Effects of Simultaneous Intake of Soybean Protein and Diacylglycerol on Lipid Profiles and Body Fat Accumulation in Rats

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Soybean protein (SPI) and diacylglycerol (DAG) are functional components with benefits for lipid metabolism. Since simultaneous intake of such components is expected to exert effects additively and/or synergistically in lifestyle-related diseases, we examined the effects of simultaneous intake of SPI and DAG on lipid profiles. Five-week-old male Wistar rats were fed experimental diets with and without cholesterol for 28 d. In the rats fed cholesterol-free diets, significant interactions between dietary oil and protein were observed in the serum triacylglycerol (TG), hepatic cholesterol, and TG concentrations, whereas in the rats fed cholesterol diets, the serum and hepatic lipid concentrations were significantly lower in rats fed SPI than in those fed casein. Although our results suggest that simultaneous intake of SPI and DAG has slightly ameliorating effects on lipid profiles in rats, simultaneous intake of foods or food components with similar functions are not necessarily effective.

Key words: soybean protein (SPI); diacylglycerol (DAG); simultaneous intake; lipid profiles; rats

Recently, from the viewpoint of improvement or prevention of life-style related diseases, such as obesity, hypertension, hyperlipidemia, and diabetes, much attention has been paid to the beneficial effects of functional foods and food components.1 Numerous studies have been conducted in humans and animals on the bioavailability of various food components, particularly to clarify the physiological impact of bioactive components. Of many functional food components, soybean protein,2 n-3 polyunsaturated fatty acids,3 phytosterols,4 diacylglycerol (DAG),5 and oligo-peptide derived from globin protein digest6 are thought to be potentially beneficial in modulating the lipid profiles.

However, to date, there is little information on the physiological (additive, synergistic, or negative) impacts of simultaneously given functional foods or food components, which have similar benefits, although the physiological benefits of individual components have been examined in detail in humans and animals. Hence, in the present study, we focused in particular on the combination of SPI and DAG, which have regulatory functions in serum cholesterol and TG concentrations. Both food components are recognized as ingredients in food for specified health uses (FOSHU) in Japan.

Soybean and its products are representative traditional foods in Japan. SPI intake has been shown to exhibit hypocholesterolemic effects in various species, including humans and rats.7 These effects were attributed to a reduction in cholesterol absorption and increases in fecal steroid excretions in rabbits and rats.8,9 It is also assumed that SPI has cholesterol-lowering effects by stimulating cholesterol 7α-hydroxylase, the key enzyme converting cholesterol to bile acid.10

On the other hand, DAG, which consists mainly of sn-1,3 species, has been shown to suppress body fat accumulation in mice much more than dietary TG with a similar fatty acid composition,11 and also to prevent increases in postprandial serum TG concentrations in healthy humans.12 In addition, long-term intake of DAG also lowered the serum TG concentration in type II diabetic patients with hypertriglyceridemia.13 These physiological effects of DAG depend on different metabolic functioning due to structures differing from TG.14 Simultaneous intake of components with physiologically similar functions involved in lipid metabolism appears to be more beneficial for reduction of the risks of life-style related diseases. In this study, therefore, we examined the physiological impact of the simultaneous intake of SPI and DAG on lipid profiles and fecal bile acid excretion in rats fed cholesterol-free and cholesterol diets. In addition, body fat accumulation of rats was also assessed.

Materials and Methods

Test oils. Corn oil was used as a common dietary oil. DAG was a commercially available dietary oil containing sn-1,2 and sn-1,3 DAG in a ratio of approximately 3:7 (Kao, Co., Tokyo). The fatty acid compositions of the test oils were analyzed by GC equipped with an HR-Theromon-3000 (0.25 mm × 25 m) column (Shinwa Chemical Industries, Tokyo), under the following analytical conditions: injection temperature 250 °C, detector temperature 250 °C, column temperature raised from 150 °C to 210 °C at 2 °C/min, split ratio 30:1, and N2 was used as the carrier gas. The fatty acid compositions of the dietary oils are shown in Table 1.

Animals and experimental design. Four-week-old male Wistar rats (Japan SLC, Shizuoka, Japan) weighing 60–80 g were housed individually in a light-controlled room (12-h light-dark cycle) at an ambient temperature of 23 ± 2 °C. Tap water and commercially available laboratory chow (CE-2; Clea Japan, Tokyo) were provided ad libitum for 1 week before feeding of the experimental diets. They were randomly divided into 4 groups of 5 animals each to average the

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Abbreviations: SPI, soybean protein; DAG, diacylglycerol; TG, triacylglycerol; FOSHU, food for specified health uses
initial body weight. The rats were allowed free access to water and the experimental diets for 28 d. The contents of the basal diet were as follows (g/kg diet): protein, 200; test oil, 70; AIN-93G mineral mixture; 10; AIN-93 vitamin mixture; 10; cellulose, 50; 1-cystine, 3; and α-corn starch to 1,000. Soybean protein isolate (SPI, Fujipro R, Fuji Oil, Osaka, Japan) or casein was used as the nitrogen source. The diets containing SPI were added at 3 g/kg diet with dl-methionine at the expense of α-corn starch. Cholesterol and sodium cholate were added at levels of 0.5% and 0.125% in the cholesterol diets respectively. At the end of the experiment period, after overnight fasting, the rats were anesthetized and blood was withdrawn from the abdominal aorta. The tissues (liver, epididymal, and perirenal adipose tissues) were excised, washed with saline, and weighed. Feces were collected for 24 h at the end of the experimental period, and were freeze-dried. The care and use of animals was in accordance with the guidelines of the Incorporated Administrative Agency, National Institute of Health and Nutrition of Japan.

Blood sample collection and chemical analysis. Blood samples obtained from the rat abdominal aorta were centrifuged at 3,000 rpm for 15 min. Sera were stored at −30°C until analysis. Serum lipids, such as total cholesterol, HDL-cholesterol, TG, and phospholipids, indices of liver function, and other biochemical parameters were analyzed with commercially available assay kits (Cholesterol E-Test, Triglyceride E-Test, Phospholipid E-Test, GOT-UV Test, GPT-UV Test, γ-GTP C-Test, alkaline phospha K-Test, Albumin/globulin B-Test, and Glucose B-Test Wako, Wako Pure Chemicals, Osaka, Japan). Serum insulin levels were measured with a commercially available ELISA kit (Amersham Biosciences, Amersham, UK).

Assay of liver lipids. Liver lipids were extracted with chloroform-methanol (2:1, v/v), as described by Folch et al. The concentrations of lipids in the liver were determined using enzymatic reagent kits (Cholesterol E-Test and Triglyceride E-Test Wako, Osaka, Japan) after drying the lipid extracts and dissolving the lipids with Triton X-100 with minor modifications. Liver phospholipids concentrations were assayed by the method of Nagata et al.

Extraction of fecal total lipids and assay of fecal bile acids. Fecal total lipids were extracted from lyophilized feces 3 times with ethanol at 70°C for 1 h. The contents of fecal bile acids were assayed enzymatically using hydroxy steroid dehydrogenase, as described elsewhere, with minor modifications. The standard used for this assay was cholic acid.

Statistical analysis. Values are shown as means ± SEM (standard error of the mean). The statistical significance of the effects of dietary oil (corn oil vs. DAG) and protein (casein vs. SPI) was assessed by two-factor factorial ANOVA, and then the statistical significance of the differences between groups was determined by Fisher’s PLSD test when the effects of dietary oil and/or protein were significant. Statistical significance was defined at P < 0.05.

Results

Body weight gain, food intake, and relative tissue weights

The body weight gain, food intake, and relative tissue weights of rats fed the experimental diets are shown in Table 2. In the rats fed the cholesterol-free diet, there were no significant differences in these parameters among the groups. In addition, no significant interaction between dietary protein and oil was observed. In the case of the cholesterol diet, body weight gain, food intake, and relative liver weights in the rats fed the DAG and casein diets were significantly higher than those of other groups (P < 0.05), although there were no significant differences in epididymal or perirenal adipose tissue weights among the groups. The increases in body weight gain, food intake and relative liver weights were significantly influenced by dietary oil (P = 0.04), protein (P = 0.02), and both factors, P < 0.0001 and P < 0.0001 respectively. Furthermore, there was significant interaction (P = 0.004) between the two food components in relative liver weights.

Serum lipid concentrations

The serum lipid concentrations of the rats fed the experimental diets are listed in Table 3. In the rats fed cholesterol-free diets, serum total cholesterol concentration was significantly influenced by dietary protein (P = 0.005). In particular, the serum total cholesterol concentrations of the rats fed the SPI diets were significantly lower (P < 0.05) than in those fed the casein diets, regardless of the kind of dietary oil. Moreover, significant interaction (P = 0.039) between the two components was observed in the serum TG concentrations, but there were no significant differences in the serum TG and phospholipid concentrations among the groups. In the rats fed the cholesterol diets, the serum total cholesterol and TG concentrations were significantly influenced by dietary protein (P < 0.001 and P = 0.03 respectively), and the serum total cholesterol concentrations of the rats fed the SPI diets were significantly lower (P < 0.05) than those fed the casein diets. However, there were no significant interactions between dietary oil and protein.

Biological parameters in serum

In the rats fed the cholesterol-free diets, there were no significant differences in total protein or indices of hepatic functions, such as AST, ALT, γ-GTP, and alkaline phosphatase. Significant interactions between dietary oil and protein were observed (P = 0.003) in the serum glucose concentrations. In the rats fed the corn oil and casein diet, serum glucose concentration (mean value, 5.14 mmol/l) was significantly lower (P < 0.05) than in those fed the other diets (corn oil and SPI, DAG and casein, and DAG and SPI were 135, 153 and 138 mg/dl respectively). However, the serum insulin concentrations were not affected by the diets. In the rats fed the cholesterol diets, although the serum γ-GTP concentration (0.87 IU/l) was significantly lower (P < 0.05) in the rats fed the combined corn oil and SPI diet than the corn oil and casein diet (2.79 IU/l), neither significant influences nor interactions of the individual food factors were observed in the other biological parameters.

Hepatic lipid concentrations

Table 4 shows the liver lipid concentrations of the rats fed the experimental diets. In the rats fed the cholesterol-free diet, significant interactions between dietary oil and protein were detected in the cholesterol

| Table 1. Fatty Acid Composition of Dietary Oil (weight %) |
|-----------|-----------|
|           | Corn oil  | Diacylglycerol |
| 16:0      | 11.6      | 3.18           |
| 16:1      | 0.10      | —              |
| 18:0      | 1.79      | 1.18           |
| 18:1      | 31.9      | 41.7           |
| 18:2      | 53.8      | 48.5           |
| α-18:3    | 0.84      | 5.40           |


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and TG concentrations ($P = 0.012$ and 0.021 respectively), but no significant influences of individual dietary factors were observed. The cholesterol concentration of the rats fed the diet of combined DAG and SPI was significantly lower ($P < 0.05$) than in those fed the diets of corn oil and SPI, or DAG and casein. The TG concentration of the rats fed the diet of corn oil and casein was significantly lower ($P < 0.05$) than in those fed the diets of corn oil and SPI, or DAG and casein. In the rats fed the cholesterol diets, the results of two-way ANOVA did not show any significant interactions between dietary oil and protein in the liver lipid concentrations. The cholesterol concentrations were significantly affected by dietary oil and protein ($P < 0.001$ and $P < 0.001$ respectively), suggesting that DAG significantly increases liver cholesterol concentrations even though SPI significantly decreases those concentrations. The liver cholesterol concentration of the rats fed the DAG and casein diet was significantly higher ($P < 0.05$) than those of the other groups, and that of the corn oil and SPI diet was significantly lower ($P < 0.05$) than the other groups. The TG and phospholipid concentrations were significantly influenced by dietary protein (TG, $P = 0.002$, and phospholipids, $P = 0.023$). Although the TG concentration in the rats fed the DAG and casein diet was significantly higher ($P < 0.05$) than those of the other dietary groups, no significant differences in phospholipid concentrations were observed among the groups.

**Fecal bile acid concentrations**

The fecal bile acid concentrations of the rats fed the experimental diets are shown in Table 5. In the rats fed the cholesterol-free diets, neither significant influence by the individual factor nor significant interaction between the factors were observed in fecal bile acid concentrations. In contrast, in the rats fed the cholesterol diets, a significant influence ($P = 0.0003$) of dietary protein was observed. SPI intake significantly increased fecal bile acid excretion ($P < 0.05$), but no significant interaction between dietary oil and protein was observed in fecal bile acid concentrations in the rats fed the cholesterol diets.

**Discussion**

SPI and DAG are representative functional food components in FOSHU products, which have health claims related to the improvement in the area of lifestyle-related disease. Therefore, simultaneous intake of components of similar function would be expected to reduce the risk factors for lifestyle-related disease. Hence, in this study, we examined the effects of the simultaneous intake of these functional components on growth, lipid profiles, and body fat accumulation. In general, it seems that the feeding of these components does not induce malnutrition or prevent normal growth of humans and animals.\(^\text{21,22}\) In fact, although simultaneous intake of DAG and casein resulted specifically in increases in body weight gain, food intake, and relative liver weight on the cholesterol diets, these alterations were not significant alterations to the growth of the experimental animals even in this study.

We observed significant increases in liver weights in the rats fed the DAG and casein diets. Similar responses in liver weight in rats fed the DAG diet were also detected in our previous study.\(^\text{23}\) Since alteration in relative liver weight is associated with accumulation of liver lipids, we discuss these alterations cautiously. Moreover, separate or combined intake of SPI and DAG did not affect adipose tissue weights. Murase et al. reported that a 5-month feeding of a high DAG and high sucrose diet resulted in significant reductions in body fat accumulation in mice as compared with a high fat and high sucrose diet.\(^\text{24}\) These results indicate that it requires a certain period to suppress body fat accumulation by functional food components. In our study, the rats were fed the experimental diets only for 28 d. Thus, DAG might not have acted functionally act on body fat accumulation in the short experimental period.
With respect to the lipid profiles of the rats fed the experimental diets, we found that simultaneous intake of SPI and DAG showed interactive significance on the serum and liver lipids profiles in the rats fed the experimental diets. In particular, these combinations showed ameliorating effects, but not sufficient, on serum and liver TG concentrations even in the rats fed the cholesterol-free diets. Although the physiological functions of individual components could not be statistically detected by modulating the lipid profiles of rats, it seems that the physiological impact might be enhanced by a combination of components that lower serum TG concentrations. In the case of the cholesterol diets, however, the physiological function of SPI was thought to be characteristic. That is, not only serum and liver cholesterol but also the TG concentrations of the rats fed the SPI diets were improved. Generally, it is considered that soybean protein decreases serum cholesterol and TG levels and thus reduces the incidence of cardiovascular disease as compared with animal protein. Recently, it was reported that β-conglycinin contributes at least in part to the TG-lowering effects of SPI. Thus it is thought that SPI has physiological functions that improve both cholesterol and TG levels.

Contrary to the physiological function of SPI, the DAG and casein diet increased serum and liver cholesterol and TG concentrations (Tables 3 and 4). The accumulation of liver TG concentrations might have been responsible in part for the increase of liver weights in rats fed the DAG and casein diet. Although Hara et al. indicate that DAG intake is generally thought not to promote liver lipid accumulation, we observed liver lipid accumulation in rats fed the DAG diets, as reported in our previous paper. Thus, our data are not necessarily consistent with the results obtained by Hara et al. It seems that a fatty liver causes hepatic cirrhosis, hepatic inflammation, and certain lifestyle-related disease risk factors, such as high blood pressure, coronary heart disease, and stroke. Therefore, these are the important problems that should be elucidated as to why the accumulation of liver TG was observed in our studies. To clear up such problems, first, we attempted to examine the influence of DAG on lipid metabolism due to strain differences.

In addition, we also observed significant excretion of fecal bile acid in the rats fed the SPI diets (Table 5). Increase in the fecal bile acid concentration is a primary mechanism of the cholesterol-lowering effects of SPI. These mechanisms were also determined with sufficient reproducibility in our study. Thus, SPI effectively exerted potent cholesterol- and TG-lowering effects even in this experimental design.

As to the individual functionality of SPI and DAG, SPI showed some ameliorative effects in the lipid profiles of the rats to varying degrees, while DAG did not show sufficient physiological benefits, such as an improvement in the serum TG level and the prevention of body fat accumulation, reported previously. Since the physiological benefits and the mechanisms of SPI and DAG have been established, the physiological impacts of simultaneous intake of foods and food components of similar function are expected.
by a number of consumers. However, we did not detect obvious additive or synergistic significances. The physiological functions of DAG appear to be defined by obvious additive or synergistic significances. The physiological functions of SPI rather than dietary oil. In addition, our results indicate that simultaneous intake of SPI and DAG showed statistical interactions, but not sufficiently, in serum TG, and liver cholesterol and TG levels in the rats fed the cholesterol-free diets. On the other hand, in the rats fed cholesterol diets, the lipid profiles might have been affected by the physiological function of SPI rather than dietary oil. In conclusion, in this study, we investigated the effects of simultaneous intake of SPI and DAG on lipid profiles and the body fat accumulation in rats. Simultaneous intake of SPI and DAG showed statistical interactions, but not sufficiently, in serum TG, and liver cholesterol and TG levels in the rats fed the cholesterol-free diets. On the other hand, in the rats fed cholesterol diets, the lipid profiles might have been affected by the physiological function of SPI rather than dietary oil. In addition, our results indicate that simultaneous intake of food components of similar functions does not exert effects additively and/or synergistically, and that these functions depend on dietary conditions. Moreover, one should pay attention to liver lipid accumulation in the rats fed the DAG diets in the future. Therefore, further studies are needed to clarify the effective application of functional foods and food components and the mechanism of liver lipid accumulation in the rats fed the DAG diets.

References


