Influence of diets containing casein, soy isolate, and soy concentrate on serum cholesterol and lipoproteins in middle-aged volunteers[1, 2]

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ABSTRACT Fifty-seven healthy volunteers (mean age 46 yr) were fed for 45 days on diets containing 16% of energy as protein, 35% as fat (polyunsaturated/saturated fat ratio = 0.5) and about 375 mg cholesterol/day. Of the protein in the diets 60% was provided as caseinate, as soy protein isolate, or as soy protein concentrate. After a control period of 17 days during which all the subjects received the casein diet, 17 subjects continued on this diet for the next 28 days (test period), 20 subjects switched to the soy isolate diet, and the remaining 20 subjects switched to the soy concentrate diet. Serum cholesterol levels at the end of the control period were 207 ± 36, 205 ± 40, and 199 ± 35 mg/dl (mean ± SD) for the casein, isolate, and concentrate groups, respectively. Mean changes over the test period were −2 ± 10, −8 ± 12, and +1 ± 10 mg/dl, respectively. Compared with the casein diet, the isolate diet caused a small, nonsignificant decline in both serum total cholesterol and low-density lipoprotein cholesterol (−6.5 mg/dl) and an increase in high-density lipoprotein cholesterol (+5.8 mg/dl) ($p < 0.05$). These effects may have been more obvious if there had been no differences between groups in weight loss. No correlation was found between the response and the initial cholesterol level. No differences in lipoprotein composition were found between the casein and soy concentrate groups. Our data suggest that soy protein preparations do not have dramatic effects on the serum total cholesterol concentration in healthy subjects. However, pure soy protein might have some beneficial effect on the distribution of cholesterol over the lipoproteins. The lack of effect of the less refined soy protein concentrate suggests that the dietary fiber and other nonprotein components of soy concentrate do not have, at least in the short-term, a favorable effect on serum cholesterol and lipoproteins in healthy adults. *Am J Clin Nutr* 1982;35:925–934.

KEY WORDS Casein, soy protein, serum cholesterol, serum lipoproteins, high-density lipoproteins, dietary fiber

Introduction

Several clinical investigations in hypercholesterolemic patients have shown that replacement of animal proteins in the diet by textured soy protein can lower the concentration of serum low-density lipoprotein (LDL) cholesterol to a marked degree (1–3). This had led to the expectation that the replacement of animal protein by vegetable protein in the human diet might aid in lowering serum total cholesterol levels, and hence, be of benefit in the prevention of atherosclerosis (4–6). However, we were unable to confirm the hypocholesterolemic effect of soy protein compared with casein in a strictly controlled nutritional trial with 69 volunteers aged 18 to 28 yr. We did observe a small but significant increase in the ratio of high-density lipoprotein (HDL) over low-density lipoprotein cholesterol on the soy protein diet (7).

Several factors, such as the lipid status of the subjects and the type of soy protein used, may explain the difference in results between the studies of other workers and ourselves. This is supported by the results of Sirtori et

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al. (8, 9) who found that the therapeutic response was related to the initial serum cholesterol level of their patients. In our previously reported study, the volunteers were students with low serum cholesterol levels. Thus it seemed appropriate to examine whether the results of our earlier experiment could be confirmed in subjects with a higher mean serum cholesterol level. As serum cholesterol increases with age in Western countries we decided to repeat out previous experiment with middle-aged volunteers. We also decided to examine if different types of soy preparation vary in effectiveness in lowering serum cholesterol. In addition to the highly purified soy protein preparation that we used previously (soy isolate), a diet containing soy preparations with substantial amounts of nonprotein material (soy concentrate) was now also tested. It has been suggested that the nonprotein material from legumes such as dietary fiber and saponins may lower serum cholesterol and might be responsible for the observed effects in some studies (10–14).

Subjects and methods

Subjects

Thirty-two male and 29 female volunteers were recruited from the town of Wageningen and surrounding areas through advertisements in local newspapers. The subjects ranged in age from 29 to 60 yr (mean 46 ± 9 yr). They were apparently healthy as determined by a medical questionnaire and were found to have normal values for blood Hb and no detectable glucose or protein in their urine. Erythrocyte sedimentation rate was slightly but persistently elevated (20 to 30 mm/h) in five subjects, while another six subjects showed a slightly elevated blood pressure (diastolic between 95 and 105 mm Hg or systolic between 150 and 160 mm Hg). Four men and eight women were overweight (weight/height^2 more than 27 and 26 kg/m^2, respectively). Serum total cholesterol in casual blood samples ranged from 135 to 305 mg/dl (mean 215 mg/dl) and HDL-cholesterol from 34 to 86 mg/dl (mean 55 mg/dl). None had received any medication known to affect serum lipids for at least 2 months before the study.

Before approval for the study was obtained from the Ethical Committee of the Department of Human Nutrition of the Agricultural University. The experimental protocol was fully explained to the participants and informed consent was obtained. The subjects were not offered payment and were free to end their participation at any moment wanted. They were seen by one of the investigators (JMA van R) and by four research dietitians each day during the week. During the experiment, two subjects decided to withdraw. Participants were asked to note in diaries any illness, drugs use, and departures from the diets. On the basis of this information, it was decided to eliminate the data of another two subjects from the analysis, so that data on 57 subjects were used.

Diets and control of food intake

The amount (and type) of fat, cholesterol, protein, and carbohydrates in the test diets were planned to simulate an average Western diet, but with slightly higher intakes of protein and polyunsaturated fatty acids. Of the protein 60% was replaced by protein from caseinate (casein diet), from soy protein isolate (isolate diet), or from soy protein concentrate (concentrate diet). Details of these proteins are given in **Table 1**. To keep the diets palatable, it was necessary to provide 40% of the protein from other sources (mainly from wheat, rice, potatoes, and other vegetables). The caseinates, isolates, and concentrates were incorporated into highly similar specially developed products. Brown bread and gluten-free bread and analogues of milk and yogurt were prepared as described previously (7). Half of the protein in brown breads consisted of test protein; the protein in the gluten-free breads and in the milk-like beverages consisted of test protein alone. The analogues of milk and yogurt were incorporated into soups, sauces, puddings, and sandwich spreads. Cheese was used in the casein diet because it contains casein as practically the only protein. A gelated product was prepared from soy isolate as a counterpart for cheese in the isolate diet (7), and a commercial textured product was used in the concentrate diet. Butter fat was added to both soy diets to balance the fat in cheese. The gelated and textured soy products were added to sauces and sandwich spreads. Fresh egg yolk was used in all diets to adjust cholesterol intakes, and each subject consumed one multivitamin tablet (Davitamon 10, N.V. Organon, 5349 AB Oss) per day.

TABLE 1
Composition of the protein preparations used

	Caseinate*	Soy isolate†	Soy concentrate‡
	g/100 g	*g/100 g*	*g/100 g*
Protein	92.4§	79.7§	57.2§
Moisture	3.0	5.5	5.9
Fat	0.3	0.5	1.6
Ash	4.2	4.0	6.4
Carbohydrates	0.2‖	10.3¶	28.9¶

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

† Soy protein isolate PP500E and PP610, Purina Protein Europe, B-1050 Brussels, Belgium. Data expressed as mean of the values for PP500E and PP610.

‡ Soy protein concentrates Unico (powder) and Dubit (textured, prepared from Unico), Unimills BV, 3330 AA Zwijndrecht. Data expressed as mean of the values for Unico and Dubit.

§ Kjeldahl nitrogen-to-protein factors of 6.38 for casein and 5.70 for soy protein were used. The convential factor of 6.25 would have given values of 90.5 for casein, 87.4 for soy isolate and 62.7 for soy concentrate.

‖ Lactose.

¶ By difference.

Only small amounts of meat (providing about 7% of total protein intake) and no egg white, fish, legumes, or dairy products apart from cheese were used in the preparation of the diets. Each diet met the recommended nutritional allowances for adults (15). The daily menu is given in **Table 2**. As the purity of the protein preparations differed (see Table 1), it was necessary to incorporate different amounts of the protein preparations in the diets in order to keep the proportion of protein constant. The diets were completely identical except for the type of protein and the amount of nonprotein material derived from the preparations used, because the diets consisted of the same regular foodstuffs and similar special products.

Total diets were provided for the participants except for 240 kcal/day, which the subjects were free to choose from a list of foodstuffs not containing protein. All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs. Hot meals were served on weekdays in the evening at the Department.

TABLE 2
Example of a daily menu for a subject consuming 2240 kcal/day*

	Item	Amount: Total	Amount: Test protein
		g	g
Breakfast and lunch	Brown bread†	245	18
	Gluten-free bread†	80	6
	Margarine	30	
	Sandwich spread†	75	6
	Meats	30	
	Sugar‡	25	
Dinner	Soup†	200	6
	Potatoes (or rice)	100	
	Other vegetables	175	
	Soy sauce†	150	13
	(or Casein sauce†	125	3
	plus cheese	40	10)
	Egg yolk	20	
	Salad	30	
	Pudding†	175	6
Snacks	Fruit§		
	Juice‡		
Total (kcal)		2000	55

* Food provided for subjects. In addition to these items subjects were allowed to choose 240 kcal/day of foodstuffs from a list of low-protein products. Subjects were allowed unlimited tea, coffee, selected low-calorie beverages, and up to 6 g of coffee-whitener/day.

† Special product containing test protein.

‡ Sugar was provided as sugar, apple syrup, honey, apple juice, or grapefruit juice, as determined by individual preference.

§ The amount of fruit varied with the type of fruit provided.

All other foods were provided as packages daily; food for the weekend including ingredients for the hot meals was provided each Friday. Nonadherence to the diets was recorded in diaries. The special products were well accepted and adherence to the diets was excellent. Each subject weighed and recorded his or her food intake on 2 random days during the control period and on 4 random days during the test period. The actual nutrient intake was calculated for each individual from these food records using a computerized food composition table supplemented with analyses of special products (16). In addition, double portions of each diet were collected and analyzed as described earlier (17, 18).

Experimental design

All subjects consumed the casein diet for a control period of 17 days after which they were divided into three groups; the groups were matched for initial serum total cholesterol, HDL-cholesterol, energy intake, and sex. During the test period of 28 days, the soy isolate group (n = 20) received the soy isolate diet, the soy concentrate group (n = 20) received the soy concentrate diet, and the casein group (n = 17) continued on the casein 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 0.8, 0.7, and 0.4 kg in the casein, isolate, and concentrate groups, respectively. These weight losses continued during the test period, with mean weight losses of 1.9, 1.4, and 1.2 kg, respectively. The mean weight reduction in the casein group was significantly higher than in the concentrate group ($p < 0.01$). Sixteen subjects lost more than 3 kg of weight over the entire experimental period; two of them, both from the casein group, lost more than 5 kg (5.4 and 5.6 kg, respectively).

Blood sampling and analysis

Blood samples were obtained fortnightly from an arm vein after an overnight fast. At the end of the control period, and also at the end of the test period, two samples were taken at 1-day intervals (Fig. 1). Serum was obtained by low-speed centrifugation and all serum samples were assayed for total cholesterol and HDL-cholesterol. At the end of both periods, lipoproteins in sera were separated by density gradient ultracentrifugation, and recovered by aspiration into very low-density lipoprotein ($d < 1.006$ g/ml), LDL + sinking pre-β lipoproteins ($1.006 < d < 1.075$ g/ml), HDL-2 ($1.075 < d < 1.125$ g/ml) and HDL-3 ($d > 1.125$ g/ml). The mean recovery of cholesterol was 97.2 ± 5.0%. All methods used were those described for our previous experiment (7). Comparison of HDL-levels determined by ultracentrifugation and by the Mn-heparin procedure yielded a correlation coefficient of $r = 0.91$. Values obtained by the ultracentrifugation method were about 3% higher than those obtained by the precipitation procedure. The sera of 16 of the 57 subjects showed a sinking pre-β lipoprotein band with a mean density of 1.064 ± 0.005 g/ml [calculated as described (7)]. In order to partition this lipoprotein band completely into the LDL-fraction, we used a boundary between HDL and LDL of 1.075 g/ml instead of the usual density of 1.063 g/ml.

Statistical evaluation

The response to the test diet was calculated per subject as the change from the end of the control to the end of the test period. Differences in diet effects were examined by comparing the mean responses of the groups by unpaired *t* tests. The effects of sex, initial cholesterol levels, and weight changes were examined by analysis of covariance and by correlation analysis (19).

Results

Nutrient intake

The nutrient intake as measured by food records is shown in **Table 3**. Food records and chemical analysis of double portions indicated that there were essentially no differ-

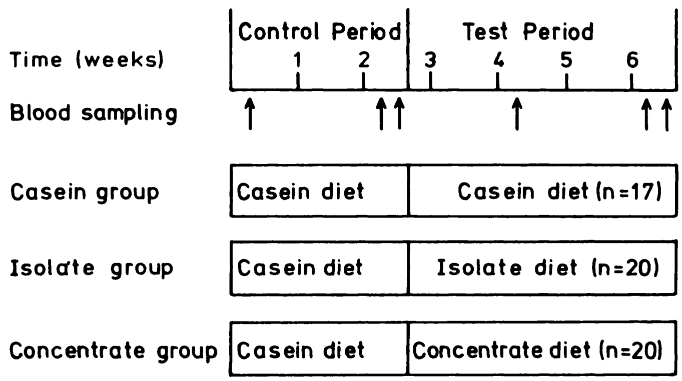

FIG. 1. Experimental design.

TABLE 3
Mean daily intake of nutrients before and during the experiment according to individual food records

	Habitual intake before experiment*	Control period	Test period		
		Casein diet	Casein diet	Isolate diet	Concentrate diet
Energy (kcal/day)	2265	2140	2116	2166	2244
(mJ/day)	9.5	9.0	8.9	9.1	9.4
Protein (% of energy)	13.8	16.2	15.9	16.2	16.1
Casein	†	9.8	9.6	0.0	0.0
Soy protein	†	0.0	0.0	9.8	9.8
Other animal proteins	8.3	1.4	1.4	1.2	1.1
Other vegetable proteins	5.5	5.0	4.9	5.2	5.2
Total fat (% of energy)	36.6	34.1	34.5	34.5	34.9
Saturated	15.9	14.8	15.3	15.0	14.9
Monounsaturated	13.1	11.7	11.5	11.6	12.0
Polyunsaturated	7.6	7.6	7.7	7.9	8.0
Carbohydrates (% of energy)	45.0	46.2	48.0	45.1	45.6
Sugars	21.5	21.2	22.3	20.0	20.7
Starch	23.5	25.0	25.7	25.1	24.9
Alcohol (% of energy)	4.6	3.5‡	1.6	4.2	3.4
Cholesterol (mg/day)§	297	369	374	381	382
Dietary fiber (g/day)	32	36	37	48‖	69¶

* 3-day records.
† Not measured.
‡ Percentages of energy during the control period were 2.1, 4.5, and 3.8 for the casein, isolate and concentrate groups, respectively.
§ Mean plant sterol intake was 320 mg/day (β-sitosterol, 50%; campesterol, 22%; stigmasterol, 6%; others, 22%).
‖ Included 6.8 g of unavailable carbohydrates from soy protein preparations.
¶ Included 27.8 g of unavailable carbohydrates from soy protein preparations.

ences between the experimental diets with respect to the amount of protein, fat, carbohydrate, and cholesterol and to the composition of the fatty acids, carbohydrates, and plant sterols. The amount of cholesterol in the experimental diets was about 75 mg/day higher than in the preexperimental diet. The differences between groups in alcohol consumption during the test period were similar to those during the control period. From the food records it could be calculated that about 60% of the total dietary protein consisted of either casein or soy protein. Analysis of the soy isolates and soy concentrates used revealed only negligible differences in amino acid composition. Although the amino acid composition of the whole diet was diluted by those proteins that were common to all diets, the calculated differences between the casein and soy diets were still pronounced and similar to those described previously (7). As expected the test diets differed greatly in dietary fiber content. The mean protein consumption was about 90 g/day, of which about 55 g were derived from the test protein. This corresponded to about 60 g caseinate, 70 g soy isolate, or 100 g soy concentrate. Therefore the isolate and concentrate provided the subjects daily with about 7 and 28 g of dietary fiber (Table 1), including about 0.1 and 0.5 g of polygalacturonic acid (pectin), respectively. The calcium content of the casein diet was slightly higher than that of the soy diets, and the concentrate diet had a lower sodium content and a higher potassium content than the casein and isolate diet. However, it is unlikely that these slight differences in mineral composition influenced the level of serum lipids. Chemical analysis of double portions revealed a slightly higher fat content and a lower oligosaccharide and cholesterol content in all diets than did the calculations based on records plus food tables. This was in agreement with our previous findings (7, 17, 18).

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 mean concentrations at the end of the control and test period are given in **Table 4**. During the casein control period of 17 days serum cholesterol concentration decreased in nearly all subjects compared with the uncontrolled preexperimental situation by on average 12 mg/dl (0.30 mmol/l). Changes in serum cholesterol were negatively correlated with the initial levels ($r = -0.40$; $p < 0.001$); thus the largest declines in cholesterol were found in the subjects with the highest initial levels. It is difficult to explain the cholesterol changes by differences between the nutrient composition of the test diets and the preexperimental self-selected diets (Table 3). It may well be that the controlled nutritional conditions and/or the habituation to the new menu- and

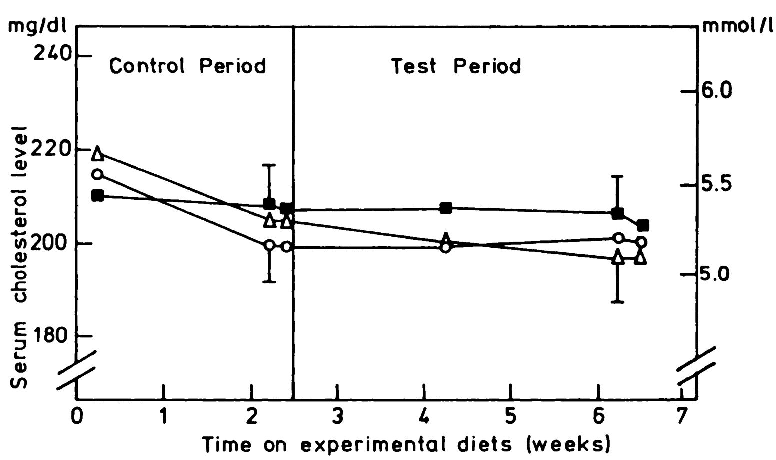

FIG. 2. Effects of casein and soy protein diets on mean serum total cholesterol concentrations in humans throughout the experiment. Vertical bars indicate 1 SEM. ■——■, casein group (17 subjects); △——△, soy isolate group (20 subjects); ○——○, soy concentrate group (20 subjects).

TABLE 4
Effects of casein and soy protein diets on cholesterol concentration in whole serum (mean ± SD); each individual value represents the average of two separate determinations at the end of the respective period

	Casein group	Isolate group	Concentrate group
	*mg/dl**	*mg/dl*	*mg/dl*
	(n = 17)	(n = 20)	(n = 20)
All subjects			
Control period	207 ± 36	205 ± 40	199 ± 35
Test period	205 ± 35	197 ± 43	200 ± 38
Change	−2.3 ± 10.1	−7.7 ± 12.0†	+1.2 ± 10.4
Initial cholesterol level			
Highest 25%	(n = 5)	(n = 5)	(n = 5)
Control period	240 ± 34	248 ± 24	235 ± 30
Test period	235 ± 39	243 ± 26	244 ± 31
Change	−5.3 ± 15.1	−4.3 ± 18.8	+9.2 ± 15.7
Lowest 25%	(n = 5)	(n = 5)	(n = 5)
Control period	168 ± 19	157 ± 23	164 ± 37
Test period	170 ± 15	145 ± 19	159 ± 31
Change	+1.6 ± 6.5	−11.5 ± 8.3‡	−4.6 ± 7.4
Wt loss during study			
Less than 3 kg	(n = 10)	(n = 15)	(n = 16)
Control period	214 ± 35	205 ± 39	204 ± 35
Test period	214 ± 38	199 ± 44	205 ± 38
Change	−0.3 ± 7.2	−6.3 ± 12.5	+1.5 ± 11.3
More than 3 kg	(n = 7)	(n = 5)	(n = 4)
Control period	197 ± 38	202 ± 45	180 ± 32
Test period	192 ± 29	189 ± 41	179 ± 34
Change	−5.4 ± 13.3	−12.6 ± 9.0†	−0.8 ± 7.7

* 100 mg/dl = 2.59 mmol/l.
† Significantly different from concentrate group ($p < 0.05$).
‡ Significantly different from casein group ($p < 0.05$).

eating-pattern and the special products played a role.

The control group that continued on the casein diet did not show any further change in serum total cholesterol which showed that levels had stabilized at the end of the baseline period of 17 days. The soy concentrate diet also induced hardly any change in average cholesterol concentration, but the soy isolate diet caused a small decline in total cholesterol of about 8 mg/dl (0.20 mmol/l). Analysis of the individual responses during the test period failed to show effects of sex, but revealed a significant diet effect. It appeared that the decline on the soy isolate diet was significantly different from the small increase on the concentrate diet ($p < 0.05$) but not from the small decline on the casein diet. In the casein group changes in serum cholesterol during the test period were negatively correlated with initial levels ($r = -0.37$; $p = 0.07$). However, in the isolate and concentrate group the relationships were positive ($r = +0.25$; $p = 0.14$ and $r = +0.35$; $p = 0.07$, respectively). Thus, in both soy groups the largest declines in serum cholesterol were observed in subjects with the lowest initial levels (see also Table 4).

Although every effort was made to adjust energy intake as required to control body weight, weight losses were observed in nearly all subjects. It has been reported that weight loss can decrease serum total cholesterol (20) and increase HDL-cholesterol (21) but that a considerable amount of weight must be lost (10 to 15%) to produce noticeable effects. Although in our study none of the correlations between changes in either serum total HDL-cholesterol and variation in body weight was significant at the 5% confidence level, Table 4 does suggest some relation between weight loss and decrease of serum cholesterol. Changes in HDL-cholesterol for those subjects who lost less than 3 kg of body weight over the whole experiment are given in a footnote to **Table 5**. Weight loss was

TABLE 5
Effects of casein and soy protein diets on cholesterol concentration in lipoprotein fractions of serum (mean ± SD)

	Casein group (n = 16)	Soy isolate group (n = 20)	Soy concentrate group (n = 20)
	*mg/dl**	*mg/dl*	*mg/dl*
Ultracentrifugation:			
Very low-density lipoprotein			
Control period	14 ± 7	15 ± 10	15 ± 9
Test period	14 ± 8	14 ± 8	16 ± 9
Change	+0.0 ± 5.3	−0.9 ± 5.4	+0.3 ± 3.9
LDL			
Control period	127 ± 31	128 ± 33	119 ± 29
Test period	130 ± 33	124 ± 33	126 ± 31
Change	+2.8 ± 12.0	−3.7 ± 15.1†	+7.6 ± 11.5
Total HDL			
Control period	62 ± 14	58 ± 11	58 ± 15
Test period	58 ± 11	59 ± 11	56 ± 14
Change	−4.5 ± 9.2	+1.3 ± 5.9‡	−1.7 ± 5.5
HDL-2			
Control period	37 ± 13	30 ± 9	31 ± 12
Test period	33 ± 10	31 ± 9	29 ± 12
Change	−4.0 ± 8.4	+0.3 ± 5.0‡†	−2.8 ± 4.3
Mn-heparin precipitation:			
HDL§			
Control period	58 ± 11	56 ± 10	57 ± 13
Test period	58 ± 10	58 ± 10	55 ± 13
Change	−0.4 ± 4.6	+1.9 ± 5.8†	−1.9 ± 3.1

* 100 mg/dl = 2.59 mmol/l.
† Significantly different from concentrate group ($p < 0.05$).
‡ Significantly different from casein group ($p < 0.05$).
§ For those subjects who lost less than 3 kg of wt for the whole experiment average changes over the test period amounted to −1.2 mg/dl (n = 10) in the casein group, +2.0 mg/dl (n = 15) in the isolate group, and −1.8 mg/dl (n = 16) in the concentrate group.

most serious in subjects in the casein group. Therefore, it is possible that the effects of soy protein on serum total cholesterol and HDL-cholesterol would have been somewhat larger if there had been no differences between groups in weight loss.

Distribution of cholesterol between the lipoprotein fractions

The mean cholesterol concentrations in the lipoprotein fractions are given in Table 5. In the casein group and soy isolate group there were no appreciable changes in concentrations in any of the fractions. In the soy concentrate group, however, there was an increase in LDL-cholesterol (+7.6 mg/dl or +0.20 mmol/l). Comparison of the changes in LDL-cholesterol concentration on the various diets showed that there had been a decline in the soy isolate group of 11.3 mg/dl ($p < 0.05$) relative to the soy concentrate group, and of 6.5 mg/dl (NS) relative to the casein group. HDL-cholesterol results after ultracentrifugation revealed that the average HDL-cholesterol concentration had increased by 5.8 mg/dl relative to the casein group ($p < 0.5$) and had not changed significantly relative to the concentrate group. Changes in HDL-cholesterol occurred mainly in the HDL-2 fraction. The HDL-cholesterol results after Mn-heparin-precipitation were similar to those found after ultracentrifugation. With the precipitation method there was no noticeable difference in response between the isolate and the casein group, but the difference between the isolate and the concentrate group now reached statistical significance (+3.8 mg/dl or +0.10 mmol/l). No differences in distribution of cholesterol over the serum lipoprotein classes were found between the casein and concentrate group.

TABLE 6
Effects of casein and soy protein diets on serum HDL-cholesterol/total cholesterol ratio (mean ± SD)*

	Casein group (n = 17)	Isolate group (n = 20)	Concentrate group (n = 20)
Start of control period	0.27 ± 0.08	0.26 ± 0.08	0.26 ± 0.06
End of control period	0.29 ± 0.08	0.28 ± 0.07	0.29 ± 0.07
End of test period	0.29 ± 0.07	0.30 ± 0.07	0.28 ± 0.06
Change over			
Control period	+0.02 ± 0.02	+0.02 ± 0.03	+0.03 ± 0.03
Test period	−0.00 ± 0.02	+0.02 ± 0.02†	−0.01 ± 0.02

* HDL-cholesterol values obtained by the Mn-heparin precipitation method were used for this calculation.
† Significantly different from casein and concentrate group ($p < 0.01$).

Analysis of the individual responses failed to show a sex effect.

The overall changes in the distribution of cholesterol over the lipoproteins can be summarized by the HDL-cholesterol/total-cholesterol ratio. The mean of the individual ratios at the start and at the end of the control period and at the end of the test period are presented for each group in **Table 6**. During the control period there was a significant increase in the ratio in all three groups, mainly because of a decrease in LDL-cholesterol. During the test period the ratio remained unchanged in most subjects from the soy groups and in nearly all subjects from the casein group. However, nine subjects from the isolate group showed an increase of the ratio by more than 10%, and six subjects from the concentrate group showed a similar decline of the ratio. The mean response on the soy isolate diet was significantly different from the response on either the casein or the concentrate diet. No differences were found between the casein and concentrate group.

Discussion

In our present study with middle-aged volunteers we failed to find a marked effect on serum total cholesterol of diets containing either soy isolate or soy concentrate when compared with a diet containing casein. These results confirm our previous study with younger volunteers (aged 18 to 28 yr) who had lower serum cholesterol concentrations (7). Our results are in marked contrast with those of Sirtori et al. (1, 2), who observed dramatic decreases in serum cholesterol levels in hypercholesterolemic patients on diets containing soy protein. Other studies on hyper- and normocholesterolemic subjects have revealed smaller (22, 23), inconsistent (24), or no effects (25–27) when soy protein diets were fed in comparison with control diets containing animal protein. Consumption of plant proteins other than those from soybean has not been demonstrated to have a hypocholesterolemic effect in humans either (28–31).

In our earlier work (7), we suggested that one explanation for the discrepancy between our results and those of the Italian workers might be that our subjects had comparatively low initial levels of serum cholesterol. However, our present study fails to confirm this explanation. Although clinical hypercholesterolemic subjects were not involved, the average initial cholesterol level was appreciably higher (55 mg/dl) than in our previous study and we still failed to observe dramatic changes in total serum cholesterol concentrations. In fact, those subjects from the soy isolate group with the highest initial levels showed less of a decline than did the subjects with lower initial levels (Table 4). The patients studied by Sirtori et al. (1) had still higher values (mean 342 mg/dl), so we still cannot exclude a specific cholesterol-lowering effect of soy protein in overt type II hypercholesterolemic patients. However, we prefer to think that other properties of the diets used by Sirtori et al. (1) apart from the nature of the protein were responsible for the remarkable efficacy of these diets in combating hypercholesterolemia. A case in point might be the almost complete absence of cholesterol from these diets.

Earlier we suggested a role for the nonprotein components in soy products in lowering cholesterol levels (7). For this reason, our present experiment compared not only a relatively pure soy protein product (soy isolate) with casein, but also a more crude product (soy concentrate) that still contained most of the dietary fiber of the soybean. However, serum cholesterol levels on the concentrate diet were actually increased when compared with the isolate diet. A cholesterol-lowering action has been described for viscous fiber compounds such as pectin and guar gum but not for those consisting mostly of cellulose and hemicelluloses (32). Earlier work from our laboratory demonstrated that fiber from wheat bran raised cholesterol levels in humans (17). It should be noted that fiber in bran, like that in soy concentrate, consists mostly of hemicelluloses and contains little or no pectin; the main fiber types in soy are arabinogalactans (33, 34), while in bran they are arabinoxylans (32). Decreases of serum cholesterol concentrations after addition of legumes to the diet have been reported (32). However, dietary constituents such as amount and type of fat may also have changed in these experiments (32). Thus the lack of a specific cholesterol-lowering action of the dietary fiber in soy protein preparations is not in conflict with other experimental evidence in humans on this point.

Although we did not find a marked change in total cholesterol concentrations, our data do show a small decline in LDL-cholesterol and a small increase in HDL-cholesterol on the soy isolate diet when compared with the casein control group, but there was no effect with the soy concentrate diet. These effects of the soy isolate diet are identical with our previous findings in young adults with low serum cholesterol levels (7). Changes in HDL were confined mainly to the HDL-2 subfraction. This agrees with the role of HDL-2 as the variable component of HDL (35, 36). Because an increase in the ratio of serum HDL to total cholesterol could be associated with a lower risk of coronary artery disease (37, 38) the significant increase in this ratio on the isolate diet suggests that soy protein could have a small beneficial effect even when the concentration of total cholesterol remains constant. Yet, it must be noted that in our studies these favorable effects were only observed with the rather pure soy protein isolate.

In summary, we believe that there is as yet no evidence that a moderate replacement of animal by vegetable protein in diets of otherwise normal composition will have an effect on the concentration of serum total cholesterol comparable to that observed with changes in the fat composition of the diet. However, a small favorable effect on the distribution of cholesterol over the various lipoprotein classes cannot be excluded. Moreover, one would expect that in practical situations the replacement of animal protein by vegetable protein containing foodstuffs would lead to changes in the concentration of serum lipids because the main sources of animal protein are often also rich in saturated fat and cholesterol.

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