Dietary Fats Modulate Age-Dependent Effects of Dietary Proteins on Cholesterol Metabolism in Rats

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Summary

Different aged male rats, 4 weeks or 9 months old, were fed diets containing either casein (CAS), milk whey protein (WHY) or soybean protein (SOY) with corn oil or sardine oil for 4 weeks. The hypocholesterolemic effect of SOY, compared to CAS, was more evident in rats fed corn oil than in those fed sardine oil, and in young rats than in adult rats, irrespective of dietary cholesterol. In contrast, the liver cholesterol-lowering effect of SOY was more marked in adult than in young rats in all experiments. WHY exerted an intermediate effect on the concentration of liver cholesterol. At both ages, the response of liver 3-hydroxy-3-methylglutaryl (HMG) CoA reductase activity was diverse and dependent upon the source of dietary fat and age of the animal. Fecal steroid excretion was significantly higher in rats fed SOY than in those fed either CAS or WHY, especially in adult rats. The results showed a diverse interaction of the protein type, fat and age in respect to lipid metabolism.

Key Words: dietary protein, dietary fat, cholesterol metabolism, age, rats

The importance of diet in the regulation of cholesterol metabolism has long been stressed (1). Ageing causes an increase in the serum cholesterol level, and the reduction in the cholesterol turnover rate with age may be the cause for an elevation of the cholesterol pool in the body (2). We showed that age-related degeneracy of cholesterol metabolism could be manipulated by the type of dietary fat (3).

Dietary protein influences the serum cholesterol level by modifying cholesterol dynamics (4), and in young rats there appears to be an interaction of dietary protein and fats on lipid metabolism (5, 6). Since dietary fat modifies the effect of dietary protein on the concentration of serum cholesterol, it seems likely that this interaction may also be influenced by age. In the present study, we examined the combined effect of dietary proteins and fats on cholesterol metabolism in rats of different ages.

Experimental

Young (4 weeks old) and adult (9 months old) male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka) were divided into groups of 6 animals each and fed ad libitum AIN-formular diets for 4 weeks (7). Casein (CAS), whey protein (WHY) or soybean protein (SOY) were used as a protein source. Corn oil or sardine oil (containing 0.56% cholesterol) were used as a fat source in experiments 1 and 2, or in experiment 3, respectively (8, 9). In experiment 2, cholesterol (0.5%) was added at the expense of sucrose, since dietary cholesterol intensifies the differential effects of dietary proteins or fats (10). These experiments were conducted separately. Feces were collected for 2 days beginning 5 days before killing. Rats were killed by decapitation at 1:00.

Analyses

The activity of HMG-CoA reductase was measured as described previously (3). Fecal steroids were analyzed by gas-liquid chromatography (8, 9). Serum and liver lipids were extracted, and cholesterol was measured (9). Microsomal protein was determined by the method of Lowry et al. (11).

Results

The concentration of serum cholesterol in rats fed different proteins is summarized in Table 1. In experiment 1 with corn oil diets free of cholesterol, the concentration of serum cholesterol was signif-
Table 1. The concentrations of serum and liver cholesterol in young and adult rats fed diets containing different proteins.

| Diet | Serum (mg/100 ml) | Liver (mg/g) |
|------|------------------|-------------|
|      | Young            | Adult       | Young          | Adult          |
|      |                  |             |                |                |
| Exp. 1 |                  |             |                |                |
| CAS   | 116.9±8.3*       | 114.3±5.1  | 3.12±0.32      | 5.58±0.90**    |
| WHY   | 112.1±4.6*       | 117.4±7.0  | 3.22±0.24      | 4.06±0.47**    |
| SOY   | 82.6±8.5b        | 104.1±4.3* | 2.79±0.22      | 2.69±0.24b     |
| Exp. 2 |                  |             |                |                |
| CAS   | 142.3±7.5*       | 190.2±18.2**| 18.3±0.24*     | 20.2±2.1      |
| WHY   | 101.8±4.7b       | 130.7±5.1**| 10.4±1.5b     | 19.4±2.1*     |
| SOY   | 105.2±2.3b       | 98.3±10.2b | 10.3±1.2b     | 16.6±2.3*     |
| Exp. 3 |                  |             |                |                |
| CAS   | 84.7±6.7         | 137.2±7.0* | 3.09±0.41      | 4.65±0.38**    |
| WHY   | 89.7±10.2        | 131.2±9.3* | 2.81±0.17      | 4.18±0.48**     |
| SOY   | 72.4±6.4         | 105.4±11.0*| 2.80±0.56      | 2.85±0.15b     |

Values are mean ± SE of 6 rats. *b In age-matched rats, values without common superscript letters denote significant difference (p<0.05) between diets in the same experiment. * Significant difference (p<0.05) versus the corresponding young rats.

icantly lower in the SOY group than in the two milk protein groups in young, but not adult rats. As a result, an age-related increase in serum cholesterol was observed only in the SOY group. In experiment 2 with cholesterol-enriched diets containing corn oil, the concentration of serum cholesterol was significantly lower in the SOY group than in the other groups, especially in adult rats. An age-related increase in serum cholesterol was observed in the CAS and WHY groups but not in the SOY group. On the other hand, in experiment 3 with diets containing fish oil, no significant protein-effect was observed in the concentration of serum cholesterol, although it tended to be lower in the SOY group. This was true even in adult rats whose cholesterol level was significantly higher than in young rats.

In experiment 1, there was a significant protein-dependent difference in the liver cholesterol in adult rats; it was significantly lower in the SOY group than in the CAS groups. The effect in young rats was not so marked. In experiment 2 using cholesterolemic diets, the concentration of liver cholesterol was significantly lower in the SOY groups especially in young rats, and a significant age-related increase was found in the SOY and WHY groups. In experiment 3 using fish oil, there was no difference in the liver cholesterol level in young rats, whereas in the adult rats it was higher in the two milk protein groups than in the SOY group. Consequently, the age-effect on liver cholesterol was observed only in these two groups.

Figure 1 shows the specific activities of HMG-CoA reductase of liver microsomes. In experiment 1, the HMG-CoA reductase activity of young rats tended to be lower in the SOY group than in the CAS or WHY groups. However, since the age-related decrease in the activity was observed only in the two milk protein groups, it tended to be higher in the SOY group in adult rats. In experiment 2, the response of the reductase in young rats was similar to that observed in experiment 1, although the activity was markedly low due to dietary cholesterol (12). In experiment 3, the activity tended to be higher in the SOY group than in the two animal protein groups in rats of both ages, and the age-effect was not clear.

Table 2 shows the total amounts of fecal steroids in experiments 2 and 3. In experiment 2 with the cholesterolemic diet, total amount of steroids excreted tended to be higher in the SOY group than in the WHY and CAS groups at both ages, particularly in adult rats. In experiment 3, it was significantly higher in the SOY group than in the two animal protein groups, and the excretion

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Fig. 1. Effects of dietary proteins on the activity of HMG-CoA reductase in young and adult rats fed diets containing different fat type. *Significantly different (p<0.05) between age groups on the same diet. □, casein; □, whey protein; ■, soybean protein.

Table 2. Fecal total steroid excretion in young and adult rats fed diets containing different proteins.

| Diets | Steroid excretion (mg/day) |
|-------|---------------------------|
|       | Young                     | Adult                    |
| Exp. 2|                            |                          |
| CAS   | 81.4±3.4                  | 89.3±2.7*                |
| WHY   | 90.4±3.8                  | 81.3±4.1*                |
| SOY   | 93.2±4.6                  | 105±4*                   |
| Exp. 3|                            |                          |
| CAS   | 9.96±0.44*                | 12.6±0.4*                |
| WHY   | 9.04±0.46*                | 11.0±1.1*                |
| SOY   | 12.6±0.4*                 | 16.1±1.3**               |

Values are mean±SE of 6 rats. * In age-matched rats, values without common superscript letters denote significant difference (p<0.05) between diets in the same experiment. * Significant difference (p<0.05) versus the corresponding young rats.

tended to increase significantly in the SOY group with age. In both experiments, SOY compared to CAS resulted in the higher output of bile acids, regardless of fat type or age (data not shown).

Discussion

The present study showed that age influences an interrelated effect of SOY, vegetable oil and cholesterol. The protein effect on cholesterol metabolism depends largely on dietary cholesterol (10). When the cholesterol-free diet was fed, the susceptibility of the serum cholesterol concentration to dietary protein appears to be marked in young rats as reported in rabbits (13). However, when the cholesterol-enriched diet was given, the magnitude of the reduction in the concentration of serum cholesterol caused by SOY was greater in adult rats than in young rats. In contrast, when fish oil was the source of dietary fat, the differential effects of dietary proteins on serum cholesterol was obscured. Thus, the age-dependent hypocholesterolemic effect of SOY compared to CAS seems to be influenced more by cholesterol in the diet rather than the type of dietary fats.

In the present study, significant modification by the fat type was observed in the response of HMG-CoA reductase activity, especially in young rats; the reductase activity tended to be lower in the SOY group than in the CAS group fed a corn oil diet free of cholesterol, whereas the adverse effect was shown in young rats fed fish oil. Therefore, the fat type modified the response of cholesterol synthesis to dietary protein, especially in young rats. The response of HMG-CoA reductase to dietary cholesterol degenerated with age (12). Nagata et al. (4) have shown in rats that the stimulation of the cholesterol synthesis by the SOY compared to CAS is possibly secondary to an increased output of fecal steroids. Indeed, SOY enhanced the excretion of fecal total steroids regardless of different fat types. The reduction of cholesterol synthesis, concomitant with an enhancement of fecal steroid

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output, should strengthen the cholesterol-lowering effects of SOY (14). The different responses of cholesterol synthesis to the different fat type may in part explain the diverse effects of proteins on the serum cholesterol level (4).

Hepatic cholesterol accumulation caused by dietary cholesterol was marked in adult rats as observed in the previous study (10), and the liver cholesterol-lowering effect of SOY was weakened with age. Alternatively, it seems that the dramatic reduction of the serum cholesterol level in adult rats fed SOY may in part be secondary to the decreased secretion of cholesterol into the blood circulation. Dietary casein induces higher rates of very low-density lipoprotein secretion by the rat liver (4), and ageing may modulate the process. In this context, it is interesting that SOY does not increase liver cholesterol in adult rats even when the fat source was fish oil.

In conclusion, an appropriate combination of soybean protein with fat strengthens their beneficial effects on cholesterol metabolism and leads the metabolic parameters to a desirable direction in adult rats. The fat type appears to determine the magnitude of the change in cholesterol synthesis induced by dietary protein.

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