

Activation of Catalase and Other Enzymes by Corn Oil Intake

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ABSTRACT The effects of linoleic acid intake on catalase and other enzymes were investigated by feeding 0, 1, 5 or 10% corn oil diet to rats previously fed a fat-free diet. Rats fed more than 1% corn oil for 2 weeks showed significant increases of glutathione peroxidase and superoxide dismutase in liver cytosol when compared to the controls fed no corn oil. Peroxisomal catalase activity especially was increased. The catalase activity was markedly increased also by ethyl linoleate intubation. Thus, it was demonstrated that the peroxide elimination mechanisms were activated by linoleic acid intake. The elevation of peroxisomal catalase by linoleic acid intake might be related to the hypolipidemic effect, similar to the possible relation between peroxisome induction and the hypolipidemic effect of many hypolipidemic compounds, which has been reported. *J. Nutr.* 112: 2235-2239, 1982.

INDEXING KEY WORDS linoleic acid · corn oil · glutathione peroxidase · superoxide dismutase · P-450 · catalase · peroxisomes

It is well known that the intake of a linoleic acid-rich oil such as corn oil prevents hyperlipidemia. On the other hand, the values of thiobarbituric acid-reactive substances in serum, liver and adipose tissue were increased by feeding a peroxide-free corn oil diet in a previous experiment (1). When the liver microsomes were incubated with Fe^{+3} -ADP and NADPH, the relative chemiluminescence emission in the visible region with peroxide cleavage of endogenous lipids was higher in the group fed 5 or 10% corn oil diet than in that fed 0.5% corn oil (1). This suggests that linoleic acid undergoes peroxidation physiologically to give thiobarbituric acid-reactive substances. To give an example, prostaglandins are formed also by peroxidation of arachidonic acid derived from linoleic acid. However, as the production and accumulation of lipid peroxides cause cell damage, the defense mechanisms against the products of lipid peroxidation are possibly activated simultaneously. In the present experiment, the effects of corn oil or linoleate intake on peroxide elimination were investigated.

MATERIALS AND METHODS

Male Wistar rats (Awadzu Animals, Osaka, Japan) 5 weeks old were previously fed a fat-free diet for 2 weeks, and then fed 10% fat diets containing 0, 1, 5 or 10% corn oil for another 2 weeks. All diets were adjusted to 10% fat by adding hydrogenated beef tallow. The fat-free diet contained 68.5% sucrose, 18% casein, 9.5% cellulose, 4% salt mixture (1), 0.1% choline chloride, and vitamin mixture (1). The 10% fat diets were substituted sucrose with fat. For the vitamin E requirement 40 mg DL- α -tocopherol per kilogram diet was added. The fatty acid components of corn oil were (by percent): palmitic acid, 8.5; stearic acid, 3.8; oleic acid, 40.1; linoleic acid, 46.1 and others, 1.5. The fatty acid components of hydrogenated beef tallow were (by percent): myristic acid, 5.2; palmitic acid, 33.5; stearic acid, 58.2 and others, 3.1. Freshly prepared diets were given each day in an amount sufficient for one night. In another experiment, male Wistar rats 3

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weeks old were fed the fat-free diet for 6 weeks and then orally given ethyl linoleate (0.5 g/day per 100 g) by stomach tube once a day for 6 days.

The rats were decapitated. The homogenization of livers was carefully carried out essentially according to the method of de Duve et al. (2). The livers were homogenized with 3 volumes of 0.25 M sucrose in a smooth-walled glass tube fitted with a teflon pestle. The tube, kept in an ice bucket, was given a single run upward and down against the pestle. The resulting slurry was centrifuged at $600 \times g$ for 10 minutes. The sediment was rehomogenized and centrifuged again. This operation was repeated twice. Details of fractionation to nuclear, heavy and light mitochondrial, microsomal and cytosol were the same as those described by de Duve et al. (2). To separate peroxisomes, the light mitochondrial fractions were layered on discontinuous sucrose density gradients and centrifuged (3).

The activities of superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) were assayed and expressed according to the methods of McCord et al. (4) and Aebi (5), respectively. Glutathione peroxidase (EC 1.11.1.9), NADPH-cytochrome *c* reductase (EC 1.6.2.4) and cytochrome P-450 were determined by the methods of Little et al. (6), Williams et al. (7) and Omura et al. (8), respectively. The data are expressed as nanomoles of NADPH oxidized to NADP per minute per milligram cytosolic protein for glutathione peroxidase and nanomoles reduced cytochrome *c* per minute per milligram microsomal protein for NADPH-cytochrome *c* reductase. P-450 was defined and expressed according to Omura et al. (8). The activity of urate oxidase (EC 1.7.3.3) was determined according to the procedure of Leighton et al. (9) and expressed as nanomoles oxidized urate per minute per milligram peroxisomal protein.

Liver lipids were extracted and separated to obtain total phospholipids by thin-layer chromatography as described previously (10). After saponification of the phospholipids with 10% ethanolic KOH at 60° for 1 hour, the aqueous phase was washed with petroleum ether and acidified, and then fatty acids were extracted with petroleum ether. The

fatty acids were methylated with diazomethane and injected on a Hitachi K-53 gas chromatograph (Hitachi Co., Tokyo, Japan) equipped with a hydrogen flame detector. A column of diethyleneglycol succinate, 10% on chromosorb W (column packing Shimadzu, Kyoto), 80–100 mesh and packed in a tube 2 m long, was maintained at 180° with a nitrogen flow.

RESULTS AND DISCUSSION

Rats previously fed a fat-free diet for 2 weeks were used to demonstrate the effects of corn oil intake. After feeding the experimental diets containing 0–10% corn oil for another 2 weeks, the animals were killed; catalase and other enzymes were measured. Table 1 shows the fatty acid compositions (in percent) of liver phospholipids. The total phospholipid concentrations per milligram protein of liver were not significantly different among all the groups; 107 ± 9.0 , 109 ± 7.7 , 114 ± 3.5 and 107 ± 6.6 (nmol/mg, mean \pm SD of 4 rats) for the groups fed 10% fat diets containing 0, 1, 5 or 10% corn oil, respectively. Therefore, the relative concentrations of each fatty acid per milligram protein would be parallel to the fatty acid compositions (in percent) among the groups. In the rats fed no corn oil, phospholipid linoleic acid and arachidonic acid are remarkably decreased as compared to those of rats fed 1, 5 or 10% corn oil diet, and eicosatrienoic acid appears in place of the fatty acids. Holman reported that a triene:tetraene ratio of approximately 0.4 is the minimum requirement of linoleic acid (1% of calories) (11). The 1.88 ratio of liver phospholipids in the rats not fed corn oil (shown in table 1) may indicate essential fatty acid deficiency. For these rats, however, the body weights were not significantly different from those of rats fed corn oil, and no symptoms of essential fatty acid deficiency were observed.

The activities of enzymes related to peroxidation in liver are shown in table 2. Glutathione peroxidase and superoxide dismutase in liver cytosol fraction were increased by corn oil feeding. Cytochrome P-450 in liver microsomes also was increased. The specific activities of glutathione peroxidase, superoxide dismutase and P-450 were significantly elevated by 1% corn oil diet as com-

TABLE 1
Effect of corn oil feeding on phospholipid fatty acid in rat liver^{1,2}

Dietary corn oil	16:0	16:1	18:0	18:1	18:2	20:3	20:4
	%						
0	24.7 ± 3.1	8.83 ± 2.2	19.4 ± 1.8	22.3 ± 3.0	4.71 ± 1.4	13.0 ± 2.0	6.92 ± 1.6
1	23.1 ± 2.1	4.79 ± 1.6 ^a	24.3 ± 1.4 ^b	18.1 ± 3.2	6.80 ± 1.6	1.90 ± 0.5 ^b	17.3 ± 2.8 ^b
5	25.7 ± 1.6	3.22 ± 0.9 ^b	25.0 ± 1.8 ^b	14.8 ± 0.4 ^b	10.4 ± 1.3 ^b		21.1 ± 2.6 ^b
10	24.6 ± 1.6	2.01 ± 0.4 ^{bc}	27.4 ± 2.0 ^{bc}	11.1 ± 0.7 ^{bd}	14.9 ± 0.3 ^{bd}		19.8 ± 2.2 ^b

¹ Male Wistar rats, 5 weeks old, were fed a fat-free diet for 2 weeks and separated into four groups, then fed 10% fat diets containing 0, 1, 5 or 10% corn oil, respectively, for 2 weeks. All diets were adjusted to 10% fat by addition of hydrogenated beef tallow. Minor components of phospholipid fatty acid are not shown. Results are means ± SD ($n = 6-8$). ² Significantly different from group fed no corn oil: ^a $P < 0.01$; ^b $P < 0.001$. Significantly different from group fed 1% corn oil diet: ^c $P < 0.02$; ^d $P < 0.001$.

pared to those of rats fed no corn oil and not much further elevated by increasing the amount of dietary corn oil. NADPH-cytochrome *c* reductase was not changed by corn oil feeding.

The specific activities of catalase in both peroxisomal and cytosol fractions of liver were markedly increased by corn oil feeding, as shown in table 3. The peroxisomal marker enzyme, urate oxidase was rather increased. The catalase activity found in the cytosol fraction might be partially released from peroxisomes. Even if it were, since urate oxidase activity was not detectable in the cytosol fraction, only a little catalase would be released. The catalase activities of peroxisomal and cytosol fractions were still lower in the fat-free diet group (fed a fat-free diet for

4 weeks) than in the group fed hydrogenated fat. Total units of catalase and urate oxidase activities in all subcellular fractions of liver are shown in table 4. The total units of catalase in the cellular fractions was also higher in the group 10% fed corn oil than in the group fed 10% hydrogenated fat, whereas the total units of urate oxidase was slightly higher only in the light mitochondrial fraction.

Also when rats were fed ethyl linoleate by stomach tube instead of dietary corn oil, the specific activities of catalase in peroxisomal and cytosol fractions were markedly increased, as shown in table 5. It is demonstrated that catalase is induced by linoleate intake. However, peroxisomal urate oxidase was not increased by the linoleate intubation. The results of tables 3, 4 and 5 confirm that

TABLE 2
Effect of corn oil feeding on enzymes in rat liver^{1,2}

Dietary corn oil	Glutathione peroxidase	Superoxide dismutase	NADPH-cytochrome <i>c</i> reductase	P-450
	<i>nmol/(min · mg)</i>	<i>μmol/(min · mg)</i>	<i>nmol/(min · mg)</i>	<i>nmol/mg</i>
0	224 ± 20.9	23.0 ± 2.08	10.7 ± 1.29	0.78 ± 0.03
1	342 ± 30.0 ^a	32.6 ± 4.50 ^a	11.7 ± 1.53	1.20 ± 0.14 ^a
5	404 ± 24.0 ^{ac}	52.0 ± 5.67 ^{ad}	9.75 ± 0.82	1.05 ± 0.07 ^a
10	361 ± 51.5 ^a	51.0 ± 9.13 ^a	12.5 ± 2.30	1.03 ± 0.13 ^b

¹ The rats described in the legend of table 1 were killed and livers removed. The liver homogenates were fractionated into microsomes and cytosol to measure (in milligrams protein of cytosol) glutathione peroxidase and superoxide dismutase and to measure (in milligrams protein of microsomes) NADPH-cytochrome *c* reductase and P-450. Results are means ± SD ($n = 6-8$). ² Significantly different from group fed no corn oil: ^a $P < 0.001$; ^b $P < 0.01$. Significantly different from group fed 1% corn oil diet: ^c $P < 0.01$; ^d $P < 0.001$.

TABLE 3
Effect of corn oil feeding on activity of catalase and urate oxidase

Dietary fat ¹	Catalase	Urate oxidase
	unit/mg	munitt/mg
<i>Peroxisomal fraction</i>		
10% hydrogenated fat	1.32 ± 0.25 ^{2,3,a}	66.3 ± 8.02
10% corn oil	2.70 ± 0.66 ^{bc}	79.8 ± 6.81 ^d
Fat free	0.94 ± 0.07	67.3 ± 7.10
<i>Cytosol fraction</i>		
10% hydrogenated fat	0.20 ± 0.01	Not detected
10% corn oil	0.41 ± 0.03 ^{bc}	Not detected
Fat free	0.18 ± 0.01	Not detected

¹ See the footnotes to table 1 for explanation of 10% diets. Another group of rats were fed a fat-free diet instead. ² Means ± SD (n = 6-8). ³ Significantly different from group fed fat-free diet: * P < 0.01; ^b P < 0.001. Significantly different from group fed 10% hydrogenated fat: ^c P < 0.001; ^d P < 0.02.

liver catalase activity is increased by linoleate feeding. Catalase may be specifically induced in liver peroxisomes.

The effects of linoleate intake on glutathione peroxidase, superoxide dismutase, P-450 and catalase were clear when compared

to controls (not fed linoleate). When rats are fed corn oil in the diet, linoleic acid seems to undergo lipid peroxidation (1) and simultaneously to activate the defense mechanism for lipid peroxidation. The peroxide elimination mechanisms are possibly activated by linoleate intake. Chow et al. (12) reported that the glutathione peroxidase increased in the adipose tissues (but not in the liver) of animals fed 15.7% tocopherol-stripped corn oil diet, as compared to the animals fed the corn oil diet with α -tocopherol. This also suggests that lipid peroxidation occurred in the adipose tissues of animals fed corn oil, and the tissues possess the ability to respond to oxidative stress by increasing the activity of glutathione peroxidase. In the present experiment, this is also the case in liver, when compared to controls (not fed linoleate).

A potent hypolipidemic drug, clofibrate (ethyl- α -p-chloro-phenoxyisobutyrate) and a number of potent hypolipidemic analogues of clofibrate were shown to induce catalase activity and peroxisome proliferation in liver cells (13-16). It has been suggested that the hypolipidemic and peroxisome proliferative effects are two common properties of some potent hypolipidemic drugs (13-19), although there is conflicting discussion (20). Similarly, the hypolipidemic effects of linoleic acid-rich oil such as corn oil are well

TABLE 4
Effect of corn oil feeding on total activities of catalase and urate oxidase in subcellular fractions of rat liver^{1,2}

Dietary fat	Subcellular fraction				
	Nuclei	Heavy mitochondria	Light mitochondria	Microsomes	Cytosol
<i>untt/g liver</i>					
<i>Catalase</i>					
10% hydrogenated fat	6.26 ± 0.55	3.13 ± 0.90	7.58 ± 0.94	0.91 ± 0.28	7.44 ± 0.77
10% corn oil	7.70 ± 0.97	5.12 ± 0.37 ^a	13.5 ± 1.05 ^b	1.51 ± 0.38	13.1 ± 1.15 ^b
<i>Urate oxidase</i>					
10% hydrogenated fat	0.47 ± 0.07	0.64 ± 0.15	0.93 ± 0.10	0.23 ± 0.07	Not detected
10% corn oil	0.47 ± 0.04	0.57 ± 0.09	1.28 ± 0.10 ^c	0.29 ± 0.07	Not detected

¹ The livers of the groups fed the 10% hydrogenated fat diet and the 10% corn oil diet were fractionated according to de Duve et al. (2). Results are means ± SD (n = 3). ² Significantly different from hydrogenated fat group: * P < 0.05; ^b P < 0.01; ^c P < 0.02.

TABLE 5
Effect of linoleate intubation on catalase^{1,2}

Intubation	Catalase	Urate oxidase
	unit/mg	munit/mg
<i>Peroxisomal fraction</i>		
None	1.32 ± 0.20	80.6 ± 0.20
Linoleate	2.46 ± 0.13*	85.5 ± 3.27
<i>Cytosol fraction</i>		
None	0.25 ± 0.04	Not detected
Linoleate	0.38 ± 0.01*	Not detected

¹ After male Wistar rats, 3 weeks old, were fed a fat-free diet for 6 weeks, ethyl linoleate (0.5 g/day per 100 g) was given to them by stomach tube once a day for 6 days. Results are means ± SD ($n = 3$). ² Significantly different from no intubation of linoleate: * $P < 0.01$.

known and the induction of peroxisomal catalase activity by linoleate intake has been demonstrated in the present experiment. Therefore, it may be reasonable to suggest that peroxisomal catalase induction by feeding linoleic acid is closely related to the hypolipidemic effect.

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