Effects of Dietary Protein Levels and Cholesterol on Tissue Lipids of Rats

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Summary The effects of the levels (10, 20, and 30%) of dietary proteins, soybean protein or casein, on various lipid parameters were examined in rats. The plasma cholesterol (CHOL) level tended to decrease with an increasing dietary protein level, in particular when diets contained CHOL. The hypocholesterolemic effect of soybean protein was evident with CHOL-enriched diets. The fecal excretion of acidic but not neutral steroids increased with an increasing protein level and it was higher in rats fed soybean protein than in those fed casein. The ratio of arachidonate to linoleate in plasma and liver phosphatidylcholine tended to increase with an increasing dietary protein level, although it was considerably higher in casein than in soybean protein diets. The results indicated that the hypocholesterolemic effect of soybean protein is influenced not only by the amount but also by the presence or absence of dietary CHOL. In addition, the degree of conversion of linoleate to arachidonate also depended on the type and level of dietary protein. Key Words cholesterol, dietary protein, plasma cholesterol, fatty acids of phosphatidylcholine

The source of dietary protein influences the plasma cholesterol (CHOL) level in experimental animals and humans (1, 2), and in general soybean protein is hypocholesterolemic compared to casein. The amount of dietary protein is also a determinant of the plasma cholesterol level. Despite numerous studies, the mechanism underlying the hypocholesterolemic effect of vegetable protein is not clear. The type of dietary protein also appears to modify the desaturation of linoleate to arachidonate (3). Since several lines of studies suggest the possible involvement of eicosanoids in the regulation of CHOL metabolism (4), it seems likely that changes in the metabolism of polyunsaturated fatty acids (PUFA) may be relevant to the protein-dependent changes in the plasma CHOL level. Alternatively, studies relating the interaction of dietary protein with metabolism of PUFA may provide a useful clue to the mechanism of the hypocholesterolemic action of vegetable proteins.

In the present study, the effects of differences in the types and levels of dietary proteins on the plasma and liver CHOL level, steroid excretion, and fatty acid composition of tissue phosphatidylcholine were examined in rats fed either casein or soybean protein with or without CHOL.

Materials and methods

Animals and diets Male Wistar rats, weighing 130–160 g, obtained from Kyudo Co., Kumamoto, were randomly divided into 6 groups of 6 animals each in experiments 1 (CHOL-free diets) and 2 (CHOL-enriched diets). The composition of experimental diets is shown in Table 1. Ten, 20, and 30% levels of casein or soybean protein isolate were used, with or without CHOL and sodium cholate. Animals were fed their respective diets for 30 days ad libitum. At the end of the feeding period, the animals were starved overnight and blood were collected by heart puncture under Na-pentobarbital anesthesia into tubes containing heparin.

Plasma and liver lipids were extracted and analyzed for CHOL (5). Plasma and liver phosphatidylcholine (PC) were separated by thin-layer chromatography and the fatty acid composition was analyzed by gas-liquid chromatography (GLC) (6). Feces were collected for the last 3 days of the experiments and freeze-dried. Neutral and acidic steroids were analyzed by GLC (7, 8).
Table 1. Composition of diets.

| Components     | C10 | C20 | C30 | S10 | S20 | S30 |
|----------------|-----|-----|-----|-----|-----|-----|
| Casein         | 10  | 20  | 30  |     |     |     |
| Soybean protein|     |     |     | 10  | 20  | 30  |
| Corn oil       | 5   | 5   | 5   | 5   | 5   | 5   |
| Mineral mixture | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Cellulose      | 4   | 4   | 4   | 4   | 4   | 4   |
| Corn starch    | 75.5| 65.5| 55.5| 75.5| 65.5| 55.5|

Cholesterol and sodium cholate were added at 0.5 and 0.125% levels, respectively, at the expense of corn starch when the diets were CHOL-enriched diets. 1Haper mixture.

Results

Plasma and liver CHOL The concentrations of plasma CHOL is shown in Fig. 1. The concentration of plasma CHOL decreased with an increasing level of dietary protein irrespective of the presence or absence of dietary CHOL when rats were fed soybean protein diets. When rats were fed CHOL-enriched diets, the plasma CHOL level tended to be lower in the soybean protein group than in the casein group.

Fig. 2. Concentration of liver CHOL of rats fed diets containing varying levels of casein and soy protein with or without CHOL. Means of 6 rats. *Significantly different (p<0.05) between the casein and soy protein groups. ——, Casein; …——, Soy protein.

The concentration of liver CHOL is shown in Fig. 2. The concentration of liver CHOL decreased with an increasing dietary protein level irrespective of the presence or absence of dietary CHOL. Although the CHOL concentration in the soybean group tended to be higher than that of casein group in CHOL-free diets, it was much lower in the CHOL-enriched diet, especially on the high-protein regimen.

Fecal neutral and acidic steroid excretion The results of the neutral and acidic steroid excretion are shown in Figs. 3 and 4. The dietary protein level had little influence on neutral steroid excretion although excretion tended to be higher in soybean protein than in casein diets enriched with CHOL. Except for rats fed CHOL-enriched soybean protein diets, acidic steroid excretion was influenced by the dietary protein level in both casein and soybean protein, it increased with an increasing dietary level of protein. In addition, in CHOL-free but not CHOL-enriched diets acidic steroid excretion was considerably higher in the vegetable protein groups than in their casein counterparts.
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Fig. 3. Neutral steroid excretion of rats fed diets containing varying levels of the casein and soy protein with or without CHOL. Means of 6 rats. *Significantly different (p<0.05) between the casein and soy protein groups. □, Casein; □, Soy protein.

Fig. 4. Acidic steroid excretion of rats fed diets containing varying levels of the casein and soy protein with or without CHOL. Means of 6 rats. *Significantly different (p<0.05) between the casein and soy protein groups. □, Casein; □, Soy protein.

Fatty acid composition of liver phosphatidylcholine  Fatty acid compositions of liver PC are shown in Tables 2 and 3. When rats were fed diets free of CHOL, the percentage of linoleate in liver PC decreased but that of arachidonate increased with an increasing dietary casein level. When CHOL was added, the percentages of these PUFA remained unchanged in both protein diets. However, rats fed CHOL-enriched soybean protein diets had a higher proportion of linoleate than those fed the corresponding casein diets. A similar response was observed in plasma PC (data not shown).

Since the proportion of linoleate was high and that of arachidonate was low in soybean protein
Table 2. Fatty acid composition of liver PC in rats fed CHOL-free diets.

| Group | 16:0     | 18:0     | 18:1     | 18:2     | 20:4     |
|-------|----------|----------|----------|----------|----------|
| C10   | 23.1 ± 0.5a | 20.2 ± 0.2a | 6.6 ± 0.3a | 8.2 ± 0.4a | 28.5 ± 1.0a |
| C20   | 21.1 ± 0.5b | 21.0 ± 0.5ab | 5.5 ± 0.3b | 7.0 ± 0.4b | 31.2 ± 1.0b |
| C30   | 20.7 ± 0.3b | 22.9 ± 0.7b | 5.9 ± 0.2ab | 6.8 ± 0.2b | 31.4 ± 1.1b |
| S10   | 34.9 ± 1.2** | 24.5 ± 0.5** | 7.3 ± 0.2a | 9.5 ± 0.3a | 22.9 ± 1.3** |
| S20   | 19.1 ± 1.6c | 21.1 ± 0.4c | 4.9 ± 0.2c | 7.7 ± 0.7c | 27.3 ± 1.0** |
| S30   | 21.1 ± 0.3c | 21.2 ± 0.4c | 4.6 ± 0.2** | 8.0 ± 0.3** | 30.4 ± 0.8c |

F-values

| Level | Type | Level × type |
|-------|------|--------------|
| s     | s    | s            |
| s     | s    | s            |
| s     | ns   | ns           |

Values are means±SE of 6 rats per group. a–b and x–z: Values in the same column not sharing common superscript letters are significantly different at p<0.05. *Significantly different between the casein and soybean protein groups with the same protein level. s, Significant; ns, not significant.

Table 3. Fatty acid composition of liver PC in rats fed CHOL-enriched diets.

| Group | 16:0     | 16:1     | 18:0     | 18:1     | 18:2     | 20:4     |
|-------|----------|----------|----------|----------|----------|----------|
| C10 + CHOL | 27.2 ± 0.6a | 4.7 ± 0.2a | 16.0 ± 0.7ab | 14.4 ± 0.5a | 12.2 ± 0.6a | 23.8 ± 0.8a |
| C20 + CHOL | 27.1 ± 0.9a | 4.6 ± 0.4a | 13.8 ± 0.8b | 14.6 ± 0.6a | 12.8 ± 0.7a | 25.0 ± 1.0a |
| C30 + CHOL | 24.2 ± 0.7a | 4.5 ± 0.4a | 18.0 ± 1.2a | 14.5 ± 0.9a | 11.6 ± 0.7a | 24.6 ± 1.4a |
| S10 + CHOL | 27.2 ± 0.5a | 4.8 ± 0.2a | 14.8 ± 0.4a | 14.4 ± 0.4a | 14.3 ± 0.6** | 22.8 ± 1.3* |
| S20 + CHOL | 26.5 ± 0.8a | 4.3 ± 0.3a | 15.7 ± 0.6a | 13.0 ± 0.6* | 15.3 ± 0.6** | 24.0 ± 1.0a |
| S30 + CHOL | 25.7 ± 0.9a | 3.8 ± 0.3a | 16.5 ± 1.5a | 13.1 ± 0.5a | 15.0 ± 0.2** | 24.2 ± 1.1a |

F-values

| Level | Type | Level × type |
|-------|------|--------------|
| s     | s    | ns           |
| ns    | s    | ns           |
| ns    | ns   | ns           |

Values are means±SE of 6 rats per group. a–b and x–z: Values in the same column not sharing common superscript letters are significantly different at p<0.05. *Significantly different between the casein and soybean protein groups with the same protein level. s, Significant; ns, not significant.

Group, the ratio of arachidonate to linoleate was considerably higher in the casein groups than in the soybean protein groups (Fig. 5). In rats fed diets free of CHOL the ratio increased with increasing dietary protein level both in liver and plasma PC. When CHOL was added to the diet, the protein level-dependent change disappeared, but the ratio still tended to be higher in the casein groups than in the soybean protein groups. In plasma PC, the ratio decreased linearly in rats fed the vegetable protein in response to the increase in the dietary protein level but it did not change in liver PC.

Discussion

The results of these studies indicated the possibility that the response of plasma CHOL to different dietary proteins is at least partly related to the ratio of arachidonate to linoleate of tissue PC; there was a negative correlation between these 2 parameters. Thus, the dietary protein-dependent change in the plasma CHOL concentration presumably is attributable to the activity Δ6-desaturase (9), because an increase in the concentration of arachidonate in plasma PC may stimulate the metabolism of CHOL by increasing the formation of cholesteryl arachidonate in plasma by lecithin : CHOL acyltransferase (10). In addition, the hypocholesterolemic effects of soybean protein and the ratio of arachidonate to linoleate in tissue PC were both dependent on the dietary protein level.

In this study, the hypocholesterolemic effect of soybean protein was observed in rats fed CHOL-
Fig. 5. Ratio of arachidonate to linoleate of phosphatidylcholine in liver of rats fed diets containing varying levels of casein and soy protein with or without CHOL. Means of 6 rats. *Significantly different ($p<0.05$) between the casein and soy protein groups. □, Casein; ◯, Soy protein.

enriched but not CHOL-free diets. The hypocholesterolemic effect of soybean protein compared to casein depends on the dietary CHOL level, and the difference becomes clear when CHOL-enriched diets were fed (11). The dietary fat level also modifies the effect of dietary protein and the hypocholesterolemic action of soybean protein was evident in low-fat diets (12).

In CHOL-free diets, bile acid excretion was higher in the soybean protein groups than in the casein groups, but the plasma CHOL concentration was not different between the 2 groups. Since feeding soybean protein stimulates hepatic CHOL synthesis (13), it seems likely that increased fecal steroid excretion is compensated by the enhanced synthesis. In addition, a similar phenomenon has been observed when cholestyramine was given to rats fed CHOL-free diets (14).

There is evidence supporting that the hypocholesterolemic effects of soybean protein in relation to casein is attributable to the increased fecal steroid excretion (15). In the present study, fecal excretion of steroids, in particular acidic steroids, was dependent more on the type of dietary protein rather than on the dietary level. The increased fecal steroid excretion may stimulate CHOL metabolism in the liver and because dietary CHOL interferes with the desaturation of linoleate (16) may lead to the modification of the ratio of arachidonate to linoleate in tissue PC.

The reduction of the arachidonate/linoleate ratio of tissue phospholipid by soybean protein compared to casein was mainly ascribed to the reduced conversion of linoleate to arachidonate. There is a possibility that soybean protein may reduce the production of eicosanoids which lower the plasma CHOL level. In fact, the production of PGI$_2$ and TXA$_2$ was clearly lower in rats fed soybean protein than in those casein (17). Alternatively, the reduction by soybean protein of the PUFA ratio may exclude the possibility that eicosanoids are involved in the hypocholesterolemic effect of the vegetable protein.

There was a trend for the plasma CHOL level to decrease as the level of dietary protein increased, particularly for soybean protein, and this was accompanied by increasing fecal acidic steroid excretion. The relationship was more evident in liver than in plasma CHOL. It is thus plausible that the increase in fecal bile acid excretion is the factor at
least responsible for the hypocholesterolemic effect of soybean protein (13, 14). In conclusion, this study offered evidence that both the type and the level of dietary protein modify not only the plasma CHOL level but also the arachidonate/linoleate ratio of tissue PC.

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