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Hypocholesterolemic Effects of Eicosapentaenoic Acid, Phospholipids, and Phytosterols in Rats*1

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Abstract

The feeding trials were conducted to examine the effects of eicosapentaenoic acid methylester (EPA-ME), β-sitosterol, fucosterol, *Tapes* PL(phospholipids of a short-necked clam), soybean lecithin, and chicken-egg lecithin on cholesterol levels of the serum and liver in rats. EPA-ME, β-sitosterol, and fucosterol suppressed the elevation of cholesterol levels in both the serum and liver. However, the simultaneous addition of EPA-ME and either β-sitosterol or fucosterol did not increase the hypocholesterolomic effects on the serum and liver. *Tapes* PL, soybean lecithin, and chicken-egg lecithin elevated the serum cholesterol level markedly. On the other hand, *Tapes* PL suppressed the increase in liver-cholesterol levels in contrast with soybean lecithin and chicken-egg lecithin.

Previously, we have demonstrated that eicosapentaenoic acid methylester (EPA-ME)*5 lowered cholesterol levels of both the serum and liver of rats[1]. On the other hand, phytosterols such as β-sitosterol[2,3] and fucosterol[4,5] have been known to have a hypocholesterolemic activity. Therefore, the present study was planned to examine the effects of β-sitosterol and fucosterol on the lowering of cholesterol level by EPA-ME in rats.

In marine fish and invertebrate lipids, EPA is generally present more abundantly as the constituents of polar lipids than those of neutral lipids[6]. This gives us an interest in whether phospholipids (PL) rich in highly unsaturated fatty acid (HUFA) are effective in lowering cholesterol levels in rats. Therefore, the feeding experiments were carried out to resolve the above question. Phospholipids (*Tapes* PL) were isolated from a short-necked clam, *Tapes philippinarum,* and their hypocholesterolemic effect was compared with those of EPA-ME, soybean lecithin, and chicken-egg lecithin. This paper deals with these results and discussion.

Materials and Methods

EPA-ME, Phytosterols, and Phospholipids Used

EPA-ME was prepared from a squid-liver oil by reversed phase high performance liquid chromatography on Bondapak C_{18}/Corasil (50-100 μm) as described previously[7]. The purity of EPA-ME was about 95% by gas-liquid chromatography (GLC) on 10% DEGS[8]. Soybean lecithin

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*5 The IUPAC Rule recommended the use of icosapentaenoic acid intead of eicosapentaenoic acid. In this paper, however, the name of eicosapentaenoic acid was used according to the traditional designation.

(Merck, West Germany), chicken-egg lecithin (Merck), and β-sitosterol (Nakarai Chemical Co., Japan) were purchased from commercial suppliers and used without purification. *Tapes* PL was isolated from the short-necked clams as mentioned previously[9]. Fucosterol was isolated from a brown alga, *Sargassum fulvellum,* by the similar manners to those described previously[10]: single peak in GLC on OV-17[11]; Mass spectrum, m/e 394 (M^+), 379, 296 (base peak), 281, 255, 253, 228, 227, 55; NMR(δ), 0.69 (C-18 CH_3), 1.02 (C-19 CH_3), 0.99 (C-21 CH_3), 0.98 (C-26,27 CH_3), 1.68 (C-29 CH_3).

Feeding Experiments and Determination of Lipid and Cholesterol Contents

Male Wistar-strain rats were obtained from a commercial breeder and maintained on diet 2 containing 1.0% cholesterol and 4.0% butter as lipid sources (Table 1) for 1 week. The rats, about 200 g in body weight, were grouped (each group, 6 rats) and reared on 12 test diets for 2 weeks. The rats were fed the diets daily at a level of 10% body weight and water *ad lib.* The basal diet (diet 1) was the essentially same as that reported previously[1] and contained the following ingredients (g/100 g): Sucrose 60, casein 18.5, gelatin 5, starch 5, α-cellulose 3, minerals 4, sodium cholate 0.25, choline hydrochloride 0.25, vitamins 0.071, and butter 4.0

Lipid and cholesterol concentrations were determined by the methods of Bragdon[12] and Sperry and Webb[13], respectively.

Results and Discussion

In the present study, two feeding experiments were carried out. Table 1 shows the body weight gain, liver weight, hepatosomatic index, and liver-lipid content of the rats fed the test diets. Table 2 shows cholesterol levels of the serum and liver of rats after the feeding trials.

In experiment I, the feeding trials were conducted to examine the effects of EPA-ME and phytosterols, β-sitosterol and fucosterol, on cholesterol levels of the serum and liver. The addition of 1.0% cholesterol to the basal diet containing 4.0% butter as lipids elevated the liver-lipid content (Table 1) as observed in the previous study[1]. The increase in the liver-lipid content was suppressed by the supplement of 0.3% EPA-ME (diet 3) and 0.1% β-sitosterol (diet 5) but not by that of 0.1% fucosterol (diet 7). However, the simultaneous addition of 0.3% EPA-ME and 1.0% β-sitosterol (diet 4) gave no additional effect on the lowering of liver-lipid level. The cholesterol levels of serum and liver also rose markedly in the rats fed diet 2. The supplement of 0.3% EPA-ME (diet 3), 0.1% β-sitosterol (diet 5), or 0.1% fucosterol (diet 7) suppressed the elevation of cholesterol levels in both the serum and liver (Table 2). However, the simultaneous addition of EPA-ME and either β-sitosterol (diet 4) or fucosterol (diet 6) did not enhance the hypocholesterolemic effects on the serum and liver.

In experiment II, the feeding trials were carried out to examine the cholesterol-lowering effects of 3 types of phospholipids. The levels of liver-lipid, serum-cholesterol, and liver-cholesterol were again elevated in the rats fed diet 2. The increase in the liver-lipid levels were slightly suppressed by the supplement of 0.9% *Tapes* PL (diet 10), 0.6% soybean lecithin (diet 11), or 0.6% chicken-egg lecithin (diet 12). Literatures have shown that unsaturated fatty acids and fats generally lower the serum-cholesterol level in man and experimental animals[14,15], Although *Tapes* PL, soybean lecithin, and chicken-egg lecithin contained considerably high levels of unsaturated fatty acids (Table 3), they did not lower the serum-cholesterol level but elevated it markedly (Table 2). We assume that the phospholipids examined might promote the absorption of cholesterol from the

Table 1. Effects of phospholipids, phytosterols, and EPA-ME on the hepatosomatic index and liver lipid content in rats.

Exptl.	Diet No.	Composition of diet	Body wt. gain (%)	Liver wt. (g)	Hepatosomatic index (%)*1	Liver lipid (mg/g tissue)
	1	Basal diet (BD)*2	21.4	16.5	5.6	60.5
	2	BD + 1.0% CH (cholesterol)	31.7	16.0	5.9	90.0
	3	BD + 1.0% CH + 0.3% EPA-ME*3	34.2	17.3	5.6	77.4
I	4	BD + 1.0% CH + 0.3% EPA-ME + 0.1% Sito*4	22.5	17.5	5.7	73.6
	5	BD + 1.0% CH + 0.1% Sito.	19.8	15.4	5.4	71.8
	6	BD + 1.0% CH + 0.3% EPA-ME + 0.1% Fuco*5	32.5	15.8	5.5	91.1
	7	BD + 1.0% CH + 0.1% Fuco.	13.5	16.1	5.4	91.1
	1	BD	24.3	8.1	5.9	78.7
	2	BD + 1.0% CH	22.4	8.5	6.5	106.3
	3	BD + 1.0% CH + 0.3% EPA-ME	18.8	9.5	7.5	89.3
II	8	BD + 1.0% CH + 0.3% *Tapes* PL	22.8	8.4	6.7	101.4
	9	BD + 1.0% CH + 0.6% *Tapes* PL	18.2	9.1	7.4	98.8
	10	BD + 1.0% CH + 0.9% *Tapes* PL	20.0	9.1	7.5	88.1
	11	BD + 1.0% CH + 0.6% Soybean lecithin	15.3	8.0	6.7	91.8
	12	BD + 1.0% CH + 0.6% Chicken-egg lecithin	18.0	7.0	7.0	86.3

* 1 Liver wt./Body wt.
* 2 Basal diet contained 4.0% butter as lipid sources.
* 3 Eicosapentaenoic acid (20: 5ω3) methylester
* 4 β-Sitosterol
* 5 Fucosterol

Table 2. Effects of phospholipids, phytosterols, and EPA-ME on the hepatosomatic index and liver lipid content in rats.

Exptl.	Diet No.	Composition of diet	Serum cholesterol		Liver cholesterol	
			Total (mg/100 mℓ)	Free (mg/100 mℓ)	Total (mg/g)	Free (mg/g)
	1	Basal diet (BD)	187.5 ± 12.1*	22.5 ± 4.5*	24.7 ± 3.0*	3.7 ± 0.2*
	2	BD + 1.0% CH (cholesterol)	268.8 ± 25.3	38.4 ± 6.0	42.8 ± 5.1	6.1 ± 0.4
	3	BD + 1.0% CH + 0.3% EPA-ME	221.2 ± 10.0	42.8 ± 3.2	27.0 ± 3.1	4.4 ± 0.2
I	4	BD + 1.0% CH + 0.3% EPA-ME + 0.1% Sito	190.8 ± 15.2	42.4 ± 5.1	32.6 ± 7.1	4.9 ± 0.3
	5	BD + 1.0% CH + 0.1% Sito	192.8 ± 20.4	42.4 ± 8.2	33.6 ± 4.0	4.0 ± 0.2
	6	BD + 1.0% CH + 0.3% EPA-ME + 0.1% Fuco	218.6 ± 21.1	53.0 ± 7.0	31.0 ± 3.0	4.7 ± 0.4
	7	BD + 1.0% CH + 0.1% Fuco	213.9 ± 27.4	52.8 ± 4.4	37.8 ± 5.3	5.0 ± 0.4
	1	BD	164.6 ± 11.1	30.3 ± 3.0	17.9 ± 3.1	
	2	BD + 1.0% CH	323.4 ± 40.0	76.6 ± 9.9	39.1 ± 7.7	
	3	BD + 1.0% CH + 0.3% EPA-ME	271.6 ± 25.5	76.5 ± 9.9	22.3 ± 6.0	
II	8	BD + 1.0% CH + 0.3% *Tapes* PL	393.4 ± 50.1	97.1 ± 9.8	30.8 ± 6.0	
	9	BD + 1.0% CH + 0.6% *Tapes* PL	344.2 ± 45.0	79.8 ± 8.1	30.0 ± 5.3	
	10	BD + 1.0% CH + 0.9% *Tapes* PL	384.9 ± 43.3	108.6 ± 9.7	24.2 ± 4.4	
	11	BD + 1.0% CH + 0.6% Soybean lecithin	372.6 ± 32.7	102.0 ± 9.5	42.2 ± 7.1	
	12	BD + 1.0% CH + 0.6% Chicken-egg lecithin	468.4 ± 53.2	115.8 ± 9.7	40.5 ± 6.0	

* S.E.

Table 3. Main fatty acids of phospholipids examined

Fatty acid	Composition (%)*		
	Soybean lecithin	Chicken-egg lecithin	*Tapes* PL
14: 0	1.0	0.2	2.1
16: 0	16.3	36.4	18.6
16: 1	0.4	1.3	4.1
18: 0	3.8	13.3	8.4
18: 1ω9	13.6	26.0	4.3
18: 2ω6	51.6	13.9	0.4
18: 3ω3	8.2	—	0.2
20: 1ω9	0.3	—	9.1
20: 4ω6	0.9	3.9	2.7
20: 5ω3	0.7	0.7	19.0
22: 6ω3	—	—	12.5

* Determined by GLC on 10% DEGS

intestines and consequently elevate the serum-cholesterol level, because the absorption rate of dietary cholesterol is generally increased by coexistent phospholipids and triglycerides[15]. As for the liver-cholesterol, soybean lecithin and chicken-egg lecithin showed a hypercholesterolemic effect, whereas *Tapes* PL suppressed the increase in liver-cholesterol levels. Especially, the supplement of 0.9% *Tapes* PL (diet 10) resulted in a marked decrease in liver-cholesterol level as comparable to that of 0.3% EPA-ME (diet 3). Since EPA-ME and docosahexaenoic acid (DHA) methylester reduced the liver-cholesterol level of rats[1], we assume that the effect of *Tapes* PL on the lowering of liver-cholesterol may be attributable mainly to that of EPA and DHA. Thus, *Tapes* PL were shown to have a hypocholesterolemic effect on the liver but not on the serum.

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