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Effect of Different Dietary Proteins on Plasma and Liver Fatty Acid Compositions in Growing Rats (42271)

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Abstract. Weanling male rats were fed semipurified diets containing 20% protein derived from casein, soy protein, or wheat gluten for 5 weeks. The fat source was 2% safflower oil (containing 79.4% 18:2n–6). In comparison with the casein-fed rats, the growth rate of rats fed plant proteins was slower, particularly that of rats fed the wheat gluten diet. The plasma cholesterol levels were significantly lower in rats fed wheat gluten than in those fed the other dietary protein regimens. The fatty acid compositions of plasma and liver lipids were also examined. The ratios of 16:1/16:0, 18:1/18:0 and 20:4n–6/18:2n–6 in both plasma and liver phospholipids and cholesteryl esters were consistently reduced in animals fed plant protein suggesting a reduced activity of fatty acid desaturation. The reduction of fatty acid desaturation may possibly be attributed to the low lysine/arginine ratio in plant proteins.

The source of dietary protein plays an important role in the regulation of the concentration of plasma cholesterol (CH) in experimental animals. Plant protein, in particular soybean protein, exerts a hypocholesterolemic effect as compared with animal protein such as casein (1–5). Although the mechanism has not been completely elucidated, reports have shown that the fecal excretion of neutral and acidic steroids in animals given soybean protein is greater than those given casein (6–12). The CH-lowering effect of polyunsaturated fats (13–15) has also been suggested by many investigators to be based on a similar mechanism. Since the hypocholesterolemic responses to intakes of plant proteins have been demonstrated when the animals were fed a CH-free diet with a low fat or a low essential fatty acid (EFA) content (3, 4, 9), it is possible that plant proteins might have altered the metabolism of fatty acids, in particular EFA, and possibly potentiated their effects. The aim of this report was to obtain basic information regarding the effect of dietary protein from different sources on the metabolism of plasma and liver fatty acids in animals receiving a semipurified diet containing 2% safflower oil.

Materials and Methods. Weanling male Sprague–Dawley rats (approximately 50 g) obtained from Charles River Breeding Laboratories (Montreal, Quebec, Canada) were randomly separated into four groups of six animals each and were fed *ad libitum* a semipurified diet containing 20% protein of different sources (vitamin-free casein, soy assay protein, and wheat gluten) for 5 weeks. The fat source was 2% safflower oil. The composition of the diets is shown in Table I. A separate group of rats ($n = 6$) was maintained on regular chow and was used as reference group only. All animals were weighed weekly. The food consumption was also recorded.

At the end of the experiment, animals were sacrificed by exsanguination under light ether anesthesia. Blood was collected by heart puncture into a test tube containing EDTA (1 mg/ml blood). Plasma was separated by centrifugation at 4°C. Livers were rapidly excised, rinsed with ice-cold saline, blotted, weighed, and frozen. Liver lipids were extracted with a chloroform–methanol mixture (16). Concentrations of liver CH were measured by gas liquid chromatography (GLC) using 5-α-cholestane as an internal standard (17). Liver triglycerides (TG) were measured colorimetrically by the method of Van Handel and Zilversmit (18). Plasma CH and TG were measured enzymatically using a Cobas-Bio centrifugal analyzer (Hoffman–La Roche). Plasma and liver phospholipids (PL) were measured by the method of Stewart (19). Cholesteryl esters (CE), TG, and PL were separated by thin-layer chromatography and the fatty acid compositions were determined as previously described (20). Data were compared statistically by the Student–Neuman–Keuls multiple range test (21).

Results and Discussion. The body weight

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TABLE I. COMPOSITION OF DIETS (g/100 g FEED)

Component	Diet		
	Casein	Soy	Wheat gluten
Casein[a]	20	—	—
Soy protein[b]	—	20	—
Wheat gluten[c]	—	—	20
Sucrose	67.3	67.3	67.3
Safflower oil[d]	2	2	2
Cellulose	5	5	5
Mineral mix[e]	5	5	5
Vitamin mix[f]	0.5	0.5	0.5
Choline chloride	0.2	0.2	0.2

[a] Containing 90.6% protein ($N \times 6.38$).
[b] Containing 86.8% protein ($N \times 6.25$).
[c] Containing 75.7% protein ($N \times 5.70$).
[d] Containing 79.4% 18:2n − 6, 10.7% 18:1, 6.9% 16:0, and 2.1% 18:0.
[e] AIN-76 providing as g/kg of diet, $CaHPO_4$, 500; NaCl, 74; K_2SO_4, 52; MgO, 24; KIO_4, 0.01; $CrK(SO_4) \cdot 12H_2O$, 0.55; $MnCO_3$, 3.5; $ZnCO_3$, 1.6; $CuCO_3$, 0.3; potassium citrate, 220; ferric citrate, 6.0; and sucrose, 118.03.
[f] Providing as g/kg of diet, choline dihydrogen citrate, 349.7; ascorbic acid, 101.7; vitamin E acetate, 24.2; inositol, 11.0; *p*-aminobenzoic acid, 11.0; niacin, 9.9; calcium panthothenate, 6.6; menadione, 5.0; vitamin A palmitate, 4.0; vitamin B_{12}, 3.0; pyridoxine, 2.2; riboflavin, 2.2; thiamine HCl, 2.2; vitamin D_2, 0.4; folic acid, 0.2; biotin, 0.044; and corn starch, 466.7.

gain of the casein-fed rats was not different from that of the chow-fed rats (data not shown) indicating an adequate nutritional state of these animals. On the other hand, the body weight gain of animals fed the wheat gluten diet was significantly lower than that in the animals fed the other diets. The growth rate of animals fed on soy protein was lower than that of casein-fed animals but was significantly greater than that of wheat-gluten-fed animals (Table II). Signs of alopecia and scaly tail which are similar to the symptoms of EFA deficiency were also observed in animals fed the wheat gluten diet. However, these symptoms could not be attributed to the deficiency of EFA as the analyses of fatty acid profiles in plasma and liver show that high levels of EFAs were present in animals fed all three diets. The liver weight was greater in the casein-fed animals than that of other dietary groups (Table II). However, there were no differences in the concentrations of liver CH and PL in animals fed different dietary proteins.

Table II also shows the effect of dietary proteins on plasma lipids. The plasma CH levels in wheat-gluten-fed animals were significantly lower than the others, whereas the plasma TG levels were highest in casein-fed animals. Mokady and colleagues (22–24) have demonstrated that the growth retardation of wheat-gluten-fed animals was due to protein malnutrition, but the hypocholesterolemic effect of wheat gluten was independent of the protein malnutrition. In the present study, the food

TABLE II. EFFECT OF DIETARY PROTEINS ON BODY AND LIVER WEIGHTS, FOOD INTAKES, AND LIVER AND PLASMA LIPID LEVELS

	Protein source		
	Casein	Soy	Wheat gluten
Body wt (g)	288.2 ± 25.0[a]	225.6 ± 14.5[b]	78.8 ± 9.2[c]
Liver wt (g)	16.4 ± 2.0[a]	11.5 ± 0.6[b]	4.0 ± 0.4[c]
(% BW)	5.7 ± 0.2[a]	5.1 ± 0.2[b]	5.1 ± 0.6
Food intake (g/100 g BW/day)	11.8 ± 0.9	12.2 ± 1.0	10.3 ± 1.7
Plasma (mg/dl)			
CH	60.8 ± 15.0[a]	59.4 ± 13.8[a]	33.4 ± 6.3[b]
TG	216.0 ± 50.7[a]	124.4 ± 26.0[b]	119.5 ± 49.9[b]
PL	130.5 ± 38.6	116.0 ± 35.1	100.2 ± 45.2
Liver (mg/g)			
CH	2.4 ± 0.6	2.0 ± 0.2	2.2 ± 0.8
TG	8.0 ± 1.0	8.0 ± 2.0	7.0 ± 1.0
PL	24.0 ± 5.4	24.4 ± 2.6	23.3 ± 1.8

Note. Abbreviations: CH = cholesterol, TG = triglycerides, PL = total phospholipids. Values (means ± SD) with different superscripts are significantly different at $P < 0.05$ or better.

TABLE III. EFFECT OF DIETARY PROTEINS ON MAJOR FATTY ACID COMPOSITIONS (mg/100 mg TOTAL FATTY ACIDS) OF PLASMA AND LIVER CHOLESTERYL ESTERS

Fatty acid	Protein source		
	Casein	Soy	Wheat gluten
Plasma			
16:0	5.5 ± 0.8[a]	5.7 ± 0.2[a]	8.7 ± 0.5[b]
16:1	4.0 ± 1.7[a]	2.9 ± 1.1	1.6 ± 0.5[b]
18:0	0.9 ± 0.5	0.9 ± 0.2	2.0 ± 1.4
18:1	9.6 ± 1.1	12.0 ± 3.7	8.2 ± 0.7
18:2n−6	11.5 ± 0.8[a]	20.5 ± 2.2[b]	18.8 ± 2.5[b]
20:4n−6	66.4 ± 2.9[a]	55.1 ± 4.2[b]	59.0 ± 1.6[b]
Liver			
16:0	24.5 ± 4.1[a]	37.8 ± 3.0[b]	32.1 ± 5.5
16:1	15.2 ± 1.9[a]	8.1 ± 1.4[b]	6.6 ± 1.9[b]
18:0	4.3 ± 1.7[a]	7.9 ± 1.2[b]	8.2 ± 1.7[b]
18:1	32.3 ± 5.5[a]	25.0 ± 2.1[b]	22.5 ± 4.7[b]
18:2n−6	8.7 ± 1.3[a]	10.0 ± 2.4	12.3 ± 2.5[b]
20:3n−6	—	0.8 ± 0.5	0.8 ± 0.4
20:4n−6	13.3 ± 1.8[a]	8.8 ± 2.0	8.7 ± 1.3[b]

Note. Values (means ± SD) with different superscripts are significantly different at $P < 0.05$ or better.

intake in relation to body size was not significantly different in any of the groups (Table II). Although the actual protein content in the three diets varied slightly (75.7–90.6%), the actual protein consumption calculated (2.1, 2.1, and 1.6 g/100 g body wt/day, respectively, for casein, soy protein, or wheat-gluten-fed rats) was also not different. Therefore, the slower growth of wheat-gluten-fed rats was unlikely to be a result of the relative protein deficiency and was more likely due to the difference of protein quality in the diets.

The fatty acid composition of plasma and liver CE from rats fed on the different diets is shown in Table III. The proportions of 16:1 and 20:4n−6 in plasma and in liver CE and that of 18:1 in liver CE were significantly higher, whereas the levels of 18:2n−6, 16:0, and 18:0 were significantly lower in rats fed the casein diet in comparison with those fed the soybean or wheat gluten diets. As a result, the ratios of 16:1/16:0, 18:1/18:0, and 20:4n−6/18:2n−6 in rats fed the casein diet were significantly higher than those in rats fed the plant proteins. Between the two plant protein-fed groups, the ratios of 16:1/16:0 and 18:1/18:0 in plasma CE were much lower in wheat-gluten-fed animals (Table III).

Table IV shows the fatty acid compositions of plasma and liver TG. Feeding the rats casein caused a significant increase in 16:0 and 16:1 levels and a decrease in 18:2n−6 and 20:4n−6 levels. Among the three groups, the levels of 16:0 and 16:1 were inversely related to the levels of 18:2n−6 and 20:4n−6.

Distribution of fatty acids in plasma and liver PL are shown in Table V. In animals fed soy protein, the proportions of 16:0 and 18:2n−6 were significantly higher whereas those of 20:4n−6 and 22:5n−6 were significantly lower in comparison with the animals fed casein. Similar to soy protein feeding, wheat gluten feeding also significantly increased the levels of 18:2n−6, and increased to a greater extent the levels of 22:5n−6 and 22:6n−3. However, the levels of 20:4n−6 in wheat-gluten-fed animals were not different from those in casein-fed rats.

Table VI shows the distribution of TG fatty acids in epididymal fat pad. The levels of 16:1 and 18:1 in different protein groups were decreased in the following order: casein > soy protein > wheat gluten. On the other hand, the levels of 18:2n−6 were increased and followed a reverse order of casein < soy protein < wheat gluten.

TABLE IV. EFFECT OF DIETARY PROTEINS ON MAJOR FATTY ACID COMPOSITIONS (mg/100 mg TOTAL FATTY ACIDS) OF PLASMA AND LIVER TRIGLYCERIDES

Fatty acid	Protein source		
	Casein	Soy	Wheat gluten
Plasma			
16:0	26.5 ± 3.8[a]	20.9 ± 6.3[b]	12.6 ± 3.5[c]
16:1	15.1 ± 2.5[a]	9.3 ± 1.7[b]	3.0 ± 1.4[c]
18:0	2.4 ± 1.4[a]	2.5 ± 1.6[a]	7.9 ± 3.3[b]
18:1	35.3 ± 2.5[a]	36.8 ± 4.4[a]	24.0 ± 2.0[b]
18:2n−6	16.0 ± 4.2[a]	27.3 ± 9.0[b]	43.2 ± 4.4[c]
20:4n−6	1.5 ± 0.4	3.1 ± 1.5[a]	8.4 ± 5.6[b]
Liver			
16:0	32.6 ± 1.9	35.2 ± 1.6	32.1 ± 2.1
16:1	15.3 ± 1.9[a]	13.7 ± 1.1[a]	6.4 ± 1.6[b]
18:0	2.3 ± 0.6[a]	2.1 ± 0.4[a]	5.3 ± 1.1[b]
18:1	38.1 ± 1.8[a]	35.8 ± 2.5	32.4 ± 2.1[b]
18:2n−6	3.9 ± 1.6[a]	8.2 ± 3.4[b]	17.0 ± 2.4[c]
20:4n−6	0.7 ± 0.4[a]	1.6 ± 0.6	2.3 ± 0.7[b]

Note. Values (means ± SD) with different superscripts are significantly different at $P < 0.05$ or better.

Our results suggest that plant protein feeding might have affected the distribution of long chain fatty acids between TG and other lipid fractions as shown by an increase of 20:4n−6 levels in TG and a decrease of 20:4n−6 levels in PL. The levels of 22:5n−6 and 22:6n−3, products of elongation and desaturation of 18:2n−6 and 18:3n−3, respectively, were found to be increased in plasma and liver PL of wheat-gluten-fed rats, suggesting the metabolism of both n-6 and n-3 fatty acids were active. On the other hand, the ratios of 16:1/16:0, 18:1/18:0, 20:4n−6/18:2n−6, and 22:5n−6/22:4n−6 in lipids of plant protein-fed rats were significantly reduced suggesting that the activities of fatty acid desaturation in these animals were decreased. The reduction was generally greater in animals fed wheat gluten than in animals fed soy protein.

The cause of this difference, however, is not clear. Tomas *et al.* (25) have reported that protein restriction during early development in the rat reduced the activities of Δ^9-, Δ^6-, and Δ^5-desaturases in liver and the levels of 20:4n−6 in liver PL. In this study, the reduced ratios of 20:4n−6/18:2n−6 in wheat-gluten-fed rats were the result of elevated 18:2n−6 levels rather than a reduced 20:4n−6 level. Furthermore, the actual protein consumption in relation to body size was not different from one to another group. Therefore, it is unlikely that protein deficiency was responsible for the reduced ratios of 20:4n−6/18:2n−6. Kritchevsky and colleagues (2, 6, 26, 27) and others (28) have attributed the hypocholesterolemic effect of soybean in rabbits to its low ratio of lysine/arginine. Although this effect has not been demonstrated in rats (29), the ratio of 20:4n−6/18:2n−6 in animals fed casein, soy protein, and wheat gluten appeared to be in line with the respective dietary lysine contents (0.72, 0.63, and 0.12%) and the dietary lysine/arginine ratios (2.12, 0.84, and 0.39). It is possible therefore that the same reasoning may also be applicable to the present results and that reduced ratios of 20:4n−6/18:2n−6 in rats fed plant protein are a function of low lysine/arginine ration in the diet.

TABLE V. EFFECT OF DIETARY PROTEINS ON MAJOR FATTY ACID COMPOSITIONS (mg/100 mg TOTAL FATTY ACIDS) OF PLASMA AND LIVER PHOSPHOLIPIDS

	Protein source		
Fatty acid	Casein	Soy	Wheat gluten
Plasma			
16:0	18.2 ± 1.8[a]	22.2 ± 2.0[b]	12.6 ± 4.4[c]
16:1	2.1 ± 0.6	2.0 ± 0.6	—
18:0	12.2 ± 1.4	10.9 ± 0.6	12.4 ± 2.3
18:1	10.5 ± 0.3	11.9 ± 2.6	9.1 ± 4.0
18:2n−6	18.6 ± 1.0[a]	25.7 ± 2.6[b]	25.7 ± 4.8[b]
20:3n−6	1.1 ± 0.2	1.0 ± 0.1	—
20:4n−6	32.8 ± 1.6[a]	22.0 ± 3.2[b]	32.2 ± 2.3[a]
22:4n−6	—	0.6 ± 0.03[a]	1.4 ± 0.2[b]
22:5n−6	4.6 ± 0.9[a]	2.4 ± 0.5[b]	6.5 ± 0.8[c]
22:6n−3	—	1.6 ± 0.3	2.6 ± 1.0
Liver			
16:0	17.3 ± 0.6[a]	19.5 ± 0.7[b]	18.8 ± 0.6[b]
16:1	4.5 ± 1.0	3.8 ± 0.6	2.8 ± 1.4
18:0	15.1 ± 2.6	14.9 ± 3.2	15.4 ± 3.5
18:1	10.8 ± 1.0[a]	9.1 ± 0.5[b]	5.4 ± 0.7[c]
18:2n−6	9.6 ± 0.7[a]	13.2 ± 0.4[b]	11.8 ± 2.0
20:3n−6	1.7 ± 0.2[a]	2.4 ± 0.4[b]	0.8 ± 0.4[c]
20:4n−6	30.9 ± 2.4[a]	26.7 ± 1.9[b]	30.7 ± 2.2[a]
22:4n−6	0.7 ± 0.2[a]	0.7 ± 0.1[a]	1.6 ± 0.4[b]
22:5n−6	6.2 ± 0.9[a]	4.4 ± 0.6[b]	8.4 ± 2.7[a]
22:5n−3	—	0.5 ± 0.07[a]	0.7 ± 0.1[b]
22:6n−3	1.3 ± 0.2[a]	3.0 ± 0.2[a]	4.4 ± 0.7[c]

Note. Values (means ± SD) with different superscripts are significantly different at $P < 0.05$ or better.

TABLE VI. EFFECT OF DIETARY PROTEINS ON MAJOR FATTY ACID COMPOSITIONS (mg/100 mg TOTAL FATTY ACIDS) OF TRIGLYCERIDES IN EPIDIDYMAL FAT PADS

	Protein source		
Fatty acid	Casein	Soy	Wheat gluten
Plasma			
14:0	4.4 ± 0.9	3.4 ± 0.3[a]	5.0 ± 0.5[b]
16:0	27.9 ± 0.9	28.2 ± 1.8	27.6 ± 3.3
16:1	17.2 ± 1.8[a]	13.9 ± 0.6[b]	10.4 ± 2.6[c]
18:0	1.7 ± 0.1[a]	2.3 ± 0.1[b]	2.7 ± 0.3[c]
18:1	31.5 ± 1.2[a]	29.5 ± 1.2[b]	23.0 ± 2.4[c]
18:2n−6	15.7 ± 2.2[a]	20.0 ± 3.1[b]	27.1 ± 7.3[b]
18:3n−3	—	0.3 ± 0.04[a]	0.6 ± 0.1[b]
20:4n−6	0.4 ± 0.1[a]	0.4 ± 0.05[a]	1.1 ± 0.4[b]
22:6n−3	—	—	0.2 ± 0.1

Note. Values (means ± SD) with different superscripts are significantly different at $P < 0.05$ or better.

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