

Further studies on the hypocholesterolaemic effect of soya-bean protein in rats*

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1. The hypocholesterolaemic effect of soya-bean protein compared with that of casein, when fed at 200 g/kg diet for 3 weeks, was studied in rats.
2. Using a cholesterol-free low-fat (10 g maize oil/kg) diet, the hypocholesterolaemic effect was demonstrated not only with soya-bean protein but also with an amino acid mixture simulating this protein. The addition of lysine to these diets did not modify their intrinsic effects on serum cholesterol.
3. By feeding the vegetable protein or its amino acid mixture, both high-density-lipoprotein and very-low-density-lipoprotein plus low-density-lipoprotein-cholesterol decreased to a similar extent, and the concentration of serum apolipoprotein A-I decreased and that of apolipoprotein B increased.
4. Although rats given soya-bean protein excreted more neutral and acidic steroids, the difference disappeared when the amino acid mixture was fed, indicating that the hypocholesterolaemic effect of the vegetable protein is not ascribed only to the increased elimination of steroids.
5. It is likely that the difference in the amino acid composition between animal and vegetable proteins is in fact responsible for the different response of serum cholesterol.

Available knowledge regarding the effects of dietary proteins on plasma cholesterol levels in humans and several experimental animals suggests that plant proteins, in particular soya-bean protein, generally exert a hypocholesterolaemic effect as compared with animal protein such as casein (Carroll & Hamilton, 1975; Hamilton & Carroll, 1976; Huff & Carroll, 1977; Huff *et al.* 1977; Yadav & Liener, 1977; Carroll *et al.* 1977, 1978; Sirtori *et al.* 1977, 1979; Belton & Truswell, 1978; Fumagalli *et al.* 1978; Kim *et al.* 1978; Hevia & Visek, 1979; Hevia *et al.* 1979; Nosedá *et al.* 1979; Nagata *et al.* 1980), though Neves *et al.* (1980) failed to demonstrate such an effect of plant proteins.

It has been pointed out that the effect of soya-bean protein on plasma cholesterol is easily modified by the type of diet (Hamilton & Carroll, 1976; Hevia & Visek, 1979; Kritchevsky *et al.* 1977). In our previous experiments with rats, the hypocholesterolaemic effect of soya-bean protein isolate compared with that of casein could be demonstrated only when the animals were given a cholesterol-free low-fat diet (Nagata *et al.* 1980). Neves *et al.* (1980) have reported recently that pure and crude plant proteins, when compared with the corresponding animal proteins, do not have a hypocholesterolaemic potential in rats. The diets of Neves *et al.* (1980) contained 100 g soya-bean oil/kg, and this level is suspected to be too high to demonstrate the cholesterol-lowering effect of plant proteins. Even at the level of 50 g maize oil/kg, the hypocholesterolaemic effect of the soya-bean protein isolate was greatly reduced (Nagata *et al.* 1980). Previous studies have shown that in rats plant proteins were hypocholesterolaemic only when they were already made hypercholesterolaemic with possibly essential-fatty-acid-deficient diets (Yadav & Liener, 1977).

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Only limited evidence is available concerning the mechanism of the hypocholesterolaemic effect of soya-bean protein. Since there is still controversy as to whether the plant protein itself or the non-protein contaminants (Oakenfull *et al.* 1979; Potter *et al.* 1979; Sautier *et al.* 1979) present in the preparation are responsible for the cholesterol-lowering potential, the effect of an amino acid mixture simulating soya-bean protein isolate or casein on serum cholesterol levels of rats was compared in the present paper. Simultaneously, the effect on serum high-density lipoprotein-cholesterol and several apolipoproteins as well as on the excretion of steroids in faeces were examined. In some trials, the study was also designed to investigate the effect of supplementary amino acids on the cholesterol metabolism.

METHODS

Animals and diets

Male Wistar rats (Kyudo Co., Kumamoto) were used throughout. The animals were given a commercial diet (Type NMF; Oriental Yeast Co., Tokyo) for at least 1 week before the start of the experiments. When they weighed 150–160 g they were transferred to individual stainless-steel cages and maintained at 20–21° with alternating 12 h periods of light (08.00–20.00 hours) and dark and they were given the experimental diets *ad lib.* for 3 weeks. Food consumption and body-weight were recorded three times each week. At the end of each experiment, rats were fasted (18.00–09.00 hours), killed by decapitation and blood collected. The composition of the basal diet was (g/kg): nitrogen source 200, maize oil 10, mineral mixture 40, vitamin mixture (water-soluble) 10, choline chloride 1.5, cellulose powder 20, and sucrose to 1 kg. The N sources were casein (vitamin-free containing 145 g N/kg dry matter; ICN Pharmaceuticals Inc., Cleveland Ohio), soya-bean protein isolate (Fujipro R containing 144 g N/kg dry matter; Fuji Oil Co., Osaka) (Nagata *et al.* 1980) and amino acid mixtures (Ajinomoto Co., Tokyo and Kyowa Hakko Kogyo Co., Tokyo) simulating casein and soya-bean protein isolate. The compositions of the amino acid mixtures are shown in Table 1, which were based on analyses by the manufacturer, Yadav & Liener (1977) and that of the present study. The vitamin mixture (Oriental Yeast Co.) was prepared according to Harper (1959). Mineral mixtures according to Harper (1959) or Ebihara *et al.* (1979) were used for protein diets or amino-acid-mixture diets respectively; the latter mixture contained an extra amount of zinc compared with the former. Fat-soluble vitamins which were dissolved in maize oil contained (/kg): retinyl palmitate 4000 µg, cholecalciferol 50 µg, DL- α -tocopheryl acetate 100 mg. Supplementation of lysine or arginine was achieved at the expense of sucrose. In order to make the arginine:lysine value in these N sources identical, 8.6 g arginine or 17.2 g lysine/kg were added to diets containing animal or vegetable proteins respectively.

Lipid analysis

Blood serum was obtained by centrifugation. Serum and liver lipids were extracted and analyzed for cholesterol, triglyceride and phospholipid as described previously (Nagata *et al.* 1980). Serum high-density-lipoprotein (HDL)-cholesterol was measured in the supernatant fraction obtained after dextran sulphate (Daiichi Seiyaku Co., Tokyo) precipitation of very-low-density (VLDL) and low-density-lipoproteins (LDL) (Burnstein *et al.* 1970). The concentrations of apolipoproteins A-I, B and E were determined by rocket immunoelectrophoresis (Imaizumi *et al.* 1978). Hydroperoxide was measured by fluorometry according to Yagi (1976). Faeces were collected for 2 d at the end of each experiment and analysed for neutral (Miettinen *et al.* 1965) and acidic steroids (Grundy *et al.* 1965) by gas-liquid chromatography.

Statistical analysis

Data were analysed by the Student's *t* test (Fisher, 1970).

Table 1. *Composition of amino acid mixtures simulating soya-bean protein or casein*

L-Amino acids	Composition (g/kg diet)*	
	Casein	Soya-bean protein
Ala	4.78	8.44
Arg	6.88	15.18
Asp	13.82	22.80
Cys	2.36	2.96
Glu	43.20	38.40
Gly	3.62	8.36
His	5.98	4.96
Ileu	8.52	7.36
Leu	17.32	14.30
Lys	14.38	14.16
Met	4.68	2.46
Phe	10.08	10.16
Pro	20.20	12.90
Ser	11.56	11.24
Thr	8.50	8.02
Trp	1.66	2.84
Tyr	10.84	7.38
Val	11.62	8.04

* The values are based on values provided by Yadav & Liener (1977), the manufacturer and the present study.

RESULTS

Serum and liver lipid concentration

Table 2 summarizes weight gain, food intake, liver weight and the concentration of serum and liver triglyceride and phospholipid. In each experiment, there were no differences in food intake and weight gain in relation to the type of dietary proteins or amino acid mixtures. On feeding a diet containing protein as the N source, liver weight was slightly but significantly higher in rats given casein than in the animals given soya-bean protein. This was not the situation when the animals were given amino-acid-mixture diets. Differences in the nature of the dietary N sources, either proteins or amino acid mixtures, did not cause uniform changes in the concentrations of serum and liver triglyceride and phospholipid, although in some instances there were small differences between the two groups. The addition of specific amino acids to the diet displayed no additional effects on these lipid components, except for the increase in serum triglyceride of rats given diets containing soya-bean protein or its amino-acid-mixture supplemented with lysine.

Cholesterol levels in serum and liver are shown in Table 3. Both soya-bean protein and its amino acid mixture exhibited a hypocholesterolaemic effect by comparison with the corresponding casein diets. The cholesterol-lowering effect of the vegetable protein was mainly attributable to the decrease in HDL-cholesterol, the net decrease in VLDL plus LDL-cholesterol being considerably lower. However, the proportion of the HDL-cholesterol relative to total cholesterol in rats given diets containing soya-bean protein or its amino-acid-mixture was similar to or even higher than that in rats given the corresponding casein diets. The addition of lysine did not modify greatly the hypocholesterolaemic effect of soya-bean protein or its amino-acid-mixture. Also, no changes in serum cholesterol were observed even after the addition of arginine to casein or its amino-acid-mixture. Although the liver cholesterol level was higher in the casein than in soya-bean protein groups, replacing the amino acid mixture for the protein obscured the difference.

Table 2. *Body-weight gain, liver weight and serum and liver lipids of rats given soya-bean protein or casein*
(Mean values with their standard errors for six rats/dietary group)

Expt no.	Dietary regimens	Body-wt gain (g)		Food intake (g/d)		Liver wt (g/kg body-wt)		Serum lipids (mg/l)				Liver lipids (mg/g)			
		Mean	SE	Mean	SE	Mean	SE	Triglyceride	Phospholipid	Triglyceride	Phospholipid	Triglyceride	Phospholipid	Triglyceride	Phospholipid
								Mean	SE	Mean	SE	Mean	SE	Mean	SE
(1)	Soya-bean protein	114	6	20.2	0.5	31.5	0.5 ^a	1090	95	1140	60	19.6	0.6	39.8	2.6
	Casein	114	5	19.0	0.2	34.2	1.1 ^b	1250	145	1240	66	23.2	2.3	40.5	2.4
	Soya AA mix	102	4	19.8	0.5	31.8	1.0 ^{ab}	1460	228	1130	52	23.2	2.0	41.5	3.8
(2)	Casein AA mix	108	10	19.8	0.9	29.2	0.3 ^a	916	73	1270	48	24.6	3.3	38.4	1.7
	Soya-bean protein	112	6	20.0	0.6	28.7	0.5 ^a	815	50 ^a	1200	83 ^{ac}	22.4	1.0	39.7	0.4 ^a
	Soya-bean protein + Lys	101	7	18.3	0.9	28.8	0.3 ^a	1230	52 ^b	1270	71 ^{ab}	20.5	1.9	42.2	0.4 ^b
(3)	Casein	108	6	18.8	0.7	30.8	0.6 ^b	1350	102 ^b	1350	83 ^{ab}	19.2	1.6	40.2	0.6 ^a
	Casein + Arg	106	5	18.3	0.6	31.5	0.9 ^b	1320	128 ^b	1450	56 ^b	18.2	1.4	39.3	0.4 ^a
	Soya AA mix	88	3	19.8	0.5	30.4	0.8	1280	102 ^a	987	83 ^a	20.8	1.4	35.2	1.2
	Soya AA mix + Lys	94	7	20.1	1.1	31.0	1.0	1810	146 ^b	997	38 ^a	19.6	0.7	36.1	1.1
	Casein AA mix	90	2	19.8	0.2	29.5	0.8	1610	158 ^{ab}	1330	109 ^a	20.8	2.5	35.7	1.2
	Casein AA + Arg	87	3	19.4	0.4	30.2	0.8	1800	188 ^b	1430	68 ^b	20.4	1.5	37.8	0.9

^{a, b, c}. In each experiment, values in the same vertical column not sharing common superscript letters were significantly different; $P < 0.05-0.01$.
AA mix, amino acid mixture simulating soya-bean protein or casein; Lys, lysine; Arg, arginine.

Table 3. Concentration of serum and liver cholesterol in rats given soya-bean protein or casein

(Mean values with their standard errors for six rats/dietary group)

Expt no. Dietary regimes	Serum cholesterol (mg/l)						Liver cholesterol (mg/g)	
	Total cholesterol		HDL-cholesterol		LDL-cholesterol		Mean	SE
	Mean	SE	Mean	SE	Mean	SE		
(1) Soya-bean protein	693	50 ^a	508	25 ^a	185	41	3.2	0.1 ^a
Casein	872	54 ^{bc}	596	26 ^{bc}	277	46	3.7	0.2 ^b
Soya AA mix	726	59 ^{ab}	522	31 ^{ab}	205	32	3.5	0.1 ^{ab}
Casein AA mix	873	29 ^c	645	19 ^c	228	35	3.2	0.1 ^a
(2) Soya-bean protein	536	45 ^a	381	30 ^a	155	20 ^a	2.9	0.1 ^a
Soya-bean protein + Lys	626	49 ^{ab}	357	25 ^a	269	25 ^b	2.9	0.1 ^a
Casein	757	74 ^{bc}	471	50 ^{ab}	286	26 ^b	3.3	0.1 ^b
Casein + Arg	813	40 ^c	487	33 ^b	326	28 ^b	3.3	0.1 ^b
(3) Soya AA mix	666	57 ^a	427	32 ^a	239	29 ^a	3.9	0.1
Soya AA mix + Lys	674	33 ^a	406	23 ^a	269	17 ^{ab}	3.5	0.1
Casein AA mix	938	78 ^b	557	25 ^b	372	67 ^{ab}	3.6	0.1
Casein AA mix + Arg	980	87 ^b	579	29 ^b	401	62 ^b	3.4	0.2

^{a, b, c.} In each experiment, values in the same vertical column not sharing common superscript letters were significantly different; $P < 0.05-0.01$.

AA mix, amino acid mixture simulating soya-bean protein or casein; Lys, lysine; Arg, arginine; HDL, high-density-lipoprotein; LDL, low-density-lipoprotein (including very-low-density-lipoprotein).

Serum apolipoprotein concentration

Table 4 summarizes the results obtained for the concentration of apolipoproteins A-I, B and E. The concentration of apo A-I was significantly lower in rats given soya-bean protein than in those given casein. The same pattern of response was observed with the amino acid mixture simulating these proteins. Soya-bean protein and its amino acid mixture produced an increase in apo B content by comparison with the response to the corresponding casein diets. The concentration of apo E was affected only when the animals were fed on the amino acid mixture and it tended to increase in the casein-amino acid group. The addition of lysine or arginine did not influence these responses.

The effect of dietary proteins on the concentration of serum hydroperoxide was less well defined, although feeding the casein-amino-acid diet supplemented with arginine caused an increase in the hydroperoxide level as compared with the soya-bean protein-amino-acid-mixture diet supplemented with lysine.

Faecal excretion of steroids

Table 5 shows the excretion of neutral and acidic steroids. Rats given soya-bean protein excreted larger amounts of faeces and neutral and acidic steroids than those given casein. Interestingly, these differences disappeared when the animals were given the amino acid mixture as an N source; there was a marked decrease in faecal weight with the soya-bean protein-amino-acid-mixture diet, but not with the casein-amino-acid diet when compared with the corresponding protein diets. Due to this decrease, faecal output of steroids was apparently similar for the two amino acid mixture groups. Neither lysine nor arginine supplementation modified the pattern of responses observed with proteins or amino acid mixtures.

Table 4. Concentration of serum apolipoproteins ($\mu\text{g/ml}$) and hydroperoxide (nmol/ml) in rats given soya-bean protein or casein

(Mean values with their standard errors for six rats/dietary group)

Expt no.	Dietary regimens	Apo A-I		Apo B		Apo E		Hydroperoxide	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
(1)	Soya-bean protein	588	56 ^a	340	12 ^a	311	22	2.1	0.1 ^a
	Casein	838	42 ^b	205	16 ^b	265	20	1.7	0.2 ^{ab}
	Soya AA mix	647	25 ^a	305	7 ^a	285	28	1.7	0.2 ^{ab}
	Casein AA mix	1015	69 ^b	238	22 ^b	314	14	1.6	0.2 ^b
(2)	Soya-bean protein	602	29 ^a	239	19 ^a	245	27	2.6	0.8
	Soya-bean protein + Lys	612	19 ^a	212	35 ^a	258	14	2.0	0.9
	Casein	1037	122 ^b	101	10 ^b	239	19	2.2	0.6
	Casein + Arg	977	37 ^b	106	23 ^b	266	10	2.9	1.2
(3)	Soya AA mix	695	35 ^a	139	13	321	30 ^a	1.8	0.1 ^{ab}
	Soya AA mix + Lys	834	54 ^{ac}	130	8	329	22 ^a	1.6	0.1 ^a
	Casein AA mix	1091	75 ^b	112	12	412	24 ^b	1.8	0.2 ^{ab}
	Casein AA mix + Arg	915	76 ^{bc}	120	14	473	33 ^b	2.3	0.3 ^b

^{a, b, c}. In each experiment, values in the same vertical column not sharing common superscript letters were significantly different; $P < 0.05-0.01$.

AA mix, amino acid mixture simulating soya-bean protein or casein; Lys, lysine; Arg, arginine.

Table 5. Faecal excretion of endogenous neutral and acidic steroids in rats given soya-bean protein or casein

(Mean values with their standard errors of six rats/dietary group)

Expt no.	Dietary regimens	Faeces excreted* (g/d)		Neutral steroids (mg/d)						Acidic steroids (mg/d)	
				Coprostanol		Cholesterol		Total		Mean	SE
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
(1)	Soya-bean protein	1.2	0.1 ^a	6.30	0.69 ^a	2.45	0.16 ^a	8.75	0.81 ^a	12.6	0.92 ^a
	Casein	0.9	0.1 ^b	3.53	0.39 ^b	1.41	0.17 ^b	4.94	0.44 ^b	4.84	1.01 ^b
	Soya AA mix	0.9	0.1 ^b	5.93	0.74 ^a	1.93	0.35 ^{ab}	7.86	0.61 ^a	7.19	1.01 ^b
	Casein AA mix	0.8	0.1 ^b	5.99	0.71 ^a	1.50	0.17 ^b	7.34	0.80 ^a	6.19	0.69 ^b
(2)	Soya-bean protein	1.0	0.1 ^a	5.84	0.48 ^a	2.07	0.29 ^a	7.91	0.73 ^a	9.32	0.45 ^a
	Soya-bean protein + Lys	0.9	0.1 ^{ab}	5.40	0.47 ^a	1.56	0.21 ^{ab}	6.96	0.73 ^{ab}	11.5	0.96 ^a
	Casein	0.8	0.1 ^b	3.64	0.23 ^b	1.17	0.23 ^b	4.80	0.45 ^b	4.42	0.59 ^b
	Casein + Arg	0.7	0.0 ^b	3.55	0.44 ^b	1.03	0.23 ^b	4.59	0.61 ^b	3.67	0.62 ^b
(3)	Soya AA mix	0.7	0.1	4.02	0.85	1.61	0.72	5.64	0.60	5.32	0.46
	Soya AA mix + Lys	0.8	0.1	5.54	0.25	1.19	0.21	6.73	0.30	5.64	0.77
	Casein AA mix	0.7	0.1	4.59	0.49	0.89	0.13	5.47	0.52	5.55	0.25
	Casein AA mix + Arg	0.7	0.0	5.09	0.06	0.98	0.18	6.07	0.40	6.08	0.46

^{a, b}. In each experiment, values in the same vertical column not sharing common superscript letters were significantly different; $P < 0.05-0.01$.

AA mix, amino acid mixture simulating soya-bean protein or casein; Lys, lysine; Arg, arginine.

* Weight of lyophilized faeces.

DISCUSSION

Although we have previously reported that the hypocholesterolaemic effect of the soya-bean protein isolate, as compared with casein, can be demonstrated only when the rats were fed a low-fat diet free of cholesterol (Nagata *et al.* 1980), it was not obvious if the difference in the amino acid composition of these two proteins could be responsible for causing different responses in serum cholesterol levels. The results obtained with the amino-acid-mixture diet apparently indicated that the difference in the amino acid composition did in fact account for the cholesterol-lowering action of soya-bean protein. Yadav & Liener (1977) came to a similar conclusion, though they possibly ignored the effect of essential-fatty-acid deficiency on the cholesterol metabolism. In rabbits, the cholesterol-lowering action became obscure when the amino acid mixture replaced the protein (Huff *et al.* 1977). However, the present results support the view that the amino acid composition itself is responsible for the hypocholesterolaemic effect of soya-bean protein.

If the difference in the amino acid composition is a major factor regulating the concentration of serum cholesterol of rats, then the question arises as to which amino acid(s) has most influence on the serum cholesterol level. Recently Kritchevsky (1979) and Czarnecki & Kritchevsky (1979) studied the effect of the difference in arginine:lysine (1.13 in soya-bean protein and 0.49 in casein) on serum cholesterol of rabbits. The results indicate that arginine:lysine was responsible for controlling serum cholesterol levels, and they concluded that the anti-atherogenicity of soya-bean protein could be weakened by the addition of specific amino acid, lysine in this instance. By contrast, according to the reports of Jarowski and co-workers, dietary lysine appeared to lower plasma cholesterol of rats (Jarowski & Pytelewski, 1975) and hyperlipidemic subjects (Raja & Jarowski, 1975). In our experiments, addition of lysine to soya-bean protein or arginine to casein did not modify the intrinsic effect of these proteins on serum cholesterol.

Hamilton & Carroll (1976), Gatti & Sirtori (1977) and Kim *et al.* (1978) noted the differences in methionine content because casein contains twice as much methionine as soya-bean protein and this sulphur amino acid is the first limiting amino acid in both proteins. The addition of methionine to soya-bean protein did not raise serum cholesterol levels, indicating the possible independence of the hypocholesterolaemic effect of vegetable protein to the level of this amino acid. Recently, Hermus and associates (Hermus & Dallinga-Thie, 1979; Katan *et al.* 1980) have shown that the dietary level of glycine is related to the anti-hypocholesterolaemic action of casein. From these studies it seems likely that the concentration of serum cholesterol is determined not only by specific amino acid(s) but also by the interplay of component amino acids as a whole.

Soya-bean protein decreased serum cholesterol in both the HDL and VLDL plus LDL fractions to a similar extent, whereas there was a significant decrease in serum apo A-I and increase in apo B. Though the evaluation of the changes in the apolipoprotein profiles due to feeding the vegetable protein seemed very difficult, these phenomena may relate to the difference in the supply of apo A-I from the intestine and apo B from the liver (Christophe & Verdonk, 1979). There is a possibility that the serum from rats given casein carries an extra amount of apo A-I and that the level observed with the animals given soya-bean protein is by no means subnormal. The small decrease in the concentration of HDL-cholesterol with a concomitant large decrease in cholesterol of intermediate-density lipoprotein has been observed in rabbits given soya-bean protein as compared with casein (Carroll *et al.* 1977). In the human, LDL-cholesterol appeared to decrease, but the level of HDL-cholesterol remained almost unchanged (Sirtori *et al.* 1977, 1979). A part of these discrepancies among animals may be explained by the species-specific distribution of serum

cholesterol in lipoproteins. The present observation at least indicates the characteristic modification of cholesterol metabolism by the type of dietary proteins.

It has been considered that the increase in faecal excretion of neutral steroids is a factor responsible for causing a low level of serum cholesterol after feeding soya-bean protein (Fumagalli *et al.* 1978). This factor held true in a previous experiment (Nagata *et al.* 1980). However, when the amino acid mixtures simulating soya-bean protein or casein were fed, the difference in the excretion of steroids disappeared. Thus, the dietary-protein-dependent difference in serum cholesterol cannot necessarily be explained by the increased faecal output of steroids alone. The increase in faecal steroids on feeding the vegetable protein appears to be counterbalanced partly by the decrease in the pool of hepatic cholesterol; such a reduction was not observed in rats given the amino acid mixture diet. Fumagalli *et al.* (1978) demonstrated in rabbits that soya-bean protein caused an increase in faecal excretion of neutral, but not acidic steroids. By contrast, there was no difference in the amount of faecal steroids in pigs given soya-bean protein and casein despite a difference in serum cholesterol levels (Kim *et al.* 1978). In the present study, soya-bean protein increased the excretion of both neutral and acidic steroids. These differences may be induced by species specificity in the metabolism of cholesterol.

The present results indicate that the increase in faecal excretion of steroids is not the only factor causing a low level of serum cholesterol in rats given the vegetable protein. Another possible factor associated with the hypocholesterolaemic effect of soya-bean protein may be an increased turnover of cholesterol in the rapidly-exchangeable pool. It has been known that feeding soya-bean protein to rats, in comparison with casein, results in the elevation of the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (*EC* 1.1.1.34), the rate-limiting enzyme in cholesterologenesis (Reiser *et al.* 1977).

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