Hypocholesterolemic action of dietary phosphatidylethanolamine in rats sensitive to exogenous cholesterol

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Supplementation of phosphatidylethanolamine (PE) to the cholesterol-free diet decreases the serum cholesterol level in rats. The base portion of PE is responsible for this action, but its mechanism is not clarified yet. As an initial step to understand this mechanism, some of the metabolites of the ethanolamine portion of dietary PE in the liver and blood plasma were determined in the present study by using hypercholesterolemic rats sensitive to exogenous cholesterol. The rats were fed the purified diet containing 1% cholesterol, and either PE or phosphatidylcholine (PC) as a control at 2% level each for 2 weeks. The magnitude of an elevation of the serum cholesterylester level was lower in rats fed PE than in those fed PC. The hepatic levels of triglyceride and cholesterylester tended to be higher in rats fed PE. Fecal steroid excretion was not influenced by the type of the dietary phospholipids. The level of serum ethanolamine and liver phosphoethanolamine was 1.5- and 5.8-fold higher, respectively, in rats fed PE than in those fed PC. The concentration of serum PC was lower and that of the PE was higher in rats fed PE. The level of liver phosphoethanolamine was correlated inversely to that of serum cholesterylester in rats given PE. From these results, it is suggested that inadequate utilization of PC for a component of secretory lipoproteins in the liver and for a substrate donor for cholesterylester formation in the blood serum may be relevant to the cholesterol-lowering action of the dietary PE.

Keywords: phosphatidylethanolamine; phosphoethanolamine; ethanolamine; cholesterylester

Introduction

Phosphatidylethanolamine (PE) is the second most abundant phospholipid to phosphatidylcholine (PC) in the daily foods, but an evaluation of its nutritive and physiological value is scarce.[1] A series of our previous experiments has pointed out that dietary PE takes an important part in the metabolism of cholesterol and fatty acids in rats.[2-5] Namely, the serum cholesterol level decreased in rats fed PE as compared to those fed an equivalent amount of PC or those fed a phospholipid-free diet. Furthermore, dietary PE decreased in the ratio of arachidonate to linoleate in serum and tissue phospholipids, presumably due to a suppression of Δ6-desaturase. Although the mechanism(s) underlying these effects provoked by dietary PE has (have) not been elucidated fully yet, an ethanolamine portion formed during and after absorption of PE appears to be responsible for these effects.

It is well known that cholesterol feeding profoundly affects cholesterol and fatty acid metabolsim in rats.[6,7] Hence, lipid metabolism under the condition of simultaneous intake of cholesterol and PE may provide useful information, which cannot be obtained in the normocholesterolemic state, regarding hypocholesterolemic action of dietary PE. By using the rats as an animal model that is sensitive to exogenous cholesterol (ExHC rat), but that does not require a simultaneous addition of bile acids to achieve hypercholesterolemia,[8,9] the metabolites of the base moiety of PE in the liver and the blood plasma were determined in the present experiment.

Materials and methods

Materials

Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) prepared from egg yolk were kindly provided

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Table 1 Fatty acid compositions of diets

Fatty Acids	Groups PE (Weight %)	PC
16:0	10.6 (16.6)	13.1 (34.2)
16:1	0.5 (0.3)	0.7 (1.4)
18:0	6.5 (29.6)	3.6 (10.3)
18:1	56.4 (15.5)	58.1 (28.3)
18:2	22.4 (8.1)	23.3 (15.0)
20:4	1.6 (11.2)	0.5 (3.4)
22:6	2.0 (13.8)	0.6 (4.4)

Note: The dietary lipids are composed of olive oil, safflower oil, and phospholipids. Figures in the parentheses show the fatty acid composition of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) supplemented to the diets.

Table 2 Serum and liver lipids

Lipids	Dietary groups PE	PC
Serum (mg/dl)		
TG[1]	124 ± 18	139 ± 18
CE[1]	128 ± 11*	186 ± 12
FC[1]	39.6 ± 5.2	41.0 ± 2.4
Phospholipids		
PE	2.50 ± 0.35*	0.93 ± 0.08
PC	83.5 ± 6.0*	116 ± 9.0
Sph[1]	40.2 ± 2.1	48.9 ± 5.0
Lyso-PC[1]	2.09 ± 0.88	2.06 ± 0.39
Liver (mg/g)		
TG[1]	24.0 ± 2.5†	17.2 ± 2.1
CE[1]	37.8 ± 1.2*	31.8 ± 2.0
FC[1]	3.91 ± 1.2	2.92 ± 1.0
PL[1]	23.0 ± 0.70	23.1 ± 0.62

Abbreviations: [1] TG: triglyceride; CE: cholesterylester; FC: free cholesterol; Sph: sphingomyelin; Lyso-PC: lysophosphatidylcholine; PL: phospholipid.
* Significantly different from PC group at $P < 0.05$.
† $P = 0.071$.

by Nippon Oil & Fats Co., Amagasaki. The purity of each phospholipid was more than 98% by thin-layer chromatogram. The fatty acid composition of both phospholipids is shown in the parentheses of *Table 1*. Vitamin-free casein, α-corn starch, mineral mixture (AIN-76), and vitamin mixture (AIN-76) were from Oriental Yeast Co., Tokyo. Cellulose and choline bitartrate were from Toyoroshi Kaisha, Ltd., Tokyo and Katayama Chemical Co., Osaka, respectively. DL-methionine was the product of Nakarai Tesque Co., Kyoto. Olive oil was a gift from Fuji Oil Co., Osaka. Safflower oil and sucrose were purchased from a local market. Boron trifluoro-methanol was obatained from Wako Chemical Co., Osaka.

Animals and diet

Four-week-old male ExHC rats (Takatsuki substrain, isolated from Sprague-Dawley rats: Jcl: SD, CLEA Japan Inc., Osaka), established as a strain highly susceptible to dietary cholesterol,[8] were obtained from Kyushu University Animal Facility, School of Medicine, Fukuoka. They were placed in stainless steel cages with wire-mesh floors, and allowed free access to water at all times. The room was lighted from 8:00 AM to 8:00 PM. The animals were fed a nonpurified diet (NMF, Oriental Yeast Co.) ad libitum until 8 weeks of age before starting the experiment. The composition of the experimental diet was as follows (weight %): casein; 20, corn starch; 15, cellulose; 5, vitamin mixture; 1, mineral mixture; 3.5, choline bitartrate; 0.2, DL-methionine; 0.3, olive oil; 6, safflower oil; 2, phospholipid; 2, cholesterol; 1 and sucrose; 44.0. Although the fatty acid compositions of PE and PC preparations differed obviously as shown in the parentheses of *Table 1*, those in both diets were apparently similar due to a simultaneous supplementation of olive oil and safflower oil. Rats were maintained on this diet for 14 days, and were sacrificed by withdrawing blood from the abdominal aorta under diethyl ether anesthesia. The liver and brain were excised and frozen in liquid nitrogen. Fecal samples were collected for the last two days of feeding.

Analyses

Blood serum was deproteinized with an equal part of 6% sulfosalicylic acid and spun at 10,000*g* for 10 min. The liver and brain were homogenized in four parts of 3.75% sulfosalicylic acid and centrifuged at 10,000*g* for 10 min. The deproteinized supernatant was analyzed for free amino acids using a Hitachi automated amino acid analyzer (System 835, Hitachi Koki Co., Ibaragi).[10] Lipids were determined as described previously.[3]

Statistical analyses

Results were expressed as means ± SE for five rats per group. The differences were determined by Student's *t*-test.

Results

The type of dietary phospholipids did not influence body weight, food intake, and liver weight (data not shown).

As shown in *Table 2*, dietary PE, as compared with the PC, decreased cholesterylesters in the serum and increased those in the liver. The liver triglyceride level tended to be greater in rats fed PE than in those fed PC. Among the serum phospholipids, the level of PE increased and that of PC decreased by the dietary PE. The fecal excretion of acidic and neutral steroids did not differ between both dietary groups (neutral sterol: 45.8 ± 3.6 and 46.2 ± 4.6 mg/day for PE and PC groups, respectively; acidic steroid: 18.8 ± 1.1 and 20.6 ± 1.4 mg/day for PE and PC groups, respectively.

Table 3 Concentration of ethanolamine and phosphoethanolamine in serum, liver, and brain

Amines	Serum		Liver		Brain	
	PE	PC	PE	PC	PE	PC
	(μM/l)		(μM/g)		(μM/g)	
EA[a]	53.2 ± 2.3*	36.4 ± 1.0	2.46 ± 0.10	2.45 ± 0.08	0.951 ± 0.07	0.852 ± 0.08
P-EA[a]	n.d[b]	n.d[b]	7.38 ± 0.78*	1.28 ± 0.07	0.362 ± 0.05	0.362 ± 0.04

* Significantly different from PC group at $P < 0.05$.
[a] EA: ethanolamine; P-EA: phosphoethanolamine.
[b] n.d.: not detected.

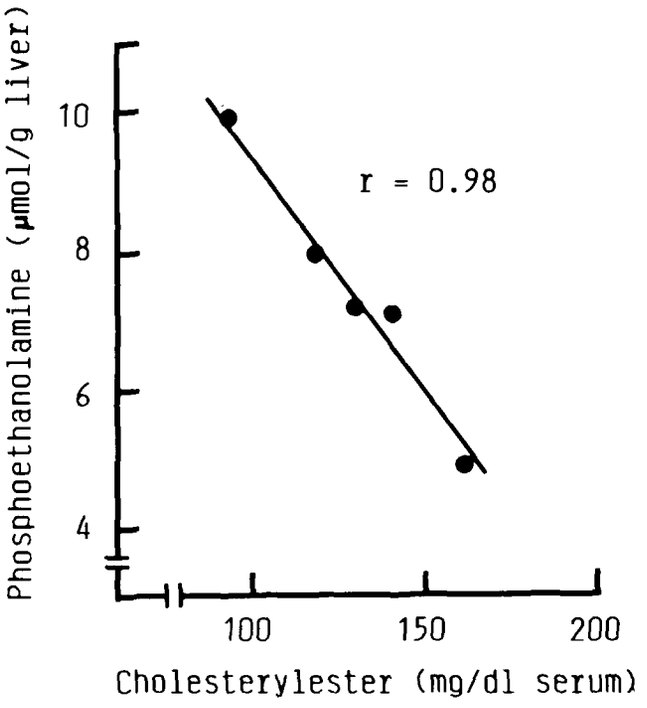

Figure 1 Correlation of serum esterified cholesterol and hepatic phosphoethanolamine. The graph was done on PE-fed rats.

As shown in *Table 3*, dietary PE increased the concentration of serum ethanolamine and liver phosphoethanolamine. Significant difference was not found in the level of other free amino acids examined at the same time (data not shown). In the brain, the level of these amines and other free amino acids was not influenced by feeding PE. As shown in *Figure 1*, the concentration of hepatic phosphoethanolamine in rats fed PE was highly inversely correlated to the level of serum cholesterylester.

Dietary PE, as compared with the PC, slightly, but significantly, increased linoleate and oleate, and instead decreased palmitate and stearate in the hepatic total lipid fraction (data not shown). The ratio of arachidonate to linoleate significantly decreased in rats fed PE (0.23 ± 0.02 and 0.35 ± 0.02 for PE and PC groups, respectively, $P < 0.05$). No difference was found in the fatty acid composition of brain lipids (data not shown).

Discussion

We have shown in the previous experiment by using normal rats fed cholesterol-free diet that supplementation of PE at 2% level or the equivalent molar amount of ethanolamine as compared with that of PC or phospholipid-free diet decreased the serum cholesterol level.[2,3] The present study further confirmed a suppressive effect of dietary PE on the serum cholesterol level in hyperresponsive rats (ExHC rat) to dietary cholesterol. Such a hypocholesterolemic effect of dietary PE appeared not to be attributed to the changes in the excretory or absorptive processes, since fecal excretion of neutral and acidic steroids was similar between the groups.

Since the rats fed PE showed a highly inversed relationship between the level of liver phosphoethanolamine and that of serum cholesteryl ester, ethanolamine originated from the dietary PE is considered to be involved in the observed changes as described above. Although the dietary PE contains more polyunsaturated fatty acids as compared to the counterparts of the dietary PC, the contribution of the fatty acid portion of the dietary PE to lowering the serum cholesterol level may be small, if any at all, since the total fatty acid composition of dietary lipids is essentially similar between both dietary groups (*Table 1*).

Ethanolamine acts as a precursor of PE in cells. The first step of this pathway involves phosphorylation by a kinase that has a broad specificity (i.e., it phosphorylates ethanolamine as well as choline).[11] The recent study by Porter and Kent showed that ethanolamine/choline kinase is the same enzyme, and choline and ethanolamine were mutually competitive inhibitors.[11] Thus, in the presence of high ethanolamine concentration, phosphorylation of choline may be diminished and less phosphorylcholine is produced, leading to a relatively high synthesis of PE versus PC. It is, therefore, possible that lower availability of newly synthesized PC would lead to slower synthesis of very low density lipoprotein (VLDL)[12] and cholesterol-rich lipoproteins such as β-VLDL under the condition of high dietary cholesterol. In fact, the concentration of hepatic cholesterylester as well as triglyceride increased in rats fed PE rather than in those fed PC, shown in *Table 2*.

It is also possible that lower concentration of serum PC may lead to a decrease in the serum cholesteryles-

ter, since the serum PC is utilized as a substrate for lecithin cholesterol acyltransferase (LCAT).[13] The fatty acid proportion of the serum PC may also influence the cholesterylester synthesis in the blood serum, since arachidonate rather than linoleate is a better substrate for LCAT in rats.[14] In fact, the dietary PE decreased the ratio of arachidonate to linoleate as compared to the dietary PC, and the Δ6-desaturase activity for linoleate was lower in rats fed PE as shown previously.[5]

Recent study by Yao and Vance indicated that the inhibitory effect of monomethylethanolamine on the secretion of VLDL and low density lipoprotein from the cultured hepatocytes obtained from rats fed choline-free diet was stronger than that of ethanolamine.[12] They also showed that the inhibitory effect of dimethylethanolamine on the secretion of lipoproteins from the cultured hepatocytes was much less as compared to that of monomethylethanolamine. By feeding experiment, Bondesson et al. showed that hypocholesterolemic as well as hypotriglyceridemic activity was more marked in rats fed monomethylethanolamine than in those fed ethanolamine when these aminoethanols were supplemented to the diets at 0.5% level.[15] Hypocholesterolemic activity of monomethylethanolamine and ethanolamine supplemented to the diets appeared to be specific, since dimethylethanolamine and butylethanolamine supplemented to the diet did not influence serum cholesterol level in rats (unpublished observation). The metabolic intermediate, phosphatidylmonomethylethanolamine (PMME), has been reported to be normally present in the liver in spite of very small amounts.[16] Thus, it is interesting to examine further if hypocholesterolemic action of dietary PE attributes to the presence of monomethylethanolamine or PMME which may be formed during the excess transformation of ethanolamine accumulated in large amounts in the liver.

Phosphoethanolamine is known to exist in most animal tissues,[17] but its biological importance is unknown, apart from its role as a possible intermediate in the metabolism of phospholipid.[18] However, the role of phosphoethanolamine as a cholinergic-enhancing factor in rat brain[19] and as a growth-promoting factor for carcinoma cells[20] now deserves attention. The present study showed a five-to-six-fold greater accumulation of phosphoethanolamine in the liver in rats fed PE than in those fed PC. Therefore, it remains to determine further possible physiological function of the metabolites of exogenous PE other than lipid metabolism.

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