Effects of Dietary Proteins and Amino Acid Mixtures on Plasma Cholesterol Levels in Rabbits[1]

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ABSTRACT Amino acid mixtures corresponding to either casein or egg yolk protein when fed to rabbits in a low fat, cholesterol-free semipurified diet, produced the same degree of hypercholesterolemia as the proteins themselves. Amino acid mixtures corresponding to soy protein isolate or sunflower protein gave low plasma cholesterol levels, but not as low as those obtained with the intact proteins. Experiments were designed to identify amino acids responsible for the differential in plasma cholesterol produced by casein or soy protein amino acid mixtures. Plasma cholesterol levels obtained with a casein amino acid mixture containing half the amounts of essential amino acids were significantly higher than the corresponding soy protein mixture. Feeding trials based on systematic modifications to the casein and soy protein mixtures indicated that interaction between essential and non-essential amino acids was important in determining plasma cholesterol levels. In further experiments, protein components were formulated by adding amino acids to casein to give a mixture corresponding to soy protein, or by adding amino acids to soy protein to give a mixture equivalent to casein. These diets failed to reverse plasma cholesterol levels which suggests differences in digestion and absorption of proteins relative to amino acid mixtures. J. Nutr. *110*, 1676–1685, 1980.

INDEXING KEY WORDS casein · soy protein · amino acid mixtures · plasma cholesterol

The prominent role of nutrition in hypercholesterolemia and atherosclerosis is generally accepted. Although most of the emphasis to date has been on dietary fat and cholesterol, investigators have recently recognized the significance of other dietary compounds (1, 2).

The nature of the dietary protein has been shown to have a significant effect on plasma cholesterol levels in both experimental animals and humans (3, 4). Rabbits become hypercholesterolemic and develop atherosclerosis when fed cholesterol-free diets containing casein or other proteins from animal sources, whereas these effects are prevented when the dietary protein requirement is supplied by soy protein isolate or other plant proteins (5–7). In human experiments, substitution of animal protein in the diet by soy protein mean analogues results in a significant lowering of plasma cholesterol levels in both normal subjects (8) and hospitalized hypercholesterolemic patients (9).

Initial experiments with rabbits demonstrated that the observed effect was at least partly due to the dietary amino acid composition (6). An enzymatic digest of casein, or a mixture of amino acids corresponding to casein, produced the same degree of hypercholesterolemia as the protein itself. An enzymatic digest of soy protein or an amino acid mixture corresponding to soy protein, gave low

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TABLE 1

Composition of diet

Ingredient[1]	%
Casein (vitamin-free)	27.0[2]
Dextrose	60.0
Celluflour	5.0
Salt mix	4.0
Molasses (50% v/v)	3.0
Vitamin mixture[3]	0.2
Corn oil	1.0

[1] The vitamins, salt mixture (Phillips and Hart salt mixture IV, plus cobalt) and "vitamin-free" casein were obtained from ICN Life Sciences Group, Nutritional Biochemicals Division, Cleveland, OH. Dextrose monohydrate was obtained from Teklad Test Diets, ARS/Sprague-Dawley Division of the Mogul Corporation, Madison, WI., and Celluflour from Chicago Dietetic Supply House, Chicago, IL. Molasses was obtained from a local feed mill. [2] Casein was added in this amount in order to supply the diet with 25% (w/w) protein. [3] Composition (mg/kg): thiamin-HCl, 7.5; pyridoxine-HCl, 7.5; biotin, 0.3; myo-inositol, 150; riboflavin, 15; nicotinic acid, 75; Ca pantothenate, 80; folic acid, 0.75; choline chloride, 1,500; vitamin A as retinyl acetate, 20,000 IU; α-tocopheryl acetate, 110 IU; menaquinone, 30. The water soluble vitamins were prepared and added to the diet as described previously (6). The fat soluble vitamins were dissolved in corn oil to provide a source of essential fatty acids.

levels of plasma cholesterol similar to that obtained with the intact protein. These experiments suggested that the amino acid composition of the diet could explain the different effects of casein and soy protein on the concentration of plasma cholesterol.

A number of human studies have demonstrated that dietary amino acid composition can influence the level of plasma cholesterol (10–13). Olsen and co-workers (10, 11), using amino acid mixtures, demonstrated that the source of non-essential nitrogen and the ratio of essential to non-essential amino acids were important with respect to plasma cholesterol levels.

This paper describes further experiments designed to investigate in more detail the relationship between dietary amino acid composition and plasma cholesterol levels in rabbits. Two additional amino acid mixtures, one corresponding to egg yolk protein and the other to sunflower seed protein, were tested. A series of experiments were conducted in which systematic modifications were made to the casein and soy protein amino acid mixtures in an attempt to identify amino acids or groups of amino acids which are important in determining the level of plasma cholesterol. The effects of adding amino acids to casein and soy protein, in order to alter the dietary amino acid pattern, were also investigated.

MATERIALS AND METHODS

The experimental diets were fed to male, New Zealand White rabbits weighing initially 1.0–1.5 kg. The rabbits were housed individually in metal cages with wire mesh bottoms, in an air-conditioned room with controlled temperature and lighting. All rabbits were fed a ground commercial diet (Master Feed Rabbit Pellets, Maple Leaf Mills Ltd., Toronto, Ontario) for 4–5 days after arrival, and were transferred gradually to the experimental diets over a 3–4-day period. Diets and water were provided ad libitum, animals were weighed weekly and feed intake was measured over the 28-day period of the experiment. Non-fasting blood samples were obtained by cardiac puncture from unanaesthetized rabbits. Plasma total cholesterol was extracted as described by Mann (14) and assayed by the method of Zlatkis and Zak (15).

The basal, low fat, cholesterol-free, semipurified diet was similar to that used in earlier experiments (5, 6) and is shown in table 1. The proteins and amino acids were incorporated into this diet in amounts calculated to provide dietary nitrogen equivalent to 25% protein. Amino acid mixtures were formulated with L-amino acids (Ajinomoto U.S.A. Inc., New York). In all diets using soy protein, the isolate Promine-R (donated by Dr. E. W. Meyer, Central Soya, Chicago, IL) was used. Amino acid mixtures corresponding to soy protein were formulated using the amino acid composition of the isolate (table 2).

The diets in this study were fed to rabbits for a 28-day period. The experiments were grouped into four series, and the diets in each series were formulated as follows.

TABLE 2

Percent composition of amino acid mixtures[1]

	Series 1				Series 2										Series 3		Series 4	
	Soy protein isolate	Casein	Sun-flower protein	Egg yolk protein	Soy protein ½ EAA	Casein ½ EAA	Soy EAA + Glu	Casein EAA + Glu	Soy EAA + Ala	Casein EAA + Ala	Soy EAA + Ser,Asp,-Pro,Glu (Soy)	Casein EAA + Ser,Asp,-Pro,Glu (Casein)	Soy EAA + Ser,Asp,-Pro,Glu (Casein)	Casein EAA + Ser,Asp,-Pro,Glu (Soy)	Casein + AA's = soy protein	Soy protein + AA's = casein	Casein + AA's ratio = 0.54	Soy protein + AA's ratio = 1.4
Essential amino acids[2]																		
L-Arginine	7.7	3.5	8.5	7.5	3.9	1.75	7.7	3.5	7.7	3.5	7.7	3.5	7.7	3.5	3.5	7.7	12.3	5.6
Glycine	4.0	2.5	5.8	3.1	2.0	1.25	4.0	2.5	4.0	2.5	4.0	2.5	4.0	2.5	2.5	4.0	5.1	2.8
L-Histidine	2.5	2.8	2.4	2.5	1.25	1.4	2.5	2.8	2.5	2.8	2.5	2.8	2.5	2.8	2.8	2.5	2.2	2.3
L-Isoleucine	4.8	5.6	4.5	5.1	2.4	2.8	4.8	5.6	4.8	5.6	4.8	5.6	4.8	5.6	5.6	4.8	4.3	6.3
L-Leucine	7.6	8.2	6.8	8.5	3.85	4.1	7.6	8.2	7.6	8.2	7.6	8.2	7.6	8.2	8.2	7.6	6.3	8.8
L-Lysine-HCl	6.0	7.3	3.8	7.5	3.1	3.6	6.0	7.3	6.0	7.3	6.0	7.3	6.0	7.3	7.3	6.0	5.7	6.5
L-Methionine	1.1	2.6	2.0	2.6	0.55	1.3	1.1	2.6	1.1	2.6	1.1	2.6	1.1	2.6	2.6	1.1	2.0	3.2
L-Phenylalanine	5.3	4.5	4.7	4.2	2.7	2.25	5.3	4.5	5.3	4.5	5.3	4.5	5.3	4.5	4.5	5.3	4.3	4.9
L-Threonine	3.7	4.2	3.8	5.5	1.85	2.1	3.7	4.2	3.7	4.2	3.7	4.2	3.7	4.2	4.2	3.7	3.3	5.0
L-Tryptophan	1.4	1.2	1.4	1.5	0.7	0.6	1.4	1.2	1.4	1.2	1.4	1.2	1.4	1.2	1.2	1.4	1.2	1.3
L-Valine	4.7	6.5	5.4	4.2	2.4	3.2	4.7	6.5	4.7	6.5	4.7	6.5	4.7	6.5	6.5	4.7	5.0	6.3
Non-essential amino acids																		
L-Alanine	3.9	2.7	4.5	5.5	5.5	3.9	—	—	51.2	50.9	—	—	—	—	3.9	2.7	2.1	5.6
L-Aspartic acid	11.7	6.5	9.8	10.2	17.3	9.5	—	—	—	—	14.0	7.8	7.8	14.0	11.7	6.5	5.0	9.1
L-Cystine	1.2	0.4	1.6	1.7	1.7	0.57	—	—	—	—	—	—	—	—	1.2	0.4	0.54	2.2
L-Glutamic acid	20.2	20.2	23.3	13.6	29.5	27.4	51.2	50.9	—	—	24.3	24.2	24.3	24.2	20.2	20.2	24.1	14.5
L-proline	5.2	10.0	4.9	4.3	7.8	14.3	—	—	—	—	6.3	12.0	12.0	6.2	5.2	10.0	7.7	4.0
L-Serine	5.4	5.8	4.7	8.8	8.0	8.2	—	—	—	—	6.5	6.9	7.0	6.5	5.4	5.8	4.5	7.6
L-Tyrosine	3.7	5.6	2.1	4.0	5.5	8.2	—	—	—	—	—	—	—	—	3.7	5.6	4.3	4.2

[1] Expressed as weight percent. The figures for soy protein were based on data provided for soy protein isolate by Dr. E. W. Meyer of Central Soya, Chicago, IL. The figures for sunflower protein were provided by Dr. F. W. Sosulski, Crop Science Department, University of Saskatchewan, Saskatoon, Sask. The values for casein and egg yolk protein are from FAO (17). [2] Amino acids determined essential for the rabbit. Glycine is considered essential as it was required for rapid growth (18).

Series 1. Two additional protein sources, sunflower and egg yolk protein were tested along with their corresponding amino acid mixtures. The dietary nitrogen in the basal diet was supplied by the following: a) sunflower seed protein isolate (donated by Dr. F. W. Sosulski; Crop Science Dept., University of Saskatchewan, Saskatoon, Canada); b) an amino acid mixture corresponding to sunflower seed protein; c) egg yolk protein [Teklad Mills, Madison, WI; delipidated as described by Hamilton and Carroll (5); final preparation contained 81% protein and less than 1% lipid], and d) an amino acid mixture corresponding to egg yolk protein.

Series 2. This series of experiments was designed to investigate which amino acids or groups of amino acids influenced the level of plasma cholesterol. In order to simplify the approach, systematic modifications were made to the casein and soy protein amino acid mixtures. The effects of these modifications on the level of plasma cholesterol were determined. In most cases, the results of one experiment determined the modification used for the following experiments.

The mixtures of amino acids were formulated as follows: a) a mixture corresponding to soy protein isolate except that the essential amino acids (EAA) were reduced by one-half and the non-essential amino acids (NEAA) increased proportionately; b) a mixture corresponding to casein except that the EAA reduced by one-half and the NEAA increased proportionately; c) a mixture in which the EAA were in the same proportions as in soy protein isolate and the non-essential nitrogen was supplied entirely by glutamic acid; d) a mixture in which the EAA were in the same proportions as in casein and the non-essential nitrogen was supplied entirely by glutamic acid; e) the same as in (c) except that alanine supplied the non-essential nitrogen; f) the same as in (d) except that alanine supplied the non-essential nitrogen; g) soy protein EAA plus the NEAA serine, aspartic acid, proline and glutamic acid in the same proportions as in soy protein; h) casein EAA plus the NEAA serine, aspartic acid, proline and glutamic acid in the same proportions as in casein; i) soy EAA plus the NEAA serine, aspartic acid, proline and glutamic acid in the same proportions as in casein, and j) casein EAA plus the NEAA serine, aspartic acid, proline and glutamic acid in the same proportions as in soy protein.

Series 3. In this series the dietary nitrogen component was formulated by combining amino acid mixtures with the intact proteins in order to alter the overall dietary amino acid pattern: a) an amino acid mixture (54% w/w of the final mixture) was added to soy protein isolate to give a mixture equivalent in amino acid composition to casein, and b) an amino acid mixture (47% w/w of the final mixture) was added to casein to give a mixture equivalent in amino acid composition to soy protein isolate.

Series 4. The diets in this series were formulated on the basis of data derived from previous experiments in which animal proteins were found to be hypercholesterolemic relative to plant proteins (5, 6). A computer program was used to correlate the amino acid composition of all the proteins tested with the plasma cholesterol level obtained after 4 weeks of feeding. No single amino acid showed either a significant positive or negative correlation with plasma cholesterol. Stronger correlations were obtained by combining groups of amino acids. When the sum of the amounts of isoleucine, leucine, threonine, tyrosine and serine was divided by the sum of arginine, glycine and glutamic acid, a higher value was obtained for animal proteins (1.0–1.5) than for plant proteins (0.5–1.0). This value was highly correlated with plasma cholesterol levels ($r = 0.82$).

To test this correlation the diets for series 4 were formulated as follows: a) an amino acid mixture (26% w/w of the final mixture) was added to soy protein isolate to give a ratio:

$$\frac{\Sigma \text{ile, leu, thr, tyr, ser}}{\Sigma \text{arg, gly, glu}} = 1.4;$$

b) an amino acid mixture (21% w/w of the final mixture) was added to casein to give a ratio:

TABLE 3

Effect of Amino Acid Mixtures on Plasma Cholesterol Levels and Growth Performance (Series 1)[1]

Diet	No. of animals	Initial wt	Weight gain	Feed consumption	Plasma cholesterol		
					Initial	14 days	28 days
		g	*g/day*	*g/day*	*mg/dl*		
Controls							
Soy protein isolate	6	1,305 ± 52	15 ± 2	61 ± 3	66 ± 14	79 ± 12	68 ± 14
Soy protein amino acid mixture[2]	10	1,254 ± 82	13 ± 2	61 ± 2	56 ± 27	127 ± 20	124 ± 30
Casein	6	1,287 ± 69	14 ± 3	63 ± 4	60 ± 15	185 ± 47	221 ± 38[a]
Casein amino acid mixture[2]	9	1,206 ± 69	13 ± 2	60 ± 2	54 ± 8	159 ± 39	213 ± 42[a]
Experimental diets							
a) Sunflower protein	6	1,308 ± 75	15 ± 2	64 ± 2	48 ± 7	57 ± 14	53 ± 12
b) Sunflower amino acid mixture	6	1,242 ± 42	11 ± 2	51 ± 1	69 ± 12	152 ± 16	140 ± 28[a]
c) Egg yolk protein	5	1,482 ± 160	11 ± 2	67 ± 4	70 ± 2	192 ± 25	286 ± 35[a]
d) Egg yolk amino acid mixture	6	1,302 ± 51	10 ± 3	53 ± 10	63 ± 9	127 ± 16	225 ± 39[a]

[1] Results expressed as mean ± SEM. [2] These experiments are the same as reported in Huff et al. (6) and are presented here for comparative purposes. [a] Significantly different from soy protein isolate ($P < 0.05$).

$$\frac{\Sigma \text{ile, leu, thr, tyr, ser}}{\Sigma \text{arg, gly, glu}} = 0.54.$$

The amino acid compositions of all the diets are listed in table 2.

Analysis of variance was performed according to a one-way classification and tests of significance were based on Duncan's New Multiple Range test which allowed for unequal numbers in different groups (16).

RESULTS AND DISCUSSION

The results of this study provide further evidence that mixtures of amino acids can influence the concentration of plasma cholesterol in rabbits. Earlier experiments (6) demonstrated that amino acid mixtures corresponding to casein or soy protein, gave similar cholesterol levels which were not significantly different from those obtained with the intact proteins. The experiments in series 1 were designed to determine whether the hypercholesterolemia obtained with egg yolk protein and the low plasma cholesterol levels obtained with sunflower seed protein could also be duplicated by amino acid mixtures. Table 3 shows that the amino acid mixture corresponding to egg yolk protein produced elevated plasma cholesterol levels as did egg yolk protein itself. The amino acid mixture corresponding to sunflower seed protein gave a significantly higher value than the intact protein ($P < 0.05$). Although the difference was non-significant, an amino acid mixture corresponding to soy protein also gave higher levels of plasma cholesterol than the intact protein. These results suggest that amino acid composition can partially explain the differential in plasma cholesterol produced by animal and plant proteins, but may not be the only factor involved.

The experiments in series 2 were designed to investigate which amino acids or groups of amino acids could influence plasma cholesterol levels. These experiments were all based on variations of the amino acid mixtures corresponding to either soy protein or casein. The results obtained were variable and somewhat difficult to explain (table 4).

It was initially thought that the essential amino acids might be important in this effect. This idea was investigated by reducing the essential amino acids by

TABLE 4

Effects of amino acid mixtures on plasma cholesterol levels and growth performance (series 2)[1]

Diet	Initial wt	Weight gain	Feed consumption	Plasma cholesterol Initial	Plasma cholesterol 14 days	Plasma cholesterol 28 days
	g	*g/day*	*g/day*	*mg/dl*		
Controls[2]						
Soy protein amino acid mixture	1,254 ± 82	13 ± 2	61 ± 2	56 ± 27	127 ± 20	124 ± 30
Casein amino acid mixture	1,206 ± 69	13 ± 2	60 ± 2	54 ± 8	159 ± 39	213 ± 42
Series 2—Modifications to amino acid mixtures[3]						
a) Soy ½ EAA	1,542 ± 67	7 ± 2	42 ± 5	79 ± 12	99 ± 20	116 ± 12
b) Cas ½ EAA	1,471 ± 52	15 ± 1	66 ± 2	86 ± 11	254 ± 37	271 ± 48[a]
c) Soy EAA + Glu	1,197 ± 65	16 ± 2	63 ± 2	98 ± 19	116 ± 17	195 ± 30
d) Cas EAA + Glu	1,186 ± 51	12 ± 1	62 ± 3	60 ± 4	119 ± 24	99 ± 14[a]
e) Soy EAA + Ala	1,574 ± 98	13 ± 2	65 ± 2	58 ± 9	229 ± 35	227 ± 42
f) Cas EAA + Ala	1,582 ± 105	10 ± 1	69 ± 6	104 ± 11	170 ± 19	253 ± 52
g) Soy EAA + Ser,Asp,-Pro,Glu (Soy)	1,353 ± 95	11 ± 1	56 ± 4	59 ± 12	153 ± 18	120 ± 12
h) Cas EAA + Ser,Asp,-Pro,Glu (Cas)	1,132 ± 84	17 ± 2	59 ± 3	68 ± 17	250 ± 25	206 ± 21[a]
i) Soy EAA + Ser,Asp,-Pro,Glu (Cas)	1,556 ± 32	8 ± 1	51 ± 2	51 ± 5	80 ± 14	76 ± 7
j) Cas EAA + Ser,Asp,-Pro,Glu (Soy)	1,494 ± 71	11 ± 3	65 ± 2	56 ± 8	80 ± 30	115 ± 22

[1] Results expressed as mean ± SEM. [2] These results are the same as in table 3 and are presented in this table for comparative purposes. The results from the soy protein amino acid mixture are from 10 rabbits and those for the casein amino acid mixture are from nine rabbits. [3] Six rabbits per dietary group. EAA = essential amino acids. See text for a detailed explanation of the diets. [a] Significantly different from the corresponding soy amino acid mixture ($P < 0.05$).

half and increasing the non-essential amino acids proportionately. It was reasoned that, by significantly altering the quantity of essential amino acids, the role of the essential amino acids as a group could be determined.

The plasma cholesterol levels obtained with the casein amino acid mixture containing half the amounts of essential amino acids, were significantly higher ($P < 0.01$) than the corresponding soy protein mixture. This suggested that the difference in plasma cholesterol may be due to the essential amino acids or an interaction between the essential and the non-essential amino acids.

Experiments in series 2 (c–f) were designed to examine these possibilities, by completely replacing the non-essential amino acids in the two mixtures by either glutamic acid or alanine. These modifications were based on experiments by Olsen et al. (10, 11) who found that using glutamic acid as the sole source of non-essential nitrogen produced a lowering of serum cholesterol in humans. The results of the present experiments using glutamic acid produced surprising results. The soy essential amino acids plus glutamic acid gave high levels of plasma cholesterol whereas low levels were obtained with the mixture of glutamic acid plus the casein essential amino acids. Use of alanine as the source of non-essential nitrogen produced a hypercholesterolemia with both mixtures. These experiments suggested several possibilities: a) the essential amino acids themselves are not responsible for the effect of casein and soy protein on plasma

TABLE 5

Modification of the amino acid composition of casein and soy protein by the addition of amino acid mixtures (series 3)[1]

Diet[2]	Amino acid composition equivalent to:	Initial wt	Weight gain	Feed consumption	Plasma cholesterol Initial	14 days	28 days
		g	*g/day*	*g/day*		*mg/dl*	
Controls							
Soy protein isolate	Soy protein	1,270 ± 50	14 ± 2	66 ± 5	51 ± 7	58 ± 9	71 ± 4
Casein	Casein	1,192 ± 45	14 ± 1	58 ± 1	92 ± 3	145 ± 16	210 ± 41[a]
Protein plus amino acid mixtures							
(a) Soy protein (46%) + amino acids (54%)	Casein	1,355 ± 92	15 ± 3	66 ± 1	61 ± 4	88 ± 15	94 ± 23
(b) Casein (53%) + amino acids (47%)	Soy protein	1,384 ± 40	12 ± 4	63 ± 3	50 ± 5	133 ± 29	185 ± 50[a]

[1] Results expressed as mean ± SEM for six rabbits per dietary group. [2] Added to the diet in amounts calculated to provide dietary nitrogen equivalent to 25% protein. [a] Signifiantly different from soy protein isolate ($P < 0.05$).

cholesterol; b) the casein mixture requires a large amount of glutamic acid to lower plasma cholesterol; c) the soy protein mixture requires non-essential amino acids other than glutamic acid or alanine to maintain low plasma cholesterol levels, or d) the interaction between the essential and non-essential amino acids is of importance.

The diets in series 2 (g and h) were formulated to determine which non-essential amino acids were required to produce the differential in plasma cholesterol observed with the complete amino acid mixtures. It was decided to include glutamic acid and serine in the mixtures since these are found in similar amounts in casein and soy protein. The two non-essential amino acids, showing the greatest difference between the two proteins, aspartic acid and proline, were also included (table 2). The resulting mixtures gave values similar to those obtained with the complete amino acid mixtures, indicating that alanine, cystine and tyrosine were not required to obtain the differential in plasma cholesterol levels. This finding strengthened the hypothesis that interaction between essential and non-essential amino acids was important.

Further modifications were made to these mixtures by combining the casein essential amino acids with the soy non-essential amino acids and vice versa (series 2, i and j). The casein-essential-plus-soy-non-essential amino acid mixture reversed the effect, as anticipated, giving low levels of plasma cholesterol. However, the mixture containing the soy-essential-plus-casein-non-essential amino acids gave an even lower level of plasma cholesterol. This finding is significant in that this mixture is the only one that has been tested that gives plasma cholesterol levels as low as the soy protein isolate itself.

The experiments in series 3 (table 5) demonstrated that the addition of amino acids to casein to give an amino acid composition equivalent to soy protein had no effect. Similarly the addition of amino acids to soy protein to give an amino acid composition equivalent to casein did not reverse the result.

The diets used in Series 4 were formulated to test the hypothesis that the dietary ratio:

$$\frac{\Sigma \text{ile, leu, tyr, thr, ser}}{\Sigma \text{arg, gly, glu}}$$

was important in regulating plasma cholesterol levels. The addition of amino acids to soy protein raised this ratio from

TABLE 6

Modification of the amino acid composition of casein and soy protein by the addition of amino acid mixtures (series 4)[1]

Diet[2]	Dietary ratio of: ΣIle,Leu, Thr,Tyr,Ser/ ΣArg,Gly,Glu	Initial wt	Weight gain	Feed consumption	Plasma cholesterol		
					Initial	14 days	28 days
		g	*g/day*	*g/day*	*mg/dl*		
Controls							
Soy protein isolate	0.78	1,270 ± 50	14 ± 2	66 ± 5	51 ± 7	58 ± 9	71 ± 4
Casein	1.1	1,192 ± 45	14 ± 1	58 ± 1	92 ± 3	145 ± 16	210 ± 41[a]
Protein plus amino acid mixtures							
a) Soy protein (74%) + amino acids (26%)	1.4	1,347 ± 56	9 ± 2	51 ± 2	74 ± 11	87 ± 19	109 ± 24
b) Casein (79%) + amino acids (21%)	0.54	1,298 ± 42	12 ± 2	60 ± 3	60 ± 9	100 ± 16	162 ± 29[a]

[1] Results expressed as mean ± SEM for six rabbits per dietary group. [2] Added to the diet in amounts calculated to provide dietary nitrogen equivalent to 25% protein. [a] Significantly different from soy protein isolate ($P < 0.05$).

0.78 to 1.40 and the addition of amino acids to casein lowered the ratio from 1.1 to 0.54. As in series 3, the results of this experiment demonstrated that the addition of amino acids failed to produce any marked alterations in the effects of intact proteins on plasma cholesterol (table 6).

These experiments indicated that the intact protein component of the diet had an overriding effect. However, this is not incompatible with the findings that plasma cholesterol levels can be markedly affected by amino acid mixtures. It is unlikely that digestion of an intact protein will provide amino acids in exactly the same proportions and time sequence as feeding an equivalent amino acid mixture (19). Therefore, the effects on plasma cholesterol obtained with the combinations of intact proteins and amino acid mixtures (Series 3 and 4) may be related to the way in which the amino acids are released during digestion. A summary of the results of these studies is shown in figure 1.

In a recently published abstract, Kritchevsky et al.[4] suggested that the differing effects of casein and soy protein on plasma cholesterol levels in rabbits may be due to their different ratios of arginine to lysine (casein, 0.49; soy protein, 1.13). Feeding casein plus arginine and soy protein plus lysine for 8 months had no consistent effect on plasma cholesterol levels, but the soy-protein-plus-lysine diet increased atherogenicity. The lack of effects of this amino acid ratio on serum cholesterol is consistent with the results of the present experiments. In series 3, amino acids were added to casein and to soy protein, which reversed the arginine/lysine ratio, but had no effect on plasma cholesterol. This ratio was also tested using the computer program mentioned earlier, which correlated the amino acid composition of all the proteins we have tested with plasma cholesterol. The arginine/lysine ratio gave a low negative correlation ($r = -0.35$).

The studies of Weigensberg et al. (20) indicated that diets high in lysine were hypercholesterolemic for rabbits. The results obtained with the correlation program did not show a strong correlation of dietary lysine with plasma cholesterol levels. Also, casein and soy protein have a similar lysine content despite their different effects on plasma cholesterol.

The experiments presented in this paper using amino acid mixtures clearly

[4] Kritchevsky, D., Tepper, S. A. & Story, J. A. (1978) Influence of soy protein and casein on atherosclerosis in rabbits. Fed. Proc. 37, 2801 (abs.).

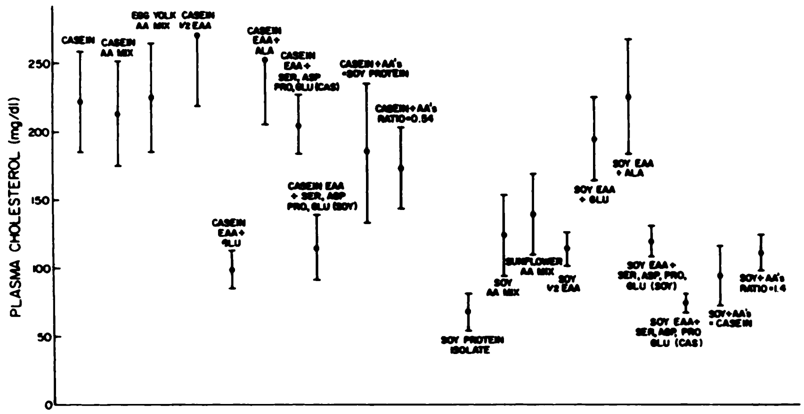

Fig. 1. Effects on plasma cholesterol of feeding low fat, cholesterol-free, semipurified diets containing amino acid mixtures. The diets were fed to rabbits for 28 days and results are given as mean ± SEM.

show that the plasma cholesterol level in rabbits can be modified by the amino acid composition of the diet. Interpretation of the experiments is difficult, but several tentative conclusions can be made. It is doubtful whether a single amino acid is responsible for the effect on plasma cholesterol levels. It is also unlikely that the essential amino acids or the non-essential amino acids themselves are a factor. The results suggest that interaction between essential and non-essential amino acids is important in determining the level of plasma cholesterol. Interaction between other groups of amino acids such as neutral, acidic, basic, branched chain, etc., could also be important. The amino acid mixtures were based on the composition of casein and soy protein isolate, which were obtained by analysis of hydrochloric acid digests. Therefore, glutamine and asparagine were included with glutamic acid and aspartic acid, respectively. It remains possible that the glutamine and asparagine content of the two proteins is a factor.

It is not clear how amino acids are able to influence plasma cholesterol levels. Whether the effect is at the level of absorption or at other stages of amino acid metabolism is not known. It is also difficult to speculate at which point in intermediary metabolism amino acids exert their effect on cholesterol metabolism.

It is also unclear whether amino acid mixtures affect plasma cholesterol levels in exactly the same way as the intact proteins. Further experiments are required to determine the effects of amino acid composition relative to the structure of the intact proteins. Also, it cannot be ruled out that non-protein constituents of the protein preparations may be partially responsible.

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