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Dietary Fat-Dependent Changes of Gene Expression in Rat Adipose Tissue

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Abstract

The gene expression of proteins involved in adipose tissue metabolism was compared among rats fed a low-fat diet (2% safflower oil), and high-fat diets containing 20% saturated fat (palm oil) or unsaturated fat rich in linoleic acid (safflower oil) for 3 weeks. High-fat diets decreased the lipoprotein lipase mRNA level in the epididymal but not in the perirenal white adipose tissue. Leptin gene expression in the perirenal but not in the epididymal white adipose tissue was significantly higher in rats fed high-fat diets than in those fed a low-fat diet. mRNA levels of glucose transporter 4, both in the epididymal and perirenal white adipose tissues, were lower in rats fed high-fat diets than in those fed a low-fat diet. High-fat diets increased the gene expression of uncoupling protein 1 and lipoprotein lipase in the interscapular brown adipose tissue, but did not affect that of leptin. Adipose tissue mRNA levels of various proteins were comparable between rats fed diets high in safflower and palm oil. We concluded that the high-fat diet influenced the gene expression in adipose tissue in a site-specific manner. The difference in the degree of unsaturation of dietary fats was rather irrelevant in modifying the level of mRNAs for proteins related to energy metabolism and expenditure in adipose tissue.

Discipline: Food
Additional key words: saturated fatty acids, linoleic acid, energy metabolism

Introduction

Adipose tissue, the energy reserve organ, plays a major role in regulating the energy metabolism in organisms[7]. Mature white adipocytes express proteins related to the regulation of the lipid and carbohydrate metabolism, and secretory cytokines and hormones that affect the energy metabolism in other tissues. Another tissue that plays a critical role in regulating the energy metabolism is the brown adipose tissue, which controls the energy balance by thermogenesis through the unique action of uncoupling protein highly expressed in this tissue[6]. Conceivably, alterations in the expression of proteins regulating the lipid and carbohydrate metabolism and energy expenditure in these tissues affect the development of obesity and related diseases.

One dietary factor affecting the energy balance and the functions of white and brown adipose tissues is the dietary fat type. Compared to saturated fats, dietary fats rich in n-6[16, 24] and n-3[19] polyunsaturated fatty acids lead to a lower body fat accumulation in the rat. Polyunsaturated fats also modify the responsiveness to insulin and catecholamine of isolated white and brown adipocytes[9, 14]. However, the information regarding the effect of dietary fat type on gene expression in white and brown adipose tissues is rather scarce. In this context, we compared the effect of a low-fat diet and high-fat diets containing saturated fat (palm oil), and polyunsaturated fat rich in linoleic acid (safflower oil) on the abundance of mRNAs for proteins regulating the lipid and carbohydrate metabolism and energy expenditure in white and brown adipose tissues. We analyzed the mRNA levels of lipoprotein lipase (LPL), leptin, glucose transporter 4 (Glut 4) and uncoupling protein 1 (UCP 1) in this study.

Materials and methods

1) Animals and diets

Male Sprague-Dawley rats purchased at 5 weeks of age (Charles River Japan, Kanagawa, Japan) were housed individually in a room with controlled temperature (20–22C°), humidity (55–60%), and lighting (light on from 07.00 to 19.00 h) and fed a commercial non-purified diet

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Table 1. Fatty acid composition of safflower oil and palm oil

(weight %)

Fatty acids	Dietary fats	
	Safflower oil	Palm oil
Myristic acid	0.1	1.1
Palmitic acid	6.9	45.3
Palmitoleic acid	–	0.1
Stearic acid	2.4	4.4
Oleic acid	13.0	39.1
Linoleic acid	77.3	9.4
α-Linolenic acid	0.2	0.3

(Type NMF, Oriental Yeast, Tokyo, Japan). After 1 week of acclimatization to the housing conditions, rats were randomly divided into 3 groups consisting of 7 animals each and fed a purified diet containing 2% safflower oil or a diet containing 20% fat either as palm or safflower oil for 3 weeks. The basal composition of the experimental diet was (in weight %): casein, 20; corn starch, 15; cellulose, 2; mineral mixture[1)], 3.5; vitamin mixture[1)], 1.0; *DL*-methionine, 0.3; choline bitartrate, 0.2 and sucrose to 100. Dietary fats were added to the experimental diets instead of sucrose. The fatty acid composition of the dietary fats is shown in Table 1. We followed the institute's guidelines for the care and use of laboratory animals.

2) Preparation of cDNA probes

Reverse transcription and polymerase chain reaction (PCR) were used to prepare cDNA probes for LPL, leptin, Glut 4, and UCP 1. First-strand cDNA synthesized from white adipose tissue RNA was used for the PCR amplification of probes for LPL, leptin, and Glut 4. Brown adipose tissue RNA was used for the substrate for reverse transcription to prepare UCP 1 cDNA. Primers used for PCR amplification of cDNA probes were selected from reported cDNA sequences[2, 3, 12, 18)] (Table 2). PCR products were purified by agarose gel electrophoresis and used for probes. cDNA probe for β-actin was provided by Wako Pure Chemicals, Osaka, Japan.

3) RNA extraction and analysis

At the end of the experimental period, rats were anesthetized with diethyl ether and killed by bleeding from the abdominal aorta. Epididymal and perirenal white adipose tissues, and interscapular brown adipose tissue were excised and homogenized in a 4 M guanidine thiocyanate solution containing 0.5% sodium *N*-lauroylsarcosianate (w/v), 25 mM sodium citrate (pH 7.0), and 0.1 M 2-mercaptoethanol (5 vol of the solution relative to tissue weight was used for white, and 10 vol was used for brown adipose tissue). Homogenates were centrifuged and infranatants below the floating fat layers were processed for RNA extraction[4)]. The infranatants to which 0.1 volume of 2 M sodium acetate (pH 4.0), an equal volume of water-saturated phenol containing 0.1% 8-quinolinol as an anti-oxidant, and 0.5 volume of a solvent mixture composed of chloroform:isoamylalcohol (49:1, v/v) were added, were vortexed for 1 min. After standing on ice for 15 min, the mixture was centrifuged at 12,000 × g for 20 min at 4°C. The upper phase was transferred to a new tube and an equal volume of isopropanol was added, and the preparation was allowed to stand overnight at –30°C. RNA in the mixture was recovered by centrifugation at 12,000 × g for 20 min and washed 3 times with 75% ethanol. RNA was dried *in vacuo* and dissolved in 0.3 mL of sterilized water and quantified by measuring the optical density at 260 nm. RNA samples were denatured and applied to a nylon membrane (Hybond N^+, Amersham International, Bucks, UK) using a dot-blot apparatus (Bio-Rad Laboratories, Hercules, CA., USA) and fixed by UV irradiation. Northern blot analysis was performed using standard procedures. RNA samples were denatured and electrophoresed on a 1.17% agarose gel containing 0.66 M formaldehyde, then transferred to a nylon membrane and fixed by UV irradiation. Twenty μg RNA was used for LPL and leptin, 40 μg for Glut 4, and 10 μg for UCP 1 to analyze the mRNA levels. Equal loading and integrity of RNA samples were con-

Table 2. Primers for the preparation by PCR of cDNA probes

Protein	Upstream primer	Downstream primer	Length of PCR products (bp)
Lipoprotein lipase	5'-TGTCTAACTGCCACTTCAACC-3'	5'-TTACTGCTTCTCTTGGCTCTG-3'	789
Leptin	5'-TCCAAGAAGAAGAAGACCCCA-3'	5'-GAGGAGTAGGAGAAACGGAC-3'	664
Glucose transporter 4	5'-GACACTGGTCCTTGCTGTAT-3'	5'-CATCAGACACATCAGCCCAG-3'	700
Uncoupling protein 1	5'-CAGACATCATCACCTTCCCG-3'	5'-AAGTCGCCTATGTGGTGCAG-3'	851

firmed by ethidium bromide fluorescence of ribosomal 18S and 28S RNAs. Nylon membranes were prehybridized for at least 5 h at 42°C in a medium containing 0.75 M NaCl, 75 mM sodium citrate, 5 × Dehardt's solution, 1% SDS (w/v), 0.05% denatured salmon sperm DNA (w/v), and 50% formamide (v/v), and then hybridized with cDNA probes labeled with [α-^{32}P] dCTP (Random Primer DNA labeling Kit Ver. 2.0, Takara Co., Kyoto, Japan was used for radio-labeling) added at concentrations exceeding 2 × 10^6 dpm/mL for 16–20 h at 42°C. Filters were washed twice with medium containing 0.3 M NaCl, 30 mM sodium citrate and 0.1% SDS at room temperature, then 4 times with medium containing 30 mM NaCl, 3 mM sodium citrate and 0.1% SDS at 65°C. Membranes were exposed to an imaging screen (Bio-Rad Laboratories, Hercules, CA, USA) for 16–20 h. RNAs hybridized with specific cDNA probes were analyzed and quantified by using an imaging analyzer (Bio-Rad Laboratories, Hercules, CA., USA).

4) Lipid analysis

Serum lipids were extracted and purified[5]. Triacylglycerol and phospholipid contents in the extracts were determined as described elsewhere[8]. Serum cholesterol, free fatty acid and glucose concentrations were determined using enzyme kits (Wako Pure Chemical, Osaka, Japan). Fatty acid composition of the white adipose tissues was determined by gas-liquid chromatography[8].

5) Statistics

The examination of significant differences of means with a pooled estimate of variance was performed according to the methods of Snedecor and Cochran[25] for one-way classifications as described elsewhere[10].

Results and discussion

1) Dietary fat effect on growth parameters and adipose tissue weight

No significant differences were observed in growth among low-fat, high-safflower, and high-palm oil groups during the experimental period (Table 3). The amount of diet consumed was comparable among the groups when expressed in terms of energy intake. Weight of both epididymal and perirenal adipose tissues was the same among the 3 groups in the present study. However, some studies indicated[16, 24] that, compared to saturated fat (at least beef tallow), dietary fat rich in linoleic acid led to a lower accumulation of body fat in rats and mice. In this context, differences in the feeding period, animal species or dietary fat (beef tallow or palm oil) should be taken into consideration. Brown adipose tissue weight also did not vary among the groups.

2) Dietary fat effect on gene expression

In a preliminary experiment, we analyzed the mRNA levels for LPL, leptin, Glut 4, and UCP 1 among epididymal white adipose tissue, brown adipose tissue, liver, and heart by Northern and dot blot hybridization using cDNA probes prepared by reverse transcription and PCR amplification (Fig. 1). Our results on tissue distribution of mRNA for the respective proteins agreed well with those reported by other authors[11, 12, 23, 26] and thus confirmed the specificity of our cDNA probes.

mRNA abundance was analyzed by dot blot hybridization (Tables 4, 5). Values were expressed by taking those for epididymal white adipose tissue in rats fed a low-fat diet as 100 except for UCP 1 mRNA. As the UCP 1 mRNA signal was detected in the brown adipose tissue but not in the white adipose tissue (Fig. 1), UCP 1

Table 3. Effect of dietary fats on growth parameters and adipose tissue weight in rats

	Dietary fats		
	Low fat	Safflower oil	Palm oil
Body weight gain (g/21days)	174 ± 6	153 ± 6	147 ± 14
Food intake (g/day)	26.0 ± 0.7[a]	19.8 ± 0.5[b]	19.4 ± 1.0[b]
Energy intake (kJ/day)	466 ± 13	434 ± 12	426 ± 21
Epididymal white adipose tissue (g/100g bw)	2.01 ± 0.11	2.29 ± 0.18	1.98 ± 0.23
Perirenal white adipose tissue (g/100g bw)	2.29 ± 0.19	2.56 ± 0.22	2.12 ± 0.34
Interscapular brown adipose tissue (g/100g bw)	0.215 ± 0.02	0.221 ± 0.01	0.192 ± 0.02

a, b): Values with different superscripts are significantly different at $p < 0.05$.

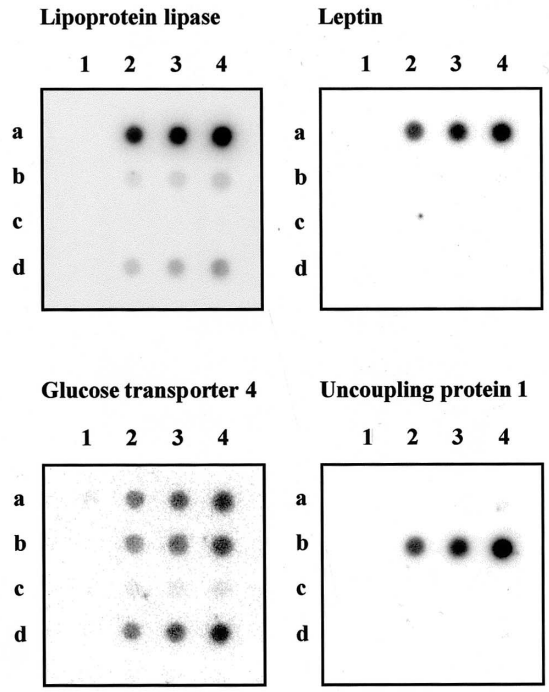

Fig. 1. Dot blot analysis of mRNA from epididymal white adipose tissue (line a), brown adipose tissue (line b), liver (line c), and heart (line d)

RNA samples were used as follows: 0 μg (column 1), 10 μg (column 2), 20 μg (column 3), and 40 μg (column 4).

mRNA abundance was expressed by taking the value in brown adipose tissue in rats fed a low-fat diet as 100. Gene expression of Glut 4 and β-actin in brown adipose tissue was too low to obtain reliable values. The mRNA levels of LPL, leptin, and Glut 4 but not of β-actin were considerably higher in the perirenal than in the epididymal white adipose tissue. The abundance of mRNA for LPL and leptin was considerably lower in the brown adipose tissue than in the white adipose tissues. Typical results obtained by Northern blot analysis of mRNA from white and brown adipose tissues are shown in Fig. 2.

Both unsaturated and saturated high-fat diets versus a low-fat diet significantly reduced the gene expression of LPL in the epididymal white adipose tissue in this study (Table 4). LPL is an enzyme that hydrolyzes serum lipoprotein triacylglycerol for the uptake of free fatty acids into the tissues[20]. In adipose tissue, free fatty acids supplied by this enzyme reaction are esterified to form triacylglycerol for storage purpose. Thus, it is reasonable to assume that high-fat diets reduce the gene expression of LPL in white adipose tissue to prevent excessive accumulation of fats in the tissue. Unexpectedly, however, high-fat diets did not modify the LPL gene expression in the perirenal white adipose tissue, in the present study. In this context, Raclot et al[19]. have recently reported that, compared to olive oil, fish oil or eicosapentaenoic and docosahexaenoic acids reduced the LPL gene expression in retroperitoneal but not in subcutaneous white adipose tissue. Therefore, it is suggested that dietary fat affects the LPL gene expression in white adipose tissue in a site-specific manner.

Leptin is highly expressed in adipocytes and acts on the hypothalamus to regulate satiety and energy balance[7]. Some studies have showed that a high-fat diet increases

Table 4. Effect of dietary fats on gene expression in rat white adipose tissue

(%)

mRNA level	Dietary fats		
	Low fat	Safflower oil	Palm oil
Lipoprotein lipase			
Epididymal	100 ± 8[a]	72.8 ± 5.0[b]	68.3 ± 10.0[b]
Perirenal	114 ± 12.0	138 ± 3.0	115 ± 20.0
Leptin			
Epididymal	100 ± 5.0	102 ± 9.0	94.9 ± 15.8
Perirenal	134 ± 13[a]	244 ± 14[b]	194 ± 26[b]
Glucose transporter 4			
Epididymal	100 ± 2[a]	53.2 ± 5.3[b]	66.9 ± 6.7[b]
Perirenal	156 ± 14[a]	92.8 ± 14[b]	100 ± 11[b]
β-actin			
Epididymal	100 ± 5	105 ± 2	107 ± 5
Perirenal	101 ± 4.6[a]	117 ± 5[ab]	126 ± 10[b]

a, b): Values with different superscripts are significantly different at $p < 0.05$.

Table 5. Effect of dietary fats on gene expression in rat brown adipose tissue

(%)

mRNA level	Dietary fats		
	Low fat	Safflower oil	Palm oil
Uncoupling protein 1	100 ± 12[a]	241 ± 14[b]	293 ± 10[b]
Lipoprotein lipase	21.4 ± 1.2[a]	29.8 ± 1.9[b]	28.2 ± 2.3[b]
Leptin	25.2 ± 3.3	31.2 ± 3.3	29.9 ± 5.1

a, b): Values with different superscripts are significantly diffent at $p < 0.05$.

the leptin gene expression in adipose tissue[15]. In the present study, we observed that both high-fat diets increased the leptin mRNA level in perirenal white adipose tissue to the same level but failed to affect this parameter in epididymal white adipose tissue. Our results suggest that a high-fat diet affects the leptin gene expression differently depending on the location of fat pads as observed for the LPL gene expression. Therefore, the previous assumption[15] that a high-fat diet increases the gene expression of leptin in adipose tissue should be carefully reexamined. Also, our study indicated that a difference in the degree of unsaturation of dietary fats does not modify the leptin gene expression in white adipose tissue.

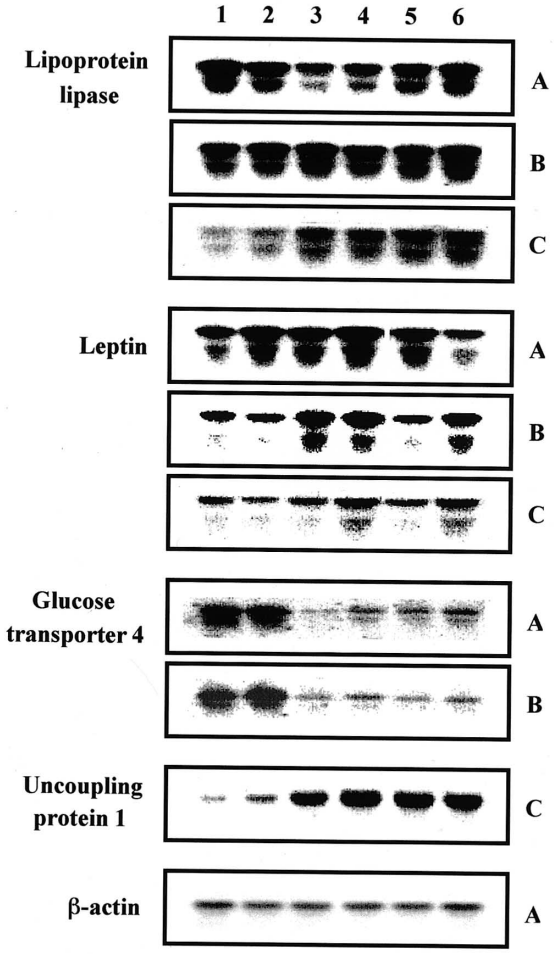

Fig. 2. Northern blot analysis of mRNA from white and brown adipose tissues of rats fed a low-fat diet (lanes 1 and 2) and high-fat diets containing safflower (lanes 3 and 4) and palm oil (lanes 5 and 6)

A: epididymal white adipose tissue,
B: perirenal white adipose tissue,
C: interscapular brown adipose tissue.

Glut 4 gene is highly expressed in skeletal muscle and adipose tissue, and plays an important role in glucose transport in response to insulin stimulation[12]. Compared to a low-fat diet, the two types of high-fat diets markedly reduced the Glut 4 gene expression both in epididymal and perirenal white adipose tissues (Table 4). These results agree with previous studies showing that high-fat feeding reduces the Glut 4 gene expression in muscle and in fat cells[13, 27]. The extent of the reduction of the Glut 4 mRNA levels was, however, comparable between rats fed high palm and safflower oil diets. This observation does not necessarily correspond to the results of previous studies showing that unsaturated fats, compared to saturated fats, markedly increase the insulin-dependent glucose utilization in isolated adipocytes[14]. It is suggested that alterations in the intracellular localization of the transporter[17] rather than the level of mRNA and thus of its translational product may be responsible for the modification by dietary fat types of the insulin responsiveness of adipocytes.

UCP 1 is specifically expressed in brown adipose tissue mitochondria, and plays a major role in the regulation of thermogenesis in this tissue[6]. Compared to a low-fat diet, both high-fat diets markedly increased the UCP 1 gene expression to a comparable extent in this experiment (Table 5). This observation agrees with a previous study[21] showing that high-fat diets caused a large increase in the UCP 1 mRNA level and suggests that high-fat diets increase the thermogenesis and thus energy expenditure in the rat. However, this consideration is not

Table 6. Effect of dietary fats on concentration of serum components in the rats
(mmol/L)

Serum components	Dietary fats		
	Low fat	Safflower oil	Palm oil
Triacylglycerol	1.24 ± 0.30^{ab}	0.65 ± 0.18^{a}	2.08 ± 0.65^{b}
Cholesterol	4.12 ± 0.34^{a}	2.82 ± 0.18^{b}	3.57 ± 0.28^{ab}
Phospholipid	3.63 ± 0.22^{a}	2.37 ± 0.15^{b}	3.47 ± 0.28^{a}
Free fatty acids	0.91 ± 0.12^{a}	0.83 ± 0.08^{a}	1.56 ± 0.27^{b}
Glucose	8.27 ± 0.48	7.77 ± 0.26	9.46 ± 0.95

a,b): Values with different superscripts are significantly different at $p < 0.05$.

supported by the fact that the weight of the white adipose tissue was comparable among rats fed a low-fat diet and two types of high-fat diets when the energy intake was the same among the groups. In this context, it is possible that alterations in the expression of UCP homologues in various tissues[22] rather than UCP 1 play a major role in the regulation of energy expenditure. Our study showed that the UCP 1 mRNA level was the same in rats fed high-palm and safflower oil diets. These results do not correspond to a previous observation[21] showing that dietary fat rich in linoleic acid, compared to saturated fats, increased the UCP 1 content in interscapular brown adipose tissue. Thus, dietary fat types may influence the efficacy of translation without affecting that of transcription and thus modify the UCP 1 content in brown adipose tissue.

Compared to a low-fat diet, both high-fat diets significantly increased the gene expression of LPL to the same level. It is reasonable to assume that high-fat diets increased the supply of free fatty acids through the up-regulation of the LPL gene expression for the consumption of extra energy by thermogenesis in brown adipose tissue. The leptin mRNA level was the same among the groups. Therefore, a previous observation showing that high-fat diets increased the gene expression of leptin in interscapular brown adipose tissue[15] was not necessarily confirmed in the present study.

3) Dietary fat effect on serum lipid and glucose concentrations

Although differences were not statistically significant, compared to a low-fat diet, a high-palm oil diet increased while a high-safflower oil diet decreased the serum triacylglycerol concentrations (Table 6). The serum cholesterol and phospholipid concentrations were significantly lower in rats fed a high-safflower oil diet than in those fed a low-fat diet, but the high palm oil diet failed to modify these parameters. The serum free fatty acid concentration was significantly higher in rats fed a high-palm oil diet than in other groups. The serum glucose concentration tended to be higher in the high-palm

Table 7. Effect of dietary fats on fatty acid composition of epididymal and perirenal white adipose tissues in rat
(weight %)

Fatty acids	Epididymal white adipose tissue			Perirenal white adipose tissue		
	Low fat	Safflower oil	Palm oil	Low fat	Safflower oil	Palm oil
Myristic acid	1.86 ± 0.04^{a}	0.83 ± 0.02^{b}	1.23 ± 0.03^{c}	1.94 ± 0.05^{a}	0.91 ± 0.04^{b}	1.30 ± 0.04^{c}
Palmitic acid	27.4 ± 0.6^{a}	16.3 ± 0.3^{b}	29.5 ± 0.6^{c}	28.9 ± 0.7^{a}	17.3 ± 0.6^{b}	30.5 ± 0.5^{a}
Palmitoleic acid	12.8 ± 0.2^{a}	3.10 ± 0.31^{b}	5.92 ± 0.39^{c}	11.9 ± 0.3^{a}	2.78 ± 0.21^{b}	5.14 ± 0.32^{c}
Stearic acid	2.95 ± 0.17^{a}	2.43 ± 0.05^{b}	2.65 ± 0.10^{ab}	3.83 ± 0.27^{a}	2.65 ± 0.07^{b}	2.79 ± 0.11^{b}
Oleic acid	37.5 ± 0.6^{a}	21.5 ± 0.3^{b}	45.0 ± 0.6^{c}	37.8 ± 0.6^{a}	21.0 ± 0.3^{b}	45.7 ± 0.6^{c}
Linoleic acid	16.3 ± 0.9^{a}	53.4 ± 0.8^{b}	14.6 ± 0.7^{a}	13.5 ± 0.8^{a}	51.7 ± 0.9^{b}	12.2 ± 0.4^{a}
Others	1.13 ± 0.12^{a}	2.04 ± 0.07^{b}	1.14 ± 0.14^{a}	0.80 ± 0.03^{a}	2.11 ± 0.12^{b}	0.74 ± 0.12^{a}

a, b, c): Values with different superscripts are significantly different at $p < 0.05$.

oil diet group than in the other groups.

4) Dietary fat effect on fatty acid composition of white adipose tissue

Fatty acid composition of epididymal and perirenal adipose tissues was similar and was similarly affected by the types of diet (Table 7).

Conclusion

Compared to a low-fat diet, diets with a high content of safflower and palm oil markedly affected the gene expression of LPL and leptin in adipose tissue in a site-specific manner. These high-fat diets decreased the gene expression of Glut 4 in white adipose tissue and considerably increased the gene expression of UCP 1 in brown adipose tissue. These observations confirmed previous findings showing that the dietary fat amount plays a major role in modifying the gene expression of these proteins that regulate the energy metabolism. In addition, it was found that the responsiveness of gene expression to dietary fats in adipose tissues differed with the location of the fat depots. Therefore, it is important to note that a physiological response observed in one fat depot can not be extrapolated to an event occurring in an another fat depot. Moreover, our results demonstrated that the difference in the degree of unsaturation of dietary fat was rather irrelevant in modulating these parameters. Alteration of gene expression therefore can not account for some physiological activities of polyunsaturated fat in modifying the function of adipocytes that have previously been reported.

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