The effect of dietary casein and soybean protein on plasma lipid levels in cebus monkeys fed cholesterol-free or cholesterol-enriched semipurified diets

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Two groups of six cebus monkeys were alternately fed semipurified diets containing either casein or soybean protein for periods of 8–10 weeks. The type of protein in the diet had little effect on the plasma cholesterol levels when cholesterol-free diets were fed. However, when 0.2% cholesterol was added to the diets, transferring the monkeys from a soybean protein diet to a casein diet resulted in a significant increase in plasma cholesterol levels (7.97 ± 1.09 versus 9.33 ± 1.31 mmol/L, n = 6, mean ± SD). On the other hand, changing the monkeys from a casein diet to a soybean protein diet significantly lowered the plasma cholesterol levels (9.64 ± 0.58 versus 7.41 ± 1.07 mmol/L, n = 6, mean ± SD). These changes in plasma cholesterol concentrations were attributed to changes in very-low and low-density lipoprotein cholesterol concentrations. The results of his study suggest that the differential cholesterolemic effect of dietary casein and soybean protein in cebus monkeys becomes more pronounced when cholesterol-enriched diets are fed.

Keywords: cholesterol; lipoproteins; dietary protein

Introduction

It has been known for a long time that the type of protein in the diet can affect plasma cholesterol levels and atherosclerosis. Studies by Ignatowski[1] as far back as 1909 indicated that feeding animal protein to rabbits resulted in arterial lesions similar to those found in humans. Later studies by Meeker and Kesten[2] in 1941 showed similar results. Rabbits fed cholesterol-free or cholesterol-enriched diets developed a higher degree of atherosclerosis on a casein diet than on a soybean protein diet. About 35 years later, Carroll and Hamilton[3] examined the cholesterolemic effect of various dietary proteins in rabbits and found that animal proteins tended to induce higher plasma cholesterol levels than plant proteins.

Recent studies in humans and various experimental animals also suggest that the type of protein in the diet can affect plasma cholesterol levels and that dietary casein induces higher plasma cholesterol levels than soybean protein. Most of these studies, however, indicated that a differential cholesterolemic effect of dietary casein and soybean protein could be observed only when cholesterol-enriched diets were used.[4] In humans, substitution of dietary soybean protein for casein resulted in a lowering of plasma cholesterol concentrations when high-cholesterol diets were used[5] or when the diets were fed to hyperlipidemic subjects.[6] The type of dietary protein had no effect when low-cholesterol diets were used and fed to normolipidemic subjects.[7-10] Similar results have been found in experimental animals. A differential cholesterolemic effect of dietary casein and soybean protein was observed in rats,[11] pigs,[12] and monkeys[13] when cholesterol-containing diets were fed, but not when cholesterol-free diets were used.[14-16] In young growing rabbits only,[17] dietary casein induces hypercholesterolemia without adding cholesterol to the diets.

In the present study, we examined the effect of dietary cholesterol on the differential cholesterolemic

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Table 1 Composition of semipurified diets

Ingredient	Casein diet		Soybean diet	
	Wt%	Energy%[a]	Wt%	Energy%[a]
Casein[b,c]	25.0	26.9	—	—
Soybean Protein[c,d]	—	—	25.0	27.6
Sucrose[b]	18.51	19.3	18.51	19.1
Dextrose[b]	18.51	19.3	18.51	19.1
Coconut oil[b]	12.33	34.2	12.33	33.9
Cellulose (alphacel)[b]	20.00	—	20.00	—
Vitamin mixture[e]	0.43	0.3	0.44	0.3
Mineral mixture[f]	4.26	—	4.26	—
Choline chloride[b]	0.26	—	0.26	—
Banana flavoring[b]	0.50	—	0.50	—
Cholesterol[b,g]	0.20	—	0.20	—

[a] For calculation of the energy percent of the various components, the following values[13] have been used: coconut oil, 38.9 kJ (9.31 kcal) per gram; dextrose and sucrose, 14.6 kJ (3.49 kcal) per gram; protein, 17.1 kJ (4.09 kcal) per gram. The casein diet had a calculated energy content of 14.01 mJ (3,352 kcal)/kg feed and the soybean protein diet of 14.16 mJ (3,380 kcal)/kg feed.
[b] Bioserve, Frenchtown, NY, USA.
[c] Casein, vitamin free, 88% protein.
[d] Soybean protein isolate, 91% protein.
[e] Newberne-Hayes vitamin mixture, Bioserve cat.# F8280. Composition (in mg/kg feed): ascorbic acid, 503; inositol, 430; taurine, 215; alpha-tocopherol acetate, 43; niacinamide, 34; calcium pantothenate, 22; retinyl acetate, 10,750 IU; riboflavin, 6.9; folic acid, 5.2; pyridoxine HCl, 3.4; thiamine-HCl, 3.4; cholecalciferol, 1066 IU; menadione, 0.4; cyanocobalamin, 0.17; biotin, 0.17; dextrin (carrier material), 3.0.
[f] Ausman-Hayes mineral mixture, Bioserve cat.# F8530. Composition (in mg/kg feed): K_2HPO_4, 13,384; $CaCO_3$, 12,354; NaCl, 6,918; $MgSO_4 \cdot 7\ H_2O$, 4,205; $CaHPO_4$, 3,093; MgO, 1,363; Fe-citrate, 1,150; Mn $(HSO_4)_2$, 52; $ZnCl_2$, 39; $Ca_3(PO_4)_2$, 21; Cu SO_4, 12; KI, 3.4; Cr-acetate, 1.7; NaF, 0.9; Na_2SeO_3, 0.2.
[g] The cholesterol-enriched diets contained 592 mg cholesterol per 4.18 mJ (1000 kcal). The cholesterol had been added at the expense of the dextrose and sucrose.

properties of dietary casein and soybean protein in cebus monkeys. Two groups of six cebus monkeys were fed semipurified diets containing either casein or soybean protein. Both diets were studied in the absence and presence of dietary cholesterol.

Materials and Methods

Animals and diets

Five male and seven female cebus monkeys that had been fed a semipurified diet containing 17% casein were divided into two groups of six monkeys on the basis of their total plasma and high-density lipoprotein (HDL) cholesterol concentrations. The total and HDL cholesterol concentrations were 7.10 ± 0.96 and 4.51 ± 0.62 (n = 6, mean ± SD) respectively, for group 1 (4 males and 2 females), and 7.09 ± 1.16 and 4.65 ± 0.68, respectively, for group 2 (1 male and 5 females). The two groups were alternatively fed cholesterol-free or cholesterol-enriched (0.2% cholesterol) semipurified diets containing either casein or soybean protein (*Table 1*) as shown in *Figure 1*. The semipurified diets were mixed with water (1 kg dry food and 0.57 liter water) and fed to the monkeys as a cake. Each animal was fed 150 grams of the wet food (96 g dry food) per day. Water was provided ad libitum.

The monkeys were housed in individual cages in a temperature-controlled room (20° C) with a 12-hour light and dark cycle. The animals were maintained in accordance with the guidelines of the Committee on Animals of the University of Lowell Research Foundation and the guidelines prepared by the Committee on Care in use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW publication no. 85-23, revised 1985).

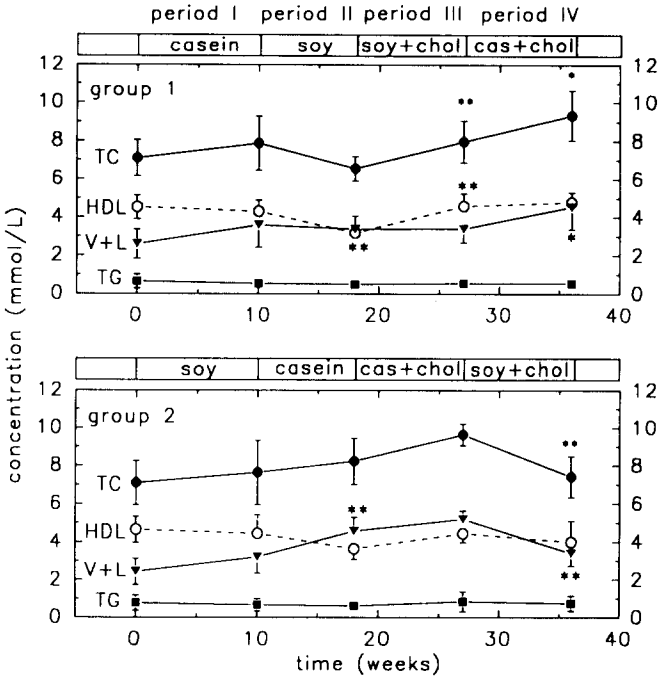

Figure 1 Concentrations of total plasma cholesterol (TC), triglycerides (TG), VLDL + LDL (V + L), and (HDL) cholesterol in two groups of six cebus monkeys fed cholesterol-free and cholesterol-enriched semipurified diets containing either casein or soybean protein. Values are expressed as mean ± SD (n = 6). Differences from the initial values of each experimental period within each group were statistically analyzed using a paired two-tailed *t*-test.
* $P < 0.05$
** $P < 0.01$

Analytical methods

Fasting monkeys were anesthetized with ketamine-HCl (5 mg/kg body weight), and blood (2.5 mL) was collected from the femoral vein into tubes containing EDTA as an anticoagulant. Cholesterol[18] and triglyceride[19] concentrations were measured using enzymatic methods. Plasma very-low-density and low-density lipoprotein (VLDL + LDL) were precipitated with heparin-$MnCl_2$[20] and the supernatant (HDL) was assayed for cholesterol. VLDL + LDL cholesterol was calculated as the difference between total and HDL cholesterol. Data were statistically analyzed using a paired or unpaired *t*-test.[21]

Results and discussion

The monkeys consumed all of the 150 grams of diet that was provided every day, and maintained a constant body weight (*Table 2*). The monkeys fed the cholesterol-enriched soybean protein diet gained some weight during the last dietary period only.

The twelve monkeys were divided into two groups that had identical initial mean plasma and HDL cho-

Table 2 Body weights of cebus monkeys fed semipurified diet containing either casein or soybean protein[a]

	Group 1		Group 2	
	diet	body weight (kg)	diet	body weight (kg)
Initial	Casein	2.34 ± 0.40	Casein	1.91 ± 0.30
Period I (10 weeks)	Casein	2.38 ± 0.31	Soy	2.08 ± 0.31
Period II (8 weeks)	Soy	2.38 ± 0.36	Casein	1.95 ± 0.31
Period III (9 weeks)	Soy + 0.2% cholesterol	2.43 ± 0.40	Casein + 0.2% cholesterol	1.98 ± 0.27
Period IV (9 weeks)	Casein + 0.2% cholesterol	2.31 ± 0.33	Soy + 0.2% cholesterol	2.38 ± 0.58

[a] Values are expressed as mean ± SD of six monkeys.

lesterol levels (*Figure 1*). Group 1 was fed a cholesterol-free casein diet and group 2 a cholesterol-free soybean protein diet. Both groups showed a slight increase in plasma cholesterol levels (*Figure 1*). Transferring the casein-fed monkeys (group 1) to a soybean protein diet resulted in a slight decrease of cholesterol levels, whereas transferring the monkeys on a soybean protein diet (group 2) to a casein diet resulted in a small increase of cholesterol levels. These changes, however, were not statistically significant. Subsequently, .2% cholesterol was added to the diets, which resulted in an elevation of the cholesterol levels in both groups. However, the monkeys fed the casein diet (group 2) had higher plasma cholesterol levels ($P < 0.01$) than the soybean protein-fed monkeys (group 1). Finally, switching the casein group to a soybean protein diet resulted in a lowering of cholesterol levels ($P < 0.01$), whereas changing the soybean protein monkeys to the casein diet increased the cholesterol level ($P < 0.05$). Again, the casein-fed animals (group 1) had higher cholesterol levels than the soybean protein-fed animals (group 2) on the cholesterol-enriched diets ($P < 0.05$). Plasma triglyceride levels did not significantly change during the studies.

Modulation in total plasma cholesterol levels were mainly reflected in changes in VLDL and LDL cholesterol levels. A significant decrease in HDL cholesterol levels, however, occurred in group 1, when the monkeys were transferred from a cholesterol-free casein diet to a soybean protein diet. Subsequently, the HDL levels increased again significantly, when cholesterol was added to the diets (*Figure 1*). A similar decrease and increase in HDL levels took place in group 2, but the changes were less pronounced. These changes might reflect a time effect, since they took place at the same time in both groups on different diets.

Major changes in plasma cholesterol levels occurred when the type of protein in the diet was changed in the presence of dietary cholesterol. Transferring the casein-soybean protein fed monkeys to a casein diet (group 1) resulted in a significant increase of plasma cholesterol levels when cholesterol-enriched diets were fed. This increase was caused by a significant increase in VLDL and LDL cholesterol levels. On the other hand, switching the casein-fed monkeys to a soybean protein diet (group 2), resulted in a steep decrease of plasma cholesterol levels, and this decrease was attributed to a decrease in VLDL and LDL cholesterol (*Figure 1*). In previous studies, we found that cebus monkeys have very low VLDL cholesterol levels. Therefore, changes in VLDL and LDL cholesterol levels in the present study probably were mainly caused by changes in LDL levels.

The results of this study suggest that a differential cholesterolemic effect of dietary casein and soybean protein becomes evident only when cholesterol-enriched diets are used. These results are in agreement with other studies indicating that both in humans[4-10] and experimental animals[11-16] dietary cholesterol enhances the differential cholesterolemic effect of dietary protein. Only in young growing rabbits[17] have significant changes in cholesterol levels been observed in the absence of dietary cholesterol.

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