Sympathetic Activity Is Lower in Rats Fed a Beef Tallow Diet Than in Rats Fed a Safflower Oil Diet

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Effects of dietary fats consisting of different fatty acids on sympathetic activity and body fat accumulation were studied in rats. Rats were meal-fed an isoenergetic diet based on either beef tallow or safflower oil for 8 weeks. Carcass fat content was greater ($P < .05$) in rats fed the beef tallow diet than in rats fed the safflower oil diet. Norepinephrine (NE) turnover rate was significantly lower ($P < .05$) in interscapular brown adipose tissue (IBAT) and pancreas in rats fed the beef tallow diet than in rats fed the safflower oil diet, resulting in a decreased ($P < .05$) diet-induced thermogenesis (DIT) and an increased ($P < .05$) serum insulin concentration in the former. To confirm the effects of dietary fats on sympathetic activity in relation to body fat accumulation, rats were chemically sympathectomized. Sympathectomy abolished the differences in body fat accumulation, DIT, and serum insulin concentration between the two dietary groups. These results suggest that the beef tallow diet promotes body fat accumulation by reducing sympathetic activity as compared with intake of the safflower oil diet.

MANY STUDIES have been performed on the role of dietary fats in the development of dietary obesity. In particular, the amount of fat in the diet is suggested to be a direct factor affecting body fat accumulation.[1] On the other hand, few studies have dealt with the effect of long-term feeding of dietary fats of different fatty acid compositions on body fat accumulation. In epidemiologic studies, Berry et al[2] suggested there was a significantly positive correlation between body mass index and saturated fatty acid intake. Romieu et al[3] reported that the body mass index of obese women was positively correlated with intakes of total fat and saturated fatty acids. These findings suggest that differences in dietary fatty acid composition affect body fat accumulation.

In recent studies, Awad et al[4] demonstrated that 4 weeks of ad libitum feeding of a diet containing 32% of energy as either safflower oil or beef tallow did not affect body fat content or fat metabolism. They reported that food consumption and food efficiency were not affected by dietary fats (data not shown); however, metabolizable energy of the experimental diets was not taken into account. Hill et al[5] reported that the fat type (lard or corn oil) in the diet had a lesser effect on body fat accumulation in rats as compared with the fat amount, but rats were not fed the same metabolizable energy of a lard diet or a corn oil diet. On the other hand, we have recently reported that when rats were meal-fed isoenergetic diets (45% of energy as fat) based on beef tallow or safflower oil for 4 months, body fat accumulation was greater in rats fed a beef tallow diet than in those fed a safflower oil diet.[6] In this experiment, consumption of experimental diets was adjusted on the basis of metabolizable energy. We suggest in this report that decreased diet-induced thermogenesis (DIT) and fat oxidation rates in animals fed the beef tallow diet are responsible for greater body fat accumulation. Furthermore, a higher level of serum insulin was found in rats fed the beef tallow diet.

DIT and insulin secretion from pancreatic β cells are regulated by the sympathetic nervous system. DIT occurs mainly in brown adipose tissue (BAT), and a decrease in sympathetic activity in BAT reduces DIT.[7-9] In the pancreas, a decrease in sympathetic activity causes an increase in insulin secretion.[10,11] Furthermore, in studies of animal models of obesity, it was demonstrated that genetically and hypothalamically obese animals have decreased sympathetic activities, which are responsible for decreased DIT and increased insulin secretion.[12] We hypothesize that sympathetic activities in BAT and pancreas are lower in animals fed a beef tallow diet than in those fed a safflower oil diet, resulting in greater body fat accumulation in the former.

In the present study, we investigated sympathetic activities in interscapular BAT (IBAT), pancreas, heart, and liver of rats fed a beef tallow diet or safflower oil diet for 8 weeks by measuring norepinephrine (NE) turnover rate as an index of sympathetic activity (experiment 1). Moreover, using sympathectomized rats, we studied the effects of these experimental diets on serum insulin levels, DIT, and body fat accumulation (experiment 2).

MATERIALS AND METHODS

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

Experiment 1: Effect of Dietary Fats on Sympathetic Activities of Various Organs and on Body Fat Accumulation in Rats

Animals and diets. Fifty male Sprague-Dawley rats (5 weeks old) were obtained from CLEA (Tokyo, Japan). Half of 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.[6] Both diets provided 45%, 35%, and 20% of energy as fat, carbohydrate, and protein, respectively. The metabolizable energy was 19.7 kJ/g for the safflower oil diet and 18.4 for the beef tallow diet. Fatty acid compositions of safflower oil and beef tallow have been described previously[6]: beef tallow consisted

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Submitted June 20, 1994; accepted September 12, 1994.

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0026-0495/95/4407-0016$03.00/0

of 44% oleic, 27% palmitic, and 18% stearic acids, and safflower oil consisted of 79% linoleic acid.

Experimental design. The animals were individually caged at 22° ± 2°C, with lights on from 7 AM to 7 PM. Each group of rats was meal-fed the diet at 8 to 9 AM and 8 to 9 PM and given free access to water 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 the energy intake between the two dietary groups. Under meal-feeding conditions, feeding one meal (within 2 hours) per day causes the food intake of the animals to decrease; however, feeding two meals per day minimized this effect of meal-feeding. The food consumption of rats listed 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, six rats in each diet group were injected with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (250 mg/kg intraperitoneally [IP]) at 0 hours (10 AM) and again with 150 mg/kg IP at 3 hours, and decapitated at 7 hours. Twelve rats in each diet group were administered 250 mg/kg IP at 0 hours and decapitated at 1 or 3 hours, whereas seven rats in each diet group received saline as controls at 0 hours and were immediately killed by decapitation. Blood was collected to obtain serum, and IBAT, pancreas, liver, and heart were quickly removed, weighed, and stored at −70°C until analyses. Carcass samples were obtained by removing the head, digestive tracts, lungs, kidneys, testes, and abdominal adipose tissues, and were stored at −20°C until analysis of carcass composition.

Analysis. NE contents of IBAT, pancreas, liver, and heart were assayed by high-performance liquid chromatography with electrochemical detection as modified by Refshauge et al.[13] Estimation of NE turnover was performed using the method reported previously.[14-16] Saline-treated rats were used for measurement of the basal organ level of NE. Since there is a monoexponential decline of organ 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.[14] Turnover rate (slope/[0.434/initial NE concentration]) was estimated from these data.

Concentrations of serum glucose[17] and insulin[18] were determined by methods reported previously. Carcass fat and protein were analyzed using the method reported by Mickelsen and Anderson.[19]

Experiment 2: Effect of Dietary Fats on Body Fat Accumulation in Sympathectomized Rats

Animals and experimental design. Male and female Sprague-Dawley rats (10 weeks old) were obtained from CLEA. Rats were fed CE-2, a commercial rodent diet (CLEA), and given free access to water. Rats were mated at age 12 weeks, and 36 male pups obtained were used in this experiment. Sympathectomy was performed according to the modification described previously.[20,21]

Table 1. Food Consumption of Rats Fed Experimental Diets

Experimental Period (wk)	Food Consumption (kJ/d)		
	Experiment 1	Experiment 2	
		Control	6-OHDA
1	335	284	216
2-3	338	354	295
4-5	322	383	327
6-7	356	349	277
8	344	332	262

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

Nineteen pups were treated long-term with 6-hydroxydopamine hydrobromide ([6-OHDA] Aldrich, Milwaukee, WI), of which 100 mg/kg was injected IP everyday for the first week and on day 14 and day 21 after birth. The remaining rats were treated with saline as controls. The validity of this protocol in producing effective sympathectomy as assessed by both functional and biochemical means has been reported previously.[22,23] NE contents of IBAT, pancreas, heart, and liver were analyzed in rats after the experiment described later, and it was confirmed that NE contents were decreased to 4% to 15% of contents in control rats by 6-OHDA treatment.

After weaning (age 3 weeks), control and sympathectomized rats were fed nonpurified diet and water ad libitum through age 5 weeks. At 5 weeks, five rats in each group were killed by decapitation. Carcass samples were collected, and initial carcass compositions were assayed as in experiment 1.

Then, control (n = 12) and sympathectomized (n = 14) rats were randomly divided into two subgroups, safflower oil diet and beef tallow diet groups. Diet compositions and housing conditions were the same as in experiment 1. Food consumption of sympathectomized rats was adjusted by the meal-feeding method to obtain the same metabolizable energy level between the safflower oil diet group and the beef tallow diet group (Table 1). Approximately 80% of the energy for control rats was provided to sympathectomized rats. It had been confirmed that sympathectomized rats could consume this amount of food under the meal-feeding conditions. Food consumption of control rats was also adjusted between the two dietary groups as described in experiment 1 (Table 1).

Whole-body oxygen consumption was measured using the method reported by Saitoh et al[24] between 6 and 7 weeks of the period of dietary manipulation. Rats used for measurement of resting oxygen consumption were placed in a glassware chamber (15 cm internal diameter and 10 cm internal height). Rats were acclimated to this apparatus for a week everyday (1 h/d) before measurement of oxygen consumption. Each group of rats was fed the appropriate diet (70% of the usual meal) within 30 minutes. Oxygen consumption was measured for 3 hours before and after the meal. Room temperature was 21° ± 1°C. Room air was continuously pumped at a flow rate of 0.7 L/min into the chamber through a small hole (1 cm internal diameter). All the air was collected into a Douglas bag (Fukuda Sangyo, Tokyo, Japan). Oxygen concentrations of the expired air were immediately analyzed by a gas mass analyzer (MGA 1100, Perkin-Elmer, St Louis, MO).

After 8 weeks of feeding the experimental diet, rats were killed by decapitation at 8 PM (before the meal). Blood, organs, and carcass samples were collected and stored as described in experiment 1.

Analysis. Serum insulin concentrations and carcass compositions were assayed as in experiment 1.

Data Analysis

In experiment 1, statistical differences in NE turnover rate, carcass composition, and serum glucose and insulin concentrations were analyzed by Student's *t* test. In experiment 2, statistical differences in carcass composition, serum glucose and insulin concentrations, and oxygen consumption were analyzed by a 2 × 2 factorial ANOVA and Scheffe's test.[25] Differences with P less than .05 were considered significant.

RESULTS

Experiment 1

Body weight, body fat, and tissue weights. Both groups of rats had the same body weight gain during the 8-week

experimental period (Table 2). Carcass fat content was significantly greater in the beef tallow diet group than in the safflower oil diet group, whereas each abdominal adipose tissue (epididymal, perirenal, and mesenteric) weight or the total weight of abdominal adipose tissues was not different between the two groups (Table 2). Carcass protein contents of the two groups were the same (Table 2). These results were similar to those reported previously.[6] IBAT, pancreas, heart, and liver weights were not different between the two dietary groups (mean values: IBAT, 0.53 g; pancreas, 1.0 g; heart, 1.1 g; liver, 13 g [both dietary groups]).

Serum glucose and insulin concentrations. Serum glucose and insulin concentrations were assayed only in rats injected with saline. The serum glucose concentration at 1 hour after the meal was significantly greater in the beef tallow diet group than in the safflower oil diet group (11.9 ± 0.5 *v* 10.0 ± 0.5 mmol/L, $P < .05$), and the insulin concentration was also greater in the beef tallow diet group (118 ± 6 *v* 94 ± 6 μU/mL, $P < .05$).

NE levels and turnover. Basal NE contents in IBAT, pancreas, and heart were significantly lower in the beef tallow diet group than in the safflower oil diet group, but contents in liver were almost the same between the two dietary groups (Fig 1). NE turnover rates in the beef tallow diet group were significantly lower in all organs (73% in IBAT, 35% in pancreas, 57% in heart, and 65% in liver) as compared with rates in the safflower oil diet group (Fig 1).

Experiment 2

Body weight, body fat, and tissue weights. The dietary regimen was initiated at age 5 weeks in both sympathectomized and control rats. The initial body weight at 5 weeks was lower in sympathectomized rats than in control rats (Table 3). During the experimental period, sympathectomized rats showed a slower growth rate as compared with control rats (Table 3). The percentage of abdominal adipose tissue was not affected by sympathectomy at either initial or final stages of the experiment (Table 3). In control

Table 2. Effects of Dietary Fats on Body Weight, Abdominal Adipose Tissue Weight, and Carcass Composition (experiment 1)

	Diet Group	
	Safflower Oil	Beef Tallow
Body weight (g)		
Initial	149 ± 1	146 ± 1
Final	422 ± 4	405 ± 4
Gain	274 ± 4	259 ± 3
Abdominal adipose tissue weight (g)	27 ± 1	29 ± 1
Carcass composition		
Weight (g)	297 ± 4	278 ± 5
Fat		
g	38 ± 2	47 ± 2*
%	13 ± 2	17 ± 2*
Protein		
g	63 ± 1	61 ± 2
%	21 ± 1	22 ± 1

NOTE. Values are the mean ± SE for 25 rats.

*Statistically significant difference ($P < .05$) *v* safflower oil diet group (Student's *t* test).

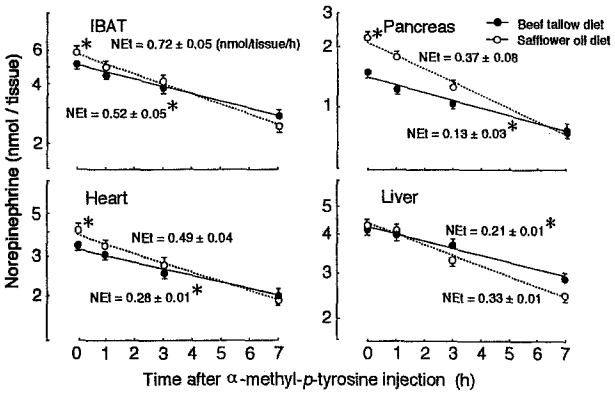


Fig 1. NE turnover rate (NEt) in IBAT, pancreas, heart, and liver of rats fed the beef tallow diet or safflower oil diet (experiment 1). NE levels were measured in saline-injected (0 hours) or α-methyl-*p*-tyrosine–injected (1, 3, and 7 hours) rats (6 to 7 at each time point). Data are the mean ± SE of NE values plotted semilogarithmically. *Statistically significant difference ($P < .05$, Student's *t* test) *v* the safflower oil diet group.

rats, the same results for carcass fat as in experiment 1 were obtained: carcass fat was greater in the beef tallow diet group than in the safflower oil diet group (Table 3). On the other hand, whereas the percentage of initial carcass fat was not different between sympathectomized and control rats, the percentage of final carcass fat was increased by sympathectomy in both dietary groups, resulting in no difference between the two dietary groups (Table 3). These results suggest that the effect of dietary fats on carcass fat content was mediated by the sympathetic nervous system. The percentage of initial or final carcass protein was not affected by either dietary fats or sympathectomy (Table 3). Weights of IBAT and pancreas at the final stage of the experiment were not affected by either dietary fats or sympathectomy (ranges of mean values: 0.48 to 0.55 g for IBAT and 0.88 to 1.11 for pancreas). Weights of heart and liver at the final stage were lower in sympathectomized rats than in control rats, but were not different between the two dietary groups (mean values: heart, 1.1 g for both dietary groups in control rats and 0.83 for both dietary groups in sympathectomized rats; liver, 13 g for both dietary groups in control rats and 9 for both dietary groups in sympathectomized rats).

Oxygen consumption. It has been reported that DIT is lower in rats fed the beef tallow diet than in rats fed the safflower oil diet, and is at least in part responsible for the greater body fat accumulation in the former.[6] Therefore, oxygen consumption before and after the meal was measured in this experiment. Preprandial oxygen consumption measured for 3 hours was not different between the two dietary groups in control rats and sympathectomized rats (Fig 2A). When measured for 3 hours after the meal, oxygen consumption of control rats was significantly lower in the beef tallow diet group than in the safflower oil diet group (Fig 2B), in agreement with results reported previously.[6] However, in sympathectomized rats, there was no difference in postprandial oxygen consumption between the two dietary groups. DIT (expressed on the basis of energy

Table 3. Effects of Dietary Fats on Body Weight, Abdominal Adipose Tissue Weight, and Carcass Composition in Sympathectomized Rats (experiment 2)

	Control		6-OHDA	
	Safflower Oil	Beef Tallow	Safflower Oil	Beef Tallow
Body weight (g)				
Initial	145 ± 3		114 ± 2†	
Final	430 ± 2	425 ± 3	326 ± 3‡	321 ± 2‡
Abdominal adipose tissue weight and percentage‖				
Initial				
g	1.13 ± 0.06		0.75 ± 0.07*	
%	0.77 ± 0.03		0.65 ± 0.05	
Final				
g	26 ± 2	27 ± 2	18 ± 2‡	20 ± 2‡
%	6.0 ± 0.5	6.2 ± 0.5	5.7 ± 0.5	6.2 ± 0.2
Carcass weight (g)				
Initial	83 ± 2		69 ± 2†	
Final	298 ± 3	291 ± 4	226 ± 2‡	222 ± 3‡
Carcass fat¶				
Initial				
g	5.6 ± 0.2		5.2 ± 0.3	
%	6.8 ± 0.2		7.7 ± 0.4	
Final				
g	34 ± 1	43 ± 1§	47 ± 2‡	46 ± 1
%	11 ± 1	15 ± 1§	21 ± 1‡	21 ± 1‡
Carcass protein				
Initial				
g	24 ± 1		19 ± 1†	
%	25 ± 1		24 ± 1	
Final				
g	66 ± 1	67 ± 2	48 ± 2‡	49 ± 1‡
%	22 ± 1	23 ± 1	22 ± 1	22 ± 1

NOTE. Values are the mean ± SE for 5 to 7 rats.

*$P < .01$, †$P < .001$: *v* control (Student's *t* test).

‡Statistically significant difference ($P < .05$) *v* control (two-way ANOVA and Scheffe's test).

§Statistically significant difference ($P < .05$) *v* safflower oil diet group.

‖Percentage of abdominal adipose tissue was calculated by dividing tissue weight by final body weight.

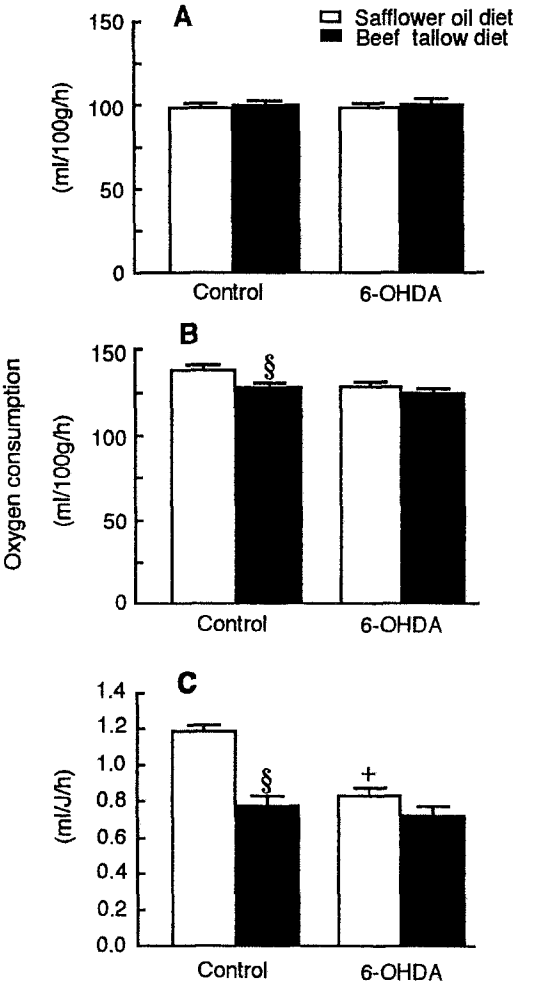
¶Carcass fat does not contain abdominal fat.

consumed) was calculated from the difference between oxygen consumption obtained before and after the meal (Fig 2C). DIT in control rats was greater in the safflower oil diet group than in the beef tallow diet group, and DIT in the safflower oil diet group was decreased by sympathectomy, resulting in no difference between the two dietary groups in sympathectomized rats. These results are compatible with the results obtained for body fat percentage (Table 3).

Serum insulin concentrations. The serum insulin concentration of control rats was significantly greater in the beef tallow diet group than in the safflower oil diet group (66 ± 4 *v* 48 ± 6 μU/mL, $P < .05$), whereas no dietary effect was observed for the concentration in sympathectomized rats (33 ± 3 *v* 32 ± 2 μU/mL).

DISCUSSION

Our previous study showed that intake of a beef tallow diet promotes body fat accumulation as compared with intake of a safflower oil diet in rats, and we suggested that this effect of a beef tallow diet is at least in part ascribed to decreased DIT.[6] Since it has been reported that the thermogenic effect of foods is mediated by the sympathetic nervous system,[8,26] NE turnover was analyzed in the present study. We clearly show here that the NE turnover rate in IBAT, a main thermogenic tissue in rats, was lower in the beef tallow diet group than in the safflower oil diet group. Because both groups of rats consumed diets with the same



ANOVA	A	B	C
Diet (D)	NS	0.01	0.01
Sympathectomy (S)	NS	0.05	0.05
DxS	NS	0.05	0.05

Fig 2. Oxygen consumption before (A) and after (B) the meal and DIT (C) in rats fed the beef tallow diet and rats fed the safflower oil diet (experiment 2). DIT was calculated by dividing the increment of whole-body oxygen consumption by energy intake. §Statistically significant difference *v* the safflower oil diet group. †Statistically significant difference *v* controls. Differences with $P < .05$ (ANOVA and Scheffe's test) were considered significant.

metabolizable energy throughout the experimental period, the difference in NE turnover rates between the two dietary groups was ascribed to the different dietary fats. NE turnover rates in liver and heart were also lower in the beef tallow diet group, and these might be related to the lower DIT in this group, because it has been suggested that tissues other than BAT are involved in thermogenesis.[27,28] The NE turnover rate in pancreas showed a great difference between the two dietary groups: the rate in the beef tallow diet group was approximately one third of that in the safflower oil diet group, in accordance with the higher serum insulin concentrations in the former than in the latter.[10,11] The higher serum insulin concentration in the beef tallow diet group might play a role in the decreased thermogenesis.[29,30]

In the sympathectomy experiment (experiment 2), the treatment almost completely abolished the effect of the beef tallow diet: body fat accumulation, DIT, and serum insulin concentration were not different between the two dietary groups in sympathectomized rats. Sympathectomized rats had lower body weights than controls and gained less weight during fat diet feeding because the former had a smaller food intake than the latter. On the other hand, sympathectomized rats had a greater percentage of final carcass fat than control rats, suggesting that the lower sympathetic activity promoted body fat accumulation. These findings strongly suggest that intake of the beef tallow diet produces greater body fat accumulation, probably due to the decreased sympathetic activity.

Mercer and Trayhurn[31] reported that energy deposition was higher and thermogenic activity of BAT was lower in both lean and genetically obese mice fed a beef tallow diet than in those fed a corn oil diet, despite isoenergetic intakes of the two diets. The results obtained in the present study are consistent with these findings, suggesting that dietary fats have effects on sympathetic activities even in genetically obese animals.

Carcass samples in this study consisted of muscles, bones, skin, and subcutaneous fat. Intake of the beef tallow diet increased carcass fats but not abdominal fats, as compared with intake of the safflower oil diet. These results were observed in a previous study[6] and confirmed in the present study, and indicate that dietary fats specifically affect carcass fat. However, sympathectomy had effects on the percentage of carcass fat but not on the percentage of abdominal fat (Table 3), suggesting that sympathetic activity may be different between carcass fat and abdominal fat. A detailed study is required to clarify the mechanism.

It has been reported that sympathetic activity is reduced in most known forms of obesity, including hypothalamic and genetic forms,[12] suggesting that the sympathetic nervous system plays an important role in the regulation of body fat accumulation. The present study demonstrated that dietary fats are modulators of sympathetic activity in relation to body fat accumulation, and suggests that saturated fats promote body fat accumulation by reducing sympathetic activity.

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