Some Metabolic Aspects of the Hypocholesterolemic Effect of Soybean Protein in Rats Fed a Cholesterol-Free Diet

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Summary The present paper shows a series of experiments carried out to elucidate the possible mechanism by which soybean protein isolate (SPI) produces a lower level of plasma cholesterol than casein in rats fed a cholesterol-free diet. When the plasma cholesterol level was in a steady state characteristic of casein and SPI, SPI in the diet was substituted for casein and vice versa. Within 3 days after substitution of dietary protein, the plasma cholesterol level in each group reached a steady state level similar to that in its previous counterpart. The inherent responses of plasma cholesterol to casein and SPI were not changed by the resection of the jejunum or the ileum and the administration of cholestyramine or β-sitosterol. The rates of the sterol synthesis in vivo of the liver and the small intestine were significantly higher in SPI-fed rats than in casein-fed rats. The hypocholesterolemic effect of SPI disappeared when Met was supplemented at a level equivalent to casein. The effects of casein and SPI were reproduced by their equivalent amino acid mixtures. The ratio of the postprandial increment of Met concentration to that of Gly concentration in the portal plasma was significantly higher when casein and its amino acid mixture were fed than when SPI and its amino acid mixture were fed. The casein-induced increase in the level of plasma cholesterol was attributed to an increase in high density lipoprotein (HDL) cholesterol. HDL-cholesterol concentration showed the positive correlation with the lecithin: cholesterol acyltransferase activity. HDL apolipoprotein level was significantly and the synthetic rate of HDL apolipoproteins in vivo was slightly higher in casein-fed rats than in SPI-fed rats. From all of these observations, the dietary protein-induced alteration of the plasma cholesterol level would arise through the change in HDL metabolism.

Key Words plasma cholesterol, casein, soybean protein isolate, ileectomy, jejunectomy, sterol synthesis, high density lipoprotein, lecithin: cholesterol acyltransferase, apolipoprotein, rat

Introduction Dietary protein plays an important role in the regulation of the plasma cholesterol level in humans and experimental animals (1–3). Carroll and Hamilton (1) systematically examined the relation between the type of dietary protein and plasma cholesterol concentration in rabbits fed a cholesterol-free diet, and found that animal proteins were more hypercholesterolemic than plant proteins. Thereafter, much attention has been paid to the regulatory mechanism of plasma cholesterol by dietary protein. Many studies have been mainly carried out in rabbits fed a diet without cholesterol and in rats fed a diet with or without cholesterol. Casein and SPI have been usually used as a representative of animal and plant protein, respectively. Huff and Carroll (4) have considered that the action of SPI would be primarily ascribed to the less intestinal steroid absorption and the more fecal steroid excretion. For the present, the hypothesis is plausible (5–7).

We, however, have doubted if the hypothesis derived from rabbits fed a cholesterol-free diet is acceptable in other animal models such as rats fed a diet with or without cholesterol, because there are some differences in lipid metabolism. When fed a cholesterol-free diet, for example, the rate of whole-animal sterol synthesis per 100 g body weight is considerably higher in the rat than in the rabbit (8). The rat has enough synthetic capacity to fully compensate for an increased loss of steroids from the body (9–11). Furthermore, the rat is different from the rabbit in cholesterol distribution among
plasma lipoproteins (8). When rats are fed a diet with cholesterol, the plasma lipoprotein cholesterol profile is similar to that of rabbits, but the sterol synthesis is strongly depressed (12). With the recognition mentioned above, it should be necessary to consider carefully whether the hypothesis proposed by Huff and Carroll (4) could be applied to other animal species and other dietary conditions.

We have studied the differential actions of casein and SPI on the plasma cholesterol level in rats fed a cholesterol-free diet (13-16). In this report we describe the present state of our knowledge for the possible mechanism.

**Plasma cholesterol concentration rapidly responds to casein and SPI**

Figure 1 indicates the time course of changes in the plasma cholesterol in rats fed a casein or SPI diet. On day 7 and day 10 of the experiment, plasma cholesterol was significantly higher in casein-fed rats than in SPI-fed rats. Within 3 days after substitution of SPI for casein and vice versa, the plasma cholesterol level in each group rapidly moved to the same level as that of its previous counterpart; the transfer of the casein-fed rats to the SPI diet produced a rapid decrease in plasma cholesterol, whereas rats switched from the SPI diet to the casein diet showed a rapid increase in plasma cholesterol. The plasma cholesterol level induced by exchanging dietary proteins remained constant to the end of the experiment on and after the diet crossover.

**Plasma cholesterol concentration is not controlled by the change in steroid absorption**

It is a well-known fact that cholesterol absorption is inhibited by jejunal resection and β-sitosterol administration (10, 17), and bile acid absorption by ileal resection and cholestyramine administration (9-11). Therefore, animals with the resection of the jejunum or the ileum and the administration of β-sitosterol or cholestyramine are suitable for animal models to test whether plasma cholesterol is regulated by the change in fecal steroid excretion induced by casein and SPI.

As shown in Fig. 2, the rapid inherent responses
of plasma cholesterol to casein and SPI remained unchanged even when the jejunum or the ileum was resected. In these experiments sham-operated rats subjected to laparotomy were prepared for a comparison. The plasma cholesterol levels in jejunectomized and ileectomized rats were almost similar to those in their sham-operated rats. As is evident in Fig. 3, the characteristic effect of each dietary protein on plasma cholesterol was also conserved even after the administration of $\beta$-sitosterol or cholestyramine. Hence, the hypothesis proposed by Huff and Carroll (4) is not applied to rats fed a cholesterol-free diet.

The plasma cholesterol concentration in a given species appears to decrease only when an output of steroids from the body exceeds the synthetic capacity of that species. The rat, however, has a high potency to maintain plasma cholesterol at a constant level (9-11). Weis and Dietschy (10) have reported that the fecal steroid excretion is much larger in ileectomized rats than in rats with an intact gastrointestinal tract and that the augmented steroid elimination induced by ileectomy is fully compensated for the increased sterol synthesis so that serum cholesterol remains constant. It has been also reported that the concurrent addition of cholestyramine or $\beta$-sitosterol to the high cholesterol diet prevents the increase in plasma cholesterol level but that the addition of them to the cholesterol-free diet fails to reduce plasma cholesterol to the lower-than-usual level (9, 17). These indicate that the interference of steroid absorption is effective only when a high cholesterol diet is fed but not when a cholesterol-free diet is fed.

Plasma cholesterol concentration is not related to the change in the rate of the sterol synthesis

The rates of $[{}^3{}H]$incorporation into digitonin-precipitable sterols in vivo in the liver and the small intestine were significantly higher in the SPI-fed rats than in the casein-fed rats (Table 1). Consequently, the dietary protein-induced change in the plasma cholesterol level is not ascribed to the difference in sterol synthesis.

Difference of amino acid composition between casein and SPI is significant for the regulation of plasma cholesterol concentration

The difference in the amino acid compositions of casein and SPI has been considered as one of main factors involved in their differential effects on plasma cholesterol (2, 18, 19). We planned to investigate which amino acid would be responsible for the different action of dietary protein. Table 2 shows the amino acid compositions of casein and SPI. The contents of 11 amino acids (11AA) such as Glu, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Tyr, and Val are less in SPI, whereas those of 7 amino acids (7AA)
Table 1. Plasma cholesterol concentration and rates of sterol synthesis \textit{in vivo} in the liver and the small intestine in casein- and SPI-fed rats.\textsuperscript{1,2,3}

| Dietary group | Plasma cholesterol (mg/dl) | \( [^3\text{H}] \text{water incorporation} \) |
|---------------|-----------------------------|-----------------------------------|
|               |                             | Liver (dpm/tissue) | Small intestine (dpm/tissue) |
| Casein        | 105.7 ± 4.5\textsuperscript{a} | 1683 ± 330\textsuperscript{a} | 2147 ± 93\textsuperscript{a} |
| SPI           | 58.8 ± 4.0\textsuperscript{b}  | 7170 ± 1266\textsuperscript{b} | 3472 ± 409\textsuperscript{b} |

\textsuperscript{1} Rats were fed the casein or SPI diet for 10 days. \textsuperscript{2} Values are mean ± SEM. \textsuperscript{3} Means not sharing a common superscript letter within the same column are significantly different (\( p < 0.05 \)).

Table 2. Amino acid compositions of diets.

| 1-Amino acid | Casein | SPI | SPI + Met | Casein + 7AA and SPI + 11AA |
|--------------|--------|-----|----------|-----------------------------|

Such as Ala, Arg, Asp, Cys, Gly, Phe, and Trp are less in casein. Casein and SPI were supplemented with 7AA and 11AA, respectively, so that the amino acid composition of casein with 7AA is the same as that of SPI with 11AA (Table 2).

Figure 4 shows the change in plasma cholesterol level in rats before and after the addition of amino acids to casein and SPI. Within 2 days after feeding the SPI + 11AA diet, plasma cholesterol concentration rapidly increased to the same level as that in casein-fed rats. On the contrary, the addition of 7AA to the casein diet did not change the casein-induced increase in the level of plasma cholesterol. Therefore, we considered that certain amino acids added to the SPI diet would elevate the plasma cholesterol level. The addition of Met alone to the SPI diet increased plasma cholesterol to the level induced by the casein diet and the SPI + 11AA diet.

The characteristic actions of casein and SPI were reproduced by casein- and SPI-type amino acid mixtures (casein AA and SPI AA) (Table 3). Plasma cholesterol levels induced by dietary proteins and amino acid mixtures were not associated with the postprandial increment of Met concentration in the portal plasma (Table 3). But the ratio of the incremental concentration of Met to that of Gly was
Table 3. Plasma cholesterol concentration and the postprandial increment of the portal amino acid concentration.1,2,3

| Dietary group | Plasma cholesterol (mg/dl) | Met (nmol/ml) | Gly (nmol/ml) | Met/Gly |
|---------------|---------------------------|---------------|---------------|---------|
| Casein        | 128.2 ± 8.2a              | 26.3 ± 3.8b   | 47.0 ± 9.4b   | 0.61 ± 0.07a |
| SPI           | 99.1 ± 2.3b               | 15.0 ± 3.1b   | 94.3 ± 17.2b  | 0.17 ± 0.04a |
| Casein AA     | 118.5 ± 5.6a              | 52.7 ± 5.9a   | 95.0 ± 16.8b  | 0.60 ± 0.06a |
| SPI AA        | 92.8 ± 4.6b               | 24.4 ± 3.0b   | 206.0 ± 20.9a | 0.12 ± 0.01b |

1 Plasma cholesterol concentration was determined on day 9 of the experimental feeding. Then rats were fasted overnight, and refed a diet. Thirty minutes after refeeding, blood was taken from the portal vein under anesthesia. The postprandial increment of amino acid concentration in the portal plasma was determined by the difference in the concentration between refed and fasted rats. 2 Values are mean ± SEM. 3 Means not sharing a common superscript letter within the same column are significantly different (p<0.05).

Fig. 4. Change in plasma cholesterol concentration in rats fed the casein or SPI diet with or without amino acids. After rats were fed a casein or SPI diet for 10 days, the casein-fed rats were divided into 2 subgroups, and the SPI-fed rats into 3 groups. For the subsequent 7 days they were fed respective diets. Each point is the mean ± SEM. The plasma cholesterol levels in rats fed the casein diet or the casein + 7AA diet were significantly higher than those in rats fed the SPI diet throughout the experimental period (p<0.05). Values with an asterisk are significantly different from those in rats fed the SPI diet (p<0.05). O—O, the casein diet; ●—●, the casein + 7AA diet; ○—○, the SPI diet; ⋄—⋄, the SPI + 11AA diet; ▲—▲, the SPI + Met diet.

significant higher in casein- and casein AA-fed rats than in SPI- and SPI AA-fed rats (Table 3).

These results indicate that the amino acid composition of dietary protein is responsible for the difference in the plasma cholesterol level. Judging from the postprandial amino acid pattern in the portal plasma, we have tentatively considered that the Met-induced increase of plasma cholesterol concentration in SPI-fed rats would be produced by the change in balance of amino acids absorbed into the portal plasma.

Casein-fed rats are different from SPI-fed rats in high density lipoprotein (HDL) metabolism

As shown in Fig. 5, the rapid responses of plasma cholesterol to casein and SPI are almost ascribed to the change in HDL-cholesterol. Figure 6 indicates the change in lecithin: cholesterol acyltransferase (LCAT) activity. LCAT activity showed the positive correlation with HDL-cholesterol concentration (r = 0.525, p < 0.001, n = 60). LCAT which is located in the HDL-fraction is the plasma enzyme for esterification of free cholesterol in HDL, and its activity is regulated by HDL apolipoproteins (20). Therefore, we considered that casein and SPI affected HDL apolipoprotein metabolism. In fact, HDL apolipoprotein level was significantly higher in casein-fed rats than in SPI-fed rats (Table 4). The rate of [3H]Leu incorporation in vivo into HDL-apolipoproteins was slightly higher in casein-fed rats than in SPI-fed rats (Table 4). The disturbance of apolipoprotein metabolism might be the cause for the phenomena.

From the results described above, we concluded that the inherent effects of casein and SPI on plasma cholesterol are not produced by the differences in steroid absorption and sterol synthesis. It is plausible that the dietary protein-dependent modification of the plasma cholesterol level might be produced as a result of the change in HDL metabolism. But the
mechanism observed in other animals and other dietary conditions could not be ruled out, because our hypothesis was derived from experiments on rats fed a cholesterol-free diet.

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