



Effects of casein versus soy protein diets on serum cholesterol and lipoproteins in young healthy volunteers^{1, 2}

Joop M. A. van Raaij, M.Sc., Martijn B. Katan,³ Ph.D.,
Joseph G. A. J. Hautvast, M.D.D.N. (Cantab), and Ruud J. J. Hermus,⁴ Ph.D.

ABSTRACT The effects of casein and soy protein on serum cholesterol levels and lipoprotein composition were studied in 69 healthy volunteers (18 to 28 yr of age) under strict dietary control. Subjects were fed for 6 wk on diets containing 13% of energy as protein, 38% as fat (P/S ratio = 0.6) and about 380 mg cholesterol per day. Of the protein in the diets 65% consisted of casein or soy protein or a 2:1 mixture of casein and soy protein. After a control period of 10 days during which all the subjects received the casein-soy diet, 20 subjects continued on this diet for the next 4 wk as a base-line control, 25 subjects switched to the casein diet, and the remaining 24 subjects switched to the soy diet. Both food records and chemical analysis of double portions revealed that the diets were completely identical except for the type of protein. Average serum cholesterol levels at the end of the control period were 152 ± 27 mg/dl (3.93 ± 0.69 mmol/l) and 153 ± 23 mg/dl (3.95 ± 0.60 mmol/l) (mean \pm SD) for the casein and soy group, respectively. At the end of the test period the levels were 149 ± 24 and 150 ± 23 mg/dl, respectively; thus there was no significant change on either diet. On the casein diet there was no change in the low-density lipoprotein cholesterol concentration, and only a slight, nonsignificant increase in the high-density lipoprotein cholesterol concentration. On the soy diet, however, there was a significant decline in low-density lipoprotein-cholesterol (-6.6 mg/dl; -0.17 mmol/l) and a significant increase in high-density lipoprotein-cholesterol ($+5.8$ mg/dl; $+0.15$ mmol/l). The decline in low-density lipoprotein cholesterol in the soy group was significantly different from the small change in the casein group, but the difference in increase in high-density lipoprotein cholesterol in the soy and the casein group was only weakly significant. This suggests that soy protein could have a slight beneficial effect on the distribution of cholesterol over the various lipoprotein fractions, even at constant total cholesterol concentration. *Am. J. Clin. Nutr.* 34: 1261-1271, 1981.

KEY WORDS Cholesterol, serum lipids, casein, soy protein

Introduction

Diet is thought to play an important role in provoking hypercholesterolemia and atherosclerosis. The influence of nutritional factors such as type and amount of dietary fat, carbohydrate, and dietary fiber on plasma cholesterol concentrations has been widely emphasized. Recently more attention has been devoted to the role of the type of dietary protein.

In rabbits proteins derived from animal sources as part of semipurified diets are gen-

erally found to produce hypercholesterolemia, whereas usually little or no elevation of

¹ From the Department of Human Nutrition, Agricultural University, 6703 BC Wageningen, The Netherlands.

² Supported in part by the Netherlands Institute for Dairy Research (NIZO), P.O. Box 20, 6710 BA Ede, The Netherlands.

³ Author to whom request for reprints should be addressed.

⁴ Present address: Central Institute for Nutrition and Food Research (CIVO-TNO), P.O. Box 360, 3700 AJ Zeist, The Netherlands.



serum cholesterol is observed with proteins from plant sources (1-5). Similar effects have been observed in rats (6) and pigs (7); the data for chickens are conflicting (8, 9).

In man, epidemiological data show a strong positive correlation between animal protein consumption and mortality from coronary heart disease (10). Furthermore, in several countries the trend toward increasing mortality from coronary heart disease during this century coincides with a doubling in the ratio of animal to vegetable protein in the diet (11-13). In nutritional studies of vegetarians, Hardinge and Stare (14) found lower serum cholesterol levels among those who were pure vegetarians than among lacto-ovo-vegetarians. Both groups had significantly lower levels than non-vegetarians. Sacks et al. (15) found similar effects in vegetarians.

In humans, only a few controlled trials relating dietary protein to serum cholesterol have been carried out. While differences in the concentrations of serum cholesterol have been consistently produced in rabbits (2-5, 16), the results of studies on humans are conflicting (17-19). We have, therefore, investigated the effect of casein and soy protein on the concentrations of serum cholesterol and lipoproteins in healthy young volunteers using highly purified proteins and with strict control of nutrient intake.

Subjects and methods

Subjects

Forty-six male and 30 female university students, aged 18 to 28 yr, volunteered and were selected for the study. All subjects were healthy and had normal values for serum cholesterol, serum triglycerides, blood pressure, and percentage of body fat as described for previous experiments (20). In addition the subjects were found to have normal values for blood hemoglobin and erythrocyte sedimentation rate and to have no detectable glucose or protein in the urine; none had received any medication known to affect serum lipids for at least 2 months before the study.

The experimental protocol was fully explained to the participants and informed consent was obtained. The subjects were not offered payment and were free to stop participating at any moment. They were seen daily by one of the investigators (J.M.A.v.R.) and by two research dietitians. During the experiment two subjects became ill, two decided to withdraw, and three did not fully adhere to the dietary protocol; their data were eliminated.

Diets and control of food intake

The amount (and type) of fat, cholesterol, protein, and carbohydrates in the diets were planned to simulate

an average Western diet (11). The diets were completely identical except for the type of dietary protein. Of the protein 65% was replaced by either casein (casein diet), soy protein (soy diet), or a 2:1 mixture of casein and soy protein (cassoy diet). To keep the diets acceptable 35% of the protein came from other sources (mainly from wheat, rice, potatoes, and other vegetables).

The 65% replaceable protein was incorporated as caseinate or soy isolate (Table 1) into specially developed products. Milk like beverages were prepared from butter concentrate, lactose, milk minerals and test protein (caseinate or soy isolate, 4.4% by weight); these beverages were in part fermented to yoghurts; both were developed by Unilever Research Laboratory, 3130 AC Vlaardingen, and by DMV-Veghel, and manufactured by the Department of Food Technology of the Agricultural University. The Institute for Cereals, Flour and Bread (IGMB-TNO), 6701 AN Wageningen, prepared brown breads from flour, bran, and test protein (75, 10, and 15 parts, respectively) and small amounts of minerals, yeast, etc.; gluten-free breads from gluten-free flour and test protein (85 and 15 parts, respectively) and small amounts of minerals, yeast, etc; cookies from gluten-free flour, test protein, sugar, and fat (51, 10, 22, and 17 parts, respectively). Sandwich spreads were manufactured by DMV-Veghel and by Unilever Research, and consisted of test protein (14% by weight), fat (16% by weight) and either vegetables or fruits plus sugar. Cheese was used in the casein and the cassoy diets because it contains casein as practically the only protein. For the soy diet, a gelled product was prepared by Unilever Research from soy isolate (20% by weight) flavoring and spices (2.5% by weight) and water; butterfat was also added to the soy diet to balance the fat in cheese. Fresh egg yolk was used in all diets to adjust cholesterol intakes and each subject consumed one multivitamin tablet per day. Each diet met the nutritional requirements for adults (21). Special margarines were prepared by VanDenBerg & Jurgens B.V., 3000 AD Rotterdam. All supplies were purchased in bulk except for fresh products which were purchased from the same distributors throughout the experiment.

TABLE 1
Composition of casein and soy protein isolate used in the experimental diets

	Casein*	Soy isolate†
	g/100 g	
Protein (N × 6.25)‡	90.5	92.8
Water	3.0	3.1
Lactose	<0.2 [§]	
Fat	<0.8 [§]	<0.1 [§]
Ash	4.2	3.5

* Calcium and sodium caseinate (spray dried, bland), DMV Milk Industries, 5460 BA Veghel. Data expressed as mean value for calcium and sodium caseinate.

† UNISOL NH 70, UNIMILLS B.V., 3330 AA Zwijndrecht.

‡ The true Kjeldahl nitrogen-to-protein conversion factors for casein and soy protein are 6.38 and 5.70, respectively (52). Using the figure of 5.70 the protein content of soy isolate is 84.6 g/100 g, which by difference gives an unavailable carbohydrate content of about 8.8 g/100 g.

[§] Data provided by manufacturer.

The three diets consisted of the same regular foodstuffs and of highly similar special products. Breakfast and evening meal consisted of breads, margarine, spreads, sweetenings, fruit and fruit juices. The hot meal at noon consisted of soup, potatoes (or rice), other vegetables, sauces, egg yolk, cheese (or gelated soy product), and dessert. The soups, sauces, and desserts were prepared from the analogs of milk and yoghurt. None of the following products was used: dairy products (except for cheese), meats, egg white, fish, legumes, onions, and garlic.

All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs, except for 500 kJ (120 kcal) per day, which the subjects were free to choose from a list of foodstuffs not containing protein. Individuals were allowed unlimited tea, coffee and selected low-calorie beverages. Sugar was provided and up to 6 g/day of coffee whitener was allowed. Hot meals were served on weekdays at noon in the department. All other foods were provided as packages daily; food for the weekend including ingredients for midday hot meals, was provided each Friday afternoon. Food rejections or deviations of any kind were recorded in diaries. Most of the special products were well accepted and adherence to the diets was excellent.

During the control and test period the actual intake of nutrients was calculated for each individual on 2 and 4 separate days, respectively, by multiplying the weights of foods on the questionnaire by values on a computerized food composition table (22). In addition, double portions of each diet were analyzed as described earlier (20, 27).

Experimental design

All subjects consumed the cassoy diet for a control period of 10 days, after which they were divided into three groups; the groups were matched for initial serum cholesterol, energy intake, and sex. During the test period of 28 days, the casein group (n = 25) received the casein diet, the soy group (n = 24) received the soy diet, and the cassoy group (n = 20) continued on the cassoy diet, as shown in Figure 1.

Body weight was recorded weekly. For each individual, energy intake was adjusted to avoid changes in body weight of more than 2 kg/2 wk. During the control period body weights decreased by 1.1, 0.8, and 1.1 kg in the casein, cassoy, and soy group, respectively. These slight weight losses continued during the test period, with mean weight reductions of 1.3, 1.0, and 1.6 kg, respectively. The largest declines were found in three subjects

on the soy diet (3.1, 4.0, and 4.1 kg). There were no differences in weight loss between the groups during the test period.

The subjects were asked to note in diaries illness, drug use and departures from the diets. Hemoglobin and erythrocyte sedimentation rate were measured fortnightly and found to remain normal.

Blood sampling and analysis

Specimens of blood were obtained from an arm vein after an overnight fast. Blood was collected weekly and serum was obtained by low-speed centrifugation. All the samples were assayed for total cholesterol and high-density lipoprotein (HDL)-cholesterol. At the end of the control and test period two samples were taken at 1-day intervals (Fig. 1), and the results for total cholesterol and HDL-cholesterol were averaged. Serum cholesterol was measured with the reagent of Huang et al. (23) using serum calibrators as described earlier (24). Reproducibility for blind control sera provided by the Center for Disease Control, Atlanta, GA, was $\pm 1.4\%$ (CV) and accuracy was within 1.8% of the "true" (target) values. HDL-cholesterol was determined after Mn-heparin precipitation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) (24, 25) at an Mn-concentration of 92 mmol/l. At the end of both periods, lipoproteins in sera of the subjects from the casein and soy group were separated by density gradient ultracentrifugation (26-28). Before preparing the gradient prior to the ultracentrifugation, Sudan Black was mixed with the serum in order to stain the lipoproteins (28). After centrifugation the lipoprotein bands were photographed and then separated by use of a tube slicer into VLDL ($d < 1.015$ g/ml), LDL ($1.015 < d < 1.060$), and HDL ($d > 1.060$ g/ml). For each individual the mean LDL density (center of the colored LDL band) was calculated from the photograph with the aid of the known density gradient in the centrifuge tubes (A. H. M. Terpstra, C. J. H. Woodward, and F. J. Sanchez-Muniz, unpublished data). Lipoprotein fractions were assayed for cholesterol by an enzymatic method (29) using a kit (No. 124087, Boehringer-Mannheim GmbH, West Germany) and using serum calibrators as described earlier (24).

The mean recovery was 97.8% (CV = 4.6%). Day-to-day reproducibility was $\pm 1.0\%$ (CV) and bias was less than 1.0% compared with values obtained by the Abell method (30). Apolipoprotein-B was measured in whole sera by rocket immunoelectrophoresis as described previously (27). For one person the samples obtained at the

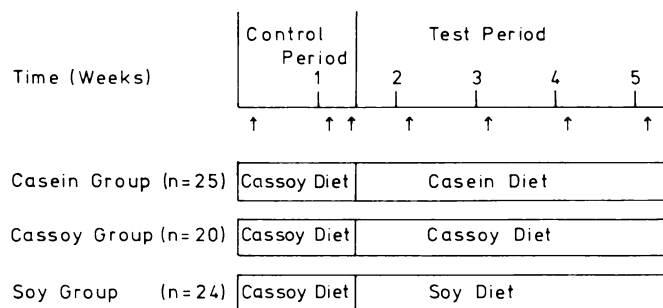


FIG. 1. Experimental design. Blood sampling is indicated by arrows.

start and at the end of the test period were always analyzed on one plate in order to minimise the effect of interassay variability. Analytical error was monitored by analysing a sample from a control pool on each plate. The combined within-day and between-day coefficient of variation for this control serum was 7%.

Statistical evaluation

The major hypothesis to be tested was that soy protein would lower serum cholesterol concentration, if compared with casein. A minor hypothesis was that a 2:1 mixture of casein and soy protein would also lower serum cholesterol, when compared with casein. The effect of diet was examined by testing the difference between the mean responses of the groups by unpaired one tailed *t* tests (31). The experiment was designed to protect against an error of the second type, i.e., missing a significant difference through chance fluctuations: it was calculated that the casein and the soy group would each need to contain at least 24 subjects in order to detect differences between groups in total cholesterol response of at least 0.25 mmol/l (10 mg/dl) at a significance level α of 5% and with a power β of 80%. The effect of sex was examined by analysis of variance (31).

Results

Nutrient intake

The results of nutrient intake, as measured by food records, are shown in Table 2. Food records and the chemical analysis of double portions indicated that there were essentially no differences between the experimental diets

with respect to the amount of protein, fat, carbohydrate, alcohol, cholesterol, and dietary fiber and to the composition of the fatty acids, plant sterols and carbohydrates. The calcium content of the casein diet was slightly higher than that of the soy diet, but it is unlikely that this influenced serum lipids. The analysis did reveal a slightly higher total protein content and a lower sugar content than the calculations based on the records for all diets. The amino acid composition of casein was, as expected considerably different from that of soy protein (Table 3). The amino acid composition of the whole diet was diluted by those proteins that were already present and similar for all diets. However, the differences between the diets were still pronounced (Table 3) and established beyond doubt that the planned differences in protein amino acid composition had been achieved. Both from the food records and from the amino acid analysis it could be calculated that about 65% of the total dietary protein consisted of soy protein or casein.

Serum total cholesterol

The time course of the mean serum total cholesterol concentration is presented for each group in Figure 2. The values of the

TABLE 2
Mean daily intake of nutrients before and during the experiment according to individual food records*

	Habitual intake before experiment†	Control period		Test period	
		Casoy diet‡	Casein diet§	Casoy diet¶	Soy diet¶
Energy (kcal)	2710	2595	2515	2670	2565
(MJ)	11.3	10.8	10.5	11.2	10.7
Total fat (energy %)	33.1	38.5	38.2	38.6	38.4
Saturated	15.7	13.8	13.2	13.8	12.9
Mono-unsaturated	12.0	17.0	17.0	17.0	17.4
Poly-unsaturated	5.4	7.7	7.9	7.8	8.1
Carbohydrates (energy %)	49.3	47.4	47.2	47.5	47.4
Sugars	22.6	23.3	23.2	23.4	22.4
Polysaccharides	26.7	24.1	24.0	24.1	25.0
Protein (energy %)	13.7	12.6	12.9	12.3	12.6
Casein		5.6	8.8	5.4	
Soy protein		2.6		2.5	8.4
Alcohol (energy %)	3.9	1.6	1.7	1.6	1.7
Cholesterol (mg/day)	334	394	387	398	365
Dietary fiber (g/day)	38.2	28.8	28.7	29.4	28.2

* The food records were elaborated using Netherlands food composition tables supplemented with analyses of special products.

† Three-day records.

‡ Two-day records.

§ Four-day records.

|| 1000 kcal = 4.2 MJ.

TABLE 3
Amino acid composition of casein, soy protein isolate, and the duplicate portions of the diets* †

Amino acid	Pure protein		Complete diets		
	Casein*	Soy Isolate	Casein	Cassoy	Soy
			<i>g/100 g amino acid</i>		
L-alanine	2.8	4.1	3.2	3.5	4.0
L-arginine	3.3	7.6	3.8	4.8	6.8
L-aspartic acid [‡]	6.5	11.3	7.5	8.5	10.8
L-glutamic acid [‡]	21.4	19.6	23.2	22.9	22.1
Glycine	1.7	4.0	2.3	2.9	3.9
L-histidine	2.8	2.5	2.7	2.7	2.5
L-isoleucine	5.1	5.0	4.7	4.6	4.7
L-leucine	8.8	7.8	8.3	8.0	7.6
L-lysine	7.6	6.2	6.4	6.1	5.5
L-methionine	2.5	1.2	2.0	1.6	1.2
L-phenylalanine	4.7	5.2	4.6	4.7	4.9
L-proline	9.5	4.9	9.0	8.1	5.8
L-serine	5.5	5.3	5.5	5.6	5.1
L-threonine	3.9	3.7	3.7	3.7	3.6
L-tyrosine	5.2	3.6	4.1	3.7	3.3
L-valine	6.4	5.0	6.0	5.5	5.0
L-cystine [‡] plus L-tryptophan [‡]	3	3	3	3	3
	100	100	100	100	100

* Amino acid analysis of a hydrochloric acid digest of the lyophilized material using a Jeol JLC-5AH amino acid analyzer.

† Expressed as g amino acid per 100 g amino acid.

‡ Cystine and tryptophan were not analyzed; their total contribution was estimated (52) as 3 g/100 g.

§ Average value for sodium and calcium caseinate.

¶ Including amide.

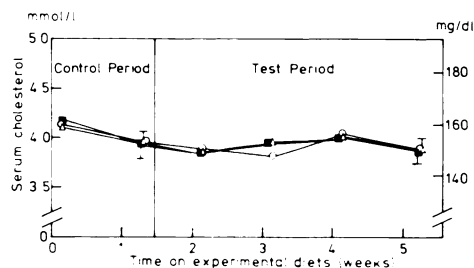


FIG. 2. Effects of casein and soy protein diets on mean total serum cholesterol concentrations in humans throughout the experiment. Vertical bars indicate 1 SEM. ■—■, casein group (25 subjects); ○—○, cassoy group (20 subjects); △—△, soy group (24 subjects).

mean concentrations at the end of both periods are shown in **Table 4**. During the cassoy control period of 10 days serum total cholesterol concentration decreased slightly in all groups compared with the uncontrolled preexperimental situation. The control group that continued on the cassoy diet did not show any change in serum total cholesterol during the test period, proving that levels had stabilized already at the end of the base-line

TABLE 4
Effects of casein and soy protein diets on serum total cholesterol concentrations (Mean ± SD)*

	Casein group (n = 25)	Cassoy group (n = 20)	Soy group (n = 24)
	<i>mg/dl†</i>		
Control period	152 ± 27	153 ± 24	153 ± 23
Test period	149 ± 24	150 ± 25	150 ± 23
Change	-3 ± 14	-3 ± 13	-3 ± 10

* Each value represents the average of two separate determinations at the end of the respective period.

† 100 mg/dl = 2.59 mmol/l.

period of 10 days. The soy and the casein diets also induced hardly any change in average cholesterol concentrations, except for minor oscillations of about 4 mg/dl (0.1 mmol/l). No sex effects were found.

Distribution of cholesterol over the lipoprotein fractions

The mean cholesterol concentrations in the lipoprotein fractions are given in **Table 5**. Comparison of HDL-levels determined by ultracentrifugation and by the Mn-heparine

TABLE 5
Effects of casein and soy protein diets on cholesterol concentrations in lipoprotein fractions and on serum apolipoprotein-B levels (mean \pm SD)

	Casein group (n = 23)	Soy group (n = 23)
	<i>mg/dl*</i>	
Cholesterol		
Ultracentrifugation:		
VLDL control period	8 \pm 3	10 \pm 3
test period	10 \pm 5	10 \pm 5
change	+1.5 \pm 5.8	+0.8 \pm 5.0
LDL control period	80 \pm 17	88 \pm 19
test period	79 \pm 16	81 \pm 19
change	-0.4 \pm 12.4	-6.6 \pm 9.3 ^{†‡}
HDL control period	59 \pm 16	57 \pm 10
test period	62 \pm 15	62 \pm 12
change	+2.3 \pm 8.5	+5.8 \pm 5.0 [†]
Mn-heparin precipitation:		
HDL control period	58 \pm 12	55 \pm 9
test period	60 \pm 12	58 \pm 8
change	+1.9 \pm 5.4	+3.1 \pm 3.5 [†]
Serum apoprotein-B	<i>mg/l</i>	
control period	488 \pm 119	462 \pm 107
test period	441 \pm 99	447 \pm 107
change	-47 \pm 52 ^{†‡}	-15 \pm 51 [‡]

* 100 mg/dl = 2.59 mmol/l.

[†] Significantly different from 0 (paired *t* test; *p* < 0.05).

[‡] Significantly different from casein group (unpaired *t* test; *p* < 0.05).

procedure yielded a correlation coefficient of $r = 0.91$, which is similar to published values (32, 33). Values obtained by the ultracentrifugational method were in general a few percent higher than those obtained by the precipitation procedure (cf. Table 5). This is a common finding (32, 33) and is probably explained by the presence of small amounts of sinking pre- β lipoprotein (lipoprotein (a)) in the ultracentrifugal HDL fraction when isolated within the classical density limits of 1.063 and 1.210 g/ml. In the casein group there were no significant changes in concentrations in any of the lipoprotein fractions. In the soy group, however, there was a significant decline in LDL-cholesterol and a significant increase in HDL-cholesterol.

There were no changes in VLDL-cholesterol concentration. The decline in LDL-cholesterol in the soy group is significantly different from the small change of LDL-cholesterol in the casein group, but the difference in increase in HDL-cholesterol in the soy and

the casein group is only weakly significant according to the ultracentrifugal data and not significant according to the precipitation method. Analysis of variance failed to show significant sex effects on the changes during the test period. The individual changes in serum total cholesterol, HDL- and LDL-cholesterol were not correlated with the initial values (at the end of the control period).

Serum apolipoprotein-B concentration

In both the casein and the soy group the apoprotein-B concentration declined during the test period (Table 5), but the decline in the casein group was significantly greater than that in the soy group. In the casein group the LDL-cholesterol/apoprotein-B ratio increased significantly from 1.64 to 1.79 g/g ($p < 0.01$), and in the soy group this ratio declined from 1.90 to 1.81 g/g ($p < 0.05$).

It should be noted that at the end of the control period the soy group happened to have a lower mean concentration of apo-B

and a higher concentration of LDL-cholesterol than the casein group. This resulted in a significantly higher initial LDL-cholesterol/apo-B ratio in the soy group than in the casein group. Such a difference is not surprising, because we did not explicitly match the groups for this ratio.

During the test period the mean densities of the LDL particles of the subjects in the casein group declined significantly from 1.039 ± 0.005 to 1.036 ± 0.003 g/ml (mean \pm SD; paired *t* test; $p < 0.05$). In the soy group, the slight increase in mean densities from 1.036 ± 0.002 to 1.037 ± 0.002 g/ml was not significant.

Discussion

Recent experiments, both in various animal species (2, 3, 6, 7) and in humans (17, 18) have shown profound effects on serum cholesterol levels of soy versus meat and/or milk proteins. This has led to the expectation that replacement of animal by vegetable protein in the human diet might aid in lowering serum total cholesterol levels, thereby providing an extra tool in the prevention of atherosclerotic vessel disease. Our results do not provide support for these expectations: in a carefully controlled short-term trial using nearly 70 healthy young subjects we found no effect on serum total cholesterol of either casein or soy protein compared with a 2:1 mixture of these proteins. However, when duplicate portions of the same diets were fed to 12 New Zealand White rabbits the casein diet caused much higher serum cholesterol concentrations than the soy diet; the difference was already significant after 2½ wk (Fig. 3; Reference 34).

There are several ways to explain the differences in response between humans and animals. It is known that animal species differ greatly in resistance to diet-induced hypercholesterolemia. Apparently, healthy humans are less sensitive to changes of dietary protein than rabbits, rats, or pigs. Furthermore, most animal experiments have used young animals which have spent a considerable part of their lifespan on the test diets. Our results do not rule out the possibility that a long-term intake of animal protein, starting at an early age, will reveal similar cholesterol-raising effects in normocholesterolemic humans. All the

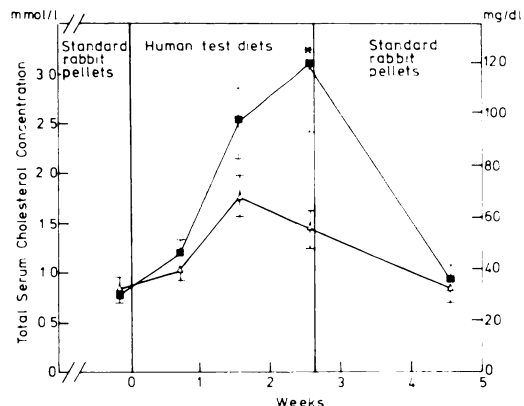


FIG. 3. Effects of casein and soy protein diets on mean total serum cholesterol concentrations of rabbits. Vertical bars indicate 1 SEM. Throughout the experiment on humans, duplicate portions of the casein and soy diets were collected and stored at -40°C . These were homogenized, freeze-dried, and pelleted afterward, and fed to 12 New Zealand White rabbits (3 months of age). Before and after the test period of 2½ wk the rabbits were fed a commercial rabbit pellet diet. The increase in serum total cholesterol on the casein diet was significantly greater than that on the soy diet ($p < 0.05$). As the groups had unequal variances *t* was tested at 5 instead of 10 degrees of freedom, as suggested by Cochran (Reference 31, p. 115). After switching back to the commercial diet serum cholesterol concentrations returned to baseline values. ■—■, casein group ($n = 6$); Δ — Δ , soy group ($n = 6$).

same, our results do stress the risk of extrapolating too readily animal data concerning the effect of protein on serum cholesterol directly to man. However, these species differences may provide clues to the mechanism of cholesterol homeostasis.

Epidemiological studies have suggested, when comparing nations or whole populations, a relation between intake of animal protein, serum cholesterol level, and cardiovascular disease (10).

Epidemiological data, however, should be interpreted with caution as differences in other nutritional factors may be present. As an example, the main sources of animal protein (meat and dairy products) also contain much saturated fat and cholesterol.

Very few strictly controlled experiments in man relating the source of dietary protein to the concentration of cholesterol in serum have been carried out, and the sparse results are contradictory (Table 6). No clear effects of the type of dietary protein were found by Walker et al. (35), Campbell et al. (36), An-

TABLE 6
Summary of studies with humans on the effect of dietary protein on the serum (plasma) cholesterol concentration

Subjects	n	Protein source	Composition of test diets			Duration of test periods days	Effect of plant diet mg/dlt	Authors
			Protein*	Fat [†]	P/S			
Healthy women (17-22 yr)	6	Mixed animal	8	36		42	Walker et al. (35)	
	6	Mixed plant	8	36		42		-13 NS
Healthy men (53-70 yr)	6 [‡]	Casein/Lactalb. ‡ (75%) [§] Gluten (75%)	7	40	low	25	Campbell et al. (36)	
			7	40	low	25		-18 NS
Healthy men (21-26 yr)	11 [‡]	Casein/lactalb. (75%) Gluten (75%)	7	40	high	25	Anderson et al. (37)	
			7	40	high	25		+9 NS
Hypercholesterolemic patients (22-68)	20 [‡]	Egg white (50%) Gluten (50%)	16	37	0.5	28	Sirtori et al. (17)**	
			16	37	0.5	28		+4 NS
Healthy women (19-25 yr)	10 [‡]	Mixed animal (63%) Soy (63%)	21	21	2.2	21	Carroll et al. (18)	
			21	26	2.7	21		-50 ^{††}
Mildly hypercholesterolemic young men	?	Mixed animal (58%) Soy (58%)	15	34	0.4	37-41	Shorey and Davis (19)	
			16	33	0.4	37-41		-9 ^{††}
Healthy men and women (18-28 yr)	25	Mixed animal Soy	13-16	30-35	0.4	42	present results	
			13-16	30-35	0.4	42		+6 NS
Healthy men and women (18-28 yr)	24	Casein (65%) Soy (65%)	13	37	0.6	28	present results	
			13	37	0.6	28		0

* Percentage of total energy intake.

[†] 100 mg/dl = 2.59 mmol/l.

[‡] Casein/lactalbumin mixture: 80% casein, 20% lactalbumin.

[§] The values within parentheses indicate the percentage of the total protein provided by the indicated protein.

^{||} Low P/S ratio: 12% of total fat as linoleic acid; high P/S ratio: 40% of total fat as linoleic acid.

[†] In this study a cross-over design was used.

** Addition of 500 mg cholesterol per day to the soy diet did not modify the hypocholesterolemic response (17). The soy diet was less effective at a P/S ratio of 0.1 (38).

^{††} p < 0.01.

^{‡‡} p < 0.05.

derson et al. (37), and Shorey and Davis (19). On the other hand, small but significant effects have been observed by Carroll et al. (18), while striking cholesterol-lowering properties of soybean protein have been reported by Sirtori et al. (17, 38). How can one explain these different results?


Many soy products contain appreciable amounts of nonprotein material and it has been suggested that this is partly responsible for the observed effects in some experiments (39–45). Even the soy protein isolate used in our experiment, which is representative of the purest commercially available material, still contains about 10% of nonprotein material, mainly carbohydrates. The customary Kjeldahl nitrogen-to-protein conversion factor of $N \times 6.25$ obscures this fact (Table 1). It has also been suggested that the form in which the soy protein is incorporated in the diets is important (46). The test diets in some studies differed not only in the source of protein but also in cholesterol content and the amount and composition of fat because of the strong coupling of protein with other nutrients in ordinary foodstuffs (17). The lipid status of the subjects could be an important factor: our subjects had low to very low serum cholesterol levels, while Sirtori et al. (17, 38) studied hypercholesterolemic patients. Normocholesterolemic subjects are possibly less sensitive to changes in dietary protein than hypercholesterolemic individuals (38), which remains to be established. All these factors may have played a role in producing conflicting results in the different studies with humans.

Another important factor is the use in the studies of widely different “animal” and “vegetable” proteins. It has been shown that animal or vegetable proteins cannot be grouped in that way regarding their cholesterol action in rabbits (1, 3, 41, 43). Animal experiments suggest that effects of dietary protein on serum cholesterol can be explained, at least partly, by the amino acid composition of the proteins (2, 3, 34, 43, 45, 47–49). The role of the individual amino acids or of specific combinations of amino acids, however, still remains unclear. Attention has been particularly focused upon the lysine/arginine ratio (3, 49, 50), glycine (43, 49), glutamic acid (51), and methionine (39) but the results are not conclusive (49). The

amino acid composition of soy protein is intermediate in many regards between casein and meat protein (52). As a result, our soy protein diet contained more alanine, arginine and glycine and less glutamic acid, tyrosine, and valine than the casein diet, but the reverse was true for the plant protein diet (containing soy protein) in Carroll's experiment (18); there was less alanine, arginine, and glycine and more glutamic acid, tyrosine, and valine in their plant protein diet than in their meat/milk protein diet. Therefore, it is conceivable that had we chosen other plant and animal proteins, we might have been able to demonstrate an effect on the concentration of cholesterol in serum.

Our data suggest a small decline in LDL-cholesterol and a small increase in HDL-cholesterol concentration on soy protein but not on casein. In view of the inverse relation between HDL-cholesterol concentration and obesity, the lipoprotein data were reanalyzed after exclusion of possible interference by weight loss. Deletion of the results of the subjects who lost more than 2 kg of body weight during the test period (six subjects from the casein and nine from the soy group) left the conclusions essentially unchanged.

The effect on HDL- and total cholesterol remained the same after exclusion of those people who lost more than 2 kg. Only the difference in the effect on LDL-cholesterol became less pronounced (3 instead of 6 mg/dl). Sirtori et al. (38) found that soybean protein exerted its hypocholesterolemic effect mainly by lowering LDL-cholesterol, while only a minor decline of HDL-cholesterol was observed. Our results for the LDL-cholesterol/apoprotein-B ratio suggest that ingesting the casein diet caused a shift to LDL-particles richer in cholesterol, while consuming the soy diet caused a shift to LDL-particles containing less cholesterol. The interpretation of these changes is complicated, however, because persons randomized into the soy group happened to have higher LDL-cholesterol/apoprotein-B ratios than the subjects in the casein group; thus the starting levels were not comparable, although the direction of the individual diet effects was unmistakable. These findings are confirmed by the changes in the density of the LDL. The data on the LDL composition and density are

preliminary only and this finding should be investigated further. However, it seems clear that the absence of an effect on total cholesterol levels by dietary casein and soy protein does not rule out the possibility that changes in composition and/or concentration of individual cholesterol-transporting lipoproteins do occur. Although it has not definitely been "proven" that an increase of the HDL/LDL cholesterol ratio by dietary manipulation will decrease the incidence of coronary artery diseases, it is a fact that high HDL/LDL cholesterol ratios are predictive for lower risk for these diseases (53, 54). If our results can be confirmed, this would suggest that soy protein could have qualitatively a beneficial effect, even at constant total cholesterol concentrations. 

The authors thank the volunteers for their excellent cooperation and keen interest. We wish to acknowledge for developing and preparing the special products: DMV-Milk Industries (F. Visser and J. Schut), Unilever Research (P. van Stratum, H. Vonkeman, C. Andreae, H. Herstel, G. G. Nijholt, and P. Westdorp before his untimely death), IGMB-TNO (P. Sluimer), VanDenBergh & Jurgens (J. W. Van der Kamp), The Department of Food Technology of the Agricultural University (H. J. van der Stege and G. Kleter), and UNIMILLS (L. Schutte and A. Visser). We thank S. Visser (NIZO) for the amino acid analyses; P. H. E. Groot (Erasmus University, Rotterdam) for providing us with the methodology and materials for apolipoprotein assays; A. H. M. Terpstra for supervising the rabbit experiment; C. E. West for help in preparing the manuscript; the dietitians Marita Engelen, Jeanne de Vries, Annelies Geenen and Lenie Lamers for preliminary dietary planning and for dietary supervision; the analysts Mrs. J. Barendse, Mrs. J. Bos, Mrs. C. Germing, F. Schouten, P. van de Bovenkamp, H. Verwey; Gisèle Snelder, Marja Markusse and Anke Ekkes for their excellent assistance; and all the other workers of our Department. Workers from NIZO (Mrs. E. C. H. van Beresteijn), CIVO-TNO, Unilever Research and the Department of Statistics of the Agricultural University generously gave helpful advice and discussion.

References

1. Carroll KK, Hamilton RMG. Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. *J Food Sci* 1975;40:18-23.
2. Hamilton RMG, Carroll KK. Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis* 1976;24:47-62.
3. Hermus RJJ. Experimental atherosclerosis in rabbits on diets with milk fat and different proteins. *Agric Res Report* 838. Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation, 1975.
4. Kritchevsky D, Tepper SA, Williams DE, Story JA. Experimental atherosclerosis in rabbits fed cholesterol-free diets. Part 7. Interaction of animal or vegetable protein with fiber. *Atherosclerosis* 1977;26:397-403.
5. Kritchevsky D, Tepper SA, Story JA. Influence of soy protein and casein on atherosclerosis in rabbits (abstr). *Fed Proc* 1978;37:747.
6. Yadav NR, Liener IE. Reduction of serum cholesterol in rats fed vegetable protein or an equivalent amino acid mixture. *Nutr Rept Int* 1977;16:385-9.
7. Kim DN, Lee KT, Thomas WA. Effect of soy protein on serum cholesterol in swine fed fat and cholesterol. *Exp Mol Pathol* 1978;29:385-99.
8. Kritchevsky D, Kolman RR, Guttmacher RM, Forbes M. Influence of dietary carbohydrate and protein on serum and liver cholesterol in germ-free chickens. *Arch Biochem Biophys* 1959;85:444-51.
9. Hevia P, Visek WJ. Dietary protein and plasma cholesterol in chickens. *J Nutr* 1979;109:32-8.
10. Stamler J. Population studies. In: Levy RI, Rifkind BM, Dennis BH, Ernst RD, eds. *Nutrition, lipids and coronary heart disease*. New York: Raven Press, 1979;25-88.
11. Bosman W. De voeding in Nederland in 1976. *Voeding* 1979;40:351-360.
12. Mulder T. De voeding in Nederland gedurende de jaren 1960-1969. *Voeding* 1973;34:54-97.
13. Kritchevsky D. Diet and cholesteremia. *Lipids* 1977;12:49-52.
14. Hardinge MG, Stare FJ. Nutritional studies of vegetarians. 2. Dietary and serum levels of cholesterol. *Am J Clin Nutr* 1954;2:83-8.
15. Sacks FM, Castelli WP, Donner A, Kass EH. Plasma lipids and lipoproteins in vegetarians and controls. *N Engl J Med* 1975;292:1148-51.
16. Belton EA, Truswell AS. Effect of casein compared with soya-bean protein isolate on plasma cholesterol in the rabbit. *Proc Nutr Soc* 1978;37:12A.
17. Sirtori CR, Agradi E, Conti F, Mantero O, Gatti E. Soy bean-protein diet in the treatment of Type-II hyperlipoproteinaemia. *Lancet* 1977;1:275-7.
18. Carroll KK, Giovannetti PM, Huff MW, Moase O, Roberts DCK, Wolfe BM. Hypocholesterolemic effect of substituting soy-bean protein for animal protein in the diet of healthy young women. *Am J Clin Nutr* 1978;31:1212-21.
19. Shorey RL, Davis JL. Effects of substituting soy for animal protein in the diets of young mildly hypercholesterolemic males (abstr). *Fed Proc* 1979;38:551.
20. Stasse-Wolthuis M, Albers HFF, Jeveren JGC van, et al. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids and colonic function. *Am J Clin Nutr* 1980;33:1745-56.
21. Nederlandse Voedingsmiddelen Tabel. Aanbevolen hoeveelheden energie en voedingsstoffen. 1979, 32e druk.
22. Hautvast JGAJ. Ontwikkeling van een systeem om gegevens van voedingsenquetes met behulp van de computer te verwerken. *Voeding* 1975;36:356-61.
23. Huang TC, Chen CP, Wefler V, Raftery A. A stable reagent for the Liebermann-Burchard reaction. *Ap-*

- plication to rapid serum-cholesterol determination. *Anal Chem* 1961;33:1405-7.
24. Haar F van der, Gent CM van, Schouten FM, Voort HA vd. Methods for the estimation of high density cholesterol, comparison between two laboratories. *Clin Chim Acta* 1978;88:469-81.
 25. Burstein M, Samaille J. Sur un dosage rapide du cholesterol lié au α - et β - lipoproteins du serum. *Clin Chim Acta* 1960;5:609.
 26. Redgrave TG, Roberts DCK, West CE. Separation of plasma lipoproteins by density gradient ultracentrifugation. *Anal Biochem* 1975;65:42-9.
 27. Brussaard JH, Dallinga-Thie G, Groot PHE, Katan MB. Effects of amount and type of dietary fat on serum lipids, lipoproteins and apolipoproteins in man: a controlled 8-week trial. *Atherosclerosis* 1980;36:515-27.
 28. Deleted in proof.
 29. Röschlau P von, Bernt E, Gruber W. Enzymatische Bestimmung des Gesamt-Cholesterins im Serum. *Z Klin Chem Klin Biochem* 1974;12:403-7.
 30. Abell LL, Levy BB, Brody BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and a demonstration of its specificity. *J Biol Chem* 1952;195:357-66.
 31. Snedecor GW, Cochran WG. *Statistical methods*. 6th ed. Ames: Iowa State University Press, 1967.
 32. Warnick GR, Cheung MC, Albers J. Comparison of current methods for high-density lipoprotein cholesterol quantitation. *Clin Chem* 1979;25:596-604.
 33. Demacker PNM, Vos-Jansen HE, Jansen AP, Laar A van't. Evaluation of the dual-precipitation method by comparison with the ultracentrifugation method for measurement of lipoproteins in serum. *Clin Chem* 1977;23:1238-44.
 34. Raaij JMA van, Katan MB, Hautvast JGAJ. Casein, soya protein, serum-cholesterol. *Lancet* 1979;11:958.
 35. Walker GR, Morse EH, Overley VA. The effect of animal protein and vegetable protein diets having the same fat content on the serum lipid levels of young women. *J Nutr* 1960;72:317-21.
 36. Campbell AM, Swendseid ME, Griffith WH, Tuttle SG. Serum lipids of men fed diets differing in protein quality and linoleic acid content. *Am J Clin Nutr* 1965;17:83-7.
 37. Anderson JT, Grande F, Keys A. Effect on man's serum lipids of two proteins with different amino acid composition. *Am J Clin Nutr* 1971;24:524-30.
 38. Sirtori CR, Gatti E, Mantero O, et al. Clinical experience with the soybean protein diet in the treatment of hypercholesterolemia. *Am J Clin Nutr* 1979;32:1645-58.
 39. Helms P, Gatti E, Sirtori CR. Soybean-protein diet and plasma-cholesterol. *Lancet* 1977;1:805-6.
 40. Sautier C, Doucet C, Flament C, Lemonnier D. Effects of soy protein and saponins on serum, tissue and feces steroids in rat. *Atherosclerosis* 1979;34:233-41.
 41. Hermus RJJ, Stasse-Wolthuis M, Hautvast JGAJ. Soybean-protein diet and plasma-cholesterol. *Lancet* 1977;1:905.
 42. Oakenfull DG, Fenwick DE, Hood RL, Topping DL, Illman RL, Storer GB. Effects of saponins on bile acids and plasma lipids in the rat. *Br J Nutr* 1979;42:209-16.
 43. Hermus RJJ, Dallinga-Thie GM. Soya, saponins and plasma-cholesterol. *Lancet* 1979;1:48.
 44. Potter JD, Topping DL, Oakenfull D. Soya, saponins and plasma-cholesterol. *Lancet* 1979;1:223.
 45. Huff MW, Hamilton RMG, Carroll KK. Effects of dietary proteins and amino acids on the plasma cholesterol concentrations of rabbits fed cholesterol-free diets. In: Manning GW, Haust MD, eds. *Adv Exp Biol Med*. Vol. 82. *Atherosclerosis: metabolic, morphologic and clinical aspects*. New York: Plenum Press 1977:275-277.
 46. Sirtori CR, Descovich G, Nosedà G. Texturated soy protein and serum-cholesterol. *Lancet* 1980;1:149.
 47. Huff MW, Hamilton RMG, Carroll KK. Plasma cholesterol levels in rabbits fed low fat, cholesterol-free, semipurified diets: effects of dietary proteins, protein hydrolysates and amino acid mixtures. *Atherosclerosis* 1977;28:187-95.
 48. Hermus RJJ, Stasse-Wolthuis M. Lipids and lipoproteins in rabbits fed semisynthetic diets containing different proteins. In: Peeters H, eds. *Protides of the biological fluids*. Vol. 25. Oxford: Pergamon Press, 1978:457-60.
 49. Hermus RJJ. Dietary proteins, amino acids and serum cholesterol. XIth International Congress of Nutrition, 1978. Rio de Janeiro-Brasil, abstr. 124.
 50. Kritchevsky D. Vegetable protein and atherosclerosis. *J Am Oil Chem Soc* 1979;56:135-40.
 51. Bazzano G, Olson RE. Effect of glutamic acid on sterol metabolism in man. *Am J Clin Nutr* 1969;22:667-8.
 52. Amino acid content of foods and biological data on proteins. Rome: Food Policy and Food Science Service, Nutrition Division, Food and Agriculture Organization of the United Nations, 1970.
 53. Blackburn H, Chapman J, Dawber TR, et al. Revised data for 1970 ICHD report. *Am Heart J* 1977;94:539-40.
 54. Miller GJ, Miller NE. Plasma-high-density lipoprotein concentration and development of ischaemic heart-disease. *Lancet* 1975;1:16-9.