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Supplementation of orotic acid to the casein, but not to egg protein, soy protein, or wheat gluten diets, increases serum ornithine carbamoyltransferase activity

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Effects of dietary supplementation of orotic acid to a diet containing the casein protein were compared with diets containing egg protein, soy protein, or wheat gluten on lipid levels in the liver and serum and activities of ornithine carbamoyltransferase (OCT) and alanine aminotransferase in the serum of rats. We found that supplementation of orotic acid to each diet increased the contents of the liver total lipids, triacylglycerol, and phospholipids compared with those not supplemented. The contents of liver total lipids, triacylglycerol, cholesterol, and phospholipids in rats fed the casein diet were significantly higher than those of rats fed the other three diets when orotic acid was supplemented. The levels of triacylglycerol, cholesterol, and phospholipids in the serum of rats fed the casein diet were markedly decreased by addition of orotic acid. The supplementation of orotic acid significantly increased the activities of both serum OCT and alanine aminotransferase in rats fed the casein diet, but not in rats fed the other diets. In conclusion, liver lipid accumulation induced by dietary orotic acid depends on the type of dietary protein. The enhancement of serum OCT activity may result from liver lipid accumulation in rats fed the casein diet supplemented with orotic acid, demonstrating hepatic damage. (J. Nutr. Biochem. 11:306–310, 2000)

Keywords: dietary casein; orotic acid; fatty liver; serum lipids; serum ornithine carbamoyltransferase

Introduction

The content of liver lipids is changed by certain dietary conditions. For example, a severe fatty liver develops in rats fed a choline-deficient diet,[1,2] an orotic acid diet,[3–5] or a protein repletion diet containing fructose following a protein-free diet feeding.[6,7] As demonstrated in our previous studies, the activities of both ornithine carbamoyltransferase (OCT) and alanine aminotransferase in serum significantly increased in rats fed either a choline-deficient or an orotic acid diet.[8,9] However, these serum enzymatic activities did not change in rats fed a protein repletion diet containing fructose although the liver lipid levels were similar among these three types of fatty liver.[8] Thus, addressing an issue concerning activities of the enzymes may help us gain an insight into the pathologic mechanisms underlying fatty liver formation as well as liver damage induced by dietary orotic acid.

Casein has generally been used as a sole dietary protein source in the formation of fatty liver in rats fed an orotic acid diet.[3–5] This study was designed to compare supplementation of orotic acid to the diet containing casein with other diets containing egg protein, soy protein, or wheat gluten to study the differences in combinations of orotic acid diets on liver lipid levels and serum OCT and alanine aminotransferase activities.

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Table 1 Composition of the diets

	Casein (g/kg)	Egg protein (g/kg)	Soy protein (g/kg)	Wheat gluten (g/kg)
Casein*	249.2			
Egg protein†		261.1		
Soy protein‡			249.7	
Wheat gluten§				282.1
Vitamin mixture (AIN-93G)‖	10	10	10	10
Choline bitartrate**	2.5	2.5	2.5	2.5
Mineral mixture (AIN-93G)‖	35	35	35	35
Soybean oil††	10	10	10	10
Sucrose‡‡	693.3	681.4	692.8	660.4

*New Zealand Dairy Board, Wellington, New Zealand.
†Taiyo Kagaku Co., Ltd., Yokkaichi, Mie, Japan.
‡Fuji Oil Co., Ltd., Izumisano, Osaka, Japan.
§Nisshin Flour Milling Co., Ltd., Tokyo, Japan.
‖Reeves, P.H., Nielsen, F.H., and Fathy, G.C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123,** 1939–1951.
**Katayama Chemical Industries Co., Ltd., Osaka, Japan.
††Wako Pure Chemical Industries, Ltd., Osaka, Japan.
‡‡Nippon Beet Sugar MGF., Co., Ltd., Obihiro, Hokkaido, Japan.

Methods and materials

Animals

This study complied with the Animal Experimental Guides according to the Committee of Experimental Animal Care, Hokkaido University. Male Wistar rats (Japan SLC, Inc., Hamamatsu, Shizuoka, Japan), weighing approximately 95 g as the initial body weight were housed individually in stainless-steel wire-bottomed cages in an air-conditioned room, kept at a temperature of approximately 23°C, with a 12-hr cycle of light (8:00 AM–8:00 PM) and dark (8:00 PM–8:00 AM).

Diets

The composition of the diets is shown in *Table 1*. Casein (802 g as crude protein per kilogram; New Zealand Dairy Board, Wellington, New Zealand), egg protein (766 g as crude protein per kilogram; Taiyo Kagaku Co., Ltd., Yokkaichi, Mie, Japan), soy protein (801 g as crude protein per kilogram; Fuji Oil Co., Ltd., Izumisano, Osaka, Japan), and wheat gluten (709 g as crude protein per kilogram; Nisshin Flour Milling Co., Ltd., Tokyo, Japan) were used as dietary protein sources and supplemented by the amount of 200 g of protein (N × 6.25) per kilogram to the diet. Each of the four diets was then supplemented with or without orotic acid (10 g/kg diet; Wako Pure Industries, Ltd., Osaka, Japan). Sucrose was used to compensate for the dietary changes in the content of proteins and orotic acid.

Rats were allowed free access to food and water for 14 days, after which they were sacrificed by guillotine between 9:30 and 10:30 AM. The livers were immediately harvested and weighed. The blood was collected and allowed to clot at room temperature. Serum was obtained by centrifugation (3,000 × g, 10 min). Samples of the liver and serum were stored at −80°C until analysis.

Analyses

Liver total lipids, extracted and purified according to the method of Folch et al.,[10] were gravimetrically estimated after removing the solvent. Liver triacylglycerol[11] and cholesterol[12] were measured by the enzymatic methods, and the content of phospholipids in the liver was calculated by subtracting triacylglycerol and cholesterol from total lipids. Serum triacylglycerol[11] and cholesterol[12] were similarly determined. Serum phospholipids were measured by the enzymatic method.[13] For calculation of the concentrations of triacylglycerol and phospholipids in liver and serum, 885.4 for triolein and 786.1 for L-α-phosphatidylcholine dioleoyl were used as the molecular weights of triacylglycerol and phospholipids, respectively. Activities of serum OCT (EC 2.1.3.3)[14] and alanine aminotransferase (EC 2.6.1.2.)[15] were measured as described previously.

Statistical methods

Data were subjected to Student's *t*-test[16] and Duncan's multiple range test.[17] A *P*-value of less than 0.05 was considered significant.

Results

Food intake

Food intake was similar among rats fed the casein, egg protein, and soy protein diets. Supplementation of orotic acid to the casein, egg protein, and soy protein diets did not affect food intake. However, food consumption was significantly lower in rats fed the wheat gluten diet than in the other three groups. Supplementation of orotic acid to the wheat gluten diet further decreased the content (*Table 2*).

Body weight gain

There was no significant difference in body weight gain among rats fed the casein, egg protein, and soy protein diets, whereas it was markedly decreased in rats fed the wheat gluten diet compared with those fed the other three diets. Body weight gain in rats fed the egg protein diet was higher than in those fed the other three diets when orotic acid was supplemented. Supplementation of orotic acid to the egg protein, soy protein, and wheat gluten diets did not change the body weight gain. However, it was significantly decreased in rats fed the casein diet supplemented with orotic acid compared with that of the unsupplemented casein diet (*Table 2*).

Liver weight

Without supplementation of orotic acid, liver weight in the casein, egg protein, and soy protein groups was significantly increased compared with that in rats fed the wheat gluten diet. When orotic acid was supplemented, liver weights of rats fed the egg and soy protein diets was lower than those of rats fed the casein diet and higher than those of animals fed the wheat gluten diet. Supplementation of orotic acid in each of four diets markedly increased liver weight compared with the unsupplemented diets (*Table 2*).

Liver lipids

The contents of total lipids in the unsupplemented diets were 52.1 ± 1.1, 46.3 ± 1.8, 39.7 ± 1.2, and 50.6 ± 2.0 mg/g of liver for the casein, egg protein, soy protein, and wheat gluten diets, respectively. When orotic acid was supplemented these values were 308 ± 12, 112 ± 14,

Table 2 Food intake, body weight gain, and liver weight

	Orotic acid added (g/kg)	Casein	Egg protein	Soy protein	Wheat gluten
Initial body weight (g)	0	94.9 ± 2.2	94.9 ± 2.6	94.8 ± 1.8	94.9 ± 0.9
	10	94.9 ± 2.2	94.9 ± 1.6	94.9 ± 1.3	94.8 ± 0.9
Food intake (g/14 days)	0	200 ± 6[a]	204 ± 5[a]	219 ± 9[a]	143 ± 4[b]
	10	192 ± 7[e]	201 ± 5[e]	201 ± 8[e]	128 ± 5[f]
P		NS	NS	NS	<0.05
Body weight gain (g/14 days)	0	95.0 ± 4.0[a]	90.5 ± 2.4[a]	86.8 ± 5.1[a]	24.1 ± 2.1[b]
	10	75.7 ± 2.0[e]	89.8 ± 1.2[d]	78.8 ± 5.0[e]	21.3 ± 2.6[f]
P		<0.01	NS	NS	NS
Liver weight (g/kg body wt)	0	57.2 ± 1.5[a]	55.4 ± 2.1[a]	55.2 ± 2.5[a]	40.0 ± 1.4[b]
	10	87.0 ± 2.1[d]	68.5 ± 1.1[e]	70.1 ± 1.1[e]	58.0 ± 3.2[f]
P		<0.001	<0.001	<0.001	<0.001

Data represent means ± SEM for six rats.
[a,b,c,d,e,f]Means within the same horizontal column that do not share a common superscript letter were significantly different: $P < 0.05$.
NS–not significant.

145 ± 17, and 174 ± 35 mg/g of liver for the casein, egg protein, soy protein, and wheat gluten diets, respectively. In rats fed the diet without supplementation of orotic acid, the content of liver total lipids in the casein group was significantly higher than the content of the egg and soy protein groups and similar to that in the wheat gluten group. The values in the egg protein group were significantly higher than were those in the soy protein group. In rats fed the diets supplemented with orotic acid, the content of liver total lipids in the casein group was significantly higher than those in the egg protein, soy protein, or wheat gluten groups, the values of which were similar. The supplementation of orotic acid to each of four diets increased the content of liver total lipids.

In rats fed the diet without supplementation of orotic acid, the content of triacylglycerol in rats fed the egg protein diet was lower than that in rats fed the casein diet, similar to that in rats fed the wheat gluten diet, and higher than that in rats fed the soy protein diet. The content of phospholipids in rats fed the wheat gluten diet was higher than those in rats fed the egg protein and soy protein diets, the values of which were similar. Cholesterol contents among four groups were similar (*Table 3*).

Supplementation of orotic acid to each of the four diets increased several-fold the liver total lipid, triacylglycerol, and phospholipid contents, whereas liver cholesterol slightly increased in rats fed the casein and soy protein diets. Furthermore, there was no significant difference in liver cholesterol content with and without supplementation of orotic acid in rats fed the egg protein and wheat gluten diets. When orotic acid was supplemented, the contents of total lipids and three each lipid (triacylglycerol, cholesterol, and phospholipids) were significantly higher in rats fed the casein diet than in those fed other three diets (*Table 3*).

Serum lipids

The levels of serum triacylglycerol, cholesterol, or phospholipids were similar to, higher than, or higher than ($P < 0.1$) those in rats fed the casein diet compared with those fed other three diets without supplementation of orotic acid. However, supplementation of orotic acid in rats fed the casein diets resulted in a profound reduction of all three serum lipid levels, which were significantly lower than those in rats fed the egg protein, soy protein, and wheat gluten diets, although supplementation also decreased se-

Table 3 Liver lipids

Liver lipids	Orotic acid added (g/kg)	Casein	Egg protein	Soy protein	Wheat gluten
Triacylglycerol (μmol/g)	0	18.1 ± 1.4[a]	14.6 ± 1.2[b]	8.6 ± 0.5[c]	12.9 ± 0.7[b]
	10	124 ± 7[d]	45.4 ± 5.6[e,f]	62.1 ± 6.1[e]	40.3 ± 8.4[f]
P		<0.001	<0.001	<0.001	<0.01
Cholesterol (μmol/g)	0	5.05 ± 0.41[a]	4.95 ± 0.13[a]	4.33 ± 0.23[a]	5.08 ± 0.47[a]
	10	10.2 ± 0.4[e]	6.01 ± 0.57[f]	7.54 ± 0.26[f]	6.45 ± 0.77[f]
P		<0.001	NS	<0.001	NS
Phospholipids (μmol/g)	0	43.4 ± 2.2[a,b]	40.0 ± 1.0[b]	38.7 ± 1.4[b]	47.2 ± 3.0[a]
	10	247 ± 14[d]	88.1 ± 12.7[f]	110 ± 16[e,f]	171 ± 34[e]
P		<0.001	<0.01	<0.001	<0.01

Data represent means ± SEM for six rats.
[a,b,c,d,e,f]Means within the same horizontal column that do not share a common superscript letter were significantly different: $P < 0.05$.
NS–not significant.

Table 4 Serum lipids

Serum lipids	Orotic acid added (g/kg)	Casein	Egg protein	Soy protein	Wheat gluten
Triacylglycerol (mmol/L)	0	1.14 ± 0.14[a]	1.19 ± 0.16[a]	0.90 ± 0.10[a]	0.52 ± 0.07[b]
	10	0.34 ± 0.06*	1.06 ± 0.12[d]	0.54 ± 0.10[e,f]	0.86 ± 0.16[d,e]
P		<0.001	NS	<0.05	NS
Cholesterol (mmol/L)	0	2.90 ± 0.13[a]	2.51 ± 0.10[b]	2.09 ± 0.16[c]	2.35 ± 0.10[b,c]
	10	0.83 ± 0.10[f]	1.91 ± 0.08[d]	1.32 ± 0.13[e]	1.47 ± 0.16[e]
P		<0.001	<0.001	<0.01	<0.001
Phospholipids (mmol/L)	0	2.61 ± 0.10[a]	2.42 ± 0.08[a,b]	2.13 ± 0.10[b]	2.25 ± 0.11[b]
	10	0.88 ± 0.5[f]	1.78 ± 0.08[d]	1.32 ± 0.14[e]	1.55 ± 0.16[d,e]
P		<0.001	<0.001	<0.001	<0.01

Data represent means ± SEM for six rats.
[a,b,c,d,e,f]Means within the same horizontal column that do not share a common superscript letter were significantly different: $P < 0.05$.
NS–not significant.

rum lipid levels except for the values of triacylglycerol in rats fed the egg protein or wheat gluten diets (*Table 4*).

Serum enzyme activities

The activities of serum OCT and alanine aminotransferase were roughly similar among rats fed four (or three) different diets without supplementation of orotic acid. Supplementation of orotic acid to the egg protein, soy protein, and wheat gluten diets did not change these activities. However, the casein diet supplemented with orotic acid caused a marked increase in the activities of these two serum enzymes, which were significantly higher than those in rats fed other three (or two) diets (*Table 5*).

Discussion

The present study reconfirmed that dietary supplementation of orotic acid, a common component in milk,[18,19] caused lipid accumulation in the liver.[3–5] The content of liver total lipids was markedly higher in rats fed the casein diet compared with those fed the egg protein, soy protein, and wheat gluten diets when supplemented with orotic acid. Similarly, liver triacylglycerol, cholesterol, and phospholipid levels were highest in rats fed the casein diet supplemented with orotic acid (*Table 3*). It seems unlikely that such differences resulted from food consumption because the food intake was similar among the casein, egg protein, and soy protein groups (*Table 2*). On the other hand, lipid accumulation in the liver due to supplementation of orotic acid depended on the types of dietary proteins (*Table 3*). The cystine, arginine, and glycine levels were markedly lower in rats fed the casein diet than in those fed the egg protein and soy protein diets,[20] suggesting that a difference in the amino acid components, possibly lowering these three amino acids, might account for the profound lipid accumulation in the liver induced by orotic acid.

Decrease in transportation of triacylglycerol by the very low density lipoproteins from the liver to the serum is one of the reasons explaining lipid accumulation in the liver induced by dietary orotic acid.[21,22] In fact, when supplemented with orotic acid, the serum triacylglycerol level was significantly decreased by 70% in rats fed the casein diet, whereas it decreased by only 40% in rats fed the soy protein diet (*Table 4*). Supplementation of orotic acid to the egg protein and wheat gluten diets did not change the serum triacylglycerol levels. In addition, when supplemented with orotic acid, the serum cholesterol and phospholipid concentrations were reduced by 71% and 67%, respectively, in rats fed the casein diet, whereas the levels of these two lipids decreased by only 24% and 26% in the egg protein diet

Table 5 Serum ornithine carbamoyltransferase and alanine aminotransferase activities

Serum enzyme	Orotic acid added (g/kg)	Casein	Egg protein	Soy protein	Wheat gluten
Ornithine carbamoyltransferase (IU/L)					
	0	1.79 ± 0.10[a,b]	2.11 ± 0.43[a,b]	1.46 ± 0.40[b]	2.66 ± 0.33[a]
	10	16.5 ± 2.1[c]	3.88 ± 0.71[d]	2.40 ± 0.22[d]	4.02 ± 0.60[d]
P		<0.001	NS	NS	NS
Alanine aminotransferase (U/L)					
	0	9.90 ± 0.65[b]	12.2 ± 0.7[a]	11.7 ± 0.4[a,b]	ND
	10	28.3 ± 4.4[c]	13.6 ± 1.1[d]	10.2 ± 0.8[d]	ND
		<0.01	NS	NS	

Data represent means ± SEM for six rats.
[a,b,c,d]Means within the same horizontal column that do not share a common superscript letter were significantly different: $P < 0.05$.
NS–not significant.
ND–not determined.

feeding group, 37% and 38% in the soy protein diet feeding group, and 37% and 31% in the wheat gluten feeding group, demonstrating that the combination of the casein diet with orotic acid resulted in the lowest serum lipid levels (*Table 4*), but the highest liver lipid accumulation (*Table 3*). Furthermore, dietary casein increased the levels of free fatty acids in serum compared with dietary soy protein,[23] allowing triacylglycerols in the liver of rats fed the casein diet to accumulate. In the present experiment, serum free fatty acid levels were not estimated. If serum free fatty acid levels in rats fed the casein diet supplemented with orotic acid were higher, this might be one of the reasons for explaining that the casein diet supplemented with orotic acid caused a marked increase in the content of triacylglycerols in the liver compared with the soy protein diet supplemented with orotic acid.

The increase in the activity of serum alanine aminotransferase is one of the signs of liver damage.[15] However, this enzyme is widely distributed not only in the liver, but also in the extrahepatic organs. On the other hand, OCT is specifically localized to the liver.[24] Therefore, estimating the activity of the latter enzyme in the serum is considered to be more appropriate in demonstrating liver damage. When supplemented with orotic acid, the activities of both serum OCT and alanine aminotransferase increased in rats fed the casein diet, whereas there were no significant increases of the serum enzymatic activities in rats fed other two or three diets, indicating that casein plays a role in aggravating liver damage.

In conclusion, although supplementation of orotic acid to each of four diets caused the accumulation of liver lipids in rats, supplementation of orotic acid to the casein diet, but not to the egg protein, soy protein, and wheat gluten diets, led to liver damage because there were no significant differences in serum enzyme activities among rats fed the supplemented and unsupplemented egg protein, soy protein, and wheat gluten diets. Therefore, it is assumed that after exceeding a critical amount of liver lipids, liver damage may suddenly occur followed by the increase in serum enzyme activities.

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