British Journal of Nutrition (1997), **78**, 459–467

Influence of dietary protein and fat on serum lipids and metabolism of essential fatty acids in rats

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(*Received 23 September 1996–Revised 12 February 1997–Accepted 5 March 1997*)

A 120 d feeding study with adult rats was conducted to evaluate the influence of two protein sources (casein and gelatin), two protein levels (50 and 300 g/kg diet) and two fat levels (50 and 150 g/kg diet) on serum lipids (total cholesterol, HDL-cholesterol and triacylglycerols) and liver polyunsaturated fatty acid levels. In general, the concentrations of serum triacylglycerols and total cholesterol and liver phospholipid levels of arachidonic acid (AA) and docosahexaenoic acid (DHA) were higher in rats fed on casein diets compared with those fed on the gelatin diets. These effects were more pronounced in rats fed on the high-casein (300 g/kg)–high-fat (150 g/kg) diet. Gelatin was hypocholesterolaemic and also suppressed the liver phospholipid levels of AA and DHA (reported for the first time). The difference in the amino acid composition between casein and gelatin may be responsible for the observed effects. Casein contains higher levels of glutamic acid, methionine, phenylalanine and tyrosine, while gelatin contains higher levels of arginine, glycine and hydroxyproline. It is suggested that a protein source which increases serum cholesterol may also increase the concentrations of AA and DHA in rat tissues.

Casein: Gelatin: Essential fatty acids

Dietary protein is known to influence cholesterol metabolism (Carroll & Kurowska, 1995; Kritchevsky, 1995). Protein of animal origin, such as casein, is more cholesterolaemic and atherogenic than plant protein, such as soyabean protein, in human subjects and a variety of animal models (Erdman & Fordyce, 1989; Carroll, 1991). Casein may exert its hypercholesterolaemic effect by mechanisms which include increased absorption and decreased turnover of cholesterol (Kritchevsky, 1995). The addition of single amino acids to casein has been reported to influence cholesterolaemia (Sugiyama & Muramatsu, 1990). In rats fed on cholesterol-free diets containing different protein sources, positive correlations between serum cholesterol and dietary glutamic acid, methionine, proline or tyrosine; and negative correlations between serum cholesterol and dietary alanine, arginine or cystine have been reported (Sautier *et al.* 1983, 1986). Information on the comparative effects of purified animal protein sources with markedly different amino acid compositions is, however, limited.

The activity of desaturases catalysing the biosynthesis of essential fatty acid metabolites such as arachidonic acid (AA) and docosahexaenoic acid (DHA) was also reported to be influenced by the quantity and quality of dietary protein (Inkpen *et al.* 1969; Peluffo *et al.* 1971, 1984). Consequently dietary protein influences the levels of AA, DHA and other long-chain *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA) in rat tissues. A high-protein diet containing 45 % energy from casein increased Δ6 desaturase activity in rat

liver microsomes compared with a low-protein diet supplying 5% energy from casein (Peluffo *et al.* 1984), suggesting that protein-deficient children may develop a notable deficiency of essential fatty acids (Brenner, 1981). High-protein diets containing 45% energy from a mixture of crystalline amino acids (simulating the amino acid composition of casein) also increased the activity of Δ6 desaturase in rat liver microsomes but decreased the activity of Δ9 desaturase compared with a high-sucrose diet (Peluffo *et al.* 1984). The elimination of phenylalanine and tyrosine from the amino acid mixture increased the Δ6 desaturase activity and massive amounts (200 g/kg) of phenylalanine or tyrosine in the diet inhibited Δ6 desaturase activity.

The studies by Peluffo *et al.* (1971, 1984) on the effects of the quantity of protein and the type of amino acids on the activity of desaturases in rat liver microsomes, were conducted with growing rats. Since the low-protein diet and the amino-acid-mixture diet devoid of phenylalanine, tyrosine and/or tryptophan did not meet the essential amino acid requirements for rat growth, the validity of these studies may be limited. Moreover, the addition of large amounts (200 g/kg) of phenylalanine or tyrosine in the study by Peluffo *et al.* (1984) may have created an amino acid imbalance.

It was, therefore, of interest to obtain further information on the influence of the quantity and quality of dietary protein on the levels of serum lipids and of PUFA in rat livers using a nutritionally balanced experimental design. A 120 d feeding study with adult rats was conducted to study the influence of two highly digestible animal protein sources with markedly different protein quality (casein and gelatin), two protein levels (50 and 300 g/kg diet) and two fat levels (50 and 150 g/kg diet) on serum lipids (total cholesterol, HDL-cholesterol and triacylglycerols) and liver PUFA levels after 30 d and 120 d of the study.

METHODS

Diets

The compositions of the eight experimental diets (high casein–low fat, HCLF; high casein–high fat, HCHF; low casein–low fat, LCLF; low casein–high fat, LCHF; high gelatin–low fat, HGLF; high gelatin–high fat, HGHF; low gelatin–low fat LGLF; low gelatin–high fat, LGHF) are shown in Tables 1 and 2. The diets were prepared weekly and stored at −4°. The low-protein casein diets and gelatin diets were supplemented with small amounts of limiting crystalline amino acids (Sigma Chemical, St. Louis, MO, USA) to ensure that experimental diets met the indispensable amino acid requirement for rat maintenance (National Research Council, 1978). Within each protein level (50 or 300 g/kg), diets were made isonitrogenous by the addition of L-glutamic acid and/or glycine. All the experimental diets also met the essential fatty acid requirements for rat maintenance (National Research Council, 1978).

The metabolizable energy (ME) per kg diet of the four low-fat diets (HCLF, LCLF, HGLF and LGLF) was 14·49 MJ and that of the four high-fat diets (HCHF, LCHF, HGHF and LGHF) was 16·58 MJ. ME was calculated using the Atwater factors of 17, 37 and 17 kJ/g for protein, fat and available carbohydrates respectively. The two dietary protein levels (50 and 300 g/kg diet) provided 5·1–5·9 and 30·7–35·0% energy from protein respectively. The two dietary fat levels (50 and 150 g/kg diet) provided 12·8 and 33·5% energy from fat respectively.

Table 1. *Composition of experimental diets (g/kg)*

Diet*†	Casein	Gelatin	Lard	Rapeseed oil	Sucrose	Maize starch
HCLF	333·3	—	45·0	5·0	345·8	172·9
HCHF	333·3	—	135·0	15·0	279·1	139·6
LCLF	55·6	—	45·0	5·0	526·6	263·5
LCHF	55·6	—	135·0	15·0	524·2	262·1
HGLF	—	306·1	45·0	5·0	364·3	182·1
HGHF	—	306·1	135·0	15·0	297·3	148·6
LGLF	—	51·1	45·0	5·0	531·5	265·7
LGHF	—	51·1	135·0	15·0	459·0	229·5

H, high; L, low; C, casein; G, gelatin; F, fat.

* Each diet also contained (g/kg): AIN-76 mineral mixture (American Institute of Nutrition, 1977) 35, AIN-76A vitamin mixture (American Institute of Nutrition, 1980) 10, choline bitartrate (Sigma Chemical, St. Louis, MO, USA) 2 and cellulose (Alphacel, Teklad Test Diets, Madison, WI, USA) 50.

† Diets were supplemented as follows, to make the diets isonitrogenous (within each protein level) and to meet the indispensable amino acid requirements for rat maintenance: HCLF (g/kg): L-Glu 0·5; HCHF (g/kg): L-Glu 0·5 and Gly 0·5; LCLF (g/kg): L-Ile 0·3, L-Met 1·0, L-Glu 3·9 and Gly 3·2; LCHF (g/kg): L-Ile 0·8, L-Met 1·1, L-Glu 4·1 and Gly 4·0; HGLF (g/kg): L-Trp 0·5; HGHF (g/kg): L-Ile 0·3, L-Met 0·2, L-Trp 0·5; LGLF (g/kg): L-His 0·4, L-Ile 2·3, L-Leu 0·4, L-Met 2·0, L-Thr 0·9, L-Trp 0·5, L-Tyr 0·6 and L-Val 1·3; LGHF (g/kg): L-His 0·5, L-Ile 2·5, L-Leu 0·6, L-Met 2·2, L-Thr 1·20, L-Trp 0·6, L-Tyr 0·80 and L-Val 1·6.

Animals and feeding study

Male Sprague–Dawley rats, 14 weeks old, (Charles River, Canada Inc., St. Constant, Quebec, Canada) were housed individually in metal cages in an air-conditioned room maintained at 22° and 60% relative humidity with a 12 h day–12 h night cycle. The rats were randomly assigned to eight dietary groups of ten rats per group. Food and water were available to the animals *ad libitum*. After an overnight fast, five rats from each group were killed and blood was collected from the aorta. Liver was frozen in liquid N_2 and stored at $-80°$ under N_2 until analysed for phospholipid fatty acid composition. Blood samples

Table 2. *Dietary indispensable amino acids, arginine, glycine and glutamic acid (mg/MJ metabolizable energy (ME)) provided by experimental diets**†

Diet	His	Ile	Leu	Lys	Met + Cys	Phe	Phe + Tyr	Thr	Trp	Val
HCLF	614	1108	2103	1747	722	1133	2383	961	270	1417
HCHF	537	975	1837	1527	631	989	2082	839	237	1240
LCLF	102	208	351	289	189	189	397	160	45	237
LCHF	88	213	306	253	165	165	347	139	38	206
HGLF	164	287	573	801	217	423	494	342	48	413
HGHF	143	270	502	700	201	368	432	299	38	360
LGLF	55	208	122	134	174	69	126	122	36	158
LGHF	54	208	120	117	165	62	122	124	38	158

H, high; L, low; C, casein; G, gelatin; F, fat.

* Each diet met or exceeded the indispensable amino acid requirements for rat maintenance. The arginine, glycine and glutamic acid contents (mg/MJ ME) of the diets were: HCLF 768, 414, 4970; HCHF 671, 362, 4343; LCLF 128, 69, 828; LCHF 112, 60, 828; HGLF 1824, 5147, 1337; HGHF 1594, 4498, 1169; LGLF 304, 858, 223; LGHF 266, 750, 195.

† The indispensable amino acid requirements (mg/MJ ME) for rat maintenance were: His 53, Ile 206, Leu 120, Lys 72, Met + Cys 153, Phe + Tyr 120, Thr 120, Trp 33, and Val 153 (National Research Council, 1978).

were immediately analysed for serum total cholesterol, HDL-cholesterol and triacylglycerols. The animal feeding and treatment protocol was reviewed and approved by the Animal Care Committee of Health Canada.

Analytical methods

Total N in the diets was determined by the micro-Kjeldahl method using a Kjeltec Auto 1030 analyser (Tecator, Herndon, VA, USA). Protein was calculated by using a N-to-protein factor of 6·25. Casein and gelatin were hydrolysed for 22 h with 6 M-HCl (Sarwar & Peace, 1986) for determination of all amino acids except tryptophan and sulfur amino acids. The 4·2 M-NaOH hydrolysis was used for the quantitative measurement of tryptophan (Hugli & Moore, 1972). Hydrolysates for the determination of sulfur amino acids (methionine as methionine sulfone and cystine/cysteine as cysteic acid) were prepared by performic acid oxidation of the protein followed by the 6 M-HCl hydrolysis (Moore, 1963). Amino acids in the acid hydrolysates were determined by liquid chromatography of precolumn phenylisothiocyanate derivatives (Sarwar *et al.* 1988). Tryptophan in the basic hydrolysate was determined by a simple liquid chromatographic method requiring no derivatization (Sarwar *et al.* 1988).

Total lipids were extracted from liver with chloroform–methanol (2 : 1, v/v) (Bligh & Dyer, 1959). Phospholipids (PL) were isolated by TLC with development in hexane–diethyl ether–acetic acid (80 : 20 : 1, by vol) and transmethylated with BF_3–methanol. The fatty acid methyl esters were analysed by GLC using an SP-2560 flexible fused silica capillary column (100 m × 0·25 mm i.d.; Supelco, Inc., Bellefonte, PA, USA) in a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA). The fatty acids were identified by comparing their retention times with those of a standard mixture of fatty acids (GLC 68, NU Check Prep, Elysian, MN, USA). The fatty acids were expressed as g/100 g total fatty acids.

The blood was centrifuged at 1300 ***g*** for 20 min at 4° and the serum separated. Serum total cholesterol, HDL-cholesterol and triacylglycerols were determined enzymically using the Boehringer Mannheim cholesterol C-system (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany).

Statistical analysis

All data are reported as means and standard deviations. For each response variable, an ANOVA was done using the Statistical Systems for Personal Computers (SAS Institute, Cary, NC, USA). The factors of interest were two types of protein (casein and gelatin), two levels of protein (50 and 300 g/kg), two levels of fat (50 and 150 g/kg) and two time points (30 d and 120 d) at which data were collected. Linear contrasts were constructed to examine both the main and interaction effects (Steel & Torrie, 1980). The residuals from the model were examined for a lack of homogeneity and for outliers. Differences were considered significant when $P < 0·05$.

RESULTS

Body weights

There were no differences ($P > 0·05$) observed in the weights of rats between the eight groups at the beginning of the experiment (434 (SD 18)–456 (SD 20) g) or after 30 d of

Table 3. *Effects of feeding casein or gelatin diets on serum triacylglycerol, total cholesterol and HDL-cholesterol levels (mmol/l) in rats*

(Mean values and standard deviations for five animals per group)

	Triacylglycerols				Total cholesterol				HDL-cholesterol			
	30 d		120 d		30 d		120 d		30 d		120 d	
Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HCLF	$3{\cdot}44^{a}$	0·87	$7{\cdot}05^{a}$	1·56	$3{\cdot}34^{a}$	0·47	$4{\cdot}99^{a}$	0·73	$1{\cdot}82^{a}$	0·26	$2{\cdot}62^{a}$	0·53
HCHF	$2{\cdot}24^{b}$	0·33	$5{\cdot}76^{a}$	1·65	$2{\cdot}90^{ab}$	0·29	$4{\cdot}00^{b}$	0·26	$1{\cdot}76^{ab}$	0·19	$2{\cdot}22^{a}$	0·18
LCLF	$3{\cdot}32^{a}$	0·56	$3{\cdot}99^{b}$	0·67	$2{\cdot}64^{bc}$	0·25	$2{\cdot}89^{c}$	0·17	$1{\cdot}27^{cd}$	0·11	$1{\cdot}41^{b}$	0·10
LCHF	$1{\cdot}67^{b}$	0·20	$3{\cdot}46^{b}$	0·40	$2{\cdot}40^{bc}$	0·16	$2{\cdot}73^{cd}$	0·10	$1{\cdot}31^{cd}$	0·15	$1{\cdot}39^{b}$	0·17
HGLF	$1{\cdot}32^{b}$	0·21	$2{\cdot}25^{bc}$	0·21	$2{\cdot}27^{c}$	0·11	$2{\cdot}99^{c}$	0·08	$1{\cdot}15^{d}$	0·10	$1{\cdot}67^{b}$	0·14
HGHF	$1{\cdot}41^{b}$	0·24	$1{\cdot}67^{c}$	0·59	$2{\cdot}29^{c}$	0·21	$2{\cdot}20^{d}$	0·35	$1{\cdot}41^{cd}$	0·19	$1{\cdot}42^{b}$	0·15
LGLF	$2{\cdot}14^{b}$	0·62	$2{\cdot}71^{bc}$	0·35	$2{\cdot}72^{bc}$	0·20	$2{\cdot}99^{c}$	0·10	$1{\cdot}44^{bcd}$	0·13	$1{\cdot}72^{b}$	0·09
LGHF	$1{\cdot}72^{b}$	0·62	$3{\cdot}17^{bc}$	0·39	$2{\cdot}49^{bc}$	0·15	$2{\cdot}73^{cd}$	0·16	$1{\cdot}55^{abc}$	0·15	$1{\cdot}60^{b}$	0·13

H, high; L, low; C, casein; G, gelatin; F, fat.

[a,b,c,d] Mean values within a column not sharing a common superscript letter were significantly different, $P < 0{\cdot}05$.

feeding (438 (SD 34)–469 (SD 20) g). Similarly, the differences in the weights of rats fed on various diets for 120 d of feeding were small (about 10 %, 467 (SD 33)–518 (SD 17) g).

Serum triacylglycerols, total cholesterol and HDL-cholesterol

The serum lipid composition is shown in Table 3. On average, the eight casein groups (HCLF, HCHF, LCLF and LCHF, 30 and 120 d) had higher levels ($P < 0{\cdot}05$) of serum triacylglycerols (3.87 (SD 0·78) *v.* 1·95 (SD 0·40) mmol/l), total cholesterol (3·23 (SD 0·31) *v.* 2·58 (SD 0·17) mmol/l) and HDL-cholesterol (1·72 (SD 0·21) *v.* 1·49 (SD 0·13) mmol/l) than the eight gelatin groups (HGLF, HGHF, LGLF and LGHF, 30 and 120 d).

The high-casein groups (HCLF and HCHF) had higher average levels ($P < 0{\cdot}05$) of serum triacylglycerols (4·62 (SD 1·10) *v.* 3·11 (SD 0·46) mmol/l), total cholesterol (3·81 (SD 0·42) *v.* 2·66 (SD 0·15) mmol/l) and HDL-cholesterol (2·10 (SD 0·29) *v.* 1·34 (SD 0·13) mmol/l) compared with the low-casein groups (LCLF and LCHF). The opposite was, however, true in the case of gelatin where the low-gelatin groups (LGLF and LGHF) had higher levels ($P < 0{\cdot}05$) of the three serum variables compared with the high-gelatin groups (HGLF and HGHF) (2·43 (SD 0·50) *v.* 1·66 (SD 0·30); 2·73 (SD 0·15) *v.* 2·44 (SD 0·20); 1·58 (SD 0·13) *v.* 1·39 (SD 0·15) mmol/l respectively).

The average serum triacylglycerol, total cholesterol and HDL-cholesterol levels of all the dietary groups were higher ($P < 0{\cdot}05$) at 120 d than at 30 d (3·76 (SD 0·73) *v.* 2·16 (SD 0·46); 3·10 (SD 0·25) *v.* 2·63 (SD 0·23); 1·76 (SD 0·19) *v.* 1·47 (SD 0·16) mmol/l respectively). At both 30 d and 120 d, the HCLF group had higher ($P < 0{\cdot}05$) concentrations of serum triacylglycerols, total cholesterol and HDL-cholesterol than all other dietary groups except the triacylglycerol value at 30 d for LCLF, the triacylglycerol value at 120 d for HCHF, total cholesterol value at 30 d for HCHF, and HDL-cholesterol value at 30 d or 120 d for HCHF (Table 3).

Fatty acid composition of liver phospholipids

The levels of the nutritionally important PUFA, linoleic acid (18 : 2*n*-6), AA (20 : 4*n*-6) and DHA (22 : 6*n*-3) are shown in Table 4. Time of study had significant ($P < 0{\cdot}05$) effects on

Table 4. *Effect of feeding casein or gelatin diets on linoleic, arachidonic (AA) and docosahexaenoic acid (DHA) levels (g/100 g total fatty acids) in liver phospholipids of rats*

(Mean values and standard deviations for five animals per group)

	18 : 2*n*-6				AA				DHA			
	30 d		120 d		30 d		120 d		30 d		120 d	
Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HCLF	10·4^{a}	1·7	7·4de	1·2	23·3^{ab}	3·9	27·6^{ab}	2·7	7·8^{ab}	1·9	10·2^{abc}	2·0
HCHF	10·9^{a}	1·9	7·0^{e}	0·6	26·7^{a}	4·6	31·2^{a}	1·4	10·3^{ab}	2·2	12·4^{a}	1·5
LCLF	12·6^{a}	0·2	9·3^{b}	0·8	22·3^{a}	2·4	23·9^{b}	2·8	8·2^{ab}	1·1	8·2^{cd}	1·3
LCHF	13·9^{a}	0·9	10·9^{a}	0·9	21·7^{ab}	1·2	28·0^{ab}	1·2	8·2^{ab}	0·7	9·1^{bcd}	0·7
HGLF	13·0^{a}	1·3	8·5^{cd}	0·9	22·9^{ab}	2·7	28·1^{ab}	2·6	9·6^{ab}	1·1	10·3^{ab}	0·7
HGHF	14·6^{a}	0·6	11·5^{a}	0·8	21·0^{ab}	0·4	27·9^{ab}	1·6	7·2^{ab}	0·9	9·9^{bc}	0·7
LGLF	13·3^{a}	1·0	9·8^{bc}	0·6	20·9^{ab}	2·6	27·4^{ab}	1·4	7·6^{ab}	2·1	9·7^{bc}	0·7
LGHF	13·5^{a}	0·7	10·7^{ab}	1·1	20·3^{b}	3·9	25·9^{ab}	1·3	6·8^{b}	2·0	7·3^{d}	1·4

H, high; L, low; C, casein; G, gelatin; F, fat.

^{a,b,c,d,e} Mean values within a column not sharing a common superscript letter were significantly different, $P < 0·05$.

the concentration of all three PUFA. The average concentrations of AA (the most important metabolite of 18 : 2*n*-6) and DHA (the most important metabolite of α-linolenic acid) for all the dietary groups were higher at 120 d than at 30 d (27·5 (SD 1·9), 9·6 (SD 1·0) *v.* 22·4 (SD 2·7) and 8·2 (SD 1·5) g/100 g total fatty acids respectively) but the average concentration of 18 : 2*n*-6 was lower at 120 d than at 30 d (9·4 (SD 0·9) *v.* 12·7 (SD 1·0) g/100 g total fatty acids). The level of α-linolenic acid (not shown in Table 4), the precursor of long-chain *n*-3 PUFA, in liver PL was low (0·1–0·3 g/100 g total fatty acids), and was unaffected by diet or age.

The level of fat in the diet also significantly influenced the concentration of 18 : 2*n*-6 in the liver, the average concentration being higher ($P < 0·05$) at high levels of fat (HCHF, LCHF, HGHF and LGHF) than at low levels of fat (LCLF, HCLF, LGLF and HGLF) (11·6 (SD 0·9) *v.* 10·5 (SD 1·0) g/100 g total fatty acids). Protein source × protein levels also had a significant effect on the concentrations of 18 : 2*n*-6 and AA. The high-casein groups (HCLF and HCHF) had higher ($P < 0·05$) average concentrations of AA but lower concentrations of 18 : 2*n*-6 than the low-casein groups (LCLF and LCHF) (27·2 (SD 3·0), 8·9 (SD 1·3) *v.* 24·0 (SD 1·9) and 11·7 (SD 0·7) g/100 g total fatty acids respectively).

DISCUSSION

All the experimental diets met or exceeded the indispensable amino acid requirements for rat maintenance as specified by the National Research Council (1978). Although the requirements are based on a diet containing 48 g protein/kg from casein and 50 g fat/kg, extrapolation of the requirement to 300 g protein/kg and 150 g fat/kg diets was assumed to be safe because a constant amino acid : energy ratio was maintained (National Research Council, 1978). The fact that there were no significant differences in body weights of rats fed on different diets for 30 d would confirm the nutritional adequacy of the diets for rat maintenance. Similarly, the differences in body weights of rats fed on various experimental diets for 120 d were small.

In the present study, the average levels of serum triacylglycerols, cholesterol and HDL-cholesterol were significantly lower in rats fed on the gelatin diets compared with those fed on the casein diets. The hypocholesterolaemic effect of gelatin (an animal

protein) observed in the present study has not been reported previously. After 120 d of testing, the levels of serum triacylglycerols and cholesterol in rats fed on the HGHF diet were less than one third and half of the levels in rats fed on the HCHF diet respectively. Gelatin is an incomplete protein and the present study is not advocating the consumption of unsupplemented gelatin for human nutrition. The marked hypocholesterolaemic effect of the HGHF diet (gelatin protein supplemented with small amounts of isoleucine, methionine and tryptophan) noted in this study should, however, be investigated in human subjects to evaluate its potential pharmacological effect in lowering serum cholesterol levels. Treatment with pravastatin was reported to lower plasma cholesterol levels by 20 % and LDL-cholesterol by 26 % in men with hypercholesterolaemia (Shepherd *et al.* 1995).

A comparison of the amino acid compositions of casein and gelatin (both animal proteins) studied in this investigation revealed that casein contained higher levels of glutamic acid, methionine, phenylalanine and tyrosine (cholesterol-raising amino acids) than gelatin, while gelatin contained a higher level of arginine (cholesterol-lowering amino acid) than casein. This would lend support to the suggestion that the differences in amino acid composition of proteins (regardless of their source, animal or plant) may be partly responsible for the alterations of plasma cholesterol level in rats fed on different animal and plant proteins (Sautier *et al.* 1983, 1986; Jacques *et al.* 1986; Sugiyama & Muramatsu, 1990). Gelatin also contained about twelve times more glycine than casein. Apart from its contribution to protein synthesis, glycine is required in disproportionately large amounts for the formation of haem, creatine, collagen, nucleic acids and bile salts (Jackson, 1991). The requirement for the endogenous synthesis of glycine was reported to be between ten and fifty times the dietary intake (Neuberger, 1981). Therefore, it is possible that the extremely high level of glycine in gelatin may be partly responsible for its hypocholesterolaemic activity. Further experiments involving amino acid supplementation of casein and gelatin are required to prove conclusively that the widely different cholesterolaemic responses of the two animal protein sources noted in the present study are due to differences in their amino acid profiles.

The present study also shows that the elevation of the serum lipids (triacylglycerols, total cholesterol and HDL-cholesterol) is accompanied by changes in the liver PL fatty acid profile. Feeding of the HCHF diet to rats resulted in reduced levels of 18 : 2*n*-6 but increased levels of AA and DHA in liver phospholipids. This increase in the *n*-6 and *n*-3 metabolites of 18 : 2*n*-6 and α-linoleic acid might indicate an increase in Δ6 desaturase activity. Similar observations about the influence of a high-casein diet on the metabolism of 18 : 2*n*-6 have been made previously (Koba & Sugano, 1990; Koba *et al.* 1990, 1991, 1993).

Koba & Sugano (1990) showed that casein (200 g/kg diet) promoted desaturation of 18 : 2*n*-6 in rat liver phosphatidylcholine (PC) compared with soyabean protein isolate (200 g/kg diet). The effect of protein sources was detected within 4 d after feeding different proteins and persisted even after an overnight fast. The addition of arginine to the casein diet tended to increase linoleic acid and to decrease AA in rat liver PC, while the effect of lysine addition was inconclusive. The magnitude of linoleic desaturation (18 : 3*n*-6 + 20 : 3*n*-6 + 20 : 4*n*-6)/18 : 2*n*-6) was similar in rats fed on casein or potato protein but was significantly lower in rats fed on soyabean protein (Koba & Sugano, 1990). Soyabean protein contained twice as much arginine as casein or potato protein, while the lysine : arginine ratios for casein, potato protein and soyabean protein were 2·1, 1·5 and 0·9 respectively, suggesting that the arginine content rather than the lysine : arginine ratio was at least one of the factors for the protein-dependent regulation of linoleic acid metabolism (Koba & Sugano, 1990). In the present investigation, the lowest levels of AA and DHA

were found in rats fed on the LGHF diet while highest levels of AA and DHA were found in rats fed on the HCHF diet. Since gelatin contained twice the level of arginine than casein, it is possible that arginine may have some influence on the metabolism of essential fatty acids.

In the present study, the HCHF diet not only increased the concentration of serum cholesterol but also increased the liver PL levels of AA and DHA compared with the gelatin diets. This observation is in general agreement with the data of Koba *et al.* (1993) who observed in rats that dietary protein modulates microsomal cholesterol levels, cholesterol : phospholipid ratio and membrane fluidity, and subsequently the activity of Δ6 desaturase in rat liver microsomes. Based on the present study and on the observations of Koba *et al.* (1993), it is suggested that a protein source which increases serum cholesterol may also promote the biosynthesis of essential fatty acids, AA and DHA.

The authors thank Laurent Samure, Kevin Kittle and Dominique Patry for the care of the animals. The technical assistance of G. Pelletier, H. G. Botting and J. Matte is greatly appreciated.

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