Elevation of the Mitochondrial Oxidative Activity of Rat Liver by Long-Term Intake of a High-Fat Diet Based on Beef Tallow or Safflower Oil

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Summary Using rats we examined the effects on liver mitochondrial functions of the long-term intake of a high-fat diet based on beef tallow or safflower oil. Forty-eight rats were divided into three dietary groups: a low-fat diet group and two high-fat ones (beef tallow and safflower oil). The low-fat diet provided 7, 73, and 20% of the total energy as fat, carbohydrate, and protein, respectively; and two high-fat diets, 45, 35, and 20% of energy, respectively. Rats were isoenergetically fed the appropriate diet for 4 months. The state-3 respiration of the mitochondria was significantly higher in the two high-fat diet groups than in the low-fat diet group, when both succinate and β -hydroxybutyrate were used as substrates for the respiration. However, there was no difference between the two high-fat diet groups. The groups were ranked in terms of cytochrome *a* content in their mitochondria as follows: low-fat diet group < beef tallow diet group < safflower oil diet group. The citrate synthase activity of the liver was also significantly higher in the two high-fat diet groups than in the low-fat one, but no difference was obtained between the two former. However, the mitochondrial DNA concentration in liver was not different among the three dietary groups. From these results, we conclude that long-term intake of high-fat diets increases the oxidative activity of liver mitochondria and that differences in the type of dietary fatty acids do not markedly alter mitochondrial functions.

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It is well known that the biogenesis of membrane lipids is affected by exogenous fatty acids derived from dietary fats [1]. The composition of fatty acids of membrane phospholipids is affected by dietary fats and the phospholipids create the environment for membrane proteins, suggesting that dietary fats affect the functions of organelles and cells.

Recent studies indicate that the mitochondrial function is affected by modification of membrane phospholipids with dietary fats [2–5]. Feeding rats a high-fat diet enriched with corn oil increased the contents of cytochrome *c*, *c*₁, and *aa*₃ in liver mitochondria [2] and the activity of cytochrome oxidase in heart mitochondria [3, 4]. Also, oxygen consumption of liver mitochondria was greater in rats consuming a diet enriched with safflower oil for 3–4 weeks than in the animals consuming a high-fat diet enriched with beef tallow [5]. On the other hand, Yamaoka *et al.* [3] reported that oxygen consumption in rat heart mitochondria was decreased but that mitochondrial F₀F₁-ATPase was activated by dietary sardine oil compared with dietary corn oil. These results suggest that the fatty acid composition of dietary fats affects the mitochondrial oxidative activity in rat liver and heart, but all of the experiments were limited to the evaluation of the relatively short-term effects (up to 30 days) of dietary fats.

In the present study, we examined the effects of long-term (4 months) intake of a high-fat diet enriched with safflower oil or beef tallow on the oxidative activity of liver mitochondria compared with those obtained with a low-fat control diet. Here we provide evidence suggesting that the long-term intake of either high-fat diet increases the oxidative activity of liver mitochondria compared with the intake of the low-fat control diet but that the fatty acid composition of the high-fat diet has less effect on the mitochondrial oxidative activity under the long-term feeding conditions than it does under short-term feeding.

MATERIALS AND METHODS

Animal care and experimental design. Forty-eight male Sprague-Dawley rats (4 weeks old) were obtained from CLEA Japan, Tokyo. All procedures involving animals were approved by the Experimental Animal Care Committee of the University of Tsukuba. The rats were divided into three dietary groups: a low-fat diet (control) group and two high-fat ones (beef tallow or safflower oil). The compositions of the experimental diets are shown in Table 1. Digestible energy (kJ/g), calculated as gross energy fed minus energy in feces, was 15.9, 19.2, and 20.5 for low-fat, beef tallow, and safflower oil diets, respectively. The metabolizable energy of each diet was calculated as digestive energy multiplied by 0.96: 15.3, 18.4, and 19.7, respectively. Fatty acid compositions of the beef tallow and

Table 1. Composition of the experimental diets.

Ingredient	Diet		
	Low fat	High fat	
		Beef tallow	Safflower oil
		(g/kg diet)	
Corn starch	568	278	278
Sucrose	90	112	112
Casein	182	224	224
Fat			
Safflower oil	29	—	224
Beef tallow	—	224	—
Cellulose	50	61.9	61.9
Vitamin mixture ¹	10	12.4	12.4
Mineral mixture ¹	70	86.5	86.5
L-Methionine	1	1.2	1.2
α -Tocopherol	0.25	0.25	0.25
Tert-butylhydroquinone	0.2	0.2	0.2
		(% of calorie)	
Fat	7	45	45
Carbohydrate	73	35	35
(Sucrose)	(10)	(10)	(10)
Protein	20	20	20
		(kJ/g diet)	
Digestible energy ²	15.9	19.2	20.5
Metabolizable energy ³	15.3	18.4	19.7

¹Vitamin and mineral mixtures were purchased from CLEA Japan, Tokyo.

²Digestible energy was calculated as gross energy fed minus energy in feces.

³Metabolizable energy of each diet was calculated as digestible energy multiplied by 0.96.

safflower oil used were described previously [6]. Each diet was freshly prepared every day throughout the experimental period. The animals were individually caged at $22 \pm 2^\circ\text{C}$, with light from 0700 h to 1900 h. Each group of rats was meal-fed the diet at 0800–0900 h and 2000–2100 h for 4 months. All groups of rats were offered the appropriate diet in amounts such that the three groups consumed equal metabolizable energy during the experimental period. The food consumption of rats in the high-fat groups was approximately the maximal energy amount of diet that rats in the low-fat diet group could consume under the meal-feeding conditions, because the energy density of the low-fat diet was the lowest among the experimental diets, as described above.

On the final day of the experiment, half of the rats in each diet group were killed by decapitation at 0800 h (before the meal), and the remaining ones were killed at 1000 h (after meal). Liver, heart, and soleus muscle were removed, weighed, frozen in liquid nitrogen, and stored at -50°C until used. Before the liver was frozen, mitochondria were prepared from a part of the tissue by the method of Schnaitman and Greenawalt [7].

Although rats in each diet group were killed at two time points (before and

after meal), no difference was found between the time points in the biochemical properties measured in this study. Therefore, data obtained at the two time points for the same diet group were combined.

Analyses. Phospholipids in liver mitochondria were extracted by the method of Kate *et al.* [8]. After extraction, individual phospholipids were separated by thin-layer chromatography [9], and phospholipid phosphorus was determined by the method of Chen *et al.* [10]. Fatty acid compositions of dietary fats and phospholipids in liver mitochondria were analyzed according to the method of Nelson *et al.* [11] using a gas-liquid chromatograph system (Shimadzu Corp., Kyoto, Japan, Model GC-7AG). The system was equipped with a fused silica wall-coated capillary column, 0.25 mm inner diameter $\times$ 25 m long, coated with ULBON HR20 (Shimadzu). Respiratory activity of isolated liver mitochondria was measured polarographically with an oxygen electrode (Rank Brothers, Cambridge, England) [12]. Cytochrome *a* content in liver mitochondria was measured by difference spectrum (reduced-oxidized) analyses [13]. The activity of citrate synthase (EC. 4.1.3.7) in liver, heart, soleus muscle, and isolated liver mitochondria was measured by the method of Srere [14]. For assays of the enzyme activity in tissues, the frozen tissues were powdered with a mortar and pestle cooled in liquid nitrogen, weighed, and homogenized at 4°C in 0.1 M phosphate buffer (pH 7.2) containing 1% Triton X-100 and 5 mM EDTA with 20-s bursts of a motor-driven homogenizer (Polytron). After centrifugation at 4°C for 15 min at 600 $\times$ g, the supernatant fraction was used for the assay. One unit of citrate synthase catalyzed the formation of 1 μ mol of free CoA/min. The protein concentration of these tissues and of isolated liver mitochondria was determined by the method of Smith *et al.* [15]. Mitochondrial DNA (mtDNA) in liver was determined by slot blot analysis using human cytochrome *b* cDNA as a probe, as described previously [16].

Statistical analysis. Data are presented as means $\pm$ SEM and analyzed by a one-way analysis of variance and Tukey's test. Differences with $p < 0.05$ were considered to be significant.

RESULTS

Body weight and tissue weights of rats

Body weights of the rats were similar among the three diet groups, but were slightly greater in the safflower oil diet group than in the low-fat diet group (Table 2). Weights of liver, heart, and soleus muscle were not different among the three diet groups (Table 2).

Respiratory activity of isolated liver mitochondria

The value of state-3 respiration reflects the ability of mitochondria to produce ATP. The state-3 respiration of liver mitochondria using succinate and β -hydroxybutyrate as substrates for the respiration was significantly higher in the two

Table 2. Body weight, and liver, heart, and soleus muscle weights of rats.¹

Item	Diet group		
	Low fat (n=16)	High fat	
		Beef tallow (n=16)	Safflower oil (n=16)
Body		(g)	
Initial	95.9±1.0	95.9±1.0	95.8±1.1
Final	492.4±4.7 ^a	501.5±3.6 ^{ab}	511.4±3.3 ^b
Gain	396.5±4.6 ^a	405.6±3.9 ^{ab}	415.6±3.4 ^b
Liver	14.7±0.4	14.0±0.2	13.8±0.2
Heart	1.06±0.02	1.08±0.02	1.09±0.02
Soleus muscle	0.35±0.01	0.37±0.01	0.37±0.01

¹Values are means±SEM. The values not sharing the same superscript letters are significantly different ($p < 0.05$).

Table 3. Respiration and oxidative phosphorylation of isolated liver mitochondria.¹

	Diet group		
	Low fat (n=16)	High fat	
		Beef tallow (n=16)	Safflower oil (n=16)
Succinate as a substrate			
State 3 (natoms O/min/mg protein at 30°C)	141.6±6.7 ^a	163.7±5.7 ^b	176.9±9.2 ^b
RCI	4.3±0.23	5.0±0.20	4.8±0.27
ADP/O	1.7±0.04	1.8±0.05	1.7±0.05
β -Hydroxybutyrate as a substrate			
State-3 (natoms O/min/mg protein at 30°C)	58.3±2.0 ^a	73.2±2.5 ^b	74.6±2.0 ^b
RCI	3.9±0.3	4.2±0.34	4.6±0.35
ADP/O	2.4±0.08	2.5±0.08	2.4±0.05

¹Values are means±SEM. The values not sharing the same superscript letters are significantly different ($p < 0.05$). RCI, Respiratory control index.

high-fat diet groups than in the low-fat one, but was not different between the two high-fat groups (Table 3). As also shown in Table 3, the ADP/O ratio and respiratory control index obtained for mitochondria indicated that the mitochondria used appeared to be appropriate preparations for the experiments.

Cytochrome a content in liver mitochondria

Cytochrome *a* is a redox component of the terminal part of the mitochondrial electron-transport chain. In terms of cytochrome *a* content in liver mitochondria, the groups were ranked, as follows: safflower oil diet > beef tallow diet > low-fat diet groups (Table 4).

Citrate synthase activity in liver, heart, and soleus muscle

Citrate synthase is one of the key enzymes in the mitochondrial tricarboxylic acid cycle, and its activity is used as a marker of mitochondrial oxidative activity.

The citrate synthase activity in liver tissue was significantly higher in the two high-fat diet groups than in the low-fat diet group, but no difference was observed between the two high-fat diet groups (Table 5). When the enzyme activity was measured in mitochondria isolated from liver, the same trend was observed (Table 5).

In contrast to the citrate synthase activities in liver, the activities in heart and

Table 4. Cytochrome *a* contents in liver mitochondria.¹

	Diet group		
	Low fat (<i>n</i> =16)	High fat	
		Beef tallow (<i>n</i> =16)	Safflower oil (<i>n</i> =16)
Cytochrome <i>a</i>	0.199±0.01 ^a	0.236±0.01 ^b	0.256±0.01 ^c

¹Values are means±SEM. The values not sharing the same superscript are significantly different (*p*<0.05).

Table 5. Citrate synthase activity of liver, heart, and soleus muscle.¹

Tissue	Diet group		
	Low fat (<i>n</i> =16)	High fat	
		Beef tallow (<i>n</i> =16)	Safflower oil (<i>n</i> =16)
Liver	192.4±6.5 ^a	215.8±4.7 ^b	210.9±3.7 ^b
	61.9±1.8 ^a	73.3±1.3 ^b	74.3±1.5 ^b
Heart	440.3±14.3	426.5±15.6	420.2±13.1
Soleus muscle	406.9±12.3	382.4±11.0	398.8±9.3

¹Values are means±SEM. The values not sharing the same superscript letters are significantly different (*p*<0.05).

Table 6. Phospholipid composition of rat liver mitochondria.¹

Phospholipid	Diet group		
	Low fat (<i>n</i> =16)	High fat	
		Beef tallow (<i>n</i> =16)	Safflower oil (<i>n</i> =16)
PC	67.0±3.3	69.5±2.7	62.5±1.4
PE	46.4±2.0 ^a	43.8±1.1 ^a	37.7±1.4 ^b
CL	27.6±1.5	29.2±1.1	29.9±1.4

¹Values are means±SEM. The values not sharing the same superscript letters are significantly different (*p*<0.05). PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; CL, Cardiolipin.

soleus muscle were almost the same among the three diet groups (Table 5). Therefore, further analysis was not carried out on heart or soleus muscle.

Mitochondrial DNA content in liver

Mitochondria have mtDNA, which has been regarded as a marker of mitochondrial density in tissues [16]. Slot blot analyses determined mtDNA in liver as 12.9 ± 1.0 ng/ μ g total DNA for the low-fat diet group, 12.4 ± 0.9 for the beef tallow diet group, and 11.2 ± 0.8 for the safflower oil diet group. These data indicate that the mtDNA contents in liver were not different among three diet groups, suggesting that the mitochondrial density in liver was not affected by the dietary fats.

Phospholipid content and fatty acid composition of phospholipids in liver mitochondria

The contents of major three phospholipids in liver mitochondria were almost

Table 7. Effects of the low-fat and high-fat diets on fatty acid composition of mitochondrial phosphatidylcholine, phosphatidylethanolamine, and cardiolipin.¹

Fatty acid	Diet group		
	Low fat (n = 16)	High fat	
		Beef tallow (n = 16)	Safflower oil (n = 16)
Phosphatidylcholine		(%)	
16 : 0 ²	23.5 ± 0.8^a	19.6 ± 1.4^b	19.3 ± 0.7^b
16 : 1	2.6 ± 0.2	Trace	ND ³
18 : 0	18.5 ± 0.7^a	27.3 ± 0.5^b	26.7 ± 0.5^b
18 : 1	9.2 ± 0.3^a	10.5 ± 0.2^b	3.0 ± 0.2^c
18 : 2	9.0 ± 0.3^a	4.6 ± 0.1^b	7.7 ± 0.2^c
20 : 4	31.2 ± 0.7^a	25.3 ± 0.7^b	35.6 ± 0.5^c
22 : 6	1.1 ± 0.0^a	3.8 ± 0.2^b	2.0 ± 0.3^c
Phosphatidylethanolamine			
16 : 0	18.1 ± 0.4^a	15.2 ± 0.4^b	14.3 ± 0.4^c
16 : 1	1.8 ± 0.3	Trace	ND
18 : 0	20.5 ± 0.7^a	26.3 ± 0.7^b	28.7 ± 0.5^c
18 : 1	8.9 ± 0.3^a	9.0 ± 0.6^a	4.2 ± 0.2^b
18 : 2	5.8 ± 0.7^a	3.1 ± 0.6^b	5.2 ± 0.4^a
20 : 4	34.5 ± 0.7^a	30.3 ± 0.6^b	31.7 ± 0.5^b
22 : 6	2.8 ± 0.2^a	9.0 ± 0.4^b	4.2 ± 0.1^c
Cardiolipin			
16 : 0	8.4 ± 0.9	8.6 ± 0.7	8.7 ± 0.8
16 : 1	10.2 ± 0.5	4.9 ± 0.3	Trace
18 : 0	2.6 ± 0.4	3.3 ± 0.5	2.9 ± 0.5
18 : 1	15.7 ± 0.5^a	29.4 ± 1.2^b	11.1 ± 0.4^c
18 : 2	50.0 ± 1.7^a	33.4 ± 1.3^b	61.7 ± 1.7^c
20 : 4	4.7 ± 0.7	4.8 ± 0.4	4.9 ± 0.3
22 : 6	Trace	3.5 ± 0.5	Trace

¹Values are means $\pm$ SEM. The values not sharing the same superscript letters are significantly different ($p < 0.05$). ²Number of C atoms : number of double bonds. ³ND, not detected.

the same among the three diet groups (Table 6), except that the content of phosphatidylethanolamine was slightly lower in the safflower oil diet group than in the other two diet groups.

On the other hand, the fatty acid composition of the phospholipids, especially that of cardiolipin, in liver mitochondria was greatly affected by the diets (Table 7). Characteristic features were that the content of linoleic acid (18:2) in all the phospholipids examined was less in the beef tallow diet group than in the other two diet groups and that the content of oleic acid (18:1) was greater in the beef tallow diet group than in the other two groups. These results indicate that the mitochondrial fatty acid composition of the beef tallow diet group was different from that for the other two diet groups, suggesting that the fatty acid composition of liver mitochondrial phospholipids reflects the dietary fats because the beef tallow diet contained a fat different from that of the other two diets (see Table 1).

DISCUSSION

In the present study, feeding of the high-fat diet based on beef tallow or safflower oil for 4 months increased the mitochondrial state-3 respiration rate, cytochrome *a* concentration, and citrate synthase activity in rat liver compared with the feeding of the low-fat diet. Although the fatty acid composition of liver mitochondrial phospholipids was different between the two high-fat diet groups, the mitochondrial oxidative activity based on the state-3 respiration and citrate synthase activity were similar between the two groups. These results suggest that the mitochondrial oxidative activity in liver is affected by the fat content in the diet, but not by the fatty acid composition of the dietary fats, when rats are fed the diet for a long period. The mitochondrial density estimated from the concentration of mtDNA was not altered in the liver of rats fed the high-fat diets, suggesting that heightened enzyme expression in the liver mitochondria is responsible for the elevated oxidative activity.

Abuirmeileh and Elson [5] reported that feeding rats a safflower oil diet for 3–4 weeks increased the oxygen consumption of their liver mitochondria compared with the feeding of a beef tallow diet. Also, they [2] reported that the concentrations of cytochrome *c*, *c*₁, and *aa*₃ in the liver were increased concomitantly with an increase in cytochrome oxidase activity by feeding rats a corn oil diet for 3 weeks compared with the levels obtained by feeding them a beef tallow diet. From these results, they suggested that the increase in the mitochondrial cytochrome components was responsible for the elevated oxidative capacity of liver mitochondria obtained by feeding the animals unsaturated fatty acid for a relatively short period. In contrast, no difference was observed in either the mitochondrial state-3 respiration rate or the citrate synthase activity between rats fed the safflower oil diet and those given the beef tallow diet in the present study. Only the cytochrome *a* concentration was slightly higher in the former than in the latter. Our results may be a consequence of long-term feeding of the high-fat diets; i.e., the rats may have

adapted to the diets, suggesting that it may take a longer period for rats to adapt to the beef tallow diet than to the safflower oil diet.

In summary, we provided evidence that the long-term feeding of high-fat diets based on saturated or unsaturated fatty acids increases mitochondrial oxidative activity compared with the feeding of a low-fat diet. These results suggest that fat content, but not fatty acid composition, in the diets is a modulator for the oxidative capacity of rat liver mitochondria under long-term feeding conditions.

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