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Role of dietary lysine, methionine, and arginine in the regulation of hypercholesterolemia in rabbits

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These experiments were conducted to see whether the hypercholesterolemia produced by a diet enriched in lysine (Lys) and methionine (Met) can be reproduced by feeding these amino acids separately, and whether dietary arginine (Arg) counteracts their hypercholesterolemic effects. Another aim was to investigate the mechanisms involved in modulations of serum cholesterol levels by these amino acids. The results of this study, which were in agreement with the results of earlier experiments in our laboratory, showed that feeding a low-fat, cholesterol-free, semipurified amino acid diet enriched with Lys + Met to rabbits caused a marked increase in serum total and low density lipoprotein cholesterol and apolipoprotein B levels, whereas a similar diet enriched in essential ketogenic amino acids (EketoAA) resulted in a more moderate increase in these parameters. Supplementing the diet with either Lys or Met alone was also less effective in inducing hypercholesterolemia than increasing levels of both amino acids. Dietary Arg partially counteracted the hypercholesterolemic effect of Lys + Met but not that of the EketoAA or of Lys and Met fed separately. The growth performance of rabbits fed the Lys + Met diet was inferior to that of those fed the other diets. Liver total phospholipid levels and the ratio of phosphatidylcholine to phosphatidylethanolamine were higher in rabbits fed the Lys + Met-enriched diet than in those animals fed a diet in which Arg was supplemented. In conclusion, our results indicate that high levels of both Lys and Met are needed to cause a maximum elevation of serum cholesterol and that the moderately antihypercholesterolemic effect of Arg is seen only when both amino acids are supplemented. They also suggest that these essential amino acids may affect cholesterol metabolism partly through alteration of liver phospholipids. (J. Nutr. Biochem. 10:166–171, 1999)

Keywords: dietary amino acids; hypercholesterolemia; lysine; methionine; arginine; rabbits

Introduction

It is well known that semipurified, cholesterol-free diets containing casein or other animal proteins induce an elevation of serum total and low density lipoprotein (LDL) cholesterol levels in rabbits that can be prevented by using a vegetable protein such as soy protein.[1] Earlier studies in our laboratory showed that the amino acid composition of dietary proteins is an important determinant of their cholesterolemic properties. Thus, the hypercholesterolemia produced by casein could be reproduced by feeding a corresponding mixture of amino acids, and an amino acid mixture corresponding to soy protein gave a higher level of serum cholesterol.[2]

The hypercholesterolemia produced by casein or casein amino acids also was found to increase with increasing levels in the diet.[3] This provided a means of determining which amino acids were affecting the level of serum cholesterol. By selectively increasing the levels of only the essential amino acids (EAA) or the nonessential amino acids (NEAA) of casein, it was shown that the hypercholesterolemia was due primarily to the EAA.[4] Increasing all EAA except arginine (Arg) produced an even more pronounced hypercholesterolemia, indicating that Arg was counteracting the hypercholesterolemic effects of other EAA.[5]

Further experiments along these lines showed that all the

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Table 1 Amino acid composition (in g/kg diet) of the semipurified diets*

Amino acid	Control	NEAA	Eketo AA	Eketo AA + Arg	Lys + Met	Lys + Met + Arg	Lys	Lys + Arg	Met	Met + Arg
Essential										
Arg	5.1	5.1	.51	**15.7**	5.1	**15.7**	5.1	**15.7**	5.1	**15.7**
Gly	3.7	3.7	**11.3**	**11.3**	3.7	3.7	3.7	3.7	3.7	3.7
His	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Ile	8.3	8.3	**25.3**	**25.3**	8.3	8.3	8.3	8.3	8.3	8.3
Leu	12.1	12.1	**37.0**	**37.0**	12.1	12.1	12.1	12.1	12.1	12.1
Lys	10.8	10.8	**32.9**	**32.9**	**32.9**	**32.9**	**32.9**	**32.9**	10.8	10.8
Met	3.8	3.8	3.8	3.8	**11.6**	**11.6**	3.8	3.8	**11.6**	**11.6**
Phe	6.6	6.6	**20.1**	**20.1**	6.6	6.6	6.6	6.6	6.6	6.6
Thr	6.2	6.2	**18.9**	**18.9**	6.2	6.2	6.2	6.2	6.2	6.2
Trp	1.7	1.7	**5.2**	**5.2**	1.7	1.7	1.7	1.7	1.7	1.7
Val	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6
Total	71.9	71.9	173.2	183.9	101.9	112.5	94.1	104.7	79.8	90.4
Nonessential										
Ala	4.0	11.8	6.4	5.9	10.3	9.6	10.6	10.0	11.4	10.8
Asp	9.6	28.3	15.5	14.1	24.6	23.2	25.5	24.2	27.3	26.0
Cys	0.6	1.7	1.0	0.9	1.5	1.4	1.6	1.5	1.7	1.6
Glu	29.7	88.0	48.1	43.8	76.2	72.0	79.2	75.1	84.9	80.7
Pro	14.7	43.6	23.8	21.7	37.7	35.6	39.2	37.2	42.0	39.9
Ser	8.5	25.3	13.8	12.6	21.8	20.7	22.8	21.5	24.4	23.2
Tyr	8.2	24.4	13.3	12.1	21.0	20.0	22.0	20.8	23.5	22.4
Total	75.3	223.1	121.8	111.1	193.1	182.5	200.9	190.3	215.2	204.6
Total	147.2	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0

*The control diet had an amino acid composition equivalent to 14.7% casein. The nonessential amino acid (NEAA) diet contained 14.7% casein amino acids plus a supplement of casein NEAA added up to 29.5%. In the essential amino acid (EAA) supplemented diets, the EAA indicated in **bold** were present at three times the level in the control diet, and the total level of amino acids was adjusted to 29.5% with casein NEAA. EketoAA–essential ketogenic amino acids. Arg–arginine. Lys–lysine. Met–methionine. Gly–glycine. His–histidine. Ile–isoleucine. Leu–leucine. Phe–phenylalanine. Thr–threonine. Trp–tryptophan. Val–valine. Ala–alanine. Asp–aspartic acid. Cys–cysteine. Glu–glutamylo. Pro–proline. Ser–serine. Tyr–tyrosine.

essential ketogenic amino acids (EketoAA: glycine, isoleucine, leucine [Leu], lysine [Lys], phenylalanine, threonine, and tryptophan) produced a moderate hypercholesterolemic response, whereas a combination of Lys + methionine (Met) produced an even higher level of serum cholesterol.[6] These results help to explain why animal proteins were more hypercholesterolemic than plant proteins in our earlier experiments, because animal proteins tend to have higher levels of Lys and Met, whereas plant proteins are generally higher in Arg.[7]

The present experiments were undertaken to determine whether a high level of dietary Arg could counteract the hypercholesterolemia caused by diets containing high levels of Lys + Met or the EketoAA. A second objective was to investigate the hypercholesterolemic properties of Lys and Met fed separately at high levels and to test whether Arg could counteract their hypercholesterolemic effects.

Another goal of these experiments was to learn more about the mechanism by which dietary amino acids modulate cholesterolemia. In a recent experiment, a higher incorporation of injected radiolabeled Lys in liver polar lipids was observed with the hypercholesterolemic Lys + Met diet than with a normocholesterolemic Lys + Leu diet.[8] Consistently, animal proteins have been reported to increase the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in liver[9,10] and erythrocytes[11] compared with soy protein, and the dietary content of Met is thought to influence cholesterolemia, at least in part through alteration of hepatic phospholipid metabolism.[9] Because PC is the main phospholipid of very low density lipoproteins (VLDL) and is required for its assembly by hepatocytes,[12] and because Met can provide methyl groups for the synthesis of PC,[13] we investigated effects of Met, Lys, and Arg on liver PC level.

Methods and materials

Animals and diets

Male New Zealand white rabbits (Reimen's Fur Ranches, Guelph, Ontario, Canada), weighing approximately 1.8 ± 0.1 kg (mean ± SD) were housed individually in stainless steel cages in an animal room maintained at 21 to 24°C with 12-hour light/dark cycles. Upon arrival, they were fed for 5 days with high fiber rabbit pellets (Agway, Syracuse, NY USA). They then were randomly divided into groups and gradually transferred to the experimental amino acid diets over a 6-day period. The diets were pair-fed over a period of 3.5 weeks. Water was provided ad libitum. Body weights and food consumption were monitored.

The composition of the experimental low-fat, cholesterol-free, semipurified amino acid diets was similar to that used in earlier experiments.[4] The control diet contained all casein EAAs and NEAAs at the levels equivalent to 14.7% casein, as shown in *Table 1*. It also contained 59.5% dextrose, 13.0% cellulose, 4.0% mineral mixture, 3.0% molasses, 2.2% soy oil, 2.0% vitamin mixtures, and 1.3% palm oil. The amino acid supplemented diets were enriched with amino acids (29.5%) at the expense of dextrose (45.0%). In these supplemented diets, selected EAAs were increased to three

times the level in the control diet. The total level of amino acids was adjusted to 29.5% with casein NEAA, which have previously been shown to have little effect on cholesterolemia in rabbits.[5] The NEAA diet contained 14.7% casein amino acids plus a supplement of casein NEAA added up to 29.5%.

Because of similarities in cholesterolemic responses between the control and NEAA diets, only the control diet was included in Experiment 1. The comparison with NEAA diet was made separately in Experiment 3 in order to determine whether the liver phospholipid responses were similar for both diets.

Serum and lipoprotein measurements

Following the period on experimental diets, the rabbits were fasted overnight and blood samples were collected from a marginal ear vein. VLDL ($d < 1.006$ g/mL), LDL (1.006 g/mL $< d <$ 1.063 g/mL), and high density lipoprotein (HDL; 1.063 g/mL $< d <$ 1.21 g/mL) fractions were collected after ultracentrifugation of fresh serum.[14] Cholesterol concentration was measured in serum and lipoprotein fractions using an enzymatic-colorimetric test (CHOD-PAP kit, Boehringer-Mannheim, Montreal, Quebec, Canada). Triacylglycerol (TAG) levels were determined in serum with an enzymatic-colorimetric assay (GPO-PAP kit, Randox, Ardmore, Crumlin, UK). Apolipoprotein B (apoB) concentration in LDL was determined by isopropanol precipitation.[15]

Liver analysis

In vivo incorporation of [^{14}C]-acetate into liver lipids. Three rabbits from each of the groups fed high levels of Lys + Met and Lys + Met + Arg in Experiment 1 were injected intravenously with [^{14}C]-acetate (10 μCi/kg, SA = 56 Ci/Mol, Amersham, Oakville, Ontario, Canada) and euthanized with an overdose of Euthanyl (Canada Packers, Cambridge, Ontario, Canada) 3 hours later. Liver total lipids were extracted according to Folch et al.[16] Saponifiable (polar) lipids were precipitated with cold acetone and separated from nonsaponifiable (neutral) lipids.[17] Neutral lipids were then separated into sterols and fatty acids by precipitation of the fatty acid fraction with concentrated hydrochloric acid.[18] Liver lipid fractions were assayed for radioactivity.

Quantification of liver lipids. Liver total and free cholesterol, and TAG contents were measured after evaporation of Folch extraction solvents under gaseous nitrogen, using enzymatic-colorimetric methods (CHOD-PAP and F-CHOL kits, Boehringer-Mannheim; GPO-PAP kit, Randox). The liver esterified cholesterol content was determined by subtracting free cholesterol from total cholesterol. Liver phospholipids were digested with perchloric acid[18] prior to phosphorus determination (Inorganic phosphorus kit, Sigma, St. Louis, MO USA). PC and PE were separated by thin-layer chromatography with the solvent system chloroform: methanol:ammonia:water (65:35:4:4, v/v)[19] and the phosphorus contents of the PC and PE fractions were determined enzymatically (Inorganic phosphorus kit from Sigma), after extraction with chloroform:methanol (2:1, v/v).

Statistical analysis

A general analysis of variance (ANOVA) was done to compare the growth performance results of the rabbit groups. The lipid parameters measured were then analyzed by Student's *t*-tests when two groups were compared or by ANOVA when more than two groups were compared.[20] When the ANOVA was significant, a Student-Newman-Keuls procedure was used to locate the differences between the groups. Correlation tests were done[20] between the Lys + Met to Arg ratio in the diet and the LDL cholesterol, LDL-apoB, and the liver phospholipids (total phospholipids, PC, PE, and the PC to PE ratio). Other correlation tests also were done between the LDL cholesterol and apoB levels and the liver phospholipids. These analyses were performed using the Sigma Stat Statistical Software (Jandel Corporation, San Rafael, CA USA). The effects of amino acid diets were considered to be significant at a *P*-value of less than 0.05. Data are expressed as means ± SEM.

Table 2 Growth performance of rabbits fed experimental diets*

Diet	*N*	Initial weight (kg)	Food intake (g/day)	Weight change (g/day)
Experiment 1				
Control	5	1.9 ± 0.1	109 ± 15	2 ± 2
Lys + Met	6	2.0 ± 0.1	88 ± 11	−5 ± 3
Lys + Met + Arg	6	2.0 ± 0.1	97 ± 17	−9 ± 3
EketoAA	6	2.1 ± 0.1	91 ± 11	2 ± 3
EketoAA + Arg	6	2.0 ± 0.1	93 ± 10	−2 ± 4
ANOVA[a]		*P* = NS	*P* = NS	*P* = NS
Experiment 2				
Lys + Met	5	2.0 ± 0.1	51 ± 12	−19 ± 3[c]
Lys	6	2.0 ± 0.1	60 ± 2	2 ± 2[b]
Lys + Arg	6	2.0 ± 0.0	62 ± 4	−3 ± 3[b]
Met	5	2.0 ± 0.1	58 ± 2	5 ± 2[b]
Met + Arg	6	2.0 ± 0.0	61 ± 1	6 ± 2[b]
ANOVA[a]		*P* = NS	*P* = NS	*P* < 0.01
Experiment 3				
Control	6	2.0 ± 0.1	64 ± 1	12 ± 3
NEAA	5	2.0 ± 0.1	64 ± 1	14 ± 1
Students *t*-test[a]		*P* = NS	*P* = NS	*P* = NS

*Mean ± SEM.
[a]NS–no statistical difference ($P \geq 0.05$). Values bearing a different letter are statistically different at $P < 0.05$.
Lys–lysine. Met–methionine. Agr–arginine. EketoAA–essential ketogenic amino acids. ANOVA–analysis of variance. NEAA–nonessential amino acids.

Results

Effects on growth

In Experiment 1, there were no differences in weight change between the different dietary groups (*Table 2*). In Experiment 2, although the rabbits were pair-fed, the group on the Lys + Met diet lost considerable weight ($P < 0.01$), whereas the other groups showed only small gains or losses. In Experiment 3, the weight gains were better than in experiments 1 and 2.

Effects on serum lipids and lipoproteins

Table 3 shows the results of serum total and LDL cholesterol, and LDL-apoB for the three experiments. In Experiment 1, supplementing the diet of rabbits with Lys + Met markedly increased their serum total and LDL cholesterol levels ($P < 0.05$) and their LDL-apoB levels ($P < 0.01$) compared with the control, whereas supplementing the diet with EketoAA only tended to elevate these parameters. The addition of Arg to the Lys + Met diet partially counteracted the rise in serum total and LDL cholesterol levels and

Table 3 Effect of amino acid diets on serum total and LDL cholesterol and LDL-apoB levels*

Diet	Cholesterol (mmol/L)		LDL-apoB (mg/mL)
	Total	LDL	
Experiment 1			
Control	3.3 ± 0.1[c]	1.9 ± 0.1[c]	0.6 ± 0.1[c]
Lys + Met	7.6 ± 0.8[b]	6.2 ± 0.8[b]	1.5 ± 0.2[b]
Lys + Met + Arg	5.0 ± 0.9[b,c]	3.6 ± 0.8[b,c]	0.9 ± 0.2[c]
EketoAA	5.7 ± 0.5[b,c]	4.2 ± 0.3[b,c]	1.0 ± 0.1[b,c]
EketoAA + Arg	6.0 ± 0.8[b,c]	4.4 ± 0.7[b,c]	1.1 ± 0.2[b,c]
ANOVA[a]	$P < 0.05$	$P < 0.05$	$P < 0.01$
Experiment 2			
Lys + Met	11.4 ± 1.7[b]	7.9 ± 1.5[b]	2.0 ± 0.2[b]
Lys	6.7 ± 0.9[c]	4.2 ± 1.1[c]	0.9 ± 0.2[c]
Lys + Arg	7.0 ± 0.9[c]	4.6 ± 0.8[c]	0.9 ± 0.2[c]
Met	5.2 ± 0.4[c]	3.4 ± 0.4[c]	0.8 ± 0.1[c]
Met + Arg	6.4 ± 1.4[c]	3.8 ± 0.7[c]	0.9 ± 0.1[c]
ANOVA[a]	$P < 0.05$	P < 0.05	$P < 0.01$
Experiment 3			
Control	3.0 ± 0.2	1.3 ± 0.1[c]	0.5 ± 0.1
NEAA	4.2 ± 0.6	2.2 ± 0.4[b]	0.7 ± 0.1
Student's *t*-test[a]	P = NS	$P < 0.05$	P = NS

*Means ± SEM of 5 to 6 rabbits.
[a]NS = no statistical difference ($P \geq 0.05$). Values bearing a different letter are significantly differenct at $P < 0.05$.
Lys–lysine. Met–methionine. Agr–arginine. EketoAA–essential ketogenic amino acids. ANOVA–analysis of variance. NEAA–nonessential amino acids.

significantly counteracted the rise in LDL-apoB levels ($P < 0.01$). However, addition of Arg did not counteract the moderately hypercholesterolemic effect of the EketoAA diet. The various amino acid diets had no significant effect on VLDL and HDL cholesterol concentrations (data not shown).

In Experiment 2, the high levels of Lys + Met produced a greater rise in serum total and LDL cholesterol levels ($P < 0.05$), as well as LDL-apoB levels ($P < 0.01$) than the high levels of either amino acid alone. Lys + Met diet also raised VLDL cholesterol compared with Lys and Met diets but did not affect HDL cholesterol (data not shown). The addition of Arg to the moderately hypercholesterolemic Lys or Met diets did not alter total and LDL cholesterol and LDL-apoB. It also did not influence VLDL or HDL cholesterol levels (data not shown). In both experiments, the diets had no effect on serum TAG levels (data not shown).

In Experiment 3, feeding the NEAA diet resulted in low levels of serum total, VLDL, and HDL cholesterol, as well as LDL-apoB and total TAG, which were similar to the control diet. However, the NEAA diet produced moderately higher LDL cholesterol than the control diet ($P < 0.05$).

Effect on liver lipids

In Experiment 1, the Lys + Met diet induced a significantly higher in vivo incorporation of intravenous-injected radio-labeled acetate into liver polar lipids than the Lys + Met + Arg diet ($P < 0.05$), but the incorporation into neutral sterols and free fatty acids was not significantly different (*Table 4*). The higher hepatic [^{14}C]-polar lipid levels were associated with moderately increased levels of liver total phospholipids in rabbits fed the Lys + Met diet ($P < 0.05$) and with greater PC to PE ratio than the Lys + Met + Arg diet ($P < 0.01$; *Table 5*, Experiment 1).

Table 4 Effect of amino acid diets on incorporation of injected ^{14}C-acetate into liver lipids*

Diet	Polar lipids (dpm/g liver)	Neutral sterols (dpm/g liver)	Free fatty acids (dpm/g liver)
Experiment 1			
Lys + Met	777 ± 34[b]	115 ± 18	144 ± 20
Lys + Met + Arg	551 ± 67[c]	82 ± 12	126 ± 22
Student's t-test[a]	$P < 0.05$	P = NS	P = NS

*Means ± SEM of 3 rabbits.
[a]NS–no statistical difference ($P \geq 0.05$). Values bearing a different letter are significantly different at $P < 0.05$.
Lys–lysine. Met–methionine. Arg–arginine.

In Experiment 2, the Lys + Met diet nonsignificantly increased the levels of liver total phospholipids and PC and nonsignificantly reduced the level of PE compared with the Lys or Met diets. Similarly, the addition of Arg to the Lys or Met diets did not significantly reduce the level of liver total phospholipids or PC, but the addition of Arg to the Lys diet did significantly increase the level of liver PE ($P < 0.01$), which resulted in a significant decrease in the PC to PE ratio ($P < 0.01$, *Table 5*). The Lys + Arg and Met + Arg diets gave significantly lower liver PC levels and the Lys + Arg diet a significantly higher PE level than the Lys + Met diet ($P < 0.01$). Thus, the PC to PE ratio was significantly lower on the Lys + Arg and Met + Arg diets than on the Lys + Met diet ($P < 0.01$).

In Experiment 3, the NEAA diet induced similar hepatic total phospholipid concentrations and PC to PE ratio, as did the control diet. However, higher PC levels and lower PE levels were observed in the NEAA diet than in the control diet ($P < 0.05$).

The liver content of total, free, and esterified cholesterol and of TAG was not modulated by dietary amino acids in any experiment (data not shown). In Experiment 1, the ratio of liver esterified to free cholesterol was identical with Lys + Met diet (0.5 ± 0.1) and with Lys + Met + Arg diet (0.5 ± 0.2).

Analysis of regressions

When combining the data from all experiments ($N = 51$, as in *Table 5*), we found a positive correlation between the Lys + Met to Arg ratio in the diet and the LDL cholesterol ($P < 0.01$, $r = 0.54$) and apoB ($P < 0.01$, $r = 0.57$) levels. In addition, this ratio was positively correlated with liver total phospholipid ($P < 0.05$, $r = 0.36$) and PC ($P < 0.01$, $r = 0.55$) levels, and with the PC to PE ratio ($P < 0.01$, $r = 0.57$). Moreover, the LDL cholesterol and apoB levels also were positively correlated with the liver total phospholipid

Table 5 Effect of amino acid diets on liver phospholipids levels*

Diet	Total phospholipids (mg/g liver)	PC (mg/g liver)	PE (mg/g liver)	PC/PE (mol/mol)
Experiment 1				
Lys + Met	21.9 ± 0.6[b]	5.9 ± 0.3	5.2 ± 0.2	1.1 ± 0.04[b]
Lys + Met + Arg	19.2 ± 0.6[c]	5.0 ± 0.6	5.0 ± 0.5	0.9 ± 0.03[c]
Student's *t*-test[a]	$P < 0.05$	P = NS	P = NS	$P < 0.01$
Experiment 2				
Lys + Met	25.2 ± 1.5	7.1 ± 0.3[b]	3.7 ± 0.5[c]	1.9 ± 0.2[b]
Lys	24.3 ± 1.6	6.1 ± 0.5[b,c]	3.9 ± 0.5[c]	1.6 ± 0.1[b]
Lys + Arg	21.6 ± 1.7	5.0 ± 0.5[c]	6.9 ± 0.6[b]	0.7 ± 0.04[d]
Met	24.1 ± 2.2	6.5 ± 0.3[b,c]	5.5 ± 0.8[b,c]	1.2 ± 0.2[b,c]
Met + Arg	22.9 ± 1.6	5.1 ± 0.4[c]	5.7 ± 0.4[b,c]	0.9 ± 0.1[c,d]
ANOVA[a]	P = NS	$P < 0.01$	$P < 0.01$	$P < 0.01$
Experiment 3				
Control	18.1 ± 1.2	3.4 ± 0.3[c]	3.2 ± 0.1[c]	1.1 ± 0.1
NEAA	18.0 ± 1.1	4.5 ± 0.2[b]	3.8 ± 0.2[b]	1.2 ± 0.03
Student's *t*-test[a]	P = NS	$P < 0.05$	$P < 0.05$	P = NS

*Means ± SEM of 5 to 6 rabbits.
[a]Values bearing a different letter are significantly different. NS = no statistical difference ($P \geq 0.05$).
PC–phophatidylcholine. PE – phosphatidylethanolamine. Lys–lysine. Met–methionine. Arg–arginine. ANOVA–analysis of variance. NEAA–nonessential amino acids.

($P < 0.01$, $r = 0.39$ and $P < 0.01$, $r = 0.45$, respectively) and PC levels ($P < 0.05$, $r = 0.28$ and $P < 0.01$, $r = 0.45$, respectively), and with the PC to PE ratio ($P < 0.01$, $r = 0.45$ and $P < 0.01$, $r = 0.49$, respectively).

Discussion

The results of these experiments confirm earlier observations that high levels of dietary Lys + Met produce marked hypercholesterolemia in rabbits. They also show that a high level of dietary Arg can partially counteract the hypercholesterolemia produced by Lys + Met, but not that due to high levels of EketoAA. Furthermore, enriching the diet with excess of either Lys or Met alone was less effective in increasing the cholesterolemic responses in rabbits than enriching with high levels of both Lys and Met. Similarly, the supplementation with Arg did not reduce the moderate hypercholesterolemia produced by either the Lys or Met enriched diets.

Thus, it is clear that the diet needs to be enriched in both Lys and Met to induce substantial hypercholesterolemia in rabbits. This is consistent with the finding that the EketoAA diet, which is enriched with Lys but not with Met, produced only a moderate hypercholesterolemia. It is also in agreement with other observations that the combinations of Leu + Lys[6,8] or Leu + Met[6] did not increase the level of LDL cholesterol as much as Lys + Met. It is similarly in accordance with earlier work on chow-fed rabbits, which showed that supplementing with a combination of Lys, Met, Leu, isoleucine, phenylalanine, and tryptophan gave a higher level of serum cholesterol than supplementation with either Lys, Leu, and isoleucine or Met, phenylalanine, and tryptophan.[21] Furthermore, the moderate antihypercholesterolemic effect of Arg was observed only at the high levels of serum cholesterol produced by the combination of Lys + Met and not at the lower levels produced by the EketoAA or by Lys or Met alone.

The diet enriched in Lys + Met caused a marked weight loss, which was not matched by the other diets, even though the animals were pair-fed. This weight loss in rabbits on the Lys + Met diet has been observed before.[6] Lower weight gains also were reported in rats fed a diet enriched with Lys + Met.[22] It has been proposed that decreased weight gain in kittens fed diets enriched with EAA is due to an adverse effect of excess Met and possibly other EAA.[23] In earlier experiments,[6,8] the weight loss in rabbits fed diets enriched in Lys + Met was not consistently correlated with the degree of hypercholesterolemia, and we concluded that it seems unlikely that the overall cholesterolemic responses to dietary amino acids are influenced by failure to gain weight.[5]

Liver phospholipid levels were measured in these experiments as a means of investigating possible mechanisms involved in the elevation of serum cholesterol by dietary EAA. The results show that Lys + Met diet increased the levels of liver total phospholipids and the ratio of PC to PE more than when Arg was added (*Table 4* and *Table 5*, Experiment 1). In other experiments in mice, liver PC and the ratio of PC to PE were higher on a casein than a soy protein diet.[10] In rats, a casein diet gave a lower level of liver microsomal PE and a higher PC to PE ratio than a soy protein diet.[24] In the latter experiments, the addition of Met to the soy diet reduced the level of PE and increased the PC to PE ratio. The results of Sugiyama et al.[9] also showed a positive correlation between dietary Met intake and the liver microsomal PC to PE ratio in rats fed seven different dietary

proteins. It seems possible that the high dietary levels of Lys + Met are influencing enzymes involved in the biosynthesis of PC in the liver.[9] In particular, excess of methyl groups from Met[25] could stimulate a conversion of PE to PC via the enzyme phosphatidylethanolamine N-methyltransferase, which could lead to an increased secretion of apoB-containing lipoproteins.[26]

Conclusion

Our results indicate that high levels of both Lys and Met are required for maximum elevation of serum total and LDL cholesterol levels and that the modulating effect of Arg is observed only when both amino acids are supplemented. Furthermore, our observations suggest that the hypercholesterolemic effects of dietary casein and casein EAA may be mediated, at least in part, by an increase in some liver phospholipids, including possibly PC.

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