**Soy Protein and Fish Oil Independently Decrease Serum Lipid Concentrations but Interactively Reduce Hepatic Enzymatic Activity and Gene Expression Involved in Fatty Acid Synthesis in Rats**

Yoko TAKAHASHI

Nutritional Function Laboratory, National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305–8642, Japan

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**Summary** The degree of interaction between dietary protein and fat sources to modulate hepatic lipid metabolism was investigated. Male rats were fed diets containing either casein or soy protein isolate as the protein source and either palm or soy oil as the fat source. After 3 wk, the activity and mRNA expression of enzymes involved in hepatic fatty acid synthesis were significantly lower with soy protein than casein when palm oil was the fat source. The same values for the same enzymes were greatly lowered regardless of the protein source when fish oil was the fat source. Both enzymatic activity and mRNA expression for fatty acid oxidation were significantly stimulated by fish oil, but only the former was increased by soy protein. Although both soy protein and fish oil reduced serum lipid concentrations, they worked independently. In soy protein-fed rats, mRNA levels of key enzymes related to cholesterol and bile acid synthesis were decreased and increased, respectively, compared with levels in casein-fed animals. Instead, fish oil strongly induced the mRNA expression of biliary cholesterol transporters, ATP-binding cassette sub-family G, member 5 (ABCG5) and ATP-binding cassette sub-family G, member 8 (ABCG8). Therefore, dietary soy protein and fish oil generally exerted independent hypolipidemic actions in rats. However, the reduction of hepatic fatty acid synthesis caused by the simultaneous ingestion of soy protein and fish oil was smaller than their expected additive reduction, because fish oil strongly decreased the synthesis.

**Key Words** fish oil, interaction, lipid, liver, soy protein

Soy protein and fish oil, in addition to their macronutrient content, contain bioactive components that directly or indirectly interact with hepatic genes to modulate serum lipid levels. Soy protein has been shown to reduce serum lipid levels in experimental animals and humans (1–3). Animal studies have demonstrated that soy protein increased the fecal excretion of bile acids and sterols (1), thus directing cholesterol synthesis toward replenishing the cholesterol and bile acid pools. Compared with casein, soy protein increased the mRNA and protein levels of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (1, 2, 4) and the mRNA expression and activity of cholesterol 7α-hydroxylase (CYP7A1) in the liver of rats (3, 4). Soy protein increased the mRNA expression of ATP-binding cassette, sub-family G, member 5 (ABCG5) and ATP-binding cassette, sub-family G, member 8 (ABCG8) (5), putative membrane transporters that excrete biliary cholesterol in liver (6). It is also reported that soy protein had a hypotriglyceridemic effect in liver through the easing of sterol regulatory element-binding protein (SREBP)-1-dependent lipogenesis (2, 3).

Dietary lipids, as well as proteins, have a considerable impact on the modulation of lipid metabolism. n-3 polyunsaturated fatty acids (PUFAs), especially abundant in fish oil, have been implicated in lowering blood lipid levels compared with saturated fats (7, 8). n-3 PUFAs were identified as a putative dietary factor for modulating fatty acid metabolism through the activation of peroxisome proliferator-activated receptor (PPAR) α, a nuclear receptor that regulates fatty acid oxidation, and the suppression of SREBP-1c-mediated lipogenesis in liver (7, 9). Several studies have reported that consumption of fish oil lowered serum cholesterol levels in rats (8). Although the effects of n-3 PUFAs on hepatic cholesterol metabolism are less well documented than those of fatty acid metabolism, fish oil suppressed the activity of HMG-CoA reductase in animals (10).
In Asian populations, epidemiological surveys and intervention studies have confirmed that the consumption of soy products and fish lowers mortality from cardiovascular disease (11). Soy and fish are staples of Asian diets but it is not known whether these foods when consumed together interact to regulate hepatic lipid metabolism in a manner different from the ingestion of the individual foods. Dietary protein and fat sources interacted to change serum and hepatic triglyceride concentrations in rats (12), but the molecular mechanisms related to hepatic fatty acid and cholesterol metabolism were not determined. The present study was performed to investigate the potential interactive effects of dietary protein sources (casein and soy protein) with fat sources (palm oil and fish oil) on the activity and mRNA expression of enzymes involved in the metabolism of fatty acid and cholesterol in the liver of rats.

MATERIALS AND METHODS

Materials. Soy protein isolate (FUJIPRO) was donated by Fuji Oil Co. Ltd., Osaka, Japan. The isoflavone content of the protein was 1.93 g/kg. Casein was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Palm oil and fish oil were obtained from NOF Corporation (Tokyo, Japan). The fish oil contained 27.8% docosahexaenoic acid and 9.2% eicosapentaenoic acid, according to the manufacturer. The total cholesterol (TC) content of the palm oil and fish oil was determined as 0.006 and 0.281 mg/g, respectively, by enzymatic methods (13).

Animals and experimental diets. Male Sprague-Dawley rats (Charles River, Kanagawa, Japan) at 4 wk of age were housed individually in a room with controlled temperature (20–22°C) and humidity (55–65%), and a 12-h lighting cycle (07:00–19:00), and fed a commercial non-purified diet (Type NMF; Oriental Yeast Co., Ltd., Tokyo, Japan). After 7 d of acclimatization to the conditions, the rats were randomly divided into 4 groups consisting of 7–8 animals each with similar mean body weights and assigned experimental diets. The diets contained 200 g/kg of protein and 150 g/kg of fat: casein-palm oil diet (CP), casein-fish oil diet (CF), soy protein-palm oil diet (SP), and soy protein-fish oil diet (SF). CF and SF contained 100 g/kg of fish oil and 50 g/kg of palm oil. The basal composition of the experimental diet was (in g/kg): protein, 200; fat, 150; corn starch, 150; cellulose, 20; mineral mixture (AIN-93G-MX), 35 (14); vitamin mixture (AIN-93-VX), 10 (14); L-cystine, 3; choline bitartrate, 2.5; and sucrose, 429.5. Animals had free access to diet and water during the experiment. After 3 wk of feeding, the animals were sacrificed by bleeding from the abdominal aorta under diethyl ether anesthesia, and the liver was quickly excised. Animals were handled according to the guidelines of the Ministry of Agriculture, Forestry and Fisheries for experiments with laboratory animals. The animal studies were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization, Japan.

Analyses of serum and liver components. Serum triacylglycerol (TG), TC, high-density lipoprotein cholesterol (HDL-C), phospholipid, and free fatty acid concentrations were determined using commercial enzyme kits (Wako Pure Chemical Industries, Ltd.). Serum insulin concentrations were assayed using an ELISA kit for rat insulin (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan). Hepatic lipid was extracted (15), and levels of TG (16) and TC (13) were analyzed as previously reported.

Analyses of enzymatic activities. Immediately after the excision, approximately 1.5 g of liver was homogenized with 10 mL of 0.25 M sucrose buffer containing 1 mM EDTA and 3 mM Tris-HCl (pH 7.2) on ice. An aliquot of the homogenate was centrifuged at 200,000 × g for 30 min at 4°C. The activities of enzymes involved in fatty acid synthesis such as fatty acid synthase (FAS), ATP-citrate lyase (ACL), glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase, and malic enzyme (ME) were measured spectrophotometrically using the supernatant fraction as an enzyme source (17). The activities of enzymes involved in fatty acid oxidation, acyl-CoA oxidase (ACOX), carnitine palmitoyltransferase (CPT), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-keotacyl-CoA thiolase, were analyzed using a whole liver homogenate as an enzyme source (17).

Analysis of mRNA levels. Total RNA was extracted from liver, and cDNA was synthesized by reverse transcription (18). We measured mRNA levels by quantitative real-time PCR (17) of genes involved in fatty acid synthesis (acyetyl-CoA carboxylase, FAS, ACL, G6PD, ME, and SREBP-1c), fatty acid desaturation (stearoyl-CoA desaturase 1), fatty acid oxidation (carnitine octanoyltransferase, ACOX, bifunctional enzyme, carnitine palmitoyltransferase 2, trifunctional enzyme subunit α, trifunctional enzyme subunit β, and PPARα), cholesterol synthesis (HMG-CoA reductase, farnesyl diphosphate synthase, and squalene synthase) (19), bile acid synthesis (CYP7A1) (19), and sterol excretion into the bile (ABCG5, ABCG8) (6). The nucleotide sequences of primers and probes used are listed in Table 1 and reported elsewhere (17, 20). mRNA abundance was calculated as a ratio to the value for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each cDNA sample and expressed as a percentage assigning the value in rats fed CP as 100. Because the expression of reference genes varies depending on the experimental conditions such as individual differences, dietary status, and stress (21), the validation of GAPDH as a reference gene was confirmed by the correlation (p<0.001, r=0.959) of the threshold cycles between GAPDH and β-actin, another popular housekeeping gene, in individual hepatic total RNA samples as previously reported (22).

Statistical analysis. Values were expressed as the mean±SE. Statistical analyses were performed using SPSS 13.0J for Windows (SPSS Japan Inc., Tokyo, Japan). To determine the significance of the effects of
dietary protein and lipid sources and interactions between the two factors, data were analyzed with a 2×2 factorial ANOVA at a level of \( p < 0.05 \). If the interaction was significant, a simple main effect test by applying the Bonferroni correction was performed to identify whether the effect of dietary protein (fat) types changes the parameters of lipid metabolism depending on the fat (protein) types. The correlation between the activity and mRNA expression of the hepatic enzymes was estimated with Pearson’s product-moment correlation coefficient.

**RESULTS**

**Serum and liver lipids and growth**

There were significant physiological differences due to protein and fat sources in serum and hepatic lipids, but no significant protein×lipid interactions (Table 2). The soy diets SP and SF lowered body weight gain 6% and 14% compared to the casein diets CP and CF, respectively, despite a similar food intake and liver weight. The fish oil diets CF and SF decreased serum TG (69–74%) and TC (46–55%) levels compared to the palm oil diets CP and SP, respectively. Soy protein also lowered serum TC concentrations by 31–43% and TG concentrations by 9.0–23%. The soy protein and fish oil diets resulted in lower serum HDL-C and phospholipid levels than the casein and palm oil diets, respectively. Fish oil decreased the ratio of TC: HDL-C. The ratios in the CP, CF, SP and SF groups were 2.03, 1.65, 2.48, and 1.68, respectively. Fish oil decreased serum free fatty acid levels by 70–72%. Overall, SF had the lowest serum lipid concentrations among the groups. Serum insulin concentrations were not affected by dietary protein or fat sources. Dietary fat source had a significant impact on hepatic TG and TC concentrations, which were lower in rats fed fish oil diets than in those fed palm oil diets. Hepatic TG levels were higher in rats fed soy protein than casein, but the difference was not significant. Protein source did not alter the hepatic cholesterol concentration.

**Activity of enzymes and expression of genes for hepatic lipogenesis**

In contrast to the lack of interaction of serum and hepatic lipid levels with dietary protein and fat sources, we observed significant interaction in the genes and enzymes involved in fatty acid synthesis. The activities of FAS, ACL, G6PD, 6-phosphogluconate dehydrogenase, and ME were considerably reduced by fish oil compared to palm oil in the rats fed the casein diets (Fig. 1). Soy protein significantly reduced the activities of all these enzymes compared with casein in the rats fed the palm oil diets, whereas fish oil strongly decreased the enzymatic activities except ME regardless of protein source (Fig. 1A–D). Soy protein maintained lower levels of ME activity in the rats on both the fat diets, although fish oil clearly decreased the activity in rats on the casein diets (Fig. 1E).

When the diet was SF, relative mRNA expression was the lowest for the fatty acid synthesis-related enzymes that were examined in this study. As observed for most of the enzyme activities, there was significant

### Table 1. Nucleotide sequences of primers and probes for real-time PCR.

| Genes                        | Sense primer | Antisense primer | Probe    | GenBank accession no. |
|------------------------------|--------------|------------------|----------|-----------------------|
| ABCG5                        | 5'-GGGACAGGAAAATCCTCAAAGA-3' | 5'-TGAGCTACCTAAGATGCACATGGT-3' | 5'-CTCCTTGTACATCGAGAGTGGCCA-3' | NM_053754 |
| ABCG8                        | 5'-GGCAAGATGAAATCAGGACAAAT-3' | 5'-CATGTGCCACACACTTCTGTATCA-3' | 5'-AAACGGGCAACCCAGCACGC-3' | NM_130414 |
| CYP7A1                       | 5'-GCATTTGGACACAGAAGCATTG -3' | 5'-GGAGGGTTTTGGTAAAAGTGTTGT-3' | 5'-CCCAAATGATGGAAATACCACGGAA-3' | NM_012942 |
| Farnesyl diphosphate synthase| 5'-CATCAACGATGCTCTGCTTCTG-3' | 5'-GGGCTGCTCCCTGCAGTAG-3' | 5'-CCGCTATCTACCGCCTGCTTAAG-3' | BC059125 |
| HMG-CoA reductase             | 5'-CGTCTTCAGCACTGTCGTCATT-3' | 5'-GAAAAAAGGGCAAAGCTTCATTT-3' | 5'-TTCCTCGACAAAGAATTGACAGGCT-3' | BC064654 |
| Squalene synthase            | 5'-ACCGGGTCCCCAACTCA-3' | 5'-CTCTGCGTCCTGATGTTGGA-3' | 5'-CCGTCAGCTAGCAAGGCCAAGCA-3' | BC081810 |
| Stearoyl-CoA desaturase 1     | 5'-TTGCCAGAGGGAATAGGGAAA-3' | 5'-CTCTCCCATCCTTACTTACAAACCA-3' | 5'-ACCGTGCGTGCTAATTCTTCTCTCAAGGT-3' | AF509569 |
Table 2. Effect of dietary protein and fat types on food intake, body and liver weights, serum lipid and insulin concentrations, and liver lipid levels in rats.

|                  | CP     | CF     | SP     | SF     | Protein | Fat   | P×F  |
|------------------|--------|--------|--------|--------|---------|-------|------|
| Food intake (g/d)| 19.5±0.5 | 19.5±0.6 | 19.3±0.2 | 18.4±0.7 | 0.294   | 0.406 | 0.420|
| Body weight (g)  | 330±7  | 339±9  | 318±6  | 311±12 | 0.031   | 0.866 | 0.358|
| Body weight gain (g/d) | 9.13±0.25 | 9.59±0.40 | 8.54±0.20 | 8.25±0.53 | 0.019   | 0.830 | 0.336|
| Liver (g/100 g body weight) | 4.98±0.16 | 4.85±0.13 | 5.33±0.17 | 4.91±0.19 | 0.231   | 0.119 | 0.383|

Values are shown as mean±SE for 7–8 rats. The “Two-way ANOVA” column represents the main and interactive effects of protein of two types (casein and soy protein) and fat of two types (palm oil and fish oil) and their interaction (P×F). Values of p<0.05 were accepted as statistically significant.

Fig. 1. Interactive effects of dietary protein and fat sources on the activities of hepatic lipogenic enzymes. (A) fatty acid synthase, (B) ATP-citrate lyase, (C) glucose 6-phosphate dehydrogenase, (D) 6-phosphogluconate dehydrogenase, (E) malic enzyme. Values are shown as the mean±SE for 7–8 rats. The values accompanied by different capital letters differ significantly between the same protein diets, and accompanied by different small letters differ significantly between the same fat diets at p<0.05.
protein/lipid interaction for mRNA expression by the genes for G6PD and stearoyl-CoA desaturase 1 (Fig. 2A and B). Fish oil reduced the mRNA level of ME in casein diets by approximately 60%, while expression levels were considerably low and almost identical in palm and fish oil diets when soy was the protein source (Fig. 2C). Genes for acetyl-CoA carboxylase, FAS, and ACL did not show significant interactions (Table 3) although the interactions in the activities of their respective enzymes were observed (Fig. 1A and B for FAS and ACL, respectively). The correlations between the relative mRNA expression of enzyme, ACL, G6PD and MEs and enzymatic activity were fairly linear (Fig. 2A–D). The mRNA expression of SREBP-1c, which regulates these lipogenic enzymes (23), was decreased significantly with fish oil in the diet (Table 3). Soy protein also lowered the expression when compared with casein, although the reduction was not significant.

Activity of enzymes and expression of genes for hepatic fatty acid oxidation

The enzymatic activities for hepatic fatty acid oxidation were significantly higher in rats fed soy protein than in those fed casein (Table 4). Fish oil also increased these activities but decreased the activity of 3-hydroxyacyl-CoA dehydrogenase compared to casein. In contrast to the enzymes for fatty acid synthesis, no significant protein/lipid interactions were observed, except for 3-ketoacyl-CoA thiolase.

Similar to the enzyme activity, mRNA expression was clearly increased in fish oil-fed rats compared with palm oil-fed rats (Table 4). However, the protein source had different effects on the expression of these enzymes. Soy protein increased the mRNA expression of carnitine palmitoyltransferase 2 and trifunctional enzyme α unit...
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independent of fat type. In contrast, it did not modify the mRNA level of bifunctional enzyme or trifunctional enzyme $\beta$, and actually reduced the mRNA levels of carnitine octanoyltransferase and ACOX. However, no significant interaction between protein and fat sources was observed. The mRNA level of PPAR$\alpha$ was significantly higher in rats fed fish oil than in those fed palm oil, but protein source had no effect. The activities of ACOX and CPT were linearly related to mRNA expression levels (Fig. 3E and F).

**Expression of genes for hepatic cholesterol metabolism**

Soy protein reduced the mRNA expression of genes encoding enzymes for cholesterol synthesis compared with casein (Table 5). Fish oil consistently decreased the gene expression for these enzymes but the reductions were not significant. The mRNA abundance of CYP7A1 was significantly increased by soy protein compared to casein. In contrast to genes encoding enzymes for cholesterol and bile acid synthesis, fish oil strongly increased the mRNA expression of the genes for ABCG5 and ABCG8 that have been presumed to play a role in biliary cholesterol secretion in hepatocytes (6). Dietary soy protein also increased the mRNA expression of ABCG8 compared with casein, although the differences were not significant. No interactions between dietary protein and lipid were observed.

**DISCUSSION**

This study examined whether soy protein and fish oil interactively affect lipid metabolism in rats. A strong
Table 4. Effect of dietary protein and fat types on the activities and mRNA levels of enzymes involved in fatty acid oxidation and mRNA level of PPARα in liver of rats.

| Enzyme activity (nmol/min/mg protein) | CP    | CF    | SP    | SF    | Two-way ANOVA |
|--------------------------------------|-------|-------|-------|-------|---------------|
| Acyl-CoA oxidase                      | 0.93±0.064 | 2.53±0.21 | 1.01±0.04 | 3.06±0.07 | 0.029 <0.001 0.093 |
| Carnitine palmitoyltransferase        | 2.40±0.07 | 6.47±0.32 | 3.21±0.09 | 7.95±0.29 | <0.001 <0.001 0.192 |
| Enoyl-CoA hydratase                   | 5.51±120  | 6.72±119 | 8.18±217  | 8.53±344  | <0.001 0.004 0.090  |
| 3-Hydroxyacyl-CoA dehydrogenase      | 28±5    | 111±2  | 325±14  | 130±9    | 0.006 <0.001 0.291 |
| 3-Ketoacyl-CoA thiolase               | 144±7   | 267±17 | 171±16  | 359±12   | 0.001 <0.001 0.044 |

Relative mRNA level (%)

| Enzyme activity          | CP    | CF    | SP    | SF    | Two-way ANOVA |
|--------------------------|-------|-------|-------|-------|---------------|
| Carnitine octanoyltransferase | 100±11 | 378±51 | 67.3±10.0 | 242±17 | 0.005 <0.001 0.067 |
| Acyl-CoA oxidase          | 100±9 | 180±14 | 87.5±4.5  | 144±8   | 0.016 <0.001 0.220 |
| Bifunctional enzyme       | 100±15 | 218±19 | 75.3±4.1  | 243±15  | 0.994 <0.001 0.100 |
| Carnitine palmitoyltransferase 2 | 100±9 | 149±9  | 110±6  | 178±6   | 0.016 <0.001 0.228 |
| Trifunctional enzyme subunit α | 100±7 | 153±9  | 122±4   | 195±6   | <0.001 <0.001 0.129 |
| Trifunctional enzyme subunit β | 100±8 | 169±9  | 104±6   | 190±8   | 0.138 <0.001 0.299 |
| PPARα                     | 100±5 | 137±9  | 124±14  | 138±8   | 0.193 0.011 0.238 |

Table 5. Effect of dietary protein and fat types on the mRNA levels of enzymes and proteins involved in cholesterol synthesis and efflux in the liver of rats.

| Enzyme activity          | CP    | CF    | SP    | SF    | Two-way ANOVA |
|--------------------------|-------|-------|-------|-------|---------------|
| HMG-CoA reductase         | 100±15 | 89.7±9.3 | 75.6±2.6 | 58.0±2.6 | 0.003 0.120 0.677 |
| Farnesyl diphosphate synthase | 100±28 | 72.5±13.1 | 45.3±1.5 | 30.0±2.1 | 0.004 0.171 0.392 |
| Squalene synthase         | 100±18 | 95.4±11.2 | 78.0±1.9 | 66.2±3.5 | 0.023 0.446 0.738 |
| CYP7A1                    | 100±12 | 119±28 | 173±17  | 183±34  | 0.012 0.557 0.861 |
| ABCG5                     | 100±25 | 796±101 | 130±8   | 640±88  | 0.380 <0.001 0.197 |
| ABCG8                     | 100±18 | 5.293±440 | 489±40  | 9.631±2.822 | 0.149 <0.001 0.225 |

Values are shown as mean±SE for 7–8 rats. The “Two-way ANOVA” column represents the main and interactive effects of protein of two types (casein and soy protein) and fat of two types (palm oil and fish oil) and their interaction (P×F). Values of p<0.05 were accepted as statistically significant.

Hypolipidemic effect was observed in the serum of rats fed fish oil and a substantial effect in those fed soy protein (Table 2). However, an interactive contribution of these dietary factors to modulate serum and hepatic lipid levels and other physiological parameters was not observed. Similar hypolipidemic effects were examined in rats by Demonty et al. (12); they observed interactive diminution of serum TG levels when menhaden oil was combined with soy protein but not with either casein or cod protein. To further characterize the interaction of protein and fat sources in the modulation of lipid metabolism, we investigated the changes of mRNA levels and activities of hepatic lipid metabolism-related enzymes. In contrast to serum and hepatic lipid concentrations, we did observe dietary protein and fat interactions in the activities of the enzymes and the expression of some genes for lipid synthesis.

When soy protein and fish oil were concomitantly given, the significant interactions of these dietary factors were found in reducing hepatic lipogenesis as indicated in Figs. 1 and 2. Since the level of fish oil that was used inhibited hepatic lipogenesis maximally, any further reduction by soy protein was very limited. The low activity of lipogenic enzymes may have been due to saturation of down-regulation in mRNA expression of hepatic lipogenic genes. Hepatic lipogenesis-related genes are preferentially regulated by SREBP-1c (23). In the present study, the mRNA expression of SREBP-1c was reduced by fish oil (Table 3) as previously reported (7, 9). In contrast, soy protein only marginally reduced this mRNA expression (p<0.1). Several studies have shown that the consumption of soy protein significantly decreased the mRNA expression of SREBP-1 as well as lipogenic enzymes in the liver of rats, probably related to lower plasma insulin level (1, 2, 4). Meanwhile, the present and other (24, 25) studies did not reveal a clear
reduction of SREBP-1c mRNA levels or serum insulin concentration in soy protein-fed groups (Table 2). Therefore, dietary soy protein may not directly regulate the mRNA expression of SREBP-1c, but regulates it through an insulin-related mechanism, or decreases protein or signaling levels of this transcription factor. Our results suggest that hepatic lipogenesis is reduced by fish oil primarily through decreased mRNA expression of SREBP-1c, whereas the reduction by soy protein appears to be mediated by other factors as well as a slight reduction in SREBP-1c expression. Therefore, there is a possibility that attenuated reduction in hepatic lipogenesis caused by concomitant ingestion of soy protein and fish oil was observed because of the partly competitive suppression of hepatic lipogenesis through SREBP-1c.

On the other hand, dietary protein and lipid sources regulated the mRNA levels and activities of fatty acid oxidation-related enzymes independently, rather than interactively (Table 4). We found fish oil substantially increased the mRNA level and the activity of fatty acid oxidative enzymes; however, the relative expression of PPARα was relatively weak. Since n-3 PUFAs are able to directly bind to PPARα and stimulate its activity (9), the increased mRNA expression of PPARα by fish oil is not essential to stimulate the activities of fatty acid oxidative enzymes. Soy protein also increased the activities of enzymes related to fatty acid oxidation in the present study, but the increase was not always accompanied by a change in the mRNA expression of these enzymes. The increase in the mRNA expression of PPARα caused by soy protein was not significant, suggesting the soy protein was unlikely to directly stimulate PPARα activity. Rather, the isoflavone component of soy protein possibly stimulates the activity of fatty acid oxidative enzymes. It was reported that a soy protein concentrate enriched with isoflavones significantly stimulated the activities of ACOX and CPT2 but not the mRNA expression of CPT2 in the liver of obese Zucker rats compared with a casein diet (26). Our previous study also demonstrated that the activities, but not the mRNA levels, of enzymes involved in fatty acid oxidation were dose-dependently increased by isoflavone supplementation in rats fed a soy protein diet (17).

Dietary protein and lipid sources also regulated the mRNA levels of cholesterol metabolism-related enzymes and proteins independently (Table 5). In addition to the down-regulation of mRNA expression of enzymes involved in cholesterol synthesis in the soy protein-fed rats compared with the casein-fed animals, we found a significant induction of CYP7A1 mRNA expression in rats fed soy protein. Even though we did not determine the excretion of fecal bile acids and steroids, these changes of mRNA expression are characteristics of the excretion of bile acids and the conversion of sterol into bile acid. It is commonly recognized that the increasing bile steroid secretion is one of the major hypolipidemic effects of soy protein (3, 4). In fact, Madani et al. reported that soybean protein rather than casein had higher CYP7A1 activity in the presence of 0.1% cholesterol in the diet, accompanying higher bile steroid excretion into feces (27). Since fish oil did not have a significant effect on these levels, the decrease in cholesterol synthesis and the increase of CYP7A1 in liver may be due entirely to the soy protein. By contrast, a strong up-regulation of ABCG5 and ABCG8 by fish oil compared with palm oil was observed in the present study. Recently, Nishimoto et al. demonstrated that a fish oil diet significantly increased the mRNA expression of hepatic ABCG5 and ABCG8 and excreted cholesterol into feces compared with a soy or coconut oil diet in mice (28). The facilitation of cholesterol secretion in bile acid by fish oil was also reported in rats (29). Thus, the substantial up-regulation in the expression of these cholesterol transporters that excrete cholesterol into bile acid may account for the reduction in serum cholesterol concentrations caused by consumption of fish oil. Although the precise mechanism of the regulation is still controversial at present, the expression of ABCG5/ABCG8 was mediated by several nuclear receptors, such as liver X receptor (30) and PPARα (31) that were able to interact with fish oil (7, 9). Collectively, soy protein and fish oil may independently reduced serum cholesterol levels by facilitating the transportation of cholesterol into bile acid through different mechanisms.

In the present study, although soy protein and fish oil significantly interacted to decrease hepatic lipogenesis, the interaction did not reflect the physiological parameters such as serum lipid concentrations. This is probably because serum lipid concentrations are modulated by multiple mechanisms. We found the significant interaction of dietary protein with fat sources only in hepatic lipogenesis, but homeostasis of lipid metabolism was maintained by various metabolic pathways, i.e., hepatic lipogenesis and fatty acid oxidation, lipoprotein clearance by liver and peripheral tissues. Further studies throughout the body are needed to investigate possible mechanisms underlying the regulation of serum lipid levels.

In conclusion, the present study demonstrated that soy protein and fish oil independently lowered serum lipid levels in rats. Soy protein and fish oil independently decreased the activity and mRNA expression of enzymes related to lipogenesis. The hepatic lipogenic activities by fish oil were maximally suppressed and overwhelmed the modest reduction in lipogenesis derived from soy protein. There were no interactions between dietary protein and fat sources that affected hepatic fatty acid oxidation or cholesterol metabolism.

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