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Dietary Methionine Level Affects Linoleic Acid Metabolism Through Phosphatidylethanolamine *N*-Methylation in Rats

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ABSTRACT: The effects of dietary methionine level on the profiles of fatty acids and phospholipids and on the plasma cholesterol concentration were investigated to confirm whether the methionine content of dietary proteins is one of the major factors that cause differential effects on lipid metabolism. The effect of dietary supplementation with eritadenine, which is shown to be a potent inhibitor of phosphatidylethanolamine (PE) *N*-methylation, was also investigated. Rats were fed six diets containing casein (100 g/kg) and amino acid mixture (86.4 g/kg) differing in methionine content (2.5, 4.5, and 7.5 g/kg) and without or with eritadenine supplementation (30 mg/kg) for 14 d. The ratio of arachidonic to linoleic acid of liver microsomal and plasma phosphatidylcholine (PC) was significantly increased as the methionine level of diet was elevated, indicating that dietary methionine stimulates the metabolism of linoleic acid. The PC/PE ratio of liver microsomes and the plasma cholesterol concentration were also increased by dietary methionine. These effects of methionine were completely abolished by eritadenine supplementation The *S*-adenosylmethionine concentration in the liver reflected the methionine level of diet. These results support the idea that the differential effects of dietary proteins on lipid metabolism might be ascribed, at least in part, to their different methionine contents, and that methionine might exert its effects through alteration of PE *N*-methylation.
Lipids 33, 235–242 (1998).

It is confirmed that lipid metabolism can be modified by the type of dietary proteins. For instance, plant proteins compared with animal proteins generally lead to lower plasma cholesterol concentrations (1–4). The metabolism of fatty acids, especially linoleic acid, also has been shown to be affected by dietary protein types (5,6). Additionally, the profile of liver microsomal phospholipid classes, as represented by the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE), could be altered by the type of dietary proteins (7). A number of studies so far reported have suggested that both the difference in the physicochemical properties and the difference in the amino acid composition of dietary proteins participate in the differential effects on the plasma cholesterol concentration. In contrast, the mechanism underlying the differential effects of dietary proteins on the metabolism of fatty acids appears to be less understood. Recently we demonstrated that methionine supplementation of a low-methionine diet containing soybean protein brought about a significant increase in the ratio of arachidonic to linoleic acid of liver microsomes and plasma in rats, suggesting that dietary methionine stimulates the metabolic conversion of linoleic acid into arachidonic acid (8). The PC/PE ratio of liver microsomes and the plasma cholesterol concentration also were increased by methionine supplementation (8). These results suggest that the methionine content of dietary proteins may have a wide range of effects on lipid metabolism, and that metabolism of linoleic acid, phospholipids, and cholesterol may not be independent of each other. Furthermore, previous studies have suggested that dietary methionine level may affect the PC/PE ratio of liver microsomes through alteration of PE *N*-methylation (7,8). If this is true, the effect of dietary methionine on lipid metabolism would be abolished or suppressed by the inhibitor of PE *N*-methylation.

In this study, the effects of dietary methionine level on the composition of fatty acids and molecular species of PC on the microsomal PC/PE ratio and on the plasma cholesterol concentration were investigated to confirm whether the methionine content of dietary proteins is one of the major factors that cause differential effects on lipid metabolism. The effect of supplementation with eritadenine, which strongly depresses PE *N*-methylation (9), also was investigated to see whether the effect of methionine can be abolished by eritadenine.

MATERIALS AND METHODS

Animals. Thirty-six male 5-wk-old rats of the Wistar strain were received at 90–100 g body weight from Japan SLC (Hamamatu, Japan). The rats were individually housed in hanging stainless-steel wire cages kept in an isolated room at

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Abbreviations: GLC, gas–liquid chromatography; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; LDL, low density lipoprotein; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; TLC, thin-layer chromatography; VLDL, very low density lipoprotein.

a controlled temperature (23–25°C) and humidity (40–60%). Lights were maintained on a 12-h cycle (lights on from 0600 to 1800 h). For 5 or 6 d, rats were fed the powdered laboratory stock diet as described previously (10). Then, they were divided into six groups of six rats each with similar mean weights (~121 g) and allowed free access to the experimental diets and water for 14 d. The experimental plan was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University.

Diets. Six diets differing in methionine content (2.5, 4.5, and 7.5 g/kg diet) and without or with eritadenine were utilized in this study. Table 1 shows the composition of experimental diets without eritadenine. Casein (100 g/kg) and casein-simulating amino acid mixture (86.4 g/kg) were used as protein sources. Therefore, the diets were comparable to 20% casein diet in the amino acid composition except for sulfur amino acids. The amount of total sulfur amino acids (methionine and cystine) in the diet was maintained to a constant level on a molar basis, which was equivalent to the amount of methionine of 8 g/kg diet. Eritadenine was added to the diet at a level of 30 mg/kg diet at the expense of lactose; this dose level was shown to be sufficient to elicit maximal effect (10). The eritadenine was isolated from dried *Lentinus edodes* mushroom as reported previously (10). The fatty acid composition of corn oil used was as follows (wt%): 16:0, 12.2; 18:0, 1.8; 18:1, 32.8; 18:2n-6, 51.4; and 18:3n-3, 1.6.

Tissue collection and fractionation. At the end of the feeding period, non-fasted rats were killed by decapitation under light anesthesia with diethyl ether between 1200 and 1300 h. Plasma was separated from heparinized whole blood by centrifugation at 2000 × *g* for 20 min at 4°C. An aliquot of the plasma was stored at 4°C until subsequent analyses for plasma lipid concentrations, and the residual plasma was stored at −80°C until analyses for phospholipids. After collection of blood, the whole liver was quickly removed, rinsed in ice-cold saline, blotted on filter paper, and weighed. The liver was homogenized in 4 vol (vol/wt) of an ice-cold 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M KCl, a modified solution of Nguyen *et al*. (11). An aliquot (2 mL) of the homogenate was stored at −30°C until subsequent analyses for liver lipid concentrations. Another aliquot (12 mL) of the homogenate was centrifuged at 10,000 × *g* for 12 min at 4°C, and the resultant supernatants were further centrifuged at 105,000 × *g* for 60 min at 4°C to obtain the microsomal fraction as a precipitate. The microsomes obtained were resuspended in the homogenizing buffer and stored at −80°C until analyses for phospholipids.

Lipid analysis. The plasma concentrations of total cholesterol, high density lipoprotein (HDL) cholesterol, free cholesterol, triglycerides, and phospholipids were measured enzymatically with kits (Cholesterol C-Test, HDL Cholesterol-Test, Free Cholesterol C-Test, Triglyceride G-Test, and Phospholipid B-Test, respectively; Wako Pure Chemical, Osaka, Japan). The difference between total cholesterol and HDL cholesterol or free cholesterol was assumed to be very low density lipoprotein (VLDL) + low density lipoprotein (LDL) cholesterol or esterified cholesterol, respectively. The total lipids of liver homogenate, liver microsomes, and plasma were extracted by the method of Folch *et al*. (12). The cholesterol, triglycerides, and phospholipids in the extract of liver homogenate were measured according to Zak (13), Fletcher (14), and Bartlett (15), respectively. For the determination of phospholipid class composition, the phospholipids in the extract of liver microsomes were separated into each class by thin-layer chromatography (TLC) on silica gel 60 (E. Merck, Darmstadt, Germany) using chloroform/methanol/water (65:25:4, by vol) as a developing solvent. The bands of each phospholipid class were visualized in iodine vapor, scraped off the plate, and analyzed directly for inorganic phosphorus (15). For the determinations of fatty acid and molecular species composition, PC was likewise separated by TLC from liver microsomes and plasma, visualized with dichlorofluorescein, scraped off the plate, and extracted with chloroform/methanol (1:2, vol/vol). An aliquot of PC was treated with 14% (w/w) BF_3/methanol reagent (Wako Pure Chemical), and fatty acid methyl esters formed were analyzed by gas–liquid chromatography (GLC) on a Model GC-17A (Shimadzu, Kyoto, Japan), equipped with a TC-FFAP capillary column (0.25 mm × 30 m; GL Sciences, Tokyo, Japan). Another aliquot of PC was converted to diacylglycerol benzoates according to the method of Blank *et al*. (16). The diacylglycerol benzoates were analyzed by high-performance liquid chromatography (HPLC) on a Model LC-6A system (Shimadzu), equipped with an ODS column (4.6 × 250 mm, Lichrosorb RP-18; E. Merck), essentially according to the method of Blank *et al*. (16). Since some peaks consisted of two molecular species, the ratio of two molecular species was determined by the analysis of fatty acids by GLC. A representative chromatogram of PC molecular species by HPLC was shown previously (17).

TABLE 1
The Composition of the Experimental Diets

	Diet		
	2.5M4.4C	4.5M2.8C	7.5M0.4C
Ingredient (g/kg)			
Casein	100	100	100
Amino acid mix[a]	86.4	86.4	86.4
Cornstarch	489.6	489.2	488.6
Sucrose	200	200	200
Corn oil	50	50	50
Mineral mix[b]	35	35	35
Vitamin mix[b]	10	10	10
Choline·Cl	4	4	4
Cellulose	20	20	20
Lactose	1	1	1
L-Methionine	0	2	5
L-Cystine	4	2.4	0
Concentration (g/kg)			
Methionine	2.5	4.5	7.5
Cystine	4.4	2.8	0.4

[a]The composition was similar to that of casein except that sulfur amino acids were omitted. The mixture contained the following amino acids (g/86.4 g): L-Ile, 4.51; L-Leu, 7.75; L-Lys·HCl, 8.27; L-Phe, 4.25; L-Tyr, 4.63; L-Thr, 3.38; L-Trp, 1.05; L-Val, 5.51; L-His, 2.51; L-Arg·HCl, 3.61; L-Ala, 2.51; L-Asp, 5.75; L-Glu, 17.51; Gly, 1.49; L-Pro, 9.38; L-Ser, 4.25.
[b]AIN-76 (Oriental Yeast, Tokyo, Japan).

TABLE 2
Growth, Food Intake, Liver Weight, and Liver Lipid Concentrations in Rats Fed Experimental Diets[a]

	Diet					
	–Eritadenine			+Eritadenine		
	2.5M4.4C	4.5M2.8C	7.5M0.4C	2.5M4.4C	4.5M2.8C	7.5M0.4C
Body weight gain (g/14 d)	68 ± 3	67 ± 2	64 ± 2	64 ± 2	70 ± 2	63 ± 3
Food intake (g/14 d)	192 ± 4	192 ± 2	186 ± 3	188 ± 3	193 ± 3	187 ± 4
Liver weight (g/100 g body weight)	4.6 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
Liver lipids (μmol/g liver)						
Cholesterol	6.8 ± 0.2^{c}	7.0 ± 0.1^{b,c}	7.3 ± 0.1^{b,c}	7.0 ± 0.3^{b,c}	7.1 ± 0.2^{b,c}	7.4 ± 0.2^{b}
Triglycerides	15.5 ± 1.3^{b}	17.0 ± 0.5^{b}	17.1 ± 1.0^{b}	11.4 ± 0.6^{c}	12.2 ± 0.7^{c}	14.3 ± 1.5^{b}
Phospholipids	28.7 ± 0.4^{c}	28.7 ± 0.6^{c}	29.8 ± 0.6^{c}	31.8 ± 0.7^{b}	30.5 ± 0.6^{b,c}	32.1 ± 0.8^{b}

[a]Values are mean ± SEM for six rats. Values in a row with different superscripts are significantly different at $P < 0.05$.

Assay of methionine metabolites and protein. The concentrations of *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) in the liver were measured with HPLC according to Cook *et al*. (18) with some modifications. Frozen livers were thawed and homogenized in 4 vol (vol/wt) of ice-cold 0.5 M perchloric acid, and the homogenates were centrifuged at 10,000 × *g* for 10 min at 4°C. The resultant supernatants were filtered through a 0.45-μm Millipore filter (Nihon Millipore, Tokyo, Japan) and applied to an HPLC column (Shim-pak CLC-ODS, 6 × 150 mm; Shimadzu) . The mobile phase was a 100 mM KH_2PO_4 solution containing 10 mM sodium heptane sulfonate and 3% (vol/vol) methanol. The flow rate was 1.5 mL/min, and the elution was monitored at 254 nm. Liver microsomal protein was measured according to Lowry *et al*. (19).

Statistical analysis. Data were analyzed by one-way analysis of variance, and the differences between means were tested using Duncan's multiple range test (20) when the *F* value was significant. Simple correlation between variables was calculated by linear regression analysis using mean values. A *P* value of 0.05 or less was considered significant.

RESULTS

Growth, food intake, liver weight, and liver lipid concentrations. There was no significant difference in the body weight gain, food intake, and relative liver weights of animals among the six groups (Table 2). Dietary methionine level had little effect on liver lipid concentrations. Eritadenine supplementation tended to decrease the triglyceride concentration and inversely to increase the phospholipid concentration in the liver.

Plasma lipid concentrations. The plasma total cholesterol concentration was significantly increased by elevating dietary methionine level (Fig. 1). In the same manner, plasma concentrations of VLDL + LDL cholesterol, HDL cholesterol, and cholesteryl esters were significantly affected by dietary methionine level. The plasma concentrations of triglycerides and phospholipids were not affected by dietary methionine level. Eritadenine supplementation brought about marked decreases in the plasma concentrations of cholesterol and phospholipids, but not triglycerides. In those rats fed eritadenine-supplemented diets, the hypercholesterolemic effect of dietary methionine disappeared completely.

Methionine metabolites and microsomal phospholipids. The concentration of SAM in the liver was significantly increased by elevating dietary methionine level, whereas the concentration of SAH was unchanged (Fig. 2). Consequently, the SAM/SAH ratio was significantly increased by dietary methionine. Eritadenine supplementation significantly decreased the SAM/SAH ratio, which was mainly due to the increase in SAH concentration. The concentration of PC in liver microsomes was unaffected by dietary methionine level, whereas the concentration of PE was significantly decreased by elevating dietary methionine level. Eritadenine supplementation significantly increased the PE concentration and

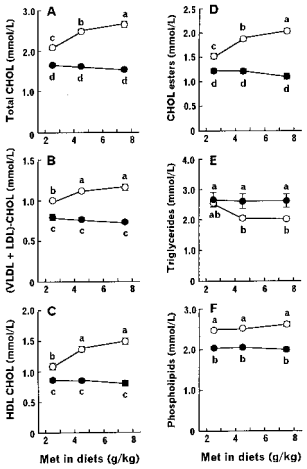

FIG. 1. Effects of dietary methionine (Met) level and eritadenine (Er) supplementation on plasma lipid concentrations in rats. The circle and its bar represent mean and SEM, respectively, for six rats. Values with different letters are significantly different at $P < 0.05$. CHOL, cholesterol; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein. ○, –Er; ●, + Er.

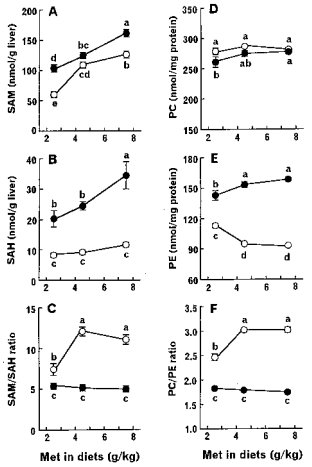

FIG. 2. Effects of dietary Met level and Er supplementation on the concentration of Met metabolites (A–C) and microsomal phospholipids (D–F) in the liver of rats. The circle and its bar represent mean and SEM, respectively, for six rats. Values with different letters are significantly different at $P < 0.05$. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine. See Figure 1 for other abbreviations and symbol legend.

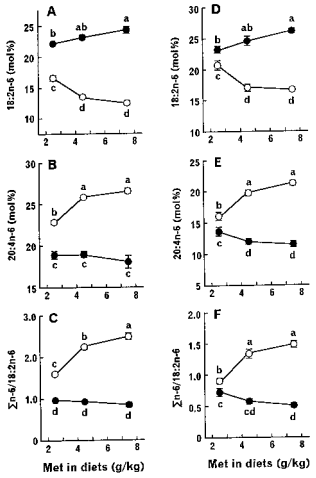

FIG. 3. Effects of dietary Met level and Er supplementation on the proportion of linoleic and arachidonic acids in liver microsomal (A–C) and plasma (D–F) PC in rats. The circle and its bar represent mean and SEM, respectively, for six rats. Values with different letters are significantly different at $P < 0.05$. Abbreviations: Σn-6, metabolites of 18:2n-6. See Figures 1 and 2 for other abbreviations. See Figure 1 for symbol legend.

consequently significantly decreased the PC/PE ratio. Eritadenine abolished the effect of dietary methionine on the PC/PE ratio.

Fatty acid and molecular species composition of PC. The proportion of linoleic acid in liver microsomal PC was significantly decreased, and conversely the proportion of arachidonic acid was significantly increased by elevating dietary methionine level (Fig. 3). Consequently, the ratio of linoleic acid metabolites to linoleic acid was significantly increased by dietary methionine. In the same manner, the proportion of linoleic and arachidonic acids in plasma PC was influenced by dietary methionine level. Table 3 shows the fatty acid composition of liver microsomal and plasma PC in rats fed diets without eritadenine. The diets higher in methionine brought about a decrease in the proportion of palmitic acid and increases in the proportion of 22:5n-6 and 22:6n-3, in addition to the suppression of linoleic acid metabolism. Table 4 shows the molecular species composition of plasma PC in rats fed diets without eritadenine. The diets higher in methionine caused a significant decrease in the proportion of 16:0-18:2 PC and a significant increase in the proportion of 18:0-20:4 PC. The effects of dietary methionine level on the other PC molecular species were relatively small. Figure 4 shows the effects of dietary methionine level and eritadenine supplementation on the proportion of plasma 16:0-18:2 and 18:0-20:4 PC molecular species. Eritadenine supplementation significantly increased the proportion of 16:0-18:2 PC and conversely significantly decreased the proportion of 18:0-20:4 PC. In the presence of dietary eritadenine, higher levels of dietary methionine potentiated the effect of eritadenine on plasma PC molecular species. There was a significantly negative or positive correlation between the concentration of plasma total cholesterol and the proportion of plasma 16:0-18:2 or 18:0-20:4 PC, respectively (Fig. 5).

DISCUSSION

Effects on fatty acid and phospholipid profiles. The conversion of linoleic acid into arachidonic acid is one of the most important metabolisms of fatty acids quantitatively and qualitatively in higher animals, since arachidonic acid exists in membrane phospholipids at relatively high levels, and since arachidonic acid is the predominant precursor for various types of eicosanoids. In rats, the metabolism of linoleic acid can be modified by dietary proteins types (5,6), in addition to the other dietary constituents, such as n-3 fatty acids (21) and cholesterol

TABLE 3
Effect of Dietary Methionine Level on the Fatty Acid Composition (mol%) of Phosphatidylcholine[a]

	Liver microsomes			Plasma		
	Diet (–eritadenine)			Diet (–eritadenine)		
Z						
14:0	0.6 ± 0.0[b]	0.5 ± 0.0[c]	0.4 ± 0.0[c]	0.9 ± 0.1[b]	0.9 ± 0.1[b]	0.6 ±0.0[c]
16:0	22.6 ± 0.6[b]	20.5 ± 0.2[c]	20.4 ± 0.2[c]	24.6 ± 0.3[b]	23.5 ± 0.3[c]	22.3 ± 0.4[d]
16:1n-7	2.8 ± 0.1[b]	2.3 ± 0.1[c]	2.2 ± 0.1[c]	3.1 ± 0.2[b]	2.1 ± 0.2[c]	2.7 ± 0.2[b]
18:0	16.5 ± 0.5[c]	18.4 ± 0.6[b]	19.0 ± 0.3[b]	14.6 ± 0.7[c]	17.8 ± 0.7[b]	16.4 ± 0.4[b,c]
18:1n-9	5.2 ± 0.1[b]	4.9 ± 0.1[c]	4.9 ± 0.1[c]	11.1 ± 0.8	9.1 ± 0.5	10.0 ± 0.8
18:1n-7	5.4 ± 0.2	5.7 ± 0.0	5.4 ± 0.1	3.6 ± 0.2	3.3 ± 0.0	3.4 ± 0.2
18:2n-6	16.6 ± 0.4[b]	13.5 ± 0.3[c]	12.4 ± 0.4[c]	20.7 ± 0.7[b]	17.0 ± 0.6[c]	16.7 ± 0.3[c]
18:3n-6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
20:1n-9	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:1n-7	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
20:2n-6	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
20:3n-9	0.3 ± 0.0[b]	0.2 ± 0.0[c]	0.2 ± 0.0[c]	0.3 ± 0.1[c]	0.5 ± 0.1[b]	0.2 ± 0.0[c]
20:3n-6	1.2 ± 0.1[b]	0.9 ± 0.0[c]	0.8 ± 0.0[c]	0.7 ± 0.0[b]	0.5 ± 0.0[c]	0.5 ± 0.0[c]
20:4n-6	22.8 ± 0.2[b]	25.8 ± 0.2[c]	26.5 ± 0.3[c]	16.0 ± 0.7[c]	19.7 ± 0.5[b]	21.3 ± 0.4[b]
22:4n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1[c]	0.5 ± 0.1[b,c]	0.7 ± 0.1[b]
22:5n-6	1.4 ± 0.1[c]	2.3 ± 0.1[b]	2.3 ± 0.1[b]	0.9 ± 0.2[c]	1.6 ± 0.1[b]	1.7 ± 0.1[b]
22:5n-3	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
22:6n-3	2.6 ± 0.1[d]	3.1 ± 0.1[c]	3.4 ± 0.1[b]	1.6 ± 0.1	2.0 ± 0.2	2.1 ± 0.1
∑n-6/18:2n-6	1.6 ± 0.0[d]	2.2 ± 0.1[c]	2.5 ± 0.1[b]	0.9 ± 0.0[c]	1.3 ± 0.1[b]	1.5 ± 0.1[b]

[a]Values are mean ± SEM for six rats. Within a row of each tissue, values with different superscript letters (b–d) are significantly different at $P < 0.05$. ∑n-6, metabolites of 18:2n-6.

(22). It has been shown that dietary soybean protein as compared with casein suppresses the metabolism of linoleic acid and consequently decreases the ratio of arachidonic to linoleic acid of phospholipids (6). However, by what factor of dietary proteins linoleic acid metabolism is differentially affected has not yet been fully elucidated. The present study clearly demonstrated that linoleic acid metabolism is significantly affected by the dietary level of methionine; low methionine diet led to suppressed metabolism of linoleic acid and conversely high methionine diet stimulated the metabolic conversion of linoleic acid into arachidonic acid. It is well recognized that the methionine content of soybean protein is considerably lower than that of casein. Our previous study showed that methionine supplementation of a soybean protein diet caused a significant increase in the arachidonic/linoleic acid ratio of liver microsomal and plasma PC in rats (8). Thus, the results obtained here, together with previous results, strongly suggest the idea that the differential effect of different types of dietary proteins on linoleic acid metabolism might be attributable to the difference in their methionine contents.

TABLE 4
Effect of Dietary Methionine Level on the Molecular Species Composition (mol%) of Plasma Phosphatidylcholine[a]

Molecular species	Diet (–eritadenine)		
	2.5M4.4C	4.5M2.8C	7.5M0.4C
16:0-18:1[b]	10.8 ± 0.3[c]	9.1 ± 0.5[d]	8.4 ± 0.1[d]
18:0-18:1	1.4 ± 0.3	1.3 ± 0.2	1.3 ± 0.3
18:1-18:1	0.5 ± 0.1[d]	0.9 ± 0.1[c]	0.8 ± 0.1[c]
16:0-18:2	22.2 ± 0.4[c]	16.8 ± 0.3[d]	16.3 ± 0.4[d]
18:0-18:2	9.4 ± 0.1[c]	7.5 ± 0.3[e]	8.4 ± 0.1[d]
18:1-18:2	2.4 ± 0.1[c]	1.7 ± 0.3[d]	1.0 ± 0.2[e]
16:0-20:4	15.7 ± 0.3	16.1 ± 0.6	16.2 ± 0.2
18:0-20:4	18.2 ± 0.4[e]	21.5 ± 0.4[d]	24.2 ± 0.7[c]
18:1-20:4	3.6 ± 0.3	4.1 ± 0.3	4.4 ± 0.2
16:0-22:5	2.1 ± 0.3[e]	3.9 ± 0.2[c]	3.0 ± 0.4[d]
18:0-22:5	2.2 ± 0.2[d]	4.3 ± 0.4[c]	3.1 ± 0.3[c,d]
16:0-22:6	4.5 ± 0.1	4.5 ± 0.2	4.4 ± 0.1
18:0-22:6	3.5 ± 0.1[d]	4.3 ± 0.2[c]	4.8 ± 0.2[c]

[a]Values are mean ± SEM for six rats.
[b]Fatty acids in the *sn*-1 and *sn*-2 positions of phosphatidylcholine are indicated at the left and right sides, respectively. Values in a row with different superscript letters (c–e) are significantly different at $P < 0.05$.

The conversion of linoleic acid into arachidonic acid is thought to be regulated by the activity of Δ6-desaturase, the rate-limiting enzyme of linoleic acid metabolism. Actually, it was shown that the Δ6-desaturase activity was significantly lower in rats fed a soybean protein diet than in rats fed a casein diet (22). Although Δ6-desaturase activity was not measured in the present study, fatty acid profiles, as represented by the arachidonic/linoleic acid ratio, suggest that dietary methionine level might affect the enzyme activity. This may be further supported by the fact that 22:5n-6 and 22:6n-3 were increased by elevating dietary methionine level, despite 22:4n-6 and 22:5n-3 being unchanged. The enzyme Δ6-desaturase is also thought to participate in the formation of 22:5n-6 and 22:6n-3 from 22:4n-6 and 22:5n-3, respectively (23). It is interesting to know by what mechanism dietary methionine level affects the metabolism of linoleic acid. With regard to this, there are several reports to provide available information. For instance, Leikin and Brenner (24–26) have reported that the activity of Δ5 or Δ6 desaturase might be regulated by the PC/PE or cholesterol/PC ratio of liver microsomal membranes. Consistent with this, She *et al.* (27) have

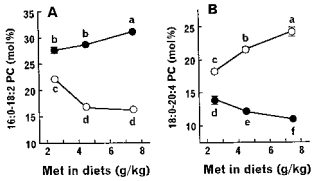

FIG. 4. Effects of dietary Met and Er supplementation on the proportion of 16:0-18:2 (A) and 18:0-20:4 (B) molecular species of plasma PC in rats. The circle and its bar represent mean and SEM, respectively, for six rats. Values with different letters are significantly different at $P < 0.05$. See Figures 1 and 2 for abbreviations. See Figure 1 for symbol legend.

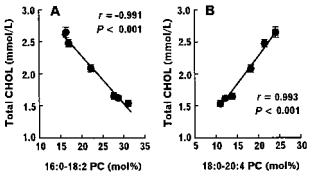

FIG. 5. Relationship between the plasma total CHOL concentration and the proportion of 16:0-18:2 (A) or 18:0-20:4 (B) molecular species of plasma PC in rats fed six experimental diets. The circle and its bar represent mean and SEM, respectively, for six rats. See Figures 1 and 2 for abbreviations.

shown that feeding a vitamin B_6-deficient diet caused decreases in both the PC/PE ratio and Δ6-desaturase activity of rat liver microsomes. Sugiyama *et al*. (10) also showed that there was a significantly positive correlation between the PC/PE ratio and the activity of Δ6 desaturase in rats fed graded levels of eritadenine. Furthermore, dietary supplementation with PE or ethanolamine (28,29) and diabetes caused by streptozotocin (30) have been shown to decrease both the PC/PE ratio and linoleic acid metabolism in rats. These results suggest that the activity of Δ6 desaturase can be affected by various types of dietary treatment through alteration of liver microsomal phospholipid profile, as represented by the PC/PE ratio. Previously we demonstrated that in rats fed different types of dietary proteins, the liver microsomal PC/PE ratio was significantly correlated with the methionine content of dietary proteins (7). In support of this, the present study showed that the PC/PE ratio of liver microsomes could be influenced by the level of dietary methionine. Taken together, it seems reasonable to consider that dietary methionine level might affect the metabolism of linoleic acid through alteration of the PC/PE ratio of liver microsomes in rats.

In the liver of rats, PC is synthesized either by the CDP-choline pathway or the PE *N*-methylation pathway, although the former is thought to be the main pathway (31). On the other hand, it appears that the PC/PE ratio of liver microsomes is regulated mainly by the extent of PE *N*-methylation (8). The decreased PC/PE ratio evoked by vitamin B_6-deficiency (27) or eritadenine supplementation (9) could be attributable to the increase in hepatic SAH concentration, since SAH is known to be a potent inhibitor of various types of methyltransferases, including PE *N*-methyltransferase. In contrast, the decreased PC/PE ratio caused by a low methionine diet cannot be ascribed to the change in SAH concentration (Fig. 2). It is confirmed that PE *N*-methylation is also influenced by the hepatic concentration of SAM, the substrate of the reaction. The K_m values for SAM of PE *N*-methyltransferase in rat liver microsomes for sequential methylation of PE were estimated to be 51, 58, and 79 μM, respectively (32). On the other hand, concentrations of SAM in the liver of rats fed the low- and high-methionine diets were found to be 60 and 126 μmol/g liver, respectively (Fig. 2). Taking these K_m values and hepatic SAM concentrations into consideration, it is reasonable to consider that the low-methionine diet might decrease the extent of PE *N*-methylation by decreasing hepatic SAM concentration, thereby leading to an increase in microsomal PE concentration and a resultant decrease in the PC/PE ratio. One of the most important findings of this study is that all of the effects tested of dietary methionine level on lipid metabolism disappeared completely under the condition of eritadenine supplementation. Since eritadenine strongly depresses the PE *N*-methylation (9), these results are taken to support the idea that dietary methionine level affects the metabolism of a variety of lipids (e.g., phospholipids, fatty acids, and cholesterol) through the depression of PE *N*-methylation.

Effect on plasma cholesterol concentration. Several reports have shown that methionine supplementation of a low-methionine diet, e.g., soybean protein diet, increases the plasma cholesterol concentration in rats (8,33–35). These results suggest that methionine has a hypercholesterolemic action at least under the experimental conditions used, although methionine also has been shown to have a hypocholesterolemic action under different dietary conditions. For instance, it was shown that methionine supplementation of a cholesterol-enriched low-protein diet, unlike standard or high-protein diet, resulted in a significant decrease in the plasma cholesterol concentration in rats (36). The hypercholesterolemic effect of methionine in rats fed a cholesterol-free diet was confirmed by the present study. However, the mechanism by which dietary methionine exerts its hypercholesterolemic action is not yet fully elucidated. The present study clearly demonstrated that the plasma cholesterol-elevating effect of methionine could be abolished by eritadenine supplementation, suggesting that the stimulation of PE *N*-methylation and a resultant increase in the PC/PE ratio of liver microsomes might participate in the enhancement of plasma cholesterol by methionine. In support of this, there was a significantly positive correlation between the PC/PE ratio and the plasma total cholesterol concentration in rats fed different

types of dietary proteins (7). However, it is possible that the stimulation of linoleic acid metabolism and a resultant decrease in the proportion of linoleic acid in phospholipids are more directly associated with the hypercholesterolemic action of methionine than the increase in the PC/PE ratio, since dietary linoleic acid is known to have a hypocholesterolemic action. In the present study, the depression of linoleic acid metabolism by the low-methionine diet was found to reflect mainly in the increase in the proportion of 16:0-18:2 molecular species of plasma PC (Table 4). This was also the case for the eritadenine-induced alteration of PC molecular species, although the extent of the effect of eritadenine was greater than that of dietary methionine. It was shown that the uptake rate of reconstituted HDL cholesterol by perfused rat livers was most stimulated by 16:0-18:2 PC of five PC molecular species used (16:0-18:2, 16:1-16:1, 18:0-18:2, 18:1-16:0, or 20:1-20:1) for the reconstitution of HDL (37). This PC molecular species-dependent differential uptake of lipoprotein cholesterol is thought to be mediated by hepatic lipase, since the hydrolysis of HDL phospholipids by the phospholipase A_1 activity of hepatic lipase is shown to be necessary for the subsequent uptake of HDL constituents (38), and since the 16:0-18:2 PC is the most preferred substrate for hepatic lipase (37). The uptake of the other lipoproteins, such as remnants of chylomicron (39) and VLDL (40), is also supposed to be mediated by hepatic lipase. Therefore, it is expected that the treatment to increase the proportion of 16:0-18:2 molecular species of plasma lipoprotein PC should enhance the cholesterol uptake of certain types of lipoproteins by the liver. Along this line, it seems reasonable to consider that dietary methionine level affects the phospholipid molecular species composition of plasma lipoproteins, thereby altering the uptake rate of lipoprotein cholesterol by the liver. This is also the case for the hypocholesterolemic action of eritadenine. In support of this, a significantly negative correlation was observed between the proportion of plasma 16:0-18:2 PC and the plasma total cholesterol concentration in rats fed six diets differing in methionine level and with or without eritadenine supplementation (Fig. 5).

Although 18:0-20:4 PC also had a significantly positive correlation with the plasma cholesterol concentration, it is currently unclear whether 18:0-20:4 PC exerts as a hypercholesterolemic factor. On the other hand, it has been shown that soybean protein increased the activity of lipoprotein receptor for β-VLDL in liver cells of rats fed a cholesterol-enriched diet (41). Hence, another mechanism is that a low-methionine diet may enhance the activity of lipoprotein receptors, thereby accelerating the uptake of plasma lipoprotein cholesterol by the liver. With regard to this, it is interesting that increased fluidity of LDL receptor membranes has been postulated to participate in the increase in LDL receptor activity in cynomolgus monkeys fed a diet rich in linoleic acid (42). Therefore, the possibility that dietary methionine level may affect the proportion of linoleic acid in lipoprotein receptor membrane phospholipids, in addition to plasma lipoprotein phospholipids, thereby possibly affecting lipoprotein receptor activities, cannot be ruled out. The effect of dietary methionine level on the activity of lipoprotein receptors in liver cell membranes remains to be further investigated.

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