Original Article

Body fat accumulation is greater in rats fed a beef tallow diet than in rats fed a safflower or soybean oil diet

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The effects of dietary fats, consisting of different fatty acids, on body fat accumulation and uncoupling protein (UCP) in interscapular brown adipose tissue were studied in rats. Metabolisable energy in experimental diets based on safflower oil, soybean oil or beef tallow was measured strictly (experiment 1). Male Wistar rats were then meal-fed an isoenergetic diet for 8 weeks (experiment 2). Each group of rats showed the same weight gain during the 8-week experimental period. Carcass fat content was greater in rats fed the beef tallow diet than in those fed the with the safflower or soybean oil diets, whereas the weight of abdominal adipose tissue was the same for all three dietary groups. Gene expression of *UCP1* and the UCP content of the interscapular brown adipose tissue was lower in the beef tallow diet group than in the other dietary groups. A negative correlation was observed between carcass fat content and n-6 unsaturated fatty acid content in dietary fats. These results suggest that the greater body fat accumulation in rats fed the beef tallow diet results from lower expression of *UCP1* mRNA and lower UCP content in brown adipose tissue. n-6 Polyunsaturated fatty acids may be the most effective fatty acids in limiting body fat.

Key words: body fat, high-fat diet, metabolisable energy, rat, uncoupling protein.

Introduction

Considerable interest has arisen concerning the effect of dietary fatty acid composition on body fat accumulation.[1–3] We have reported in a series of studies that sympathetic activities in peripheral tissues are lower in rats fed a beef tallow diet than in rats fed a safflower oil diet when all rats are fed isoenergetic (metabolisable energy) diets based on beef tallow-enriched saturated fatty acid or safflower oil-enriched n-6 polyunsaturated fatty acid for 8 weeks.[4–6] Rats fed the beef tallow diet show higher body fat accumulation induced by a decrease in diet-induced thermogenesis[6] and an increase of serum insulin and triacylglycerol concentrations.[4–6] Moreover, we have suggested that intake of other animal fats (lard) rich in saturated and monounsaturated fatty acids, as compared with intake of vegetable oils rich in polyunsaturated fatty acids, decreases diet-induced thermogenesis by inhibiting sympathetic activity in brown adipose tissue, resulting in greater body fat accumulation.[7,8]

It is well known that ageing influences the ability to digest macronutrients.[9–12] In our series of experiments, consumption of experimental diets was adjusted on the basis of metabolisable energy (digestible energy calculated as gross energy fed minus energy in faeces) measured in 4-week-old rats.[3,6] However, because our studies used rats fed experimental diets for 8–16 weeks (rats 4–20 weeks old), our experiments could not be performed under strictly isoenergetic conditions. Indeed, rat growth was greater in rats fed a safflower oil diet than in those fed a beef tallow diet, but the difference was not significant.[3,6] It is necessary to determine if metabolisable energy changed during the experimental period in order to validate our earlier studies.[4–6]

On the other hand, safflower oil (over 70% linoleic acid) is not a popular dietary oil or standard experimental fat. The source of fat in the AIN-76 diet (standard purified diet for rats reported by the American Institute of Nutrition) is corn oil enriched with linoleic acid to the same extent as safflower oil.[13] Recent evidence has shown this to be unacceptable because it does not provide sufficient linolenic acid (n-3 polyunsaturated fatty acid) to meet requirements.[14] Lee *et al.* suggested that a n-6 : n-3 ratio of 5 is the point of greatest influence on tissue lipids.[15] Bourree *et al.* suggested that the optimal n-6 : n-3 ratio is between 1 and 6.[16] Soybean oil (n-6 : n-3 ratio = 7) is the only single source of dietary fat that comes close to meeting this criteria.[14]

In the present study, we measured metabolisable energy in experimental diets every week for an 8-week experimental period in order to validate our previous studies (experiment 1).

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We reinvestigated body fat accumulation in rats fed a beef tallow diet compared to rats fed a safflower or soybean oil diet under isoenergetic conditions (experiment 2). Uncoupling protein (UCP) 1 gene expression and UCP content in the interscapular brown adipose tissue were taken as indices of sympathetic activity in brown adipose tissue (experiment 2).

Materials and methods

All procedures involving animals were approved by the Experimental Animal Care Committee of Kagawa University.

Experiment 1: estimation of metabolisable energy in experimental diets using rats fed ad libitum

Animals and diets. Fifteen male Wistar rats (3 weeks old) were obtained from Japan SLC (Shizuoka, Japan) and randomised into three groups. Rats were fed CE-2, commercial rodent diet (CLEA Japan, Tokyo, Japan) and water ad libitum through to age 4 weeks. The rats were then fed a synthetic diet based on beef tallow, safflower oil or soybean oil. These diets contained the following ingredients (in g/kg): corn starch, 260; sucrose, 120; casein, 255; fat (beef tallow, safflower oil or soybean oil), 250; cellulose, 50; vitamin mixture,[13] 13; mineral mixture,[13] 45; DL-methionine, 4; choline chloride, 2.5; and *t*-butylhydroquinone, 0.5. The fatty acid compositions of the beef tallow, safflower oil and soybean oil are given in Table 1.

Experimental design. Rats were caged individually at 23 ± 2°C, with light from 08:00 to 20:00 h. Each group of rats (n = 5/group) was given free access to the beef tallow, safflower oil or soybean oil diets and water for 8 weeks. Body weight and food intake were measured every day. Food consumption is shown in Table 2. Faeces were collected during the last four days of every week to determine the metabolisable energy. On the final day of the experiment, the rats were fasted overnight and killed by decapitation. Abdominal adipose tissues (epididymal, perirenal and mesenteric) and interscapular brown adipose tissue were removed and weighed.

Analyses. Measurements of the gross energy in the experimental diets and faeces collected every week was requested from the Japan Food Analyses Center (Tokyo, Japan). Digestible energy was calculated as energy fed minus energy in faeces. The metabolisable energy of each diet was calculated as digestible energy multiplied by 0.96.[2]

Table 1. Fatty acid composition of experimental fats

	Experimental fats (g/100 g total fatty acid)		
Fatty acid†	Safflower oil	Soybean oil	Beef tallow
14:0	0.2	ND	2.9
14:1 (n-9)	ND	ND	0.7
16:0	7.9	10.4	24.0
16:1 (n-9)	0.1	0.1	3.2
18:0	2.7	4.0	16.6
18:1 (n-9)	15.5	23.9	46.5
18:2 (n-6)	72.0	52.9	2.8
18:3 (n-3)	ND	7.8	0.3
20:0	0.4	0.3	0.2
Others	1.2	0.6	2.8

†Number of C atoms : number of double bonds. ND, not detected.

Table 2. Food consumption of rats fed experimental diets

Experimental period (weeks)	Food consumption (kJ/d)			
	Experiment 1			Experiment 2
	Safflower oil	Beef tallow	Soybean oil	
1	187 ± 5	204 ± 6	194 ± 5	154
2	215 ± 4	215 ± 4	202 ± 3	168
3	250 ± 4	250 ± 5	236 ± 7	176
4	278 ± 5	277 ± 4	266 ± 6	196
5	289 ± 5	299 ± 7	293 ± 1	204
6	281 ± 3	297 ± 7	288 ± 9	204
7	279 ± 6	290 ± 5	287 ± 5	204
8	270 ± 2	268 ± 2	278 ± 2	204
Mean	256 ± 6	263 ± 7	255 ± 8	216

Values were calculated from the metabolisable energy of the diet.

Experiment 2: Effect of dietary fats on body fat accumulation and UCP1 gene expression in pair-fed rats

Animals and experimental design. Eighteen male Wistar rats (3 weeks old) were obtained from Japan SLC and randomised into three groups (n = 6/group). Rats were fed CE-2, commercial rodent diet, and water ad libitum through to age 4 weeks. Diet compositions and housing conditions were the same as in experiment 1. The metabolisable energy of each diet was previously determined in experiment 1. Each group of rats was meal-fed at 07:00–08:00 h and 19:00–20:00 h for 8 weeks. Rats were offered the appropriate diet in amounts such that the three groups consumed equal metabolisable energy during the experimental period. The meal-feeding method was used to adjust the energy intake among the three dietary groups. Normal meal-feeding conditions (one meal, 2 h/day) decrease the food intake of the animals. Two meals per day minimised this effect of meal feeding. The food consumption shown in Table 2 was approximately the maximal amount the rats could consume under the meal-feeding conditions. On the final day of the experiment, each dietary group was fed a meal at 07:00–08:00 h, then killed by decapitation at 09:00 h. Abdominal adipose tissues (epididymal, perirenal and mesenteric) and interscapular brown adipose tissue were removed and weighed. Carcass samples were obtained by removing the head, tail, digestive tract, lungs, kidneys and testes.[3] These were stored at –20°C until the carcass composition was analysed.

Analyses. Carcass fat and protein were analysed by the method of Mickelsen and Anderson.[17]

Total RNA was extracted from the interscapular brown adipose tissue with a guanidium thiocynate water-saturated phenol extraction method.[18] First-stand cDNA synthesis was performed on 5 μg total RNA using oligo(dT) as described in the manufacture's instructions (Gibco BRC Super Script Kit; Life Technologies, Gaithersburg, MD, USA). The sequences of the primers of UCP1 and β-actin used for polymerase chain reaction (PCR) amplification were described previously.[19] The PCR reactions were carried out in a DNA Thermal Cycler (PC-700, ASTEC, Fukuoka, Japan) using the following cycle conditions: initial denaturation at 94°C for 1 min, annealing at 58°C for 1.5 min and extension at 73°C for 8.5 min. PCR products (10 μL) were dyed with ethidium bromide and analysed by electrophoresis in 2% agarose gels. The amount of mRNA in each sample was quantified by densitometry using an analysis program for Macintosh (NIH image, US National Institutes of Health, Springfield, VA, USA). The PCR cycles were kept within their respective exponential and linear ranges.

Brown adipose tissue mitochondria were prepared by a method described previously.[20] Briefly, interscapular brown adipose was homogenised in 5 mL of 0.01 mol/L phosphate-buffered saline (PBS) (pH 7.2), then centrifuged at 700 g for 10 min. The supernatant below the fat layer was sucked out carefully, then centrifuged at 6000 g for 10 min. The pellet was resuspended in 0.01 mol/L PBS (pH 7.2). The mitochondrial fraction was analysed immediately. Protein was determined by the Bradford method.[21]

The UCP immunological determination was made by Western blot analysis. Rabbit antirat UCP was donated by Dr Kawada, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto, Japan. Protein from the brown adipose tissue (2 mg/mL) was subjected to SDS-polyacrylamide gel electrophoresis in 11% polyacrylamide slab gels, according to the method of Laemmli.[22] Following electrophoresis, the proteins were electrophoretically transferred to a nitrocellulose membrane using the method of Burnette.[23] The immunoreactivity of the UCP was detected by the ECL Western blotting system (Amersham Pharmacia Biotech, Buckinghamshire, England).

Data analysis

Statistical differences were analysed by one-way factorial analysis of variance (ANOVA) and the Newman–Keuls test.

Results

Experiment 1

Body weight and adipose tissue weights. Each group of rats showed the same body weight gain during the 8 week experimental period (Table 3). Abdominal adipose tissue (epididymal, perirenal and mesenteric) weight and total weight of abdominal adipose tissues were not different among the three dietary groups (Table 3). Interscapular brown adipose tissue weight was not different among the three groups (Table 3).

Metabolisable energy in the experimental diets. The metabolisable energy of each experimental diet remained constant during the experimental period because digestible energy

Table 3. Effects of dietary fats on body weight and adipose tissue weight (experiment 1)

	Diet group		
	Safflower oil	Soybean oil	Beef tallow
Body weight (g)			
Initial	82 ± 3	82 ± 3	82 ± 3
Final	326 ± 6	331 ± 5	318 ± 1
Gain	244 ± 6	249 ± 5	236 ± 1
Abdominal adipose tissue weight (g)	24 ± 1	26 ± 2	24 ± 2
Interscapular brown adipose tissue weight (mg)	399 ± 6	416 ± 1	402 ± 2

Values are the mean ± SE for five rats.

(energy in the experimental diets fed minus energy in faeces) was constant. The mean metabolisable energy of the diets were 20.4 ± 0.03 kJ/g (range: 20.3–20.5 kJ/g) for the safflower oil diet, 20.4 ± 0.04 kJ/g (range: 20.1–20.5 kJ/g) for the soybean oil diet and 19.5 ± 0.1 kJ/g (range: 18.9–19.8 kJ/g) for the beef tallow diet.

Experiment 2

Body weight, adipose tissue weights and carcass composition. Each group of rats showed the same weight gain during the 8-week experimental period (Table 4). Carcass fat content was significantly greater ($P < 0.05$) in the beef tallow diet group than in the safflower oil diet group or the soybean oil diet group, whereas neither abdominal adipose tissue (epididymal, perirenal and mesenteric) weight or the total weight of abdominal adipose tissues differed among the three dietary groups (Table 4). Carcass protein contents for the three groups were the same (Table 4). Interscapular brown adipose tissue weight was not different among the three groups (Table 4).

The relationship between carcass fat content and palmitic, stearic, oleic or linoleic acid in dietary fats (Fig. 1) was analysed. A positive correlation was observed between carcass fat content and palmitic, stearic and oleic acid, whereas a negative correlation was observed between carcass fat and linoleic acid (Fig. 1).

Gene expression of UCP1 and UCP content in the brown adipose tissue. The UCP1 mRNA level in the brown adipose tissue was normalised to β-actin mRNA content, which did not differ among groups (data not shown). The mRNA level was significantly lower ($P < 0.05$) in rats fed a beef tallow diet than in rats fed a safflower oil diet or a soybean oil diet (Fig. 2). The UCP content in the brown adipose tissue was lower ($P < 0.05$) in the beef tallow diet group than in the other groups (Fig. 2).

Discussion

The ability to digest macronutrients can be affected by animal ageing.[9–12] However, in the present study metabolisable energy, calculated from digestible energy, did not change during the 8-week experimental period (experiment 1). This discrepancy may be due to use of young rats (4–12 weeks old). The mean metabolisable energy in the beef tallow diet was about 5% lower than in the safflower oil diet or the soybean oil diet. These levels are approximately the same as the metabolisable energy values reported previously.[3,6] For this reason, our previous experiments were performed strictly under pair-feeding conditions.[4–6]

We have shown here that body fat accumulation was greater in rats fed the beef tallow diet compared with the safflower oil or soybean oil diets. The difference in carcass fat content was especially significant between the beef tallow diet group and the safflower oil diet group (experiment 2). Because all rat groups consumed the same amount of metabolisable energy throughout the experimental period, the difference in body fat accumulation can be ascribed to the different dietary fats. In this context, it should be noted that *UCP1* mRNA levels and UCP content in brown adipose tissue were significantly lower in the beef tallow diet group than in the safflower oil diet group or soybean oil diet group. We previously reported that a beef tallow diet promotes less diet-induced thermogenesis and norepinephrine turnover in brown adipose tissue than does a safflower oil diet.[3,6]

Diet-induced thermogenesis is a major component of overall energy expenditure.[24] Particularly in rodents, thermogenesis in brown adipose tissue plays an important role as an energy buffer in resistance to dietary obesity.[25] Brown adipose tissue is morphologically distinguished from white fat mainly by its multilocular fat droplets, its strong vascularisation and its extensive innervation by the sympathetic nervous system.[25,26] Thermogenesis in the brown adipose tissue is activated by catecholamines via β_3-adrenergic receptor.[27] An increase in cyclic adenosine 5'-monophosphate (AMP) leads to increased lipolysis providing a supply of oxidisable substrate for mitochondria. *UCP1* activation leads to the generation of heat involving free fatty acids as second messengers.[28] Besides these effects, stimulation by catecholamines causes a marked increase in *UCP1* mRNA and

Table 4. Effects of dietary fats on body weight, adipose tissue weight and carcass composition (experiment 2)

	Diet group		
	Safflower oil	Soybean oil	Beef tallow
Body weight (g)			
Initial	58 ± 3	58 ± 3	58 ± 3
Final	236 ± 4	243 ± 3	231 ± 3
Gain	178 ± 4	185 ± 3	173 ± 1
Abdominal adipose tissue weight (g)	17 ± 1	17 ± 1	18 ± 1
Interscapular brown adipose tissue weight (mg)	335 ± 12	335 ± 9	337 ± 1
Carcass composition			
Weight	145 ± 4	149 ± 2	144 ± 2
Fat (g)	16 ± 1[c]	23 ± 1[b]	32 ± 1[a]
Percentage fat	11 ± 1[c]	14 ± 1[b]	22 ± 1[a]
Protein (g)	35 ± 1	35 ± 1	33 ± 1
Percentage protein	24 ± 1	21 ± 1	23 ± 1

Values are the mean ± SE for six rats. Within a row, values with different superscripts are significantly different ($P < 0.05$).

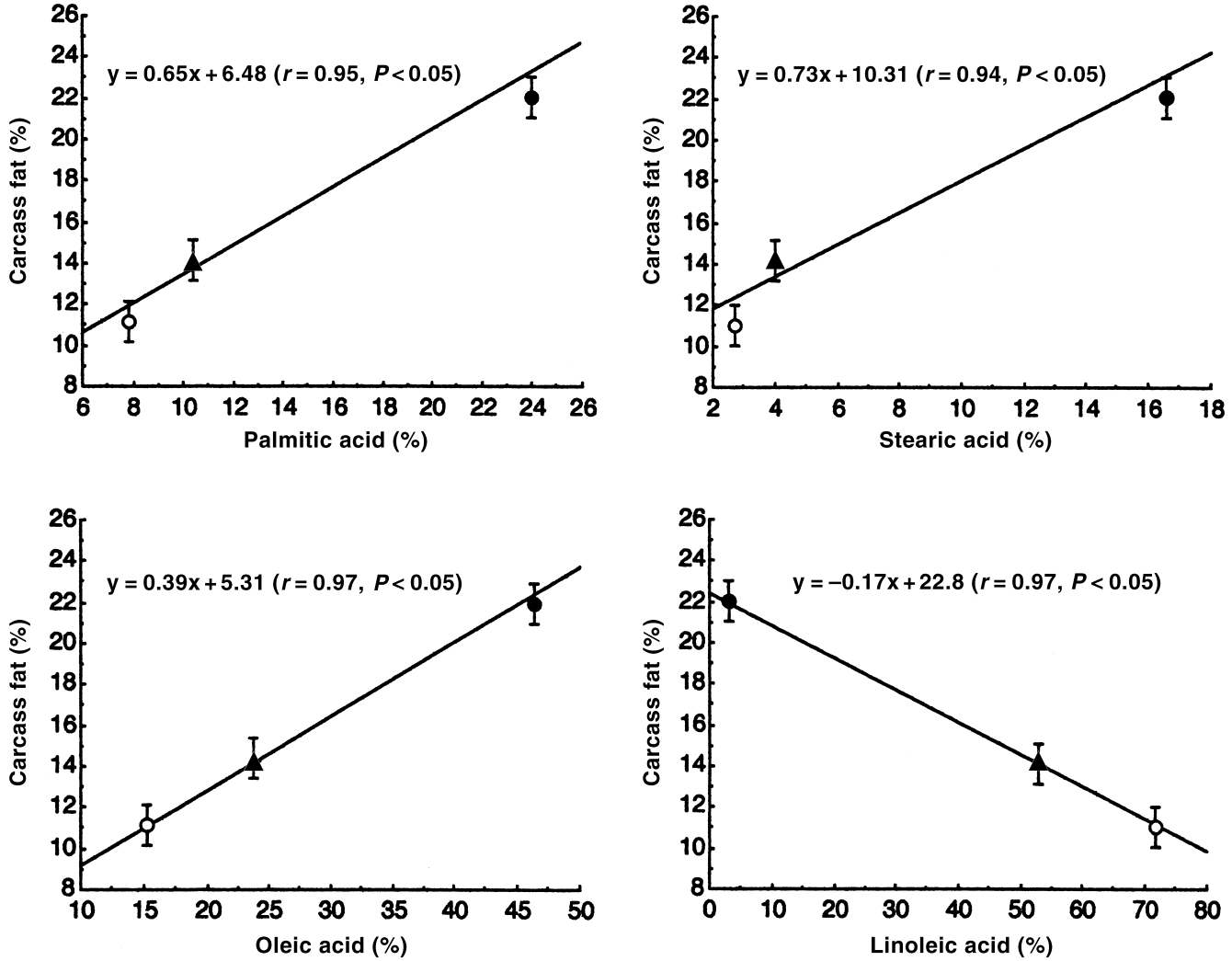

Figure 1. Relationship between carcass fat content and dietary fatty acids in rats fed (○) a safflower oil diet, (▲) a soybean oil diet and (●) a beef tallow diet. Each point represents the mean ± SE for six rats.

protein levels in brown adipose tissue.[29] Due to the unique capacity of brown adipose tissue for uncoupled metabolism, it is ideally situated to play an important role in the regulation of energy balance. Bouillaud *et al.* reported that norepinephrine increased the level of *UCP1* mRNA, consequently increasing UCP content.[30] Ricquier and Mory[31] and Rehnmark *et al.*[32] demonstrated that the expression of *UCP1* was accelerated by sympathetic stimulation. Lower sympathetic activity in the brown adipose tissue of rats fed a beef tallow diet may reduce the level of *UCP1* mRNA, thus resulting in the reduction of UCP content. Sadurkis *et al.*[33] demonstrated that both the metabolic response to norepinephrine and the amount of UCP were lower in mice fed a low-polyunsaturated fatty acid diet compared to those fed a high-polyunsaturated fatty acid diet (2-week experimental diet). Our present findings support those results.

Recently, safflower oil and corn oil enriched in n-6 polyunsaturated fatty acid have proven to be unacceptable as standard experimental oils because they do not provide sufficient linolenic acid (n-3 polyunsaturated fatty acid) to meet requirements.[14] It is suggested that soybean oil (n-6 : n-3 ratio = 7) is the only appropriate single source of dietary fat.[14] However, in this experiment, carcass fat content was lower in the safflower oil diet than in the soybean oil diet (experiment 2). A positive correlation was observed between carcass fat content and palmitic, stearic and oleic acids, whereas a negative correlation was observed between carcass fat and linoleic acid. These findings suggest that n-6 polyunsaturated fatty acid may be the most effective of the fatty acids tested in limiting body fat. According to some reports,[34,35] fatty acids such as linoleic acid, arachidonic acid and eicosapentaenoic acid in food directly become ligands for the peroxisome proliferator-activated receptor (PPAR) α and δ, which is a ligand-dependent receptor-type transcription factor. UCP genes are target genes of PPAR.[35] Polyunsaturated fatty acids function not only as adipocyte differentiation regulators but also as PPAR molecular sensors in lipid metabolism where they play a significant role in continuing the obese state.[35] On the other

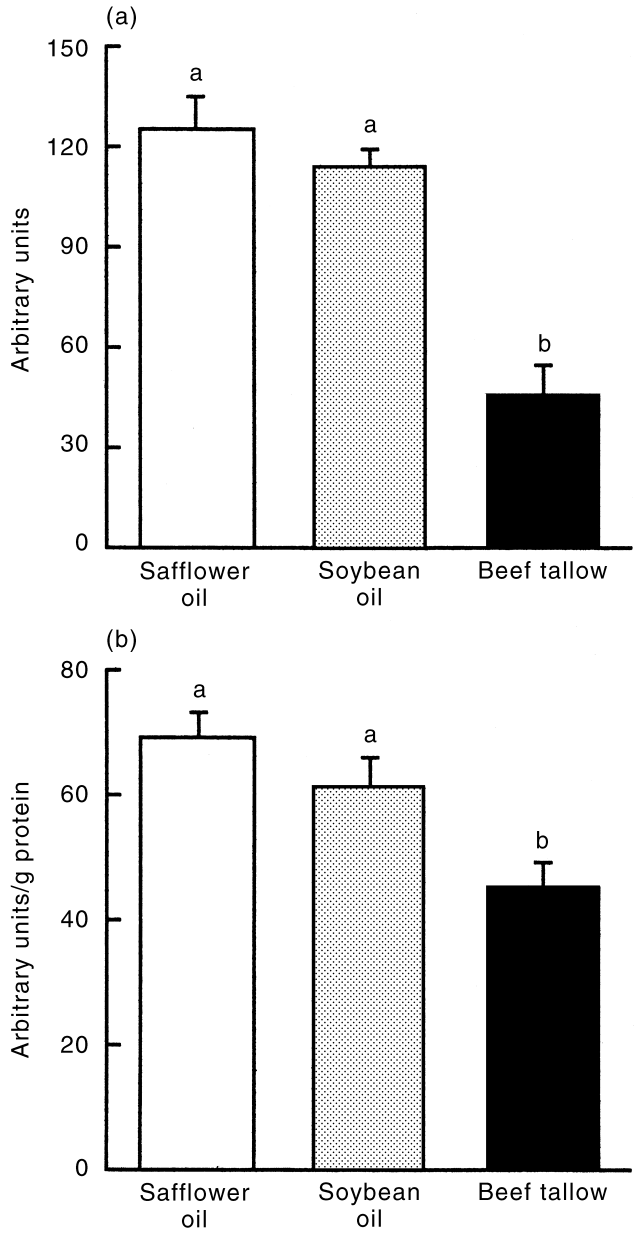

Figure 2. (a) *UCP1* mRNA levels normalised to β-actin mRNA content and (b) the relative content of mitochondrial uncoupling protein (UCP) in the interscapular brown adipose tissue of rats fed a safflower oil diet, a soybean oil diet or a beef tallow diet. Values are means and SE for six rats. Within a row, values with different superscripts are significantly different ($P < 0.05$).

hand, saturated and n-9 monounsaturated fatty acids in dietary fats may promote body fat accumulation. However, Takeuchi *et al.*[7,8] reported that body fat accumulation did not differ among rats fed a safflower oil (rich in linoleic acid) diet, a high oleic acid safflower oil (rich in oleic acid) diet and a linseed oil (rich in linolenic acid) diet. The cause of this disagreement is not known. Detailed studies are required to clarify the mechanisms by which particular fatty acids in dietary fats affect body fat accumulation.

In this study, abdominal adipose tissue weight was the same for all diet groups. These results were also observed in previous studies.[3–6] Shimomura *et al.*[36] suggest that physical exercise has a greater effect on abdominal fat than on subcutaneous fat. In contrast to the effects of physical exercise, the effect of dietary fatty acid composition may be greater on subcutaneous fats. Lipid composition of the plasma membrane was strongly site specific and was greatly affected by dietary fatty acids.[37] Because the plasma membrane may play an important role in the regulation of lipid metabolism,[38] the differential effect of fat accumulation in carcass and abdominal adipose tissues might be explained by site-specific alterations in the fatty acid composition of the plasma membrane.

Mercer and Trahurn[39] reported that energy deposition is higher and thermogenic activity in brown adipose tissue is lower in both lean and genetically obese mice fed a beef tallow diet than in those fed a corn oil diet, despite the isoenergetic intakes of the two diets. The results obtained in the present study are consistent with these findings, suggesting that dietary fats affect the expression of UCP and sympathetic activities, even in genetically obese animals.

In conclusion, the present study demonstrated that the metabolisable energy of each experimental diet did not change during the experimental period, which proved that our previous experiments were strictly and accurately performed. Body fat accumulation was greater in rats fed a beef tallow diet than in those fed safflower oil or soybean oil diets, resulting from lower expression of *UCP1* mRNA and UCP content in the brown adipose tissue. n-6 Polyunsaturated fatty acid may be the most effective fatty acid in limiting body fat.

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