Effects of Dietary Proteins on Plasma Lipoprotein Levels in Normal Subjects: Interaction with Dietary Cholesterol

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Summary We have compared the effects of dietary soy protein and casein in diets low in cholesterol (less than 100 mg/d) and in diets enriched in cholesterol (500 mg/d) to examine whether the level of cholesterol intake affects the response of plasma lipoproteins to dietary proteins of plant and animal origin. Normal men and women consumed formula diets containing 20% of calories as soy protein or casein, 27% as fat and 53% as carbohydrate in 2 crossover studies. The dietary periods lasted for 31 days and were separated by a month-long interim period on self-chosen food. Following an initial reduction of plasma total cholesterol and low-density lipoprotein-cholesterol (LDL-C) levels on all diets, the plasma lipid and lipoprotein concentrations stabilized. On low-cholesterol diets the concentration of each of the major lipoprotein classes were similar during the soy and the casein dietary periods. On cholesterol-enriched diets, the concentration of LDL-C stabilized at a 16% lower level on soy protein than on the casein diet ($p < 0.02$), while the concentration of high-density lipoprotein-cholesterol (HDL-C) was 16% higher ($p < 0.01$). Since the difference in LDL-C ($p < 0.05$) and in HDL-C ($p < 0.025$) levels on casein and on soy protein diets were significantly greater on the high than on the low cholesterol intake, the findings indicate that the level of dietary cholesterol may determine whether plant and animal dietary proteins have similar or different effects on plasma LDL-C and HDL-C concentrations.

Key Words soy protein, casein, dietary cholesterol, liquid-formula diets, plasma lipids, plasma lipoproteins, normolipidemic subjects

Introduction

Studies of the effects of dietary proteins on

plasma lipids and lipoproteins in humans have yielded variable and apparently contradictory findings. Both hyperlipidemic patients and normolipidemic subjects have in some investigations shown identical effects of animal and plant proteins (1–4), whereas other studies have demonstrated beneficial effects of vegetable proteins by inducing either lower levels of plasma total cholesterol, triglycerides, LDL-C, and very low density lipoprotein-cholesterol (VLDL-C), or higher levels of HDL-C, compared to those obtained with animal proteins (1, 2, 5–8). The cause of these discrepancies is not known, but one possibility is that the proteins interact with other dietary components. Thus, it has been shown that the type of dietary fiber (9), addition of certain amino acids to diets (10), and the degree of saturation of dietary fats (11) affected both plasma lipid levels and extent of arterial lesions in rabbits fed casein and soy protein. In human studies also, dietary saturated and unsaturated fats affect the response of hypercholesterolemic patients to dietary soy protein (6). Cholesterol is another dietary component which in a number of animals, such as rat, mouse, pig and chicken generally must be present in order that dietary casein and soy protein display different effects on plasma cholesterol levels (12). In a study of hypercholesterolemic patients, however, no effect was observed on cholesterol-lowering by dietary soy protein, whether the intake of cholesterol was low or high (6).

We wanted to examine whether dietary cholesterol plays a role in the effects of animal and vegetable proteins on circulating lipoproteins. For this purpose we conducted 2 studies on normolipidemic subjects using diets with a low (13) and with a high (14) cholesterol content, but otherwise identically composed. The present report is based on

Table 1. Comparison of participants receiving low-cholesterol and cholesterol-enriched diets.

	Low-cholesterol diets	Cholesterol-enriched diets
Women, <i>n</i>	5	6
Men, <i>n</i>	5	5
Age, years	41 ± 14*	38 ± 10
Body weight, kg	62 ± 10	64 ± 12
Body weight, % of ideal**	93 ± 6	96 ± 10
	mg/100 ml	
Plasma cholesterol	171 ± 32	170 ± 41
Plasma triglyceride	68 ± 32	56 ± 20
Plasma VLDL-C	13 ± 7	8 ± 8
Plasma LDL-C	110 ± 25	105 ± 37
Plasma HDL-C	47 ± 13	57 ± 11

* Mean ± SD of 10 and 11 subjects on low-cholesterol and cholesterol-enriched diets, respectively. There were no significant ($p > 0.05$) differences between the 2 groups.

** Calculated from the initial body weight percent of the midpoint for ideal body weight for height and medium frame according to the Metropolitan Life Insurance Tables, 1983.

both of those investigations.

Methods

Subjects The participants were all healthy, active women and men of normal body weight, who were normolipidemic (Table 1). They also had normal liver and kidney function and normal plasma levels of glucose, uric acid, albumin and electrolytes. The 2 groups of subjects participating in the low-cholesterol and in the cholesterol-enriched dietary studies, respectively, did not differ significantly in any of the characteristics listed in the table. Six subjects participated in both studies. The protocol, which was in accord with the Helsinki Declaration, was explained to each individual and informed consent was obtained.

Diets The low-cholesterol and the cholesterol-enriched diets were identically composed, except that cholesterol (USP, Sigma) was added to the latter. The major ingredients are listed in Table 2. They also contained various salts, and calcium was added to the soy protein diets to compensate for the calcium contained in the casein preparation (Casec, Mead Johnson Laboratories). Both the casein and the soy protein isolate (Purina 660, The Ralston Purina

Table 2. Major ingredients of diets.

	Casein diets (g)	Soy protein diets (g)
Protein (20%)*		
Calcium caseinate	139	—
Soy protein isolate	—	140
Fat (27%)		
Safflower oil (high oleinate)	54	54
Carbohydrate (53%)		
Corn starch hydrolysate	260	260
Water	1100	1100

* Percent of energy content.

Company) were 92% pure on a dry weight basis according to the manufacturers. Further details on dietary ingredients and composition have been published (13, 14). The participants received, in addition, a daily supplement of vitamins and trace minerals to satisfy minimal daily requirements. They were allowed calorie-free beverages and were specifically asked not to drink alcohol.

The diets were analyzed as previously described (13) for protein content and total fat, while the carbohydrate content was obtained by difference. The fatty acid composition as well as the sterol content and composition of the diets were analyzed by gas-liquid chromatography. The calcium content was determined by flame atomic absorption photometry.

Experimental design All subjects received both the soy protein and the casein diets, with or without cholesterol enrichment, in a crossover design for 31 days each, interrupted by a 30 day period on a diet of self-chosen food. Half of the participants started on the soy diet and the other half on the casein diet (Fig. 1).

Analysis of plasma lipids and lipoproteins For lipoprotein fractionation, freshly prepared EDTA-plasma was centrifuged separately at densities 1.006, 1.063, and 1.125 g/ml for 24 h at 180,000 × *g*. After centrifugation, the tubes were sliced, and top and bottom fractions were isolated quantitatively. Plasma concentration of VLDL-C was determined from the cholesterol content of the $d < 1.006$ g/ml fraction. LDL-C plasma concentration was de-

terminated in the following ways: 1) as the difference in cholesterol content of the $d > 1.006$ and the $d > 1.063$ g/ml fractions, 2) from the cholesterol content of the $d > 1.006$ g/ml fraction, before and after heparin-MnCl₂ precipitation (15) of LDL, 3) as the difference in cholesterol content of the $d < 1.063$ and the $d < 1.006$ g/ml fractions, and 4) according to the formula of Friedewald *et al.* (16) from plasma total cholesterol, triglyceride and HDL-C (method 2; see below). Plasma concentration of HDL-C was determined in the following ways: 1) as the cholesterol content of the $d > 1.063$ g/ml, 2) as the cholesterol content of plasma after precipitation of LDL and VLDL by heparin-MnCl₂ (15), and 3) as the cholesterol content of the $d > 1.006$ g/ml after precipitation of LDL. HDL₃-C was estimated as the cholesterol content of the $d > 1.125$ g/ml fraction, and the

plasma concentration of HDL₂-C as the difference between the cholesterol content of the $d > 1.063$ g/ml fraction and HDL₃-C. Recoveries of cholesterol in the ultracentrifugal lipoprotein fractions and comparisons of the different procedures for determination of LDL-C and HDL-C concentrations have been described (14). Cholesterol (17) and triglycerides (18) were assayed by established procedures.

Statistical analysis Statistical evaluations were done by paired or unpaired Student's *t*-test.

Results

Intake of energy and nutrients Table 3 shows the mean daily intake of calories and nutrients calculated from the chemical composition of the diets and the amount of liquid formula consumed by each individual during the particular dietary period. The caloric intake on soy protein and casein diets were similar, and so were the modest decreases in body weight, namely 1.4 ± 0.98 kg (mean $\pm$ SD) (soy protein) and 1.2 ± 1.03 kg (casein) on the low-cholesterol diets, and 1.1 ± 0.78 kg (soy protein) and 1.1 ± 0.81 kg (casein) on the cholesterol-enriched diets.

The fatty acid composition of the different diets were closely similar, oleic acid constituting about 72%, linoleic acid about 17% and the saturated palmitic and stearic acids together about 11%. Also the plant sterol composition of the different diets were virtually identical with beta-sitosterol, the main component, constituting about 64%. The cholesterol intake was always less than 100 mg/d on the low-cholesterol diets, while it was about 500 mg/d on the average on the cholesterol-enriched diets. The

Fig. 1. Time course of plasma lipid concentrations during the two dietary periods on low-cholesterol diets, separated by a 30 day interval on a self-chosen diet. Each point and vertical bar represent mean and SD of 5 subjects.

Table 3. Daily intake of energy and nutrients.

	Low-cholesterol diets		Cholesterol-enriched diets	
	Casein	Soy protein	Casein	Soy protein
Energy (kcal/d)	2327 $\pm$ 497*	2199 $\pm$ 463	2294 $\pm$ 475	2477 $\pm$ 560
Protein (g/d)	122 $\pm$ 25	111 $\pm$ 23	121 $\pm$ 25	112 $\pm$ 25
Fat (g/d)	67 $\pm$ 14	70 $\pm$ 15	69 $\pm$ 14	75 $\pm$ 17
Carbohydrate (g/d)	307 $\pm$ 66	283 $\pm$ 60	296 $\pm$ 61	338 $\pm$ 77
Cholesterol (mg/d)	72 $\pm$ 15	12 $\pm$ 3	549 $\pm$ 114	486 $\pm$ 110
Plant sterols (mg/d)	214 $\pm$ 46	251 $\pm$ 53	224 $\pm$ 46	265 $\pm$ 60
Calcium (mg/d)	620 $\pm$ 133	631 $\pm$ 133	579 $\pm$ 120	637 $\pm$ 144

* Mean $\pm$ SD of average daily intakes of 10 and 11 subjects on low-cholesterol and cholesterol-enriched diets, respectively.

Fig. 2. Time course of low-density and high-density lipoprotein-cholesterol concentrations. Each point and bar represent mean and SD; $n = 10$ for the low-cholesterol diets, and $n = 11$ for the cholesterol-enriched diets.

mean daily cholesterol intake on the casein diets was always about 60 mg higher than on the corresponding soy protein diet due to the presence of small amounts of cholesterol in the casein preparation.

Plasma concentrations of lipids and lipoprotein
All experimental diets caused an initial fall in plasma total cholesterol of more than 20%, whereas triglyceride levels were unaffected (Fig. 1). The reduction in plasma cholesterol was due primarily to a lowering of LDL-C levels, but also HDL-C concentrations decreased (Fig. 2). After about 2 weeks on the formula diets, plasma cholesterol and lipoprotein levels stabilized, and blood samples obtained after 24, 28, and 32 days of each dietary period was used to compare the effects of the 2 dietary proteins. During the month-long interval between the 2 dietary periods, when the participants consumed self-chosen food, plasma lipid and lipoprotein levels returned to prestudy values (Fig. 1).

During the last part of the experimental dietary periods, when plasma lipid and lipoprotein concentrations had become stable, plasma total cholesterol, triglyceride and VLDL-C levels were similar on the casein and the soy protein diets, regardless of the magnitude of cholesterol intake (Table 4). Correction for the predicted increase in plasma cholesterol on either low-cholesterol or cholesterol-enriched diets, due to the higher daily

intake while on casein than on the soy protein diets (19), does not affect this result. In contrast, the response of LDL-C and HDL-C plasma levels to the 2 dietary proteins depended on the cholesterol content of the diets. On low-cholesterol diets, the mean plasma levels of LDL-C and HDL-C, determined by several different methods, were virtually identical whether the dietary protein was casein or soy protein. On cholesterol-enriched diets, however, LDL-C levels were significantly lower (15–16%) on the soy protein than on the casein diet, while HDL-C concentrations were significantly higher (12–16%) (Table 4). The difference in LDL-C levels on soy protein and casein diets was 1 ± 10 mg/100 ml plasma (Mean $\pm$ SD) on low-cholesterol diets and 13 ± 16 mg/100 ml on cholesterol-enriched diets, and these differences were significantly ($p < 0.05$) different by unpaired comparison. For HDL-C the corresponding differences were 2 ± 4 and 8 ± 6 mg/100 ml, respectively, and they likewise were significantly different ($p < 0.025$).

Subfractionation of HDL revealed that the difference in HDL-C concentrations observed on cholesterol-enriched soy protein and casein diets was due mainly to differences in HDL₂-C levels, which were 19% higher on the soy protein diet, whereas HDL₃-C concentrations were similar. On low-cholesterol diets plasma levels of HDL subfractions were unaffected by the kind of dietary protein (Table 4).

Table 4. Plasma lipid and lipoprotein-cholesterol concentrations on days 24, 28, and 32 of the dietary periods.

	Low-cholesterol diets			Cholesterol-enriched diets		
	Casein (mg/100 ml)	Soy protein (mg/100 ml)		Casein (mg/100 ml)	Soy protein (mg/100 ml)	<i>p</i>
Cholesterol	125 ± 24 ^{*1}	127 ± 24	NS ^{*2}	138 ± 29	133 ± 19	NS
Triglyceride	56 ± 14	63 ± 25	NS	47 ± 16	51 ± 18	NS
VLDL-C	7 ± 2	9 ± 3	NS	6 ± 2	7 ± 2	NS
LDL-C ^{*3}						
1)	67 ± 19	68 ± 21	NS	83 ± 28	70 ± 14	<0.02
2)	66 ± 18	64 ± 21	NS	84 ± 27	73 ± 16	<0.025
3)	68 ± 19	63 ± 18	NS	74 ± 26	63 ± 14	=0.07
4)	68 ± 23	66 ± 18	NS	81 ± 29	68 ± 17	<0.025
HDL-C ^{*4}						
1)	46 ± 10	48 ± 9	NS	47 ± 9	56 ± 12	<0.01
2)	46 ± 10	47 ± 11	NS	48 ± 10	54 ± 13	<0.02
3)	42 ± 9	43 ± 10	NS	47 ± 10	53 ± 11	<0.02
HDL ₂ -C ^{*5}	21 ± 12	21 ± 9	NS	32 ± 11	38 ± 12	<0.01
HDL ₃ -C ^{*6}	26 ± 4	26 ± 3	NS	16 ± 7	18 ± 6	NS

^{*1} Mean ± SD of 10 and 11 subjects on low-cholesterol and cholesterol-enriched diets, respectively; the value for each subject was calculated as the mean of concentrations on day 24, 28, and 32 of each dietary period. ^{*2} No significant ($p > 0.05$) difference between values obtained on casein and on soy protein diets. ^{*3} LDL-cholesterol concentrations were determined from 1) the $d > 1.006$ minus the $d > 1.063$ g/ml fractions, 2) the $d > 1.006$ minus the $d > 1.006$ g/ml fraction after LDL precipitation, 3) $d < 1.063$ minus the $d < 1.006$ g/ml fraction, and 4) the plasma concentrations of total cholesterol, triglyceride, and the HDL-C according to Ref. (16). ^{*4} HDL-cholesterol concentrations were determined from 1) the $d > 1.063$ g/ml fraction, 2) plasma after precipitation with heparin-MnCl₂, and 3) the $d > 1.006$ g/ml fraction after precipitation of LDL. ^{*5} HDL₂-cholesterol concentrations were determined from the $d > 1.063$ minus the $d > 1.125$ g/ml fraction. ^{*6} HDL₃-cholesterol concentrations were determined from the $d > 1.125$ g/ml fraction.

Table 5. Plasma lipoprotein-cholesterol concentrations in female and male subjects, taking cholesterol-enriched diets, on days 24, 28, and 32 of each dietary period.^{*1}

	Casein diet	Soy protein diet	<i>p</i>
Women (<i>n</i> = 6)			
Cholesterol intake			
mg/d	482 ± 84 ^a	419 ± 73 ^b	
mg/kg/d	8.8 ± 2.1	7.7 ± 1.7	
LDL-C, ^{*3} mg/100 ml	75 ± 27	65 ± 10	NS ^{*2}
HDL-C, ^{*3} mg/100 ml	53 ± 9	61 ± 12	NS
HDL ₂ -C, ^{*3} mg/100 ml	34 ± 13	41 ± 15	NS
Men (<i>n</i> = 5)			
Cholesterol intake			
mg/d	629 ± 93 ^a	567 ± 93 ^b	
mg/kg/d	8.5 ± 1.0	7.7 ± 1.1	
LDL-C, mg/100 ml	93 ± 28	75 ± 16	<0.05
HDL-C, mg/100 ml	43 ± 7	50 ± 10	<0.02
HDL ₂ -C, mg/100 ml	29 ± 8	34 ± 9	<0.02

^{*1} Mean ± SD. ^{*2} No significant ($p > 0.05$) difference between the dietary periods. ^{*3} For procedures used to determine LDL-C (method 1), HDL-C (method 1) and HDL₂-C see Table 4, footnotes.

^{a,b} Cholesterol intake by men significantly greater than that by women ($p < 0.05$, ^a, $p < 0.02$ ^b).

Both women and men had lower plasma concentrations of LDL-C and higher levels of HDL-C and HDL₂-C on cholesterol-enriched diets containing soy protein than casein. But only in the men did the differences between the two proteins achieve statistical significance (Table 5). Although the greater effects observed in men might be due to a significantly greater intake of cholesterol, the intake was the same for the 2 sexes when adjusted for differences in body weight. The data therefore suggest a greater sensitivity of men to the differential effects of the two dietary proteins.

Discussion

The results of the present study show that dietary casein and soy protein can have similar or quite different effects on plasma lipoprotein levels in normolipidemic subjects, depending on the level of cholesterol intake. With a daily intake of 500 mg, which is average for the adult Danish population, soy protein caused both a lowering of LDL-C and an increase in HDL- and HDL₂-C when substituted for casein. But when the cholesterol intake was less than 100 mg/d, the 2 dietary proteins had indistinguishable effects on lipoprotein concentrations. Since the chemical composition of the major lipoprotein classes remained constant under the various dietary regimens (13, 14), the differences observed in lipoprotein-cholesterol concentrations represent equivalent differences in plasma levels of total lipoprotein mass of the various lipoproteins.

Our finding that cholesterol-enriched diets are necessary to bring out the different effects of animal and plant dietary proteins on plasma lipoproteins is in agreement with studies in experimental animals. Thus, in several animal species, such as rat, pig and chicken, cholesterol-rich diets are generally required to reveal a hypocholesterolemic effect of dietary plant proteins (12). The only previous attempt to study interaction of dietary protein and cholesterol in humans involved hypercholesterolemic patients on low cholesterol, soy protein diets. Addition of cholesterol (500 mg/d) to the soy diet did not affect its marked LDL-lowering effect (6).

The mechanism behind the interaction of dietary proteins and cholesterol is unknown. An increased intestinal uptake of cholesterol and bile acids on casein diets, as compared to soy protein diets, has been proposed to be the primary cause of the hypercholesterolemic effect of casein in experi-

mental animals (20). In this view, effects of dietary proteins on hepatic synthetic rates of cholesterol and bile acids and on lipoprotein receptor activity are secondary to the effects on intestinal steroid absorption. Our finding, that an increased cholesterol intake, as opposed to a low intake, brings out different effects of the 2 dietary proteins on lipoprotein levels, would appear to agree with this hypothesis. However, measurements of neutral and acid fecal steroid excretion by hypercholesterolemic, hypertriglyceridemic and normolipidemic individuals showed no significant differences between soy protein and casein or mixed protein diets on these parameters (2, 21). It should be added, however, that the daily cholesterol intake in these 2 studies was 200 mg or less, and thus the lack of effects on fecal steroid excretion may not be relevant to the findings of the present study.

If dietary casein causes an increased intestinal uptake of cholesterol and bile acids, compared to soy protein, this would be expected to reduce both hepatic LDL-receptor activity and fractional catabolic rates of LDL and consequently increase plasma LDL-C levels. A recently published study in rabbits showed indeed that casein both increased LDL-C concentrations and decreased fractional catabolic rates of LDL, compared to soy protein (22). Preliminary observations in human studies, employing the cholesterol-enriched diets described in the present report, showed however identical fractional catabolic rates on casein and soy protein diets, of both native and modified LDL (23). These findings thus suggest that the differences in LDL-concentrations in man may be due to diet-induced differences in LDL-production rates rather than catabolic rates. Since LDL is derived mainly from small VLDL (Sf 20–60) and IDL, soy protein and casein may in man affect production of these precursors or their transformation into LDL. Another possibility is that *de novo* hepatic LDL production is affected.

The increased plasma levels of HDL on cholesterol-enriched soy protein diet, compared to casein, may due either to an increased synthesis or a decreased rate of elimination. Observations in rabbits suggest that dietary soy protein, compared to casein, accelerates VLDL turnover (24), which might bring about an increased HDL production. This possibility is in agreement with a human turnover study of apo B in large VLDL (Sf 60–400) in hypercholesterolemic patients (25) showing a

significant increase in fractional catabolic rate of VLDL-apo B on soy protein compared to a mixed protein diet. This effect on VLDL turnover thus might explain how an increased HDL production could come about, although it does not explain why the level of cholesterol intake should influence HDL production.

We thank Ninn Buch Petersen, Hance Merete Olsen, Grethe Sejersen and Anne Trolle-Christensen for expert technical assistance; Kirsten Andersen for help in designing and making the diets; and the participants for their dedication.

This work was supported by the Danish Heart Foundation.

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