Effects of Different Hypocaloric Diets on Protein Secretion From Adipose Tissue of Obese Women

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Little is known about common factors (e.g., macronutrients and energy supply) regulating the protein secretory function of adipose tissue. We therefore compared the effects of randomly assigned 10-week hypoenergetic (−600 kcal/day) diets with moderate-fat/moderate-carbohydrate or low-fat/high-carbohydrate content on circulating levels and production of proteins (using radioimmunoassays and enzyme-linked immunosorbent assays) from subcutaneous adipose tissue in 40 obese but otherwise healthy women. Similar results were obtained by the two diets. Body weight decreased by ~7.5%. The secretion rate of leptin decreased by ~40%, as did that of tumor necrosis factor-α (TNF-α), and interleukin (IL)-6 and -8 decreased by 25–30%, whereas the secretion of plasminogen activator inhibitor 1 (PAI-1) and adiponectin did not show any changes. Regarding mRNA expression (by real-time PCR), only that of leptin and IL-6 decreased significantly. Circulating levels of leptin and PAI-1 decreased by 30 and 40%, respectively, but there were only minor changes in circulating TNF-α, IL-6, or adiponectin. In conclusion, moderate caloric restriction but not macronutrient composition influences the production and secretion of adipose tissue–derived proteins during weight reduction, leptin being the most sensitive and adiponectin and PAI-1 the least sensitive. *Diabetes* 53:1966–1971, 2004

Adipose tissue secretes a number of proteins with auto-, para-, and endocrine actions, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and -8, plasminogen activator inhibitor 1 (PAI-1), leptin, and adiponectin (1,2). Little is known about the regulation of the protein secretory function of human adipose tissue except for leptin (3). The effect of energy restriction is of particular importance for obesity treatment (4). Dietary-induced weight loss normalizes plasma levels and adipose tissue gene expression of several adipocyte-derived proteins (5–12). Whether the same is true for protein secretion is unknown, except for TNF-α (5,10). Furthermore, the relative roles of loss of body fat, energy restriction per se, and changes in macronutrient supply are not known. It is also unknown which proteins are more or less sensitive to nutritional changes regarding their production by adipose tissue. These questions were investigated by studying the release of leptin, adiponectin, IL-6 and -8, TNF-α, and PAI-1 in subcutaneous adipose tissue of 40 obese women before and after 10 weeks on moderate hypoenergetic diets with either low-fat/high-carbohydrate content or moderate-fat/moderate-carbohydrate content.

RESEARCH DESIGN AND METHODS

All subjects participated in a European multicenter study termed Nutrient-Gene Interactions in Human Obesity: Implications for Dietary Guidelines (NUGENOB, www.nugenob.com), which examines the interaction between hypoenergetic diets and genes. Our Swedish center included 40 obese women aged 21–49 years with a BMI of 30.9–47.7 kg/m^2 who were otherwise healthy and free of regular medication, except 1 woman who was treated with thyroid hormone for goiter (unchanged during the intervention). The study was approved by the committee on ethics at Huddinge University Hospital, and informed consent was obtained from all subjects. None had undergone surgical treatment for their obesity. They were randomly assigned to either a moderate-fat/moderate-carbohydrate (n = 20) or a low-fat/high-carbohydrate (n = 20) diet by the coordinating center in Copenhagen. Five women (four in the moderate-fat group and one in the low-fat group) were postmenopausal.

Energy expenditure and composition of diets. The daily energy requirement was estimated as follows: resting energy expenditure was measured by indirect calorimetry (Deltatrac II; Datex-Ohmeda, Helsinki, Finland) and multiplied by 1.3 for physical activity level. The subjects were then prescribed a daily energy intake 600 kcal lower than the estimated energy requirement. The dietary target for fat content was 20–25 energy percent (E%) for the low-fat diet and 40–45 E% for the moderate-fat diet. Both diets contained 15–20 E% from protein and the rest (60–65 and 40–45 E%, respectively) from carbohydrates. The subjects were given dietary instructions based on an education system consisting of isoenergetic interchangeable units (13). Alcohol was not allowed. The subjects kept food diaries and recorded everything they ate or drank during the study to facilitate compliance. Instructions were given to minimize differences in the amount of fruit and vegetables eaten, type of fat, amount and type of fiber, type of carbohydrate, and meal frequency between the two groups.

Adipose tissue biopsies and dietary intervention. Before and after the dietary intervention, the subjects were investigated at 8:00 A.M. after an overnight fast. Biopsies (~2 g) of subcutaneous abdominal adipose tissue were obtained under local anesthesia (14), venous blood samples were collected, and measurements of weight, circumference of waist and hips, and bioimpedance were taken (QuadScan 4000; Bodystat, Isle of Man, U.K.). Approximately one-half of the tissue was snap frozen for later mRNA analysis.

During the dietary intervention, the subjects visited or had telephone contact with the dietitian every week. The dietitian assessed the compliance of the subjects and checked the content of their diets from the food diaries. The subjects also performed a 3-day weighed food record of 2 weekdays and 1 weekend day before starting the dietary intervention and at the end of the 10-week diet to estimate their habitual diet and compliance with the diet. In

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HOMA, homeostasis model assessment; IL, interleukin; PAI-1, plasminogen activator inhibitor 1; TNF-α, tumor necrosis factor-α.

TABLE 1
Clinical data before and after 10 weeks of dietary intervention

	Moderate fat (n = 20)			Low fat (n = 20)		
	Before	After	P	Before	After	P
Age (years)	35.3 ± 2.0	—	—	35.1 ± 1.6	—	—
BMI (kg/m^2)	37.6 ± 1.1	34.9 ± 1.1	<0.0001	36.6 ± 0.9	33.7 ± 0.9	<0.0001
Weight (kg)	103 ± 3.3	95.4 ± 3.1	<0.0001	102 ± 2.7	94.4 ± 2.7	<0.0001
Waist-to-hip ratio	0.95 ± 0.01	0.94 ± 0.01	0.30	0.94 ± 0.009	0.93 ± 0.01	0.038
Body fat (%)	45.9 ± 1.0	43.2 ± 1.0	0.0001	44.5 ± 1.0	42.1 ± 1.2	0.0002
Glucose (mmol/l)	5.72 ± 0.28	5.57 ± 0.37	0.029	5.31 ± 0.09	5.18 ± 0.08	0.055
Insulin (μU/ml)	13.7 ± 1.9	11.4 ± 1.6	0.044	10.7 ± 1.1	9.08 ± 0.82	0.13
Fat cell volume (pl)	818 ± 32	707 ± 30	0.0005	837 ± 33	703 ± 38	0.0001

Values are means ± SE (Wilcoxon's signed-rank test). Plasma levels of glucose were determined by the routine chemistry laboratory of the hospital.

addition, 1-day weighed food records were completed in the 2nd, 5th, and 7th week of the intervention. The food records were analyzed using a food-nutrient database. The subjects were weighed when they visited the center (usually every 2nd week).

Adipose tissue. The adipose tissue was washed in physiological saline and cut into small pieces (~10 mg). Then ~500 mg were subjected to collagenase isolation (15), and mean fat cell weight and volume were determined (16). The total lipid content in the incubate was measured gravimetrically after organic extraction, and the number of adipocytes was calculated by dividing the total lipid weight by the mean cell weight, as described (16,17). The remaining 400 mg were incubated in 4 ml of Krebs-Ringer phosphate buffer (pH 7.4) supplemented with 40 g/l of BSA and 1 g/l of glucose for 2 h at 37°C in a shaking water bath with air as the gas phase. Then, 2 ml of the medium were removed, frozen in liquid nitrogen, and stored at −70°C to be used for the determination of TNF-α, adiponectin, and IL-6 and -8 as described previously for TNF-α (18). The remaining 2 ml of medium were frozen in liquid nitrogen, freeze dried, and redissolved in 250 μl of distilled water for the determination of leptin and PAI-1 as described (19,20). The incubated adipose tissue was homogenized, and total lipid was extracted as described (21). The secretion of proteins was related to 10^7 fat cells in the incubated tissue. The methods to determine TNF-α, leptin, and PAI-1 release from human subcutaneous adipose tissue in vitro have been evaluated (5,19,20). For IL-6 and -8 and adiponectin, methodological experiments revealed that *1*) recovery from incubation medium was >90% and *2*) secretion increased in a linear fashion for at least 3 h.

Protein and metabolite determinations. Human leptin and adiponectin radioimmunoassay kits from Linco (St. Charles, MO) were used for the determination of leptin and adiponectin concentrations in medium and serum. Quantikine human immunoassays (R&D Systems, Abingdon, U.K.) were used for the quantification of IL-6 and -8 in medium. Quantikine HS (high sensitivity) human immunoassays (R&D systems, Abingdon, U.K.) were used for the determination of IL-6 and TNF-α plasma levels and the concentration of TNF-α in medium. TintElize PAI-1 immunoassay (Biopool, Ventura, CA) was used to determine the PAI-1 levels in medium. Plasma enzymatic activity of PAI-1 was determined as described (19). Plasma insulin was determined by radioimmunoassay (Insulin RIA 100; Kabi-Pharmacia, Uppsala, Sweden). The kits were used according to instructions from the manufacturers. The technology needed for the determination of plasma IL-8 levels was not available in the laboratory. Plasma glucose, cholesterol, and triglycerides were determined by the hospital's routine chemistry laboratory. Measures for insulin resistance were obtained using the homeostasis model assessment (HOMA): [fasting plasma insulin (mU/l) × fasting plasma glucose (mmol/l)] ÷ 22.5 (22).

mRNA quantitation. Total RNA was extracted from subcutaneous adipose tissue biopsies using the RNeasy total RNA Mini Kit (Qiagen, Courtaboeuf, France). Agarose gels were used to check the RNA quality, and the samples from 6 of the 40 subjects (3 from each diet group) were excluded due to inadequate quality. An amount of 1 μg of total RNA was reverse transcribed using random hexamers as primers and Superscript II reverse transcriptase (Invitrogen, Cergy Pontoise, France). Real-time quantitative PCR was performed on a GeneAmp 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Then, 10 ng of cDNA was used as template for real-time PCR. The thermal cycler parameters for the real-time PCR were 2 min at 50°C, followed by 40 cycles with 10 s at 95°C and 1 min at 60°C. For leptin and adiponectin, a set of primers (Genset-Proligo, Paris, France) was designed using the software Primer Express 1.5 (Applied Biosystems) and used at a final concentration of 300 nmol/l with SYBR Green–based chemistry. A dissociation curve was generated at the end of the PCR cycles to verify that a single gene product was amplified. For PAI-1, TNF-α, and IL-6 and -8, the TaqMan approach was used. Both primers and TaqMan probes were obtained from Applied Biosystems. The probes were labeled with a reporter dye (FAM) on the 5′ end. The probe for 18S ribosomal RNA was labeled with the reporter dyes VIC and TAMRA on the 5′ end and the 3′ end, respectively. For TaqMan assays, because of the very high specificity of the method, checking for nonspecific product formation with dissociation curves is not needed. For each primer pair, a standard curve was obtained using serial dilutions of human adipose tissue cDNA before mRNA quantitation. We used 18S rRNA as control to normalize gene expression using the Ribosomal RNA Control TaqMan assay kit (Applied Biosystems).

Statistical analysis. Data were compared between and within the two groups by the Mann-Whitney U test, Wilcoxon's signed-rank test, Spearman's correlation, and repeated-measures ANOVA using StatView 5.0 (SAS Institute, Cary, NC). The variables in the study were analyzed for normality, and nonnormally distributed variables were log transformed when appropriate. Standard error was used as measure of dispersion. The level of significance was $P \leq 0.05$. Because of assay failures, the total number of subjects in each analysis was between 34 and 40.

RESULTS

Dietary composition and compliance. The self-reported baseline dietary intake was similar in the two groups (data not shown). All subjects completed the dietary intervention. The total amount of calories from fat during the intervention was significantly different from baseline ($P <$ 0.01 for both groups) and was within the targeted 40–45 E% in the moderate-fat diet group (41.5 E%) and close to the targeted 20–25 E% in the low-fat diet group (26.5 E%). The reduction in energy intake was almost identical in the two groups (~500 kcal/day).

There was a marked difference in the percentage of total calories from carbohydrate and fat between the groups and also in the amount of dietary fiber ($P < 0.001$ for all of these factors). The percentage of total calories from protein also differed between the groups ($P < 0.01$). Values for the moderate- and low-fat diets, respectively, were: carbohydrate: 38.9 ± 0.90 vs. 52.4 ± 0.85 E%; dietary fiber: 13.7 ± 0.78 vs. 18.8 ± 0.97 g/day; and protein: 19.6 ± 0.36 vs. 21.1 ± 0.32 E%. The ratio of saturated to monounsaturated to polyunsaturated fatty acids was ~2:2:1 in the habitual diet and the two intervention diets. There was no difference in compliance between the groups.

Clinical findings. There was no statistical difference in age, resting energy expenditure, anthropometric measurements, blood pressure, fat cell volume, or fasting plasma levels of glucose, insulin, triglyceride, and cholesterol between the groups at baseline (data not shown). The

TABLE 2
Messenger RNA levels of leptin, TNF-α, IL-6 and -8, adiponectin, and PAI-1 before and after the diet

	Moderate fat (n = 17)			Low fat (n = 17)			Total		
	Before	After	P	Before	After	P	Before	After	P
Leptin (10^{-3} AU)	1.54 ± 0.32	1.18 ± 0.27	0.0003	1.99 ± 0.34	1.68 ± 0.29	0.076	1.77 ± 0.23	1.43 ± 0.20	0.0004
TNF-α (10^{-7} AU)	3.42 ± 0.30	3.19 ± 0.29	0.49	3.02 ± 0.20	3.11 ± 0.41	0.69	3.22 ± 0.18	3.15 ± 0.25	0.47
IL-6 (10^{-8} AU)	6.07 ± 0.94	3.69 ± 0.39	0.013	4.89 ± 0.63	3.36 ± 0.45	0.028	5.48 ± 0.57	3.53 ± 0.30	0.0011
IL-8 (10^{-7} AU)	5.42 ± 1.1	5.16 ± 0.81	0.83	4.10 ± 0.57	3.95 ± 0.56	0.80	4.76 ± 0.63	4.56 ± 0.50	0.50
Adiponectin (10^{-3} AU)	3.31 ± 0.44	3.03 ± 0.34	0.18	2.85 ± 0.48	2.67 ± 0.47	0.46	3.08 ± 0.32	2.85 ± 0.29	0.15
PAI-1 (10^{-6} AU)	3.74 ± 0.63	4.26 ± 1.4	0.23	2.42 ± 0.38	2.33 ± 0.55	0.46	3.08 ± 0.38	3.29 ± 0.76	0.16

Values are means ± SE (Wilcoxon's signed-rank test). Messenger RNA values are expressed relative to the reference gene 18S.

average weight reduction for all subjects was 7.7 ± 0.4 kg, i.e., a ~7.5% decrease in body weight. There was a continuous loss of weight in both groups ($P < 0.0001$) (Table 1). The reduction in percent body fat, BMI, and fat cell volume was similar for both groups.

Plasma glucose and plasma insulin levels decreased after both diets. The changes were significant in the moderate-fat diet group (Table 1) and when all subjects were analyzed together. HOMA index decreased significantly in the entire group ($P = 0.018$) but not when the two diet groups were analyzed separately.

Adipose tissue mRNA quantitation. Messenger RNA data are shown in Table 2. At baseline there was no difference between the two diet groups in expression levels for any of the genes. After the diet, leptin mRNA decreased significantly by ~25% in the moderate-fat diet group but not in the low-fat diet group, although a tendency was observed ($P = 0.076$). When both groups were considered together, leptin decreased significantly by ~20% ($P = 0.0004$). IL-6 gene expression decreased significantly in both groups by 10–20%. There was no diet effect on TNF-α, IL-8, adiponectin, or PAI-1 mRNA levels when the groups were considered separately or together. No statistically significant interactions between diet, weight change, and changes in mRNA levels were found in ANOVA analysis.

Adipose tissue protein secretion. Table 3 shows protein secretion before and after the diet. Leptin secretion decreased by ~40% for all subjects put together and in both dietary groups ($P \leq 0.005$).

Secretion of TNF-α and IL-6 and -8 decreased by 20–30% when all subjects were considered together ($P \leq 0.02$). Significant changes were found for IL-8 in both groups ($P = 0.040$ and 0.010 for the moderate- and low-fat diet groups, respectively), for TNF-α in the low-fat diet group ($P = 0.030$), and for IL-6 in the moderate-fat diet group ($P = 0.040$). There was a trend toward a reduction of TNF-α and IL-6 after both diets. The secretion of adiponectin and PAI-1 did not change in the entire cohort or in either of the dietary groups. There were no statistically significant interactions between diet, weight change, and changes in protein secretion in ANOVA analysis.

To test the effect of weight loss on adipose tissue secretion of PAI-1 and adiponectin, we put both groups together and analyzed those 20 who lost the most weight (range 8–12 kg). In this group adiponectin secretion was 2.4 ± 0.2 μg/10^7 cells/2 h before and 2.7 ± 0.4 μg/10^7 cells/2 h after the diet ($P = 0.33$), and PAI-1 secretion was 17.4 ± 2.3 ng/10^7 cells/2 h before and 21.0 ± 4.7 μg/10^7 cells/2 h after the diet ($P = 0.77$).

The correlation between changes in insulin sensitivity and protein secretion was analyzed in the 20 subjects on either diet with the largest decrease in HOMA index (range 0.5–4.6). Only the secretion of leptin changed significantly, from 65 ± 7 ng/10^7 cells/2 h before to 33 ± 4 ng/10^7 cells/2 h after the diet ($P = 0.0013$).

Circulating levels. The serum and plasma levels of leptin, TNF-α, IL-6, and adiponectin and the plasma PAI-1 activity are shown in Table 4. There was a 20–35% decrease of the serum levels of leptin in the entire group of subjects as well as in both groups after the diet. In Spearman's correlation analysis, this decrease correlated with the change in leptin secretion ($P = 0.022$). No other correlations were found between changes in mRNA expression, secreted proteins, and circulating levels.

There was a small change in plasma levels of IL-6 in the entire cohort ($P = 0.046$) but not in the two groups after the diet. The levels of TNF-α and adiponectin were not influenced by diet in all subjects or in either group. However, there was a 40% decrease in plasma PAI-1 activity in

TABLE 3
Amounts of secreted proteins from 400 mg of fresh adipose tissue before and after dietary intervention

	Moderate fat (n = 20)			Low fat (n = 20)			Total		
	Before	After	P	Before	After	P	Before	After	P
Leptin (ng)	56.7 ± 4.8	36.7 ± 4.7	0.0051	61.3 ± 7.2	33.3 ± 4.3	0.0040	59.0 ± 4.3	35.0 ± 3.2	<0.0001
TNF-α (ng)	0.88 ± 0.16	0.65 ± 0.10	0.35	1.42 ± 0.21	0.95 ± 0.18	0.030	1.15 ± 0.14	0.80 ± 0.10	0.023
IL-6 (ng)	10.7 ± 1.1	8.2 ± 1.1	0.040	14.6 ± 2.8	10.0 ± 1.8	0.062	12.7 ± 1.5	9.1 ± 1.1	0.0050
IL-8 (ng)	42.8 ± 5.8	33.8 ± 5.2	0.040	52.3 ± 6.9	42.0 ± 8.6	0.010	47.8 ± 4.6	38.1 ± 5.2	0.0010
Adiponectin (μg)	2.32 ± 0.24	2.72 ± 0.35	0.16	2.27 ± 0.20	2.37 ± 0.22	0.42	2.29 ± 0.15	2.53 ± 0.20	0.12
PAI-1 (ng)	18.4 ± 2.2	17.6 ± 4.3	0.26	20.6 ± 3.3	22.6 ± 5.8	0.94	19.5 ± 2.0	20.1 ± 3.6	0.39

Values are means ± SE (Wilcoxon's signed-rank test). The amount of secreted protein is given per 10^7 cells/2 h.

TABLE 4
Circulating levels of leptin, TNF-α, IL-6, and adiponectin and PAI-1 activity before and after the diet

	Moderate fat (n = 20)			Low fat (n = 20)			Total		
	Before	After	P	Before	After	P	Before	After	P
Serum leptin (ng/ml)	38.8 ± 3.1	29.9 ± 2.6	0.0080	36.5 ± 4.0	24.4 ± 2.5	0.0012	37.6 ± 2.5	27.2 ± 1.8	<0.0001
Plasma TNF-α (pg/ml)	4.80 ± 1.6	4.84 ± 1.5	0.65	2.79 ± 0.72	2.16 ± 0.53	0.059	3.79 ± 0.87	3.50 ± 0.79	0.56
Plasma IL-6 (pg/ml)	3.82 ± 0.41	3.54 ± 0.48	0.064	3.56 ± 0.22	3.38 ± 0.37	0.31	3.69 ± 0.23	3.46 ± 0.30	0.046
Serum adiponectin (μg/ml)	16.4 ± 1.7	16.9 ± 1.6	0.88	18.3 ± 1.8	20.6 ± 1.9	0.30	17.3 ± 1.3	18.7 ± 1.3	0.40
Plasma PAI-1 (IU/ml)	28.6 ± 4.1	19.4 ± 4.1	0.0016	27.0 ± 4.1	14.2 ± 3.1	<0.0001	27.8 ± 2.9	16.8 ± 2.6	<0.0001

Values are means ± SE (Wilcoxon's signed-rank test).

all subjects and in both groups after the diet ($P \leq 0.002$) but no difference between the groups ($P = 0.094$). We found no statistically significant interactions between diet, weight change, and changes in circulating protein values.

DISCUSSION

Adipocyte-derived proteins may play a role in the pathophysiology of obesity and type 2 diabetes (1,2,23,24). Because these disorders are often treated with weight reduction, we have performed a discriminative study comparing the effects of energy restriction per se versus dietary macronutrient composition on protein secretion from adipose tissue in obese humans. We used fresh intact adipose tissue pieces and short-time incubation because collagenase isolation and prolonged incubation alter adipocyte gene expression and TNF-α secretion (25,26).

The role of dietary fat in the development of obesity is controversial (27,28). Some evidence indicates that moderate-fat diets cause obesity, whereas low-fat diets prevent weight gain (29). In contrast, to succeed in losing weight, it could be of paramount importance to restrict the intake of carbohydrates (30). Very-low-carbohydrate diets (20–30 g/day) without energy restriction cause weight loss and improve metabolic profiles (31–33) even more efficiently than low-fat, energy-restricted diets (32,33).

The results of this study clearly show that the macronutrient composition, at least the fat and carbohydrate contents, of hypoenergetic diets was not of major importance for the outcome of dietary treatment. The moderate-fat/moderate-carbohydrate and low-fat/high-carbohydrate diets caused a similar weight loss of ~7.5% and had similar effects on insulin sensitivity, fat cell volume, circulating and secreted proteins, and mRNA levels. There are differences in circulating levels of leptin and adiponectin between pre- and postmenopausal women (34,35). Five menopausal women were included in this study. We also calculated all results without these women. However, the findings from these calculations did not differ from those obtained with the entire cohort (n = 40).

The effects of the dietary intervention on protein secretion varied, but three clear patterns could be distinguished. First, the weight reduction resulted in a marked reduction in leptin secretion by ~40% after both diets. Second, the hypoenergetic diets had a moderate effect on TNF-α and IL-6 and -8 secretion (20–30% decrease). Last, PAI-1 and adiponectin were not affected by the diets. It is likely that a more extensive weight reduction is required to cause changes in the secretion of PAI-1 and adiponectin, whereas a rather small weight loss is enough to cause a marked reduction in leptin secretion and a small reduction in the secretion of TNF-α and ILs. Apparently, adiponectin and PAI-1 release is very resistant to weight changes induced by caloric restriction because there was no change in secretion even in the women who had the most marked weight loss on either diet (on average ~10% weight loss). It is also possible that a more rapid weight loss during a shorter period of time could give other types of results. Indeed, more rapid or more marked weight reduction than in our study has been shown to induce a change in circulating or adipose PAI-1 and adiponectin (8,9,36,37).

Adipose tissue is the major source of circulating leptin levels (20). Therefore, it is expected that the marked decrease in secretion of leptin after dieting would lead to an almost equally marked decrease in serum leptin levels. It is less clear to what extent human adipose tissue contributes to circulating cytokine levels. TNF-α appears above all to be produced and act locally in human fat tissue (18), and there is no in vivo release of TNF-α from subcutaneous adipose tissue to the blood (38). However, human subcutaneous adipose tissue releases IL-6 in vivo (38), which suggests that it acts as an endocrine signal. A diet-induced weight loss of ~14 kg in 2 years' time caused decreased serum concentrations of IL-6 (37). IL-6 levels in serum and in subcutaneous adipose tissue decreased after a reduction of 2.1 kg/m^2 in BMI induced by a 3-week very-low-calorie diet, but TNF-α levels did not change in serum or adipose tissue (6). These results are partly in agreement with the present study: secretion of TNF-α and IL-6 decreased by 20–30%, but there was no effect on plasma TNF-α levels and only a small but significant decrease in plasma IL-6 levels. Taken together, the data suggest that adipose tissue has a minor effect on the regulation of circulating TNF-α levels.

A surprising finding is the marked decrease in plasma PAI-1 activity after the energy restriction, despite no change in adipose tissue PAI-1 secretion. This strongly suggests that factors other than adipose tissue regulate the whole-body biological activity of PAI-1, perhaps hepatocytes or endothelial cells (36). In 15 obese subjects after a 3-week very-low-calorie diet causing a weight reduction of ~5.8 kg, plasma PAI-1 antigen decreased, but mRNA and protein levels increased (36). However, present and previous studies are not really comparable because Bastard et al. (36) analyzed PAI-1 in homogenized frozen tissue and not PAI-1 secretion from fresh adipose tissue. Although the extent of the weight loss was similar to the weight reduction in the present study, it was induced in a much

shorter time. It is quite possible that compensatory effects occur during long-term and slower weight loss, as in our study. In theory, reduced PAI-1 secretion from visceral fat could contribute to the fall in plasma PAI-1 activity. There are regional differences in several adipose-secreted proteins, as reviewed (39), and at least some studies suggest higher secretion from visceral compared with subcutaneous fat (40,41). It is, however, unlikely that visceral fat is responsible for the fall in plasma PAI-1 activity because this depot is relatively small in obese women. For example, the major omentum constitutes <1% of total body fat in very obese subjects (42).

The only major changes in gene expression were decreased leptin and IL-6 mRNA. The expression of IL-6 showed a significant decrease in both diet groups. The mRNA levels of leptin decreased significantly in the moderate-fat diet group and when all subjects were considered together. It also tended to decrease in the low-fat diet group. Thus, leptin and IL-6 are at least in part regulated at the transcriptional level during energy restriction. The mRNA levels of TNF-α and IL-8 did not change, although the secretion rates of these proteins decreased in the whole group after diet, suggesting that TNF-α and IL-8 production is subject to posttranscriptional regulation. Recent studies have shown that the expression of TNF-α is limited almost exclusively to macrophages in the stromal-vascular fraction of murine adipose tissue, whereas IL-6 is expressed in both the adipocyte and stromal-vascular fractions (43,44). Whether this is true also for human adipose tissue is not yet clear.

Weight loss improves obesity-associated insulin resistance (45). This was confirmed in the present study and could be a factor behind decreased leptin production (23,46). It is, however, not important for the other secreted proteins because there was no dietary effect on IL-6 or -8, TNF-α, adiponectin, or PAI-1 when the subjects who improved their insulin sensitivity the most were analyzed separately (data not shown).

In summary, this study suggests that energy supply per se and not the macronutrient composition is of importance for the regulation of the protein secretory function and gene expression of human adipose tissue, at least during energy restriction. Leptin is most sensitive for energy restriction, followed by cytokines and chemokines, whereas PAI-1 and adiponectin are not affected by a moderate weight decrease/reduction in energy intake. However, other sources for PAI-1 besides subcutaneous adipose tissue are very sensitive to nutritional changes.

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