Effects of dietary fat type and energy restriction on adipose tissue fatty acid composition and leptin production in rats

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Abstract **To investigate whether dietary fatty acid (FA) composition and energy restriction (ER) interactively influence *obese* (*ob*) gene expression, rats consumed diets containing beef tallow, safflower, or fish oil ad libitum (AL) or at 60% AL intake. Circulating leptin concentrations were higher ($P < 0.0001$) after AL feeding, but were not influenced by dietary fat. ER decreased ($P < 0.0001$) weight gain and visceral adipose weight, which were positively correlated ($r = 0.40$ $P < 0.001$, $r = 0.58$ $P < 0.0001$) with circulating leptin levels. Visceral adipose *ob* mRNA levels were greater in animals fed unsaturated fats, particularly safflower oil, which had the highest *ob* mRNA levels. Circulating leptin levels did not parallel *ob* mRNA levels, except for the greater abundance detected in AL adipose in comparison to ER animals. In addition, visceral FA profiles reflected dietary fat source and were influenced by an interaction of dietary fat and energy. These data demonstrate that dietary fat, particularly from a plant or marine source, and ER interactively influence *ob* mRNA levels; however, alterations in *ob* mRNA do not confer changes in circulating leptin, with the exception of ER, which is a key determinant. Thus, dietary intake is an important regulator of leptin production; however, the significance of these modest changes in diet-induced obese animals requires further study.**—Hynes, G. H., J. Heshka, K. Chadee, and P. J. Jones. **Effects of dietary fat type and energy restriction on adipose tissue fatty acid composition and leptin production in rats. *J. Lipid Res.* 2003.** 44: **893–901.**

Supplementary key words gene regulation • *obese* gene • beef tallow • safflower oil • fish oil • visceral adipose

Leptin, the 16 kDa protein product of the *obese* (*ob*) gene, is primarily produced and secreted by white adipocytes (1). Leptin interaction with hypothalamic receptors controls body fat stores through anorexic and thermogenic effects (1, 2). Animal studies have shown reductions in body weight upon administration of leptin protein in both lean and obese animals (3). Emerging evidence supports a unique function of leptin in reducing triglyceride content in adipocytes and nonadipocytes through stimulation of lipid oxidation (4–7). Furthermore, circulating leptin levels correlate strongly with the amount of adipose tissue (8–11); however, a large variability in leptin levels has been reported in humans with similar fat stores (9).

Dietary fatty acid (FA) profile influences adipose tissue FA composition in humans and animals (12–14), as well as membrane FA composition and membrane protein function (15–17). In particular, feeding animals diets with a high ratio of polyunsaturated-saturated FAs improved insulin action and glucose transport, as well as other cellular processes (15–17). Moreover, consumption of the PUFA of marine sources rich in n-3 long-chain FAs has been shown to reduce adipose tissue growth in comparison to diets rich in saturated FAs (SFAs) (12, 18, 19). Furthermore, these reductions in adipose tissue following n-3 PUFA can be explained by differences in adipocyte lipid content (20, 21). Recently, it has been shown that a shift toward increased dietary n-3 PUFA in humans reduced plasma leptin concentrations and leptin expression in rat epididymal (Ep) adipose tissue (22, 23).

Raclot et al. (24) observed reduced retroperitoneal (Rp) *ob* mRNA levels with docosahexanoic acid (22:6 n-3) as the sole fat source, compared with a lard-olive oil mixture. Moreover, *ob* mRNA levels were positively correlated with fat cell size, suggesting a role for n-3 PUFA in regulating fat cell size and leptin expression. The reduction in *ob* mRNA levels was tissue specific, as demonstrated by a lack of response in subcutaneous (Sc) adipose tissue; thus, regulation of leptin production may be dependent upon the anatomic location of the tissue. Due to the increased metabolic activity of visceral adipose, it is considered to be the primary contributor to overall circulating leptin levels

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(25). Whether there is a unique tissue-specific response to dietary FA composition and an inherent effect on leptin production is not fully understood.

Apart from the size and location of adipose stores, energy intake is also an important determinant of leptin production. Fasting in human subjects has been shown to reduce circulating leptin, whereas refeeding increases leptin levels (26). Moreover, fasting in animals (27) and humans (26, 28, 29) has been shown to significantly reduce leptin mRNA and plasma levels independent of changes in weight or fat mass; however, it is unknown whether mild energy restriction (ER) in combination with dietary FA composition influences leptin production or action.

Whether dietary FA composition and ER influence adiposity through changes in leptin production is not yet established. In addition, the variability in leptin expression (30, 31), as well as any correlations between *ob* mRNA and circulating leptin levels (31) related to the anatomic location and metabolic differences of the fat tissue, are not well understood in relation to the FA composition and energy intake. Hence, the aim of the present study was to examine *ob* mRNA and leptin levels in animals fed diets differing in type of fat and level of energy. Furthermore, the second aim of this study was to investigate whether the regulation of *ob* mRNA levels is tissue specific by comparing *ob* mRNA levels from visceral adipose tissue.

MATERIALS AND METHODS

Animals and diets

Seventy-two male Sprague-Dawley rats (275 ± 4.0 g) purchased from Charles River, Inc. (Quebec, Canada) were housed individually in stainless steel hanging cages at 22 ± 1°C with a 12 h light-dark cycle. After habituation to commercial rat chow for 7 days, animals were stratified into one of three dietary fat groups and fed high-fat diets (20% wt/wt) containing either menhaden fish oil (FO), safflower oil (SO), or beef tallow (BT) as the sole fat source. Animals within each fat group were further subdivided to receive, for 10 weeks, ad libitum (AL) diet or 60% of AL daily intakes, yielding 12 rats per diet treatment group. Diets were prepared every 3 days and were stored at −20°C. The FA composition of each dietary fat was determined prior to feeding by gas chromatography (**Table 1**). BT diets were supplemented with 1% SO to maintain adequate intakes of 18:2 (n-6). With the exception of cornstarch, diets consumed by food-restricted animals were modified to supply equal quantities of all nutrients, including fat compared with the AL-fed diets (**Table 2**). Diets met the National Research Council requirements for growing rats, and body weights were monitored weekly throughout the feeding trial to ensure adequate nutritional intake. After 10 weeks, animals were fasted overnight and killed by CO_2 asphyxia. Serum was isolated and immediately stored at −80°C until analysis. Ep, perirenal (Pr), and Rp fat pads were weighed and snap frozen in liquid N_2 followed by storage at −80°C. Ethical approval for the study was obtained from the McGill University Animal Ethics Committee.

Serum analysis

Serum analyses were performed in duplicate using commercially available radioimmunoassay kits for rat leptin (Linco Research Diagnostics, MO) and insulin (ICN Pharmaceuticals, Inc., Costa Mesa, CA) utilizing HPLC-purified ^{125}I-rat leptin (32) or insulin as tracer. Radioactivity was determined by γ counting (LKB Wallac, 1282 compugamma CS, Fisher Scientific, Montréal, QC, Canada). Serum values were quantified using standard curves and automated data reduction procedures. Leptin and insulin values are expressed as ng/ml and μU/ml, respectively.

TABLE 1. Major fatty acid composition of the experimental fats

FA	BT	SO	FO
		%	
14:0	4.1	ND	8.4
16:0	27.7	6.0	15.2
16:1 (n-7)	6.0	ND	11.6
18:0	14.0	4.0	2.7
18:1 (n-9)	45.5	19.0	9.5
18:2 (n-6)	1.9	69.7	1.8
18:3 (n-3)	0.2	0.4	1.8
20:0	0.6	ND	0.2
20:1 (n-9)	ND	ND	1.3
20:4 (n-6)	ND	ND	2.3
20:5 (n-3)	ND	ND	16.0
22:6 (n-3)	ND	ND	10.8
Other	ND	0.9	18.4

BT, beef tallow; FA, fatty acid; FO, menhaden oil; ND, nondetectable; SO, safflower oil.

Lipid extraction and FA analysis

An aliquot from each adipose depot was used for total lipid extraction using a modified method according to Folch et al. (33) for analysis of stored triglycerides. Briefly, 50 mg of tissue was homogenized for 15 sec at 12,000 rpm in chloroform-methanol, 2:1 (v/v), with heptadecanoic acid as an internal standard. FA methyl esters were prepared with the addition of 1 ml boron trifluoride-methanol (14% solution, Sigma)-hexane-methanol, 7:6:7 (v/v/v) after incubation for 55 min at 90°C (34). Butylated hydroxytolulene was added to all solvent mixtures to the final concentration of 0.01%. FA methyl esters were analyzed in duplicate

TABLE 2. Dietary composition

g/100 g Diet	Energy Intake	
	100% of AL	*60% of AL*
Fat[a]	20.0	33.3
Casein (vitamin free)	15.0	25.0
Corn starch (dextrinized)	45.0	8.5
Sucrose	10.0	16.7
Cellulose	5.0	8.3
AIN-93G vitamin mix[b]	1.0	1.7
AIN-93M mineral mix[c]	3.5	5.8
L-Cysteine	0.18	0.3
Choline bitartrate (98% choline)	0.25	0.42
Tert-butylhydroquinone	0.0004	0.007

AL, ad libitum.

[a] Dietary sources of fat included BT, SO, or menhaden fish oil (FO).

[b] Vitamin mix (g/kg): nicotinic acid 3.0, D-calcium pantothenate 1.6, pyridoxine HCL 0.70, thiamin HCL 0.60, riboflavin 0.60, folic acid 0.20, D-biotin 0.02, vitamin B_{12} (0.1% titrated in mannitol) 2.50, α tocopherol powder (250 U/g) 30.0, vitamin A palmitate (250,000 U/g) 1.60, vitamin D_3 (400,000 U/g) 0.25, phylloquinone 0.075, powdered sucrose 959.655.

[c] Mineral mix: calcium carbonate 35.7%, monopotassium phosphate 25.0%, sodium chloride 7.4%, potassium sulfate 4.66%, potassium citrate monohydrate 2.8%, magnesium oxide 2.4%, ferric citrate 0.606%, zinc carbonate 0.165%, manganese carbonate 0.063%, copper carbonate 0.03%, potassium iodate 0.001%, sodium selenate, anhydrous 0.001025%, ammonium molybdate, ^4H_2O 0.000795%, sodium metasilicate, ^9H_2O 0.145%.

using a Hewlett-Packard 5890 gas liquid chromatograph (Palo Alto, CA) equipped with a 30 m × 0.2 mm SP 2330 column (Supelco, Bellafonte, PA). FA methyl esters were identified based on the retention times of known FA standards (Supelco).

RNA isolation and Northern blotting

Adipose tissue was homogenized in TRIzol Reagent (Invitrogen, Burlington, ON, Canada) as per the manufacturer's protocol. Subsequent isolation, precipitation, and washing yielded total RNA from each tissue sample stored in Tris-EDTA buffer (pH 8.0). The optical density was measured using a spectrophotometer at 260–280 nm to ensure the integrity and concentration of isolated RNA. Agarose gel (1%) electrophoresis was performed using 20 μg total RNA along with LS174T cell total RNA as negative control with epadipose total RNA from an untreated animal as positive control. Following transfer onto nitrocellulose membranes (Schleicher and Schuell Inc., Keene, NH) using 20× SSC (sodium chloride, 17.5%; sodium citrate, 8.8%) solution overnight, RNA was cross-linked by UV exposure (Stratalinker, Bio-Rad, Life Sciences, Mississauga, ON, Canada).

Prehybridization and hybridization of the membranes were performed using a solution of 5× SSPE, 5× Denhardt's, 50% Formamide, 0.5% SDS, and 250 μg/ml ssDNA. Specific cDNA probes labeled with [^{32}P]dCTP by random priming using a Nick translation kit (Invitrogen-Life Technologies, Burlington, ON, Canada). The cDNA fragment (320 bp) for rat adipose tissue leptin was synthesized using 3′ primer TCATTGGCTATCTGCAGCAC and 5′ primer sequence AAGAAGATCCCAGGGAGGAA, and ligated into a pCRT-MII vector followed by transformation into DH5α cells, which were generously donated by Dr. Ruth B. S. Harris (Pennington Biomedical Research Center, Baton Rouge, LA) (35). Rodent β-actin cDNA was used for normalization of initial RNA input. Blots were washed twice with 2× SSC at room temperature followed by three washes with 0.1× SSC and 0.1% SDS at 64°C. Autoradiography was performed by exposure of radioactive membranes to Hyperfilm at −80°C with intensifying screens. The hybridization signal of *ob* mRNA from each pooled white adipose tissue (WAT) sample (total RNA) was determined from autoradiograms using a phosphoimaging system, which measures the product of the density and area of the hybridization signal. The resultant mRNA hybridization value reported for each group was the mean of three or four individual samples of pooled total RNA. Values of *ob* mRNA for each treatment group are expressed as arbitrary units relative to β-actin.

Immunoprecipitation and Western blotting

Protein A-Sepharose beads (0.072 g) were suspended in 1 ml of 10 mM Tris-HCl buffer and rotated for 15 min at 4°C. After centrifugation and washing, beads were incubated overnight with agitation in 1% BSA for blocking. The following day the beads were incubated overnight in 10 mM Tris-HCl buffer containing 10 μg rat-specific leptin antibody (Research Diagnostics, Inc., Flanders, NJ) producing coupled antibody Protein A-Sepharose beads. Coupled antibodies were then ready for incubation with sample, allowing precipitation and SDS-PAGE. Whole-cell lysate and serum samples were isolated as described below and used for immunoprecipation studies and analysis of leptin protein quantification.

Tissue aliquots were lysed in RIPA buffer (1× PBS, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS) at a volume of 3 ml per gram of tissue using a Polytron power homogenizer at 4°C. Following homogenization, the sample was transferred to microcentrifuge tubes and centrifuged at 10,000 *g* for 10 min at 4°C. Protein concentration was determined by the Bradford method and expressed as mg/g tissue (BioRad, Life Sciences). Whole-cell lysates (50–100 μg) were resolved by 15% SDS-PAGE containing 15% acrylamide and 10% bisacrylamide, and transferred to nitrocellulose membranes. Following transfer, membranes were processed as per the manufacturer's protocol and proteins were detected by enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfe, QC, Canada) and evaluated using densitometric scanning.

Statistics

Data were analyzed by two-way ANOVA using a general linear models procedure (SAS v.8.6, SAS Institute Inc., Cary, NC). Normal distribution of all variables was ensured using the Shapiro-Wilk test for normality. Comparisons between individual diets were made using Scheffé post-hoc test after treatment effects were determined by ANOVA. Differences were considered to be significant at $P < 0.05$. Using a multivariate model in SAS, partial correlation coefficients were calculated for all variables to examine associations between selected outcome measures. Data are presented as means ± SEM.

RESULTS

Animals and measurements on WAT

Food intake, final body weight, weight gain, and visceral (Ep, Rp, and Pr) adipose tissue weight for rats fed diets differing in fat source and level of energy are shown in **Table 3**. Food intakes were similar between dietary fat types and were lower ($P < 0.0001$) for the energy-restricted animals. A main effect of energy intake ($P < 0.0001$) was seen in lowering body weight in animals pair fed the 60% AL diets. At the end of the 10 week period, all animals had gained weight, with final body weights greater ($P < 0.0001$) in animals fed AL. Accordingly, weight gain was different ($P < 0.0001$) between the level of food intake as a main effect of energy. There were no main effects for fat or the interaction of these components with respect to body weight, weight gain, and food intake.

ER decreased ($P < 0.0001$) weights of all adipose tissues studied (Table 3). When comparing experimental groups,

TABLE 3. Final body mass, weight gain, adipose depot weights, and food intake from animals fed BT, SO, and FO at graded levels of energy intake for 10 weeks

	Energy Intake	Dietary Fat Source		
		BT	SO	FO
Body weight (g)	100%	526.1 ± 14.7	537.7 ± 11.6	507.4 ± 17.2
	60%	362.6 ± 3.8[a]	383.3 ± 6.5[a]	367.9 ± 4.6[a]
Weight gain (g)	100%	238.7 ± 13.8	249.1 ± 9.0	217.8 ± 17.1
	60%	71.9 ± 3.7[a]	91.7 ± 7.1[a]	77.4 ± 5.6[a]
Ep WAT (g)	100%	12.4 ± 1.6	11.4 ± 1.2	9.7 ± 0.9
	60%	2.9 ± 0.3[a]	3.6 ± 0.3[a]	2.5 ± 0.3[a]
Rp WAT (g)	100%	14.2 ± 1.4	14.0 ± 0.9	12.4 ± 1.3
	60%	3.2 ± 0.4[a]	3.8 ± 0.4[a]	2.6 ± 0.5[a]
Pr WAT (g)	100%	3.69 ± 0.5	3.7 ± 0.4	3.46 ± 0.4
	60%	0.92 ± 0.1[a]	1.1 ± 0.1[a]	0.71 ± 0.1[a]
Food intake (g)	100%	23.0 ± 0.7	22.7 ± 0.5	21.6 ± 0.6
	60%	13.8 ± 0.1[a]	13.7 ± 0.1[a]	13.0 ± 0.1[a]
Insulin (μU/ml)	100%	68.3 ± 0.7	69.1 ± 0.7	70.8 ± 0.5
	60%	68.5 ± 0.7	68.5 ± 0.8	68.4 ± 0.5

Ep, epididymal; Pr, perirenal; Rp, retroperitoneal; WAT, white adipose tissue. Data are presented as mean ± SEM, (n = 12).

[a] Denotes statistically significantly different for main effect energy AL fed animals ($P < 0.05$).

Ep and Rp wet weights were similar and of larger mass ($P < 0.025$) than Pr WAT weights. Between dietary regimens, total abdominal adipose was larger in animals fed BT > SO > FO AL (30.5 ± 3.3, 29.2 ± 2.1, and 25.7 ± 2.4 g, respectively), although they were not different ($P = 0.2$). Restricted feeding showed a dissimilar pattern with SO > BT > FO (8.7 ± 0.8, 7.1 ± 0.8, and 5.9 ± 0.9 g, respectively) feeding stimulating larger adipose growth. There was no main effect for the dietary source of fat or interactive effects with respect to changes in abdominal adipose tissue weight in either AL or 60% AL feeding groups.

Serum leptin and insulin analysis

Fasting circulating concentrations of leptin were lower ($P < 0.0001$) in the 60% energy-restricted animals, as compared with AL-fed animals (**Fig. 1**). Leptin levels were lower in restricted (2.7 ± 0.2, 1.9 ± 0.3, and 2.6 ± 0.3 ng/ml, respectively), as compared with AL-fed rats (10.1 ± 1.6, 8.9 ± 1.3, and 11.6 ± 2.4 ng/ml, respectively) for BT-, SO-, and FO-fed animals; however, no interactive effects or main effects of fat composition were observed on changes in serum leptin levels. Fasting serum insulin levels were not different between any of the experimental groups in this study and were not influenced by an interaction of fat composition and energy intake ($P = 0.18$) (Table 3).

FA composition of WAT

The FA compositions of Pr, Ep, and Rp adipose are shown in **Tables 4**, **5**, and **6**, respectively. The adipose FA compositions for AL-fed animals were generally representative of the dietary fat consumed. Adipose tissue concentrations of 18:0 and 18:1 (n-9) were highest in the BT group ($P < 0.03$), and 18:2 (n-6) was greatest in the SO group ($P < 0.05$), thus resembling the FA composition in the diet (Table 1). Similarly, greater concentrations of 22:6 (n-3) were found in the FO group ($P < 0.0001$). Conversely, animals restricted in energy showed modified-FA compositions of adipose but were generally reflective of the fat consumed with some notable changes; specifically, an observed increase in 18:2 (n-6) concentrations. An interactive effect of fat composition and ER was observed for Pr adipose tissue concentrations of 14:0 ($P < 0.0004$, data not shown), 16:0 ($P < 0.051$), 18:0 ($P < 0.0001$), 18:2 (n-6) ($P < 0.014$), and 22:6 (n-3) ($P < 0.0001$), as shown in Table 4. In contrast, only 22:6 (n-3) concentrations were influenced by the interaction of these components of the diet ($P < 0.0001$) in Ep adipose tissue (Table 5). Independent effects of dietary fat composition and energy intake were found for 14:0 (data not shown), 16:0, 16:1 (n-7), 18:0, and 18:2 n-6 ($P < 0.05$) deposition in Ep adipose (Table 5). Rp tissue concentrations of 14:0 (data not shown), 16:0, 18:0, 18:2 (n-6), and 22:6 (n-3) were influenced ($P < 0.008$) by the interaction of dietary fat composition and ER (Table 6).

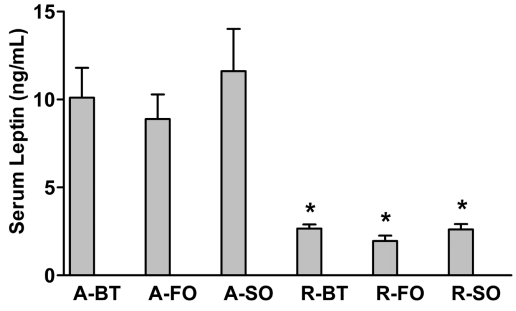

Fig. 1. Serum leptin concentrations of rats fed beef tallow (BT), safflower oil (SO), and fish oil (FO) at graded levels of energy intake.

TABLE 4. Major FAs accumulated in perirenal adipose depots of rats fed BT, SO, and FO at graded levels of energy intake for 10 weeks

FA	%	BT	SO	FO
16:0	100	26.3 ± 0.9^{a}	22.1 ± 1.3^{a}	21.5 ± 0.7^{b}
	60	23.9 ± 0.5^{a}	16.4 ± 1.1^{a,c}	14.5 ± 0.9^{a,c}
16:1 (n-7)	100	6.6 ± 0.4	4.8 ± 0.5	4.5 ± 0.3
	60	4.3 ± 0.5^{a,c}	2.1 ± 0.4^{b,c}	1.5 ± 0.3^{b,c}
18:0	100	4.5 ± 0.1^{a}	3.5 ± 0.1^{b}	3.1 ± 0.1^{b}
	60	7.4 ± 0.3^{a,c}	3.6 ± 0.1^{b}	3.7 ± 0.1^{b}
18:1	100	46.5 ± 2.2^{a}	31.5 ± 1.8^{b}	28.7 ± 1.9^{b}
	60	50.3 ± 0.4^{a}	30.4 ± 1.9^{b}	25.2 ± 0.6^{b}
18:2 (n-6)	100	11.5 ± 3.5^{a}	34.1 ± 3.5^{b}	38.8 ± 0.9^{b}
	60	9.3 ± 0.5^{a}	43.4 ± 3.4^{b}	51.1 ± 1.6^{b,c}
22:6 (n-3)	100	ND	ND	6.0 ± 0.01
	60	ND	ND	4.6 ± 0.03^{c}
Sum SFA	100	32.7 ± 1.1^{a}	27.0 ± 1.4^{b}	25.8 ± 0.9^{b}
	60	33.5 ± 0.3^{a}	21.1 ± 1.2^{b,c}	19.1 ± 0.9^{b,c}
Sum MUFA	100	53.2 ± 2.6^{a}	36.3 ± 2.3^{b}	33.2 ± 2.2^{b}
	60	54.6 ± 0.5^{a}	32.6 ± 2.6^{b}	26.7 ± 1.0^{b}
Sum PUFA	100	11.9 ± 3.5^{a}	35.1 ± 3.5^{b}	40.4 ± 1.0^{b}
	60	9.7 ± 0.5^{a}	44.3 ± 3.4^{b}	52.1 ± 1.7^{b}

ND, nondetectable; SFA, saturated FA; MUFA, monounsaturated FA. Data are presented as mean ± SEM, (n = 12).

^{a,b} Groups with different letters are statistically significantly different within the same level of food intake, $P < 0.05$.

^{c} Denotes statistically significantly different from AL-fed animals, $P < 0.05$.

TABLE 5. Major FAs accumulated in epididymal adipose depots of rats fed BT, SO, and FO at graded levels of energy intake for 10 weeks

FA	%	BT	SO	FO
16:0	100	26.0 ± 0.8^{a}	22.6 ± 0.5^{a}	20.5 ± 0.6^{b}
	60	21.7 ± 0.7^{a,c}	16.1 ± 0.7^{b,c}	14.8 ± 0.8^{b,c}
16:1 (n-7)	100	7.8 ± 0.5^{a}	5.7 ± 0.3^{b}	4.6 ± 0.2^{b}
	60	4.4 ± 0.3^{a,c}	2.8 ± 0.2^{a,c}	2.2 ± 0.3^{b,c}
18:0	100	4.5 ± 0.2^{a}	3.1 ± 0.1^{b}	2.8 ± 0.1^{b}
	60	5.4 ± 0.4^{a}	3.1 ± 0.32	3.2 ± 0.06^{b}
18:1	100	47.0 ± 1.9^{a}	31.9 ± 1.5^{b}	28.2 ± 1.8^{b}
	60	43.1 ± 2.5^{a}	29.9 ± 1.4^{b}	27.1 ± 0.4^{b}
18:2 (n-6)	100	9.9 ± 3.1^{a}	31.7 ± 2.2^{b}	39.6 ± 3.0^{b}
	60	18.0 ± 4.3^{a}	43.7 ± 2.6^{b}	48.0 ± 1.5^{b}
22:6 (n-3)	100	ND	ND	6.1 ± 0.01
	60	ND	ND	3.3 ± 0.09^{c}
Sum SFA	100	32.5 ± 1.0^{a}	27.2 ± 0.7^{b}	24.5 ± 0.8^{b}
	60	28.8 ± 1.2^{a}	21.4 ± 1.0^{b,c}	18.8 ± 0.8^{b,c}
Sum MUFA	100	54.9 ± 2.2^{a}	37.7 ± 1.7^{b}	32.8 ± 2.1^{b}
	60	47.6 ± 2.7^{a}	34.3 ± 1.5^{b}	29.4 ± 0.7^{b}
Sum PUFA	100	10.5 ± 3.1^{a}	32.9 ± 2.3^{b}	40.1 ± 3.0^{b}
	60	18.3 ± 4.3^{a}	42.1 ± 2.6^{b}	48.3 ± 1.5^{b}

Data are presented as mean ± SEM (n = 12).

^{a,b} Groups with different letters are statistically significantly different within the same level of food intake, $P < 0.05$.

^{c} Denotes statistically significantly different from AL-fed animals, $P < 0.05$.

TABLE 6. Major FAs accumulated in retroperitoneal adipose depots of rats fed BT, SO, and FO at graded levels of energy intake for 10 weeks

FA	%	BT	SO	FO
16:0	100	27.6 ± 0.3[a]	22.6 ± 0.4[b]	20.0 ± 0.3[c]
	60	23.4 ± 0.5[a,d]	15.3 ± 0.6[a,d]	13.5 ± 0.6[b,d]
16:1 (n-7)	100	6.7 ± 0.3[a]	4.3 ± 0.1[b]	3.7 ± 0.2[b]
	60	3.2 ± 0.2[a,d]	1.6 ± 0.1[b,d]	1.0 ± 0.1[b,d]
18:0	100	4.9 ± 0.1[a]	3.4 ± 0.1[b]	2.7 ± 0.08[b]
	60	7.9 ± 0.2[a,d]	3.6 ± 0.1[b]	3.8 ± 0.1[b,d]
18:1	100	51.0 ± 0.4[a]	31.1 ± 0.4[b]	26.7 ± 0.3[c]
	60	50.6 ± 0.3[a]	29.2 ± 0.5[b]	25.1 ± 0.5[c]
18:2 (n-6)	100	5.8 ± 0.2[a]	35.0 ± 1.0[b]	44.0 ± 0.7[b]
	60	10.5 ± 0.4[a,d]	47.0 ± 0.9[b,d]	53.1 ± 1.2[b,d]
22:6 (n-3)	100	ND	ND	5.1 ± 0.01
	60	ND	ND	3.7 ± 0.02[d]
Sum SFA	100	34.6 ± 0.4[a]	27.4 ± 0.6[b]	23.9 ± 0.4[c]
	60	33.5 ± 0.4[a]	19.8 ± 0.6[b,d]	18.0 ± 0.6[b,d]
Sum MUFA	100	57.8 ± 0.4[a]	35.4 ± 0.5[b]	30.4 ± 0.5[c]
	60	53.8 ± 0.4[a,d]	30.8 ± 0.2[b,d]	26.2 ± 0.5[b,d]
Sum PUFA	100	6.1 ± 0.3[a]	35.8 ± 1.0[b]	44.7 ± 0.7[c]
	60	10.9 ± 0.4[a,d]	48.0 ± 0.9[b,d]	54.5 ± 1.0[c,d]

Data are presented as mean ± SEM (n = 12).

[a,b,c] Groups with different letters are statistically significantly different within the same level of food intake, $P < 0.05$.

[d] Denotes statistically significantly different from AL-fed animals, $P < 0.05$.

TABLE 7. Correlation coefficients of serum leptin with body weight, weight gain, visceral adipose tissue, epididymal, retroperitoneal, perirenal, insulin and the sum of retroperitoneal SFA, MUFA, and PUFA

	Serum Leptin		
Body weight		Rp SFA	
r	0.39	*r*	0.40
P	0.001	*P*	0.0009
Weight gain		Rp MUFA	
r	0.40	*r*	0.27
P	0.0009	*P*	0.025
Total WAT		Rp PUFA	
r	0.58	*r*	−0.40
P	<0.0001	*P*	0.0008
Ep WAT		Insulin	
r	0.58	*r*	0.083
P	<0.0001	*P*	0.5033
Rp WAT			
r	0.48		
P	<0.0001		
Pr WAT			
r	0.36		
P	0.003		

Ep, epididymal; Rp, retroperitoneal; Pr, perirenal.

When animals were food restricted, adipose depot concentrations of 16:0 and 16:1 (n-7) were reduced ($P < 0.0001$) independent of dietary fat source consumed. Accordingly, SO and FO feeding reduced ($P < 0.0001$) the sum of SFA in all adipose depots examined. Additionally, concentrations of 18:2 (n-6) increased in response to ER in all animals, with the exception of Pr tissue from BT-fed animals, though this was only found in Rp tissue from all sources of fat ($P < 0.05$) and in Pr tissues from FO-fed animals. These changes in 18:2 (n-6) concentrations were reflected in the sum of PUFA that was greater in Rp from all sources of fat and in Pr adipose from rats fed SO and FO. Adipose from animals fed BT had greater concentrations of SFA and monounsaturated FAs (MUFAs), whereas the reverse tendency was found in SO- and FO-fed rats, consistent with the FA composition of the diet fat. Generally, Rp adipose was influenced to a greater extent than either Ep or Pr, with Rp depot FA compositions much more reflective of the dietary fat (Table 6).

Partial correlations between serum leptin levels and outcome measures

Positive associations between serum leptin, body weight ($r = 0.39$, $P < 0.0001$), and visceral adipose ($r = 0.40$, $P < 0.002$) were observed (**Table 7**). Of note was the observed positive correlation between adipose depots and leptin levels. Ep adipose was the strongest predictor of serum leptin levels. There was a positive association ($r = 0.48$, $P < 0.0001$) between leptin and Pr adipose FA concentrations of 22:6 (n-3), but not with any other FAs or sums of FAs. Examination of Ep adipose revealed positive correlations between serum leptin and concentrations of 16:0 and 16:1 (n-7) ($r = 0.26$, $P < 0.035$; and $r = 0.37$, $P < 0.0019$). Rp sums of SFA and MUFA were positively correlated ($r = 0.40$, $P < 0.0009$; and $r = 0.27$, $P < 0.0259$) with circulating leptin levels. In contrast, Rp tissue concentrations of PUFA were negatively correlated ($r = -0.40$, $P < 0.0008$) with leptin levels, which is consistent with the observed accumulation of 18:2 (n-6) and 22:6 (n-3) FAs in this depot. There was no observed association between serum leptin and insulin.

Effects of dietary fat type and ER on *obese* mRNA levels

Total RNA was isolated from six or seven animals per treatment group and pooled to provide the necessary quantity for triplicate analysis. Northern blot analysis of pooled total RNA samples (20 μg total RNA) from each treatment group and adipose depot were used for analysis of *ob* mRNA levels. The intensities of the specific signals for leptin mRNA were lower in animals fed the energy-restricted versus AL diets (**Figs. 2**, **3**) for all depots studied, which is consistent with previous reports (26, 29). In AL animals, visceral *ob* mRNA levels appeared to be higher in Rp adipose, with the exception of FO feeding, which resulted in enhanced *ob* mRNA levels in Ep. Dietary fat type also exerted different regulatory control over expression in these tissues. For example, animals fed BT had a 1.5-fold increase in *ob* mRNA levels in Rp, whereas SO-fed rats had a 3-fold increase in *ob* mRNA concentrations in Rp versus Ep adipose. FO feeding increased *ob* mRNA levels by 66% in Ep versus Rp adipose. Energy-restricted animals had reduced levels of *ob* mRNA in all tissues, with the lowest levels observed in BT- and SO-fed animals. This effect did not appear to be tissue specific, as indicated by the near identical levels in both visceral depots. In contrast, FO feeding unregulated Ep adipose *ob* mRNA by 1.5-fold compared with Rp adipose, which was also similar to BT and SO levels. Thus, ER with FO feeding was the only dietary regimen to influence *ob* mRNA and was tissue-specific to Ep adipose. Leptin expression analysis could not be performed on Pr adipose due to the limited size of this depot in these animals.

Effects of dietary fat type and ER on WAT leptin levels

To examine the effect of dietary fat type and ER on leptin concentrations in visceral adipose, we measured protein levels using an anti-rat polyclonal antibody, which was previously shown (9) to recognize plasma leptin. After several attempts, it was determined that either tissue leptin concentrations were too low to detect or the method was not sensitive enough for quantification. Immunoprecipitation attempted to concentrate levels of leptin in adipose and serum for measurement using protein A-Sepharose beads coupled to leptin polyclonal antibodies. Unfortunately, it appeared that levels could not be quantified using general antibody methods.

DISCUSSION

To our knowledge, this is the first study to demonstrate the interactive effects of dietary FA composition and ER on adipose tissue *ob* mRNA levels in rats. These findings reveal a lack of association between *ob* mRNA and circulating leptin levels as determined by the limited response to dietary regimen at an equivalent level of energy intake. In particular, serum leptin levels were similar, whereas *ob* mRNA levels were influenced by dietary treatment and depot. These parameters were profoundly influenced in response to energy intake level; in particular, AL animals had greater levels of serum leptin and *ob* mRNA as compared with 60% AL-fed animals. Thus, leptin production is tightly regulated by energy intake, apart from the size of adipose stores, consistent with previous reports in humans and animals (26–29).

Numerous authors have found reduced levels of visceral fat in animals fed diets high in PUFA (19, 21, 36). In the present study, total visceral adipose, which is the contribution from Ep, Rp, and Pr depots, was almost 4-fold heavier in AL animals compared with energy-restricted rats. Moreover, dietary fat source had a negligible effect on fat pad size. Hill et al. (36) fed rats high-fat (45% calories) diets containing BT, corn oil, and FO and observed similar body weights during a 7 month feeding trial, which is consistent with our findings after 10 weeks. Furthermore, only after 3 months of high-fat FO feeding did Hill et al. (36) observe reduced Ep and Rp tissue weight. Therefore, a failure to demonstrate a reduction in adipose depot weight after FO feeding in the present study may be attributed to the shorter length of feeding or the breed of animal. In addition, direct comparison between this study and others may be difficult due to varied percentages of fat in the diet, which supplied 40% of the calories in the present study. Moreover, a limitation in many feeding trials investigating the effects of FO on body composition is a lower food intake caused by reduced palatability of the diet. In fact, Hill et al. (36) observed reduced food intake in animals fed FO as compared with other dietary groups. Therefore, reduced adipose weight may be attributable to a lower energy intake or possibly the FA composition of the FO employed, and this difference in profiles may account for discrepancies between findings.

Consistent with our previous findings (12, 21) and those of others (36–38), adipose FA composition reflected the variations in dietary fat. Conversely, ER reduced adipose concentrations of 16:0, 16:1 (n-7), and 18:1 (n-9) in all depots studied, with the exception of Pr adipose from animals fed BT. Consequently, 18:2 (n-6) concentrations increased proportionately in these same tissues. Together, these data demonstrate a tendency for increased n-6 PUFA deposition in response to ER. The shift toward in-

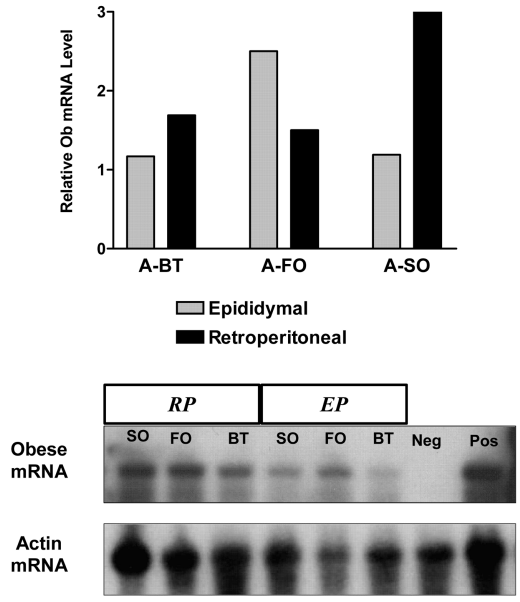

Fig. 2. Northern blot analysis of *ob* mRNA levels in epididymal (Ep) and retroperitoneal adipose tissue from rats fed BT, SO, and FO at ad libitum (AL) energy intake for 10 weeks.

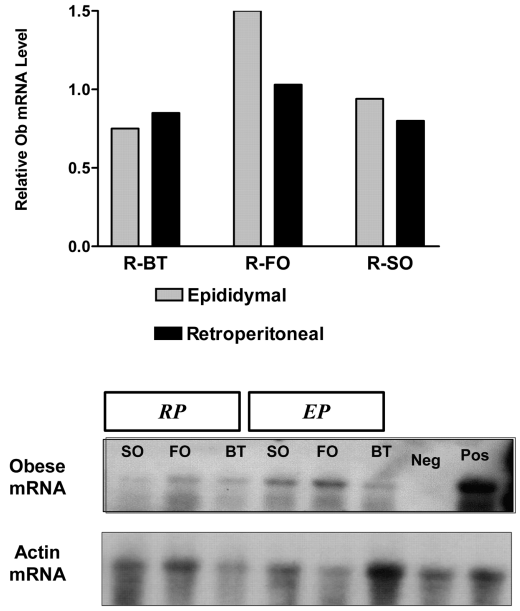

Fig. 3. Northern blot analysis of *ob* mRNA levels in Epand retroperitoneal adipose tissue from rats fed BT, SO, and FO at 60% AL energy intake for 10 weeks.

creased unsaturation can be attributed to an exchange between shorter, more common SFAs. These results are consistent with other reports demonstrating a preferable retention of 18:2 (n-6) in serum and liver triglycerides that was ascribed to large exchanges in 16:0 and 16:1 (n-7) during ER (12, 39–40). Furthermore, the greater response to FA accumulation from dietary fat within Rp adipose suggests that tissue-specific differences exist within visceral adipose.

Takahashi and Ide (41) fed rats high-fat diets (20% wt/wt) containing SO or olive oil for 3 weeks. These authors found similar weight gains and food intake, as well as Ep and Pr adipose FAs, between both diet fat treatment groups. In general, the FA composition of Pr, Rp, and Ep adipose was similar, whereas large differences were found between the sources of dietary fat, which is consistent with previous reports by Cha and Jones (12). In the present study, BT-fed rats retained more SFA and MUFA in their visceral adipose than FO- and SO-fed rats. These differences were the result of the high PUFA content of those fats. Tissue-specific differences in FA composition have been demonstrated by Bailey et al. (38) in rats fed chow AL or food-restricted by alternate day feeding for 28 months. The major FA profile of the rodent chow was as follows: 16:0, 18:0, 18:1 (n-9), and 18:2 (n-6), with concentrations of 20.5%, 9.3%, 30.5%, and 27.3%, respectively. Every 4 months, animals were selected randomly for FA analysis of Pr, Ep, and inguinal adipose tissue. As a result, these researchers found an increased concentration of SFA in Pr versus Ep adipose tissues after 12 months. The present data do not support this finding, but the shorter feeding trial may explain these differences. In contrast, ER increased the concentration of 18:2 (n-6) in adipose tissues of our animals, whereas Bailey et al. (38) found levels of 18:1 (n-9) to be elevated. Interestingly, Pr tissue from animals fed BT had a similar FA composition to those of Bailey et al. (38). This is likely related to the similarities in the FA profile of BT with the chow used in the previous study. When examining the correlations between the sum of Rp FAs, serum leptin was positively associated with SFA and MUFA, whereas a significant negative correlation was observed with the sum of PUFA. Recent intervention studies have demonstrated a similar trend between serum leptin and increased intake of these FAs in the diet (22, 39).

It remains unknown how leptin expression is regulated over the short term and long term in response to changes in physiological state such as adiposity. To further explore this issue, we examined *ob* mRNA levels in adipose tissue following dietary treatment. Ep and Rp *ob* mRNA levels were elevated in the AL and reduced in the energy-restricted animals. Tissue-specific differences were evident in the varied responses to FO and SO. Adipose tissue *ob* mRNA levels were higher after PUFA feeding, which is consistent with previous reports (22, 41), yet contrasts with the observations of Raclot et al. (24). These authors found higher *ob* mRNA levels with feeding of a lard-olive oil mix, or pure 20:5 (n-3) versus 22:6 (n-3) or FO as the sole source of fat. Interestingly, the effect of FO feeding may depend on the FA composition, more specifically, the ratio of 20:5 (n-3) to 22:6 (n-3). Furthermore, the combination of lard-olive oil yields a unique FA profile that was not explored in the present study. These results suggest that PUFA-rich fat sources influence *ob* mRNA differently by relating specifically to the source (n-3 or n-6) and the site (Ep or Rp). Taken together, it is apparent that the distinct structure of FAs is integral to the regulation of leptin expression, as only FA composition varied between energy levels.

Ep and Rp depot weights were similar after BT and SO feeding; however, *ob* mRNA levels were elevated after FO feeding versus other fat sources. Moreover, *ob* mRNA levels were greater in Ep adipose. Our data show that ER reduced the concentration of n-3 FAs in depots, which may explain the differences in *ob* mRNA levels between energy intake levels, considering that other factors were similar between adipose depots. Leptin expression was variable within similar energy levels but did not influence circulating leptin, suggesting that leptin production may be regulated by levels of leptin protein in adipose tissue. Russell and coworkers (42) have demonstrated differences in leptin secretion from adipose depots, which may contribute significantly to circulating levels. These researchers found a higher level of leptin secretion in Sc, compared with visceral, adipose. We did not explore this issue in the present study because visceral adipose *ob* mRNA levels in rodents are reported to be at least 3.6-fold higher than Sc levels, thus supporting the belief that visceral adipose is the primary source of leptin in rodents (25). When considering differences in weight gain, AL animals were ~100 g heavier than restricted animals, thus, total visceral adipose weight will account for only 25% of the difference. The contribution of Sc adipose to total fat stores may be substantial, but as demonstrated previously, greater *ob* mRNA levels can be found in visceral adipose, which is also a better predictor of circulating leptin (43).

Conflicting reports regarding the tissue-specific differences in leptin expression (24) and discordance between circulating levels (31) have made interpretation and extrapolation of leptin expression difficult. Circulating leptin levels correlated with weight gain, final body weight, and visceral adipose, thereby confirming the strong association between leptin and adipose stores in animals. In particular, Ep adipose was a better predictor of serum levels than either Rp or Pr depots. Energy intake and dietary fat did not interactively affect circulating leptin, likely attributable to the large variability in leptin values in the current study, even with similar body weight and food intake.

Supraphysiologic insulin levels, as induced during hyperinsulinemic clamp, have been shown to influence leptin mRNA expression (26, 29), and may be involved in regulating leptin production. In the present study, circulating insulin levels were similar between groups and were not associated with leptin, which is consistent with a previous report in rats that were fed a high-fat diet for 14 weeks (44). Studies demonstrating an association between leptin and insulin show that the effect is dose dependant, as hyperinsulinemia is required for an observed shift in circu-

lating leptin (45). In the present study, there was a lack of association between insulin and leptin, suggesting separate mechanisms of action for these metabolic hormones.

The differences in *ob* mRNA levels and the disparity between serum leptin values suggest that the synthesis of leptin protein may not be regulated transcriptionally, but more likely by posttranscriptional processes. It is probable that leptin levels are controlled by tissue accumulation, and thus, by leptin secretion. Examination of adipose leptin protein by Western analysis was unsuccessful in the present study. Bornstein et al. (46) required a highly sensitive analytical method employing immunohistochemistry and gold labeling to detect minute quantities of leptin; therefore, sensitivity may explain the inability to detect leptin. Furthermore, our choice of an overnight fast may have reduced concentrations beyond the level of detection (47), although confirmation will require further study.

In conclusion, these data reveal that dietary fat type affects leptin expression in a tissue-specific manner at varying levels of energy intake. Rp PUFAs were negatively correlated with circulating leptin, although distinguishing the effects of n-6 or n-3 FAs is difficult. These data confirm that energy intake is a prominent factor affecting leptin expression and production. Furthermore, results from the present study suggest that dietary FAs may have a role in determining circulating leptin levels.

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