ELSEVIER

Amino acid composition of dietary proteins affects plasma cholesterol concentration through alteration of hepatic phospholipid metabolism in rats fed a cholesterol-free diet

Kimio Sugiyama, Hironori Kanamori, Toshiyuki Akachi, and Akihiro Yamakawa

Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan

This study was designed to investigate the relationship between the amino acid composition of dietary proteins and the plasma cholesterol concentration and to examine whether the alteration of hepatic phospholipid metabolism participates in the effect of dietary proteins in rats fed a cholesterol-free diet. There was a significant positive correlation between the plasma total cholesterol concentration and the plasma concentration of methionine and valine and the hepatic concentration of valine and alanine in rats fed seven types of proteins. In contrast, the plasma cholesterol concentration exhibited a significant negative correlation with the hepatic-free ethanolamine concentration. As far as methionine and ethanolamine, their positive and negative correlation with the plasma cholesterol concentration was consistent with their known hyper- and hypocholesterolemic effects, respectively. The phospholipid profile as represented by the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in liver microsomes, but not in other tissues tested, was largely influenced by the type of dietary proteins; the PC/PE ratio exhibited a significant positive correlation with the plasma total cholesterol concentration. There was a significant correlation between the methionine content of dietary proteins and several biochemical variables including plasma cholesterol and liver microsomal PC/PE ratio. A significantly lower concentration of hepatic S-adenosylmethionine was observed with soybean protein diet than with casein diet suggesting a decrease in the PC biosynthesis via the PE N-methylation pathway in rats fed soybean protein. From these results, it is suggested that the plasma cholesterol concentration might be influenced by the methionine content of dietary proteins at least in part through an alteration of hepatic phospholipid metabolism. (J. Nutr. Biochem. 7:40–48, 1996.)

Keywords: dietary proteins; plasma cholesterol; phospholipid metabolism; methionine; ethanolamine; phosphatidylethanolamine N-methylation

Introduction

It is confirmed that the plasma cholesterol concentration can be affected by the type of dietary proteins.[1–3] The ingestion of plant proteins, as compared with animal proteins, generally results in lower plasma concentrations in various species of experimental animals. The hypocholesterolemic action of soybean protein was also demonstrated in humans.[4] A number of explanations have so far been provided for the differential effects of plant and animal proteins. These explanations can be divided into two categories: one is based on a difference in the amino acid composition of dietary proteins and the other is based on a difference in the physicochemical properties of dietary proteins or luminal digestion products. The former concerns whether specific amino acid(s) of dietary proteins affect, after being absorbed, the metabolism of cholesterol either directly or indirectly. The latter concerns whether the extent of intestinal absorption or

Address reprint requests to Dr. Kimio Sugiyama at the Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422, Japan.
Received March 20, 1995; accepted August 25, 1995.

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
SSDI 0955-2863(95)00165-4

reabsorption of cholesterol and/or bile acids is influenced by the type of dietary proteins. However, the detailed mechanism by which different types of dietary proteins differentially affect the plasma cholesterol concentration has not yet been fully elucidated.

The explanation based on the amino acid composition seems more attractive than the alternative one in terms of protein nutrition since the former can provide, if it is true, a general rule regardless of the origin of dietary proteins. Previously we reported that a significant negative correlation could be observed between the plasma cholesterol concentration and the cystine content of dietary proteins in rats fed cholesterol-enriched diets containing one of seven types of proteins.[5] The results were consistent with the observation that cystine supplementation of the diet containing exogenous cholesterol and cholate resulted in a reduction of plasma cholesterol in rats.[6,7] However, several reports have shown that cystine exhibited a hypercholesterolemic effect rather than a hypocholesterolemic effect when added to cholesterol-free diets at relatively high levels.[8,9] These results suggest that under different dietary conditions, e.g., the presence or absence of exogenous cholesterol in the diet, different mechanisms may operate in the regulation of the plasma cholesterol concentration by dietary proteins.

The importance of amino acid composition of dietary proteins has been noted not only in rats fed cholesterol-enriched diets, but also in rats fed cholesterol-free diets. Sautier et al.[10] reported that there was a significant correlation between the serum cholesterol concentration and the content of tyrosine, glutamic acid, cystine, or alanine in dietary proteins in rats fed cholesterol-free diets containing one of four types of proteins. They also reported that the serum cholesterol concentration could be significantly correlated with the content of proline, methionine, or alanine in dietary proteins when eight types of dietary proteins were used.[11] The difference in the amino acid composition of dietary proteins can be reflected in the free amino pattern of plasma and some tissues either directly or indirectly. Based on this assumption, the relationship between the concentrations of plasma cholesterol and plasma-free amino acids have been studied in humans,[12] minipigs,[13] rabbits,[14] and rats.[15] Nonetheless, it appears that conclusive results have not yet been obtained.

Recently, we provided evidence for the possibility that the alteration of hepatic phospholipid metabolism might be associated with the hypocholesterolemic action of certain substances, such as glycine,[16] *Lentinus edodes* mushroom,[17] and eritadenine[18,19] in rats fed a cholesterol-free diet. A preliminary report by our laboratory suggested that this might be also the case for the hypocholesterolemic action of soybean protein.[20]

This study was designed to determine whether the plasma cholesterol concentration can be significantly correlated with the concentration of specific amino acid(s) in the plasma and liver and whether the phospholipid profile of liver microsomes can be significantly correlated with the plasma cholesterol concentration in rats fed cholesterol-free diets containing one of seven types of plant and animal proteins. In this study, attention was given to the concentration of free ethanolamine as well as free amino acids since a previous study showed that the hepatic-free ethanolamine concentration was sensitive to certain types of hypocholesterolemic treatment.[16]

Methods and materials

Materials

S-[methyl-^{14}C]adenosyl-L-methionine and phospho[methyl-^{14}C]choline were obtained from Amersham Japan (Tokyo, Japan). S-adenosyl-L-methionine (SAM), S-adenosyl-L-homocysteine (SAH), and phosphocholine were obtained from Sigma Chemical (St. Louis, MO, USA), and silica gel plates (silica gel 60) were obtained from E. Merck (Darmstadt, Germany). The mineral and vitamin mixtures (Harper's composition[21]) were obtained from Oriental Yeast (Tokyo, Japan).

Animals and diets

Male rats of the Wistar strain weighing 90 to 100 g (5 weeks of age) were obtained from Japan SLC (Hamamatsu, Japan). They were housed individually in stainless steel wire-mesh cages in a temperature (24 ± 1°C) and humidity (50 to 60%)-controlled room with a 12 hr cycle of light (6:00 a.m. to 6:00 p.m.) and dark. Animals were acclimated to the facility for 5 to 6 days and given free access to the powdered stock diet which resembled the casein diet described below except that sucrose was replaced by corn starch and water.

In Experiment 1, 42 rats were randomly divided into seven groups of 6 rats each with similar initial mean weights and given free access to experimental diets, which contained one of seven types of proteins, and water for 14 days. The diet contained (g/kg of diet) 250 g of casein or an isonitrogenous amount of another protein, 200 g of sucrose, 50 g of corn oil, 50 g of mineral mixture, 10 g of vitamin mixture, 2 g of choline chloride, and corn starch to make up 1,000 g. The mineral mixture provided the following (g/kg of diet): $CaHPO_4 \cdot 2H_2O$, 0.22; KH_2PO_4, 17.2; NaCl, 12.5; Fe-citrate, 0.31; $MgSO_4 \cdot 7H_2O$, 5.0; $ZnCl_2$, 0.01; $MnSO_4 \cdot 4\text{~}5H_2O$, 0.061; $CuSO_4 \cdot 5H_2O$, 0.078; KI, 0.0003; $CaCO_3$, 14.7; and $(NH4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0013. The vitamin mixture provided the following (mg/kg of diet): retinyl acetate, 1.6; cholecalciferol, 0.06; all-*rac*-α-tocopheryl acetate, 120; menadione, 0.6; thiamine · HCl, 6; riboflavin, 6; pyridoxine · HCl, 3; cyanocobalamin, 0.02; L-ascorbic acid, 59; D-biotin, 0.1; folic acid, 0.2; calcium pantothenate, 24, nicotinic acid, 29; and inositol, 118.

The nitrogen content (g/100 g of protein), as measured by the Kjeldahl procedure, of casein (Nacalai Tesque; Kyoto, Japan), lactalbumin (Tokyo Kasei, Tokyo, Japan), whole egg protein (Oriental Yeast), egg albumin (Wako Pure Chemical, Osaka, Japan), sardine protein (Niigata Tekkosho, Tokyo, Japan), soybean protein (Fuji Oil, Osaka, Japan), and wheat gluten (Shinshin Shokuryo, Tokyo, Japan) was 14.2, 12.8, 14.4, 13.1, 14.4, 14.2, and 12.3, respectively. When wheat gluten was used as a protein source, the diet was fortified with lysine hydrochloride at a level of 7 g/kg of diet at the expense of protein in order to avoid growth retardation due to lysine deficiency.

In Experiment 2, the effects of selected proteins, casein, and soybean protein on phospholipid metabolism were compared in more detail since the comparative effects of these proteins on cholesterol metabolism have so far been extensively studied. Sixteen rats were divided into two groups of 8 rats each and given free access to the diets containing casein or soybean protein as described above and water for 14 days. The body weight and food consumption of the animals were measured daily in both experiments.

Tissue collection and fractionation

Rats were killed by decapitation under light anesthesia with ethylether between 11:00 a.m. and 12:00 a.m. without prior food deprivation. Blood plasma was collected from heparinized whole blood by centrifugation at 2,000*g* for 20 min at 4°C. An aliquot of the plasma was stored at 4°C until subsequent analyses for plasma lipids, and in Experiment 1 the residual plasma was stored at −30°C until it was analyzed for free amino acids.

The whole liver was quickly removed, rinsed in ice-cold saline, blotted on filter paper, and cut into two portions. One portion of the liver was quickly frozen in liquid nitrogen and stored at −80°C until analysis for free amino acids (Experiment 1) or metabolites of methionine (Experiment 2). The other portion of the liver was homogenized in 4 vol (vol/wt) of an ice-cold 10 mmol/L of Tris-HCl buffer (pH 7.4) containing 150 mmol/L of KCl. An aliquot of the liver homogenate was stored at −30°C until analyses for liver lipids. The residual portion of the homogenate was centrifuged at 10,000*g* for 10 min at 4°C, and the resultant supernatants were further centrifuged at 105,000*g* for 60 min at 4°C to obtain a microsomal fraction as a precipitate. The microsomal fraction was resuspended in the homogenizing buffer and stored at −80°C until analyses for phospholipid composition and enzyme activities (Experiment 2) were performed. In Experiment 2, several tissues, such as brain, lung, kidney, heart, and muscle (gastrocnemius), were also sampled, and microsomal fractions were similarly prepared from these tissues.

Biochemical analysis

The plasma concentrations of total cholesterol, HDL cholesterol, triglycerides, and phospholipids were measured enzymatically with kits: Cholesterol C-Test, HDL Cholesterol Test, Triglyceride G-Test, and Phospholipid B-Test, respectively (Wako Pure Chemical). The difference between total cholesterol and high density lipoprotein (HDL) cholesterol was assumed to be the cholesterol associated with very low density lipoprotein (VLDL) + low density lipoprotein (LDL). The lipids of liver homogenates and liver microsomes were extracted according to Folch et al.[22] The cholesterol, triglycerides, and phospholipids in the liver extracts were measured according to Zak,[23] Fletcher,[24] and Bartlett,[25] respectively. The phospholipids in the extracts of liver microsomes were separated into each class by thin-layer chromatography (TLC) with silica gel 60, using chloroform-methanol-water (65:25:4, vol/vol) as a developing solvent. The bands of each phospholipid class in the silica gel plate were visualized with iodine vapor, scraped off, and directly analyzed for inorganic phosphorus.[25] Hepatic concentrations of SAM and SAH were estimated by high performance liquid chromatography (HPLC) according to Cook et al.[26] with some modifications as described previously.[18]

The free amino acids and free ethanolamine in the plasma and liver were estimated as follows. An aliquot of blood plasma was deproteinized by adding an equal volume of sulfosalicylic acid solution (0.28 mol/L) and then centrifuged at 10,000*g* for 10 min. One portion of the liver was homogenized in 4 volumes of sulfosalicylic acid solution (0.17 mol/L) and likewise centrifuged. The deproteinized supernatants were analyzed for free amino acids together with free ethanolamine with an amino acid autoanalyzer (Hitachi Seisakusho, Model 835; Tokyo, Japan). The activity of phosphatidylethanolamine (PE) N-methyltransferase (EC 2.1.1.17) in liver microsomes was measured according to Tanaka et al.[27] except that a higher substrate concentration (100 μmol/L of SAM) was used. The activity of CTP:phosphocholine cytidyryltransferase (EC 2.7.7.15) in liver microsomes was measured according to Wright et al.[28] Protein was measured according to Lowry et al.[29] using bovine serum albumin as a standard.

Statistical analysis

In Experiment 1, data were analyzed by the one-way analysis of variance, and the differences between means were tested at $P < 0.05$ using Duncan's multiple range test when the F value was significant at $P < 0.05$. In Experiment 2, the Student's t-test was used to examine the significance between the two experimental groups. The correlation coefficient between variables was obtained by linear regression analysis using mean values of each group since the significance of correlation can be rigorously examined by decreasing the number of data pairs.

Results

Table 1 shows the body weight gain, food intake, liver weight, and liver lipid concentrations in rats fed seven types of dietary proteins. The egg albumin diet caused a slight depression of both growth and food intake as compared with most of the other protein diets. The relative liver weight was significantly lower with soybean protein diet than with the other protein diets. The hepatic cholesterol concentration was slightly but significantly increased with lactalbumin and wheat gluten diets. The hepatic triglyceride concentration was significantly higher in rats fed wheat gluten than in rats fed the other proteins. The hepatic phospholipid concentration was significantly higher in those rats fed lactalbumin, soybean protein, and wheat gluten than in rats fed the other proteins.

Table 2 summarizes the effects of different dietary proteins on plasma lipid concentrations. The plasma total cholesterol concentration was significantly lower in those rats

Table 1 Body weight gain, food intake, liver weight, and liver lipid concentrations in rats fed seven types of protein sources (Experiment 1)*

Dietary group	Body weight gain (g/14 days)	Food intake (g/14 days)	Liver weight (g/100 g of body weight)	Liver lipids (μmol/g of liver)		
				Cholesterol	Triglycerides	Phospholipids
Casein	77 ± 2^{ab}	186 ± 3^{b}	5.17 ± 0.10^{a}	8.4 ± 0.2^{c}	17.7 ± 0.6^{b}	28.7 ± 1.0^{c}
Lactalbumin	73 ± 2^{bc}	182 ± 2^{b}	5.18 ± 0.10^{a}	9.0 ± 0.2^{b}	14.9 ± 1.1^{bc}	33.1 ± 0.8^{b}
Whole egg protein	82 ± 2^{a}	183 ± 1^{b}	4.91 ± 0.08^{ab}	8.1 ± 0.1^{c}	16.5 ± 1.1^{b}	29.9 ± 0.6^{c}
Egg albumin	69 ± 2^{c}	169 ± 4^{c}	4.77 ± 0.07^{bc}	8.3 ± 0.1^{c}	12.5 ± 0.1^{cd}	30.5 ± 0.5^{c}
Sardine protein	82 ± 2^{a}	186 ± 2^{b}	4.95 ± 0.08^{ab}	8.0 ± 0.1^{c}	15.4 ± 0.6^{b}	30.2 ± 0.6^{c}
Soybean protein	78 ± 2^{ab}	202 ± 4^{a}	4.49 ± 0.12^{c}	8.5 ± 0.1^{bc}	9.8 ± 0.5^{d}	34.4 ± 0.5^{ab}
Wheat gluten	82 ± 3^{a}	182 ± 5^{b}	4.87 ± 0.13^{ab}	9.6 ± 0.3^{a}	31.1 ± 1.7^{a}	35.4 ± 0.4^{a}

*Values are mean ± SEM for six rats. Values in a column not sharing the same superscript letter are significantly different at $P < 0.05$.

Table 2 Effects of different dietary protein sources on plasma lipid concentrations in rats (Experiment 1)*

Dietary group	Plasma lipids (mmol/L)				
	Total cholesterol	VLDL + LDL cholesterol	HDL cholesterol	Triglycerides	Phospholipids
Casein	2.58 ± 0.06^{a}	0.82 ± 0.04^{a}	1.76 ± 0.04^{a}	1.86 ± 0.17^{ab}	2.57 ± 0.06^{ab}
Lactalbumin	1.91 ± 0.05^{b}	0.61 ± 0.04^{b}	1.30 ± 0.05^{b}	2.13 ± 0.21^{a}	2.32 ± 0.04^{c}
Whole egg protein	2.41 ± 0.08^{a}	0.68 ± 0.03^{ab}	1.73 ± 0.07^{a}	1.97 ± 0.22^{ab}	2.51 ± 0.04^{abc}
Egg albumin	2.39 ± 0.09^{a}	0.57 ± 0.07^{b}	1.82 ± 0.05^{a}	1.41 ± 0.18^{b}	2.41 ± 0.07^{bc}
Sardine protein	2.55 ± 0.09^{a}	0.81 ± 0.03^{a}	1.75 ± 0.07^{a}	1.86 ± 0.23^{ab}	2.68 ± 0.10^{a}
Soybean protein	1.44 ± 0.07^{c}	0.35 ± 0.05^{c}	1.09 ± 0.02^{c}	1.44 ± 0.16^{b}	1.90 ± 0.08^{d}
Wheat gluten	1.71 ± 0.11^{b}	0.54 ± 0.08^{b}	1.17 ± 0.03^{bc}	2.31 ± 0.15^{a}	2.26 ± 0.10^{c}

*Values are mean ± SEM for six rats. Values in a column not sharing the same superscript letter are significantly different at $P < 0.05$.

fed lactalbumin, soybean protein, and wheat gluten than in rats fed the other proteins. The plasma HDL cholesterol concentration was likewise decreased by these proteins, whereas the effect of dietary proteins on the VLDL + LDL concentration was less obvious except for the effect of soybean protein. The plasma phospholipid concentration was significantly lower with soybean protein diet than with the other protein diets.

Table 3 shows the effects of dietary proteins on the concentration of plasma-free amino acids and free ethanolamine. The concentration of cysteine could not be measured because of its low concentration in the plasma. Plasma concentrations of most of the amino acids were influenced by the type of dietary proteins. When the relationship between the plasma total cholesterol concentration and the concentration of each amino acid was analyzed by linear regression analysis, a significantly positive correlation was detected between the plasma cholesterol concentration and the plasma methionine or valine concentration.

Table 4 shows the effects of dietary proteins on the hepatic concentration of free amino acids and free ethanolamine. The arginine concentration could not be measured because of its low concentration in the liver. Hepatic concentrations of all the amino acids were significantly influenced by the type of dietary proteins. The plasma total cholesterol concentration had a significant negative correlation with the hepatic free ethanolamine concentration and a significantly positive correlation with the hepatic valine or alanine concentration.

Table 5 shows the effects of dietary proteins on the concentration and composition of major phospholipid classes in liver microsomes. Both the PE concentration and the pro-

Table 3 Effects of different dietary protein sources on plasma free amino acid concentrations in rats (Experiment 1)*

Amino acid	Dietary group (μmol/L)							r†
	Casein	Lacto albumin	Whole egg protein	Egg albumin	Sardine protein	Soybean protein	Wheat gluten	
Tau	172 ± 23^{b}	292 ± 10^{a}	309 ± 17^{a}	303 ± 19^{a}	258 ± 22^{a}	145 ± 17^{b}	246 ± 29^{a}	0.363
Asp	30 ± 1^{b}	34 ± 1^{ab}	42 ± 2^{ab}	41 ± 1^{ab}	37 ± 5^{ab}	51 ± 12^{a}	37 ± 5^{ab}	−0.541
Thr	364 ± 12^{abc}	430 ± 19^{ab}	328 ± 6^{bc}	274 ± 18^{c}	403 ± 31^{ab}	449 ± 75^{a}	153 ± 8^{d}	0.000
Ser	228 ± 7^{bcd}	196 ± 5^{d}	241 ± 1^{bc}	253 ± 17^{b}	206 ± 9^{cd}	317 ± 24^{a}	265 ± 6^{b}	−0.639
Glu	766 ± 21^{b}	676 ± 31^{b}	680 ± 23^{b}	703 ± 12^{b}	754 ± 48^{b}	799 ± 75^{b}	922 ± 13^{a}	−0.485
Pro	546 ± 16^{b}	223 ± 3^{cd}	187 ± 8^{d}	207 ± 8^{d}	236 ± 13^{cd}	264 ± 21^{c}	642 ± 33^{a}	−0.152
Gly	85 ± 3^{c}	105 ± 5^{c}	129 ± 1^{b}	153 ± 4^{a}	162 ± 3^{a}	176 ± 17^{a}	163 ± 5^{a}	−0.458
Ala	585 ± 29^{bc}	584 ± 20^{abc}	565 ± 17^{bc}	601 ± 40^{abc}	687 ± 39^{a}	530 ± 41^{c}	654 ± 20^{ab}	0.341
Val	389 ± 17^{ab}	239 ± 13^{cd}	379 ± 8^{ab}	418 ± 15^{a}	335 ± 42^{b}	259 ± 7^{c}	192 ± 9^{d}	0.827‡
Met	68 ± 2^{b}	54 ± 5^{c}	80 ± 2^{a}	81 ± 3^{a}	83 ± 5^{a}	36 ± 1^{d}	44 ± 3^{cd}	0.936§
Ile	160 ± 6^{a}	127 ± 5^{bc}	169 ± 2^{a}	173 ± 5^{a}	159 ± 18^{a}	148 ± 6^{ab}	116 ± 6^{c}	0.693
Leu	256 ± 9^{a}	266 ± 13^{a}	231 ± 3^{ab}	228 ± 9^{ab}	232 ± 28^{ab}	195 ± 8^{bc}	170 ± 7^{c}	0.599
Tyr	154 ± 3^{ab}	93 ± 3^{d}	144 ± 5^{bc}	155 ± 2^{ab}	121 ± 6^{c}	170 ± 16^{a}	137 ± 9^{bc}	−0.082
Phe	64 ± 3	55 ± 2	60 ± 1	61 ± 1	61 ± 6	62 ± 2	59 ± 2	0.341
EA	43 ± 3	47 ± 2	47 ± 3	41 ± 3	44 ± 7	55 ± 6	41 ± 5	−0.565
Lys	626 ± 12^{b}	784 ± 27^{a}	556 ± 7^{b}	575 ± 8^{b}	792 ± 63^{a}	567 ± 38^{b}	358 ± 10^{c}	0.388
His	86 ± 3^{a}	76 ± 3^{ab}	76 ± 2^{ab}	72 ± 1^{ab}	68 ± 8^{b}	74 ± 6^{ab}	79 ± 2^{ab}	0.015
Arg	108 ± 2^{c}	110 ± 7^{c}	182 ± 2^{a}	156 ± 1^{b}	188 ± 14^{a}	195 ± 15^{a}	133 ± 2^{bc}	−0.078

*Values are means ± SEM for three determinations of the pooled samples from two rats. Values in a row not sharing the same superscript letter are significantly different at $P < 0.05$.
†Correlation coefficient with the plasma total cholesterol concentration.
‡§A significant correlation at $P < 0.05$ and $P < 0.01$, respectively.
EA, ethanolamine.

Table 4 Effects of different dietary protein sources on hepatic free amino acid concentrations in rats (Experiment 1)*

Amino acid	Dietary group (nmol/g of liver)							r†
	Casein	Lact albumin	Whole egg protein	Egg albumin	Sardine protein	Soybean protein	Wheat gluten	
Tau	10,500 ± 574^{c}	14,800 ± 471^{a}	15,100 ± 238^{a}	16,000 ± 164^{a}	13,500 ± 454^{b}	3,400 ± 329^{d}	12,300 ± 704^{b}	0.602
Asp	1,380 ± 36^{ab}	1,070 ± 31^{c}	1,210 ± 113^{bc}	1,610 ± 125^{a}	1,310 ± 26^{bc}	1,210 ± 51^{bc}	1,270 ± 106^{bc}	0.482
Thr	363 ± 12^{cd}	422 ± 30^{ab}	333 ± 5^{d}	276 ± 12^{e}	388 ± 13^{bc}	456 ± 18^{a}	132 ± 19^{f}	0.006
Ser	478 ± 16^{c}	333 ± 19^{d}	449 ± 12^{c}	428 ± 24^{c}	423 ± 19^{c}	1,160 ± 25^{a}	590 ± 32^{b}	–0.708
Glu	8,950 ± 219^{a}	6,480 ± 141^{d}	7,590 ± 91^{c}	8,080 ± 187^{bc}	7,470 ± 168^{c}	8,240 ± 139^{b}	8,570 ± 326^{ab}	0.021
Pro	272 ± 20^{b}	106 ± 7^{c}	102 ± 12^{c}	95 ± 8^{c}	95 ± 14^{c}	141 ± 15^{c}	358 ± 3^{a}	–0.241
Gly	875 ± 54^{c}	617 ± 21^{d}	922 ± 29^{c}	883 ± 57^{c}	1,300 ± 22^{b}	1,920 ± 86^{a}	903 ± 28^{c}	–0.428
Ala	3,880 ± 107^{ab}	3,550 ± 183^{bc}	3,790 ± 187^{bc}	4,000 ± 216^{ab}	4,460 ± 112^{a}	3,220 ± 205^{c}	3,630 ± 227^{bc}	0.843‡
Val	334 ± 6^{a}	219 ± 12^{bc}	341 ± 7^{a}	361 ± 10^{a}	266 ± 10^{b}	179 ± 33^{cd}	148 ± 27^{d}	0.856‡
Cys	66 ± 6^{b}	58 ± 4^{b}	56 ± 4^{b}	52 ± 2^{b}	90 ± 8^{a}	66 ± 2^{b}	56 ± 4^{b}	0.282
Met	32 ± 1^{cd}	47 ± 3^{a}	47 ± 2^{a}	39 ± 1^{b}	31 ± 1^{d}	27 ± 1^{d}	37 ± 2^{bc}	0.153
Ile	155 ± 4^{bc}	124 ± 5^{d}	175 ± 5^{a}	180 ± 6^{a}	140 ± 6^{c}	157 ± 4^{b}	107 ± 6^{e}	0.445
Leu	279 ± 5^{ab}	297 ± 14^{a}	285 ± 5^{a}	287 ± 9^{a}	248 ± 8^{c}	257 ± 6^{bc}	197 ± 10^{d}	0.404
Tyr	123 ± 5^{a}	72 ± 3^{c}	131 ± 2^{a}	127 ± 3^{a}	86 ± 4^{bc}	143 ± 15^{a}	101 ± 3^{b}	–0.067
Phe	65 ± 1^{a}	63 ± 2^{ab}	66 ± 1^{a}	60 ± 3^{abc}	52 ± 1^{c}	62 ± 4^{ab}	56 ± 4^{bc}	0.012
EA	124 ± 21^{c}	257 ± 28^{b}	150 ± 11^{c}	145 ± 12^{c}	160 ± 18^{c}	405 ± 48^{a}	317 ± 22^{b}	–0.983§
Lys	473 ± 9^{c}	477 ± 22^{c}	565 ± 11^{b}	633 ± 13^{a}	549 ± 31^{b}	609 ± 24^{ab}	466 ± 23^{c}	0.012
His	761 ± 4^{a}	628 ± 16^{c}	690 ± 9^{b}	715 ± 15^{ab}	683 ± 12^{b}	698 ± 18^{b}	629 ± 27^{c}	0.546

*Values are mean ± SEM for three determinations of the pooled samples from two rats. Values in a row not sharing the same superscript letter are significantly different at $P < 0.05$.
†Correlation coefficient with the plasma total cholesterol concentration.
‡§A significant correlation at $P < 0.05$ and $P < 0.001$, respectively.
EA, ethanolamine.

portion of PE to the total phospholipids were significantly higher in those rats fed lactalbumin, soybean protein, and wheat gluten than in rats fed the other proteins. Inversely, the proportion of phosphatidylcholine (PC) to the total phospholipids was significantly decreased by these proteins although the effects of dietary proteins on the concentration of PC and total phospholipids were not obvious. Consequently the PC/PE ratio was significantly lower in those rats fed lactalbumin, soybean protein, and wheat gluten than in rats fed the other proteins. The plasma total cholesterol concentration had a significant negative correlation with the concentration or composition of PE and a significantly positive correlation with the composition of PC or with the PC/PE ratio.

Table 6 shows the comparative effects of casein and soybean protein diets on the composition of each phospholipid class in blood plasma and microsomes from various tissues. The proportions of PC and PE to the total phospholipids were differentially influenced by the two protein diets only in the liver of various tissues tested. The PC/PE ratio was also differentially influenced only in the liver. The other phospholipid classes were least affected by the two diets in all the tissues tested.

Table 7 shows the hepatic concentrations of SAM and SAH, and the activities of rate-limiting enzymes for the PC biosynthesis via the PE N-methylation and CDP-choline pathways in liver microsomes of rats fed casein and soybean protein diets. Feeding the soybean protein diet, as compared

Table 5 Effects of different dietary protein sources on the concentration and composition of phospholipids in liver microsomes of rats (Experiment 1)*

Dietary group	Concentration (nmol/mg of protein)			Composition (mol %)		PC/PE (mol/mol)
	PC	PE	Total	PC	PE	
Casein	252 ± 9^{ab}	66 ± 4^{b}	372 ± 15^{b}	67.7 ± 0.5^{a}	17.7 ± 0.4^{e}	3.84 ± 0.12^{a}
Lactalbumin	259 ± 12^{ab}	107 ± 5^{a}	428 ± 21^{a}	60.5 ± 0.2^{b}	25.0 ± 0.2^{c}	2.42 ± 0.02^{c}
Whole egg protein	266 ± 5^{a}	73 ± 2^{b}	396 ± 6^{ab}	67.1 ± 0.5^{a}	18.5 ± 0.4^{de}	3.64 ± 0.09^{ab}
Egg albumin	264 ± 9^{ab}	76 ± 2^{b}	395 ± 12^{ab}	66.9 ± 0.3^{a}	19.2 ± 0.3^{d}	3.49 ± 0.07^{b}
Sardine protein	252 ± 10^{ab}	72 ± 4^{b}	377 ± 16^{b}	66.9 ± 0.5^{a}	19.0 ± 0.5^{d}	3.54 ± 0.12^{b}
Soybean protein	239 ± 11^{ab}	107 ± 6^{a}	410 ± 19^{ab}	58.2 ± 0.2^{c}	26.1 ± 0.2^{b}	2.23 ± 0.02^{cd}
Wheat gluten	236 ± 5^{b}	114 ± 3^{a}	410 ± 8^{ab}	57.5 ± 0.4^{c}	27.9 ± 0.5^{a}	2.07 ± 0.05^{d}
r†	0.671	–0.941§	–0.785‡	0.966¶	–0.944§	0.955¶

*Values are mean ± SEM for six rats. Values in a column not sharing the same superscript letter are significantly different at $P < 0.05$.
†Correlation coefficient with plasma total cholesterol concentration.
‡§¶A significant correlation at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
PC, phsphatidylcholine; PE, phosphatidylethanolamine.

Table 6 Effects of casein and soybean protein diets on the phospholipid composition of plasma and microsomes from various tissues in rats (Experiment 2)*

Tissue	Dietary group	Phospholipid composition (mol %)						PC/PE (mol/mol)
		PC	PE	PI + PS	SM	LPC	CL	
Liver	Cas	63.0 ± 0.4	19.4 ± 0.2	10.3 ± 0.1	3.4 ± 0.1	ND	ND	3.25 ± 0.06
	Soy	52.8 ± 0.6‡	27.9 ± 0.4‡	10.2 ± 0.3	4.1 ± 0.3	ND	ND	1.89 ± 0.04‡
Kidney	Cas	41.7 ± 0.3	28.4 ± 0.2	11.6 ± 0.1	15.3 ± 0.5	ND	ND	1.47 ± 0.02
	Soy	42.4 ± 0.5	28.9 ± 0.2	11.5 ± 0.2	15.0 ± 0.5	ND	ND	1.47 ± 0.01
Heart	Cas	41.8 ± 0.3	36.2 ± 0.3	5.2 ± 0.1	3.5 ± 0.2	1.9 ± 0.2	7.6 ± 0.1	1.15 ± 0.01
	Soy	41.6 ± 0.3	36.6 ± 0.3	5.0 ± 0.1	3.3 ± 0.1	1.7 ± 0.2	7.5 ± 0.1	1.14 ± 0.01
Lung	Cas	57.4 ± 0.7	19.5 ± 0.3	8.2 ± 0.2	9.4 ± 0.2	ND	ND	2.95 ± 0.06
	Soy	57.8 ± 0.6	19.5 ± 0.2	8.3 ± 0.2	9.1 ± 0.2	ND	ND	2.96 ± 0.03
Muscle	Cas	58.4 ± 0.3	27.9 ± 0.3	6.7 ± 0.1	3.2 ± 0.1	1.4 ± 0.2	ND	2.09 ± 0.02
	Soy	58.0 ± 0.6	27.3 ± 0.3	6.5 ± 0.1	3.4 ± 0.1	1.4 ± 0.1	ND	2.13 ± 0.03
Brain	Cas	38.8 ± 0.3	35.1 ± 0.1	13.9 ± 0.2	5.4 ± 0.1	ND	1.4 ± 0.1	1.11 ± 0.01
	Soy	38.5 ± 0.5	34.7 ± 0.3	12.8 ± 0.7	5.0 ± 0.1†	ND	1.6 ± 0.1	1.11 ± 0.02
Plasma	Cas	56.7 ± 1.1	5.9 ± 0.3	3.8 ± 0.3	6.7 ± 0.4	19.8 ± 0.5	ND	9.78 ± 0.48
	Soy	55.4 ± 0.7	5.5 ± 0.2	3.2 ± 0.2	7.7 ± 0.3	21.9 ± 0.6†	ND	10.25 ± 0.59

*Values are mean ± SEM for eight rats. Raws do not total 100% as unidentified phospholipids (2 to 7%) were not included.
†‡A significant difference from the casein group at $P < 0.05$ and $P < 0.001$, respectively.
Cas, casein diet; Soy, soybean protein diet; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phassphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; CL, cardiolipin; ND, not detected.

with the casein diet, significantly decreased the hepatic SAM concentration by about 40%. However, both the SAH concentration and the SAM/SAH ratio were not significantly different between the two groups. The activity of PE N-methyltransferase was significantly higher in rats fed the soybean protein diet than in rats fed the casein diet. On the contrary, a lower activity of CTP:phosphocholine cytidyryltransferase was observed with the soybean diet than with the casein diet.

Discussion

One of the objectives of this study is to identify the amino acid(s), if any, by which the plasma cholesterol concentration is predominantly influenced in rats fed cholesterol-free diets containing different types of dietary proteins. The present study could demonstrate that the plasma cholesterol concentration had a significant correlation with some amino acids and free ethanolamine. These results, however, must be carefully interpreted together with the results obtained by other methods since the existence of a significant correlation does not necessarily indicate a sequence of cause and effect and since the plasma and liver concentrations of free amino acids measured at one point in time may be influenced by most recent ingestion of food. Several reports have shown that methionine elicits its hypercholesterolemic effect when added to cholesterol-free diets.[30–32] The hypocholesterolemic action of dietary ethanolamine was also demonstrated in rats.[33] Thus, as far as methionine and ethanolamine are concerned, the results of the correlation experiment in the present study appear to coincide with the results of other experiments reported previously.

The plasma methionine concentration is considered to reflect, if not entirely, the methionine content of dietary proteins, whereas the hepatic free ethanolamine concentration is considered to be influenced by the amino acid composition of dietary proteins indirectly. Since ethanolamine is a water-soluble precursor for PE biosynthesis via the CDP-ethanolamine pathway, the hepatic free ethanolamine concentration can be regarded as reflecting the synthetic rate or the amount of PE in the liver. A considerable part of PE synthesized in the liver further undergoes sequential methylation reactions catalyzed by PE N-methyltransferase, which uses SAM as the donor of methyl group, to yield PC.

Table 7 The hepatic concentrations of S-adenosylmethionine and S-adenosylhomocysteine and the activities of certain enzymes participating in phosphatidylcholine biosynthesis in liver microsomes of rats fed casein and soybean protein diets (Experiment 2)*

Dietary group	Concentration in the liver (nmol/g of liver)			Enzyme activity (pmol/min/mg of protein)	
	SAM	SAH	SAM/SAH	PEMT	CPCT
Casein	105 ± 5	14 ± 1	7.5 ± 0.6	118 ± 4	618 ± 28
Soybean protein	60 ± 4†	10 ± 2	6.2 ± 1.1	179 ± 2†	438 ± 15†

*Values are mean ± SEM for eight rats.
†A significant difference from the casein group is indicated at $P < 0.001$.
SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; PEMT, phosphatidylethanolamine N-methyltransferase; CPCT, CTP: phosphocholine cytidyryltransferase.

S-adenosylmethionine is the first metabolite of methionine metabolism via the trans-sulfuration pathway, and free ethanolamine can be liberated from PE by the reaction catalyzed either by base-exchange enzyme or phospholipases. Thus, ethanolamine is metabolically linked to methionine through a PE N-methylation reaction. These considerations, together with the experimental results obtained, support the idea that of all the amino acids constituting dietary proteins, methionine is the most effective amino acid at influencing the plasma cholesterol concentration in rats fed a cholesterol-free diet.

Figure 1 depicts the relationship between the methionine content of diets and certain biochemical variables. When the correlation was analyzed by linear regression analysis, there was a significant correlation between the dietary methionine content and each variable at $P < 0.05$. However, it is probable that the effect of dietary methionine content on these variables is not linear but is saturable as indicated by dotted lines in the figure. In any case, *Figure 1* suggests the possibility that these variables fluctuate in response to the methionine content of dietary proteins regardless of the origin of proteins.

At least two mechanisms are considered for the increase in PE concentration in liver microsomes induced by certain dietary proteins: a decrease in the consumption of PE due to the depression of the PE N-methylation reaction and a stimulation of PE biosynthesis via the CDP-ethanolamine pathway. The activity of PE N-methyltransferase is thought to be regulated by the enzyme substrates SAM and PE rather than the enzyme mass.[35,36] The K_m values for SAM of PE N-methyltransferase in rat liver microsomes for sequential three methylation reactions at pH 9.2 were estimated to be 51, 58, and 79 μmol/L, respectively.[37] Taken into consideration of these K_m values, it seems reasonable to consider that a decreased hepatic SAM concentration in rats fed soybean protein brought about a decrease in the activity of PE N-methyltransferase in vivo. This appears to be the case also for lactalbumin and wheat gluten since these proteins were found to decrease the hepatic SAM concentration in another experiment in our laboratory (unpublished data). It is considered that the decrease in hepatic SAM concentration by these proteins is due to the low methionine content of proteins.

The finding that PE was significantly increased by a soybean protein diet only in the liver, where an exclusively higher activity of PE N-methyltransferase is known to exist as compared with extrahepatic tissues, also supports that the activity change of PE N-methyltransferase is associated with the alteration of liver microsomal PE concentration. Although the in vitro activity of PE N-methyltransferase was rather increased with the soybean protein diet than with the casein diet, this is possibly ascribed to the increased PE concentration in liver microsomes as discussed previously.[18,36,38] The expanded pool size of hepatic free ethanolamine in rats fed certain types of dietary proteins can be taken to indicate that PE biosynthesis via the CDP-ethanolamine pathway is stimulated in these rats. Thus dual and accelerating mechanisms, i.e., a depression of PE N-methyltransferase followed by a stimulation of de novo PE biosynthesis, are postulated for the increase in liver microsomal PE induced by certain types of dietary proteins.

It was shown that the depression of PE N-methylation caused an increase in PC biosynthesis via the CDP-choline pathway in cultured rat hepatocytes treated with 3-deazaadenosine and that this compensatory increase in PC biosynthesis was followed by an increase in the activity of CTP-:phosphocholine cytidyryltransferase in rat liver microsomes.[39] Further, the microsomal cytidyryltransferase activity was shown to be increased in rats fed a choline-deficient diet.[40,41] These findings have been accounted for in terms of a feedback inhibition of cytidyryltransferase by PC.[42] The fact that the liver microsomal PC concentration, as compared with the PE concentration, was less influenced by the type of dietary proteins appears to be accounted for by the compensatory stimulation of PC biosynthesis via the CDP-choline pathway. Although the interpretation of the results obtained that soybean protein, as compared with casein, led to a lower activity of cytidyryltransferase is complicated, the results at least suggest that PC deficiency was not caused by the soybean protein diet.

Another objective of this study is to examine whether the alteration of the hepatic phospholipid profile is associated with the hypo- or hypercholesterolemic effect of dietary proteins in rats fed a cholesterol-free diet. Previously we provided a hypothetical rule that a variety of treatments to decrease the PC/PE ratio of liver microsomes necessarily results in a reduction of plasma cholesterol, although all of the hypocholesterolemic treatments do not necessarily decrease the PC/PE ratio.[18] The present study suggests that such a rule is applicable to the hypocholesterolemic effect of certain dietary proteins at least in rats fed a cholesterol-free diet. However, it is still unclear how the microsomal phospholipid profile participates in the regulation of the plasma cholesterol concentration except in the case of PC deficiency.

Since PC is the exclusively major phospholipid class of plasma lipoproteins, a decrease in the PC/PE ratio caused by a shortage of net PC biosynthesis impairs the assembly and secretion of VLDL from the liver[43] and thereby induces the development of fatty liver with overall decreases in plasma lipids including cholesterol. In the present study, lactalbumin and soybean protein did not cause fatty liver, suggesting that the hypocholesterolemic effects of these proteins are independent of the PC deficiency. In contrast, feeding a wheat gluten diet increased the liver triglyceride concentration. This increase in liver fat appears to be associated, at least in part, with threonine imbalance caused by lysine supplementation of the diet judging from decreased threonine concentrations in the plasma and liver.

Currently at least two processes are thought to participate in the hypocholesterolemic action of soybean protein: the secretion of lipoprotein cholesterol from the liver into the blood circulation and the uptake of plasma lipoprotein cholesterol by tissues. It has been shown that the secretion rate of lipoprotein cholesterol from the liver in rats fed a soybean protein diet is significantly lower than in rats fed a casein diet.[44,45] Further, it was pointed out that a decrease in the amount of hepatic cholesterol due to an enhanced loss of steroids into feces might be associated with the decrease in hepatic secretion of lipoprotein cholesterol.[44] In fact, a number of studies have shown that feeding soybean protein diets increases the fecal excretion of both neutral and acidic ste-

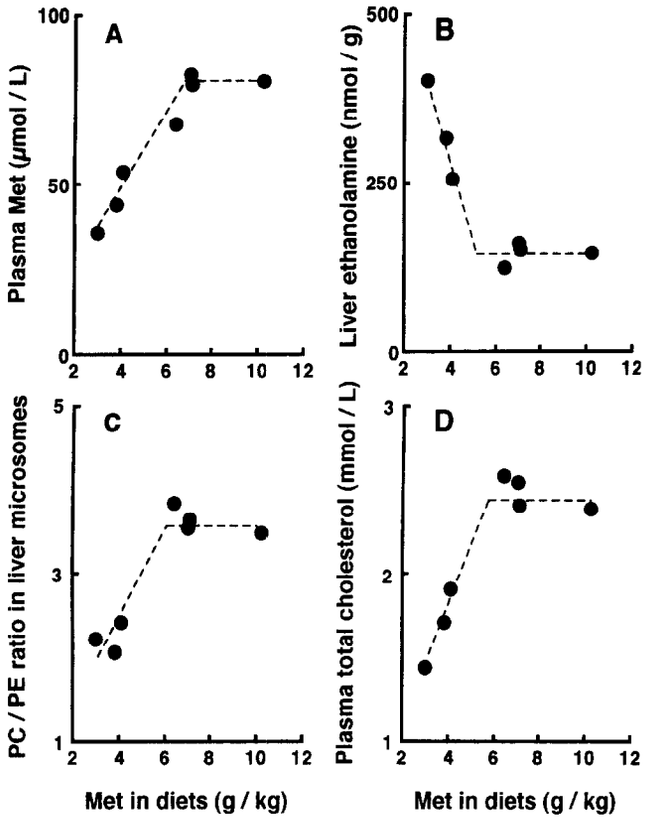

Figure 1 The relationship between the dietary methionine content and the plasma methionine concentration (A), the hepatic free ethanolamine concentration (B), the ratio of phosphatidylcholine to phoaphatidylethanolamine in liver microsomes (C), or the plasma total cholesterol concentration (D) in rats fed diets containing one of seven types of proteins (Experiment 1). The methionine content of diets (g/kg of diet) that was calculated based on the data from protein suppliers and a reference as described previously[34] was as follows: soybean protein diet, 3.0; wheat gluten diet, 3.8; lactalbumin diet, 4.1; casein diet, 6.4; sardine protein diet, 7.0; whole egg protein diet, 7.1; egg albumin diet, 10.2. Each circle denotes the mean value of each group. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

roids in rats. In contrast, Oda et al.[46] have shown that feeding a soybean protein diet caused a decrease in the biosynthesis of apolipoprotein A-I and thereby led to a reduction of HDL secretion from the liver in rats. In addition to these explanations, it seems possible that an altered hepatic phospholipid profile may be associated, in some way, with the decreased secretion of lipoprotein cholesterol in rats fed a soybean protein diet.

It was demonstrated that feeding a soybean protein diet, as compared with a casein diet, resulted in an increase in the turnover rate of plasma cholesterol in rabbits.[47] With regard to this, a higher activity of hepatic receptor for β-VLDL was observed with a soybean protein diet than with a casein diet in rats.[48] These findings suggest that the soybean protein diet brings about an increased uptake of plasma lipoprotein cholesterol by tissues. In addition to lipoprotein receptor activities, the nature of lipoprotein itself is thought to be important in influencing the uptake rate of lipoprotein cholesterol by tissues. Although the phospholipid class composition of plasma lipoproteins is known to be highly resistant to dietary manipulation, the molecular species composition of plasma phospholipids can be altered by certain types of dietary treatment. For instance, it was reported that the molecular species composition of plasma PC was significantly modified by dietary supplementation with clofibrate, a hypolipidemic drug, in rats.[49]

Kadowaki et al.[50] have demonstrated that the uptake rate of reconstituted HDL cholesterol by perfused rat livers was largely influenced by the difference in the PC molecular species included in that lipoprotein. In this context, it is interesting to note that the fatty acid composition of plasma PC and the activity of Δ6-desaturase in liver microsomes could be significantly influenced by dietary protein types in rats fed cholesterol-free diets.[51–53] Additionally, it was shown that the Δ6-desaturase activity in liver microsomes was modified by the microsomal concentration of PC in rats fed control and vitamin B6–deficient diets.[54] These findings suggest the possibility that the PC molecular species composition of plasma lipoproteins may be influenced by dietary protein types through an alteration of the PC/PE ratio of liver microsomes. The correlation between the plasma PC molecular species composition and the hypo- or hypercholesterolemic effect of dietary proteins appears to deserve further investigation.

References

1 Carroll, K.K. and Hamilton, R.M.G. (1975). Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. *J. Food Sci.* **40,** 18–23

2 Kritchevsky, D. (1979). Vegetable protein and atherosclerosis. *J. Am. Oil Chem. Soc.* **56,** 135–140

3 Terpstra, A.H.M., Hermus, R.J.J., and West, C.E. (1983). The role of dietary protein in cholesterol metabolism. *World Rev. Nutr. Diet* **42,** 1–55

4 Sirtori, C.R., Agradi, E., Mantero, O., Conti, F., and Gatti, E. (1977). Soybean protein diet in the treatment of type II hyperlipoproteinemia. *Lancet* **i,** 275–277

5 Sugiyama, K., Ohkawa, S., and Muramatsu, K. (1986). Relationship between amino acid composition of diet and plasma cholesterol level in rats fed a high cholesterol diet. *J. Nutr. Sci. Vitaminol.* **32,** 413–423

6 Sugiyama, K., Kushima, Y., and Muramatsu, K. (1985). Effects of methionine, cystine and taurine on plasma cholesterol level in rats fed a high cholesterol diet. *Agric. Biol. Chem.* **48,** 2897–2899

7 Sugiyama, K., Mizuno, M., and Muramatsu, K. (1986). Effect of individual amino acids on plasma cholesterol level in rats fed a high cholesterol diet. *J. Nutr. Sci. Vitaminol.* **32,** 623–633

8 Serougne, C. and Rukaji, A. (1983). Plasma and lipoprotein cholesterol in rats fed *L*-amino acid-supplemented diets. *Ann. Nutr. Metab.* **27,** 386–395

9 Aoyama, Y., Matsumoto, H., Tsuda, T., Ohmura, E., and Yoshida, A. (1988). Effect on liver and serum lipids in rats of dietary additions of fibers and cholestyramine to a cystine-excess diet. *Agric. Biol. Chem.* **52,** 2811–2816

10 Sautier, C., Dieng, K., Flament, C., Doucet, C., Suquet, J.P., and Lemonnier, D. (1983). Effect of whey protein, casein, soya-bean protein and sunflower proteins on the serum, tissue and fecal steroids in rats. *Br. J. Nutr.* **49,** 313–319

11 Sautier, C., Flament, C., Doucet, C., and Suquet, J.P. (1986). Effects of eight dietary proteins and their amino acid content on serum, hepatic and fecal steroids in rats. *Nutr. Rep. Int.* **34,** 1051–1061

12 Sanchez, A., Hubbard, R.W., Smit, E., and Hilton, G.F. (1988). Testing a mechanism of control in human cholesterol metabolism: Relation of arginine and glycine to insulin and glucagon. *Atherosclerosis* **71,** 87–92

13 Hagemeister, H., Scholz-Ahrens, K.E., Schulte-Coerne, H., and Barth, C.A. (1990). Plasma amino acids and cholesterol following

consumption of dietary casein or soy protein in minipigs. *J. Nutr.* **120,** 1305–1311
14 Kurowska, E.M. and Carroll, K.K. (1991). Studies on the mechanism of induction of hypercholesterolemia in rabbits by high dietary levels of amino acids. *J. Nutr. Biochem.* **2,** 656–662
15 Horigome, T. and Cho, Y.S. (1992). Dietary casein and soybean protein affect the concentrations of serum cholesterol, triglyceride and free amino acids in rats. *J. Nutr.* **122,** 2273–2282
16 Sugiyama, K., Kanamori, H., and Tanaka, S. (1993). Correlation of the plasma cholesterol-lowering effect of dietary glycine with the alteration of hepatic phospholipid composition in rats. *Biosci. Biotech. Biochem.* **57,** 1461–1465
17 Sugiyama, K., Akachi, T., and Yamakawa, A. (1993). The hypocholesterolemic action of *Lentinus edodes* is evoked through alteration of phospholipid composition of liver microsomes in rats. *Biosci. Biotech. Biochem.* **57,** 1983–1985
18 Sugiyama, K., Akachi, T., and Yamakawa, A. (1995). Eritadenine-induced alteration of hepatic phospholipid metabolism in relation to its hypocholesterolemic action in rats. *J. Nutr. Biochem.* **6,** 80–87
19 Sugiyama, K., Akachi, T., and Yamakawa, A. (1995). Hypocholesterolemic action of eritadenine is mediated by a modification of hepatic phospholipid metabolism in rats. *J. Nutr.* **125,** 2134–2144
20 Sugiyama, K., Saeki, S., Kanamori, H., and Muramatsu, K. (1991). Relationship between plasma cholesterol-lowering effect of soybean protein isolate and phospholipid biosynthesis in rats. *Nutr. Sci. Soy Protein, Jpn.* **12,** 56–62
21 Harper, A.E. (1959). Amino acid balance and imbalance. Part I. Dietary level of protein and amino acid imbalance. *J. Nutr.* **68,** 405–424
22 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509
23 Zak, B. (1957). Simple rapid microtechnic for serum total cholesterol. *Am. J. Clin. Pathol.* **27,** 583–588
24 Fletcher, M.J. (1968). A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta* **22,** 393–397
25 Bartlett, G.R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.* **234,** 466–468
26 Cook, R.J., Horne, D.W., and Wagner, C. (1989). Effect of dietary methyl group deficiency on one-carbon metabolism in rats. *J. Nutr.* **119,** 612–617
27 Tanaka, Y., Doi, O., and Akamatsu, Y. (1979). Solubilization and properties of a phosphatidylethanolamine-dependent methyltransferase system for phosphatidylcholine synthesis from mouse liver microsomes. *Biochem. Biophys. Res. Commun.* **87,** 1109–1115
28 Wright, P.S., Morando, J.N., and Kent, C. (1985). Regulation of phosphatidylcholine biosynthesis in chinese hamster ovary cells by reversible membrane association of CTP:phosphocholine cytidyryltransferase. *J. Biol. Chem.* **260,** 7919–7926
29 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
30 Yagasaki, K., Okada, K., Takagi, K., and Irikura, T. (1984). Effect of 4-(4′-chlorobenzyloxy)benzyl nicotinate (KCD-232) on cholesterol metabolism in rats fed an amino acid imbalance diet. *Agric. Biol. Chem.* **48,** 1417–1423
31 Tanaka, K. and Sugano, M. (1989). Effects of addition of sulfur-containing amino acids and glycine to soybean protein and casein on serum cholesterol levels of rats. *J. Nutr. Sci. Vitaminol.* **35,** 323–332
32 Oda, H., Okumura, Y., Hitomi, Y., Ozaki, K., Nagaoka, S., and Yoshida, A. (1989). Effect of dietary methionine and polychlorinated biphenyls on cholesterol metabolism in rats fed a diet containing soy protein isolate. *J. Nutr. Sci. Vitaminol.* **35,** 333–348
33 Murata, M., Imaizumi, K., and Sugano, M. (1982). Effect of dietary phospholipids and their constituent bases on serum lipids and apolipoproteins in rats. *J. Nutr.* **112,** 1805–1808
34 Sugiyama, K., Ohkawa, S., and Muramatsu, K. (1985). Dietary sulfur-containing amino acids and glycine as determinant factors in plasma cholesterol regulation in growing rats. *J. Nutr. Sci. Vitaminol.* **31,** 121–125
35 Ridgway, N.D., Yao, Z., and Vance, D.E. (1989). Phosphatidylethanolamine levels and regulation of phosphatidylethanolamine *N*-methyltransferase. *J. Biol. Chem.* **264,** 1203–1207
36 Tijberg, L.B.M., Geelen, M.J.H., and Golde, L.M.G. (1989). Regulation of the biosynthesis of triacylglycerols, phosphatidylcholine and phosphatidylethanolamine in the liver. *Biochim. Biophys. Acta* **1004,** 1–19
37 Audubert, F. and Vance, D.E. (1983). Pitfalls and problems in studies on the methylation of phosphatidylethanolamine. *J. Biol. Chem.* **258,** 10695–10701
38 Loo, G. and Smith, J.T. (1986). Effect of pyridoxine deficiency on phospholipid methylation in rat liver microsomes. *Lipids* **21,** 409–412
39 Pritchard, P.H., Chiang, P.K., Cantoni, G.L., and Vance, D.E. (1982). Inhibition of phosphatidylethanolamine *N*-methylation by 3-deazaadenosine stimulates the synthesis of phosphatidylcholine via the CDP-choline pathway. *J. Biol. Chem.* **257,** 6362–6367
40 Yao, Z., Jamil, H., and Vance, D.E. (1990). Choline-deficiency causes translocation of CTP:phosphocholine cytidyryltransferase from cytosol to endoplasmic reticulum in rat liver. *J. Biol. Chem.* **265,** 4326–4331
41 Yanagita, T., Yamamoto, K., Ide, T., and Enomoto, N. (1990). Effect of choline deficiency on CTP:phosphocholine cytidyryltransferse and choline kinase activities in rat liver subcellular fractions. *J. Nutr. Sci. Vitaminol.* **36,** 287–290
42 Jamil, H., Yao, Z., and Vance, D.E. (1990). Feedback regulation of CTP:phosphocholine cytidyryltransferase translocation between cytosol and endoplasmac reticulum by phosphatidylcholine. *J. Biol. Chem.* **265,** 4332–4339
43 Yao, Z. and Vance, D.E. (1988). The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* **263,** 2998–3004
44 Sugano, M., Tanaka, K., and Ide, T. (1982). Secretion of cholesterol, triglyceride and apolipoprotein A-I by isolated perfused liver from rats fed soybean protein and casein or their amino acid mixtures. *J. Nutr.* **112,** 855–862
45 Pfeuffer, M. and Barth, C.A. (1986). Modulation of very low density lipoprotein secretion by dietary protein is age-dependent in rats. *Ann. Nutr. Metab.* **30,** 281–288
46 Oda, H., Fukui, H., Hitomi, Y., and Yoshida, A. (1991). Alteration of serum lipoprotein metabolism by polychlorinated biphenyls and methionine in rats fed soybean protein diet. *J. Nutr.* **121,** 925–933
47 Huff, M.W. and Carroll, K.K. (1980). Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *J. Lipid Res.* **21,** 546–558
48 Sirtori, C.R., Galli, G., Lovati, M.R., Carrare, P., Basisio, E., and Galli-Kienle, M. (1984). Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. *J. Nutr.* **114,** 1493–1500
49 Kawashima, Y., Nishizawa, H., and Kozuka, H. (1992). Reduction by clofibric acid of serum arachidonic acid in rats. *Biochem. Pharmacol.* **43,** 2321–2326
50 Kadowaki, H., Patton, G.M., and Robins, S.J. (1993). Effect of phosphatidylcholine molecular species on the uptake of HDL triglycerides and cholesteryl esters by the liver. *J. Lipid Res.* **34,** 180–189
51 Huang, Y.S., Cunnane, S.C., and Horrobin, D.F. (1986). Effect of different dietary proteins on plasma and liver fatty acid compositions in growing rats. *Proc. Soc. Exp. Biol. Med.* **181,** 399–403
52 Sugano, M., Ishida, T., and Koba, K. (1988). Protein-fat interaction on serum cholesterol level, fatty acid desaturation and eicosanoid production in rats. *J. Nutr.* **188,** 548–554
53 Ikeda, A., Koba, K., and Sugano, M. (1993). Impact of dietary protein on polyunsaturated fatty acid desaturation in rats fed diets rich in α-linolenic acid. *Biosci. Biotech. Biochem.* **57,** 61–64
54 She, Q.-B., Hayakawa, T., and Tsuge, H. (1994). Effect of vitamin B6 deficiency on linoeic acid desaturation in the archidonic acid biosynthesis of rat liver microsomes. *Biosci. Biotech. Biochem.* **58,** 459–463