

## Communications to the Editor

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## EFFECT OF HIGH CHOLESTEROL DIET ON RAT LIVER PEROXISOMAL ENZYMES

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The effect of a high cholesterol diet on rat liver peroxisomal enzymes was investigated. Chow containing 2% cholesterol was given for 14 days to rats initially weighing *ca.* 200 g. The cholesterol levels of the blood and liver were elevated and the activities of the hepatic peroxisomal fatty acyl-CoA  $\beta$ -oxidizing system (FAOS) and fatty acyl-CoA oxidase (FAO), a rate-limiting enzyme, were significantly increased (approximately 1.4- and 1.5-fold, respectively). Other groups of the rats were fed a normal diet or a 2% cholesterol diet for 7 days. Then clofibrate (40 mg) was intraperitoneally administered daily to both groups for 7 days, all the while the rats continued to receive the same diet as before. Though clofibrate treatment alone enhanced the peroxisomal FAOS and FAO activities of the liver (4.3-fold for FAOS and 13.5-fold for FAO), both activities were further increased significantly in rats on the 2% cholesterol diet (approximately further 1.4- and 1.3-fold, respectively). The high cholesterol diet failed to increase catalase and urate oxidase activities, which are not directly related to fatty acid  $\beta$ -oxidation. Cholesterol is a probable physiological substrate for liver peroxisomes, and thus the particles are considered to be induced by various substrates: not only long-chain fatty acids, but also cholesterol.

KEYWORDS—peroxisome;  $\beta$ -oxidation; hypercholesterolemia; fatty acyl CoA oxidizing system; catalase; urate oxidase

Recent work on peroxisomal fatty acid  $\beta$ -oxidation in rat liver may be summarized as follows. Liver peroxisomes have a fatty acyl-CoA  $\beta$ -oxidizing system (FAOS) which is specific for long-chain fatty acids.<sup>1,2)</sup> The FAOS activity is strongly induced by administration of clofibrate<sup>2)</sup> or phthalate plasticizers<sup>3)</sup> and by administration of long-chain fatty acids.<sup>4,6)</sup> Though the physiological significance of the peroxisomal FAOS is still not fully understood, the FAOS can metabolize cholesterol *in vitro*.<sup>7,8)</sup> That is to say, it participates in the biosynthesis of bile acids not only *in vitro*<sup>9,10)</sup> but also *in vivo*.<sup>11,12)</sup> In the present study, we investigated whether or not a high cholesterol diet enhances rat liver peroxisomal enzyme activities, since cholesterol itself<sup>7,8)</sup> or its metabolite<sup>9,10)</sup> may be a substrate for peroxisomal FAOS.

The experiments were conducted in two parts: first the effects of a high-cholesterol diet only; then the effects of that diet plus clofibrate treatment. In the first part, Wistar male rats weighing about 200 g were fed a standard chow (Clea CE-2) containing 2% cholesterol for two weeks. Control rats were kept on standard diet for the same time. In the second part, for last one week clofibrate was intraperitoneally administered to the rats daily at a dose of 40 mg. Then the blood was collected, the liver was homogenized with 0.25 M sucrose, and a large-granule fraction was prepared by centrifugation from 600 × g for 10 min to 20,000 × g for 30 min. The fraction was suspended in 0.25 M sucrose and the peroxisomal enzyme activities of the suspension were determined by the methods described in the cited references: peroxisomal FAOS,<sup>1)</sup> peroxisomal fatty acyl-CoA oxidase (FAO),<sup>3)</sup> catalase,<sup>14)</sup> and urate oxidase.<sup>15)</sup> The cholesterol contents in the serum and liver were also determined.<sup>16)</sup>

Table I shows the hepatic peroxisomal enzyme activities and the liver and serum cholesterol contents after the rats were fed on high cholesterol diet for 14 days. Serum cholesterol was slightly but significantly increased in rats given the high cholesterol diet. Liver cholesterol of the treated rats was approximately twice the control value. Peroxisomal FAOS activity of rats fed on the high cholesterol diet was increased to 1.4 times and FAO (a rate-limiting enzyme)<sup>17)</sup> was also enhanced to 1.5 times the control, while catalase activity was decreased significantly and urate oxidase was not affected.

Table I. Effect of High Cholesterol Diet on Cholesterol Content and Hepatic Peroxisomal Enzymes<sup>1)</sup>

Parameter	(Units)	Normal	Cholesterol <sup>2)</sup>
Serum			
cholesterol	(mg/dl)	72.5 ± 6.8	83.5 ± 10.8 <sup>a)</sup>
Liver			
cholesterol	(mg/g)	4.58 ± 0.43	9.05 ± 1.24 <sup>a)</sup>
FAOS	(U/g liver)	255 ± 32	360 ± 25 <sup>a)</sup>
FAO	(mU/g liver)	130.6 ± 30.1	199.0 ± 39.7 <sup>a)</sup>
Catalase	(U/g liver)	5942 ± 497	4437 ± 597 <sup>b)</sup>
Urate oxidase	(U/g liver)	2.44 ± 0.46	2.56 ± 0.30

1) Two groups of 5 rats were fed normal or 2% cholesterol diet for 14 days. Peroxisomal enzyme activities were determined using a large granule fraction of the liver. Each value is the mean ± S.E.

2) Statistically significant changes: a)  $p < 0.01$ ; b)  $p < 0.02$ .

The effect of the high cholesterol diet on the hepatic enzyme activities of the rats treated with clofibrate are shown in Table II. Administration of clofibrate to normal rats decreased the serum cholesterol level to 40%. The liver

cholesterol content was almost unaffected. The peroxisomal FAOS and FAO activities of the rats treated with clofibrate were *ca.* 4.3 and 13.5 times those observed in normal rats, respectively. Catalase activity was also elevated 1.2 times by clofibrate. On the other hand, when clofibrate was administered to the rats fed high cholesterol diet, the rats had higher FAOS and FAO levels in the liver peroxisomes (1.4- and 1.3-fold increases, respectively) than the rats treated with clofibrate alone. The proportionate increases were almost the same as those in cholesterol-treated rats over normal ones (Table I). The catalase and urate oxidase activities of liver peroxisomes of clofibrate-treated rats were unchanged by the high cholesterol diet.

Table II. Effect of High Cholesterol Diet on Peroxisomal Enzyme Activities of Rats Treated with Clofibrate<sup>1)</sup>

Parameter	(Units)	Normal	Clofibrate	Clofibrate + cholesterol <sup>2)</sup>
Serum				
cholesterol	(mg/dl)	74.5 ± 2.9	44.8 ± 2.8	59.8 ± 7.3 <sup>a)</sup>
Liver				
cholesterol	(mg/g)	4.20 ± 0.37	4.02 ± 0.45	8.75 ± 1.34 <sup>a)</sup>
FAOS	(U/g liver)	250 ± 35	1068 ± 207	1468 ± 428 <sup>b)</sup>
FAO	(mU/g liver)	120 ± 29	1626 ± 230	2130 ± 306 <sup>a)</sup>
Catalase	(U/g liver)	4456 ± 355	5391 ± 376	5617 ± 497
Urate oxidase	(U/g liver)	2.44 ± 0.16	2.08 ± 0.07	2.13 ± 0.11

- 1) Three groups of 5 rats were fed on the normal (two groups) or 2% cholesterol diet for 7 days, and then the 2% cholesterol-diet groups and one of the normal-diet groups were treated with clofibrate (40 mg) daily for 7 days. The rats continued to receive the same diets until sacrifice. Peroxisomal enzyme activities were determined using a large-granule fraction of the liver. Each value is the mean ± S.E.
- 2) Statically significant increase with respect to clofibrate alone:  
 a)  $p < 0.01$  ; b)  $p < 0.02$ .

As Tables I and II show, the high cholesterol diet activated peroxisomal FAOS activity *ca.* 1.4-fold. This enhancement of FAOS activity is in conformance with the increase of FAO activity, a rate-limiting enzyme in the system.<sup>17)</sup> The cholesterol diet did not increase the peroxisomal enzyme activities unrelated to  $\beta$ -oxidation, such as urate oxidase and catalase. Krisans *et al.*<sup>7,8)</sup> showed that cholesterol can be a substrate of liver peroxisomal  $\beta$ -oxidation. Other substrates of peroxisomal  $\beta$ -oxidation are also known to induce FAOS activity. With increasing amounts (5-25% w/w) of hydrogenated soya bean oil in the diet, for example, an increase of 1.5-fold was found in liver peroxisomal  $\beta$ -oxidation.<sup>18)</sup> Further, diet containing 20% hydrogenated marine oil produced a 4- to 6-fold increase in the oxidation. These oils contain large amounts of higher fatty acids.<sup>18)</sup> A more

direct investigation using C<sub>6</sub> - C<sub>18</sub> fatty acids with cultured hepatocytes has also been reported.<sup>19)</sup> Fatty acids of chain length C<sub>6</sub> - C<sub>8</sub> did not induce  $\beta$ -oxidation, but fatty acids with a chain length longer than C<sub>12</sub> resulted in a significant increase in peroxisomal FAO (1.5-fold). It has been reported that peroxisomal FAOS can degrade longer chain fatty acids than C<sub>12</sub>,<sup>1,17)</sup> but has little effect on C<sub>6</sub> - C<sub>8</sub> fatty acids. Therefore, it seemed reasonable to assume that when a substance which can be metabolized by peroxisomal FAOS accumulates in the blood or liver, FAOS activity is increased in response. In this experiment, when high cholesterol diet was given to the rats, the liver peroxisomal FAOS activity increased slightly but significantly, and a similar increase was also induced by the high cholesterol diet in the rats receiving the potent peroxisome proliferator, clofibrate. Therefore, cholesterol, which is known to be substrate of peroxisomes *in vitro*, may also directly or indirectly act as a substrate of the peroxisomal  $\beta$ -oxidation system *in vivo*. In other words, liver peroxisomes may be involved in the *in vivo* metabolism of cholesterol and the biosynthesis of bile acids.

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