		DWL (n = 14)		EWS (n = 14)		EWL (n = 16)	
	Abs	%	Abs	%	Abs	%	Abs	%
AT	−0.6 ± 0.2	−2.2 ± 0.7	−4.8 ± 0.3*,†,‡	−17.0 ± 1.0*,†,‡	−1.5 ± 0.3‡,§	−5.2 ± 0.8‡,§	−6.1 ± 0.4*,†,§	−18.7 ± 1.2*,†,§
SAT	−0.2 ± 0.1	−0.9 ± 0.5	−3.4 ± 0.3*,†	−15.7 ± 1.0*,†	−0.8 ± 0.2‡,§	−3.6 ± 0.8‡,§	−4.2 ± 0.3*,†	−17.0 ± 1.0*,†
VAT	−0.003 ± 0.1	−1.9 ± 2.7	−0.8 ± 0.1*,†	−25.2 ± 2.0*,†	−0.4 ± 0.1*,‡,§	−13.6 ± 2.3*,‡,§	−1.1 ± 0.1*,†	−27.5 ± 1.9*,†

Values are means ± SE; absolute changes are in kg. AT, total adipose tissue; SAT, subcutaneous AT; VAT, visceral AT. $P < 0.05$: *vs. Control, †vs. EWS, ‡vs. EWL, §vs. DWL.

(Table 3). Detailed MRI results are presented elsewhere (23).

Relationship of leptin to adipose tissue, regional fat distribution, and exercise parameters. Baseline plasma leptin was positively correlated with AT ($r^2 = 0.33$, $P < 0.001$; Fig. 2*A*) and SAT mass ($r^2 = 0.35$, $P < 0.001$; Fig. 2*B*), and these relationships remained significant after either exercise or weight loss (Fig. 2, *A* and *B*). Plasma leptin was not significantly correlated with VAT before treatment ($r^2 = 0.002$, $P = 0.76$), but a positive correlation was observed after treatment ($r^2 = 0.09$, $P = 0.03$; Fig. 2*C*). Weight loss-induced reductions in leptin in DWL and EWL were positively correlated with reductions in AT mass ($r^2 = 0.32$, $P < 0.001$) and with reductions in SAT (r^2=0.35, $P < 0.001$); however, a correlation between changes in leptin and VAT was not found (r^2=0.02, $P = 0.46$). On the other hand, when the C group was included in the regression analysis, a significant correlation was observed between changes in leptin and changes in VAT ($r^2 = 0.09$, $P = 0.03$). Plasma leptin was not significantly correlated with $\dot{V}O_{2\,max}$ ($r^2 = 0.02$, $P = 0.27$). Changes in leptin in EWS and EWL were not significantly correlated with the duration of the exercise sessions ($r^2 = 0.003$, $P = 0.77$). This relationship remained nonsignificant ($r^2 = -0.24$, $P = 0.21$), even after adjusting for changes in adipose tissue mass. Similarly, exercise intensity ($r^2 = 0.27$, $P = 0.17$) and energy expended ($r^2 = 0.20$, $P = 0.28$) via exercise were not significantly correlated with plasma leptin with or without normalizing for changes in adipose tissue mass.

DISCUSSION

Leptin, the *ob* gene product, circulates in proportion to adipose tissue size, thus providing a feedback mechanism in the regulation of body weight homeostasis (5, 17, 21, 31). Short-term fasting decreases or overfeeding increases leptin levels without any noticeable changes in weight (1, 7, 15). Similarly, weight loss induced by caloric restriction reduces circulating leptin levels (4, 5, 30). These findings suggest that short-term and chronic changes in energy balance can modulate *ob* gene expression and leptin secretion. However, reports on leptin response to exercise in humans have been conflicting (4, 10, 11, 16, 20, 22). Previous studies of the effects of exercise on leptin response have been hindered by the inability to dissociate the effects of exercise itself from the confounding effects of energy balance.

In the present study, the treatment groups were well matched, and in particular, the negative energy balance induced by energy expended via exercise was carefully matched with that induced by caloric restriction. Thus the tight control of the present study permitted us to dissociate the leptin response to exercise itself from its response to energy balance and changes in fat mass. *Ob* gene expression and leptin secretion are higher in subcutaneous than in visceral adipocytes. In addition to adipocyte size, intrinsic differences between regional fat depots, including lipolytic response to β-adrenergic stimuli and insulin, must also contribute to *ob* gene expression (18, 19). The present study also permitted us to examine the effects of exercise in the presence and absence of weight loss on leptin response in relation to that of subcutaneous and visceral adipose tissues.

In the present study, weight loss (~7.5 kg) induced by caloric restriction was similar to that achieved by energy expended via exercise. Our results indicate that, in moderately obese men, a collateral decrease in plasma leptin was observed with weight loss regardless of the manner in which it was achieved. Observations of decreases in circulating leptin levels after diet-induced weight loss concur with those previously reported in the literature (4, 5, 30). In the present study, chronic exercise (12 wk of aerobic conditioning), independent of its effects on weight loss, had no effect on circulating leptin levels in moderately obese men. The results of our study are supported by numerous reports that acute and chronic exercise has no effect on circulating leptin levels in men (10, 11, 22) and in postmenopausal women (4).

On the other hand, Hickey et al. (11) reported a 17.5% reduction in serum leptin selectively in women but not in men after 12 wk of aerobic training without a concomitant change in adipose tissue mass. It is possible that gender differences, including the gender-dependent lipolytic regulation of adipose tissue by the hormonal milieu, could explain the selective response of leptin to exercise in their female subjects. However, in contrast, Christensen et al. (4) found no effect of addition of exercise to diet-induced weight loss compared with weight loss achieved by caloric restriction alone in postmenopausal women. Furthermore, in the study by Hickey et al., although the increased energy expended via exercise was compensated for by a concomitant increase in caloric intake in males, there was virtually no change in caloric intake in the females after exercise training, and yet, paradoxically, the fe-

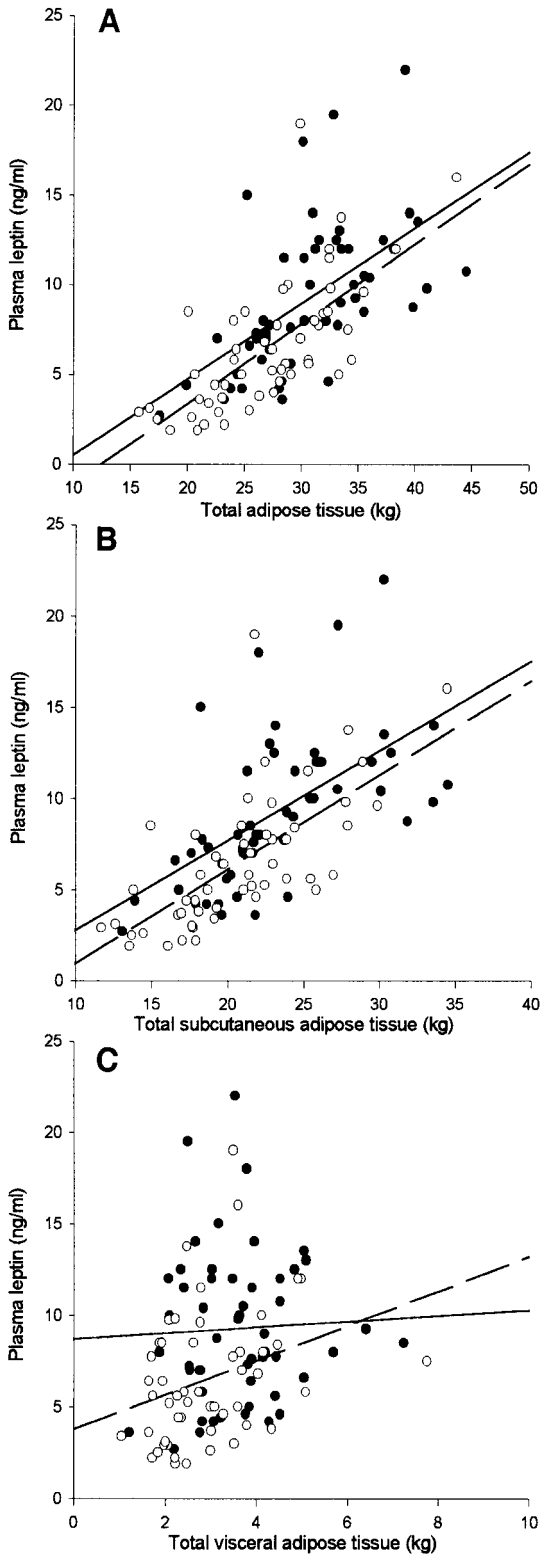

Fig. 2. Regression analyses of plasma leptin pretreatment (pre-, ●, solid lines) and posttreatment (post-, ○, broken lines) with adipose tissue in 52 obese men. *A*: total adipose tissue (pre-, $y = 0.42\,x - 3.75$, $r^2 = 0.33$, $P < 0.001$; post-, $y = 0.45\,x - 5.57$, $r^2 = 0.49$, $P < 0.001$); *B*: subcutaneous adipose tissue (pre-, $y = 0.49\,x - 2.14$, $r^2 = 0.35$, $P < 0.001$; post-, $y = 0.52\,x - 4.20$, $r^2 = 0.46$, $P < 0.001$); *C*: visceral adipose tissue (pre-, $y = 0.16\,x + 8.72$, $r^2 = 0.002$, $P = 0.76$; post-, $y = 0.94\,x + 3.76$, $r^2 = 0.09$, $P = 0.03$).

males remained weight stable. It is possible that basal metabolic rate might be suppressed in response to negative energy balance to compensate for the insufficient energy intake to meet the increased energy expenditure after exercise training. Thus the possibility cannot be excluded that leptin response to exercise training observed in the female subjects might be secondary to energy balance and not to the exercise itself. Further studies are warranted to clarify whether leptin response to exercise independent of its effects on energy balance is indeed gender specific.

Pasman et al. (20) reported that the number of hours of exercise training per week correlated significantly with changes found in leptin when corrected for changes in insulin and body fat percentage, which led the authors to conclude that exercise decreased leptin levels. It is difficult to interpret the results of the study by Pasman et al. The number of hours of exercise training reflects energy expenditure and not necessarily exercise per se. In our study, there was no correlation between plasma leptin and exercise time, intensity, or energy expended via exercise, despite normalizing for adipose tissue mass. Furthermore, in that study by Pasman et al., it is possible that the significantly lower plasma leptin in the trained compared with the control group may not be related to exercise itself, but rather to changes in energy balance induced by exercise. However, it is also possible that the leptin response to exercise observed in that study could have resulted from a significantly longer training protocol (16 mo) compared with the protocol used in our study (12 wk) and that (4–5 mo) by Pérusse et al. (22). However, results from numerous studies, including ours, suggest that the leptin response to exercise results from the secondary effects of exercise on energy balance and not from exercise per se.

The mechanism by which changes in energy balance modulate circulating leptin levels remains unclear, but it is likely mediated by changes in *ob* gene expression within adipose tissue. However, adipose tissue must be able to detect the overall energy balance and its lipid content and, in turn, to adjust *ob* gene expression accordingly (8). Furthermore, recent studies have found higher o*b* gene expression and leptin secretion in subcutaneous compared with visceral adipocytes, suggesting that subcutaneous adipocytes are major sources of leptin (18). In the present study, reductions in leptin correlated positively with those of total ($r^2 = 0.32$) and subcutaneous ($r^2 = 0.35$) adipose tissue. Because visceral adipose tissue accounted for only a relatively small variation in leptin response ($r^2 = 0.09$), it is likely not an important contributor to the long-term regulation of leptin secretion (18). Our findings imply that weight loss-induced leptin reduction results predominantly from a diminution in *ob* gene expression within subcutaneous adipocytes. The absence of leptin response to exercise itself, despite a reduction in visceral adipose tissue, could be attributed to a minimal loss of subcutaneous adipose tissue.

Increasing evidence supports the notion that insulin is a critical regulator of *ob* gene expression and is a

leptin secretagogue (14, 28). Thus insulin may provide a mechanism by which adipose tissue detects changes in overall energy balance and in turn up- or downregulates *ob* gene expression accordingly. In accordance with numerous studies, a significant correlation between changes in leptin and changes in insulin in all subjects was observed ($r^2 = 0.15$, $P < 0.05$), suggesting that decreased leptin levels may occur via an insulin-dependent regulatory mechanism. Insulin sensitivity (assessed by a euglycemic hyperinsulinemic clamp) and plasma insulin were significantly improved after weight loss induced by caloric restriction or exercise. In the absence of weight loss, exercise resulted in a trend toward improvement in insulin sensitivity ($P = 0.09$) but not in plasma insulin (detailed results are presented in Ref. 23). It is possible that the absence of leptin response to exercise in the EWS group, despite a significant though minimal loss of adipose tissue, is associated with unchanged plasma insulin and a lack of improvement in insulin sensitivity.

It is interesting to note that, in the present study, there were wide interindividual differences in leptin response to exercise in the absence of weight loss, with the majority of individuals showing no change, whereas several individuals showed increases and others decreases in plasma leptin in response to exercise. This finding is in agreement with that found in the lean men in the study by Pérusse et al. (22). It is unclear why a wide interindividual response of leptin to exercise exists; however, several factors may have contributed to this variation. It is possible that changes in the ratio between bound and free forms of leptin or alterations in leptin secretion and/or clearance induced by exercise might account for these observations (22). It is also possible that exercise could have altered the lipolytic response to β-adrenergic stimuli or the antilipolytic response to insulin in some but not all individuals. The alterations in regional fat regulation could have up- or downregulated *ob* gene expression and leptin secretion. It is unlikely that diurnal variation could be responsible for this variation, because blood samples from our subjects were obtained at the same time of day before and after exercise training. Moreover, in accordance with Pérusse et al., the interindividual variation in leptin response to exercise in the absence of weight loss in our study was also within the confidence intervals of the leptin assay.

In the current study, plasma leptin levels were measured after an overnight fast in two blood samples (pre- and posttreatment), and the posttraining samples were obtained 4 days after the last exercise sessions. It is possible that exercise acutely altered leptin secretion or its diurnal rhythm. Similarly, a readaptation in the leptin axis could have occurred during the 4-day period when leptin levels were not measured, and if indeed leptin secretion was transiently altered by exercise, it would have been undetected. However, exercise has been shown to have no acute effects on leptin secretion (16, 22) or its diurnal rhythm (12). The exercise-induced decrease in leptin, which was secondary to an exercise-induced negative energy balance, returned to baseline levels 18–24 h after exercise (16). Moreover, the diurnal rhythm of leptin is entrained to meal timing rather than to the true circadian clock (26). Thus there is no convincing evidence to date that would suggest that the diurnal leptin rhythm in our subjects or its secretion during or immediately after exercise or in the 4-day period was altered by an "exercise factor," independently of the energy cost of exercise. However, it is possible that an overnight fast could have exaggerated the leptin response to weight loss and exercise in our subjects.

In summary, we have clearly demonstrated that weight loss resulting from caloric restriction or from an increase in energy expenditure via exercise results in a collateral decrease in circulating leptin in moderately obese men. In addition, our results reinforce the notion that exercise, independent of its effects on energy balance, has no profound effect on leptin secretion. The weight loss-induced decrease in circulating leptin is associated with a reduction in total and subcutaneous adipose tissue. It remains to be elucidated whether an exercise training protocol that improves insulin sensitivity could uncouple the leptin-energy balance relationship and in turn alter *ob* gene expression and circulating leptin independently of changes in adipose tissue size.

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