Beef Tallow Diet Decreases β-Adrenergic Receptor Binding and Lipolytic Activities in Different Adipose Tissues of Rat

Tatsuhiro Matsuo, Hiroshi Sumida, and Masashige Suzuki

The effects of dietary fats consisting of different fatty acids on lipolytic activity and body fat accumulation were studied in rats. Sprague-Dawley male rats were meal-fed an isoenergetic diet based on either beef tallow or safflower oil for 8 weeks. Lipolytic activities in epididymal and subcutaneous adipose tissues were lower in the beef tallow diet group than in the safflower oil diet group. Body fat accumulation was greater in rats fed the beef tallow diet versus the safflower oil diet. Norepinephrine (NE) turnover rates used as an index of sympathetic activities in adipose tissues were lower in the beef tallow diet group. β-Adrenergic receptor binding was determined with [^{3}H]dihydroalprenolol. Binding affinities of β-receptors in adipose tissues were significantly lower in the beef tallow diet group. Membrane fluidities of adipose tissues were also lower in the beef tallow diet group. Membrane fluidities were correlated with the affinities of the β-receptor. We believe from these correlations that the decreases in β-receptor binding affinities are due to the changes in membrane fluidities. The results of the present study suggest that intake of the beef tallow diet promotes body fat accumulation by reducing lipolytic activities resulting from lower β-receptor binding and sympathetic activity in adipose tissues.

CONSIDERABLE INTEREST has arisen concerning the effect of dietary fatty acid composition in long-term feeding on body fat accumulation.[1-3] We have recently reported that sympathetic activities in the interscapular brown adipose tissue and pancreas were lower in rats fed a beef tallow diet than in rats fed a safflower oil diet when rats were fed isoenergetic diets (45% of energy as fat) based on beef tallow–enriched saturated fatty acid or safflower oil–enriched n-6 polyunsaturated fatty acid for 8 weeks.[4] Rats fed the beef tallow diet have shown higher body fat accumulation induced by the decrease of diet-induced thermogenesis and the increase of serum insulin concentration.[4] Moreover, we have suggested that intake of animal fats rich in saturated fatty acids, as compared with intake of other vegetable oils (high-oleic safflower oil and linseed oil) rich in monounsaturated or n-3 polyunsaturated fatty acids, decreases diet-induced thermogenesis by a decline of sympathetic activity in brown adipose tissue, resulting in promotion of body fat accumulation.[5]

It is well known that intake of a high-fat diet causes changes in the fatty acid composition of the plasma membrane.[6] Higher levels of saturated fatty acid in plasma membranes produce lower membrane fluidity by reducing polyunsaturated to saturated fatty acid (P/S) ratios in plasma membranes.[7,8] The change of membrane fluidity affects the capacity of adrenergic receptor binding[6] and enzyme activity in the plasma membrane.[9] Plasma membrane fluidity would affect the activity of tyrosine hydroxylase, a key enzyme of norepinephrine (NE) synthesis in the plasma membrane.[6] Fatty acids released from adipose tissues (fat catabolism) are regulated by adrenergic function, β-adrenergic receptor binding, and NE turnover rates. We have recently suggested that β-adrenergic receptor binding and NE turnover rates in brown adipose tissue,[10] heart,[10] soleus muscle,[10] and liver[11] were decreased in rats fed the beef tallow diet, resulting from the decrease of membrane fluidities of those organs.

From these points of view, we speculated that an increase of saturation in the fatty acid composition of adipocyte plasma membrane by the beef tallow diet would result in a decrease of β-adrenergic receptor binding and NE turnover rates, and consequently decreased lipolytic activities in adipose tissues. It is possible to discern that a decrease of lipolytic activity with the beef tallow diet results in higher body fat accumulation in rats.

On the other hand, we have reported that dietary fats affect body fat accumulation to a greater extent in subcutaneous adipose tissue than in intraabdominal adipose tissues.[3,4] These results suggest that lipolytic activities and adrenergic function (NE turnover rates and β-adrenergic receptor binding) may differ between subcutaneous adipose tissue and intraabdominal adipose tissue.

The aim of this study was to examine (1) whether intake of a beef tallow diet decreases lipolytic activities and adrenergic function (NE turnover rates and β-adrenergic receptor binding) in adipose tissues as compared with intake of a safflower oil diet, (2) whether dietary fats affect the composition and fluidity of the adipocyte plasma membrane, and (3) whether NE turnover rates and β-adrenergic receptor binding are different between epididymal adipose tissue and abdominal subcutaneous adipose tissue in rats fed the beef tallow diet and the safflower oil diet.

MATERIALS AND METHODS

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

Animals and Diets

Sixty-two male Sprague-Dawley rats (5 weeks old) were obtained from CLEA Japan (Tokyo, Japan). Half the animals were fed a safflower oil diet, and the other half were fed a beef tallow diet. The compositions of both diets have been described previously.[3] Both diets provided 45%, 35%, and 20% of energy as fat, carbohydrate, and protein, respectively. The metabolizable energy

From the Nutrition and Biochemistry Division, Sanyo Women's College, Hiroshima; and the Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.

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Address reprint requests to Tatsuhiro Matsuo, Nutrition and Biochemistry Division, Sanyo Women's College, Sagatahonmachi, Hatsukaichi, Hiroshima 738, Japan.

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was 19.7 kJ/g for the safflower oil diet and 18.4 kJ/g for the beef tallow diet. Fatty acid compositions of safflower oil and beef tallow have been described previously[3]; beef tallow consisted of 44% oleic, 27% palmitic, and 18% stearic acids, and safflower oil consisted of 79% linoleic acid. Total cholesterol content in safflower oil and beef tallow was measured using a kit (T-cholesterol test) purchased from Wako Pure Chemical (Osaka, Japan).

Experimental Design

Animals were individually caged at 22° ± 2°C, with light from 7 AM to 7 PM. Each group of rats were meal-fed the diet at 8 to 9 AM and 8 to 9 PM and given water with free access for 8 weeks. Both groups of rats were offered the appropriate diet in amounts such that the two groups consumed equal metabolizable energy during the experimental period. The meal-feeding method was used to adjust energy intake between the two dietary groups. Under meal-feeding conditions, feeding one meal (within 2 hours) per day causes food intake of the animals to decrease; however, feeding two meals per day, as in this study, minimized the decrease in food intake. The food consumption of rats shown in Table 1 was approximately the maximal amount of diet that rats could consume under meal-feeding conditions. On the final day, rats in each diet group were fed a meal at 8 to 9 AM. Then, 14 rats in each diet group were injected with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (300 mg/kg intraperitoneally), at 0 hours (10 AM) and decapitated at 1.5 or 3 hours, and seven rats in each diet group received saline as controls at 0 hours, and were immediately killed by decapitation. Ten rats in each diet group were not treated and were decapitated at 8 AM (before meals). Blood was collected to obtain serum. Intraabdominal and abdominal subcutaneous adipose tissues, except for parts of epididymal and subcutaneous adipose tissues, were quickly removed as follows. Samples of epididymal adipose tissue were obtained at surgery from the bilateral epididymides except for blood vessels, and samples of subcutaneous adipose tissue (~2 g) were obtained at surgery from bilateral subcutaneous epigastric abdominal depots. The removed adipose tissues were washed in saline, weighed, and stored at −80°C until analysis. Pieces of epididymal and subcutaneous adipose tissues weighing 100 to 120 mg were used for assay of lipolytic activity. Carcass samples were obtained by removing the head, liver, heart, lungs, kidneys, spleen, testes, pancreas, and digestive tracts, and were stored at −20°C until analysis of carcass composition.

Serum and Carcass Analyses

Serum free fatty acid levels were measured enzymatically using kits (NEFA C Test) purchased from Wako Pure Chemical (Osaka, Japan). Serum glycerol level was measured[12] enzymatically using a kit (F-kit, glycerol) purchased from Boehringer Mannheim-Yamanouchi (Tokyo, Japan). Carcass fat and protein were analyzed by the method reported by Mickelsen and Anderson.[13]

Table 1. Food Consumption of Rats Fed Both Experimental Diets

Experimental Period (wk)	Food Consumption (kJ/d)
1	242
2	332
3	346
4	360
5	343
6	355
7	355
8	355

NOTE. Values were calculated as metabolizable energy of the diets.

Lipolytic Activity of Epididymal and Subcutaneous Adipose Tissues

Lipolytic activity of epididymal and subcutaneous adipose tissues was measured by the methods reported by Suzuki et al[14] as modified by Oscai et al.[15] Briefly, small pieces of epididymal or subcutaneous fat pads weighing 100 to 120 mg were incubated with mild shaking at 37°C for 60 minutes in 2 mL Krebs-Ringer bicarbonate buffer containing 3% bovine serum albumin (Fraction V, essential fatty acid–free; Sigma, St Louis, MO), pH 7.4. l-Norepinephrine bitartarate (Sigma) at 10 μg/mL was present in the incubation medium. After incubation, the reaction was stopped by cooling the flasks in ice water for 5 minutes. Lipolytic activity was expressed as the release of glycerol per hour under basal and NE-stimulated conditions in which glycerol content was measured[12] using a kit (F-kit, glycerol) purchased from Boehringer Mannheim-Yamanouchi and the method as modified by Vaughan.[16] We previously confirmed reproducibility of the measurement (data not shown).

Rodbell[17] established the technique for isolation of fat cells. However, we have used adipose tissue slices to measure lipolysis. The reason for this was, first, the possibility of some cell loss during the isolation process,[15] and second, Oscai et al[15] reported that if a higher level of enzyme activity was an important consideration, then adipose tissue slices should be used. Since rats were fasted in this experiment for 12 hours before decapitation, the activity level of hormone-sensitive lipase in adipose tissue must have been higher. Third, in our previous experiments, lipolytic activities were not significantly different with the method using adipose tissue slices and isolated fat cells (T. Matsuo, unpublished observation, March 1994).

Preparation of Adipocyte Plasma Membranes

To prepare adipocyte plasma membranes for analysis of fatty acid composition, membrane fluidity, and adrenergic receptor binding assay, adipose tissues from noninjected rats were prepared using methods reported previously.[18] Briefly, adipose tissues were homogenized with a glass homogenizer in 10 vol 50-mmol/L Tris hydrochloride buffer containing 5 mmol/L $MgCl_2$ and 145 mmol/L NaCl (pH 7.6) and centrifuged at 900 × *g* for 10 minutes at 4°C. The supernatant was centrifuged at 11,500 × *g* for 20 minutes at 4°C. Then the supernatant was centrifuged at 49,000 × *g* for 60 minutes at 4°C. The pellet was resuspended in 50 mmol/L Tris hydrochloride buffer, and membranes were immediately used for analysis. Protein content was determined by the method reported by Bradford.[19]

Lipid Extraction

The membrane suspensions were freeze-dried, and then total lipid content was extracted from membranes by the method reported by Folch et al.[20] The fatty acid composition of total lipid was determined after hydrolysis and methylation according to the method reported by Nelson et al[21] using a gas-liquid chromatograph system (Model GC-7AG; Shimadzu, Kyoto, Japan). The system was equipped with a fused-silica, wall-coated capillary column, 0.25 mm inner diameter × 25 m long, coated with ULBON HR20 (Shimadzu).

Fluorescence Polarization

Membrane fluidity was assessed by fluorescence polarization using the lipid-soluble fluorophore, 1,6-diphenyl-1,3,5-hexatriene

(DPH), as modified by the method reported by Nicolas et al.[7] A diluted aliquot containing 50 μg protein/mL from the membrane fraction in Tris hydrochloride buffer (pH 7.6) was incubated at 37°C for 30 minutes with 1 μL DPH. The probe was initially dissolved in tetrahydrofran. Final concentration in the incubating medium was 2 μmol/L.

Steady-state fluorescence polarization studies were performed at 25°C using a fluorescence spectrophotometer (Model F-2000; Hitachi, Tokyo, Japan). The excitation wavelength was 361 nm, with emission being detected at 431 nm. The degree of fluorescence polarization (P) was calculated by the method reported by Shinitzky and Barenholz.[22]

β-*Adrenergic Receptor Binding Assay*

Radioligands were incubated with adipocyte membrane preparations in a total volume of 0.25 mL. Incubations were stopped by cooling on ice, followed by rapid vacuum filtration onto Whatman GF/C filters, which were then washed with 20 mL Tris hydrochloride buffer. Nonspecific binding was defined as binding of radioligand in the presence of an excess concentration of a specific displacing agent, 1 μmol/L propranolol (Wako Chemical Industries). β-Adrenergic receptor binding was evaluated with 0.05 to 5 nmol/L [^{3}H]dihydroalprenolol (specific activity, 62 Ci/mmol; Amersham, Buckinghamshire, England) as modified by Bylund and Snyder[23] and a 60-minute incubation at 25°C. Nonspecific binding of [^{3}H]dihydroalprenolol constituted 10% to 30% of total binding. β-Adrenergic receptor number and binding affinity were determined from Scatchard analysis.[24]

NE Content and Turnover

NE content of epididymal and subcutaneous adipose tissues was assayed by high-performance liquid chromatography with electrochemical detection (Model LC-6A; Shimadzu) as modified by Refshauge et al.[25] Estimation of NE turnover was performed by the method reported previously.[26] Saline-treated rats were used for measurement of basal tissue NE level (NE_0). Since there is a monoexponential decline of tissue NE levels after α-methyl-*p*-tyrosine treatment, these data were then subjected to a least-square linear regression analysis of log NE concentration versus time. Turnover rates (slope/[0.434 × initial NE concentrations]) were estimated from these data.

Statistical Analysis

Statistical differences in body weight, tissue weights, carcass composition, and serum components were analyzed by Student's unpaired *t* test. Statistical differences in fatty acid composition of plasma membrane, lipolytic activities of adipose tissues, fluorescence polarization, NE turnover rate and β-receptor binding were analyzed by two-way ANOVA and Scheffe's test.[27]

RESULTS

Body Weight and Body Composition

Both groups of rats consumed diets with the same metabolizable energy (Table 1) and had the same body weight gain during the 8-week experimental period (Table 2). However, body fat weights were partially different between the two groups. Epididymal and perirenal adipose tissue weights and total weight of abdominal adipose tissues were significantly greater ($P < .01$) in the beef tallow diet group than in the safflower oil diet group. Carcass fat content was significantly greater ($P < .01$) in the beef tallow diet group versus the safflower oil diet group, whereas carcass protein contents of the two diet groups were of similar levels (Table 2). Mesenteric adipose tissue weight was not different between the two groups (Table 2).

Table 2. Effect of Dietary Fats on Body Weight, Abdominal Adipose Tissue Weight, and Carcass Composition

Parameter	Diet Group	
	Safflower Oil	Beef Tallow
Body weight (g)		
Initial	140 ± 1	137 ± 1
Final	427 ± 3	422 ± 4
Gain	287 ± 3	285 ± 4
Abdominal adipose tissue weight (g)		
Epididymal	6.8 ± 0.3	8.4 ± 0.3*
Perirenal	9.9 ± 0.4	12.0 ± 0.4*
Mesenteric	5.7 ± 0.2	5.5 ± 0.3
Total	23 ± 1	26 ± 1*
Carcass weight (g)	280 ± 3	274 ± 2
Carcass fat		
g	41 ± 1	56 ± 2*
%	15 ± 1	20 ± 1*
Carcass protein		
g	70 ± 1	66 ± 1
%	25 ± 1	24 ± 1

NOTE. Values are the mean ± SEM for 31 rats.

*Statistically significant difference ($P < .01$) from the safflower oil diet group (Student's *t* test).

Serum Glycerol and Free Fatty Acid Concentrations

Serum components were assayed only in rats that were not injected with α-methyl-*p*-tyrosine or saline. Glycerol concentration was 5.33 ± 0.01 μmol/dL for the safflower oil diet group and 5.26 ± 0.02 μmol/dL for the beef tallow diet group, and these values were significantly different ($P < .05$). Serum free fatty acid concentration was lower in the beef tallow diet group versus the safflower oil diet group (920 ± 34 *v* 1,013 ± 86 μmol/L, $P < .1$).

Fatty Acid Composition of Adipocyte Plasma Membrane

Fatty acid composition of adipocyte plasma membrane is shown in Table 3. Whatever the tissue, linoleic acid (18:2n-6) was significantly lower ($P < .01$) and myristic acid (14:0), myristoleic acid (14:1n-9), palmitic acid (16:0), palmitoleic acid (16:1n-9), stearic acid (18:0), and oleic acid (18:1n-9) were significantly higher ($P < .01$) in the beef tallow diet group versus the safflower oil diet group. In subcutaneous adipose tissue, arachidonic acid (20:0) in plasma membranes was significantly higher ($P < .01$) in the beef tallow diet group. Fatty acid compositions of adipocyte plasma membranes in the two dietary groups were similar between epididymal and subcutaneous adipose tissues except for myristic and stearic acids. P/S ratios of plasma membranes in epididymal and subcutaneous adipose tissues were significantly lower ($P < .01$) in the beef tallow diet group versus the safflower oil diet group (Table 3).

Lipolytic Activity of Epididymal and Subcutaneous Adipose Tissues

Lipolytic activity was determined by the release of glycerol per hour (Fig 1). Both basal and NE-stimulated

Table 3. Effect of Dietary Fats on Fatty Acid Composition (%) of Adipocyte Plasma Membrane

Fatty Acid	Epididymal		Subcutaneous	
	Safflower Oil	Beef Tallow	Safflower Oil	Beef Tallow
14:0	6.2 ± 0.2	13.9 ± 0.3*	9.8 ± 0.3†	15.8 ± 0.3*
14:1(n-9)	0.5 ± 0.1	1.3 ± 0.1*	0.5 ± 0.1	1.6 ± 0.1*
16:0	13.0 ± 0.1	16.4 ± 0.1*	11.7 ± 0.1	17.8 ± 0.2*
16:1(n-9)	1.3 ± 0.1	3.8 ± 0.1*	0.7 ± 0.1†	3.2 ± 0.1*
18:0	6.7 ± 0.1	10.3 ± 0.1*	5.6 ± 0.1	7.7 ± 0.1*†
18:1(n-9)	16.3 ± 0.1	35.9 ± 0.2*	14.8 ± 0.1	35.5 ± 0.2*
18:2(n-6)	46.7 ± 0.2	6.9 ± 0.2*	48.2 ± 0.1	7.9 ± 0.2*
18:3(n-3)	0.5 ± 0.1		0.4 ± 0.1	
20:0	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1*
20:1(n-9)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
20:3(n-6)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
20:4(n-6) ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.3 ± 0.2	
Other	1.0 ± 0.2	3.5 ± 0.1	0.8 ± 0.1	2.9 ± 0.3
Saturated	26.5 ± 0.5	41.0 ± 0.6*	27.3 ± 0.5	41.7 ± 0.7*
Monounsaturated	18.4 ± 0.4	41.3 ± 0.6*	16.4 ± 0.4	40.8 ± 0.8*
Polyunsaturated	54.1 ± 0.8	13.9 ± 0.4*	55.7 ± 0.9	14.6 ± 0.4*
P/S ratio	2.04 ± 0.12	0.34 ± 0.02*	2.04 ± 0.11	0.35 ± 0.02*

NOTE. Values are the mean ± SEM for 10 rats.

*Statistically significant difference ($P < .01$) from the safflower oil diet group.

†Statistically significant difference ($P < .01$) from epididymal adipose tissue (2-way ANOVA and Sheffe's test).

lipolytic activities of epididymal and subcutaneous adipose tissues were lower in the beef tallow diet group than in the safflower oil diet group, although the difference in basal lipolytic activities of epididymal adipose tissue was not recognized at the 5% significance level ($P < .1$). Significantly high basal and NE-stimulated lipolytic activities were recognized in epididymal adipose tissue as compared with subcutaneous adipose tissue.

Membrane Fluidity

Fluidity of adipocyte plasma membrane was assessed by fluorescence polarization (P) of DPH probes (Fig 2). Whatever the tissue, membrane fluidities were lower ($P < .05$) in the beef tallow diet group than in the safflower oil diet group.

β-Adrenergic Receptor Binding

β-Adrenergic receptor binding was determined with [^{3}H]dihydroalprenolol. Adipocyte plasma membranes from the two different adipose tissues of rats fed the beef tallow diet or the safflower oil diet were incubated with increasing concentrations of radioligands. The binding process was saturable, and Scatchard plots are shown in Fig 3. Mean maximal binding sites (B_{max}) and dissociation constants (K_d) are shown in Table 4. β-Adrenergic receptor binding affinity for [^{3}H]dihydroalprenolol in epididymal and subcutaneous adipose tissues was significantly lower (55% in epididymal adipose tissue and 45% in subcutaneous adipose tissue) in the beef tallow diet group versus the safflower oil diet group (Table 4). However, maximal numbers of β-adrenergic receptors in the two different adipose tissues were not different between the two diet groups (Table 4).

NE Content and Turnover

Basal NE content in subcutaneous adipose tissue was significantly lower ($P < .05$) in the beef tallow diet group than in the safflower oil diet group, whereas that in epididymal adipose tissue was not different between the two dietary groups (Table 5). Whatever the tissue, NE turnover rates were significantly lower ($P < .05$) in the beef tallow diet group than in the safflower oil diet group (Table 5). In the beef tallow diet group, NE turnover rate was significantly higher ($P < .05$) in epididymal adipose tissue than in subcutaneous adipose tissue (Table 5).

DISCUSSION

We have shown here that lipolytic activities of epididymal and subcutaneous adipose tissues were lower in rats fed the beef tallow diet versus the safflower oil diet, and especially the difference in subcutaneous adipose tissue was significant between the two dietary groups. In this context, it should be noted that serum glycerol and free fatty acid concentrations were lower in the beef tallow diet group versus the safflower oil diet group. We previously reported that intake of a beef tallow diet promotes more body fat accumulation as compared with intake of a safflower oil diet.[3,4] Fat accumulation with a beef tallow diet was due to deposition of fat in the carcass rather than intraabdominal adipose tissues. It has been reported that metabolism of adipose tissues is different between the two sites.[28,29]

We have clearly shown here that binding affinity of the β-adrenergic receptor for [^{3}H]dihydroalprenolol in both epididymal and subcutaneous adipose tissues was lower in the beef tallow diet group than in the safflower oil diet group. β-Adrenergic receptor binding in the adipocyte

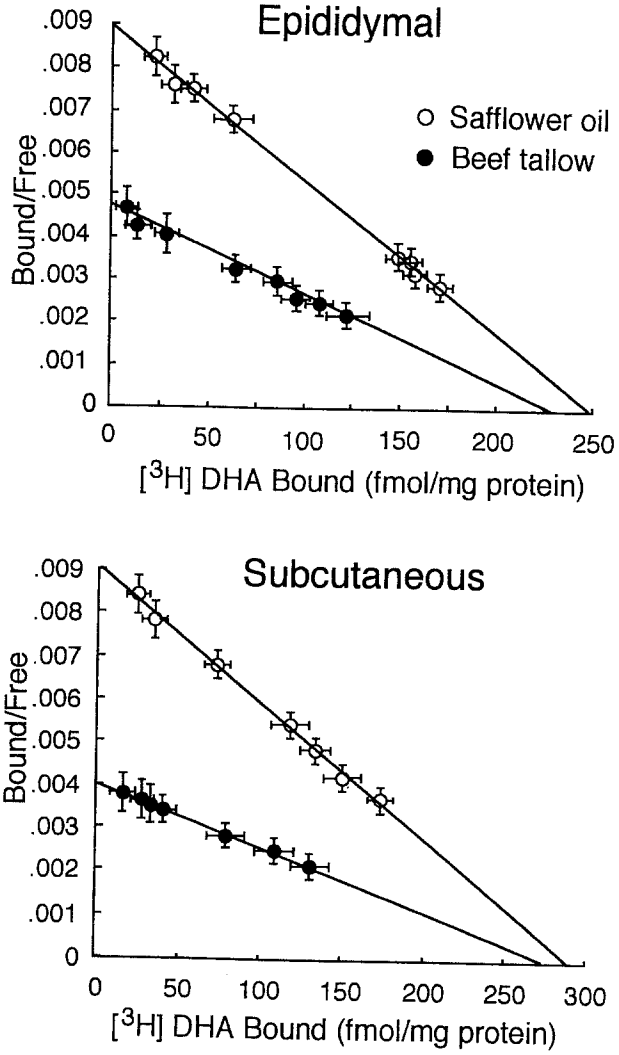

Fig 1. Basal (oblique line bar) and NE-stimulated (complete bar) lipolytic activity of epididymal and subcutaneous adipose tissues in rats fed the beef tallow diet or safflower oil diet. Data are the mean ± SEM. *Statistically significant difference from the safflower oil diet group. §Statistically significant difference from epididymal adipose tissue. Differences with $P < .05$ (ANOVA and Scheffe's test) were considered significant.

plasma membrane plays an important role as a lipolytic function in adipose tissues.[30] β-Adrenergic receptor coupled on the plasma membrane with adenylate cyclase activated by binding of catecholamine to the β-adrenergic receptor.[30] Adenylate cyclase catalyzes the production of an intracellular messenger, cyclic adenosine monophosphate, and it in turn activates hormone-sensitive lipase and modifies lipolytic activities in adipose tissues.[30] Because both groups of rats consumed diets with the same metabolizable energy throughout the experimental period, the difference in β-adrenergic receptor binding affinity between the two dietary groups was ascribed to different dietary fats.

Compared with the difference in β-adrenergic receptor binding, maximal numbers of β-adrenergic receptors in epididymal and subcutaneous adipose tissues were not

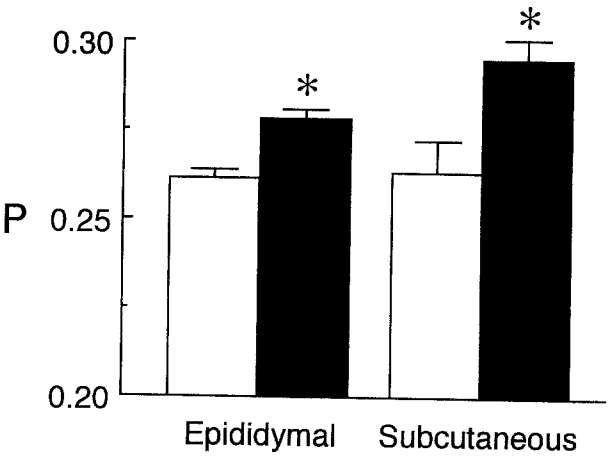

Fig 2. Fluorescence polarization (P) of DPH in adipocyte plasma membrane of rats fed the beef tallow diet (■) or safflower oil diet (□). Data are the mean ± SEM. *Statistically significant difference from the safflower oil diet group. Differences with $P < .05$ (ANOVA and Scheffe's test) were considered significant.

significantly different between the two dietary groups. It was suggested that the dietary fats induced alteration in receptor binding affinity rather than in receptor number. Nicolas et al[8] reported that whatever the diet (sunflower oil diet or control diet), the maximal number of β-adrenergic receptors for [125I]iodocyanopindolol was higher in perirenal adipose tissue than in subcutaneous adipose tissue in pigs. Our results with the present study are not consistent with their findings. The cause of this disagreement is unclear. In this study, despite a lower NE turnover rate in both adipose tissues in the beef tallow diet group, maximal

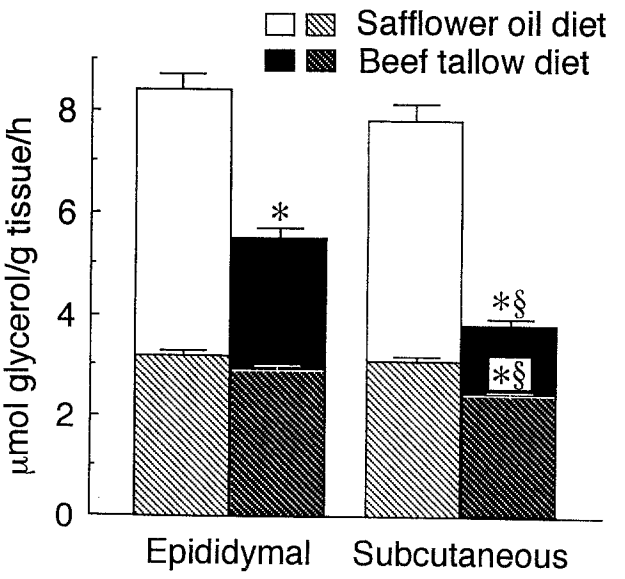

Fig 3. Scatchard plots of [3H]dihydroalprenolol to plasma membranes isolated from epididymal and subcutaneous adipose tissues of rats fed the beef tallow diet or safflower oil diet. Lines were determined by regression analysis. Values are the mean ± SEM. K_d and B_{max} are shown in Table 5.

Table 4. Effect of Dietary Fats on β-Adrenoceptor Binding in Adipose Tissues

Parameter	Epididymal		Subcutaneous	
	Safflower Oil	Beef Tallow	Safflower Oil	Beef Tallow
K_d (nmol/L)	3.4 ± 0.5	6.2 ± 0.7*	3.9 ± 0.4	8.6 ± 2.0*
Bmax (fmol/mg protein)	249 ± 40	238 ± 30	293 ± 29	288 ± 70

NOTE. Values are the mean ± SEM for 10 rats.

*Statistically significant difference ($P < .05$) from the safflower oil diet group (2-way ANOVA and Sheffe's test).

numbers of β-adrenergic receptors did not change. Similar results were found in our previous study.[10,11] Although it is not known why β-adrenergic receptors are upregulated in the beef tallow diet group, presynaptic change may not always be accompanied by changes in receptor number.[31] As one explanation, there may be some receptors in the adipocyte that fail to function in the beef tallow diet group despite upregulation, since B_{max} cannot directly show the receptor proteins. Another experiment, such as an immunohistochemical or genetic study, is required to clarify this phenomenon.

Our previous studies have suggested that decreasing membrane fluidity was caused by the beef tallow diet.[10,11] It is well known that dietary fatty acid composition affects membrane fatty acid composition of various tissues (eg, adipose tissue,[7] atria,[37] and brain[33]), particularly by intake of a high-fat diet. Membrane fluidity is affected by membrane fatty acid composition.[7-9] The presence of polyunsaturated fatty acid in the lipid bilayer contributes to its fluidity, whereas, in contrast, saturated fatty acids are rigidifying molecules because of the absence of double bonds. Consequently, the P/S ratio is known to be representative of the contribution of fatty acids to membrane fluidity. In this experiment, P/S ratios of adipocyte plasma membranes in epididymal and subcutaneous adipose tissues were lower in the beef tallow diet group than in the safflower oil diet group. The results of P/S ratios were parallel to those of membrane fluidity. On the other hand, membrane cholesterol and phospholipids influence physical characteristics of the membrane.[7,9] Nicolas et al[7] reported that increases of the membrane phosphatidylcholine to phosphatidylethanolamine ratio and cholesterol to phospholipid ratio had a fluidifying effect on the membrane. In this study, we did not measure membrane cholesterol content and distribution of phospholipid classes. However, since cholesterol contents in the beef tallow and safflower oil used here were 96 and 0 mg/100 g, respectively, cholesterol content of adipocyte membrane must be higher in the beef tallow diet group than in the safflower oil diet group. In the future, we should examine the effects of dietary fats on membrane cholesterol and phospholipids regarding the change in membrane fluidity.

Table 5. Effect of Dietary Fats on NE Turnover in Adipose Tissues

Parameter	Epididymal		Subcutaneous	
	Safflower Oil	Beef Tallow	Safflower Oil	Beef Tallow
NE_0 (ng/g tissue)	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.8 ± 0.1*†
k (%/h)	11.2 ± 0.1	7.5 ± 0.1*	11.1 ± 0.2	6.5 ± 0.1*
$t_{1/2}$ (h)	6.2 ± 0.1	9.3 ± 0.1*	6.3 ± 0.1	10.7 ± 0.1*
NE_t (ng/g tissue/h)	0.14 ± 0.01	0.09 ± 0.01*	0.13 ± 0.01	0.05 ± 0.01*†

NOTE. Values are the mean ± SEM for 7 rats.

Abbreviations: NE_0, basal NE level; k, fractional turnover (slope/0.434); $t_{1/2}$, half-life (0.693/k); NE_t, NE turnover rate ($NE_0 \times k$).

*Statistically significant difference ($P < .05$) from the safflower oil diet group.

†Statistically significant difference ($P < .05$) from epididymal adipose tissue (2-way ANOVA and Sheffe's test).

β-Adrenergic receptor binding is altered by membrane fluidity.[3,9] In the present study, we observed a correlation between K_d of the β-adrenergic receptor for [^{3}H]dihydroalprenolol and fluorescence polarization ($r = .594$, $P < .05$ for epididymal adipose tissue and $r = .623$, $P < .05$ for subcutaneous adipose tissue). Wince and Rutledge[33] suggested that the lower binding of β-adrenergic receptor to [^{3}H]dihydroalprenolol was caused by lower membrane fluidity. Their study supports our present findings. Many studies have been performed on the role of lipid modification in the properties of membrane receptors.[9] Particularly, changes in saturated or unsaturated fats in the membrane are suggested to be a main factor affecting alterations of the receptor complex.[9] These mechanisms are not completely understood; however, membrane fluidity is one of the important effectors of receptor functions.[9]

In addition to β-adrenergic receptor binding, sympathetic activities in adipose tissues also play roles in lipolytic activities of adipose tissues.[30] Sympathetic activities in white adipose tissues are not frequently studied because of the limited content of catecholamines. On the other hand, there is a sufficient quantity of NE to activate lipolysis, since the amount of NE in adipose tissues is approximately 10^{-9} mol/L in this experiment. Because epinephrine was not detected in adipose tissues in this experiment, it suggested that epinephrine from the adrenal gland is less concerned with lipolytic activities in adipose tissues, especially in the nonexercise state. In this study, whatever the adipose tissue, NE turnover rates were lower in the beef tallow diet group than in the safflower oil diet group. Those findings suggested that sympathetic activities in adipose tissues were lower in the beef tallow diet group than in the safflower oil diet group, and that it was one of the factors inhibiting lipolytic activities in adipose tissues of the beef tallow diet group.

In conclusion, the present study demonstrates that dietary saturated fats are modulators of body fat accumulation. They induce lower lipolytic activities in adipose tissues, resulting from lower β-adrenergic receptor binding and NE turnover rates. The present study suggests that saturated fats induce lower membrane fluidities by reducing P/S ratios in rat adipose tissues; however, a detailed study is required to clarify this mechanism.

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