Interaction of dietary protein, cholesterol and age on lipid metabolism of the rat

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(*Received 19 September 1988 – Accepted 20 December 1988*)

1. Male rats at 1 (young) and 9 months (adult) of age were fed on purified diets, supplemented with or without cholesterol, containing 200 g protein/kg (casein (CAS), whey protein (WHY) or soya-bean protein (SOY)) for 4 weeks.

2. SOY exerted a hypocholesterolaemic effect in young rats regardless of dietary cholesterol, whereas in adult rats it was observed only when a cholesterol-enriched diet was given. WHY was also hypocholesterolaemic in rats of both ages given cholesterol. SOY tended to reduce the liver cholesterol both in young and adult rats.

3. The liver hydroxymethylglutaryl-CoA reductase (NADPH) (*EC* 1.1.1.34) activity tended to be lower in the vegetable-protein groups than in the animal-protein groups in young rats, but the age-related reduction was observed only in the latter groups.

4. There was no significant age-related difference in the activity of liver cholesterol 7α-monooxygenase (*EC* 1.14.13.17) in response to diets. However, when cholesterol was given, activity tended to decrease with age. Rats given SOY excreted more faecal steroids than those given casein, particularly adult rats.

5. The fatty acid profile of phosphatidylcholine and the Δ6-desaturase activity of liver microsomes indicated the reduced desaturation of linoleate in the SOY and WHY groups compared with the CAS groups.

6. The results thus showed a complex interaction of protein type, cholesterol and age on cholesterol homeostasis.

The reduction in the turnover rate of cholesterol with age may lead to an elevation of the serum cholesterol level (Kritchevsky, 1980). However, age-related degeneracy of cholesterol metabolism could be manipulated by dietary polyunsaturated fatty acids (Dupont *et al.* 1972; Choi & Sugano, 1988). As dietary protein influences the serum cholesterol level in animals by modifying cholesterol dynamics (Huff & Carroll, 1980; Nagata *et al.* 1982), dietary intervention with protein may ameliorate the age-related disturbance of cholesterol metabolism. The protein effect on cholesterol metabolism appears to be dependent on dietary cholesterol (Nagata *et al.* 1980, 1981; Eklund & Sjöblom, 1986). In addition, dietary protein alters the fatty acid profile of tissue phospholipids and eicosanoid production (Huang *et al.* 1986; Sugano *et al.* 1988), and ageing also influences these processes (Brenner, 1981).

The hypocholesterolaemic effect of soya-bean protein compared with casein in relation to age is not fully understood in rats, although results from rabbits have shown a reduced susceptibility to dietary proteins with age (Terpstra *et al.* 1983; Pfeuffer & Barth, 1986; Park *et al.* 1987). Also, it is not known how ageing influences the dietary-protein-dependent change in the desaturation system. Thus in the present study we examined the effect of dietary protein and cholesterol on various indices of lipid metabolism in the liver of rats of different ages.

* For reprints.

MATERIALS AND METHODS

Animals

Young (3 weeks old) and adult (9 months old) male Sprague-Dawley rats, obtained from Seiwa Experimental Animals, Fukuoka, were individually housed in a temperature- and light-controlled room (20–23°, lights on 08.00–20.00 hours). After acclimatization to a commercial non-purified diet (Type NMF; Oriental Yeast Co., Tokyo) for 1 week, rats were divided into three groups of six animals each and fed *ad lib.* on one of three experimental diets containing casein (CAS; Wako Pure Chemicals, Osaka), whey protein (WHY; Meiji Milk Co., Tokyo) or soya-bean protein (SOY; Fujipro R, Fuji Oil Co., Osaka) as the protein source. The actual crude protein contents (nitrogen × 6·25) of these commercial protein sources were 860, 900 and 860 g/kg respectively. The diet was prepared according to the formula recommended by the American Institute of Nutrition (American Institute of Nutrition, 1977) and contained (g/kg): protein 200, maize oil 50, mineral mixture 35, vitamin mixture 10, choline bitartrate 2, DL-methionine 3, cellulose 50, maize starch 150 and sucrose to 1000. AIN76 vitamin and mineral mixtures were the product of Nihon Nosan Kogyo Co., Kanagawa. In one experiment (Expt 2), cholesterol (5 g/kg diet) was added at the expense of sucrose. After 4 weeks, rats were killed by decapitation at night (01.00 hours). Procedures recommended by Kyushu University for the use and care of laboratory animals were followed.

Analyses

The activity of hydroxymethylglutaryl-CoA reductase (NADPH) (*EC* 1.1.1.34; HMG-CoA reductase), cholesterol 7α-monooxygenase (*EC* 1.14.13.17) and Δ6-desaturase of liver microsomes was measured as described previously (Choi *et al.* 1987; Choi & Sugano, 1988). Serum, liver and microsomal lipids were extracted according to the method of Folch *et al.* (1957). Cholesterol, triglyceride and phospholipid were measured according to the method of Sperry & Webb (1950), Fletcher (1968) and Rouser *et al.* (1966) respectively. The fatty acid composition of microsomal phosphatidylcholine was analysed by gas–liquid chromatography (GLC) (Choi & Sugano, 1988). Faecal neutral and acidic steroids were analysed by GLC using 5α-cholestane (Nakarai Chemicals, Kyoto) (Miettinen *et al.* 1965) and 23-nordeoxycholic acid (Steraroids Inc., Wilton, NH) (Uchida *et al.* 1977; Kuriyama *et al.* 1979) as internal standards respectively. Microsomal cholesterol was determined enzymically (Choi *et al.* 1987) and protein by the method of Lowry *et al.* (1951).

Values were analysed by Student's *t* test to examine the age-effect between the same dietary group, or a one-way analysis of variance followed by Duncan's multiple-range test to inspect all differences within the same age-group.

RESULTS

In both experiments with (Expt 2) or without (Expt 1) dietary cholesterol, there were no statistically significant differences in food intake, weight gain and relative liver weight among age-matched rats given different proteins (values not shown).

Table 1 summarizes the concentration of serum lipids. In Expt 1, in which diets free of cholesterol were given, the concentration of serum cholesterol was significantly lower in the SOY-fed group than in the two milk-protein-fed groups in young but not adult rats due to an age-related increase in serum cholesterol in the SOY group. The serum triglyceride concentration was comparable in the age-matched groups, but decreased with age in all groups of rats. No significant difference due to diet or age was observed in serum phospholipid. In Expt 2, with cholesterol-enriched diets, the serum cholesterol level was

Table 1. *Serum lipid concentrations (mg/l) in young and adult rats given diets containing different proteins with (Expt 2) or without (Expt 1) cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1												
TCH	1169[a]	83	1112[a]	46	826[b]	85	1143	51	1174	70	1041*	43
TG	2928	228	2446	244	2248	110	1773*	164	1714	332	1512*	119
PL	2690	69	2671	132	2452	186	2318	112	2601	145	2457	69
Expt 2												
TCH	1423[a]	75	1018[b]	47	1052[b]	23	1902[a]*	182	1307[b]*	51	983[b]	102
TG	2345	334	1826	212	1894	131	2223[a]	189	3635[b]*	476	2214[a]	261
PL	2323[a]	139	1748[b]	78	1809[b]	48	2807[a]	366	2140[ab]*	71	1762[b]	217

CAS, casein; WHY, whey protein; SOY, soya-bean protein; TCH, total cholesterol; TG, triglyceride; PL, phospholipid.

[a, b] For age-matched rats, values with unlike superscript letters were significantly different ($P < 0.05$).

* Mean values were significantly different from those for corresponding young rats ($P < 0.05$).

† For details of diets, see p. 532.

Table 2. *Liver lipid levels (mg/g) in young and adult rats given diets containing different proteins with (Expt 2) or without (Expt 1) cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1												
TCH	3·12	0·32	3·22	0·24	2·79	0·22	5·58[a]*	0·90	4·06[ab]	0·47	2·69[b]	0·24
TG	71·9[a]	7·7	64·3[ab]	7·3	39·9[b]	4·5	77·8[a]	12·4	52·6[ab]	3·5	34·6[b]	5·0
PL	31·4[a]	0·6	34·9[b]	1·2	36·0[b]	0·4	30·8[a]	0·6	35·3[b]	0·6	35·4[b]	0·5
Expt 2												
TCH	18·3[a]	2·4	10·4[b]	1·5	10·3[b]	1·2	20·2	2·1	19·4*	2·1	16·6*	2·3
TG	84·9	8·2	65·1	7·1	59·6	9·3	105[a]	16	83·4[ab]	10·3	55·6[b]	5·9
PL	30·0	0·8	30·8	0·5	30·2	0·5	28·4	1·4	25·3*	1·3	28·7	0·5

CAS, casein; WHY, whey protein; SOY, soya-bean protein; TCH, total cholesterol; TG, triglyceride; PL, phospholipid.

[a, b] For age-matched rats, values with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from those for corresponding young rats ($P < 0·05$).

† For details of diets, see p. 532.

Table 3. *The activities of liver microsomal enzymes (pmol/min per mg protein) in young and adult rats given diets containing different proteins with (Expt 2) or without (Expt 1) cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1												
HMG-CoA reductase	526	53	600	90	393	62	271*	52	292*	65	373	46
Cholesterol 7α-monooxygenase	15·3	1·1	15·4	1·5	11·4	0·9	11·1	1·6	13·7	1·0	11·6	0·5
Δ6-Desaturase	124	23	91·3	28·7	97·4	19·6	147[a]	16	93·1[ab]	17·4	84·7[b]	17·0
Expt 2												
HMG-CoA reductase	78·3	17·2	90·0	12·8	45·5	5·4	46·3	12·0	32·9*	3·8	37·6	7·8
Cholesterol 7α-monooxygenase	9·73	1·68	12·2	1·5	9·79	1·13	6·75	1·24	6·78*	0·89	7·68	0·61

CAS, casein; WHY, whey protein; SOY, soya-bean protein; HMG-CoA reductase, hydroxymethylglutaryl-CoA reductase (NADPH) (*EC* 1.1.1.34); cholesterol 7α-monooxygenase (*EC* 1.14.13.17).

[a, b] For age-matched rats, values with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from those for the corresponding young rats ($P < 0·05$).

† For details of diets, see p. 532.

higher in the CAS group than in the SOY and WHY groups at both ages. An age-related increase in serum cholesterol was observed in the CAS and WHY groups but not in the SOY group. The concentration of serum triglyceride was comparable in three groups of young rats, but it was higher in rats given WHY than in those given CAS or SOY in adult rats. There was also a protein-dependent difference in the serum phospholipid level, and it was higher in rats given CAS than in those given WHY or SOY at both ages. A significant age-related increase in serum triglycerides and phospholipids was observed only in the WHY group.

Table 2 shows concentrations of liver lipids. In Expt 1, the liver cholesterol level in adult rats was lower in the SOY group than in the CAS group, the WHY group being intermediate. There was no detectable difference in liver cholesterol in young rats. Liver cholesterol increased with age only in rats given CAS. The liver triglyceride level was highest in the CAS group and lowest in the SOY group at both ages. CAS reduced liver phospholipid compared with other proteins at both ages. However, no age-related changes were found in the concentrations of these lipids. In Expt 2, dietary cholesterol produced an accumulation of cholesterol in the liver. However, the concentration of liver cholesterol was significantly lower in young rats, but not adult rats, given WHY or SOY than in those given CAS. The significant age-effect on liver cholesterol was observed in the SOY and WHY groups. The concentration of liver triglyceride was influenced by the protein type, in particular in adult rats; it was lower in the SOY groups than in the CAS groups, the WHY groups being intermediate. Liver phospholipid was comparable in age-matched groups, but it decreased with age in the WHY group.

Table 3 shows the specific activities of HMG-CoA reductase, cholesterol 7α-monooxygenase and Δ6-desaturase of liver microsomes. When the diet free of cholesterol was given, the HMG-CoA reductase activity of young rats tended to be lower in the SOY group than in the CAS or WHY groups. However, since the age-related reduction in activity was observed only in the two milk-protein groups, it tended to be higher in the SOY group in adult rats. Although the HMG-CoA reductase activity was low in rats given cholesterol, the response in young rats resembled that observed in rats given diets free of cholesterol. The activities of cholesterol 7α-monooxygenase were in general similar regardless of age or dietary protein in both experiments, except for the WHY group in which it decreased significantly with age when cholesterol was added to the diet. In rats given diets free of cholesterol, the Δ6-desaturase activity was higher in the CAS group than in the other groups, especially for adult rats, but no age-related changes were observed for all groups.

Table 4 shows the fatty acid composition of liver microsomal phosphatidylcholine in Expt 1. At both ages, there was a protein-dependent difference in the proportion of linoleate; it was significantly higher in the SOY group than in the CAS group, and intermediate in the WHY group. Since the proportion of arachidonate was similar for all groups, the ratio of 20:3*n*-6 plus 20:4*n*-6/18:2*n*-6 was lower for rats given SOY than for those given CAS. The ratio tended to decrease with age. In general, the proportions of 20:3*n*-6 and 22:6*n*-3 increased significantly whereas that of 22:5*n*-6 decreased with age for all groups. However, there was no dietary-protein-dependent difference in the ratio of 22:5*n*-6/20:4*n*-6 in age-matched rats, although it was significantly reduced with age.

Although dietary cholesterol modified fatty acid compositions of liver microsomal phosphatidylcholine, a dietary-protein-dependent difference could be observed (Table 5); the proportions of 18:2*n*-6 and 20:3*n*-6 were higher, and those of 20:4*n*-6 were lower in the SOY group than in the CAS and WHY groups. Dietary cholesterol lowered the ratio of 20:3*n*-6 plus 20:4*n*-6/18:2*n*-6, but it was again higher in rats given CAS than in those given SOY. The difference between the CAS and WHY groups in the proportions of 22:5*n*-6 and

Table 4. *Expt* 1. *Fatty acid composition (weight %) of liver microsomal phosphatidylcholine in young and adult rats given diets containing different proteins without cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
Fatty acid‡	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16:0	18·3	0·2	18·3	0·5	20·0	0·7	17·3*	0·4	17·8	0·2	17·9*	0·5
16:1	1·3	0·1	1·3	0·1	1·2	0·1	1·2	0·1	1·6	0·2	1·3	0·1
18:0	25·2[a]	0·3	24·7[a]	0·4	22·0[b]	0·7	25·0	1·0	24·1	0·8	23·0	0·8
18:1	7·4	0·2	7·1	0·2	7·4	0·2	6·5*	0·3	7·3	0·3	6·6	0·4
18:2*n*-6	5·8[a]	0·3	6·3[ab]	0·3	7·3[b]	0·5	6·9[a]	0·4	7·5[ab]*	0·4	8·4[b]	0·4
20:3*n*-6	0·8	0·1	0·8	0·0	0·9	0·1	1·3*	0·1	1·6*	0·2	1·7*	0·1
20:4*n*-6	31·5	0·4	32·4	0·4	31·1	0·3	33·4*	0·7	32·2	0·8	32·3	0·5
22:5*n*-6	3·6	0·2	2·9	0·3	3·0	0·2	0·6*	0·1	0·5*	0·1	0·5*	0·1
22:6*n*-3	3·6	0·1	4·0	0·2	4·1	0·1	5·5*	0·1	5·2*	0·1	5·6*	0·2
20:3+20:4/18:2	5·6	0·3	5·4	0·3	4·5	0·3	5·1	0·3	4·6	0·3	4·1	0·2
22:5/20:4 ($\times 10^2$)	11·4	0·8	10·4	1·0	9·6	0·7	1·6*	0·4	1·6*	0·2	1·4*	0·2

CAS, casein; WHY, whey protein; SOY, soya-bean protein.

[a, b] For age-matched rats, values with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from those for the corresponding young rats ($P < 0·05$).

† For details of diets, see p. 532.

‡ Fatty acids present at less than 1% are not shown.

Table 5. *Expt 2. Fatty acid composition (weight %) of liver microsomal phosphatidylcholine in young and adult rats given diets containing different proteins with cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
Fatty acid‡	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16:0	19·7	0·4	20·3	0·3	19·9	0·4	19·7	0·6	20·0	0·4	19·0	0·8
16:1	2·0	0·1	1·8	0·1	2·1	0·2	2·3	0·2	2·2	0·3	1·9	0·3
18:0	19·5^{a}	0·1	20·7^{a}	0·3	16·9^{b}	0·8	19·3	1·2	19·7	0·4	17·7	1·2
18:1	10·4^{a}	0·3	9·4^{a}	0·2	12·2^{b}	0·7	9·1	1·1	8·4	0·7	10·0	1·0
18:2*n*-6	9·5^{a}	0·3	10·4^{a}	0·2	12·7^{b}	0·4	11·9	2·0	14·7*	0·4	15·4*	0·1
20:3*n*-6	1·9^{a}	0·1	1·9^{a}	0·1	2·9^{b}	0·3	2·5	0·5	2·9*	0·4	3·2	0·4
20:4*n*-6	30·2^{a}	0·3	29·9^{a}	0·3	26·7^{b}	0·7	27·7	1·9	25·9*	0·8	25·4	0·9
22:5*n*-6	1·2^{a}	0·1	0·8^{b}	0·1	0·9^{ab}	0·1	0·2*	0·0	0·1*	0·0	0·2*	0·0
22:6*n*-3	3·0^{a}	0·1	2·6^{b}	0·1	2·8^{ab}	0·0	4·6*	0·4	3·4*	0·2	4·1	0·7
20:3+20:4/18:2	3·4^{a}	0·2	3·1^{a}	0·1	2·5^{b}	0·2	2·9	0·6	2·0*	0·1	1·9*	0·1
22:5/20:4 ($\times 10^{2}$)	3·9	0·4	2·7	0·4	3·2	0·4	0·9*	0·1	0·5*	0·1	0·7*	0·1

CAS, casein; WHY, whey protein; SOY, soya-bean protein.

$^{a, b}$ For age-matched rats, values with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from those for the corresponding young rats ($P < 0·05$).

† For details of diets, see p. 532.

‡ Fatty acids present at less than 1% are not shown.

Table 6. *Expt 2. Faecal steroid excretion (mg/d) in young and adult rats given diets containing different proteins with cholesterol*†

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

	Young						Adult					
	CAS		WHY		SOY		CAS		WHY		SOY	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Faecal wt (g/d)	1·65^{ab}	0·07	1·60^{a}	0·05	1·86^{b}	0·07	2·19^{a}*	0·05	1·99^{a}*	0·07	2·54^{b}*	0·09
Acidic steroids (mg/d)	11·2^{a}	0·7	12·8^{ab}	1·2	17·0^{b}	1·9	17·8^{a}*	1·4	12·8^{b}	1·2	24·4^{c}*	1·2
Neutral steroids (mg/d)	70·6	1·6	77·7	3·5	76·3	4·3	71·6	1·7	68·0	3·9	80·5	3·5
Total steroids (mg/d)	81·8	3·4	90·4	3·8	93·2	4·6	89·3^{a}	2·7	81·3^{a}	4·1	105^{b}	4

CAS, casein; WHY, whey protein; SOY, soya-bean protein.

^{a,b} For age-matched rats, values with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from those for the corresponding young rats ($P < 0·05$).

† For details of diets, see p. 532.

22:6*n*-3 was significant. The age-related change in the fatty acid composition was most marked in the WHY group; 18:2*n*-6, 20:3*n*-6 and 22:6*n*-3 increased whereas other fatty acids tended to decrease with age.

Table 6 shows faecal excretion of steroids in Expt 2. Faecal dry weight was higher for the SOY group than for the CAS and WHY groups at both ages. Acidic steroid excretion was markedly higher in rats given SOY than in those given milk proteins at both ages. In the CAS and SOY groups, but not the WHY groups, there was an age-dependent increase in acidic steroid excretion. Neutral steroid excretion tended to be higher in rats given SOY than in those given CAS. Thus, the total amount of steroids excreted tended to be higher in the vegetable-protein group than in the two milk-protein groups at both ages, in particular in adult rats.

DISCUSSION

The present study showed the diversity of the hypocholesterolaemic action of SOY compared with CAS in rats (Nagata *et al.* 1980, 1981; Bosisio *et al.* 1981; Sugano *et al.* 1982; Eklund & Sjöblom, 1986; Pfeuffer & Barth, 1986; Park *et al.* 1987). In adult rats the hypocholesterolaemic effect of SOY was evident when the cholesterol-enriched diet was given, whereas in young rats it was evident when the cholesterol-free diet was given. Also, the liver cholesterol-lowering effect of SOY was variable. The difference in the response of serum and liver cholesterol suggests a complex interaction of both age and dietary manipulation. The difference between young and adult rats in the response of serum triglyceride to dietary cholesterol suggests a different responsiveness in lipoprotein metabolism (Pfeuffer & Barth, 1986). At both ages, WHY exerted a cholesterol-lowering effect compared with CAS when cholesterol was added to the diet. A similar finding has been reported for young rats (Sautier *et al.* 1983) and for pigs (Norton *et al.* 1987).

The effect of dietary protein on HMG-CoA reductase activity also showed a complex pattern due to age or dietary cholesterol. When rats were fed on cholesterol-free diets, SOY reduced the activity to some extent in young rats, whereas it tended to be higher in the SOY group than in the other protein groups in adult rats (Table 3). A similar pattern of response to SOY was observed even when rats were fed on cholesterol. The finding in young rats given a SOY diet free of cholesterol was inconsistent with that reported previously (Nagata *et al.* 1982). The reason for the discrepancy is not apparent, but the difference in the dietary fat level may be responsible, since both the type and amount of dietary fat modify the hypocholesterolaemic effect of SOY (Nagata *et al.* 1980; Sugano *et al.* 1988). However, the depression in cholesterol synthesis of young rats given SOY compared with those given CAS may result in a lowering of serum and liver cholesterol. The weak cholesterol-lowering effect of SOY in adult rats given a cholesterol-free diet may be compatible with that observed in aged rabbits (Terpstra *et al.* 1983). Suppression of HMG-CoA reductase activity by dietary cholesterol is accompanied by an increase in hepatic cholesterol due to the increased levels of cholesteryl esters (Dietschy & Wilson, 1970; Rodwell *et al.* 1976; Uchida *et al.* 1977). However, HMG-CoA reductase activity does not necessarily seem to be inversely correlated with liver microsomal cholesterol or cholesteryl ester contents under various conditions (Rodwell *et al.* 1976; Ide *et al.* 1978).

Although cholesterol 7α-monooxygenase activity has been considered to be regulated by microsomal cholesterol content (Balasubramaniam *et al.* 1973; Shefer *et al.* 1981), the results of recent studies do not necessarily support this concept (Einarsson *et al.* 1987; Björkhem & Åkerlund, 1988). In the present study there was no dietary-protein-dependent difference in cholesterol 7α-monooxygenase activity, in agreement with the results for young rats given cholesterolaemic diets (Bosisio *et al.* 1981). However, the significant cholesterol-lowering effect of SOY in adult rats given cholesterol may be a reflection of the

increased steroid excretion. In contrast, the age-dependent increase in the liver cholesterol pool in rats given a WHY diet supplemented with cholesterol may also, in part, be attributed to reduced steroid excretion.

The increase in the secretion of glucagon appears to be associated with metabolic changes caused by dietary SOY as compared with CAS (Noseda & Fragiacomo, 1980; Sugano *et al.* 1982). An age-related reduction in serum glucagon concentration was reported by Klug *et al.* (1979). Thus, the change in the glucagon status may at least in part be responsible both for the protein- and age-dependent difference in the response of HMG-CoA reductase, as glucagon reduces the activity of this enzyme (Rodwell *et al.* 1976).

The Δ6-desaturase activity was low in rats give SOY or WHY compared with those given CAS at both ages, suggesting that the quality of dietary protein influences the fatty acid desaturation system. Although glucagon decreases desaturase activity (Brenner, 1981), the protein-dependent difference was not necessarily attributable to the difference in amino acid composition because WHY reduced desaturase activity to the same extent as SOY. Interference of SOY with the desaturation system appears to be limited to the early steps (Sugano *et al.* 1988), as estimated by the ratios of linoleate:linoleate metabolites in tissue phospholipid.

Dietary cholesterol greatly suppressed overall desaturation in all groups, suggesting a primary role for cholesterol rather than the quality of dietary protein in the regulation of linoleate metabolism. The decrease in the activity of Δ6-desaturase by dietary cholesterol was recently reported in rats by Garg *et al.* (1988). However, the mechanism by which dietary cholesterol modifies the desaturation system is not evident at present and hence the relation between the desaturase activity and the level of hepatic cholesterol is not clear. Garda & Brenner (1985) reported evidence that suggests dependence of Δ6-desaturation of linoleate on the level of microsomal cholesterol. Further investigations are needed to elucidate the role of dietary cholesterol in fatty acid desaturation. Although the ratio of 20:3*n*-6 + 20:4*n*-6/18:2*n*-6 in microsomal phosphatidylcholine tended to decrease in all groups with age, no age-related reduction in desaturase activity was observed. The ratio of 22:5*n*-6/20:4*n*-6 decreased significantly with age regardless of dietary protein, suggesting a reduced capacity for Δ4-desaturation in relation to age (Choi & Sugano, 1988). We have recently shown that defective desaturation induced by SOY could at least be partly overcome by supplementation with γ-linolenic acid, the Δ6-desaturation product of linoleate (Sugano *et al.* 1988).

In summary, the effect of dietary protein on serum cholesterol was modified not only by dietary cholesterol but also by age. The age-related increase in serum cholesterol observed in rats given a SOY diet free of cholesterol was diminished by cholesterol supplementation. WHY in a cholesterol-enriched diet also exerted a hypocholesterolaemic effect at both ages, but it failed to ameliorate the age-related increase in serum lipids. The fatty acid desaturase system was also affected by the protein quality and cholesterol content of the diet. These results suggest a complex interaction of dietary protein, cholesterol and age on the regulation of lipid metabolism in the rat model.

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Printed in Great Britain