Studies on the mechanism of induction of hypercholesterolemia in rabbits by high dietary levels of amino acids

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Increasing the proportion of an amino acid mixture corresponding to casein in a low-fat, cholesterol-free, semipurified diet fed to rabbits causes a progressive increase in serum total and low density lipoprotein cholesterol, and the effect appears to be due primarily to the essential amino acids in the mixture. Our recent studies have also shown that the variations in serum cholesterol in response to different levels of the amino acid mixture are not associated with any changes in fecal excretion of cholesterol or bile acids. Further attempts to understand the mechanism of action of dietary amino acids on serum cholesterol levels have shown the following: (1) no correlation with levels of plasma amino acids, either in the fasting or postprandial state; (2) no correlation with serum levels of thyroid hormones; (3) no relationship to activity of hepatic or intestinal microsomal hydroxymethylglutaryl coenzyme A reductase; (4) no corresponding effects on the activities of cholesterol esterifying enzymes of intestinal mucosa; and (5) no correlation with the degree of esterification of cholesterol in very low or low density lipoproteins. Further studies are required to identify the specific amino acids responsible for the hypercholesterolemic effects and to determine the mechanisms involved.

Keywords: dietary amino acids; hypercholesterolemia; plasma amino acids; HMGCoA reductase; cholesterol esterification; rabbits.

Introduction

It is well known that casein, fed as a component of cholesterol-free, semipurified diets, can produce hypercholesterolemia in rabbits, whereas soy protein fed at the same level maintains a low, normal level of plasma cholesterol.[1] Several previous observations suggest that casein is more important than soy protein in producing this differential in plasma cholesterol. Thus, it has been shown that increasing levels of casein in the diet increase the hypercholesterolemia in a dose-related manner, whereas increasing the level of soy protein has little effect on the plasma cholesterol.[1-3] Earlier studies in our laboratory showed that feeding an amino acid mixture corresponding to casein caused an elevation of plasma cholesterol similar to that obtained by feeding an equivalent amount of casein, whereas a dietary soy protein amino acid mixture was moderately hypercholesterolemic when compared with soy protein.[1]

Recent experiments have shown that in rabbits fed the casein amino acid mixture, the plasma cholesterol increases with increasing amounts in the diet and the increase is mainly in the low density lipoprotein (LDL) fraction. This effect appeared to be due primarily to the essential amino acids in the mixture.[4]

It is still unclear how casein and soy protein in general, and certain casein amino acids in particular, affect cholesterol metabolism. Previous studies demonstrated that rabbits fed casein diet excrete less cholesterol and bile acids and have a higher level of total cholesterol and cholesterol esters in the liver than those fed the corresponding soy protein diet.[5,6] However, rabbits fed high or low levels of casein amino acids or casein essential amino acids did not show these differences in spite of the differences observed in serum and LDL cholesterol levels.[4] The above results suggest that effects of dietary protein on the en-

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*E. K. is a research associate at the University of Western Ontario.
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Supported by the Heart and Stroke Foundation of Ontario and by Protein Technologies International.
Received March 14, 1991; accepted June 19, 1991.

terohepatic circulation of sterols may account only partially for the difference in cholesterolemic response between casein and soy protein-fed rabbits. A considerable part of this difference may be due to the higher levels of essential amino acids in casein compared to soy protein.[4]

In this paper, we present the results of further attempts to better understand the mechanism of action of dietary casein amino acids. This was done by: (1) modifying the amino acid diets in an attempt to obtain a greater cholesterolemic response; (2) investigating possible effects of the amino acid diets on plasma amino acid levels, thyroid hormones, activity of hepatic and intestinal hydroxymethylglutaryl coenzyme A reductase (HMGCoA reductase) (a key enzyme responsible for cholesterol synthesis), and cholesterol esterification in serum and intestinal mucosa.

Materials and methods

Animals and diets

Young, male New Zealand White rabbits (Reimen's Fur Ranches, Guelph, Ontario, Canada), weighing approximately 1.6 kg, were used in this study. Low-fat, cholesterol-free, semipurified diets were introduced to the animals gradually as described previously[7] and were fed for 4 weeks.

The basal composition of the diets was similar to that used in our earlier experiments.[4] Differences in cholesterolemic response were induced by feeding different levels of all casein amino acids or by feeding amino acid mixtures containing a high level of essential amino acids plus a low level of nonessential amino acids and vice versa. The proportions of casein essential and nonessential amino acids in the different experimental diets are given in *Table 1*.

Table 1 Percent of casein essential and nonessential amino acid mixture used in experiments 1–3[a]

Diet	Percent of casein essential amino acid mixture	Percent of casein nonessential amino acid mixture
11.2% AA[b]	5.5	5.7
14.7% AA[cd]	7.2	7.5
20% AA[c]	9.8	10.2
25% AA[bc]	12.4	12.6
30% AA[d]	14.6	15.4
45% NESSAA + 14.7% ESSAA[d]	7.2	23.0
45% ESSAA + 14.7% NESSAA[d]	22.0	7.5

[a] The mixture of casein essential amino acids consists of L-Arg, 7.2%; Gly, 5.1%; L-His, 5.7%; L-Ile, 11.5%; L-Leu, 16.8%; L-Lys, 14.9%; L-Met, 5.3%; L-Phe, 9.2%; L-Tre, 8.6%; L-Try, 2.5%; and L-Val, 13.3%. The mixture of nonessential amino acids contains L-Ala, 5.3%; L-Asp, 12.7%; L-Cys, 0.8%; L-Glu, 39.5%; L-Pro, 19.5%; L-Ser, 11.3%; and L-Tyr, 10.9%.

[b,c,d] Diets used for experiment 1, 2, and 3, respectively.

Determination of serum and lipoprotein components

Fasting blood samples were obtained from the marginal ear vein after indicated periods of feeding the experimental diets. Very low density lipoprotein (VLDL) ($d < 1.006$ g/mL) and LDL (1.006 g/mL $< d < 1.063$ g/mL) were isolated from serum by discontinuous density gradient ultracentrifugation.[8] Cholesterol was measured in serum and LDL fractions using an enzymatic kit (CHOD-PAP obtained from Boehringer-Mannheim, Montreal, Quebec, Canada). Apoprotein B concentration in LDL was measured according to Egusa et al.[9] as modified by Huff et al.[10]

Determination of plasma amino acid composition

Amino acids were determined in heparinized plasma of rabbits fed diets containing 11.2% or 25% amino acids for 10 days. Blood samples were taken from the marginal ear vein after an 18-hour fast. Subsequently, the respective diets were fed for 5 minutes (8–10 g of diet per rabbit) and blood samples were taken postprandially after 1 hour. Amino acids were measured by ion exchange chromatography using an amino acid analyzer (Beckman model 120C) in samples deproteinized with sulfosalicylic acid, following the method of Boomgaardt and McDonald.[11]

Determination of thyroid hormones

Total thyroxine (T_4), total triiodothyronine (T_3) and free thyroxine index (FTI), which provides a measure of the thyroxine binding sites in a sample, were determined in serum, using commercial kits ($TDxT_4PLUS$, IMx Total T_3 reagent pack and TDx T-UPTAKE obtained from Abbott Laboratories Diagnostic Division, Dallas, TX, USA).

Determination of HMGCoA reductase activity in microsomal fraction of liver and intestinal mucosa

After the indicated period of feeding semipurified diets, rabbits were sacrificed in the postprandial state between 9:00 a.m. and 11:00 a.m. by an overdose of Euthanyl (Canada Packers, Cambridge, Ontario, Canada). Livers were excised, washed in cold saline, and portions were homogenized in cold buffer containing 0.3 M sucrose, 25 mmol/L mercaptoethanol and 10 mmol/L EDTA, pH 7.0, according to Kovanen et al.[12] The microsomal fraction was prepared as described previously[13] and stored at −70° C. The upper 30 cm of the intestine was removed, washed with cold saline, cut lengthwise, and the mucosa scraped off. Scrapings were homogenized and microsomes were isolated according to Field et al.[14] and frozen at −70° C.

Microsomal protein was determined according to Lowry et al.[15] after precipitation of protein with 5% TCA. Within 2 weeks after isolation, the microsomal fractions were incubated with 15 nmol of DL-3-[glutaryl-3-^{14}C]-HMGCoA (SA 15,000 dpm/nmol) in

Table 2 Comparison of levels of single amino acids in fasting and postprandial plasma of rabbits fed 11.2% AA or 25% AA diet

Amino acid	Fasting levels [μmol/L]		Postprandial levels [μmol/L]	
	11.2% AA diet	25% AA diet	11.2% AA diet	25% AA diet
Asp	37 ± 4	33 ± 4	38 ± 4	48 ± 8
Thr	531 ± 67	451 ± 25	545 ± 114	690 ± 123
Ser	257 ± 46	233 ± 16	280 ± 53	356 ± 33
Glu	138 ± 19	115 ± 18	132 ± 10	160 ± 32
Pro	151 ± 9	127 ± 23	317 ± 59	533 ± 79
Gly	1493 ± 252	1010 ± 141	1524 ± 313	1160 ± 238
Ala	375 ± 75	254 ± 37	572 ± 134	502 ± 48
Val	97 ± 14	92 ± 11	117 ± 32	223 ± 49
Met	36 ± 16	26 ± 5	38 ± 16	34 ± 10
Ile	41 ± 7	40 ± 6	41 ± 13	74 ± 24
Leu	58 ± 8	56 ± 6	54 ± 17	107 ± 35
Tyr	47 ± 11	47 ± 4	62 ± 18	112 ± 18
Phe	23 ± 7	39 ± 3	17 ± 7	47 ± 8
His	101 ± 13	117 ± 21	141 ± 32	167 ± 38
Lys	133 ± 23	96 ± 21	195 ± 63	238 ± 62
Arg	125 ± 21	110 ± 11	133 ± 39	154 ± 28

Each result is a mean ± SEM. Four rabbits per group.

buffer containing 25 mmol/L glucose-6-phosphate, 3 mmol/L NADP, 70 mmol/L NaCl, 0.174 units of glucose-6-phosphate dehydrogenase, 15 mmol/L dithiothreitol, 0.04 M KH_2PO_4, 0.05 M KCl, 0.25 M sucrose, 0.03 M EDTA and 0.2 mg of trypsin inhibitor, pH 7.2, in a total volume of 170 μL.[14]

The reaction was terminated by addition of 25 μL of concentrated HCl and 2 nmol of RS-[5-^{3}H]mevalonolactone (SA 20,000 dpm/nmol) was added as internal standard. After allowing 1 hour for lactonization at 37° C, the mixture was separated on thin layer chromatography (TLC) plates (Silica Gel 60), basically according to Shapiro et al.[16] Mevalonolactone was visualized in iodine vapor and scraped into scintillation vials. Radioactivity was counted on an LKB 1214 Rackbeta liquid scintillation counter. The activity of HMGCoA reductase was expressed as nmol of [^{14}C]mevalonate formed per minute per mg of microsomal protein.

Determination of activity of cholesterol esterifying enzymes in microsomal fraction from intestinal mucosa

Mucosal scrapings from the middle jejunum were prepared as for the HMGCoA reductase assay and the microsomal fraction was obtained following the method of Chautan et al.[17] The microsomal supernatant was saved for determination of cholesterol ester hydrolase (CEH). Both microsomes and supernatants were stored at −70° C and assayed within 2 weeks. For determination of activity of acyl-CoA:cholesterol acyltransferase (ACAT), 500 mg of microsomal protein was incubated for 10 minutes at 37° C with buffer containing 0.1 M KH_2PO_4 and 2 mmol/L dithiothreitol, pH 7.4, containing 1.34 mg bovine serum albumin and 20 nmol/L [1-^{14}C]oleoyl CoA (SA 12000 dpm/nmol) in a total volume of 250 μL, as described by Chautan et al.[17] At the end of the incubation period, the reaction was stopped with chloroform:methanol 2:1 (vol/vol) and radioactive cholesteryl oleate was determined in the presence of internal standard after separation by TLC, according to the method of Stange et al.[18] Cholesterol ester hydrolase activity was determined with [1-^{14}C]cholesterol as substrate, following the method of Heider et al.[19] Esterification rates of both enzymes were calculated in pmol of radioactive products formed per mg microsomal protein per minute.

Determination of total and free cholesterol in lipoprotein fractions and in intestinal microsomes

Portions of the intestinal microsomal preparations and of VLDL and LDL fractions were extracted with chloroform:methanol 2:1 (vol/vol).[20] Part of each extract was subjected to mild saponification in 3% ethanolic KOH at 70° C for 1 hour and total cholesterol was quantitated in the samples either according to Zlatkis and Zak[21] as modified by Rudel and Morris[22] or by HPLC separation as described by Crick and Carroll.[23]

Free cholesterol was determined by HPLC after TLC separation of a chloroform:methanol extract in hexane:diethyl ether:acetic acid 70:30:1 (vol/vol/vol).[22]

Results

Effects on plasma amino acid composition

Table 2 shows the levels of single amino acids in plasma of rabbits fed diets containing 11.2% and 25% casein amino acids (AA) (experiment 1), both in fasting and in postprandial state. Despite the difference in plasma cholesterol response between the animals fed 11.2% and 25% AA diet (2.87 ± 0.44 mmol/L and 4.86 ± 0.52 mmol/L, respectively), the levels of single amino acids in fasting plasma remained similar on both diets.

Postprandially little or no increase in the levels of single amino acids occurred in plasma of the group fed

Table 3 Effect of increasing levels of dietary casein amino acids on growth performance, total serum and LDL cholesterol levels, and LDL apoprotein B (apoB)

Diet	Initial weight g	Weight gain g/day	Food consumption g/day	Total serum cholesterol mmol/L	LDL cholesterol mmol/L	LDL apoB g/L
14.7% AA	1662 ± 31	12.6 ± 1.6	78 ± 11	2.90 ± 0.28[a]	1.89 ± 0.28[a]	0.38 ± 0.02[a]
20% AA	1579 ± 17	16.0 ± 1.2	71 ± 4	3.10 ± 0.52[a]	2.20 ± 0.49[ab]	0.43 ± 0.10[ab]
25% AA	1613 ± 12	15.6 ± 1.2	74 ± 6	4.24 ± 0.31[a]	3.23 ± 0.23[b]	0.59 ± 0.04[b]

Values are given as means ± SEM. The results were obtained after 4 weeks of feeding semipurified diets. Each group consisted of four rabbits. The effects of diet were analyzed using a one-way ANOVA and Duncan's multiple range test and the results were verified by Kruskal-Wallis test for nonparametric values. Values in a column not sharing a common subscript letter are significantly different, $P < 0.05$.

Table 4 Triiodothyronine, thyroxine, and free thyroxine index (FTI) in serum samples of rabbits fed increasing levels of casein amino acid mixture

Diet	Total triiodothyronine nmol/L	Total thyroxine nmol/L	FTI
14.7% AA	1.2 ± 0.1	42.8 ± 2.0	0.31 ± 0.01
20% AA	1.4 ± 0.2	44.0 ± 3.0	0.31 ± 0.02
25% AA	1.1 ± 0.2	42.9 ± 4.4	0.31 ± 0.03

Values are given as means ± SEM. Four rabbits per group.

Table 5 Activity of HMGCoA reductase in microsomal fraction of liver and intestinal mucosa of rabbits fed increasing levels of casein amino acid mixture

Diet	HMGCoA reductase activity pmol/min/mg protein	
	Liver	Intestinal mucosa
14.7% AA	1.2 ± 0.3	61.8 ± 13.6
20% AA	2.2 ± 1.1	28.4 ± 10.1
25% AA	2.1 ± 0.6	26.4 ± 5.2

Values are given as means ± SEM. Four rabbits per group. Recovery rate of [^{14}C]mevalonolactone was 94 ± 2%.

11.2% AA, but greater increases were found in animals fed 25% AA, reflecting substantially higher levels of all amino acids on 25% AA diet compared to 11.2% AA diet.

Effects of increasing levels of dietary casein amino acids

The effects of feeding increasing levels of casein amino acids (experiment 2) on growth performance, total serum, and LDL cholesterol concentrations, and LDL apoprotein B content in rabbits are shown in *Table 3*. No significant differences were found among the dietary regimens in weight gain and food consumption. Total serum cholesterol, LDL cholesterol, and LDL apoprotein B increased progressively with increasing levels of casein amino acids in the diet, but only the effects of diet on LDL cholesterol and LDL apoprotein B were significant ($P < 0.05$). Additionally, the levels of total and LDL cholesterol in rabbits fed 14.7% AA diet were not higher than those obtained earlier for animals fed 11.2% AA diet;[4] therefore, in the subsequent experiment, 14.7% AA diet was used as a low cholesterol control.

Effects on thyroid hormones

The levels of total triiodothyronine, total thyroxine, and the FTI for rabbits fed increasing levels of casein AA are presented in *Table 4*. No significant effect of diet on thyroid hormones was observed.

Effects on activity of HMGCoA reductase in the liver and intestinal mucosa

Table 5 shows the effects of increasing levels of dietary casein amino acids on HMGCoA reductase activity in microsomal fractions isolated from liver and intestinal mucosa.

In hepatic microsomes the enzymatic activity was not significantly different in the different dietary groups and was much lower than the activity in intestinal mucosa. Intestinal HMGCoA reductase activity was twice as high in rabbits fed 14.7% casein amino acids as in those fed 20% and 25% casein amino acid diet, but the difference did not reach the level of significance, probably because of the small number of the animals per group.

Effect of high levels of casein amino acids and casein essential amino acids

In the next study (experiment 3) an attempt was made to increase the difference in total serum and LDL cholesterol response between the groups. To achieve that, rabbits were fed either low and high level of casein amino acid mixture (14.7% and 30% AA diet) or two modified amino acid diets formulated to maintain a high level of nonessential amino acids (NESSAA) as in the 45% amino acid diet and a low level of essential amino acids (ESSAA) as in the 14.7% AA diet (45% NESSAA + 14.7% ESSAA) and vice versa (45% ESSAA + 14.7% NESSAA). The results presented in

Table 6 Effect of different levels of dietary casein amino acids or casein essential and nonessential amino acids on growth performance, total serum, and LDL cholesterol

Diet	Initial weight g	Weight gain g/day	Food consumption g/day	Total serum cholesterol mmol/L	LDL cholesterol mmol/L
14.7% AA	1802 ± 46	11.6 ± 0.9	68 ± 3	1.79 ± 0.16	1.01 ± 0.13
30% AA	1715 ± 14	15.0 ± 1.5	47 ± 3	4.03 ± 0.88	2.56 ± 0.67
45% NESSAA + 14.7% ESSAA	1694 ± 49	20.6 ± 3.5	76 ± 9	2.54 ± 0.21	1.81 ± 0.23
45% ESSAA + 14.7% NESSAA	1660 ± 60	12.6 ± 3.7	56 ± 9	5.79 ± 1.06[ab]	4.66 ± 1.04[ab]

Values are given as means ± SEM. Five rabbits per group. Three weeks of feeding semipurified diets. The effect of diet was analyzed using one-way analysis of variance and Tukey's test.
[a] $P < 0.05$ versus 14.7% AA diet.
[b] $P < 0.05$ versus 45% NESSAA + 14.7% ESSAA.

Table 7 Intestinal mucosal cholesterol, free cholesterol, ACAT and CEH activity

Diet	Total microsomal cholesterol μg/mg protein	Free microsomal cholesterol μg/mg protein	% Free cholesterol	pmol/mg protein/minute	
				ACAT activity	CEH activity
14.7% AA	62 ± 7	54 ± 4	86 ± 5	9.3 ± 1.1	109 ± 20
30% AA	58 ± 13	54 ± 6	75 ± 3	9.8 ± 1.0	87 ± 23
45% NESSAA + 14.7% ESSAA	48 ± 5	46 ± 5	96 ± 1	8.9 ± 1.2	48 ± 13
45% ESSAA + 14.7% NESSAA	48 ± 3	43 ± 3	90 ± 6	9.8 ± 1.9	48 ± 17

Values are means ± SEM. Five rabbits per group.

Table 6 show that both total serum and LDL cholesterol were significantly higher in 45% ESSAA + 14.7% NESSAA-fed animals than in those fed 45% NESSAA + 14.7% ESSAA and in those fed 14.7% AA. The hypercholesterolemic response was the highest in 45% ESSAA + 14.7% NESSAA fed group and the lowest in rabbits fed 14.7% AA. Feeding 30% AA diet also induced substantial elevation of serum and LDL cholesterol levels, but the difference did not reach the level of significance. The serum total and LDL cholesterol levels in the animals fed 14.7% AA diet were lower than in experiment 2 (*Table 3*). This could be due to seasonal changes or to variability of the individual responses.

Effects on cholesterol esterification in intestinal mucosa and in lipoprotein particles

The effects of feeding amino acid diets that produce differing cholesterolemic responses on the activities of cholesterol esterifying enzymes (ACAT and CEH) in intestinal mucosa and on cholesterol ester accumulation in the microsomal fraction of mucosa are presented in *Table 7*. The results show that, in general, activities of CEH were 5–10-fold higher than the activities of ACAT on all experimental diets, but the cholesterolemic responses between the groups were not correlated with any differences in either the activities of the enzymes or the percent of free cholesterol in the mucosal microsomal fraction.

Further studies demonstrated that the percent of free cholesterol in VLDL and LDL fraction was also very similar in animals fed either the 14.7% AA or 30% AA diet (for VLDL it was 28 ± 1% and 27 ± 1%, for LDL it was 32 ± 1% and 30 ± 1%, respectively.

Discussion

Our first experiment clearly showed that there was no relationship between fasting and postprandial levels of plasma amino acids and fasting plasma cholesterol when rabbits were fed diets containing two different levels of the same casein amino acid mixture. Several earlier attempts to correlate serum cholesterol response with proportions of amino acids in diet and in plasma led to controversial results.[24,25] In contrast to previous studies, our approach overcame the difficulty of comparing the effect of two dietary proteins that differ both in amino acid composition and in digestibility.

The dose-related elevation of serum total and LDL cholesterol due to increasing levels of dietary casein amino acids in the second study was consistent with our results obtained for rabbits fed 11.2% and 25% casein amino acid diet.[4] A simultaneous dose-related

increase of LDL apoprotein B offered additional evidence that the lipoprotein response in rabbits fed casein amino acid diet is very similar to that obtained for animals fed intact casein.[26]

Lack of correlation between the concentration of serum cholesterol and thyroid hormones suggested that dietary amino acids do not interact with cholesterol metabolism via these hormones, although it was reported earlier that intact dietary casein induces a decrease in serum levels of thyroxine and/or triiodothyronine.[25,27]

The activity of hepatic HMGCoA reductase did not show any tendency to be suppressed in rabbits fed increasing levels of casein amino acids. This is not what would be expected by comparing effects of dietary casein and soy protein[28] but our results were in agreement with the lack of differences in liver cholesterol between the rabbits fed high and low level of casein amino acids or casein essential amino acids in our previous experiment.[4] Also, Nagata et al.[29] demonstrated that in rats fed casein amino acid mixture activity of hepatic HMGCoA reductase was even higher than in their counterparts fed a mixture of soy protein amino acids, whereas the animals fed intact casein had lower activity of this enzyme than those fed soy protein.

Intestinal mucosal HMGCoA reductase had substantially higher activity than hepatic reductase in all three experimental groups, in agreement with previous findings that sterol synthesis in intestinal tissue is higher than in the liver in rabbits,[30] but unlike the liver enzyme, it showed a tendency to be suppressed in rabbits fed higher than basic levels of casein amino acids (*Table 5*). This may, however, be of little importance for the mechanism of action of dietary casein amino acids, since it is known that almost all cholesterol synthesized in the intestine becomes incorporated into enterocyte membranes and is ultimately lost in the feces.[30-32]

The results of the third feeding experiment (*Table 6*) confirmed our previous findings that a high level of all casein amino acids and especially casein essential amino acids is important to produce hypercholesterolemia in rabbits.[4] Additionally, they demonstrated that a greater than previously reported hypercholesterolemic response can be achieved by a further increase in the level of casein amino acids or casein essential amino acids in experimental diets. In this study, we also showed that interaction between the dietary hypercholesterolemic amino acids and cholesterol metabolism is unlikely to occur at the level of esterification of endogenous cholesterol from the intestinal lumen. Since it is known that almost all cholesterol present in the intestine has to be esterified, mainly by ACAT, before being absorbed,[33] our results suggest that there would be no difference in cholesterol absorption between the groups fed high and low level of casein amino acids or casein essential amino acids. This is contrary to several previous reports demonstrating that dietary casein versus soy protein increases cholesterol absorption and decreases fecal excretion of sterols in rabbits and rats,[6,29,34] but remains consistent with the lack of difference in cholesterol absorption between rats fed casein and soy protein amino acid mixtures.[29] It is also in agreement with the lack of difference in fecal excretion of cholesterol and bile acids between rabbits fed high and low levels of casein amino acids or casein essential amino acids.[4]

Additionally, the lack of effect of a high level of dietary casein amino acids on the proportion of cholesterol esters in VLDL and LDL was consistent with the lack of accumulation of hepatic cholesterol ester in rabbits fed hypercholesterolemic amino acid diets in our previous study.[4] This indicates that the high level of cholesterol, and especially esterified cholesterol in VLDL and LDL particles, found earlier in casein-fed rabbits, is not important in hypercholesterolemia induced by feeding casein amino acids.[5,35,36]

In conclusion, our results demonstrate that the mechanism by which high levels of dietary casein amino acids, and especially casein essential amino acids, induce hypercholesterolemia in rabbits is not likely to operate via such postprandial events as changes in plasma concentration of certain amino acids or thyroid hormones, or via direct interaction with the enzymes responsible for absorption of endogenous cholesterol. It is quite possible that hypercholesterolemia produced by amino acid diets, like that produced by intact casein, is associated with down-regulation of hepatic apolipoprotein B/E receptors, responsible for LDL catabolism. Our observation that both casein and its amino acid mixtures cause cholesterol elevation mainly in the LDL fraction supports this hypothesis. A correlation may also exist between dietary amino acid-induced elevation of LDL cholesterol and the rate of intestinal synthesis of apolipoprotein B-48. This apolipoprotein is a structural component of chylomicrons and was recently found to be synthesized at a higher rate in casein- compared to soy protein-fed swine.[37] Still, there are not sufficient data to speculate what could be the metabolic linkage between high levels of certain dietary essential amino acids and activity of LDL receptors or rate of synthesis of apolipoproteins in the intestine. Further identification of particular amino acids responsible for hypercholesterolemia should help to understand their role in producing the effect.

Acknowledgments

The authors are grateful to Ajinomoto Co. USA, Inc., New York, NY, for providing L-amino acids, and to Procter and Gamble, Cincinnati, OH, USA, for the dietary fats used in this study. We also wish to thank R.G. Vanderwerf for determining the levels of thyroid hormones in serum samples and J. Shin for preparing diets and feeding rabbits.

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