Cholesterol and Dietary Protein

Papers Presented at the 76th AOCS Annual Meeting in Philadelphia, Pennsylvania, May 1985

Dietary Protein and Atherosclerosis

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Interest in the effect of protein on lipid metabolism and atherosclerosis dates back to the first decade of this century. In the 1940s Meeker and Kesten showed that soy protein was more atherogenic for rabbits than casein. Carroll and his colleagues demonstrated that, in general, proteins of animal origin were more cholesterolemic for rabbits than were plant proteins, although there was a wide range of individual effects within both classifications. Carroll also showed that partial hydrolyzates of casein or soy protein were less cholesterolemic than the intact proteins. We have hypothesized that the lysine/arginine ratio (L/A) of the protein influences its effects on lipid metabolism. The L/A of casein is about 2 and that of soy about 1. Addition of enough arginine to casein to lower its L/A to 1 reduces its atherogenicity; adding enough lysine to soy isolate to raise its L/A to 2 enhances atherogenicity. The atherogenicities of fish protein (L/A, 1.44), casein (L/A, 1.94) and whole milk protein (L/A, 2.44) are correlated directly with their L/A ($p<0.05$). Other amino acids (methionine, glycine, arginine) have been shown to affect cholesterolemia. Casein lowers the excretion of steroids in rabbits and lengthens the turnover time of cholesterol. This may be the mechanism underlying the animal/vegetable protein effects.

The first purely nutritional induction of atherosclerosis was carried out by Ignatowski over 75 years ago. He found that adult rabbits fed 0.6 to 15 g of meat daily for as long as eight mo developed nephritis, cirrhosis of the liver and atherosclerosis (1);. Weanling rabbits fed milk and egg yolk also exhibited renal disorders, cirrhosis and atherosclerosis (2,3). Several investigators (4-6) concluded that cholesterol was the atherogenic factor present in the animal protein-containing diets, and the case was considered proven in 1913 when Anitschkow and Chalatow (7) and Wacker and Hueck (8) independently showed that rabbits fed cholesterol developed atherosclerosis. Other investigators suggested that abnormal metabolites of animal protein might also play a key role in atherogenesis (9,10). Knack (11), for instance, showed that a mixture of cholesterol-containing animal products was more atherogenic than crystalline cholesterol fed at a higher level than that present in the protein.

Newburgh and his collaborators (12-15) fed dried beef or casein to rabbits and induced atherosclerosis which, they concluded, was due to the protein. Clarkson and Newburgh (16) eventually showed that the amount of cholesterol present in the atherogenic beef diet, when fed as crystalline cholesterol, was not atherogenic, suggesting that another factor must be operative.

TABLE 1

Influence of Casein or Soy Protein on Atherosclerosis in Rabbits[a] (6-mo feeding)

Regimen	Cholesterol (mg)	Aorta (% sclerotic)	Aortic atherosclerosis (avg. severity[b])
Basal	–	0	0
Casein	–	50	0.75
Soy protein	–	0	
Basal	60	70	1.24
Casein	60	75	2.08
Soy	60	35	0.44
Basal	60	56	0.89
Casein	60	77	2.08
Soy	60	33	0.33

[a]After references 17, 18 (rabbits fed diets containing 39% casein or soy protein.
[b]Graded on a 0-3 scale, with 3 being most severe.

TABLE 2

Influence of Protein on Serum Cholesterol in Rabbits[a]

Dietary protein	Serum cholesterol (mg/dl)
Animal protein	
Whole egg	235 ± 89
Skim milk	230 ± 40
Lactalbumin	215 ± 69
Beef	160 ± 60
Pork	110 ± 17
Egg white	105 ± 28
Vegetable protein	
Wheat gluten	80 ± 21
Peanut protein	80 ± 10
Soy concentrate	25 ± 5
Soy isolate	15 ± 5

[a]After reference 19. Diets contained 30 % protein and 1% corn oil, fed 28 days.

The first comparison of the atherogenic effects of animal and vegetable protein was carried out by Meeker and Kesten (17,18). Their studies (Table 1) showed clearly that soy protein was less atherogenic than casein. One difficulty with the data derived by those investigators and one that still exists is that, while casein and soy protein are the most readily available sources of animal and vegetable protein and thus have been used widely,

they might not be completely typical of animal and vegetable protein, respectively. Hamilton and Carroll (19) fed rabbits diets containing 30% of various delipidized animal and vegetable proteins for 28 days. Their results (Table 2) showed that, in general, proteins of animal origin are more cholesterolemic than those of plant origin and that within the two classes there is a wide range of cholesterolemic effect. Huff et al. (20) found that enzymatic hydrolysates of casein or soy protein were less cholesterolemic than the original protein, but that amino acid mixtures approximating the different protein compositions were not (Table 3).

On the basis of our studies comparing the effects of animal and vegetable protein on atherosclerosis in rabbits fed semipurified, cholesterol-free diets, we hypothesized that the ratio of lysine to arginine present in a protein could be an important determinant of its atherogenic capacity (21). Comparison of corn protein, wheat gluten and lactalbumin (22) showed that lactalbumin was twice as cholesterolemic as and much more atherogenic than either of the plant proteins (Table 4). To test the effects of lysine (L) and arginine (A), several studies were carried out in which rabbits were fed casein (L/A≈ 2.0), soy protein (L/A≈ 1.0), casein plus enough arginine to give an L/A≈ 1.0 and soy plus enough lysine to give an L/A≈ 2.0 (23). As expected, soy protein was less atherogenic than casein. Addition of arginine to casein did not affect cholesterolemia but reduced atherosclerosis by 26%; addition of lysine to soy protein increased average serum cholesterol by 33% and atherosclerosis by 156% (Table 5). Rabbits fed casein or

TABLE 3

Effect of Casein, Soy Protein, their Hydrolysates or Amino Acid Mixtures on Serum Cholesterol Level of Rabbits[a]

Regimen	Serum cholesterol (mg/dl)
Intact:	
Casein	213 ± 53
Soy Protein	69 ± 12
Enzymic hydrolyzate:	
Casein	178 ± 30
Soy protein	41 ± 8
Amino acid mixture resembling:	
Casein	213 ± 42
Soy protein	124 ± 30

[a]After reference 20. Diet: 27% protein, 60% dextrose, 5% fiber, 4% salt mix, 3% molasses, 1% corn oil, 0.2% vitamin mix.

TABLE 4

Effect of Corn Protein, Wheat Gluten or Lactalbumin on Atherosclerosis in Rabbits[a,b]

	Corn Protein	Wheat Gluten	Lactalbumin
Number	7	8	7
Avg. atherosclerosis			
Arch	0.4	0.6	1.1
Thoracic	0.2	0.4	0.6
Serum lipids (mg/dl)			
Cholesterol	158 ± 25	152 ± 18	312 ± 79
Triglycerides	94 ± 9	98 ± 13	122 ± 52
Serum lipoproteins μg/ml (%)			
VLDL	26(3.3)	10(2.5)	84(5.8)
IDL	130(16.3)	41(10.2)	382(26.4)
LDL	178(22.4)	145(36.1)	333(23.0)
HDL	462(58.0)	206(51.2)	648(44.8)

[a]Diet contained 40% sucrose, 25% protein, 15% cellulose, 14% coconut oil, 5% salt mix, 1% vitamin mix. Fed 10 mo.
[b]After reference 22.

TABLE 5

Serum Lipids, Lipoproteins and Atherosclerosis in Rabbits Fed Casein, Casein Plus Arginine, Soy or Soy Plus Lysine[a]

	Regimen			
	Casein(C)	C + Arginine	Soy(S)	S + Lysine
Number	20	20	25	25
Avg. atherosclerosis[b]	1.34	0.99	0.32	0.82
Serum lipids (mg/dl)				
Cholesterol	250	227	157	209
Triglycerides	105	116	63	74
Serum lipoproteins μg/ml (%)				
VLDL	21(2.3)	10(0.9)	9(1.1)	14(2.2)
IDL	130(14.4)	88(7.8)	62(7.7)	114(17.8)
LDL	288(31.9)	405(25.8)	242(30.0)	194(30.2)
HDL	465(51.4)	627(55.5)	494(61.2)	320(49.8)

[a]After reference 23.
[b](Arch plus thoracic)/2 (Increasing severity 0-4).
See Table 4 footnote for diet composition. C + arginine = 23% casein + 1.3% L-arginine, S + lysine = 23.5% soy isolate + 1.94% lysine · HCl. Diets fed 8 mo.

soy plus lysine exhibited elevated levels of very low and intermediate density lipoproteins.

Huff et al. (20) had found that, when casein and soy protein were fed in a 1:1 ratio, the resulting protein mixture was no more cholesterolemic than soy protein. We (24) compared the atherogenic effects of beef protein, casein, textured vegetable protein (TVP) and beef-TVP 1:1. As Table 6 shows, the atherogenic effects of beef and casein were similar, TVP was significantly less atherogenic than either casein or beef and beef-TVP 1:1 was 65% more cholesterolemic than TVP but only 9% more atherogenic; for comparison beef and casein were 400 and 440% more cholesterolemic than TVP and 104 and 118% more atherogenic. In another study, we compared the atherogenicity of fish protein (L/A = 1.44), casein (L/A = 1.89) and whole milk protein (L/A = 2.44) (25). The three proteins have similar levels of lysine (6.78 ± 0.09) so that the influence of lysine, per se, was not in question. If the average atherosclerotic involvement of the arteries of rabbits fed fish protein was set at 1.00, those of casein and whole milk protein were 1.26 and 1.67, respectively ($p < 0.05$) (Table 7).

The moderating influences on casein-induced cholesterolemia of amino acids other than arginine or lysine

TABLE 6

Lipids and Atherosclerosis in Rabbits Fed Beef, Casein, Textured Vegetable Protein (TVP) or Beef-TVP 1:1[a,b]

	Group			
	Beef	TVP	Beef-TVP (1:1)	Casein
Number	12	9	11	11
Serum lipids (mg/dl)				
Cholesterol	185 ± 24	37 ± 4	61 ± 6	200 ± 18
(%HDL-cholesterol)	20 ± 2	39 ± 5	43 ± 4	30 ± 4
Triglycerides	60 ± 8	59 ± 7	70 ± 13	92 ± 10
Liver lipids (g/100g)				
Cholesterol	0.77 ± 0.09	0.28 ± 0.01	0.38 ± 0.06	0.97 ± 0.05
Triglycerides	0.77 ± 0.09	1.16 ± 0.39	0.72 ± 0.12	0.51 ± 0.08
Atherosclerosis[c]				
Arch	1.3 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.2
Thoracic	0.8 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.9 ± 0.1

[a]See Table 4 footnote for diet composition. Fed 8 mo.

[b]After reference 24.

[c]Graded in order of increasing severity 0-4.

TABLE 7

Influence of Fish Protein, Casein and Whole Milk Protein (WMP) on Lipids and Atherosclerosis in Rabbits[a,b]

	Group		
	Fish	Casein	WMP
Number	10	10	9
Lysine (%)	6.81	6.91	6.61
Arginine (%)	4.74	3.65	2.71
L/A	1.44	1.89	2.44
Serum lipids (mg/dl)			
Cholesterol	283 ± 40	530 ± 76	462 ± 62
(% HDL cholesterol)	16 ± 1	12 ± 1	12 ± 2
Triglycerides	122 ± 20	177 ± 47	251 ± 56
Atherosclerosis[c]			
Arch	1.6 ± 0.2	2.1 ± 0.3	2.6 ± 0.2
Thoracic	1.0 ± 0.2	1.1 ± 0.3	1.6 ± 0.2

[a]See Table 4 footnote for dietary composition. Fed 8 mo.

[b]After reference 25.

[c]Graded in order of increasing severity 0-4.

have been studied by other investigators. Huff and Carroll (26) examined the effects of a large number of modifications of dietary amino acid content and found few to affect cholesterolemia in rabbits (Table 8). Terpstra et al. (27) summarized experiments in which rabbits were fed diets containing casein (20.8%) which gave a serum cholesterol level of 477 ± 59 mg/dl or the same level of casein plus 0.2% methionine (cholesterol, 710 ± 88) or 0.8% arginine (cholesterol, 548 ± 78). In a second study, a diet containing 20.8% casein gave a cholesterol level of 244 ± 55 mg/dl; when the casein was augmented with 1.38% glycine the cholesterol level was 106 ± 15.

Casein is more cholesterolemic than soy protein in rats (28-30), but lysine and/or arginine do not appear to affect cholesterolemia (29,30). The casein-soy difference has also been observed in swine (31).

Hodges et al. (32) studied the consequences of changing amount and type of carbohydrate and fat on cholesterolemia in men. The subjects were maintained on plant protein as the only source of protein in order to refrain from introducing yet another dietary variable. Changing from a mixed protein diet to one containing only vegetable protein resulted in a significant fall in serum cholesterol levels. When Anderson et al. (33) replaced half of the daily protein intake with egg white protein or wheat gluten, no effect on cholesterolemia was observed. True vegetarians exhibit low serum cholesterol levels, but cholesterol levels of lacto-ovo vegetarians resemble those of the general population (34).

Sirtori and his colleagues (35,36) have shown that soy protein isolate will lower serum cholesterol levels in hypercholesterolemic subjects regardless of the level of saturation of the dietary fat. Switching from a mixed diet to one containing textured vegetable protein reduces the serum L/A by 22.8% (37).

There are several mechanism(s) by which casein exerts its cholesterolemic effect. The rate of lipid absorption is faster in rats fed casein or soy plus lysine (38) (Table 9). Huff and Carroll (39) found that rabbits fed soy protein oxidized considerably more cholesterol than did those fed casein. Rabbits fed casein also exhibited a slower cholesterol turnover rate. We (23) have found that addition of lysine to soy protein does not influence the cholesterol production rate in rabbits but increases mean transit time from 18.4 days to 33.7 days. Rabbits fed casein also excrete less steroids in the feces than do rabbits fed soy protein (40). Thus, casein feeding slows turnover time of cholesterol, increases body cholesterol pools and reduces steroid excretion. The influences addition of single amino acids to proteins have or the way peptides formed by protein digestion may affect cholesterol metabolism remain to be elucidated.

TABLE 8

Serum Cholesterol Levels (mg/dl) in Rabbits Fed Amino Acid (AA) Mixtures[a]

Regimen	Serum cholesterol (28 day)
Soy AA	124 ± 30
Casein AA	213 ± 42
Soy ½EAA[b]	116 ± 30
Casein ½EAA	271 ± 48
Soy EAA-glu[c]	195 ± 30
Casein EAA-glu	99 ± 14
Soy EAA-Ala[d]	227 ± 42
Casein EAA-Ala	253 ± 52
Soy (46%) AA (54%)[e]	94 ± 23
Casein (53%) AA (47%)	185 ± 50

[a]After reference 26. (See Table 3 footnote for diet composition).
[b]Essential AA reduced by 50%, non-essential AA doubled.
[c]Essential AA; non-essential nitrogen supplied by glutamic acid.
[d]Essential AA; non-essential nitrogen supplied by alanine.
[e]Protein plus AA added to give total composition of casein in case of soy protein and soy protein in case of casein.

TABLE 9

Absorption of Cholesterol or Oleic Acid in Rats Fed Casein, Soy Protein, Casein Plus Arginine or Soy Protein Plus Lysine[a]

	Regimen			
	Casein (C)	C + Arginine	Soy (S)	S + Lysine
Oleic acid absorption (%)				
0-8 hr	44	38	20	59
8-24 hr	24	45	40	22
Cholesterol absorption (%)				
0-8 hr	24	20	14	29
8-24 hr	24	38	32	22

[a]After reference 38.

ACKNOWLEDGMENTS

Supported, in part, by Research Career Award (HL-00734) and a grant (HL-03299) from the National Institutes of Health and by funds from the Commonwealth of Pennsylvania.

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[Received January 24, 1986]