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7
Influence of Human Diets Containing Casein and Soy Protein on Serum Cholesterol and Lipoproteins in Humans, Rabbits, and Rats

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I. INTRODUCTION

In animal studies, dietary proteins derived from animal sources are generally found to be hypercholesterolemic when compared with proteins from plant sources [Carroll, 1978; Kritchevsky, 1979; Terpstra et al, 1982b]. Epidemiological studies [Stamler, 1979] and nutritional studies in vegetarians [Hardinge and Stare, 1954; Sacks et al, 1975; Burslem et al, 1978] have also suggested a relation between intake of animal protein and serum cholesterol. Such epidemiological observations, however, should be interpreted with cau-

tion as differences in other nutritional factors may be present. In humans, only a few controlled trials relating dietary protein to serum cholesterol have been carried out, and the results are conflicting [Anderson et al, 1971; Carroll et al, 1978; Sirtori et al, 1979; Shorey and Davis, 1979; Descovich et al, 1980; Vessby et al, 1980; Holmes et al, 1980; Bodwell et al, 1980; Wolfe et al, 1981].

We have recently investigated, in strictly controlled dietary studies, the effects of diets containing casein and soy protein on the concentration of serum cholesterol and lipoproteins in large groups of young students and middle-aged subjects. During our studies with humans, duplicate portions of the test diets were collected and were later fed to rabbits and rats. In this paper the results of the human and animal studies are discussed. Some of the results have been published previously [van Raaij et al, 1981, 1982].

II. MATERIALS AND METHODS

A. Human Subjects, Animals, and Diets

In the experiments to be described, three principal sources of protein have been used: casein; soy protein isolate, which is the purest form of soy protein commercially available; and soy protein concentrate, which contains more dietary fiber (Table 7-I). Six studies have been carried out and they can be divided into two groups on the basis of the diets fed (Table 7-II; Fig. 7-I).

TABLE 7-I. Composition of the Protein Preparations Used*

		Soy isolate		
	Caseinate[a]	UNISOL NH 70[b]	PP500E, PP610[c]	Soy concentrate[d]
Protein[e]	92.4	84.6	79.7	57.2
Moisture	3.0	3.1	5.5	5.9
Fat	0.3	0.1	0.5	1.6
Ash	4.2	3.5	4.0	6.4
Carbohydrates	0.2[f]	8.7[g]	10.3[g]	28.9[g]

*All values are g/100 g.

[a]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.

[b]UNISOL NH 70, UNIMILLS BV, 3330 AA Zwijndrecht (used in I-Human and I-Rabbit).

[c]Soy protein isolate PP500E and PP610, Purina Protein Europe, B-1050 Brussels, Belgium (used in II-Human, II-Rabbit, II-Rat and II-Rabbit-SP). Data expressed as mean of the values for PP500E and PP610.

[d]Soy protein concentrates Unico (powder) and Dubit (textured, prepared from Unico), UNIMILLS BV. Data expressed as mean of the values for Unico and Dubit.

[e]Kjeldahl nitrogen-to-protein factors of 6.38 for casein and 5.70 for soy protein have been used.

[f]Lactose; data provided by manufacturer.

[g]By difference.

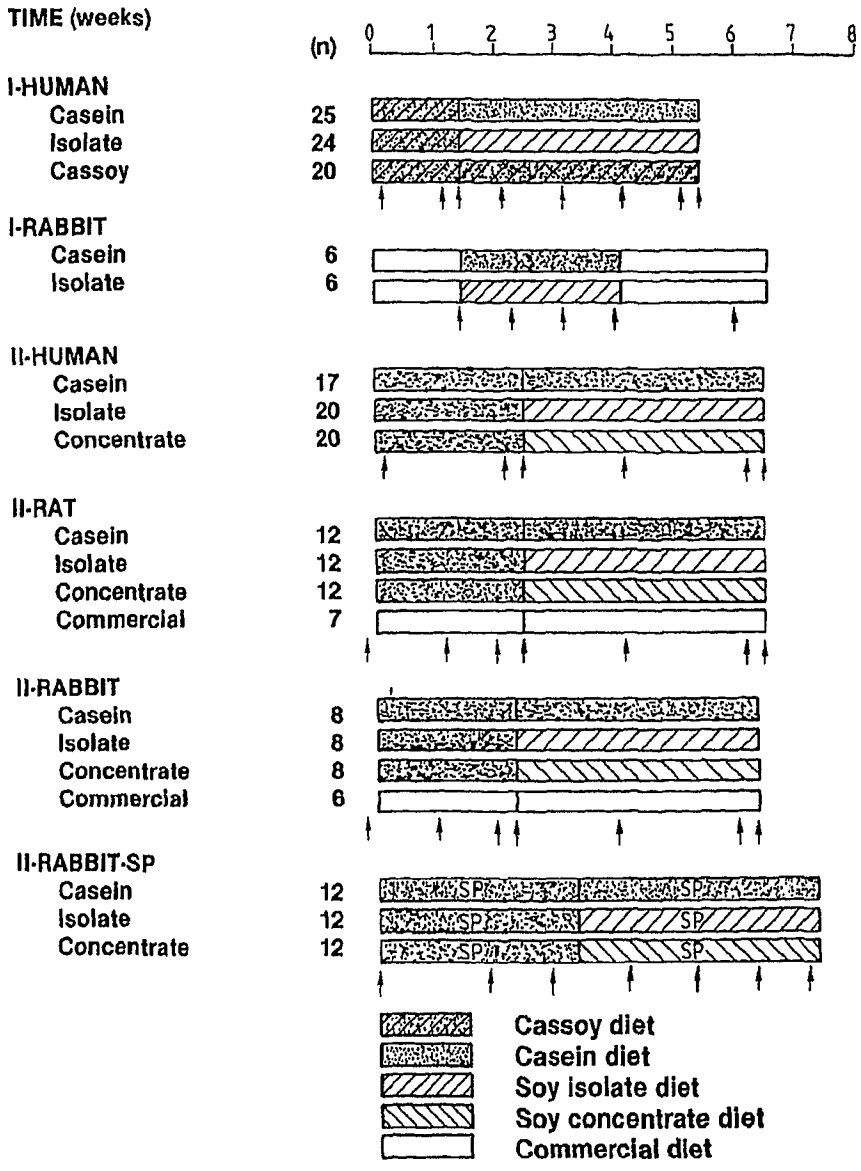

Fig. 7-1. Experimental designs. Blood sampling is indicated by arrows.

TABLE 7-II. Composition of the Diets Used (g per 100 g Dry Matter)

Experiment	Period and diet[a]	Protein[b]	Fat				Carbohydrates		Dietary fiber	Ash	Cholesterol	Fytosterols
			Total	Saturated	Mono-unsaturated	Poly-unsaturated	Total	Sugars				
I-Human, I-Rabbit	Control period											
	Cassoy	18.4[c]	21.2	8.4	9.2	3.6	41.3	14.4	9.2	4.0	0.08	0.11
	Commercial	21.3	4.8	[e]	[e]	[e]	55.4	[e]	10.6[f]	7.9	0	[e]
	Test period											
	Cassoy	17.6[c]	21.3	8.1	9.4	3.8	42.1	15.9	8.6	3.7	0.08	0.12
	Casein	18.4[c]	22.1	8.1	9.9	4.1	42.9	17.4	9.1	3.7	0.07	0.12
	Soy isolate	17.4[c]	22.2	8.0	10.1	4.1	41.8	16.5	8.6	3.6	0.07	0.12
II-Human, II-Rat, II-Rabbit	Control period											
	Casein	20.2[c]	20.6	8.4	7.7	4.5	46.2	16.0	7.8	4.5	0.07	0.07
	Test period											
	Casein	20.1[c]	20.7	8.3	7.6	4.8	45.7	15.7	8.2	4.6	0.07	0.07
	Soy isolate	19.7[c]	19.6	7.6	7.2	4.8	45.2	15.4	10.5	4.3	0.07	0.07
	Soy concentrate	19.7[c]	18.4	7.2	6.6	4.6	42.9	14.0	12.8	4.6	0.06	0.07
	Commercial (Rat)	25.2	7.3	[e]	[e]	[e]	56.7	[e]	4.7[f]	6.1	0	[e]
	Commercial (Rabbit)	21.3	4.8	[e]	[e]	[e]	55.4	[e]	10.6[f]	7.9	0	[e]

II-Rabbit-SP	Control period										
	Casein	21.6[d]	5.3	4.0	0.5	0.7	53.4	27.2	10.5	[e]	0	[e]
	Test period										
	Casein	21.6[d]	5.3	4.0	0.5	0.7	53.4	27.2	10.5	[e]	0	[e]
	Soy isolate	19.1[d]	5.3	4.0	0.5	0.8	53.1	27.0	12.8	[e]	0	[e]
	Soy concentrate	16.8[d]	5.1	3.6	0.6	0.9	47.7	24.2	17.7	[e]	0	[e]

[a]The test diets used in all experiments, except in the control period of I-Rabbit and throughout II-Rabbit-SP, were conventional human diets. These diets were freeze-dried before feeding to the animals. The composition of these diets was determined by chemical analysis [van Raaij et al, 1981, 1982]. In II-Rabbit-SP the protein preparations were incorporated into semipurified pelleted diets. The composition of these diets was calculated on basis of the ingredients used (in g/1,000, 1,006, and 1,084 g for the casein, isolate, and concentrate diet, respectively): casein, 210, or soy protein isolate, 216, or soy protein concentrate, 295; maize starch, 280; dextrose, 210; saw dust, 120; coconut oil, 40; soybean oil, 10; molasses, 50; vitamin premix, 12; mineral premix, 10; $CaHPO_4.2H_2O$, 29; NaCl, 6; $MgCO_3$, 3; MgO, 2; and $KHCO_3$, 18. As soy protein is deficient in methionine, and as the soy preparations contained more NaCl than casein, 2 g of NaCl was replaced by 2.1 and 1.4 g DL-methionine in the isolate and concentrate diets, respectively. The composition of the vitamin and mineral premixes has been described earlier [Katan et al, 1982]. In addition to the human diets, pelleted commercial diets were used in I-Rabbit, II-Rabbit, and II-Rat. Data on the composition of these diets were provided by manufacturers.

[b]The proportion of protein as test protein was 65% in I-Human and I-Rabbit, 60% in II-Human, II-Rat, and II-Rabbit, and 100% in II-Rabbit-SP.

[c]Kjeldahl nitrogen to protein factor of 6.25 was used.

[d]Kjeldahl nitrogen to protein factors of 5.70 and 6.38 were used for casein and soy protein, respectively.

[e]No data available.

[f]Crude fiber.

The first group of studies (van Raaij et al [1981] referred to in this paper as group I) consisted of a study with humans (I-Human) and a study with rabbits (I-Rabbit). In I-Human, 69 young healthy students aged 18 to 28 years were fed for 38 days on diets containing 13% of energy as protein of which 65% was replaced by protein from casein, from soy protein isolate, or from a 2:1 mixture of casein and soy protein isolate. After a control period of ten days during which all the subjects received the casein-soy diet, 20 subjects continued on this diet for a test period of 28 days, 25 subjects switched to the casein diet, and the remaining 24 subjects switched to the soy diet. Throughout the experiment duplicate portions of the diets were collected, and these were later homogenized in order to carry out a study with rabbits (I-Rabbit). Twelve male New Zealand white rabbits were maintained on a commercial diet (Cunicon I, Trouw and Co, 3881 LP Putten) until 3 months of age (control period-1). Half were transferred to the homogenized casein diet and half were transferred to the soy isolate diet for a test period of 2½ weeks before both groups were transferred back to the commercial diet for 2½ weeks (control period-2). The homogenate was supplied freeze-dried and pelleted during the first 12 days of the test period and as a wet mash during the remaining six days.

The second group of studies (group II) included a study with humans (II-Human), one study with rats (II-Rat) and two studies with rabbits (II-Rabbit and II-Rabbit-SP). In II-Human [van Raaij et al, 1982], 57 healthy subjects aged 29 to 60 years were fed for 45 days on diets containing 16% of energy as protein of which 60% was replaced by protein from casein, from soy protein isolate, or from 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 a test period of 28 days, 20 subjects switched to the soy isolate diet, and the remaining 20 subjects switched to the concentrate diet. As during the first study with humans, duplicate portions of the diets were collected, homogenized and freeze-dried in order to carry out a study with male New Zealand white rabbits (II-Rabbit) and also with female lean Zucker strain rats (II-Rat). The experimental design of these two studies was essentially the same as II-Human. Before the control periods, the rats and rabbits were maintained on commercial rat and rabbit pellets, respectively (Trouw and Co). In addition a further experiment with rabbits (II-Rabbit-SP) was carried out according to a similar design, but this time we used semipurified diets in which 100% of the protein was supplied by the same preparations of casein, soy isolate, and soy concentrate as used in II-Human, II-Rat, and II-Rabbit. At the beginning of the control periods, the rabbits and rats in the second group of experiments were 10–13 and 4 weeks of age, respectively.

For the two human experiments, food records and chemical analysis of the diets indicated that within each study there were essentially no differences between the experimental diets except for the type of protein and/or the amount

of nonprotein material derived from the protein preparations used. Apart from 120 kcal per day in I-Human and 240 kcal per day in II-Human, all of the food eaten was daily supplied to the subjects in amounts appropriate to each individual's energy requirements. The composition of the diets is given in Table 7-II, but more details about the diets have been described previously [van Raaij et al, 1981, 1982].

In the animal studies water was provided ad libitum. In I-Rabbit and II-Rat, the casein and soy diets were provided ad libitum, while in II-Rabbit and II-Rabbit-SP, the diets were fed on a restricted basis to supply the rabbits in each experiment with equal amounts of protein. The animals were maintained as described by Katan et al [1982] except that the rats as well as the rabbits were housed individually. The individual body weights were recorded weekly.

B. Sampling of Blood and Liver and Biochemical Analysis

Blood samples were collected throughout the experiments as shown in Figure 7-I. Blood was taken after an overnight fast as described earlier for humans [van Raaij et al, 1981], rabbits [Terpstra and Sanchez-Muniz, 1981] and rats [Terpstra et al, 1982a]. Serum was obtained by low-speed centrifugation, and all the serum samples were assayed for total cholesterol.

At the end of both periods, in both human studies and in II-Rat and II-Rabbit-SP, the lipoproteins of the serum were isolated by density-gradient ultracentrifugation using a modification [Terpstra et al, 1981b] of the method described by Redgrave et al [1975]. An SW50 rotor (Beckman Inc, Palo Alto, CA 94304, USA) was used for the separation of lipoprotein classes from 1-ml serum samples, as in II-Rat, and an SW41 rotor was used for the separation from 2-ml samples, as in all other studies. The lipoproteins in the gradient were visualized by prestaining the serum with Sudan black prior to ultracentrifugation. Lipoprotein fractions were removed either by tube-slicing (I-Human) or by aspiration (all other studies). In the human studies the following lipoprotein fractions were collected from individual sera: in I-Human – very low density lipoprotein (VLDL) ($d < 1.015$ g/ml), low-density lipoprotein (LDL) ($1.015 < d < 1.060$), and high-density lipoprotein (HDL) ($d > 1.060$ g/ml); and in II-Human – VLDL ($d < 1.006$ g/ml), LDL + sinking pre-beta-lipoproteins ($1.006 < d < 1.075$), and HDL ($d > 1.075$). In I-Rat, the serum samples were pooled per group at the end of the control period and again at the end of the test period. Nine lipoprotein fractions were collected, eight of which floated above the following densities (d, g/ml), respectively: 1.016, 1.032, 1.045, 1.060, 1.078, 1.102, 1.128, and 1.146, while the ninth fraction had a $d > 1.146$ g/ml. In II-Rabbit-SP analyses were carried out on pooled samples with similar cholesterol concentrations. At the end of the control period, six pools each of six sera were formed and at the end of the test period there were four pools within each group with each pool consisting of three sera. Three

lipoprotein fractions were collected: VLDL ($d < 1.006$), LDL ($1.006 < d < 1.063$), and HDL ($d > 1.063$ g/ml).

At the end of experiment II-Rabbit-SP, ie, after five weeks on the test diets, the concentration of cholesterol and the proportion present as the ester was measured in the livers of the rabbits. The animals were first stunned by a blow to the head and then killed by severing the major blood vessels in the neck and draining the blood from the body. The livers were removed immediately, dried off, and weighed. The liver was then homogenized with a Potter-Elvehjem homogenizer and lipids were extracted from a 10-g aliquot [Folch et al, 1957]. We measured total cholesterol in one part of the extract with Liebermann-Burchard reagent [Huang et al, 1961] after alkaline hydrolysis. Another part of the extract was used for separating and quantitating free and esterified cholesterol by thin-layer chromatography [West and Rowbotham, 1967].

In both human studies serum cholesterol was measured with the reagent of Huang et al [1961] with strict standardization [Katan et al, 1982]; animal sera and lipoprotein fractions were assayed for cholesterol by an enzymatic method [Röschlau et al, 1974] using a kit (Catalase kit, no. 124087, Boehringer-Mannheim GmbH, West Germany). Apolipoprotein-B (I-Human and II-Human) and apolipoprotein A_I (II-Human) were measured in whole serum by rocket immunoelectrophoresis [Laurell, 1972] largely as described previously [Brussaard et al, 1980]. Rabbit antibody against apolipoprotein A_I was kindly donated by Dr. P. Demacker.

The response to the test diet was calculated per subject or animal 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 two-tailed t-tests [Snedecor and Cochran, 1967].

III. RESULTS

A. Human Experiments

The concentrations throughout experiments I-Human and II-Human of cholesterol in whole serum, HDL, and LDL and of apolipoproteins A_I (II-Human only) and B are given in Tables 7-III and 7-IV, and in Figure 7-II. In both studies, there was no clear difference in response with respect to serum total cholesterol between the casein and soy isolate groups. In both studies a decline in LDL cholesterol and an increase in HDL cholesterol was observed on the soy isolate diet when compared with the casein diet, but only in I-Human the difference with respect to LDL cholesterol reached statistical significance (Fig. 7-II; van Raaij et al [1981, 1982]).

In I-Human an increase in apolipoprotein-B and a decline in the LDL cholesterol/apolipoprotein-B ratio was observed on the isolate diet when compared with the casein diet, suggesting that the decline in LDL cholesterol on

the isolate diet had not been caused by a decline in the number of LDL particles, but in the composition of the LDL. These findings are confirmed by the changes in the density of LDL [van Raaij et al, 1981].

In II-Human a decline in apolipoprotein-B, an increase in apolipoprotein-A_I, and no clear differences in LDL cholesterol/apo-B and HDL cholesterol/apo-A_I ratios were observed on the soy isolate diet when compared with casein. These results suggest that the decline in LDL cholesterol and the increase in HDL cholesterol were caused by a decline in the number of LDL particles and an increase in the number of HDL particles, rather than by changes in the lipoprotein composition. The correlation coefficients between LDL cholesterol and serum apo-B at the end of the control and test period were r =

TABLE 7-III. The Effect of Casein and Soy Protein Diets on Serum Cholesterol Concentrations in Humans, Rabbits, and Rats

			Serum cholesterol concentration (mg/dl, mean ± SD[b])			
Experiment	Group[a]	n	Initial	End control period	End test period	Change over test period
I-Human	Casein	25	162 ± 32	152 ± 27	149 ± 24	− 3 ± 14
	Isolate	24	159 ± 26	153 ± 23	150 ± 23	− 3 ± 10
	Cassoy	20	160 ± 27	153 ± 24	150 ± 25	− 3 ± 13
I-Rabbit	Casein	6	[c]	30 ± 7	120 ± 65	+89 ± 74
	Isolate	6	[c]	32 ± 12	55 ± 18	+24 ± 20**
II-Human	Casein	17	211 ± 38	207 ± 36	205 ± 35	− 2 ± 10
	Isolate	20	219 ± 42	205 ± 40	197 ± 43	− 8 ± 12****
	Concentrate	20	215 ± 41	199 ± 35	200 ± 38	+ 1 ± 10
II-Rat	Casein	11	96 ± 7	62 ± 7	53 ± 5	− 9 ± 5
	Isolate	12	95 ± 9	63 ± 9	51 ± 5	−13 ± 6*
	Concentrate	12	95 ± 10	63 ± 7	50 ± 6	−13 ± 5**
	Commercial	7	95 ± 10	74 ± 12*****	60 ± 7*****	−14 ± 9
II-Rabbit	Casein	8	45 ± 12	160 ± 54		
	Isolate	7				
	Concentrate	8				
	Commercial	6	45 ± 13	60 ± 17*****	62 ± 26	+ 2 ± 22
II-Rabbit-SP	Casein	12	63 ± 26	121 ± 44	278 ± 119	+157 ± 118
	Isolate	12	54 ± 17	117 ± 48	92 ± 41****	−26 ± 46***
	Concentrate	12	65 ± 38	120 ± 51	95 ± 37****	−26 ± 57***

[a]Diets indicated under Group were given in the test period; for diets given in the control period, see Figure 7-1.

[b]100 mg/dl = 2.59 mmol/l.

[c]Not determined.

Statistical comparison with the casein group by Student's t-test: *$P < 0.075$, **$P < 0.05$, ***$P < 0.001$; with the concentrate group: ****$P < 0.05$; and with all test groups: *****$P < 0.05$.

TABLE 7-IV. The Effect of Casein and Soy Protein Diets on the Concentration of Apolipoproteins in Serum in the Studies With Humans*

	Casein group		Soy isolate group		Soy concentrate group	
	End control period	Change over test period	End control period	Change over test period	End control period	Change over test period
I-Human						
Apolipoprotein-B (mg/l)	488 ± 199	−47 ± 52	462 ± 107	−15 ± 51*		
LDL cholesterol/apo-B[a] (g/g)	1.63 ± 0.19	+0.18 ± 0.25	1.92 ± 0.17	−0.08 ± 0.18*		
II-Human						
Apolipoprotein-B (mg/l)	777 ± 202	+67 ± 77	812 ± 201	+12 ± 65* **	783 ± 212	+52 ± 61
LDL cholesterol/apo-B (g/g)	1.66 ± 0.25	−0.10 ± 0.15	1.58 ± 0.19	−0.07 ± 0.21	1.54 ± 0.21	−0.01 ± 0.12
Apolipoprotein-A_I (mg/l)	1,569 ± 242	−32 ± 100	1,552 ± 183	+43 ± 131* **	1,505 ± 276	−29 ± 99
HDL cholesterol/apo-A_I[b] (g/g)	0.35 ± 0.09	0.00 ± 0.02	0.36 ± 0.07	0.00 ± 0.03	0.39 ± 0.06	−0.01 ± 0.03
Apo-A_I/Apo-B (g/g)	2.15 ± 0.64	−0.20 ± 0.17	2.01 ± 0.46	+0.04 ± 0.25* **	2.07 ± 0.73	−0.19 ± 0.24

*Results expressed as mean ± SD.

[a]LDL isolated after ultracentrifugation.

[b]HDL isolated after Mn-heparin precipitation [Burstein and Samaille, 1960; van der Haar et al, 1978].

Statistical comparison with the casein group by Student's two-tailed t-test: *$P < 0.05$; and with the concentrate group: **$P < 0.05$.

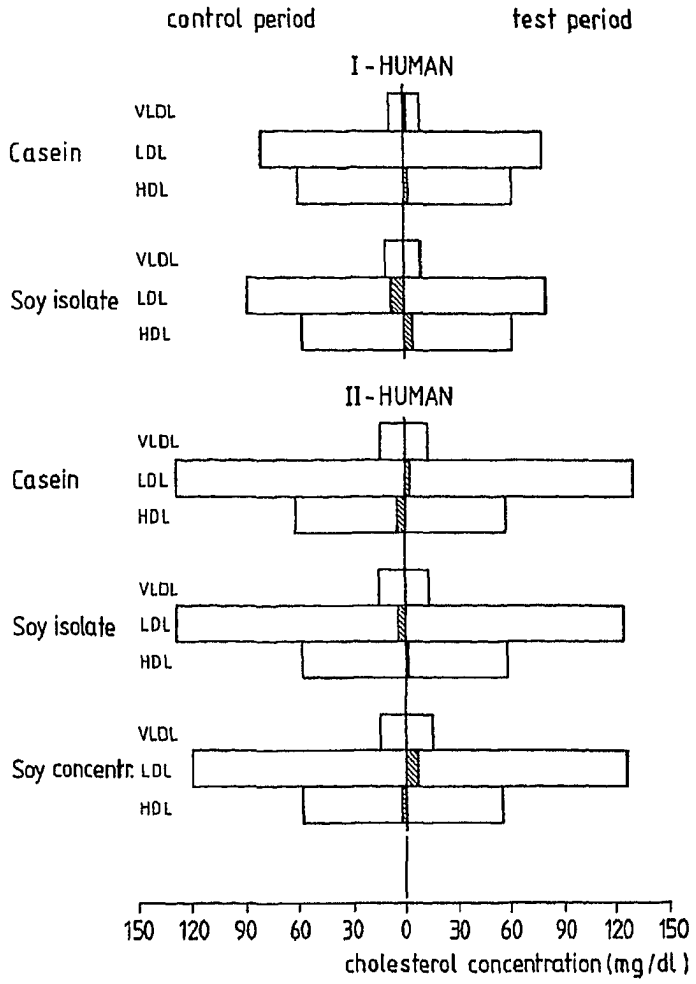

Fig. 7-II. The effect of casein and soy protein diets on the concentration of cholesterol in lipoproteins in humans (experiments I-Human and II-Human). The shaded areas right of the vertical line represent an increase over the test period, and those left of the vertical line a decrease; changes less than 1 mg/dl are not shown.

0.86 (n = 56) and r = 0.90 (n = 56), respectively. The correlation coefficients between HDL cholesterol and serum apo-A_I were r = 0.60 (n = 55) and r = 0.67 (n = 55), respectively.

Compared with soy protein concentrate, soy protein isolate produced a decrease in serum total cholesterol, LDL cholesterol, apolipoprotein-B, and LDL cholesterol/apo-B ratio, an increase in HDL cholesterol, and apolipoprotein-A_I, but had no effect on the HDL cholesterol/apo-A_I ratio. These results suggest that the increase in HDL cholesterol has been caused by an increase in the number of HDL particles, and that the decline in LDL cholesterol has been caused by a decline in both the number of LDL particles and in their cholesterol content. No differences in lipoproteins were found between the casein and the soy protein concentrate group.

The overall changes in lipoproteins can be summarized by the HDL cholesterol/total cholesterol ratio and by the apo-A_I/apo-B ratio. In experiment II-

TABLE 7-V. Feed Intake and Weight Gain in Rabbits and Rats

Experiment	Groups	Body weight[a] (g)	Feed intake[b] Control period (g/day)	Feed intake[b] Test period (g/day)	Weight gain Control period (g/day)	Weight gain Test period (g/day)
I-Rabbit[c]	Test (all)[e]	2,322	[f]	75[g]	[f]	12.5
II-Rabbit[d]	Test (all)[e]	1,481	57	57[h,i]	18.2	28.7[i]
	Commercial	1,503	85	85[i]	22.4	25.8[i]
II-Rabbit-SP[d]	Test (all)[e]	1,834	67	67[j]	[f]	17.1
II-Rat[c]	Test (all)[e]	70	9.3	10.6[k]	2.6	1.8
	Commercial	68	11.8	14.0	2.1	1.7

[a]At start control period (II-Rabbit and II-Rat) or at start test period (I-Rabbit and II-Rabbit-SP).

[b]Dry-matter contents of the diets were 94.5% for the pelleted human diets (I-Rabbit and II-Rabbit); 99.5% for the unpelleted human diets (II-Rat); 91.5% for the semipurified diets (II-Rabbit-SP); and 89.0% for the commercial rabbit and rat diets.

[c]Diets were provided ad libitum.

[d]Diets were provided restricted.

[e]Mean data of the test groups are given except for the food intake during the test period in which data for the casein group are given; there were no differences between group means for body weight and weight gain.

[f]Not measured.

[g]Food intake was 83 g per day for the isolate group.

[h]Food intake was 58 and 61 g per day for the isolate and concentrate groups.

[i]During first two weeks of test period.

[j]Food intake was 67.5 and 69.5 g per day for the isolate and concentrate groups.

[k]Food intake was 11.0 and 11.3 g per day for the isolate and concentrate groups.

Human, significant increases in both ratios were observed on the soy isolate diet when compared with the casein or concentrate diet (Table 7-IV; van Raaij et al [1982]).

B. Feed Consumption and Growth of the Experimental Animals

The feed consumption and growth of the rabbits and rats throughout the experiments are presented in Table 7-V. In all of the experiments, except in II-Rabbit, feed consumption and growth were satisfactory. In II-Rabbit, the animals consumed all their feed under the restricted feeding regime throughout the control period and for the first two weeks of the test period. However, after that, twelve rabbits failed to eat all the feed provided, their growth faltered, and their health deteriorated. Four of the rabbits lost hair. It may well be that the human diets, either before or after freeze-drying, contained insufficient vitamins and minerals for the growth of rabbits. When the experiment was completed, the rabbits were transferred to the commercial rabbit diet, and the growth and health of the less severely affected animals improved markedly. Because of the problems encountered, data obtained during the test period of experiment II-Rabbit were deleted in the subsequent analysis of the results. In all other studies, the experimental diets were well accepted, and the feed offered was consumed.

C. Serum Cholesterol and Lipoproteins and Liver Cholesterol in the Experimental Animals

The concentrations of total cholesterol in serum in the experiments with rabbits and rats are shown in Table 7-III. When rabbits were fed semipurified diets (experiment II-Rabbit-SP), the diet containing casein produced a marked increase in serum cholesterol concentration, most of the increased cholesterol being found in the LDL fraction (Table 7-VI). The declines in concentration of cholesterol after the casein control period seen on the two diets containing soy isolate and soy concentrate, respectively, were identical; these declines were observed in the LDL fraction. The higher concentration of cholesterol in the serum on the casein diet compared with the two soy diets is reflected in the concentration of cholesterol in the liver (4.5 ± 1.3 mg cholesterol/g liver, mean ± SD), although the difference was only significant ($P < 0.05$) when compared with the soy concentrate group (3.3 ± 1.0 mg/g) and not with the soy isolate group (3.6 ± 1.6 mg/g). Most of the additional cholesterol which is found in the liver at increasing serum cholesterol concentrations is in the form of cholesteryl ester. This is seen from the relationships between serum cholesterol concentration and liver cholesteryl ester concentration (Fig. 7-III) and between liver cholesterol concentration and the proportion of cholesterol in the liver that is esterified (Fig. 7-IV).

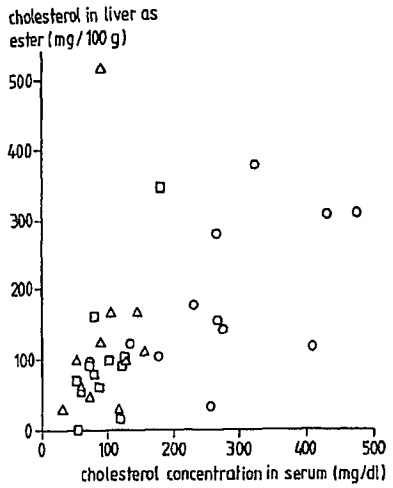

Fig. 7-III. The relationship between the cholesterol concentration in serum (mg/dl) (x) and the amount of cholesterol as ester in the liver (mg/100 g) (y) in rabbits (experiment II-Rabbit-SP); $y = 0.47x + 63.6$; $r = 0.481$, $P < 0.01$; ○, casein-fed rabbits; △, soy isolate-fed rabbits; □, soy concentrate-fed rabbits.

TABLE 7-VI. The Effect of Casein and Soy Protein Diets on the Concentration of Cholesterol in Lipoproteins in Rabbits on Semipurified Diets (Experiment II-Rabbit-SP)*

	Control period	Test period		
Lipoprotein fraction	Casein diet[a] (n = 36)	Casein diet[b] (n = 12)	Soy isolate diet[b] (n = 12)	Soy concentrate diet[b] (n = 12)
VLDL	17	33	17	18
LDL	74	223	48	55
HDL	25	41	31	31
Whole serum[c]	119	278	92	95

*All values expressed in mg/100 ml.
[a]Mean values of six pools, each of serum from 6 animals.
[b]Mean values of four pools, each of serum from 3 animals.
[c]Mean values from serum of individual animals.

In the first experiment with rabbits using the human diets (experiment I-Rabbit), the rabbits were transferred straight from the commercial diet to the test diets containing either casein or soy protein isolate. After 2½ weeks of test period, the casein diet produced significantly higher increases in cholesterol than did the soy isolate diet (Table 7-III). When the rabbits were subsequently returned to the commercial diet, the concentration of cholesterol in the serum of both groups of rabbits fell to a mean value of about 30 mg/dl [van Raaij et al, 1981]. Thus the human diet containing casein has a pronounced hypercholesterolemic effect in rabbits when compared with the soy protein diet.

In the second experiment with rabbits using the human diets (experiment II-Rabbit), the rabbits were transferred from the commercial diet to the human diets for a control period of 2½ weeks before being transferred to the test diets for a subsequent four weeks. On the casein control diet, the concentrations of cholesterol in serum increased steeply and significantly when compared with the cholesterol levels of the rabbits that continued on the commercial diet. After 4–5 weeks on the human diets, the rabbits began to show signs of ill health such as reduced appetite, reduced growth and hair loss. As discussed earlier, this may have been due to a mineral or vitamin deficiency, which did

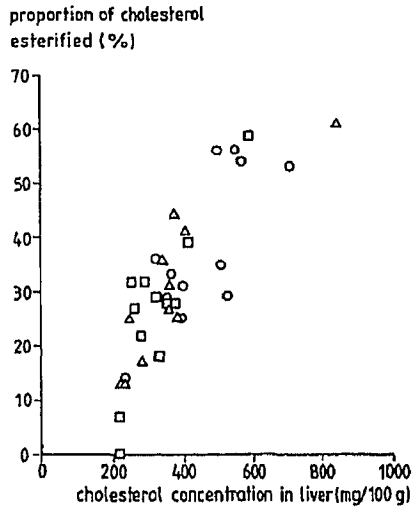

Fig. 7-IV. The relationship between the cholesterol concentration in the liver (mg/100 g) (x) and the proportion of cholesterol esterified (%) (y) in rabbits (experiment II-Rabbit-SP): $y = 0.087x - 1.46$; $r = 0.827$, $P < 0.001$; ○, casein-fed rabbits; △, soy isolate-fed rabbits; □, soy concentrate-fed rabbits.

not become manifest within the 2½-week period that the rabbits in experiment I-Rabbit were on the human diets.

In the experiment with rats using the human diets (II-Rat), the rats were transferred from the commercial diet to the human casein diet for a control period of 2½ weeks before being transferred to the human test diets. Concentration of cholesterol in serum was declining in the rats used, as shown by the rats that continued on the commercial diet for the whole experiment. On the casein control diets significant lower concentrations of cholesterol in serum were observed than on the commercial diet. During the test period the declines in serum cholesterol on the human test diets were similar to those on the commercial diet; however, the declines on both soy protein diets were more pronounced than that on the casein diet (Table 7-III). The larger declines on the soy protein diets occurred mainly in the lipoprotein fractions with densities between 1.060 and 1.128 g/ml (Table 7-VII).

IV. DISCUSSION

In our studies with young and middle-aged adults 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. When the soy preparations were incorporated into semipurified diets and fed to rabbits, we obtained results similar to those reported previously (for a review, see Terpstra et al [1982b]): Casein was found to be strongly hypercholesterolemic when compared with the soy preparations. Similar results were observed when rabbits were fed the human diets: A human diet containing soy isolate resulted in significantly lower cholesterol concentrations than the human diet containing casein (experiment I-Rabbit). In experiment II-Rabbit, the rabbits also showed a marked increase in serum cholesterol concentration after they had been transferred from the commercial diet to the casein-containing human diet (Table 7-III). As discussed before, it is unfortunate that in experiment II-Rabbit no comparisons could be made between the casein and the soy protein diets. In rats, the differences between the human diets containing either casein or soy protein were small, but marginally significant.

How can one explain these different results between humans and animals?

A. Species Effects

Animal species differ greatly in their resistance to diet-induced hypercholesterolemia. For example, rabbits are highly susceptible to hypercholesterolemia by dietary means; the replacement of protein in semipurified diets by casein and the addition of small amounts of cholesterol have both been shown to be very effective. Although most studies with rabbits have been carried out with semipurified diets, our studies clearly indicate that human diets give similar re-

TABLE 7-VII. The Effect of Casein and Soy Protein Diets on the Concentration of Cholesterol in Lipoproteins in Rats (Experiment II-Rat)*

Lipoprotein fraction (density [d] in g/ml)	Casein group			Soy isolate group			Soy concentrate group		
	Control period	Test period	Change	Control period	Test period	Change	Control period	Test period	Change
$d < 1.016$	2.9	2.5	− 0.4	2.5	2.1	− 0.4	2.9	2.1	− 0.8
$1.016 < d < 1.032$	1.5	1.5	0.0	1.4	1.4	0.0	1.4	1.2	− 0.2
$1.032 < d < 1.045$	1.7	2.1	+ 0.4	1.9	2.1	+ 0.2	1.9	1.9	0.0
$1.045 < d < 1.060$	3.5	3.5	0.0	4.6	4.1	− 0.6	3.5	3.9	+ 0.4
$1.060 < d < 1.078$	8.1	7.7	− 0.4	10.6	9.7	− 1.0	8.3	6.6	− 1.7
$1.078 < d < 1.102$	22.4	18.6	− 3.9	23.4	15.3	− 8.1	24.6	20.5	− 4.1
$1.102 < d < 1.128$	13.2	12.0	− 1.2	14.1	10.1	− 4.1	13.5	11.4	− 2.1
$1.128 < d < 1.146$	2.5	2.9	+ 0.4	2.7	3.3	+ 0.6	2.9	3.3	+ 0.4
$1.146 < d$	2.1	3.5	+ 1.4	1.9	3.7	+ 1.7	2.1	2.9	+ 0.8
Whole serum (pools)	66.0	52.6	−13.4	69.1	51.5	−17.6	65.8	51.7	−14.1

*Results expressed in mg/100 ml of serum. At the end of the control and test period, two serum samples were taken at one-day intervals. Serum samples were pooled per group. Each given value represents the average value of the two pools at the end of the respective period.

sults. In our studies the effects with the human diets were less pronounced than with the semipurified diets, but this can be explained, at least partly, by the fact that in the human diets only 65% of the protein consisted of test protein compared with 100% in the semipurified diets, while the total proportion of protein in the two types of diets was about the same. The hypercholesterolemic effect of casein increases when the amount of casein in the diet is increased [Terpstra et al, 1981a]. The amount of linoleic acid in the diet may also be a contributing factor as the casein-induced hypercholesterolemia in rabbits disappears when increased amounts of linoleic acid are included in the diets [Lambert et al, 1958; Wigand, 1959; Carroll and Hamilton, 1975; Beynen and West, 1981]. In our human diets, the proportion of polyunsaturated fat in the diet was about 4% by weight, while in the semipurified diets the proportion was less than 1%. The differences in results between the human and semipurified diets can probably not be explained by difference in duration, because after 2½ weeks on the semipurified diets the difference in effects between the casein and soy protein diets was already greater than on the human diets (data not shown).

In rats fed semipurified diets the effects of dietary proteins are only observed when considerable amounts of cholesterol (approximately 1%) are added to the diets [Terpstra et al, 1982a]. In our experiments only 0.07% of cholesterol was present, and this explains why the observed effects in rats were so small. Our studies with humans suggest that normocholesterolemic adults are also relatively insensitive to changes in dietary protein, and one might speculate that in humans, diets have to contain considerable amounts of cholesterol before the differential effect of casein and soy protein on the level of serum cholesterol is expressed. It should be noted, however, that in the experiments of Sirtori et al [1979] the cholesterol-lowering effect of the soy diet in hypercholesterolemic patients was most pronounced against a high-linoleic acid low-cholesterol background, ie, the opposite of the conditions under which the effects of dietary proteins are seen most clearly in animals.

B. Age of the Animals

Animal studies in general and our studies in particular have been carried out with young, growing animals, while in most human studies, including those reported here, adults were used. It has been reported that dietary casein-induced hypercholesterolemia in male rabbits occur only in young growing animals [West et al, 1982], and similar results have been observed in rats [McGregor et al, 1971]. Such animals spend a considerable proportion of their lives on the experimental diets. Therefore, our results on humans do not rule out the possibility that a long-term intake of animal protein starting at an early age will cause similar cholesterol-raising effects in normocholesterolemic humans.

C. Differences in Lipoprotein Metabolism

A point to keep in mind in extrapolating animal data to man concerns the differences in the distribution of cholesterol over the various lipoprotein fractions. In healthy humans, most of the serum cholesterol is transported in the LDL fraction, whereas in normocholesterolemic rabbits and rats the HDL fraction is the main carrier of cholesterol [Terpstra et al, 1982c]. Furthermore, hypercholesterolemic diets in animals often cause the appearance of an unusual lipoprotein with the density of VLDL but with a much higher cholesterol content and with a higher proportion of apolipoprotein-E [Ross and Zilversmit, 1977; Mahley et al, 1980; Terpstra et al, 1982a; Scholz et al, 1982]. In humans such a particle is seen only in type III hypercholesterolemia, a fairly rare disease. In healthy humans we did not find any marked abnormalities of the lipoprotein spectrum after six weeks on the casein diet. In comparison, however, the subjects who had received the soy isolate diet showed slightly lower LDL and higher HDL cholesterol levels. Apart from slight changes in the composition of LDL in I-Human, our apoprotein measurements did not point to marked effects of dietary proteins on lipoprotein composition in man. Thus at the level of the separate lipoproteins, casein-fed rabbits and rats appear to be different from humans.

D. Possible Mechanisms

The mechanisms underlying the cholesterolemic properties of dietary protein are not clearly understood. Probably several mechanisms are involved. The amino acid composition of the proteins plays a role [Carroll, 1978; Kritchevsky, 1979; Hermus, 1975; Terpstra et al, 1982c], but how amino acids might influence cholesterol metabolism remains to be established. Differences in amino acid composition between casein and soy protein, however, cannot fully explain the different results [Carroll, 1978; Terpstra et al, 1982c]. It has been suggested that some substances in the nonprotein part of the soy preparations such as fiber or saponins might be partly responsible for the observed effects [Potter et al, 1980; Topping et al, 1980], but our human and animal studies clearly indicate that the nonprotein part of soy concentrate did not influence serum cholesterol when compared with soy isolate. Similar results were found by Hamilton and Carroll [1976] in rabbits. As discussed previously [van Raaij et al, 1982] the type of dietary fiber present in soy concentrate is not likely to exert a marked hypocholesterolemic effect. As casein increases the concentration of cholesterol in both the liver and in serum (experiment II-Rabbit-SP), a redistribution of cholesterol between the serum and liver would not appear to be involved as is the case with the difference between rabbits which are hypo- or hyperresponsive to cholesterol [West and Roberts, 1974]. Perhaps the differential effect of casein and soy protein on serum cholesterol

levels in rabbits may be attributible to differences in the digestibility of the two proteins. As has been postulated elsewhere [Terpstra et al, 1982c] soy protein may be less digestible than casein in rabbits and the undigested protein could bind bile acids, thus facilitating their excretion and preventing their reabsorption. It may well be that in humans, soy protein is not significantly less digestible than casein or that the binding of soy protein to bile acids does not have a significant effect on the excretion of bile acids either because of the presence of other materials in the gut or because of the nature of the bile acids involved.

Although we did not find a marked change in serum total cholesterol concentrations in humans on the casein and soy diets, 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 diet, but there was no effect with the soy concentrate diet. Apart from small changes in the composition of LDL in I-Human, the apoprotein results suggest that the changes in cholesterol concentrations in the lipoprotein fractions resulted from changes in the number of lipoprotein particles.

An increase in the ratio of HDL cholesterol/total cholesterol has been associated with a lower risk of coronary heart disease [Blackburn et al, 1977; Miller and Miller, 1975], so it may well be that the increase in this ratio on the soy isolate diet could have a 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 and not with the soy concentrate. Thus the dramatic effects observed by Sirtori et al [1979] with textured soy protein not only on HDL cholesterol/total cholesterol ratio but also on the concentration of total cholesterol eluded us when we used the soy concentrate diet.

An expectation has developed that the replacement of animal proteins by vegetable proteins in human diets might aid in lowering serum total cholesterol levels, thereby providing a very useful tool in the prevention of coronary heart disease [Lewis, 1980]. This expectation is based on the many studies with animals, particularly the rabbit, and a limited number of studies with humans, particularly hypercholesterolemic patients [Sirtori et al, 1979; Descovich et al, 1980; Wolfe et al, 1981] in which diets containing animal proteins, particularly casein, have been compared with diets containing vegetable proteins, particularly soy protein. However, the results of most studies with normocholesterolemic humans, including our two studies, do not provide support for this expectation: The normocholesterolemic human appears to be relatively insensitive to changes in the type of protein in the diet. However, a small favorable effect on the distribution of cholesterol over the various lipoprotein classes cannot be excluded.

V. SUMMARY

This paper reports the results of studies in which the effect of casein and soy protein on serum cholesterol and lipoprotein concentrations were compared in humans, rabbits, and rats using human diets. In the human studies, no marked effect of diets containing either soy isolate or soy concentrate on serum total cholesterol was observed when compared with a diet containing casein. When rabbits and rats were fed the human diets, lower serum cholesterol levels were found on both the soy diets when compared with the casein diet, the differences being much more pronounced in rabbits than in rats. These results confirm differences in susceptibility between species, and that the normocholesterolemic human appears to be relatively insensitive to changes in these dietary proteins. Although in humans no effects on serum total cholesterol level were observed, the soy isolate diet did cause a small decline in cholesterol concentration in the low-density lipoprotein fraction (LDL) and a small increase in cholesterol concentration in the high-density lipoprotein fraction (HDL) when compared with the casein diet. However, there was no effect with the soy concentrate diet. Analysis of apolipoprotein concentrations suggested that the changes in cholesterol concentrations in the lipoprotein fractions resulted mainly from changes in the number of lipoprotein particles, but minor effects on the composition of the LDL could not be excluded. The lack of a cholesterol-lowering effect of the less-refined soy concentrate when compared with soy protein isolate in both the human and animal studies suggest that the nonprotein part of the soy preparations probably does not have a specific cholesterol-lowering effect. Our studies stress the risk of extrapolating animal data concerning the effect of protein on cholesterol metabolism to man.

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