Serum and Liver Cholesterol Levels of Rats and Mice Fed Soy-Bean Protein or Casein

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Summary Rats and mice were fed soy-bean protein or casein diets for 10 and 50 weeks, respectively, during which terms their serum cholesterol levels were analyzed periodically. Rats fed high-cholesterol diets containing soy protein or the amino acid mixture simulating soy protein produced lower levels of serum cholesterol throughout the experiments, as compared with those on the corresponding casein-type diets. Feeding soy protein resulted in a significant decrease in serum apoA-I and apoB, but the relative concentration of high-density lipoprotein-cholesterol was kept at the higher level. The concentration of liver cholesterol was also lower in rats fed the plant protein. In mice fed a cholesterol-free diet, the cholesterol-lowering effect of soy protein was noticeable at an early stage of the feeding periods, by 20 weeks. The extent of lipid peroxidation in rats and mice determined as TBA-reactive substances in serum was found to be the same when protein diets were given, while it was significantly higher when an amino acid mixture of the soy protein type was fed to rats. The results confirm that soy protein exhibits its hypocholesterolemic effect even when a diet rich in cholesterol is fed. The cholesterol-lowering effect of soy proteins appears to be a phenomenon common to rodents.

Key Words soy-bean protein, casein, serum cholesterol, apolipoproteins, lipid peroxidation

Dietary protein has been found to have a significant effect on serum cholesterol levels in humans as well as experimental animals. In numerous studies that provided protein-dependent changes in serum cholesterol, comparisons have been made exclusively between animal and plant proteins, particularly casein and soy-bean protein, respectively (1–10). In general, soy protein produces lower levels of serum cholesterol than casein.

The antihypercholesterolemic effect of soy protein, however, appears to be modified by the level and type of dietary fats (6, 11). As reported previously (6), the

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cholesterol-lowering effect of soy protein in rats as compared with casein was most marked when cholesterol-free low-fat diets were fed. Yadav and Liener (10) showed, utilizing high-cholesterol diets, that in rats soy protein produced serum cholesterol levels which were significantly lower than when casein was fed, but the evaluation of this result was indeed restricted due to an insufficient supply of essential fatty acids in their diets. In our preceding trials with rats (6), addition of cholesterol to the diet markedly obscured the response of serum cholesterol to dietary regimens.

Kim et al. (8, 9) fed swine a high-fat, high-cholesterol diet and observed that animals on soy protein had a lower serum cholesterol as compared to those on casein. Sirtori et al. (3) also provided evidence that in type II hyperlipoproteinemic patients vegetable protein exerted a cholesterol-lowering effect relatively independent of the content of fats and cholesterol in diets.

The aim of the present study was to confirm the hypocholesterolemic action of soy protein in rats in the presence of dietary cholesterol. In addition, in order to know the species-specific difference in response to the type of dietary proteins, the effects of soy-bean protein and casein on the serum cholesterol level of mice was investigated. In the course of these studies, the concentration of serum apolipoproteins and the extent of lipoperoxidation were also measured.

EXPERIMENTAL

Animals and diets. Three experiments were undertaken to investigate the influence of dietary protein on serum cholesterol. In experiments 1 and 2, male Wistar rats (Kyudo Co., Kumamoto) weighing approximately 80 g were used. In experiment 3, male JAC ddy mice, obtained from the same breeder as above, weighing approximately 10 g were used. All animals were housed in an air-conditioned room at 20°C with a 12-hr (lights on at 0800 to 2000 hours) light cycle (rats individually and 5–6 mice in one cage) and were given each experimental diet and water ad libitum. Rats were fed for 10 weeks during which period blood was withdrawn from the tail vein every two weeks for serum cholesterol analysis, and were killed by decapitation at termination. Mice were kept for 50 weeks and serum cholesterol was similarly determined periodically. All blood samples from both rats and mice were obtained after an overnight fast. The nitrogen sources were casein (vitamin-free, ICN Pharmaceuticals Inc., Cleveland, Ohio) or soy-bean protein isolate (Fujipro R, Fuji Oil Co., Osaka) (6). The L-amino acid (Ajinomoto Co., Tokyo) mixtures simulating casein or soy protein were also served as a nitrogen source (7). The composition of the basal diet was (%): nitrogen source 20, mineral mixture 4, vitamin mixture (water soluble, (12)) 1, choline chloride 0.15, cellulose powder (type E, Toyo Co., Tokyo) 2 and sucrose to 100. Fat (corn oil) was added at the expense of sucrose; experiments 1 and 2, 1% and experiment 3, 5%. The mineral mixtures according to Harper (12) (obtained from Oriental Yeast Co., Tokyo) or Ebihara et al. (13) were used for protein diets and amino acid mixture diets, respectively. The diet contained retinyl palmitate 400 IU, cholecalciferol 200 IU and
DL-α-tocopheryl acetate 10 mg per 100 g. In experiments 1 and 2, cholesterol and sodium cholate were added at the level of 0.5% and 0.125%, respectively.

Lipid and apolipoprotein analyses. Lipids extracted from serum and liver according to Folch et al. (14) were analyzed for triglyceride and phospholipid as described previously (6). Serum and liver cholesterol were determined by the enzyme assay (Cholesterol C-Test, Wako Pure Chemicals Co., Osaka) and the method of Sperry and Webb (15), respectively. The concentration of serum apolipoproteins was measured by rocket immunoelectrophoresis (16). Serum TBA-reactive substances were determined by fluorometry according to Yagi (17). Serum high-density lipoprotein (HDL)-cholesterol was analyzed on the supernatant obtained after precipitation of very low-density (VLDL)- and low-density (LDL)-lipoproteins with dextran sulfate-MnCl₂ (18) and VLDL- plus LDL-cholesterol by difference (Daiichi Pharmaceutical Co., Tokyo). Cholesterol in adipose tissue was determined by gas-liquid chromatography using 5α-cholestane as an internal standard (19).

RESULTS

Experiments with rats on cholesterol-enriched diets

Table 1 shows weight gain, food intake, liver weight and the concentration of serum and liver glycerolipids. In each experiment there were no differences found in growth and food intake in relation to the type of dietary proteins or amino acid mixtures. The liver weight was significantly heavier on feeding casein than on soy protein. Serum phospholipid and liver triglyceride were significantly higher in rats fed casein, while no differences were observed when the amino acid mixture diets were fed.

The concentration of serum cholesterol in the course of 10 weeks of feeding is illustrated in Fig. 1. When intact protein was used as a nitrogen source (experiment 1, Fig. 1a), the casein group showed significantly higher levels of serum cholesterol throughout the experiments. The difference was evident even after 2 weeks of feeding. In contrast to the moderate progressive decrease in serum cholesterol of rats fed soy protein with time, the time course of serum cholesterol in rats fed casein varied considerably at higher levels. In experiment 2, where amino acid mixtures replaced intact proteins, serum levels of cholesterol of rats given the mixture simulating soy-bean protein was virtually invariable and was slightly but consistently lower than those of the animals fed the casein-type amino acid mixture (Fig. 1b). However, a significant difference was observed only after 10 weeks.

The serum concentrations of cholesterol in lipoproteins and of apolipoproteins are summarized in Table 2 together with those of TBA-reactive substances. Although the concentration of HDL-cholesterol remained unchanged after feeding different proteins, the ratio of HDL- to total cholesterol was significantly higher in the soy protein group than in the casein group, since soy protein reduced serum cholesterol levels markedly. Similar, but somewhat moderate responses were demonstrated when the amino acid mixture diet was fed. The difference in type of
Table 1. Body weight gain, food intake, liver weight, and serum and liver lipids in rats fed soy-bean protein or casein.

| Dietary regimens | Body weight gain (g/10 weeks) | Food intake (g/day) | Liver weight (g/100 g body weight) | Serum lipids (mg/100 ml) | Liver lipids (mg/g) |
|------------------|-------------------------------|---------------------|-----------------------------------|--------------------------|---------------------|
|                  |                               |                     |                                   | Triglyceride            | Phospholipid        |
| Exp. 1           |                               |                     |                                   |                          |                     |
| Soy protein      | 278 ± 9                      | 20.5 ± 0.4          | 2.86 ± 0.06                      | 91.2 ± 7.42             | 127 ± 5.8          |
| Casein           | 288 ± 15                     | 20.6 ± 0.5          | 3.44 ± 0.11                      | 81.3 ± 7.53             | 171 ± 12.5         |
| Exp. 2           |                               |                     |                                   |                          |                     |
| Soy protein AA   | 262 ± 15                     | 20.9 ± 0.6          | 3.05 ± 0.08                      | 120 ± 17.4              | 110 ± 11.1         |
| Casein AA        | 262 ± 13                     | 21.0 ± 0.5          | 3.15 ± 0.09                      | 108 ± 9.23              | 141 ± 17.4         |

*Mean ± SEM of 8 rats. *AA, amino acid mixture. *Significant difference at p < 0.05.
dietary proteins greatly influenced the content of serum apoA-I and apoB. Feeding soy protein caused significantly lower levels of these apolipoproteins. The extent of lipoperoxidation was apparently the same when protein diets were fed, but it was more prominent when the amino acid mixture of the soy protein-type was the dietary source of nitrogen.

Table 3 shows the concentration of cholesterol in the liver and adipose tissue. Casein and its amino acid mixture caused a significantly greater accumulation of cholesterol in the liver as compared with the corresponding soy protein groups. There was no marked difference in the cholesterol content of adipose tissue between the two dietary regimens.

Experiment with mice on cholesterol-free diets

As shown in Table 4, food intake, weight gain and liver weight were essentially the same for mice fed a low-fat cholesterol-free casein or soy protein diet. The concentration of liver cholesterol was significantly higher in mice fed casein than in those fed soy-bean protein.

The changes in serum cholesterol and TBA-reactive substances are illustrated in Fig. 2 as a function of feeding periods. Casein caused a significant elevation of serum cholesterol as compared with soy protein during the initial 20 weeks, while at
Table 2. Concentrations of serum cholesterol, apolipoproteins and lipoperoxide in rats fed soybean protein or casein.

| Dietary regimens | Serum cholesterol (mg/100 ml) | ApoA-I | ApoA-II | ApoB | Serum apolipoprotein (μg/ml) | Lipoperoxide (nmoles/ml) |
|------------------|--------------------------------|--------|---------|------|-----------------------------|------------------------|
| Soy protein      | Exp. 1 8.4 ± 4.80***          | 39.5 ± 1.69 | 48.2 ± 2.78* | 8.88 ± 0.36* | 281 ± 0.21                  | 2.49 ± 0.39             |
|                  | Exp. 2 20.1 ± 2.70            | 42.7 ± 5.81 | 26.3 ± 4.75 | 1.006 ± 0.94 | 78.2 ± 3.32*                | 92.6 ± 3.77             |
| Casein           | 156 ± 13.6*                   | 78.7 ± 5.10 | 53.2 ± 6.51 | 516 ± 38* | 2.78 ± 0.31*                | 771 ± 60               |
| Soy protein AA   | 228 ± 29.1                    | 80.3 ± 4.56 | 39.3 ± 5.73 | N.D. | N.D.                        | N.D.                   |
| Casein AA        |                                |         |         |      |                             |                        |

*Mean ± SEM of 8 rats. 

AA, amino acid mixture. 
Significant difference at p < 0.05. N.D., not determined.
Table 3. Concentrations of liver and adipose cholesterol in rats fed soy-bean protein or casein.

| Dietary regimens | Liver cholesterol (mg/g) | Adipose cholesterol (μg/g) |
|------------------|--------------------------|-----------------------------|
| Exp. 1           |                          |                             |
| Soy protein      | 9.64 ± 1.33**a           | 449 ± 33                    |
| Casein           | 34.5 ± 5.81              | 523 ± 51                    |
| Exp. 2           |                          |                             |
| Soy protein AA    | 29.0 ± 5.22              | 447 ± 55                    |
| Casein AA        | 45.2 ± 7.68              | 524 ± 40                    |

*Mean ± SEM of 8 rats. AA, amino acid mixture. * Significant difference at p<0.05.

Table 4. Body weight gain, food intake, liver weight and liver cholesterol in mice fed soy-bean protein or casein.

| Dietary regimens | Body weight gain (g/50 weeks) | Food intake (g/day) | Liver weight (g/100 g body weight) | Liver cholesterol (mg/g) |
|------------------|-------------------------------|--------------------|-----------------------------------|--------------------------|
| Soy protein      | 41.5 ± 2.1*                  | 4.5 ± 0.1          | 3.48 ± 0.14                       | 3.68 ± 0.16*             |
| Casein           | 42.4 ± 1.8                   | 4.6 ± 0.1          | 3.52 ± 0.10                       | 5.19 ± 0.65              |

*Mean ± SEM of 15 mice. * Significant difference at p<0.05.

Fig. 2. Changes of serum cholesterol and lipoperoxide as a function of time in mice fed cholesterol-free protein-type diets. Mean ± SEM of 15 mice. ●, △, soy protein; ○, □, casein. * Significant difference at p<0.05.
40 weeks and thereafter the protein-dependent difference disappeared. There were continued increases in the concentration of TBA-reactive substances with age, but no statistically significant differences were observed with respect to the type of dietary protein throughout the experiment.

DISCUSSION

Although our previous experiments with rats fed a 1% cholesterol diet failed to demonstrate a definite hypocholesterolemic effect of soy-bean protein and its amino acid mixture as compared with the corresponding casein diets (6), the present study where the dietary level of cholesterol was reduced to one-half that of the previous trials, 0.5%, clearly showed a dietary protein-dependent difference in serum cholesterol, thus soy protein exhibiting its cholesterol-lowering effect as in the case of cholesterol-free diets. It is therefore likely that the large excess of dietary cholesterol mitigates the effect of dietary protein on serum cholesterol. Kim et al. (8, 9) and Forsythe et al. (20) showed the hypocholesterolemic action of soy protein in swine fed a high-cholesterol diet.

The difference in serum cholesterol level was more marked with the intact protein diet (experiment 1) than with the amino acid mixture diet (experiment 2), indicating that the cholesterol-lowering action may not be solely ascribed to the difference in amino acid composition, but partially relates to non-proteinous materials occurring (less than 10%) in the soy-bean protein preparation (21). However, the difference in the rate of absorption of individual amino acids when proteins or amino acid mixtures were fed should be taken into consideration.

When diets free of cholesterol were given (7), both VLDL- plus LDL-cholesterol and HDL-cholesterol decreased proportionally in rats on the soy protein diet, as compared with those on the casein diet, whereas, as observed in the present study, addition of cholesterol to the diet reduced VLDL- plus LDL-cholesterol but not the HDL-counterpart. Thus, the ratio of HDL- to total cholesterol was markedly higher in those on soy protein, showing the reduction of the so-called "atherosclerosis index." This was in essence comparable with the result obtained after feeding diets free of cholesterol; the ratio remained unchanged or increased moderately (7). These changes in the distribution of cholesterol in relation to dietary proteins obviously differed from those in humans (3) and rabbits (22), probably due to the difference in the distribution of cholesterol in serum lipoproteins in rats and these species.

The mechanism responsible for causing these changes in the distribution of cholesterol in serum lipoproteins is not apparent. Kritchevsky and his colleague directed their attention to the role of arginine in the level of serum cholesterol (23). Eklund and Sjöblom (24) recently showed a significant negative correlation between the level of dietary arginine and serum VLDL- plus LDL-cholesterol levels in female rats fed a cholesterol-free diet. Soy protein contains approximately twice as much arginine than casein. However, the situation seems not to be so simple as to be
attributable to the difference in the content of a single amino acid, arginine in this case, since the responsibility of this amino acid was not fully supported in male rats (7). Katan et al. (25) suggested the role of glycine in the hypocholesterolemic action of soy protein. Huff and Carroll (26) claimed the importance of interaction between essential and non-essential amino acids in determining plasma cholesterol.

The concentration of serum apoA-I and apoB was also altered. When rats were fed a soy protein diet free of cholesterol, serum apoA-I decreased significantly but apoB increased (6, 7). Feeding a soy protein diet containing high cholesterol resulted in the decreased level of both apoA-I and apoB. Although the decrease in apoB when cholesterol was included in the diet well paralleled that of VLDL- plus LDL-cholesterol, the reduction of apoA-I was not consistent with the response of HDL-cholesterol which was essentially unchanged by soy protein. The reduction of circulating apoA-I on cholesterol-free soy protein diets at least partly results from a decreased production of this apolipoprotein in the intestine (unpublished observation), while the rise of apoB is perhaps due to increased hepatic production. Since cholesterol feeding causes a significant reduction of hepatic cholesterogenesis, different responses are possible in the levels of circulating apoB in rats fed diets with or without cholesterol.

The antihypercholesterolemic effect of soy-bean protein was for the first time demonstrated in mice. However, the effect of dietary protein on serum cholesterol was restricted only to the relatively younger stage and it was not clear when mice became older. This age-related response to dietary protein seems to be partly relevant to the failure to observe the rise in serum cholesterol with age, particularly in rats fed casein.

Recently, the peroxidation reaction has attracted attention in relation to several prevalent diseases as well as aging (27). Though the lipoperoxide level continued to rise with age, the quality of dietary protein showed no different effect in mice. The same was apparently true for rats fed protein diets, while there was a moderate increase in the concentration of TBA-reactive substances in the serum of rats fed the soy protein-type amino acid mixture diet. Of course we measured only at one selected interval with rats, but this observation did not agree with that found in rats fed a cholesterol-free diet, where no protein-dependent difference could be seen (7). Harman (28) suggested that the type of dietary proteins might have a close connection to the level of free radical reaction which is responsible for lipid peroxidation. Anyhow, a more detailed study needs to be done in this respect.

In conclusion, soy protein, as compared with casein, was less hypercholesterolemic to rats even when a high-cholesterol diet was fed. With a low-fat cholesterol-free diet, the cholesterol-lowering effect of soy protein was also evident in mice. Available information strongly suggests that the hypocholesterolemic effect of soy protein is the phenomenon commonly observable in the rodents so far examined, i.e., rabbits, rats and mice.

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