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Serum Lipoprotein Responses to Exogenous Cholesterol in Spider Monkeys: Effect of Levels of Dietary Protein[1] (39613)

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Diet is considered important in relationship to hyperlipoproteinemia. Although exogenous cholesterol is generally related to diet-induced hyperlipoproteinemia in a number of species, including man, the extent of hyperlipoproteinemia is influenced by other components of the diet as well (1). The role of dietary fat and its interaction with dietary cholesterol has been studied extensively, whereas the effect of dietary proteins, the consumption of which varies widely among different populations, has received less attention. Studies of nonhuman primates and other species have shown conflicting results on the interrelationship of dietary cholesterol and protein in the production of hypercholesterolemia and atherosclerosis (2–8). This study demonstrates the effect of varying the protein content of cholesterol-supplemented diets on the induction of hyperlipoproteinemia in nonhuman primates.

Materials and methods. Animals and diets. The experiment was conducted in two phases. The first phase examined the effect of varying the level of protein intake on the induction of hyperlipoproteinemia in eight adult spider monkeys (*Ateles* Sp.) of mixed sexes. In the second phase, four more adult spider monkeys were added to the original group to study the effect of methionine in very low protein diets on serum lipid levels.

Prior to being fed the experimental diets, the animals received a regular diet of Purina Monkey Chow 25, which is approximately 55% carbohydrate, 25% protein, and 5% fat (w/w). The experimental diets were arbitrarily chosen to contain high [25% (w/w)], low [8% (w/w)], or very low [4% (w/w)] levels of protein (Table I), which represent 37, 12, and 6% of protein based on kilocalories. Each animal was fed 200 g of these diets once a day for 3 weeks.

Design. Each animal served as its own control. To minimize any possible influence of the order of dietary sequence (high protein followed by low protein or vice versa) on serum lipids, the animals were divided into two groups (A and B) and fed in the following sequence: Group A: diets I, II, I, III, IV, and III; Group B: diets III, IV, III, I, II, and I. The animals were then grouped together and fed diets V, VI, and V, in that order. All animals were fed regular Purina Monkey Chow 25 for several weeks before the second phase of the experiment, which was initiated based on the results of the first phase. As before, the animals were divided into two groups, and the dietary sequence in each group was started with either very low protein diets or methionine-supplemented diets. Blood (14-hr fasting) was drawn from the femoral vein twice a week under sedation with Sernylan (Bio-Ceutic Laboratories, Inc., St. Joseph, Mo.).

Serum cholesterol. Serum total cholesterol was determined in a Technicon Auto Analyzer II (Technicon Instrument Corp., Terrytown, N.Y.) (9). Cholesterol in β- and pre-β-lipoprotein was determined by a specific and quantitative precipitation procedure using heparin in the presence of Ca^{2+} (10, 11). Briefly, the method consists of mixing serum (0.2 ml), distilled water (3.2 ml), heparin (0.25% $\simeq$140 units/mg, 0.1 ml), and $CaCl_2$ (0.5 *M*, 0.5 ml) in the order given, allowing the contents to stand for 15 min, and collecting the precipitate after centrifugation. In spider monkeys, the β + pre-β-lipoprotein cholesterol obtained by the above precipitation method varied 3–10% from the preparative ultracentrifuge method (12). Consequently, this method was ap-

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TABLE I. DIETARY COMPOSITION.

Ingredients (g/100 g)	High protein (25%)		Low protein (8%)		Very low protein (4%)[a]	
	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI
Purina Chow 25[b]	41.0	38.0	–	–	–	–
Purina Chow 15[b]	–	–	53	52.5	27.0	27.0
Casein	15.0	15.0	–	–	–	–
Butter	–	5.0	–	5.0	–	5.0
Corn oil	–	–	–	–	1.0	1.0
Dextrin	–	–	–	–	30.0	20.0
Hegsted salt	–	–	–	–	1.0	1.0
Vitamins	–	–	–	–	1.0	1.0
Banana	12.0	12.0	15	10.0	11.0	13.5
Cholesterol[c]	–	0.5	–	0.5	–	0.5
Water	32.0	29.5	32.0	32.0	29.0	31.0
Gross energy (kcal/g)	2.48	2.72	2.36	2.71	2.50	2.54

[a] Methionine-supplemented diets were prepared by adding 0.4% of the amino acid to diets V and VI.
[b] Purina chow contains 25 and 15% (by weight) protein.
[c] Crystalline cholesterol was dissolved in melted butter and mixed with other ingredients.

plied in studying the serum lipoproteins of different nonhuman primate species (13). The difference between total cholesterol and β + pre-β-lipoprotein cholesterol was considered as α-lipoprotein cholesterol.

Densitometric ratios of β- and pre-β-lipoproteins. At the end of each dietary period (twenty-first day), electrophoresis of serum lipoproteins was done on an agar–agarose gel according to the method of Noble (14) with some modification (15). The lipoprotein bands were scanned in a Quick Scan densitometer (Helena Laboratories, Beaumont, Tex.), and the densitometric ratios of β- and pre-β-lipoproteins were corrected as described previously (16).

Estimation of β-, pre-β-, and α-lipoproteins. Assuming that cholesterol content per unit β- or pre-β-lipoprotein molecule in monkeys is similar to that of humans, we calculated the serum content of these two classes of lipoproteins based on the densitometric ratios of β- to pre-β-lipoprotein, and β + pre-β-lipoprotein cholesterol content (11, 15). The method was validated by comparing its results with those obtained by analytical ultracentrifuge, and the results were in agreement (11). Serum levels of α-lipoproteins were obtained by multiplying the α-lipoprotein cholesterol value by a factor of 5.9 (16).

Cholesterol response index. The serum cholesterol response to dietary cholesterol was measured in terms of cholesterol response index (mg/100 ml) as described by Bowyer *et al.* (17) with some modifications. This index was obtained by dividing the area under the curve of serum cholesterol concentration, above the basal level, by the duration (days) of feeding of cholesterol-supplemented diet. The basal value for each dietary protein level represented the mean of three determinations obtained at days 14, 17, and 21 during the corresponding basal diet period. Similar indices were calculated for β + pre-β-lipoprotein cholesterol and α-lipoprotein cholesterol.

Serum albumins. Serum albumin assay was done according to the Auto-Ref procedure manual (DADE Division of American Hospital Supply Corp, Miami, Fla.), which is a modification of the standard ether-sulfate method (18).

Histology. The liver biopsies were done using a Vim-Silverman needle. The tissues for light microscopy were fixed in 10% Formalin and processed in paraffin; sections were cut at different levels and stained with hematoxylin and eosin.

Results. The monkeys continued to maintain body weight with no observable ill effects for the duration of the study. When the animals were fed 25, 8, and 4% protein diets, their respective mean ± SD body weights were 6.18 ± 0.78, 6.05 ± 0.99, and 6.28 ± 0.75 kg.

Although the basal serum cholesterol and β + pre-β-lipoprotein cholesterol concentrations in these monkeys tended to decrease slightly with reduced intake of die-

tary protein, the differences were not statistically significant (Table II). Alpha-lipoprotein cholesterol decreased slightly but significantly ($P < 0.05$) when the protein intake was reduced from 25 to 8%. Interestingly, however, further reduction in dietary protein intake to a 4% level caused a highly significant ($P < 0.001$) increase in α-lipoprotein cholesterol. The serum concentrations of the above variables for each level of dietary protein did not change significantly by changing the dietary sequence from a high protein to a low protein diet or vice versa over these short observation periods.

As expected, the animals responded to exogenous cholesterol, but responses varied at the different levels of dietary protein (Fig. 1). For instance, the 25% protein diet gave a relatively higher serum total cholesterol response (28.1 ± 9.2 mg%) than the 8% protein diet (18.7 ± 10.7 mg%). On the other hand, further reduction in dietary protein to the 4% level markedly increased the serum total cholesterol response (51.5 ± 17.6 mg%). Serum cholesterol response to exogenous cholesterol was reflected predominantly in β + pre-β-lipoprotein cholesterol fraction when the animals were on 25% diet (25.4 ± 8.7 mg%) and 8% diet (14.6 ± 7.8 mg%), and the values differed significantly ($P < 0.05$) during the two dietary periods. Both β + pre-β-lipoprotein cholesterol (29.7 ± 12.8 mg%) and α-lipoprotein cholesterol (21.9 ± 6.1 mg%) responses increased markedly upon 4% protein intake. An analysis of variance of the responses of serum total cholesterol ($P < 0.001$), β + pre-β-lipoprotein cholesterol ($P < 0.01$), and α-lipoprotein cholesterol ($P < 0.001$) to a high cholesterol (0.5%) diet showed a significant difference due to the level of dietary protein.

TABLE II. SERUM LIPOPROTEIN CHOLESTEROL LEVELS (MEAN ± SD) IN SPIDER MONKEYS FED DIETS WITH VARYING PROTEIN CONTENT.[a]

Cholesterol fraction (mg/100 ml)	Dietary protein level (g/100 g)		
	25	8	4
Serum total cholesterol[b]	177.6 ± 34.5	167.2 ± 23.4	164.9 ± 20.8
β + pre-β-lipoprotein cholesterol[c]	139.5 ± 37.4	133.2 ± 29.9	110.4 ± 16.4
α-lipoprotein cholesterol[d]	38.1 ± 7.5[e]	34.0 ± 8.5[f]	54.5 ± 6.9[g]

[a] Represents three determinations during each dietary period (diets I, III, and V) for each animal ($N = 8$). $e > f$ ($P < 0.05$); $e < g$ ($P < 0.001$); $f < g$ ($P < 0.001$).

[b] Measured by Auto Analyzer II (9).

[c] Measured by heparin-Ca^{2+} precipitation method (10, 11).

[d] Difference between total cholesterol and β + pre-β-lipoprotein cholesterol.

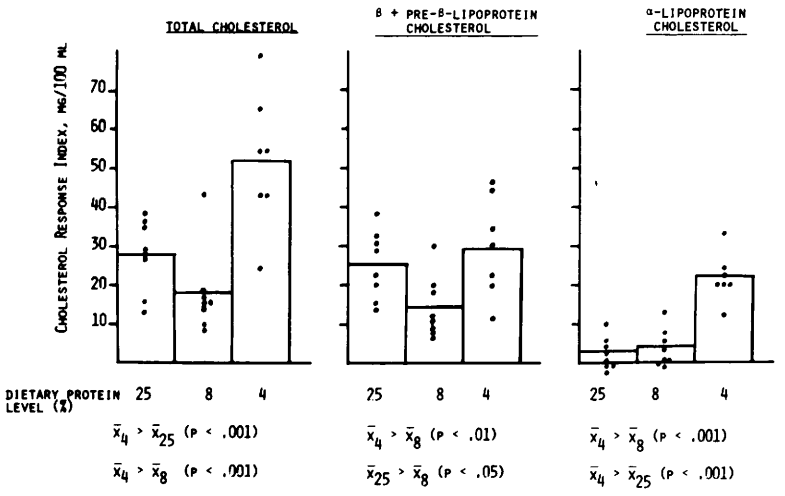

FIG. 1. Serum lipoprotein cholesterol response in spider monkeys fed high cholesterol diets containing different levels of protein.

TABLE III. SERUM CHOLESTEROL AND LIPOPROTEIN LEVELS (MEAN ± SD) IN SPIDER MONKEYS FED HIGH CHOLESTEROL DIETS[a] CONTAINING DIFFERENT LEVELS OF PROTEIN.

(mg/100 ml)[b]	Dietary protein level (g/100 g)			Analysis of variance
	25	8	4	
Total cholesterol	340.4 ± 69.1	290.1 ± 61.7	534.9 ± 140.3	$P < 0.001$
β-Lipoprotein	558.4 ± 155.5	461.6 ± 184.9	754.7 ± 251.0	$P < 0.05$
Pre-β-lipoprotein	25.1 ± 15.9	33.9 ± 19.5	27.4 ± 11.3	$P > 0.10$
α-Lipoprotein	430.4 ± 223.4	402.6 ± 203.4	1035.0 ± 363.0	$P < 0.01$

[a] Five-tenths gram of cholesterol/100 g diet (diets II, IV, and VI).
[b] Please refer to the text for the methods of measurements of these variables.

Table III shows the means of serum total cholesterol and lipoprotein concentrations at the terminus of 3-week feeding of high cholesterol diets and the different levels of protein. Unlike other serum lipid variables, pre-β-lipoprotein showed no significant change due to exogenous cholesterol and to the level of dietary protein intake. Therefore, the observed changes in serum cholesterol at different levels of dietary protein are reflected in β- and α-lipoproteins.

Although methionine-supplemented diets inhibited the increased serum cholesterol response to exogenous cholesterol by 25%, its effect was not uniform. One-third of the monkeys continued to show an increased response to dietary cholesterol in spite of methionine supplementation. Changes in serum β + pre-β-lipoprotein cholesterol and α-lipoprotein cholesterol in these animals showed trends consistent with the cholesterol responses.

Serum albumin levels were measured as a biochemical parameter for protein-calorie malnutrition. The albumin levels (g/100 ml) in monkeys fed 25% protein, 8% protein, and 4% protein with added methionine were 5.48 ± 0.36, 5.21 ± 0.68, and 5.31 ± 0.33, respectively, and the statistical analysis showed no significant differences. Furthermore, liver biopsy material examined under a light microscope showed no fatty liver characteristic of protein-calorie malnutrition.

Discussion. Although the level of dietary protein per se had little appreciable primary effect on serum lipids, different levels of protein intake clearly had a varied interacting effect on the serum lipid responses to exogenous cholesterol in spider monkeys. Strong and McGill (3) found that 25% protein diets tended to produce slightly higher serum cholesterol levels in baboons than did 10% protein diets, and they suggested a second-order interaction among dietary cholesterol and level of protein in the diet. In contrast, Middleton *et al.* (2) reported that the level of dietary protein had no interacting effects on serum cholesterol levels in squirrel monkeys fed high cholesterol diets.

As in previous studies, the serum cholesterol response to exogenous cholesterol was reflected in β- as well as α-lipoproteins during the transient phase from basal diets to ones with added cholesterol (12–19). The marked increase in α-lipoprotein cholesterol during the very low protein diet with and without cholesterol added is of particular interest. Further lipoprotein characterization studies are needed to clarify this response.

Underlying reasons for the varied effects of dietary protein intake on serum cholesterol and lipoproteins are not established. Synthesis of liver-produced plasma proteins is regulated by dietary protein and amino acid supply (20). Certain amino acids from dietary proteins are utilized for "extra protein functions" as well as for energy metabolism and protein synthesis (21–23). Methionine is required as a substrate in transmethylation reactions and production of choline for the phospholipids. Since a deficiency of sulfur amino acids impairs the efficiency of regulation of cholesterol in the blood (24), any reduction in sulfur amino acids in the diet could greatly increase the serum cholesterol response to exogenous cholesterol. Although the effect of adding methionine to the very low protein diet was obvious, it was not consistent on serum lipids in all the animals. The absence of fatty livers and no change in serum albumin levels suggest that a 4% protein level may not be low enough

to produce protein-calorie malnutrition detectable by these parameters in this period of time. Enwonwu *et al.* (25) observed that nonhuman primates develop characteristic clinical and biochemical features of protein-calorie malnutrition only at 2% protein level (w/w).

Isocaloric substitution of the bulk of the proteins with carbohydrate (dextrin) in the very low protein diets is another factor to consider in explaining the pronounced hyperlipoproteinemia (26–28). It should be noted that the very low protein diet also was lowest in nondigestible fiber. The high and the very low protein diets used in this study might represent an exaggeration of the protein level in the usual human diet in the United States, which ranges from 15 to 20% of total calories, whereas the 8% protein diet representing 12% of the calories, may be close to the optimal level. Low protein levels generally contain low levels of fat and cholesterol; populations consuming such diets show relatively low levels of serum cholesterol and a lower incidence of coronary artery disease.

Summary. The interacting effects of high (25%), low (8%), and very low (4%) dietary protein on the serum lipoprotein concentrations have been studied in spider monkeys (*Ateles* Sp.). Although the level of dietary protein per se had no appreciable main effect on the basal levels of serum total cholesterol, very low protein diet produced significant elevation in α-lipoprotein cholesterol. The serum lipoprotein responses to exogenous cholesterol varied at different levels of protein intake, the degree of response being highest at 4% protein level and lowest at 8% protein level. The changes were observed mainly in β- and α-lipoproteins.

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