

# SEPARATE EFFECTS OF DIETARY PROTEIN AND FAT ON SERUM CHOLESTEROL LEVELS: ANOTHER VIEW OF AMINO ACID CONTENT OF PROTEINS

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## SUMMARY

Casein or soy protein with vegetable or animal fat were used to determine the dietary protein or fat effects and their possible interaction on serum cholesterol levels. Young, male New Zealand white rabbits with a mean weight of 2.1 kg were divided into groups of six and fed one of four different diets containing 20% of the calories as protein, 30% as fat (according to dietary guidelines for the United States) and 50% as carbohydrate. The diets contained casein or soy (lysine/arginine ratio = 2.2 or 0.9, respectively) as the protein sources with fat from either almond oil or butter. There was no significant difference in weight gain among the diet groups. Total serum cholesterol level was highest among animals fed the diet containing butter with casein ( $177 \pm 25$  mg/dl) or soy protein ( $189 \pm 50$  mg/dl), it was intermediate in animals fed the vegetable oil with casein ( $121 \pm 14$  mg/dl), and lowest in the soy protein with vegetable oil group ( $58 \pm 12$  mg/dl). There was a significant difference in serum cholesterol levels due to the protein effect when vegetable oil was used ( $p < 0.05$ ) but not with butter. There was also a significant fat effect on

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serum cholesterol when the diet contained soy protein ( $p < 0.005$ ) but not when the protein was casein. No significant interaction was observed between the dietary fat and protein sources on serum cholesterol levels, which suggests that dietary protein and fat independently affect the levels of serum cholesterol.

Thus, dietary protein has a significant effect on serum cholesterol levels and may be a factor in the low levels of serum cholesterol observed among vegetarians and in humans of Third World countries where the diets is primarily of vegetable origin.

## INTRODUCTION

Estimates of protein quality have largely centered on the amino acid composition of proteins that provide rapid growth and nitrogen retention (1). Using these criteria of protein quality, early studies demonstrated that animal proteins elicit fast growth and have a higher biological value than plant proteins when the diet is composed of only single foods and is fed for prolonged periods of time (2-4). These data indicate that the proportion of amino acids in single foods of animal origin more nearly meets the requirements for maximum growth of animals and humans than plant proteins.

From these important experiments, the conclusion has been drawn that animal proteins are complete and plant proteins, generally, are incomplete. Moreover, animal proteins are richer sources of the essential amino acids and poorer sources of the nonessential amino acids than plant proteins (5). Subsequent studies on protein quality have shown that plant proteins can be combined to produce a protein quality similar to that found in diets containing animal proteins (6-8). Bressani and Béhar (9) have called attention to the importance of evaluating protein quality on the basis of the amino acid content of the entire meal rather than relegating proteins into poor or high quality proteins based on their growth-promoting properties when separate proteins are fed as the sole source of protein.

Recent studies on protein nutrition (10-16) show that, aside from dietary lipids and carbohydrates (17), proteins are involved in modulating the level of cholesterol in serum of animals and humans. In general, plant proteins are hypocholesterolemic and animal proteins are hypercholesterolemic. These effects of dietary proteins on serum cholesterol are attributed to their amino acid content (10-16), i.e., protein quality and not quantity of protein in the diet. Thus, this phase of research on protein nutrition is focusing on the possible role of proteins in health and disease which is distinct from protein quality based on growth and nitrogen retention. The superiority of animal proteins over plant proteins resides in an amino acid content that promotes growth and nitrogen retention in the young. The value of plant proteins seems to reside in an amino acid content that is associated with lowered serum cholesterol levels and thereby a lower risk to cardiovascular disease.

The two most studied proteins are casein and soy protein which have comparable protein quality (18) but a remarkably different effect on cholesterol levels and atherogenesis (12-16). Casein is known to be hypercholesterolemic and soy protein hypocholesterolemic. The arginine and glycine content of soy protein isolate is over two times greater than

for casein (19). Arginine and glycine have been associated with hypocholesterolemia in animals (20) and have been found to increase in fasting plasma of humans which changed from a typical meat-containing diet to a vegetarian diet (10) or who were fed a soy protein diet (21). A previous report on rabbits (19) revealed that casein is hypercholesterolemic compared to soy or almond protein when the diet contains vegetable oil. The present article compares the serum lipids levels of rabbits fed either casein or soy protein with either an animal or vegetable fat.

This study was designed to determine if there was an interaction between dietary fat and protein in their effects on the serum cholesterol levels in animals fed diets with a fat contributing 30% of the calories, as recommended (22) for human diets.

## MATERIAL AND METHODS

### *Animals and Diets*

Male rabbits of the New Zealand white strain from Red Beau Farms, Redlands, California, were kept individually in stainless steel cages with wire-mesh bases in a room with controlled illumination (12 hr/day), temperature (21-22°C) and humidity (45-50%). All animals were fed a pelleted commercial diet (Ralston Purina Chow) for 10 days after arrival. The rabbits then were divided randomly into four groups of six animals per group, with mean weights of 2.1 kg and allowed a semi-purified diet containing casein + butter (CB), casein<sup>7</sup> + almond oil<sup>8</sup> (CO), soy<sup>9</sup> + butter (SB), or soy + almond oil (SO). Nitrogen was measured by the Kjeldahl method according to Horwitz (23) and the standard nitrogen conversion factors were used (5). The composition of the diets is given in Table 1. Diets were formulated so as to contain 20% protein by weight and 14% fat (30% of calories) which is the dietary fat goal for the United States. A typical analysis of the almond oil used in this study<sup>8</sup> was 61% oleic, 29% linoleic acids and 10% saturated fatty acids. Butter, on the other hand, is rich in saturated fatty acids (50%) but low in polyunsaturated fatty acids (3%). The butter-containing diets were estimated to contain 31 mg of cholesterol per 100 g of feed as calculated (24) from the cholesterol content of butter (219 mg/100 g). The diets contained 1% AIN Vitamin Mix 76 supplemented with 1 g choline chloride/kg ration and 5% USP Salts XIV supplemented with 1.5 mg copper (as copper sulfate) and 34 mg zinc (as zinc sulfate) per kilogram of dry ration. Food and water were allowed *ad libitum*. The animals were weighed at weeks 1, 3 and 5 of the experimental period. During the experiment, one rabbit from the CB group died of an unknown cause and one from the SB group was diagnosed as having a congenitally

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<sup>7</sup> Vitamin-free casein from ICN Nutritional Biochemicals.

<sup>8</sup> Almond oil and its analyses were supplied by courtesy of John Mattei of Blue Diamond, California Almond Growers Exchange.

<sup>9</sup> Ralston Purina Supro 620T soy protein isolate with 0.1% titanium.

TABLE 1

COMPOSITION OF SEMIPURIFIED DIETS<sup>a</sup>

Ingredient	% Weight
Protein <sup>b</sup>	20.0
Lipid	14.0
Dextrin	50.0
Cellulose	10.0
Vitamins	1.0
Minerals	5.0

a See text for details.

b Purified high nitrogen casein or soy protein isolate.

malformed jaw. No relationship between diet group and mortality or morbidity was apparent.

#### *Analysis of Serum Lipids*

On day 35 of the experimental period, blood samples were drawn between 0800 and 1,200 hours after the removal of any remaining food in the cage at 2,200 hours the previous day. Approximately 5 ml of blood were collected in tubes without anticoagulant after a transverse cut of the marginal ear vein. After coagulation of blood at room temperature, serum was prepared by low-speed centrifugation also at room temperature. Cholesterol in serum was measured enzymatically according to the method of Lie *et al.* (25) and Allain *et al.* (26). The cholesterol in lipoproteins was analyzed by the electrophoresis methodology of Castelli *et al.* (27).

#### *Statistics*

The overall effects between dietary protein or fat on serum total cholesterol levels were statistically analyzed by the general linear hypothesis test. The multiple F-test analysis was used to evaluate the differences between diet groups. These statistical tests were run by use of the SPSS package of Nie *et al.* (28).

## RESULTS

There were no significant differences in weight gain between the casein and soy groups although, for unknown reasons, the group fed the soy with butter gained the least, and the soy with almond oil group gained the most weight (Table 2).

The concentration of lipids in rabbit serum is shown in Table 3. There was a significant difference in serum total cholesterol levels between

**TABLE 2**  
**WEIGHT GAIN OF RABBITS FED EXPERIMENTAL DIETS DURING Ad35-DAY**

Diet	Sample size	Weight gain
		Mean $\pm$ SE
Casein & butter	5	568 $\pm$ 135 <sup>a</sup>
Casein & oil	6	484 $\pm$ 76
Soy & butter	5	341 $\pm$ 131
Soy & oil	6	654 $\pm$ 89

a No statistical significance between any group means.

**TABLE 3**  
**THE DIETARY PROTEIN OR FAT EFFECTS ON SERUM CHOLESTEROL  
IN RABBITS DURING 5 WEEKS**

Serum lipid diet		Diet		Significance <sup>a</sup>	
		(mg/dl) Mean $\pm$ SE	(mg/dl) Mean $\pm$ SE		
A. TOTAL	CB <sup>b</sup>	177 $\pm$ 25	CO <sup>c</sup>	121 $\pm$ 14	NS
	SB <sup>b</sup>	189 $\pm$ 50	SO <sup>c</sup>	58 $\pm$ 12	p < .005
		NS		p < .05	
B. VLDL + LDL	CB	136 $\pm$ 25	CO	80 $\pm$ 8	NS
	SB	158 $\pm$ 50	SO	42 $\pm$ 11	p < .005
		NS		NS $\rho$	
C. HDL-C	CB	41 $\pm$ 1	CO	41 $\pm$ 7	NS
	SB	25 $\pm$ 3	SO	16 $\pm$ 2	p < .05
		p < .05		p < .001	

a Horizontal values (means  $\pm$  SEM) compare the fat effect, vertical values compare the protein effect; statistical analysis by the F-test.

b Sample size = 5.

c Sample size = 6.

dietary fat groups when the diets contained soy protein ( $p < .005$ ), but not when they contained casein. There was also a significant difference in total serum cholesterol levels between protein groups ( $p < .05$ ) when the diets contained almond oil but not when they contained butter.

Table 3 depicts the combined value for the cholesterol in the VLDL (very low density lipoproteins) and the LDL (low density lipoproteins) fractions of the lipoproteins in serum of rabbits. As observed, the mean values were proportionately similar to those for total cholesterol, shown

above. Again, the lowest concentration of serum lipids was in the group fed plant protein and vegetable oil.

Table 3 also indicates the results of dietary protein on high-density lipoprotein cholesterol. There was a significant decrease in HDL (high density lipoprotein) cholesterol due to soy protein when the diet included either animal ( $p < .05$ ) or vegetable fat ( $p < .001$ ). A difference in fat effect was noted only when the protein was from soy ( $p < .05$ ), not casein.

The linear hypothesis analysis of the data in Table 3, to determine 1) whether dietary protein or fat affects the serum lipid levels, and 2) whether there is an overall interaction between the dietary protein and fat on the serum lipid levels, is shown in Table 4. As the data reveal, there was a significant dietary fat effect on all the serum lipid fractions ( $p < .04$  to  $p < .006$ ) and a significant dietary protein effect on HDL cholesterol ( $p < .04$ ). No significant overall interaction between dietary protein and fat on the level of cholesterol or its lipoprotein fractions was observed.

TABLE 4

OVERALL STATISTICAL SUMMARY LEVEL OF INTERACTION AND EFFECTS OF DIETARY PROTEINS AND FATS ON SERUM LIPIDS IN RABBITS

Serum lipid	Protein-fat interaction	Nutrient effect	
		Protein	Fat
<i>Cholesterol</i>			
<u>Total</u>	NS <sup>a</sup>	NS	$p < 0.006$
<u>LDL-C</u>	NS	NS	$p < 0.007$
<u>HDL-C</u>	NS	$p < 0.001$	$p < 0.04$

a Statistical analysis by the general linear hypothesis; NS = No statistically significant difference.

## DISCUSSION

This study was designed to test the effects of dietary fat and protein and the possible interaction of these nutrients on serum cholesterol levels in rabbits fed diets containing fat at 30% of calories as suggested by the United States dietary guidelines (22). It is significant to note that the lowest serum cholesterol level was achieved in rabbits fed a diet where both the protein and fat are of plant origin (Table 3), indicating that dietary protein as well as fat, affect the serum cholesterol level. The dietary fat effect on serum total cholesterol level became significant only when the diet contained the plant protein, but not when casein was fed. Similarly, the dietary protein effect was evident only when the fat was plant origin.

Our study corroborates the hypothesis of a dietary protein effect on serum cholesterol, as reported by others in the rabbit (13-15), hamster (31, 32) mouse (33), rat (33-35), pig (29, 30, 36) and humans (12). In all these studies the serum cholesterol was lower when the diet contained plant protein as compared to animal protein. The data suggest that the

effect of dietary protein on serum cholesterol was separate from that of the effects of dietary fat, since we found no significant interaction (i.e., additive or synergistic effect) between dietary proteins and fats in their effects on the level of serum cholesterol (Table 4). This conclusion is supported by data from Walsh, Beitz and Jacobson (29) and Forsythe *et al.* (30) who found no interaction between dietary protein and fat on serum cholesterol in pigs fed diets containing 40-42% of calories as fat.

The high level of serum cholesterol in rabbits fed a diet containing soy protein and butter (Table 3) seems unusual, given the hypocholesterolemic effect of soy protein. High levels of serum cholesterol have been reported by Kattleman and Carroll (37) in rats fed diets prepared with soy protein and beef tallow or lard at 40% of calories and by Forsythe *et al.* (29) in pigs fed soy protein in a diet containing tallow at 42% of calories. The high level of serum cholesterol in rabbits (Table 3), rats (37) or pigs (30) fed soy protein diets containing different animal fats, suggest that these high levels of saturated fats which contain cholesterol appear to mask the serum cholesterol-lowering effect of soy protein. Diersen-Schade *et al.* (38) found that serum cholesterol levels were consistently low in pigs fed soy protein with soy oil, but the level of serum cholesterol was the same as in beef-fed pigs if the soy protein diets contained cholesterol in the form of egg yolk. Since cholesterol intake and excretion were similar for both diets containing cholesterol, they conclude that the soy protein is unable to inhibit cholesterol biosynthesis when dried egg yolk is added to an otherwise hypocholesterolemic diet. This effect of egg yolk is confounded by its lipid (38) or protein content (15), which are hypercholesterolemic.

Soy protein and vegetable oil have a lowering effect on serum HDL cholesterol levels (Table 3). The rabbit serum HDL-lowering effect of soy protein has been noted previously in our laboratory (19). In humans, diets that lower total serum cholesterol generally elevate HDL-cholesterol (39). Nevertheless, no correlations between rabbits and man can be made with respect to their HDL cholesterol levels, since the response of high density lipoproteins to diet in these two species appears to be markedly different. Kattleman and Carroll (37) and Forsythe *et al.* (30) found no difference in serum HDL cholesterol due to dietary protein in rats or pigs, respectively. The differences in fatty acid content of tallow, lard and butter (24), as well as species differences may partially explain the differences between our findings and those of others on HDL cholesterol levels.

The data in Table 3 indicate that the differences in amino acid composition of soy protein and casein must be responsible for the differences in levels of serum cholesterol since all other dietary factors are constant. While the possible differential digestibility of casein and soy protein has been considered (36), this does not explain the large differences exerted by these proteins on serum cholesterol levels. Supplements of arginine and glycine, which are high in soy protein, have been associated with hypocholesterolemia in animals (20), and plasma arginine and glycine concentrations are increased in human subjects who change from a typical American to a vegetarian diet (10, 11). Moreover, increases of plasma arginine and glycine in humans are associated with decreased levels of serum cholesterol. Although low serum cholesterol levels and diet-related

low mortality rates among vegetarians has been largely attributed to lipid intake (40-42), our present data show that dietary protein has a specific modifying effect on the levels of serum cholesterol aside from that of fat. Also, a review of epidemiological data (43) provides evidence for a correlation between dietary protein and incidence of coronary heart disease in 20 industrialized or developing countries where animal or plant protein predominate, respectively. The preoccupation with the role of dietary lipids in cardiovascular disease during the past three decades, has caused an oversight of the role of protein in relation to low serum cholesterol levels and the low incidence from cardiovascular disease among vegetarians and persons ingesting primarily plant proteins in Third World countries.

Research has established casein as a standard of protein quality which is directly related to the proportion of essential amino acids in proteins. Recently, however, the hypercholesterolemic effects of casein and the causal association between serum cholesterol levels and cardiovascular disease (44, 45) have been recognized. The hypocholesterolemic effects of soy protein are attributed to its nonessential amino acid content (19). Thus, high quality proteins having an essential amino acid composition that provides the best growth and development in the young may not be the best protein in adult nutrition for the purpose of maintaining low serum cholesterol levels. It now appears that our former concepts on amino acid content of protein to maximize growth and nitrogen retention must be expanded to include the entire physiological response of the body to dietary proteins.

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#### RESUMEN

##### EFFECTOS SEPARADOS DE LA PROTEINA Y DE LA GRASA DE LA DIETA SOBRE LOS NIVELES SERICOS DE COLESTEROL: OTRO PUNTO DE VISTA DEL CONTENIDO DE AMINOACIDOS EN LAS PROTEINAS

Se usó caseína o proteína de soja juntamente con grasa vegetal o grasa animal para estudiar los efectos y posibles interacciones de las proteínas con las grasas de la dieta en los niveles de colesterol sérico. Se utilizaron conejos macho jóvenes, blancos, de la cepa Nueva Zelanda, con un promedio de peso de 2.1 kg. Estos se dividieron en grupos de seis y se alimentaron con una de cuatro dietas diferentes en las que un 20% de las calorías provenía de proteínas, 30% de grasas (según las recomendaciones dietéticas de los Estados Unidos) y 50% de carbohidratos. Las dietas contenían

caseína o soja (razón de lisina/arginina = 2.2 ó 0.9, respectivamente) como fuente de proteína y aceite de almendras o mantequilla, como grasa. No hubo ninguna diferencia significativa en el aumento del peso corporal entre los grupos sujetos a las diferentes dietas. El nivel sérico de colesterol total más alto se encontró en los animales alimentados con las dietas que contenían mantequilla y caseína ( $177 \pm 25$  mg/dl) o mantequilla y proteína de soja ( $189 \pm 50$  mg/dl); ese nivel fue mediano en el grupo de animales que recibieron dietas con aceite vegetal y caseína ( $121 \pm 14$  mg/dl) y el más bajo se detectó en el grupo cuya dieta contenía proteína de soja y aceite vegetal ( $58 \pm 12$  mg/dl). Hubo una diferencia significativa en los niveles séricos de colesterol debido al efecto de las proteínas cuando se utilizó aceite vegetal ( $P < 0.05$ ), pero no así cuando se usó mantequilla. Se observó también un efecto significativo debido a la grasa en el nivel sérico de colesterol cuando la dieta contenía proteína de soja ( $P < 0.005$ ), pero no cuando la proteína era caseína. No se observó ninguna interacción significativa entre las grasas y las proteínas de la dieta, lo que sugiere que la proteína y la grasa de la dieta afectan los niveles séricos de colesterol independientemente. Por consiguiente, la proteína de la dieta tiene un efecto significativo en los niveles séricos de colesterol, y bien puede ser un factor en los bajos niveles de colesterol sérico que se observan entre los vegetarianos, así como en las personas que viven en los países del Tercer Mundo, donde la dieta es predominantemente de origen vegetal.

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