CHOLESTEROLEMIC EFFECTS OF THE LYSINE/ARGININE RATIO IN RABBITS AFTER INITIAL EARLY GROWTH

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SUMMARY

The lysine/arginine ratio has been directly associated with serum cholesterol levels. Male, New Zealand rabbits with a mean weight of 2.1 kg were fed, *ad libitum*, one of three diets containing 14o/o vegetable oil and 20o/o protein from casein, soy or almonds with lysine/arginine ratios of 2.2, 0.9, or 0.3, respectively. At the end of three weeks for phase 1, the serum cholesterol level of the casein group (154 ± 25 mg/dl, mean ± SD) was twice the level and significantly greater ($p < 0.02$) than either of the plant protein groups (soy 70 ± 7, almond 78 ± 6 mg/dl). During phase 2, the almond diet was supplemented with L-lysine to increase the lysine/arginine ratio from 0.3 to 3.0 while casein remained as the high, and soy the low lysine/arginine ratio control diets. Serum cholesterol levels remained high for the casein, and low for the soy groups, while lysine supplementation significantly increased ($p < 0.05$) the serum cholesterol level in the almond protein group (from 78 ± 6 to 101 ± 10), but not greater than the casein group. Growth was similar for rabbits fed soy or casein diets throughout the study, but lower ($p < 0.02$) for the almond group. Thus, growth rate was not related to the effect of dietary protein on levels of serum cholesterol.

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While there is a direct relationship between hypercholesterolemia and the absolute amount of dietary lysine and with the lysine/arginine ratio, the data suggest that this is only a partial explanation for the effect of proteins on the control of serum cholesterol levels.

INTRODUCTION

Protein quality is dependent on the amino acid composition of proteins and, in general, animal proteins provide the best balance of amino acids for maximum growth in animals when given as the sole source of protein in the diet (1-3). Lysine appears to be one of the amino acids most closely associated with the growth rate of weanling animals, and with the biological value of diets containing ordinary proteins of plant origin (4-6). Lysine is a common limiting amino acid in cereal grains, which are a major source of the total dietary protein intake in many countries. For this reason, lysine supplementation of cereals has been considered for improving protein quality (7-9), and foods high in lysine content have been developed (10). Recent data, however, relate the lysine content of proteins to serum levels of cholesterol (11-16).

The protein quality of soy protein and casein, the two most studied proteins in nutrition, are adequate proteins for infant and adult nutrition (17-19), have similar growth promoting qualities (20) and lysine content (21). Nevertheless, soy protein with a lysine to arginine ratio of 0.9 is hypocholesterolemic, and casein with a lysine to arginine ratio of 2.2 is hypercholesterolemic (11-14). It is becoming apparent that low lysine to arginine ratios are associated with a low atherogenic index (11), and low levels of serum cholesterol in animals (11-13) and human subjects (14). Since plant proteins are generally low in lysine relative to arginine, changing from a typical meat-containing diet of populations on a high protein intake to a vegetarian diet, results in significant reduction in serum cholesterol and triglycerides as well as a significant lowering of the lysine to arginine ratio of plasma in subjects attending a lifestyle modification center (15, 16). Thus, the amino acid composition of proteins is not only responsible for protein quality, but is also implicated in the control of serum cholesterol associated with cardiovascular disease.

The purpose of this study was to determine the level of serum cholesterol in rabbits fed animal or vegetable proteins having a lysine to arginine ratio that is high (casein = 2.2), low (soy = 0.9), or very low (almonds = 0.3). In addition, serum cholesterol was measured in rabbits fed almond protein, wherein the lysine/arginine ratio was increased to 3.0 by lysine supplementation.

MATERIAL AND METHODS

Animals

Each diet group was composed by six young male New Zealand rabbits with an average weight of 2.1 kg. They were housed in individual raised-bottom stainless steel cages in a room kept at 21-22°C, and 45-

60o/o humidity. Animals were allowed food and water *ad libitum.* After a 12 to 15 hour overnight fast, the rabbits were placed in restraining cages, the ear was swabbed with xylenol to draw the blood into the vein, and then with glycerol to prevent clotting *in situ.* Blood was removed by a transverse cut of the marginal vein in the ear and caught in a centrifuge tube without anticoagulant. The blood was then centrifuged after clotting, and the serum was frozen. All animals were weighed at the end of 1, 3 and 5 weeks.

Diet

Table 1 shows the diet composition. The dietary proteins were vitamin-free casein from ICN Nutritional Biochemicals, isolated soy protein from Ralston Purina Company[6], or partially defatted almond meal[7]. The almond powder and almond oil were prepared by hexane extraction of whole almond meal. The vitamin mix, cellulose as alphacel, and the U.S.P. Salts XIV, were from ICN Nutritional Biochemicals. The salt mix was fortified with 0.68 mg of copper sulfate, 0.338 mg of zinc sulfate and 1 g choline per kg of diet. The diets were mixed and kept in a refrigerator at 5oC. These diets contained 30o/o of the calories as fat. The lysine/arginine ratio of almonds, soy and casein was 0.3, 0.9 and 2.2, respectively (21). Diets were fed *ad libitum* for a period of three weeks during phase 1. Phase 2 was the continuation of the experiment for an additional two weeks during which L-lysine was added to the almond diet to increase the lysine to arginine ratio from 0.3 to 3.0, which is similar to whole milk, with a ratio of 2.8 (21). The casein group remained as the high lysine to arginine ratio control group, and the soy protein as the low ratio control group to the lysine-supplemented almond group. Nitrogen was analyzed by the macro Kjeldahl method (22). The standard factors for converting nitrogen to protein were used (21).

TABLE 1

WEIGHT OF RABBITS FED DIFFERENT PROTEINS

Ingredient*	o/o by weight
Protein	20
Almond oil	14
Dextrin	50
Cellulose	10
Mineral mix	5
Vitamin mix	1

* See text for complete details of ingredients.

6 Anonymous. Isolated soy protein general product description. St. Louis, MO, Ralston Purina Company, Protein Division, 1979.

7 Almond powder and its composition were kindly supplied by Dr. J.A. Mattei.

Total serum cholesterol was analyzed from the frozen serum by the enzyme methods of Lie *et al.* (23) and Allain *et al.* (24) using the Technicon SMAC I System. LDL and HDL cholesterol were measured by the same enzyme methods mentioned above, coupled with separation of the lipoproteins by electrophoresis according to Castelli *et al.* (25). The LDL cholesterol includes the VLDL cholesterol fraction. The data were statistically evaluated by analysis of covariance, paired t-test and multiple comparison F-test analyses (26).

RESULTS

The initial weight was similar for all groups, as shown in Table 2. All animals significantly increased ($p < .005$) in weight throughout the experimental period. Weight gain was similar for rabbits fed casein and soy during phase 1 and phase 2. Animals fed almond protein during phase 1, or almond protein supplemented with lysine during phase 2, grew less compared to the other two groups.

TABLE 2

WEIGHT OF RABBITS FED DIFFERENT PROTEINS

		Phase 1 (0-3 wks)	Phase 2[a] (3-5 wks)		
Protein source	N	0	3[b]	5	*Significance*
Casein	5	2161 ± 61[b1]	2431 ± 85[1,2]	2644 ± 90[1]	$p < .005$
Soy	6	2074 ± 55[1]	2571 ± 80[1]	2728 ± 107[1]	$p < .005$
Almond	6	2159 ± 92[1]	2276 ± 80[2]	2313 ± 116[2]	$p < .005$
Significance		NS	$p < .02$	$p < .002$	

a L-lysine was added to the almond diet during phase 2 to raise the lysine/arginine ratio from 0.3 to 3.0.

b Mean ± SEM reported in grams; significant differences between diet (vertical comparisons) are indicated by mean values with different number superscripts; significant growth between time periods (horizontal comparisons) is not specified, but all animals gained weight.

Table 3 depicts the serum cholesterol of rabbits fed the three different proteins. The mean total serum cholesterol was statistically similar for all three groups at the beginning of the experiment. At the end of phase 1, mean serum cholesterol was significantly higher in rabbits fed casein (154 ± 25 mg/dl, mean ± SEM) compared to soy (70 ± 7) or almond (78 ± 6). At the end of phase 2, the total serum cholesterol was also higher ($p < .02$) in the casein (109 ± 7) as compared to the soy group (58 ± 12). Adding lysine to the almond diet during the two weeks of phase 2 resulted in a higher cholesterol level (101 ± 10) than the soy group ($p < .02$), and

TABLE 3

SERUM LIPIDS IN RABBITS FED SEMIPURIFIED DIETS DIFFERING IN PROTEIN SOURCE

		Phase 1 (0-3 wks)	Phase 2[a] (3-5 wks)		
A. Total cholesterol protein source	N	0	3[b]	5	Significance
Casein	5	63 ± 4[b,1]	154 ± 25[1]	109 ± 7[1]	$p < .003$
Soy	6	56 ± 6[1]	70 ± 7[2]	58 ± 12[2]	NS
Almond	6	64 ± 5[1]	78 ± 6[2]	101 ± 10[1]	$p < .03$
Significance		NS	$p < .02$	NS	
B. LDL cholesterol					
Casein	5	40 ± 61[1]	90 ± 21[1]	74 ± 4[1]	$p < .002$
Soy	6	36 ± 6[1]	47 ± 7[2]	42 ± 10[1]	NS
Almond	6	39 ± 4[1]	43 ± 5[2]	56 ± 7[1]	NS
Significance		NS	$p < .02$	NS	
C. HDL choleterol					
Casein	5	23 ± 3[1]	46 ± 6[1]	36 ± 6[1]	NS
Soy	6	20 ± 4[1]	22 ± 2[2]	16 ± 1[2]	NS
Almond	6	25 ± 2[1]	29 ± 2[2]	44 ± 4[1]	$p < .02$
Significance		NS	$p < .05$	$p < .05$	

a L-lysine was added to the almond diet at 3 weeks to raise the lysine/arginine ratio from 0.3 to 3.0

b Mean ± SEM; significant differences between diet (vertical comparisons) are indicated by mean values with different numbers; significance between time periods (horizontal comparisons) are not specified.

statistically similar to the casein group. There were apparent, but not statistically significant differences, when comparing total serum cholesterol at the end of phase 1 to the end of phase 2 for the soy protein group, or the casein group. The tendency of cholesterol to decrease with time may be the result of a physiological adjustment to the stress of the experimental procedure.

Table 3 also indicates the LDL (low-density lipoprotein, including the very low density lipoproteins) and HDL (high-density lipoprotein) cholesterol levels. In general, the results with the LDL cholesterol follow the same pattern as those with the total cholesterol levels. LDL cholesterol was higher ($p < .02$) during phase 1 in the casein group (90 ± 21 mg/dl) as compared to the soy (47 ± 7) or the almond group (43 ± 5). After adding lysine to the almond diet in phase 2 to change the lysine to arginine ratio from 0.3 to 3.0, the LDL cholesterol was increased (43 ± 5 to 56 ± 7 mg/dl), although not significantly, compared to the unsupplemented diet period. The HDL cholesterol was significantly greater ($p < .05$) in the casein group (46 ± 6) as compared to the soy (22 ± 2) or almond groups (29 ± 2) at the end of phase 1. The addition of lysine to the almond diet resulted in an increase of HDL cholesterol ($p < .02$). The HDL cholesterol was greater ($p < .05$) in the casein group (36 ± 6), and the lysine-supplemented almond group (44 ± 4) as compared to the soy group (16 ± 1) at the end of the two weeks of phase 2.

DISCUSSION

The protein quality of casein and soy is considered to be equivalent in growing weanling animals as determined by the rat growth method (20). In the present study, weight gain in maturing young rabbits was similar for soy and casein diets (Table 2), but their effect on serum cholesterol levels was markedly different (Table 3). Interestingly, changes in HDL cholesterol levels were in opposite direction of what might be expected in humans. Growth rate was different between the groups fed plant proteins, unsupplemented almond or soy, but both had similarly low levels of serum cholesterol as compared to casein. Therefore, growth rate is not necessarily related to the level of serum cholesterol. Animal proteins are generally higher in lysine and other essential amino acids (21) and thus have higher protein quality when single foods are fed as the sole protein source (1-3, 6). On the other hand, plant proteins are generally higher in arginine and nonessential amino acids in comparison to animal proteins (21), and are associated with lower serum cholesterol levels. Soy protein has over twice as much glycine and arginine than casein, and is consistently hypocholesterolemic (11-13, Table 3). The lower quality of plant proteins when fed separately can be made comparable to animal proteins by amino acid supplementation (7) or by judicious combinations of plant proteins (6). Since plant proteins are generally low in the lysine/arginine ratio compared to animal proteins, the common practice of ingesting complementary plant proteins in Third World countries is fortuitous, because it leads not only to diets adequate in protein quality, but also to lower levels of serum cholesterol.

Lysine addition to the hypocholesterolemic almond protein to increase the lysine/arginine ratio led to elevated serum cholesterol concentrations (Table 3). However, there has been some question as to the association of dietary lysine with serum cholesterol. Gibney (27) showed that the addition of lysine to soy significantly increased serum cholesterol in rabbits fed the diet for a period of four weeks, but the level of serum cholesterol was not necessarily proportional to the content of lysine in the diet (27, 28). The association of lysine with hypercholesterolemia and atherosclerosis has been observed in man (14) and animals (11, 27). This view is challenged by Serougne and Rujak (29) who observed a lowering rather than an increase of serum cholesterol in rats fed a diet supplemented with lysine. Their diet contained 10% lysine by weight in a 23% casein diet, i.e., 30% of the total protein is free lysine. This amount of lysine is well within the toxic level, as shown by the decreased growth of the rats fed additional lysine; therefore, their data cannot be considered in the context of normal dietary effects of amino acids on serum cholesterol.

On the other hand, Mokady and Liener (30) showed that raising the lysine content from 0.18% to 0.9% in a 20% gluten protein diet significantly increased serum cholesterol in rats from 266 to 409 mg/dl. This was approaching the 637 mg cholesterol/dl of serum in rats fed casein which contained 0.7% lysine. The data of Serougne and Rujak (29) addresses the question of the toxic effect of lysine, while that of Mokady and Liener (30) shows a direct relationship between normal levels of lysine in the diet and serum cholesterol. Our data (Table 3) is consistent with a hypercholesterolemic effect of dietary lysine in mammals. There is substantive data to show that, in chicks, dietary lysine *per se* is hypercholesterolemic when added up to 4% of the diet; lysine apparently increases the biosynthesis of cholesterol (31).

The lysine/arginine ratio has been directly associated with hypercholesterolemia and atherosclerosis (11-14). The present study shows that the lowest serum cholesterol levels are achieved by a dietary lysine to arginine ratio of 0.9 in soy and is not decreased further by almond protein with a lysine/arginine ratio of 0.3 (Table 3). Also, a lysine/arginine ratio of 3.0 in the lysine supplemented almond group does not increase serum choleterol above that of casein with a lysine/arginine ratio of 2.2. Egg protein, used as the standard of protein quality (6), has a lysine/arginine ratio of only 1.0, but is hypercholesterolemic (13). These data demonstrate limitations to the possible dietary lysine/arginine ratio effect on serum cholesterol in the control of serum cholesterol levels. A 1:1 combination of soy and casein protein eliminates the hypercholesterolemic effect of casein (32) which may be due to amino acid interactions. It is possible that less almond protein is required to produce a similar effect as soy, due to its high content of arginine in relation to lysine in almond protein.

Multiple plasma amino acid relationships with serum cholesterol levels in humans, including leucine (15, 16), provide additional evidence that many plasma amino acids are related to the level of serum cholesterol in humans and not only lysine and arginine. Thus, the absolute amount of dietary lysine and the lysine/arginine ratio are directly associated with the level of serum cholesterol, but these only partially explain the effects of dietary proteins on serum cholesterol levels. Our recent studies in humans (33) suggest that the dietary amino acid effects on serum cholesterol are mediated by several plasma amino acid-induced changes in blood concentrations of insulin and glucagon, which control the rate-limiting step in cholesterol biosynthesis.

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RESUMEN

EFECTOS COLESTEROLEMICOS DE LA RAZON LISINA/ARGININA EN CONEJOS, DESPUES DEL CRECIMIENTO INICIAL

La razón lisina/arginina ha sido asociada directamente con los niveles séricos de colesterol. Se utilizaron conejos macho de la cepa Nueva Zelandia, con un peso promedio de 21 kg, los que fueron alimentados *ad libitum* con una de tres diferentes dietas que contenían 14o/o de aceite vegetal y 20o/o de caseína de proteína soja o de almendras, cuya razón lisina/árginina era de 2.2, 0.9 ó 0.3, respectivamente. Al término de tres semanas de la primera fase del estudio, el nivel sérico de colesterol del grupo de caseína era de 154 ± 25 mg/dl (promedio ± DE), o sea el doble ($P < 0.02$) que cualquiera de los grupos alimentados con proteínas de origen vegetal (soja 70 ± 7 mg/dl; almendras, 78 ± 6 mg/dl). Durante la segunda fase, la dieta con proteína de almendras se suplementó con L-lisina para aumentar la razón lisina/arginina de 0.3 a 3.0, mientras que la dieta de caseína se mantuvo como grupo control alto, y la de soja, como grupo control con una razón lisina/arginina, baja. Los niveles séricos de colesterol se mantuvieron elevados en el grupo de caseína y bajos en el grupo que recibió soya, mientras que la suplementación con lisina incrementó significativamente ($P < 0.05$) el nivel sérico de colesterol en el grupo alimentado con almendras, el que ascendió de 78 ± 6 mg/dl a 101 ± 10 mg/dl, pero sin sobrepasar el nivel del grupo de caseína. Así, el crecimiento fue similar en los conejos alimentados con soja o caseína durante todo el período del estudio, pero más bajo ($P < 0.02$) en el grupo al que se administró proteína de almendras. El crecimiento, por lo tanto, no se relaciona con el efecto de las proteínas de la dieta en los niveles de colesterol sérico.

Si bien la cantidad absoluta de lisina en la dieta y la razón lisina/arginina están asociadas positivamente con hipercolesterolemia, los datos sugieren que esto es tan sólo una explicación parcial del efecto que las proteínas ejercen en el control de los niveles séricos de colesterol.

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