Reduced Fat Mass in Rats Fed a High Oleic Acid–Rich Safflower Oil Diet Is Associated with Changes in Expression of Hepatic PPARα and Adipose SREBP-1c–Regulated Genes[1,2]

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ABSTRACT PPARs and sterol regulatory element-binding protein-1c (SREPB-1c) are fatty acid–regulated transcription factors that control lipid metabolism at the level of gene expression. This study compared a high oleic acid–rich safflower oil (ORSO) diet and a high-butter diet for their effect on adipose mass and expressions of genes regulated by PPAR and SREPB-1c in rats. Four groups of Wistar rats were fed 30S (30% ORSO), 5S (5% ORSO), 30B (29% butter + 1% ORSO), or 5B (4% butter plus 1% ORSO) diets for 15 wk. Compared with the 30B group, the 30S group had less retroperitoneal white adipose tissue (RWAT) mass and lower mRNA expressions of lipoprotein lipase, adipocyte fatty acid-binding protein, fatty acid synthase, acetyl CoA carboxylase, and SREBP-1c in the RWAT, higher mRNA expressions of acyl CoA oxidase, carnitine palmitoyl-transferase 1A, fatty acid binding protein, and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase in the liver ($P < 0.05$). The 18:2(n-6) and 20:4(n-6) contents in the liver and RWAT of the 30S group were >2 fold those of the 30B group ($P < 0.05$). These results suggested that the smaller RWAT mass in rats fed the high-ORSO diet might be related to the higher tissue 18:2(n-6) and 20:4(n-6). This in turn could upregulate the expressions of fatty acid catabolic genes through the activation of PPARα in the liver and downregulate the expressions of lipid storage and lipogenic gene through the suppression of SREBP-1c in the RWAT. J. Nutr. 136: 1779–1785, 2006.

KEY WORDS: • *High oleic acid-rich safflower oil diet* • *PPARα* • *SREBP-1c* • *obesity* • *rats*

Long-term consumption of a high-fat diet is associated with obesity and chronic diseases. The adipogenecity of a high-fat diet varies with the type of fat used (1,2). In rodents, diets high in unsaturated fat were shown to be less adipogenic than diets high in saturated fat, and was attributed to a higher oxidation rate (3) and diet-induced thermogenesis (4,5). The metabolic changes caused by dietary fat are regulated at the level of gene expression because lipid-regulated transcription factors such as PPARs, sterol regulatory element-binding protein-1c (SREBP-1c),[4] and liver-X-receptor (LXR) (6–8) were discovered.

PPARs are fatty acid–regulated nuclear hormone receptors that control lipid oxidation (9), adipocyte differentiation, glucose and lipid storage, and inflammation (10). The PPAR signaling pathway denotes the successive binding of receptors to specific ligands and then to a specific DNA sequence [peroxisome proliferator responsive element (PPRE)], which in turn activates the transcription of genes downstream of the PPRE. Genes with PPRE, i.e., target genes of PPARs, are an array of enzymes/proteins involved in fatty acid metabolism, lipid transport, and storage (9), such as acyl-CoA oxidase (ACO), fatty acid binding protein (FABP), and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS). PPARs are viewed as monitors of intracellular nonesterified fatty acid (NEFA) (11).

SREBPs are transcription factors that regulate lipid homeostasis by controlling the expressions of a range of enzymes required for endogenous cholesterol, fatty acid, triacylglycerol (TG) and phospholipid synthesis. Intracellular sterol content–dependent proteolytic cleavage of SREBP in the endoplasmic reticulum membrane and the subsequent translocation of the released active SREBP into the nucleus lead to the transcription of genes of lipid synthesis (12). As the predominant subtype in rodents and human, SREBP-1c binds sterol regulator element (SRE) in the gene promoters of many enzymes involved in lipogenesis, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (6). Transgenic mice studies suggest that SREBP-1c is involved in fatty acid synthesis and insulin-induced glucose metabolism (particularly in lipogenesis) (12,13).

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[2] Supplemental Tables 1 and 2 are available with the online posting of this paper at www.nutrition.org.

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[4] Abbreviations used: 5B, 5% butter fat; 5S, 5% oleic acid–rich safflower oil; 30S, 30% oleic acid–rich safflower oil; 30B, 30% butter fat; 30B/S, 30B diet for 12 wk and then switched to 30S diet for 3 wk; ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; aP2, adipocyte fatty acid-binding protein; CPT1A, carnitine palmitoyl-transferase 1A; EWAT, epididymal adipose tissue; FABP, fatty acid binding protein; FAS, fatty acid synthase; HMGS, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase; LPL, lipoprotein lipase; LXR, liver-X-receptor; NEFA, nonesterified fatty acid; ORSO, oleic acid–rich safflower oil; PPRE, peroxisome proliferator responsive element; RWAT, retroperitoneal adipose tissue; SREBP-1c, sterol regulatory element-binding protein-1c; SRE, sterol regulator element; TG, triglyceride.

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As a ligand, fatty acid binds directly to PPARs, and trigger the PPAR signaling pathway. In the in vitro binding assay, rat PPARα binds to fatty acids with K_d (in μmol/L) of >30 for 10:0 and 12:0, 5.4 for 14:0, 1.5 for 16:0, 1.1 for 18:0, 0.6 for 18:1 (n-9), 1.1 for 18:2 (n-6), 0.27 for 18:3 (n-6), and 1.2 for 20:4 (n-6) (14). In contrast, PUFAs regulate the nuclear abundance of nSREBP-1c and do not bind directly to SREBP. The hierarchy for fatty acid regulation of SREBP-1c mRNA level is 22:5(n-3) = 20:4(n-6) > 18:2(n-6) > 18:1(n-9) in the in vitro experiment (15). In the in vivo experiment, PUFA-rich corn oil, walnut oil, safflower oil, and fish oil markedly suppressed hepatic mRNA expression of SREBP-1 and its target gene, FAS, but olive oil and lard did not (15,16). Thus, 18:1(n-9) is as active as PUFA for PPARα ligand binding and activation (14,17), but oleic acid, triolein, and olive oil are much less effective in the suppression of lipogenic genes through SREBP-1c.

The demonstration of the fatty acid regulation of genes coded for lipid metabolism through PPARs and SREBP provides a rational basis for the early findings that diets high in unsaturated fat are less adipogenic than diets high in saturated fat (1,2). A high (n-3) PUFA-rich fish oil diet is less adipogenic (18–21) due to a coordinated induction of fatty acid oxidation genes through PPARα (22) and suppression of lipogenic genes through SREBP-1c (23). The (n-6) PUFA-rich oils were also effective in the suppression of lipogenic genes through SREBP-1c (15,16). The effect of a high oleic acid–rich fat diet, however, is controversial. A high oleic acid–rich safflower oil (ORSO) diet was shown to be as effective as high (n-3) and (n-6) PUFA diets in lowering body fat accumulation in meal-fed Sprague-Dawley rats (5). However, a high-rapeseed oil [60% 18:1(n-9) and 20% 18:2(n-6)] diet was ineffective in mice that consumed food ad libitum (21).

Compared with other fat types, ORSO has the advantages of better cooking stability, adequate essential fatty acid [~25% is 18:2(n-6)], very low saturated fatty acid content, and a lower price than olive oil. This study was designed to test the hypothesis that feeding a high-ORSO diet can increase expressions of genes regulated by PPARα and is less adipogenic than a diet high in saturated fat.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (6 wk old, n = 41) were purchased from the laboratory animal center of National Taiwan University College of Medicine (Taipei, Taiwan). Rats were housed individually in stainless steel wire cages in a room maintained at 25 ± 2°C, with a controlled 12-h light:dark cycle (light period: 0600–1800). For 2 wk of acclimation, they consumed a nonpurified diet (Laboratory Rodent Chow, Ralston Purina) and were then randomly assigned to 4 diet groups: 30S (30% ORSO), 5S (5% ORSO), 30B (29% butter and 1% ORSO), and 5B (4% butter and 1% ORSO). After 12 wk, when the 30B group had a significantly higher serum TG concentration than the 30S group, half of the 30B group was switched to the 30S diet (the 30B/S group) and fed for 3 more weeks.

The composition of the 4 test diets was modified from AIN-76 (**Table 1**) (24). The amounts of casein, and vitamin and mineral mixtures in the high-fat diets were adjusted to ensure that the nutrient/energy ratios were equivalent. Rats had free access to food and tap water. Body weight and food intake were recorded weekly. Animal care and handling conformed to accepted guidelines (25), and were approved by the Institutional Animal Care and Use Committee, National Taiwan University.

Tissue sampling and preparation. After 15 wk of feeding, rats were killed by carbon dioxide asphyxiation after overnight food deprivation. Serum were obtained by centrifugation of blood and stored at −20°C for lipid and leptin analyses. Liver, epididymal white adipose tissue (EWAT), and retroperitoneal white adipose tissue (RWAT) were excised and weighed. Liver and RWAT were quick-frozen in liquid N_2 and stored at −80°C for total RNA extraction. A portion of fresh liver was homogenized in 0.3 mol/L mannitol, 10 mmol/L HEPES, 1 mmol/L EGTA buffer (10% wt:v, pH 7.2) and centrifuged at 1000 × g for 10 min at 4°C. The resulting postnuclear supernatant was analyzed for activities of ACO according to the method of Small (26).

TABLE 1

Composition of the 5S, 5B, 30S and 30B test diets

	5S	5B	30S	30B
		g/kg		
Casein	200	200	260	260
DL-Methionine	3	3	3	3
Cornstarch	325	325	160	160
Sucrose	325	325	160	160
Cellulose	50	50	61	61
ORSO[1]	50	10	300	10
Butter[2]	—	40	—	290
AIN-76 vitamin mixture[3]	10	10	12	12
AIN-76 mineral mixture[3]	35	35	42	42
Choline	2	2	2	2
Energy, *kJ/g*	16.31	16.31	21.03	21.03
Protein, *% of energy*	21.03	20.91	21.03	20.91
Fat, *% of energy*	11.65	11.65	53.68	53.68

[1] Fatty acid composition of ORSO: 7.0% 16:0, 1.1% 16:1, 2.5% 18:0, 65.3% 18:1(n-9), 24.1% 18:2(n-6) as analyzed by GC.
[2] Fatty acid composition of butter: 0.9% 8:0, 1.9% 10:0, 4.4% 12:0, 13.0% 14:0, 33.6% 16:0, 4.7% 16:1, 10.9% 18:0, 24.7% 18:1(n-9), 1.3% 18:2(n-6), 1.3% 18:3(n-6), 1.3% conjugated linoleic acid as analyzed by GC.
[3] AIN-76 vitamin mixture, AIN-76 mineral mixture (24).

Biochemical analyses. Liver lipids were extracted with a mixture of chloroform:methanol (2:1, v:v). Both liver and serum lipids were measured by enzymatic methods using commercial kits for TG (GPO-PAP assay kit, Randox Laboratory) and NEFA (NEFA kit, Randox Laboratory). Serum leptin was measured by ELISA using a goat anti-mouse leptin antibody (R&D systems) with high affinity for rat leptin.

Semiquantitative RT-PCR and quantitative RT-PCR with endogenous standard. The mRNA expressions of PPARα and PPARγ in the liver were measured by semiquantitative RT-PCR as previously described (27). The mRNA expression of SREBP-1c in the liver and PPARγ, SREBP-1c, FAS and ACC in the RWAT were also measured by quantitative RT-PCR with endogenous standard. The sequences of primers used were shown in the supplementary Table 1. The PCR reaction system was modified as previously described (27) and all of the primers (reverse and forward) from target gene and internal control (acidic ribosomal phosphoprotein PO) were put in one tube. The PCR program was as follows: 5 min at 94°C for 1 cycle, and 1 min at 94°C, 1 min at annealing temperature, 1 min at 72°C for 30 cycles and 7 min at 72°C for 1 cycle. The cycles used were all within the linear range of the dose-response curve. The PCR product was separated by electrophoresis in 2% agarose gel with ethidium bromide and quantitated by the BioSpectrum® imaging system (UVP). Because the sizes of amplified PCR fragments were as expected, and the results of the sequence analysis of amplified PCR fragments were 100% identical to the sequence in the NCBI database, the signals detected by our RT-PCR analysis were justified to be amplified from the cDNA reverse-transcribed from PPARγ, PPARα, SREBP-1c, ACC, and FAS mRNA of the tissue extract.

Northern blot analysis for some target genes of PPARα and PPARγ. The method of cDNA preparation was as previously described (27). The sequences of primers used for cDNA preparation are shown in supplementary **Table 2**. The mRNA expressions of ACO, HMGS, carnitine palmitoyl-transferase 1A (CPT1A), and FABP in the liver and lipoprotein lipase (LPL) and adipocyte fatty acid-binding protein (aP2) in the RWAT were measured by Northern blot analysis. Total RNA was extracted from RWAT using the guanidium

TABLE 2

Body weight, food intake, liver and adipose tissue weight, concentrations of serum and liver lipid, serum leptin, and liver ACO activity in rats fed 5S, 5B, 30S, 30B or 30B/S test diets for 15 wk[1]

	Test diets, *n*					2-way ANOVA		
	5S, *8*	5B, *9*	30S, *8*	30B, *8*	30B/S, *8*	Fat type	Fat quantity	Type × Quantity
						P-value		
Final body weight, *g*	515.3 ± 24.6	506.7 ± 18.7	602.3 ± 22.1	640.9 ± 16.7	620.20 ± 20.8	0.5030	<0.0001	0.2949
Total energy intake, *kJ*	36460 ± 1542	40969 ± 1399	37678 ± 1323	43338 ± 1267	43648 ± 1563	0.2350	<0.0001	0.7001
Organ weight								
Epididymal fat, *g*	13.16 ± 1.21	12.91 ± 1.51	19.65 ± 1.65	21.82 ± 1.59	21.01 ± 1.72	0.5275	<0.0001	0.4273
Retroperitoneal fat, *g*	16.60 ± 1.94^{c}	16.39 ± 1.49^{c}	22.29 ± 1.49^{b}	30.85 ± 1.39^{a}	24.88 ± 2.05*	0.0138	<0.0001	0.01
Liver, *g*	14.36 ± 1.08	14.24 ± 0.65	17.11 ± 1.20	19.48 ± 1.03	18.47 ± 1.24	0.2670	0.0004	0.2234
Serum								
TG, *mmol/L*	2.05 ± 0.32	2.02 ± 0.10	1.05 ± 0.08*	1.58 ± 0.14	1.23 ± 0.10*	0.2509	0.0003	0.1911
NEFA, *mmol/L*	0.55 ± 0.04^{ab}	0.55 ± 0.03^{ab}	0.46 ± 0.01^{b}	0.60 ± 0.02^{a}	0.48 ± 0.02*	0.0344	0.5460	0.0265
Leptin, *nmol/L*	0.27 ± 0.06	0.28 ± 0.05	0.47 ± 0.07*	0.70 ± 0.12	0.54 ± 0.08	0.096	0.0001	0.1233
Liver								
TG, *μmol/g*	15.85 ± 0.76 ^{c}	22.47 ± 3.77^{c}	47.26 ± 3.89^{b}	93.12 ± 4.89^{a}	83.45 ± 8.92	<0.0001	<0.0001	0.0002
NEFA, *μmol/g*	38.86 ± 4.24	31.29 ± 2.96	63.74 ± 4.70	67.40 ± 5.01	81.57 ± 10.93	0.7219	<0.0001	0.1101
ACO activity, *nmol H_2O_2/(min·mg protein)*	2.31 ± 0.19 ^{b}	2.58 ± 0.14 ^{b}	4.30 ± 0.41^{a}	2.85 ± 0.17^{b}	3.13 ± 0.17	0.0246	<0.0001	0.0017

[1] Values are means ± SEM. Means for the 5S, 5B, 30S and 30B groups with superscripts without a common letter differ, $P < 0.05$. * Different from the 30B group, $P < 0.05$ (Student's *t* test).

isothiocyanate, phenol/chloroform method (28) and from liver using the TRIzol reagent (Life Technologies). The method of Northern blotting was as previously described (27). The amounts of each mRNA were quantitated using a BioSpectrum imaging system (UVP).

Fatty acid composition analysis. Total lipids were extracted from liver and RWAT by a mixture of chloroform:methanol (2:1, v:v), saponified, and treated with BF_3/MeOH. The resulted FAME and methyl pentadecanoic acid (Aldrich Chem) added as an internal standard were extracted with *n*-hexane and analyzed by GC (Varian) combined with flame ionization detection. Temperature programming of the column was as follows: 50°C hold 5 min, 50°C to 150°C (3°C/min), 150°C to 210°C (5°C/min), 210°C to 235°C (30°C/min) and then 235°C hold 10 min.

Statistical analyses. Data are expressed as means ± SEM. To test the significance of the effects of fat type, fat quantity, and their interaction, data from the 5S, 5B, 30S, and 30B groups based on a 2 × 2 factorial design were analyzed by 2-way ANOVA. When interaction ($P < 0.05$) existed between fat type and fat quantity, the significance of differences among the 5S, 5B, 30S, and 30B groups was further analyzed by Duncan's multiple range test. The significance of differences between 30B and 30S, and between 30B and 30B/S was analyzed by Student's *t* test. For all statistical analyses, data were transformed logarithmically if the variances were nonhomogeneous. The SAS 8.2 System (SAS Institute Cary) was employed for the statistical analyses.

RESULTS

Energy intake, body and tissue weights, and serum and liver biochemical data. Rats fed the high-fat diets had greater energy intake, body weight, RWAT, and liver weight. ($P < 0.05$, **Table 2**). The 30B/S and 30S groups had lower RWAT weight than the 30B group ($P < 0.05$, Table 2). Rats fed the high-fat diets had lower serum TG concentrations and higher liver TG and NEFA concentrations ($P < 0.0005$, Table 2). Moreover, the 30S group had lower serum NEFA and liver TG concentrations than the 30B group ($P < 0.05$, Table 2). Serum TG and NEFA concentrations of the 30S and 30B/S groups were lower than those of the 30B group ($P < 0.05$, Table 2). Rats fed the 30% fat diets had higher serum leptin concentrations than those fed the 5% fat diets ($P < 0.05$, Table 2). The hepatic ACO activity of rats fed the 30S diet was 150% of those fed the 30B diet ($P < 0.05$, Table 2).

Fatty acid compositions of RWAT and liver. Fat type affected the fatty acid compositions in the RWAT and liver. Rats fed the ORSO diets had lower 16:0 and 18:0 and higher 18:1(n-9), 18:2(n-6), and 20:4(n-6) in the RWAT ($P < 0.05$, **Table 3**). Rats fed the 30S diet had lower 16:0 and higher 18:2(n-6) and 20:4(n-6) than the other groups ($P < 0.05$, Table 3). Rats fed the 30B/S diet also had higher 18:2 (n-6) and lower 16:0 than those fed the 30B diet ($P < 0.05$, Table 3).

In the liver, rats fed the ORSO diets also had lower 16:0 and higher 18:2(n-6), 20:4(n-6), and 18:0 ($P < 0.05$, Table 3). In addition, liver 16:0 was lower and 18:2(n-6) and 20:4(n-6) were higher in the 30S and 30B/S groups than in the 30B group. The greatest differences in the fatty acid composition between the 30S and 30B groups were in the levels of 18:2(n-6) and 20:4(n-6). The levels of these 2 fatty acids in the liver and adipose tissue of the 30S group were >2-fold those of the 30B group ($P < 0.05$, Table 3).

mRNA expressions in the RWAT. The type and quantity of fat did not affect the expression of PPARγ mRNA. Rats fed the ORSO diets had lower LPL expression ($P < 0.05$, **Table 4** and **Fig. 1**). In addition, the expression of aP2 mRNA in the RWAT of rats fed the 30S diet was lower than that of rats fed the 30B diet ($P < 0.05$, Table 4 and Fig 1).

Rats fed the high-fat diets had lower mRNA expressions of SREBP-1c, ACC, and FAS in the RWAT ($P < 0.05$, Table 4 and Fig 1). Rats fed the ORSO diets had lower FAS mRNA expression in the RWAT ($P < 0.05$, Table 4 and Fig 1). In addition, the 30S group had lower SREBP-1c, ACC, and FAS mRNA expressions in the RWAT than the 30B group ($P < 0.05$, Table 4 and Fig 1).

mRNA expressions in the liver. Rats fed the high-fat diets had higher hepatic PPARα and SREBP-1c mRNA expressions ($P < 0.05$, Table 4 and **Fig 2**) in the liver. Rats fed the ORSO

TABLE 3

Fatty acid composition of RWAT and liver homogenates of rats fed 5S, 5B, 30S, 30B and 30B/S test diets for 15 wk[1]

	Test diets, *n*					2-way ANOVA		
	5S, *8*	5B, *9*	30S, *8*	30B, *8*	30B/S, *8*	Fat type	Fat quantity	Type × Quantity
	g/100 g total fatty acid					*P*-value		
RWAT								
16:0	20.76 ± 0.41^{b}	26.60 ± 0.95^{a}	8.65 ± 0.35^{c}	25.78 ± 1.31^{a}	21.87 ± 0.67*	<0.0001	<0.0001	<0.0001
18:0	3.77 ± 0.52	4.26 ± 0.18	3.34 ± 0.56*	5.44 ± 0.37	4.97 ± 0.24	0.0071	0.4059	0.0822
18:1(n-9)	43.31 ± 1.00	40.32 ± 0.46	47.39 ± 0.74*	38.25 ± 2.53	42.84 ± 0.60	0.0008	0.5359	0.0661
18:2(n-6)	22.49 ± 1.17^{b}	10.83 ± 0.60^{c}	36.79 ± 0.79^{a}	10.13 ± 1.09^{c}	17.49 ± 0.63*	<0.0001	<0.0001	<0.0001
20:4(n-6)	5.00 ± 1.09	1.59 ± 0.12	4.21 ± 1.46*	1.75 ± 0.25	1.30 ± 0.16	0.0029	0.7290	0.5969
Liver								
16:0	20.48 ± 1.67^{bc}	25.11 ± 1.59^{b}	19.55 ± 1.59^{c}	31.71 ± 1.62^{a}	21.34 ± 1.78*	<0.0001	0.1509	0.0557
18:0	10.30 ± 1.30	7.91 ± 0.90	7.60 ± 0.73	6.15 ± 0.37	5.60 ± 0.51	0.0359	0.0168	0.7096
18:1(n-9)	29.94 ± 3.77	27.36 ± 3.69	37.49 ± 3.93	32.74 ± 1.48	30.58 ± 1.21	0.2985	0.0762	0.7505
18:2(n-6)	14.54 ± 0.74^{b}	12.47 ± 0.72^{b}	21.75 ± 1.22^{a}	8.87 ± 0.60^{c}	25.88 ± 2.87*	<0.0001	0.0597	<0.0001
20:4(n-6)	18.75 ± 1.72	10.68 ± 0.93	11.82 ± 0.77*	4.98 ± 0.31	8.61 ± 0.40*	<0.0001	<0.0001	0.7265

[1] Values are means ± SEM. Means for the 5S, 5B, 30S and 30B groups with superscripts without a common letter differ, $P < 0.05$. * Different from the 30B group, $P < 0.05$ (Student's *t* test).

diets had higher mRNA expressions of ACO, HMGS, CPT1A, and FABP in the liver ($P < 0.05$, Table 4 and Fig 2). There were higher mRNA expressions of ACO, HMGS, CPT1A, and FABP in livers of the 30S group than in those of the 30B group ($P < 0.05$, Table 4 and Fig 2).

DISCUSSION

The 18-fold difference in the content of 18:2(n-6) between ORSO (24.1%) and butter (1.3%) seems to have led to the greatest difference in tissue fatty acid compositions between the 30S and 30B groups (Table 3). Although the 18:2(n-6) content of the ORSO (20–25%) is relatively low compared with that of common vegetable oils (>50%), the contents of 18:2(n-6) and 20:4(n-6) in the liver and RWAT of the 30S group were >2-fold those of the 30B group (Table 3). Because PUFAs are more potent than saturated fatty acids in the regulation of gene expressions (6), the differences in the mRNA expression of genes regulated by fatty acids appeared to be closely related to the differences in the contents of 18:2(n-6) and 20:4(n-6) in this study.

Results of this study indicated that high-fat diets led to obesity, but the extent of the increase in the RWAT, liver TG content, and serum leptin concentration was lower in rats fed the high-ORSO diet than in those fed the high-butter diet. These data agrees with results of Takeuchi et al. (5) that a 20% (wt:wt) ORSO diet was less adipogenic than a 20% lard diet in rats. Results of the present study further demonstrated higher mRNA expressions of hepatic PPARα target genes (ACO, CPT1A, HMGS, and FABP) and lower mRNA expressions of adipose LPL, aP2, SREBP-1c, ACC, and FAS in rats fed the

TABLE 4

The mRNA expression of PPARα, PPARγ, and SREBP-1c regulated genes in the RWAT and liver of rats fed 5S, 5B, 30S, 30B, and 30B/S test diets for 15 wk[1]

	Test diets, *n*					2-way ANOVA		
	5S, *8*	5B, *9*	30S, *8*	30B, *8*	30B/S, *8*	Fat type	Fat quantity	Type × Quantity
						P- value		
RWAT								
PPARγ	0.95 ± 0.07	1.09 ± 0.13	0.98 ± 0.17	1.29 ± 0.15	1.34 ± 0.10	0.3007	0.2997	0.8893
LPL	1.23 ± 0.23	1.28 ± 0.16	0.78 ± 0.18*	1.65 ± 0.24	1.26 ± 0.12	0.0497	0.8663	0.0771
aP2	1.41 ± 0.19	1.48 ± 0.16	1.37 ± 0.11*	2.01 ± 0.23	1.61 ± 0.12	0.0746	0.2026	0.1453
SREBP-1c	1.23 ± 0.07	1.23 ± 0.07	0.91 ± 0.06*	1.17 ± 0.08	1.04 ± 0.13	0.1256	0.022	0.0947
ACC	0.90 ± 0.07^{a}	0.79 ± 0.17^{a}	0.19 ± 0.05^{b}*	0.59 ± 0.07^{a}	0.59 ± 0.09	0.2885	0.0010	0.0474
FAS	0.83 ± 0.09	1.16 ± 0.12	0.42 ± 0.03*	0.80 ± 0.09	0.87 ± 0.13	0.0014	0.0005	0.8256
Liver								
PPARα	0.68 ± 0.06	0.64 ± 0.07	0.77 ± 0.05	0.88 ± 0.06	0.92 ± 0.04	0.6004	0.0243	0.2943
SREBP-1c	0.49 ± 0.06	0.53 ± 0.05	0.63 ± 0.03	0.74 ± 0.09	0.68 ± 0.05	0.2187	0.0063	0.6253
ACO	1.01 ± 0.99	0.88 ± 0.07	1.07 ± 0.10*	0.91 ± 0.09	1.04 ± 0.12	0.0254	0.2407	0.4191
CPT1A	0.88 ± 0.08	0.77 ± 0.06	0.95 ± 0.11*	0.78 ± 0.09	0.99 ± 0.12	0.0300	0.2965	0.3451
HMGS	1.01 ± 0.13	0.91 ± 0.07	1.26 ± 0.14*	0.80 ± 0.14	1.05 ± 0.16	0.0379	0.5960	0.1726
FABP	0.77 ± 0.15	0.64 ± 0.07	0.96 ± 0.11*	0.48 ± 0.06	0.64 ± 0.13	0.0062	0.8517	0.0962

[1] Values are means ± SEM of quantitated image analysis data. Means for the 5S, 5B, 30S, and 30B groups with superscripts without a common letter differ, $P < 0.05$. * Different from the 30B group, $P < 0.05$ (Student's *t* test).

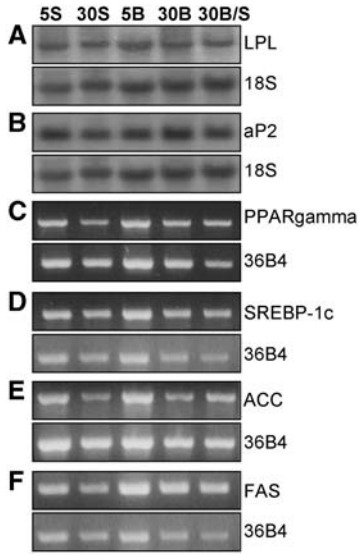

FIGURE 1 The mRNA expression of PPARγ and SREBP-1c and their target genes in the RWAT of rats fed 5S, 5B, 30S, 30B, or 30B/S for 15 wk. Total RNA were analyzed for the mRNA of LPL (*A*), and aP2 (B) by Northern blot analysis and for PPARγ (*C*), SREBP-1c (*D*), ACC (*E*), and FAS (*F*) by RT-PCR. Results of the quantitative image analysis were shown in Table 4.

high-ORSO diet than in rats fed the high-butter diet. In addition, the liver TG concentration of the 30S group was about half that of the 30B group (Table 2). These data indicated that feeding the high-ORSO diet induced a higher PPARα signaling in the liver, presumably by providing more 18:2(n-6) and 20:4(n-6) in the fatty acid pool as potent ligands for PPARα and triggering the transcription of its target genes. This in turn might enhance fatty acid oxidation and lead to less hepatic TG accumulation. In addition, the high-ORSO diet might also suppress SREBP-1c–mediated lipogenesis in the RWAT.

The activation of PPARs by fatty acids depends on site-specific availability, the metabolism of particular fatty acids, and the differences in their affinity for specific PPAR subtypes. As the predominant PPAR-subtype in rat hepatic parenchymal cells, PPARα binds 18:1(n-9) and 20:5(n-3) with comparable affinity in vitro (14). However, the addition of 20:5(n-3), but not 18:1(n-9), 18:2(n-6), and 20:4(n-6) to primary rat hepatocytes activates PPARα (29). This was thought to be due to the high relative abundance of 18:1(n-9), 18:2(n-6), and 20:4 (n-6) in the intracellular NEFA pool because it was difficult to challenge hepatocytes with exogenous 18:1(n-9), 18:2(n-6), or 20:4(n-6) to significantly increase these fatty acids in the NEFA pool (29). In the present study, the 30% fat diets were fed for as long as 15 wk, and the tissue fatty acid composition was remarkably altered by the 2 different fat sources. In the cotransfection assay using mouse PPARα, the optimal transactivation activity for PPARα was found to be with fatty acids with a chain length of ~16–20 carbons and several doubled bonds in the chain (17). Saturated fatty acids were ineffective in this assay (17). This may account, at least in part, for the lower PPARα target gene mRNA expressions in the liver of the 30B group.

High-fat diets, however, increased the hepatic mRNA expression of PPARα and SREBP-1c (Table 4 and Fig 2). The higher mRNA expression of liver PPARα in rats fed the high-fat diets may be related to the obese condition (30–33) and the resulting hyperleptinemia (Table 2). Elevated secretion of leptin from adipose tissues in the obese condition was shown to display antisteatotic and lipopenic action through an enhancement of fatty acid oxidation and suppression of lipogenesis (34,35). Hyperleptinemia in Sprague-Dawley rats fed a 60% (wt:wt) fat diet was shown to be associated with increased PPARα protein, CPT1A mRNA, and fatty acid oxidation in the liver (34). The lipopenic action of hyperleptinemia on white adipose tissue and liver is dependent on PPARα because these effects of hyperleptinemia could not be observed in PPARα-null mice (35). Based on this suggested role of leptin, it seems plausible to speculate that although a high-fat intake induced an excessive adipose tissue accumulation in genetically normal animals, the resulted hyperleptinemia may then upregulate PPARα expression in the liver.

Despite a higher mRNA expression of liver PPARα in the 30B group than the 5B group, the mRNA expressions of PPARα target genes were not higher in parallel. The lack of PUFA as a high affinity ligand might limit the PPARα signaling in the 30B group. Alternatively, a possibility that the level of PPARα protein did not increase in parallel with its mRNA level could not be excluded for the lack of change in PPARα target genes in the 30B group.

The expression of LPL gene is under multiple regulations (36) including PPARs (37) and SREBP-1c (36,38). The coordinated downregulation of SREBP-1c, LPL, ACC, and FAS in the RWAT of the 30S group might be related to the suppression of SREBP-1c mRNA expression by PUFA, more specifically, the higher content of 18:2(n-6) and 20:4(n-6) in the RWAT of the 30S group (Table 3); 18:2(n-6) and 20:4(n-6) were shown to be effective in the suppression of mature form SREBP-1c protein and mRNA (15,39,40) and SRE-dependent gene expression (41). In vivo experiment also showed that 18:2(n-6)-rich corn oil and safflower oil markedly suppressed the hepatic mRNA expression of SREBP-1c and its target genes (15,16). The lower mRNA expression of SREBP-1c and its regulated lipogenic genes (ACC and FAS) in the 30S group of the present study thus might lead to lower lipogenesis and lower RWAT weight. The SREBP-1 is expressed in the very early stage of adipocyte differentiation and was shown to promote adipocyte differentiation (38).

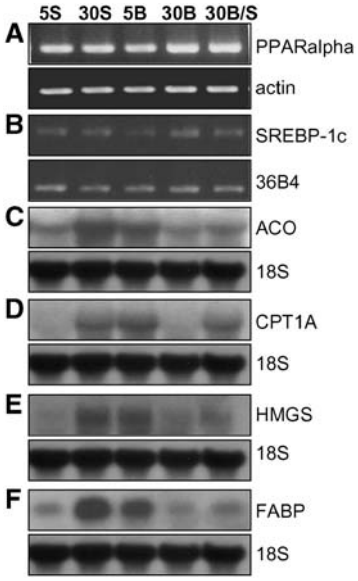

FIGURE 2 The mRNA expression of PPARα and its target genes and SREBP-1c in the liver of rats fed 5S, 5B, 30S, 30B, or 30B/S for 15 wk. Total RNA were analyzed for the mRNA of PPARα (*A*) and SREBP-1c (*B*) by RT-PCR and for ACO (*C*), CPT1A (*D*), HMGS (*E*), and FABP (*F*) by Northern blot analysis. Results of the quantitative image analysis were shown in Table 4.

In the liver, however, the mRNA expression of SREBP-1c was higher in rats fed high-fat diets (Table 4 and Fig. 2). Similarly, upregulated liver SREBP-1c was also observed in mice with high-fat diet–induced obesity (42) and mice overfed intragastrically (43). It is unclear how the liver SREBP-1c mRNA expression is upregulated in the high-fat diet–induced obesity condition. One possibility is under the regulation of LXRα or PPARα. LXRα belongs to a subclass of nuclear hormone receptors that regulates intracellular cholesterol levels by transactivating the expression of cholesterol 7α-hydroxylase (44,45), cholesterol ester transfer protein (46), and ATP-binding cassette transporter A1, which modulates cholesterol efflux and mediates reverse cholesterol transport from peripheral tissues. LXR/RXR was recently identified as a dominant activator for SREBP-1c (47). Furthermore, it was found that unsaturated fatty acids bind to LXRα (48,49) and interfere with LXR/RXR binding to DNA (50). LXRα was shown to be regulated by PPARs (51,52). Evidence for a role of PPARα in the SREBP-1c and its regulated gene expression was provided by the following observations: *1*) changes in the SREBP-1c mRNA level with the diurnal periodicity were parallel to changes in PPARα; *2*) SREBP-1c mRNA expression was lower in PPARα-null mice than in wild-type mice (53); and *3*) SREBP-1c–regulated lipogenic gene expressions were dysregulated in PPARα-null mice (54).

It is not known exactly why the fat type in high-fat diets influenced the mass of RWAT but not EWAT in this study. The cumulative growth of the EWAT was due mainly to hypertrophy, whereas the growth of the RWAT was due predominantly to hyperplasia (55). Moreover, biochemical responses to stimulations and productions of adipokines were shown to be different between subcutaneous and visceral adipose tissue (56). It was speculated that interactions among genetics, local factors (e.g., interleukin, growth factor 1, leptin), systemic factors (nutrition), vascularization, and the degree of innervation by the sympathetic nervous system might produce site-specific normal or pathological growth of adipose tissue (55).

In conclusion, feeding the high-ORSO diet resulted in less RWAT mass, higher tissue 18:2(n-6) and 20:4(n-6), higher mRNA expressions of PPARα target genes, ACO, HMGS, CPT1, and FABP in the liver and lower mRNA expressions of SREBP-1c, FAS, ACC, LPL, and aP2 in RWAT, compared with feeding the high-butter fat diet. These results suggested that the high tissue content of 18:2(n-6) and 20:4(n-6) may upregulate genes coded for enzymes of fatty acid oxidation in the liver through activating PPARα and downregulate genes coded for lipogenesis through the suppression of SREBP-1c, thus leading to lower RWAT mass.

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