Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats

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Abstract **To investigate whether dietary fat source and energy restriction interactively influence plasma leptin levels and its association of leptin with insulin action, rats were fed diets containing either fish, safflower oil, or beef tallow (20% wt/wt) for 10 weeks. Groups of rats consumed each diet ad libitum or at 85% or 70% of ad libitum energy intake in a design that held fat intake constant. Graded levels of energy restriction caused body weight to decrease ($P < 0.001$) differently according to the dietary fat provided. Plasma leptin concentrations were 60% higher ($P < 0.05$) in the groups fed fish oil and safflower oil ad libitum compared with those in the beef tallow group, despite smaller perirenal fat mass and fat cell size in the fish oil-fed animals. Energy restriction resulted in a 62% decrease ($P < 0.05$) in leptin levels in fish oil- and safflower oil-fed rats, whereas no changes were observed in beef tallow-fed animals. Plasma insulin levels were lower ($P < 0.05$) in the fish oil group fed ad libitum compared with those in the two other diet groups. These data demonstrate a hyperleptinemic effect in animals consuming diets rich in polyunsaturated fatty acid, which can be normalized to the level of saturated fat consumption by mild energy restriction. Thus, dietary fatty acid composition, independent of adipose tissue mass, is an important determinant of circulating leptin level in diet-induced obesity.—**Cha, M. C., and P. J. H. Jones. **Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats.** ***J. Lipid. Res.*** **1998.** 39: **1655–1660.**

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Leptin is a circulating protein produced by an ob gene that binds to specific hypothalamic receptors and may play a major role in control of body fat store through regulating food intake and body energy expenditure (1, 2). Leptin levels have been shown to be positively correlated with body fat both in humans (3–5) and rodents (4, 6). Thus, this protein has been suggested as a static index of the amount of triglyceride stored in adipose tissue (7). It is reported, however, that for any given amount of fat stores in obese humans, leptin levels vary markedly (4). Leptin is synthesized by and secreted from adipose tissue. Dietary fatty acid composition has been shown to modify adipose tissue and membrane phospholipid fatty acid composition and membrane protein function (8, 9). Whether the circulating levels of this protein and its action are influenced by dietary fatty acid composition is not known.

In addition to the amount of body fat, energy balance has been shown to regulate leptin levels. Circulating leptin concentrations (10–12) and ob gene expression (13) decreased in fasting obese and non-obese humans and lean rodents. Severe food restriction for 3 months in humans and 28 days in mice also reduced leptin levels (4). However, it was not clear whether mild energy restriction can influence leptin levels in diet-induced obesity.

Leptin concentration has been reported to be positively correlated with circulating insulin levels (10, 14). Insulin resistance associates with elevated leptin levels, independent of body lipid content (15). High fat intake has been shown to associate with insulin resistance (16), whereas fish oil consumption or food restriction lowered insulin levels and improved insulin action (17). We considered it would be of interest to investigate whether the previously reported correlation between insulin sensitivity and leptin level remained unchanged when dietary fatty acid composition was modified.

The present study was therefore designed to investigate effects of dietary fat type selection and graded levels of energy restriction on plasma leptin concentration and the relationship between leptin level and insulin sensitivity in diet-induced obese rats. Because leptin may act to regulate energy balance, leptin's effect on other variables related to body energy expenditure including Na^+-K^+-ATPase activity and thyroid hormone concentration were also examined. In order to investigate the action of energy deficit exclusively without decreasing intake of other nutrients, with the exception of carbohydrate, energy deficiency in the present design was achieved by removing only refined carbohydrate from the diets while keeping fat and micronutrient intakes constant.

Abbreviation: PUFA, polyunsaturated fatty acid.

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METHODS

Animals and diets

Seventy-two male Sprague-Dawley rats (209 ± 6.5 g) purchased from Charles River, Inc. (Quebec, Canada) were housed individually in stainless steel hanging cages with a 12-h light–dark cycle at 22° ± 1°C environment. After habituation to a commercial rat chow for 7 days, rats were randomized into three dietary fat groups and fed high fat diets containing either fish oil, safflower oil, or beef tallow as the fat source (**Table 1**). Three animals served as body weight controls and continued to receive rat chow. Beef tallow was supplemented with 1% safflower oil to maintain adequate intakes of linoleic acid. Animals within each fat group were either given free access to diets or were restricted to 85% or 70% of ad libitum daily intakes. Food intakes were recorded daily in the ad libitum fed animals; mean intakes in each fat group were calculated and a corresponding decreased amount of diet was then provided to the food-restricted animals. Diets consumed by the food-restricted animals were modified to supply equal quantities of all nutrients including fat each day compared with the ad libitum-fed diets, with the exception of corn starch (Table 1). Body weights were monitored weekly. At the end of the 10-week feeding trial and after a 12-h fast, animals were anesthetized using carbon dioxide gas. Blood samples were drawn by heart puncture and animals were then killed. Livers were collected and immediately frozen in liquid N_2, then stored at −80°C until further analysis. Perirenal fat pads were collected and weighed for adipocyte isolation.

Adipocyte isolation and insulin binding

Perirenal fat tissue adipocyte isolation and insulin binding analyses were performed according to methods of Olefsky and Reaven (18). Briefly, about 1 g of tissue was chopped into small pieces and incubated at 37°C in a modified Krebs-Ringer buffer with collagenase (130 mm NaCl, 10 mm HEPES, 5.2 mm KCl, 1.4 mm $CaCl_2$, 1 mm H_3PO_4, 1.4 mm $MgSO_4$, 2% BSA, 1 mm glucose, 3 mg/ml collagenase, pH 7.4) for 1 h. Isolated fat cells were washed 3 times with the same buffer without collagenase. The cell suspension was then incubated at 24°C for 45 min with ^{125}I-labeled pig insulin (New England Nuclear, DuPont Canada, QC; specific activity 80 μCi/μg). Insulin binding was terminated by centrifuging 200 μl of the above cell suspension to which 100 μl of silicon oil had been added. Cells were collected from the top of the oil phase and radioactivity was counted by γ-radiation counter (Pharmacia, Turku, Finland). Specific binding was determined by total binding corrected for nonspecific binding. Nonspecific binding was measured by incubating cells with ^{125}I-labeled insulin and non-labelled pig insulin (20 μg/ml). Cells were counted using a hematometer under an optical microscope. Fat cell size was determined after extraction of depot lipid. The cell suspension was heated at 55°C for 15 min after adding methanol. Lipids were extracted by hexane–chloroform 4:1. After shaking and centrifugation, the top organic phase was removed and air-dried to determine the total lipid. Fat cell size (mg lipid/10^5 cells) was calculated by dividing the total lipid by adipose cell number.

Na^+-K^+-ATPase activity

Hepatocyte Na^+-K^+-ATPase activity was determined by the method described by Ismail-Beigi and Edelman (19). Liver samples were homogenized with six strokes of a Teflon-glass homogenizer in 10 volumes of 0.25 m sucrose, 1.25 mm ethyleneglycol bis (beta-aminoethylether)-N,N′-tetraacetate, 10 mm Tris at pH 7.0. The homogenate was further diluted 1:2 with the same medium. Liver homogenate (100 μl) was incubated in duplicate for 5 min at 37°C in a final volume of 1 ml containing 5 mm $MgCl_2$, 120 mm NaCl, 7.5 mm KCl, 5 mm NaN_3, 5 mm ATP, 12 mm Tris buffer at pH 7.4. Na^+-K^+-ATPase activity was determined as the difference in phosphorus concentrations between this medium and a medium containing ouabain (1 mm). Phosphorus concentration was measured by the method of Fiske and SubbaRow (20). Protein concentration was determined by a colorimetric assay kit (Bio-Rad Laboratories, CA).

Other analyses

Plasma leptin concentration was measured by a leptin radioimmunoassay kit for rat (Linco Research, MO). Plasma insulin, thyroxine, and triiodothyronine concentrations were determined by radioimmunoassay kits (Immunocorp Sciences Inc. QC). All the analyses were conducted in duplicate. The fatty acid composition of the experimental fats was determined by gas–liquid chromatography as described previously (21) (**Table 2**).

Statistical methods

Data were analyzed by two-way analysis of variance using an SAS general linear model program (SAS Version 6, SAS Institute Inc., Cary, NC). Fisher's protected least significant difference procedure (Fisher's protected LSD) was used to separate means. Differences between means were considered to be significant at $P < 0.05$. Pearson correlation coefficients were calculated to examine the association of leptin concentration with the selected variables. Data are presented as means ± SEM.

TABLE 1. Composition of the experimental diets

	Energy Intake Group		
	100%	85%	70%
	g/100 g		
Casein	15	17.6	21.4
Fat	20	23.5	28.6
Corn starch	45	32.7	15
Sucrose	10	11.8	14.3
Cellulose	5	8.6	13.6
AIN-93M-MX	3.5	4.1	5
AIN-93-VM	1	1.2	1.4
l-Cystine	0.18	0.21	0.26
Choline bitartrate	0.25	0.29	0.36
Tert-butylhydroquinone	0.004	0.005	0.006

TABLE 2. Major fatty acid composition of the experimental fats

Fatty Acid	Fish Oil	Safflower Oil	Beef Tallow
		% w/w	
14:0	9.6	ND	4.1
16:0	18.7	7.1	27.7
16:1 (n–7)	13.7	ND	6.0
18:0	5.8	2.0	14.0
18:1 (n–9)	11.5	20.8	45.5
18:1 (n–7)	2.5	ND	ND
18:2 (n–6)	2.0	68.7	1.9
18:3 (n–3)	2.3	1.1	0.2
20:1 (n–9)	1.1	0.3	0.6
20:4 (n–6)	1.3	ND	ND
20:5 (n–3)	16.6	ND	ND
22:4 (n–6)	0.9	ND	ND
22:6 (n–3)	14.0	ND	ND

ND, not detectable.

RESULTS

Food intake, body weight, perirenal fat mass weight, and fat cell size of rats fed diets varying in fat source and energy intake level are shown in **Table 3**. Food consumption did not differ among groups fed different dietary fats. Body weights at time of killing of all the animals in the ad libitum-fed groups and most of the animals in the 85% of energy intake groups were over 500 g, which is 60–100 g heavier than the chow-fed animals. Body weight was not statistically different among the dietary fat groups. All groups of animals gained weight; however, the weight gains in the energy-restricted groups were proportionally decreased ($P < 0.001$) according to the energy intake level.

Perirenal fat pad weights were lower ($P < 0.01$) in fish oil-fed animals compared with other fat-fed animals. Energy restriction decreased ($P < 0.01$) the fat mass significantly. Adipocyte cell size was also influenced ($P < 0.001$) by the different diets. Cells from fish oil-fed rats were about 25% of the size of cells from safflower oil-fed and beef fat-fed animals (100% energy intake group) ($P < 0.01$). Energy deficit decreased ($P < 0.05$) the cell size in the safflower oil and beef tallow diet groups, but not in the fish oil group. There was ($P < 0.02$) a strong positive correlation ($r > 0.57$) between the perirenal fat pad weight and fat cell size in the three dietary fat groups.

Plasma leptin concentrations in rats consuming diets different in fat type and energy level are shown in **Fig. 1**. Plasma leptin concentrations were influenced by the interaction ($P < 0.03$) between fat type and energy restriction. Leptin levels were 60% lower ($P < 0.05$) in the ad libitum-fed beef tallow group (11.7 ± 1.5 ng/ml) as compared with those in the fish oil and safflower oil groups (18.8 ± 1.9 and 21.8 ± 2.5 ng/ml, respectively). Energy restriction decreased ($P < 0.01$) leptin levels in the fish oil-fed (18.8 ± 1.9 ng/ml in the ad libitum-fed vs. 7.4 ± 1.3 ng/ml in the 70% energy intake group) and the safflower oil-fed animals (21.8 ± 2.5 ng/ml in the ad libitum-fed vs. 10.6 ± 1.7 ng/ml in the 70% energy intake group). Energy deficiency had no effect on the leptin levels of the beef tallow-fed rats.

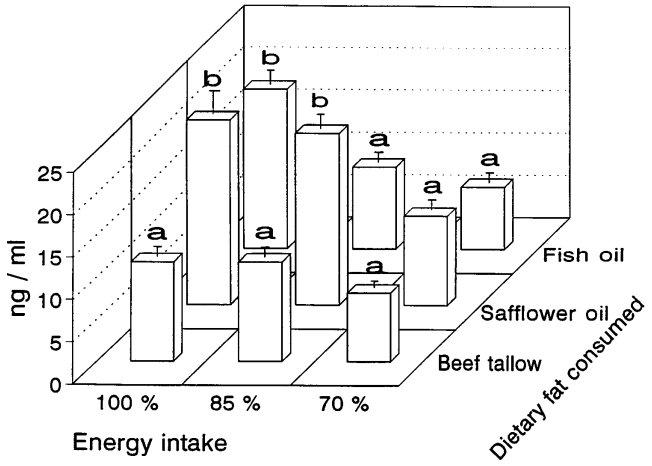

Fig. 1. Plasma leptin concentrations of rats fed fish oil, safflower oil, or beef tallow diets at graded levels of energy intake. Dietary fat source and energy restriction interactively influenced ($P < 0.03$) leptin levels. Values are means ± SEM (n = 8). Values with different letters are significantly different ($P < 0.05$, Fisher's protected LSD).

Plasma insulin and thyroid hormone concentrations in rats consuming diets different in fat type and energy level are shown in **Table 4**. An interactive effect of diet fat source and caloric restriction was found ($P < 0.001$) for plasma insulin concentrations. Insulin levels were lower ($P < 0.05$) in the ad libitum-fed fish oil group compared with the safflower oil and beef tallow diet groups. How-

TABLE 3. Food intake, body weight, fat pad weight, and fat cell size of rats fed fish oil, safflower oil, or beef tallow diets at graded levels of energy intake

	Energy Intake	Dietary Fat Consumed		
		Fish Oil	Safflower Oil	Beef Tallow
	%		g	
Average food intake/rat/day	100	19.9 ± 0.4	20.7 ± 0.6	21.8 ± 0.8
Body wt at killing	100	571.1 ± 10.2	572.8 ± 12.7	555.9 ± 26.0
	85	532.9 ± 8.2*	552.3 ± 7.5	519.9 ± 11.9*
	70	468.0 ± 11.0*#	493.5 ± 4.4*#	475.8 ± 8.3*#
Perirenal fat wt	100	6.03 ± 0.32^a	11.06 ± 0.92^b	9.01 ± 0.92^c
	85	5.10 ± 0.31^a	7.96 ± 0.71^{b*}	8.18 ± 0.36^b
	70	4.25 ± 0.26$^{a*\#}$	6.95 ± 0.33^{b*}	6.72 ± 0.37^{b*}
Perirenal fat wt/100 g body wt	100	1.06 ± 0.07^a	1.95 ± 0.18^b	1.64 ± 0.17^c
	85	0.96 ± 0.06^a	1.44 ± 0.13^{b*}	1.57 ± 0.05^b
	70	0.91 ± 0.06^a	1.41 ± 0.06^{b*}	1.41 ± 0.06^b
Fat cell size (mg lipid/10^5 cell)	100	14.0 ± 3.7^a	58.4 ± 11.2^b	52.9 ± 13.6^b
	85	14.9 ± 4.4^a	50.5 ± 8.7^b	24.8 ± 10.0^{c*}
	70	10.3 ± 4.3	22.9 ± 4.4*#	20.3 ± 4.3*

Values are means ± SEM (n = 8). Means with different superscript letters(*a*, *b*, *c*) in the same row are significantly different ($P < 0.05$, Fisher's protected LSD). For each parameter in the same column, means with an * are significantly different compared to means for the 100% fed group. Means with # are significantly different compared to the 85% fed group.

TABLE 4. Plasma concentrations of insulin and thyroid hormone in rats fed fish oil, safflower oil, or beef tallow diets at graded levels of energy intake

	Energy Intake	Dietary Fat Consumed		
		Fish Oil	Safflower Oil	Beef Tallow
	%			
Insulin (pmol/L)[1]	100	425.6 ± 22.3^{a}	536.7 ± 55.4^{b}	622.7 ± 49.7^{b}
	85	505.3 ± 42.3^{a}	411.1 ± 27.2^{a}*	294.3 ± 19.6^{b}*
	70	264.6 ± 25.6*#	228.2 ± 17.5*#	224.9 ± 37.9*
Thyroxine (nmol/L)	100	45.6 ± 1.6^{a}	74.6 ± 5.1^{b}	66.4 ± 3.0^{b}
	85	46.1 ± 2.0^{a}	65.1 ± 5.5^{b}	70.2 ± 5.0^{b}
	70	47.8 ± 2.5^{a}	75.0 ± 4.9^{b}	61.6 ± 2.5^{c}
Triiodothyronine (pmol/L)	100	471.3 ± 2.9	452.5 ± 2.5	466.3 ± 8.8
	85	470.0 ± 4.6^{a}	447.5 ± 6.5^{b}	465.0 ± 4.2^{a}
	70	480.0 ± 5.4^{a}	453.8 ± 6.0^{b}	497.5 ± 15.8^{a}*#

Values are means ± SEM (n = 8). Means with different superscript letters (*a*, *b*, *c*) in the same row are significantly different ($P < 0.05$, Fisher's protected LSD). For each parameter in the same column, means with an * are significantly different with compared to means for the 100% fed group. Means with # are significantly different compared to the 85% fed group.

[1]ANOVA: energy *fat $P < 0.001$.

ever, such differences were not observed when energy intakes were restricted to 70% of ad libitum intakes.

Plasma thyroxine concentrations were modified ($P < 0.001$) by dietary fat type, as shown by lower ($P < 0.05$) circulating levels in animals fed fish oil compared with those fed the other two fats. Energy deficit had no effect on plasma thyroxine concentrations.

Plasma triiodothyronine concentrations were influenced by dietary fat type ($P < 0.001$) and energy restriction ($P < 0.02$) independently without interaction. Triiodothyronine levels were lower ($P < 0.05$) in the safflower oil-fed rats as compared with the animals fed the other two fats in the food-restricted groups. Food restriction to 70% of ad libitum intake increased ($P < 0.05$) triiodothyronine levels in the beef tallow-fed animals. However, no change in the response of triiodothyronine levels to energy deficit was observed in the fish oil- or safflower oil-fed rats.

Insulin binding to adipocytes and hepatocyte Na^+-K^+-ATPase activities in rats consuming diets varying in fat source and energy intake are shown in **Table 5**. Neither dietary fat type nor energy restriction affected insulin binding activity to adipocytes.

Hepatocyte Na^+-K^+-ATPase activity was not influenced by dietary fat type; however, an energy deficit produced higher ($P < 0.03$) Na^+-K^+-ATPase activities. Energy restriction to 70% of ad libitum intakes increased ($P < 0.05$) Na^+-K^+-ATPase activities in the fish oil- and beef tallow-fed groups.

Correlation coefficients of plasma leptin level in response to graded levels of energy intake with body weight, perirenal fat pad weight, fat cell size, and plasma insulin concentration in rats fed diets varying in fats are shown in **Table 6**. Plasma leptin concentrations were positively correlated with body weight in all dietary fat groups. No correlations of leptin level with perirenal fat mass and fat cell size were found. Plasma insulin concentrations were positively correlated ($r > 0.46$, $P < 0.05$) with circulatory leptin levels in the fish oil- and safflower oil-fed rats, but not in the beef tallow-fed animals. No relationship was observed between the leptin level and the other parameters examined, and no significant correlation was found when the coefficient was calculated within each combination group of the dietary fat type and the energy intake level.

DISCUSSION

Circulating leptin is generally known to be correlated with body lipid stores; the concentration of this protein is markedly influenced by body energy balance (7). Present data demonstrate for the first time that leptin level is also

TABLE 5. Insulin binding to adipocyte and hepatocyte Na^+-K^+-ATPase activity in rats fed fish oil, safflower oil, or beef tallow diets at graded levels of energy intake

	Energy Intake	Dietary Fat Consumed		
		Fish Oil	Safflower Oil	Beef Tallow
	%			
Specific bound insulin (pg/2 × 10^5 cell)	100	4.23 ± 1.28	3.50 ± 1.15	3.21 ± 1.15
	85	2.20 ± 1.28	5.57 ± 0.97	2.21 ± 1.15
	70	5.10 ± 1.15	5.04 ± 1.05	2.87 ± 1.28
Na^+-K^+-ATPase activity (nmol/mg·min)	100	1.66 ± 0.16	1.21 ± 0.39	1.63 ± 0.28
	85	1.94 ± 0.40	1.62 ± 0.32	1.24 ± 0.20
	70	2.60 ± 0.37*	1.58 ± 0.31	2.81 ± 0.58*#

Values are means ± SEM (n = 5–8). For each parameter in the same column, means with an * are significantly different compared to the 100% fed group. Means with # are significantly different compared to the 85% fed group.

TABLE 6. Correlation coefficients of plasma leptin concentration with body weight, perirenal fat pad weight, fat cell size, and plasma insulin concentration in rats fed fish oil, safflower oil, or beef tallow diets when energy intake levels were pooled

	Dietary Fat Consumed		
	Fish Oil (n = 24)	Safflower Oil (n = 24)	Beef Tallow (n = 24)
Body weight			
r	0.56	0.49	0.55
P	0.02	0.02	0.008
Perirenal fat			
r	0.21	0.32	0.17
P	0.34	0.12	0.44
Fat cell size			
r	−0.23	0.34	0.34
P	0.44	0.17	0.20
Insulin			
r	0.47	0.65	0.30
P	0.048	0.0006	0.17

determined by dietary fat source and by an interactive effect of fat source with energy intake level; diets rich in PUFA can increase leptin level while such influence by dietary fat can be abolished by mild energy restriction.

The relationship between plasma leptin concentrations and body lipid stores has been investigated both in humans and animals (3–6). Maffei et al. (4) reported a highly positive correlation of plasma leptin with body mass index in both obese and lean humans and rodents. After feeding mice a high-fat Western diet for 12 weeks, Frederich and co-workers (6) found a dramatic increase in plasma leptin levels which accurately reflected body lipid content. In the present study, plasma leptin levels were positively correlated with body weight in all three diet fat groups. However, leptin concentrations were 60% higher in the ad libitum-fed fish oil diet group than those in the beef tallow-fed groups, despite smaller fat cell size and fat pad weight in the fish oil-fed animals. In a recent experiment with the same dietary regimen in rats, we reported (22) that body fat mass was 25% less in the fish oil-fed as compared with the beef tallow-fed rats. In combining those data with the present findings, our results indicate that circulating leptin did not accurately reflect the amount of body fat in our animal model. Instead, leptin levels were influenced by dietary fat source.

The possible mechanisms regarding the dissociation between circulating leptin level and body lipid content in the present study remain to be elucidated. We could speculate that n–3 PUFA consumption increases leptin synthesis and secretion, which, in turn, increases fatty acid oxidation and thus decreases body lipid store. On the other hand, large quantities of saturated fatty acid deposited in adipocytes may inhibit leptin production, resulting in lower circulating leptin level despite higher body lipid stores. Diets rich in n–6 PUFA may lack the n–3 PUFA-like action on leptin production. Over-deposition of body lipid due to the high fat intake in the safflower oil-fed rats could cause leptin resistance and thus hyperleptinemia. It would be interesting to assess whether the leptin level would increase and the body fat mass decrease in animals switched from beef tallow to a fish oil diet.

The relationship between leptin and insulin has been investigated in both humans (10, 14) and animals (23) because of the possible role of leptin in the link between obesity and pancreatic beta-cell hypersecretion. A close association of plasma leptin and insulin concentrations has been reported (10, 14, 23), which leads to the proposed action of leptin in regulating insulin secretion (24). The finding that functioning leptin receptor was expressed in pancreatic islets provides direct evidence of modification of insulin secretion by leptin (25). In the present study, hyperinsulinemia was observed in all the three dietary fat groups, although the lower level was associated with fish oil feeding, which is consistent with previous findings (17). However, contradictory to previous data, present results show that the relationship between leptin and insulin varied according to dietary fat source, i.e., low leptin in beef tallow-fed animals was associated with high insulin level while the reverse relationship was found in the fish oil-fed group. It has been shown that leptin diminishes insulin secretion by acting on the pancreatic beta cells (26). It is reasonable to postulate that the low leptin levels in the beef tallow-fed rats partially relieve its inhibition on insulin production. Therefore insulin level is increased to compensate the peripheral insulin resistance caused by high saturated fatty acid consumption as reported previously (17).

Circulating leptin has been proposed as an indicator of body energy balance under situations of negative energy balance (7). Acute food deprivation (10–12) or chronic energy restriction (4) resulted in dramatic decreases in leptin concentrations. Present results demonstrate that dietary fatty acid composition modified the response of leptin level to energy restriction. Leptin concentrations were not changed in safflower oil-fed rats when food intake was restricted to 85% of ad libitum intake; leptin levels did not respond to energy deficit in the beef tallow-fed animals. In considering that the present design excluded the possible influence of the decreased fat intake on leptin level when energy deficiency is created, it suggests that qualitative fat intake determines the response of leptin concentration to energy deficit. It was interesting to observe that when energy intake was restricted to 70% of the ad libitum intakes, even the percentage of dietary fat in the diets was increased; the effect of qualitative fat intake on leptin level was abolished. These data suggest that when energy intake was restricted to a certain level (in the present design: 70% of the ad libitum intake), caloric intake, rather than the quality of the dietary fat, was more significant in the control of leptin level.

Whether the severe decline in plasma leptin and body weight gain in fish oil-fed, energy-restricted animals indicates improved leptin action is unclear. However, Na^+-K^+-ATPase activity was increased in these animals. It is estimated that body sodium pump activity contributes 15–50% of cellular energy expenditure (27). Increased sodium pump activity associated with low leptin level may be considered a consequence of increased leptin action. If that is true, diets rich in n–3 long chain fatty acids to-

gether with mild energy restriction may be a good dietary regimen for weight loss.

In summary, dietary fatty acid composition determined the circulating leptin level which was not an accurate indicator of body fat store in diet-induced obesity. The response of leptin level to energy restriction and the relationship between leptin and insulin were dependent on dietary fatty acid composition. Results from the present study thus suggest the possibility of improving leptin action in obesity through dietary modification.

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