Fish Oil Feeding Decreases Mature Sterol Regulatory Element-binding Protein 1 (SREBP-1) by Down-regulation of SREBP-1c mRNA in Mouse Liver

A POSSIBLE MECHANISM FOR DOWN-REGULATION OF LIPOGENIC ENZYME mRNAs*

(Received for publication, May 7, 1999, and in revised form, June 16, 1999)

Hyoun-Ju Kim, Mayumi Takahashi, and Osamu Ezaki†

From the Division of Clinical Nutrition, National Institute of Health and Nutrition,
1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan

Dietary fish oil induces hepatic peroxisomal and microsomal fatty acid oxidation by peroxisome proliferator-activator receptor α activation, whereas it down-regulates lipogenic gene expression by unknown mechanism(s). Because sterol regulatory element-binding proteins (SREBPs) up-regulate lipogenic genes, investigation was made on the effects of fish oil feeding on SREBPs and sterol regulatory element (SRE)-dependent gene expression in C57BL/6J mice. Three forms of SREBPs, SREBP-1a, -1c, and -2, are expressed in liver, and their truncated mature forms activate transcription of sterol-regulated genes. C57BL/6J mice were divided into three groups; the first group was given a high carbohydrate diet, and the other two groups were given a high fat diet (60% of total energy), with the fat in the form of safflower oil or fish oil, for 5 months. Compared with safflower oil feeding, fish oil feeding decreased triglyceride and cholesterol concentrations in liver. There were no differences in amount of SREBP-1 and -2 in both precursor and mature forms between carbohydrate- and safflower oil-fed mice. However, compared with safflower oil feeding, fish oil feeding reduced the amounts of precursor SREBP-1 in membrane fraction by 90% and of mature SREBP-1 in liver nuclei by 57%. Fish oil feeding also reduced precursor SREBP-2 by 65% but did not alter the amount of mature SREBP-2. Compared with safflower oil feeding, fish oil feeding decreased liver SREBP-1c mRNA level by 86% but did not alter SREBP-1a mRNA. Consistent with decrease of mature SREBP-1, compared with safflower oil feeding, fish oil feeding down-regulated the expression of liver SREBP-1c mRNA. These data suggested that in liver, fish oil feeding down-regulated the mature form of SREBP-1 by decreasing SREBP-1c mRNA expression, with corresponding decreases of mRNAs of cholesterogenic and lipogenic enzymes.

Dietary fish oil contains n-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, which decrease blood triglyceride concentrations in hypertriglyceremic patients and are considered to have protective effects against cardiovascular diseases (1). This effect of n-3 fatty acids mainly results from the combined effects of inhibition of lipogenesis and stimulation of fatty acid oxidation in liver (2, 3). It has been shown that n-3 fatty acids in vivo or in cell culture inhibited the transcription of genes coding for lipogenesis enzymes, such as fatty acid synthase (FAS),1 acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and S14 protein (4, 5). On the other hand, n-3 fatty acids increased the transcription of the regulatory enzymes of fatty acid oxidation, such as lipoprotein lipase (LPL), fatty acid-binding protein, acyl-CoA synthetase (ACS), carnitine palmitoyltransferase 1, acyl-CoA dehydrogenase, and acyl-CoA oxidase (4, 5).

The molecular mechanisms by which n-3 fatty acids regulate gene transcription have not yet been clarified, but on the basis of in vitro assays and in comparison with peroxisome proliferators, such as fibrate compounds, it has been suggested that n-3 fatty acids can regulate gene transcription through the activation of a transcription factor, peroxisome proliferator-activated receptor (PPAR) α (6–9). In addition, a recent study to examine fish oil feeding on several genes expression in PPAR knockout mice clearly indicated that hepatic gene regulation of fish oil feeding involved at least two distinct pathways, a PPARα-dependent and a PPARα-independent pathway (10). Interestingly, enzymes for peroxisomal (cytochrome P450 4A2) and microsomal (acyl-CoA oxidase) oxidation are PPARα-dependent and are up-regulated, whereas enzymes for lipid synthesis (FAS and S14) are PPARα-independent and are down-regulated (10).

On the other hand, sterol regulatory element-binding proteins (SREBPs) are other important transcription factors that regulate fatty acid and cholesterol metabolism in liver (11). In sterol depletion, SREBPs are cleaved and become mature forms to bind sterol regulatory elements (SREs) (12, 13) and/or E-box sequences (14) and then activate the target gene expression. Thus, both expression levels and processing of SREBPs regulate the target gene expression. Furthermore, three forms of SREBPs, SREBP-1a, SREBP-1c, and SREBP-2, are expressed in liver, and they use different promoters for their own expression (15, 16). In addition, studies on transgenic mice that over-

1 The abbreviations used are: FAS, fatty acid synthase; PPAR, peroxisome proliferator-activated receptor; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein; LDL, low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LPL, lipoprotein lipase; ACS, acyl-CoA synthetase; ACC, acetyl-CoA carboxylase; SCD, stearoyl-CoA desaturase.

* This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Japanese Science and Technology Agency (Tokyo), research grants from the Japanese Ministry of Health and Welfare (Tokyo), and the Japanese Ministry of Education, Science, Sports and Culture (Tokyo). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed. Tel.: 81-3-3203-5725; Fax: 81-3-3207-3520; E-mail: ezaki@nih.go.jp.
Effects of Fish Oil on SREBP-1c

**Materials and Methods**

**Fish Oil Diet Experiment**—Female C57BL/6J mice were obtained from Tokyo Laboratory Animals Science Co. (Tokyo, Japan) at 7 weeks of age and fed a normal laboratory diet (CE2, Clea, Tokyo, Japan) for 1 week to stabilize the metabolic conditions. Mice were exposed to a 12-h light/12-h dark cycle and maintained at a constant temperature of 22 °C. Mice were divided into three groups (n = 13–16 in each group). Each group was divided into three cages, with each cage containing 4–6 mice. The first group was given a high carbohydrate diet, on a calorie basis, that contained 63% carbohydrate, 11% fat, and 26% protein. In the high carbohydrate diet, safflower oil was used as the source of fat. The second group was given a safflower oil-rich diet containing 14% carbohydrate, 60% safflower oil, and 26% protein. The third group was given a high fish oil diet containing 14% carbohydrate, 60% fish oil (mainly from tuna), and 26% protein.

**Preparation of cDNA Probe for Northern Blot**—The cDNA fragments specific to either mouse SREBP-1a or SREBP-1c were amplified by polymerase chain reaction from first strand cDNA prepared using mouse liver total RNA. The following primers were used. SREBP-1a: 5′-primer, 5′-TAGTCGGGCCTG-3′, and 3′-primer, 5′-GAGTCGGGCCTG-3′; SREBP-1c: 5′-primer, 5′-ATCGGCCGGAGGATCGTTGGGACC-3′, and 3′-primer, 5′-GAGTCGGGCCTG-3′. Amplified products were subcloned into pGEM-T Easy vector (Promega, Madison, WI), digested, and cloned into λgt11 (Stratagene, La Jolla, CA). A part of liver of each mouse was immediately homogenized to obtain membrane fractions and nuclear extracts (23), and the other portion of liver was frozen for measurement of triglyceride and cholesterol as described in previous studies (24).

**Fibrate Administration**—Female C57BL/6J mice (n = 5; 7 weeks of age) were treated for 2 weeks with fenofibrate (Sigma) mixed in high carbohydrate diet that had the same ingredient used in fish oil diet study. Control mice (n = 5) were fed under the same conditions but without the presence of fenofibrate. Because each mouse consumed approximately 1.5–2.0 g of chow/day, doses of 0.5% (w/w) mixed in diet correspond to 410–550 mg/kg of body weight/day. Mice were fed each diet for 2 weeks. Mice were sacrificed in a method similar to that of fish oil feeding experiment.

**Preparation of cDNA Probe for Northern Blot**—The cDNA fragments for mouse SREBP-1, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, apoE, and LPL were obtained by polymerase chain reaction from first strand cDNA using mouse liver total RNA. Total RNA from mouse liver was isolated by the method of Chirgwin et al. (22). A part of liver of each mouse was immediately homogenized to obtain membrane fractions and nuclear extracts, and the other portion of liver was frozen for measurement of triglyceride and cholesterol as described in previous studies (24).

**Preparation of SREBP-1a and SREBP-1c for Northern Blot**—Each group was divided into three cages, with each cage containing 4–6 mice. The first group was given a high carbohydrate diet, on a calorie basis, that contained 63% carbohydrate, 11% fat, and 26% protein. In the high carbohydrate diet, safflower oil was used as the source of fat. The second group was given a safflower oil-rich diet containing 14% carbohydrate, 60% safflower oil, and 26% protein. The third group was given a high fish oil diet containing 14% carbohydrate, 60% fish oil (mainly from tuna), and 26% protein.

**Preparation of cDNA Probe for Northern Blot**—The cDNA fragments specific to either mouse SREBP-1a or SREBP-1c were amplified by polymerase chain reaction from first strand cDNA prepared using mouse liver total RNA. The following primers were used. SREBP-1a: 5′-primer, 5′-TAGTCGGGCCTG-3′, and 3′-primer, 5′-GAGTCGGGCCTG-3′; SREBP-1c: 5′-primer, 5′-ATCGGCCGGAGGATCGTTGGGACC-3′, and 3′-primer, 5′-GAGTCGGGCCTG-3′. Amplified products were subcloned into pGEM-T Easy vector (Promega, Madison, WI). After linearization of plasmid, antisense RNA was transcribed with [α-32P]CTP (800 Ci/mmol) using bacteriophage T7 or SP6 RNA polymerase (Promega). Specific activities of the transcribed RNAs were measured in each experiment and were in the range of 0.8–1.2 × 106 cpm/μg. Aliquots of 10 μg from the transcribed RNA samples were fractionated by RPA protection assay using a RPA II™ kit (Ambion, Inc., Austin, TX). After digestion with RNase A/T1, protected fragments were separated on 8% urea/4.8% polyacrylamide gels. The gels were dried and then subjected to autoradiography. Quantitative analysis was performed with an image analyzer (BAS 2000).

**Immunoblotting**—Pooled liver membranes and nuclear extracts from 5 mice of each group were prepared by the method described by Sheng et al. (23). The same amount of protein from each fraction was applied to 7% SDS-polyacrylamide gel electrophoresis and transferred to Hybond-P membranes (Amersham Pharmacia Biotech). Immuno blot analysis was performed by using the ECL Western blotting detection system kit (Amersham Pharmacia Biotech). Membrane sheets were first incubated with antibody against SREBP-1 or SREBP-2 for 1 h at 22 °C and then several times and incubated with horseradish peroxidase-conjugated anti-mouse IgG according to the protocol supplied by the manufacturer. The bands were quantified by scanning with a Quantity One System (Bio-Rad Laboratories, Richmond, CA), and the results were expressed as the ratio of the optical density at 450 nm to the optical density at 595 nm.
Effects of Fish Oil on SREBP-1c

**TABLE I**

*Phenotypic comparison of high carbohydrate-, high safflower oil-, and high fish oil-fed mice*

Mice were killed at 5 months of feeding, and final body weight, wet parametrial white adipose tissue (WAT) and liver weight, and liver cholesterol and triglyceride were measured. Initial body weight is body weight at the beginning of diet experiments. Plasma triglycerides and cholesterol were measured under feeding conditions at 4 months of feeding. Each data point represents mean ± S.E. of 5–16 mice, and number of mice is shown in parentheses.

|                      | Carbohydrate | Safflower oil | Fish oil |
|----------------------|--------------|---------------|----------|
| Initial body weight (g) | 17.8 ± 0.3 (15) | 17.6 ± 0.3 (13) | 17.5 ± 0.3 (16) |
| Final body weight (g)   | 24.2 ± 0.6 (15) | 33.3 ± 1.2 (13)* | 22.6 ± 0.4 (16)* |
| WAT weight (g)          | 0.6 ± 0.1 (14)  | 2.1 ± 0.2 (13)* | 0.4 ± 0.0 (16)* |
| Liver weight (g)        | 1.0 ± 0.0 (15)  | 1.9 ± 0.0 (13)* | 1.5 ± 0.1 (16)* |
| Liver cholesterol (μmol/g) | 15.2 ± 1.6 (8) | 11.5 ± 0.8 (8)* | 7.5 ± 0.8 (8)* |
| Liver triglyceride (μmol/g) | 51.4 ± 7.2 (8) | 76.3 ± 2.5 (8)* | 29.3 ± 2.2 (8)* |
| Plasma cholesterol (mmol/liter) | 2.30 ± 0.08 (6) | 2.54 ± 0.09 (5) | 1.72 ± 0.09 (7)* |
| Plasma triglycerides (mmol/liter) | 0.20 ± 0.05 (6) | 0.28 ± 0.08 (5) | 0.18 ± 0.03 (7) |

* P < 0.001, safflower oil compared with carbohydrate; fish oil compared with safflower oil by Fisher’s protected least significant difference test.

Body weight (p < 0.001), a 3.5-fold increase of parametrial white adipose tissue weight (p < 0.001), and a 1.5-fold increase of triglyceride accumulation in liver (p < 0.001). In contrast, fish oil-fed mice did not develop obesity or triglyceride accumulation in liver. However, the average energy intake among these three groups was not significantly different (7.4 ± 0.5, 7.7 ± 0.9, and 8.0 ± 0.5 kcal/mouse/day in carbohydrate, safflower oil, and fish oil-fed mice, respectively; n = 5). Fish oil feeding also affected lipid metabolism. Liver cholesterol and triglyceride concentrations from fish oil-fed mice were lower by 35% (p < 0.05) and 62% (p = 0.001), respectively, than those from safflower oil-fed mice. Plasma cholesterol and triglycerides concentrations from fish oil-fed mice were also lower by 32% (p = 0.001) and 36% (p = 0.19), respectively, than those from safflower oil-fed mice. Liver weight from fish oil-fed mice was 25% greater than that from safflower oil-fed mice (p < 0.001). This might be due to the well known effects of fish oil on peroxisomal proliferation (32).

Fig. 1 shows immunoblots of the precursor and mature SREBP-1 and -2 in liver of mice fed high carbohydrate, high safflower oil, and high fish oil diets for 5 months. Because antibody to SREBP-1 reacted to both SREBP-1a and -1c forms, we could not distinguish these two forms, and we used the noncommittal term SREBP-1. However, in mouse liver, the ratio of SREBP-1c to -1a transcripts is 9:1 (31), and thus the -1c form accounted for most of SREBP-1 observed on the immunoblots. In preliminary experiments, to confirm that 125- and 68-kDa proteins we observed are really the precursor and mature SREBP-1, fasting and refeeding experiments were conducted. Both precursor and mature SREBP-1 were decreased by 48 h fasting and increased above nonfasted levels by refeeding (data not shown). This confirms not only previous finding (33) but also confirms that the bands at which we aimed were SREBP-1. There were no differences in the amount of SREBP-1 and -2 in both precursor and mature forms between carbohydrate- and safflower oil-fed mice. However, compared with safflower oil feeding, fish oil feeding reduced the amount of precursor SREBP-1 in membrane fraction by 90% and that of mature SREBP-1 in liver nuclei by 57% (Fig. 1A). Fish oil feeding also reduced the precursor SREBP-2 by 65% but did not alter the amount of mature SREBP-2 (Fig. 1B). In this experiment and others (23, 33), SREBP bands often appeared as closely spaced doublets, but the reason is not clear.

To examine whether a marked decrease of SREBP-1 protein and a moderate decrease of SREBP-2 protein level by fish oil feeding reflected their mRNA levels, SREBP-1 and -2 mRNA levels in liver were measured by Northern blotting (Fig. 2). Parallel to their protein levels, compared with safflower oil-fed mice, fish oil-fed mice showed markedly decreased SREBP-1 mRNA level by 80% (p < 0.001). As for SREBP-2 mRNA, parallel to the amount of its precursor form, it also decreased by 47% in fish oil-fed mice (p < 0.001).

The SREBP-1 gene uses two different promoters to produce two different transcripts, -1a and -1c, that differ in the first exon (15, 31). To determine whether fish oil feeding reduces the amount of one or both transcripts, an RNase protection experiment was conducted (Fig. 3). Compared with safflower oil feeding, fish oil feeding reduced the amount of SREBP-1c transcript by 86% (p < 0.001) but did not alter significantly the amount of SREBP-1a transcript. Decrease of SREBP-1c mRNA by fish oil feeding was observed in early stages of fish oil feeding, and we observed 68% decrease of SREBP-1c mRNA only after 2 days of fish oil feeding (data not shown). As noted previously (31) and also observed in this study, in normal mice, the SREBP-1c transcript in the liver was more abundant than the SREBP-1a transcript. Thus, the SREBP-1c mRNA isoform may account for decrease of total SREBP-1 mRNA by fish oil feeding. Compared with carbohydrate feeding, safflower oil feeding reduced SREBP-1c mRNA by 39% (p < 0.001), whereas there was no alteration of total SREBP-1 mRNA levels between carbohydrate and safflower oil feeding (Fig. 2A). These data indicate that there may be another isoform of SREBP-1c that is up-regulated by safflower oil feeding.

The results of Northern blots of various mRNAs involved in cholesterol and fatty acid metabolism in liver from three dietary groups are presented in Fig. 4 and Table II. We examined mRNA levels involved in cholesterologenic enzymes (LDL receptor, HMG-CoA reductase, and HMG-CoA synthase), in lipogenic enzymes (FAS, ACC, and SCD-1) and, as a negative control, mRNA level of apoE. Compared with carbohydrate-fed mice, safflower oil-fed mice showed increases of HMG-CoA reductase and HMG-CoA synthase mRNA by 43% (p < 0.05) and 102% (p < 0.05), respectively, and showed decreases of FAS and SCD-1 by 47% (p < 0.01) and 67% (p < 0.001), respectively. Compared with safflower oil-fed mice, fish oil-fed mice showed marked decreases of LDL receptor, HMG-CoA reductase, HMG-CoA synthase, FAS, and SCD-1 mRNA by 60% (p < 0.001), 80% (p < 0.001), 64% (p < 0.01), 83% (p < 0.01), and 85% (p < 0.01), respectively, and presented a moderate decrease of ACC mRNA by 42% (p < 0.01). Relative to carbohydrate-fed mice, the mRNA for HMG-CoA synthase from fish oil-fed mice was only slightly affected. It has been reported that fatty acids induced HMG-CoA synthase gene expression by PPARa activation (34). Indeed, in this study, compared with carbohydrate feeding, safflower oil-fed mice showed a 2-fold increase in HMG-CoA synthase mRNA level. Thus, in fish oil-fed mice, up-regulation of HMG-CoA synthase mRNA by PPARa activation may interfere with its down-regulation by...
SREBP-1 protein decrease. ApoE mRNA level did not differ among these three groups. Thus, corresponding with the decrease of SREBP-1 mature form, mRNA levels of cholesterologenic and lipogenic enzymes were down-regulated in fish oil feeding, relative to safflower oil feeding. It should be noted that safflower oil feeding itself decreased FAS and SCD-1 mRNA significantly. Indeed, repression of FAS and SCD-1 mRNA expression by safflower oil feeding has also been reported (35, 36). These data indicated that the amount of mature SREBP-1 protein was not involved in safflower oil feeding-induced down-regulation of FAS and SCD-1 gene expression.

To examine whether down-regulation of mRNAs of SREBP-1 and SRE-related enzymes by fish oil feeding was due to activation of PPARα by fish oil, the effects of a well known PPARα activator, fenofibrate, on expression of mRNAs for SREBP1, lipogenic enzymes, and PPARα-activated enzymes were investigated (Table III). Two weeks of administration of 0.5% (w/w) fenofibrate increased LPL and ACS mRNAs expression by 5.4-fold (p < 0.001) and 2.3-fold (p < 0.001), respectively. LPL and ACS are well known enzymes, the expression of which was increased by PPARα activator (37, 38). These data indicated that the amount of mature SREBP-1 protein was not involved in safflower oil feeding-induced down-regulation of FAS and SCD-1 gene expression.

DISCUSSION

The major finding of this study is that high fish oil feeding reduces the amount of mature, active SREBP-1 protein in nuclear extracts. This reduction was explained by a marked reduction of SREBP-1c mRNA in liver and resulted in repression of SRE-related gene expression.

Similar profiles of SREBPs were observed in other metabolic conditions. Fasting for 24 h decreased SREBP-1c mRNA expression by 60% but not SREBP-1a mRNA (33). Parallel to SREBP-1c mRNA expression, decreases of similar levels in both precursor and mature forms of SREBP-1 protein were observed in fasting mice. Although both fish oil feeding and fasting down-regulated SREBP-1c transcript, ratios of the precursor and mature forms were different. Fish oil feeding decreased the precursor SREBP-1 by 90% but its mature form by 57%. In addition, fish oil feeding also reduced the precursor SREBP-2 by 65% but did not alter the amount of mature SREBP-2. This may be explained by a reduction in the rate of mature SREBPs degradation. However, a more plausible explanation is that the differences may be due to an increase in the conversion rate of precursor SREBPs to mature SREBPs. This increase may be due to a 32% decrease of total cholesterol in fish oil-fed mice (Table I). There was no difference of the amount of total cholesterol in liver under fasting conditions (33). A decrease in cholesterol may facilitate the conversion of precursor SREBPs to mature SREBPs and may then lead to increase in their mature forms (11). Unfortunately, however, as
The free cholesterol pool responsible for regulating SREBPs conversion has not been identified; it cannot be concluded that the decrease in total cholesterol level is responsible for conversion of precursor to mature forms. A decrease of total cholesterol content in liver in docosahexaenoic acid-fed rats has also been reported (39, 40). Fish oil diet-induced decreased activity of HMG-CoA reductase, a rate-limiting enzyme of cholesterol synthesis, is considered a cause of decrease of cholesterol content in liver (41, 42). Thus, it is possible that decrease of mature SREBP-1 leads to decrease HMG-CoA reductase mRNA, and then the amount of total cholesterol in liver is decreased. Increased conversion of precursor to mature SREBPs may be a adaptive mechanism against a marked reduction of SREBP-1c mRNA.

Studies of several lines of transgenic mouse (SREBP-1a (17), -1c (18), and -2 (19)) and of knockout mice (SREBP-1 (43)) could distinguish the role of each transcript. The liver normally produces predominately 1c isoform of SREBP-1 (31), which is a very weak activator of transcription of cholesterologenic enzymes (18), whereas SREBP-1a is the most active form of SREBP-1 (17) and SREBP-2 is a relatively selective activator of cholesterol synthesis, as opposed to fatty acid synthesis. Also, SREBP-1c is important in maintaining the basal level of transcription of FAS, ACC, and SCD-1, because mice homozygous for disrupted SREBP-1 allele had decreased basal hepatic mRNA levels of FAS, ACC, and SCD-1 but not those of cholesterologenic enzymes (43). As fish oil feeding has resulted in a marked reduction of SREBP-1c mRNA, we anticipated de-
Effects of Fish Oil on SREBP-1c

Liver from high carbohydrate diet in the absence (−) and presence (+) of fenofibrate was used for preparation of total RNA. Fenofibrate (about 500 mg/kg/day) was added to the high carbohydrate diet for 2 weeks. The RNA transferred membrane sheets were probed with 32P-labeled SREBP-1, FAS, ACC, LPL, and ACS cDNAs. These mRNA levels were quantified using an image analyzer. The data for each band are shown as relative value to the mRNA level of carbohydrate diet group mice in the absence of fenofibrate. Each data point represents mean ± S.E. of 4–8 mice.

**TABLE III**

|                      | Fenofibrate (−) | Fenofibrate (+) |
|----------------------|-----------------|-----------------|
| SREBP-1              | 100 ± 10        | 108 ± 5         |
| Lipogenic enzymes    |                 |                 |
| FAS                  | 100 ± 21        | 135 ± 39        |
| ACC                  | 100 ± 18        | 200 ± 7         |
| PPARα-activated enzymes |             |                 |
| LPL                  | 100 ± 13        | 525 ± 41        |
| ACS                  | 100 ± 6         | 226 ± 10        |

*p < 0.01, *p < 0.001.

**FIG. 4.** Effects of fish oil feeding on various mRNA levels involved in cholesterol and fatty acid metabolism in livers of mice fed high carbohydrate, high safflower oil, and high fish oil diets. Total liver RNA was isolated from mice fed carbohydrate diet, safflower oil diet, and fish oil diet at 5 months of feeding. Fifteen-μg aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membrane filters were hybridized with the indicated 32P-labeled probe. A typical autoradiogram is shown. In autoradiogram, each lane represents a sample from an individual mouse.

**FIG. 5.** A mechanism for hypolipidemic effects by fish oil feeding.

creases of lipogenic enzymes but not cholesterologenic enzymes. However, fish oil feeding decreased mRNAs for both cholesterologenic and lipogenic enzymes (Table II). The reason for this discrepancy may be explained as follows. SREBP-1c has functions of maintaining not only basal lipogenic enzymes but also cholesterologenic enzymes. However, in SREBP-1 knockout mice, reduction of cholesterologenic enzymes was not detected because of induction of SREBP-2 (43). Another explanation is that because fish oil feeding decreased SREBP-2 mRNA under some conditions such that conversion of precursor form of SREBP-2 to mature form is not accelerated, functional mature form of SREBP-2 might decrease.

Recently, the effects of polyunsaturated fatty acids on SREBPs and expression of SRE-containing reporter gene in several cell lines have been reported (44). Unsaturated fatty acids treatment for 24 h caused significant down-regulation of SRE-containing reporter gene expression, and inhibition increased with fatty acid length and number of double bonds. Down-regulation of reporter gene expression was mediated by decrease of mature SREBP protein (44). Because there was no substantial decrease of precursor SREBPs, they suggested that the decrease of mature SREBPs was due to increase of intracellular regulatory pools of cholesterol but not to SREBPs mRNA levels. Thus, although polyunsaturated fatty acids decreased SRE-related gene expression both in cell lines and mice liver, their mechanisms are different. The reason for this discrepancy is not clear. It is possible that there may be metabolic differences between *in vivo* and *in vitro* studies. Indeed, SREBP-1c is predominant in most tissues, whereas SREBP-1a is predominant in most cell lines (31).

This study may provide an explanation for the mechanism of fish oil diet-induced down-regulation of lipogenic enzymes (4, 5). It has also been reported that fish oil diet decreased LDL receptor activity in rat (45), its mRNA level in rabbit (46), and HMG-CoA reductase activities in rat (42) and rabbit (41). Thus, it also may provide an explanation for fish oil diet-induced down-regulation of cholesterologenic enzymes under some conditions. It is clear from PPARα knockout mice that down-regulation of lipogenic enzymes by fish oil feeding was not mediated through PPARα activation (10). In support of this, we observed that administration of fenofibrate (a PPARα ligand) for 2 weeks failed to down-regulate mRNAs of SREBP-1 and
lipogenic enzymes (Table III). Thus, down-regulation of SREBP-1c by fish oil feeding was not mediated by activation of PPARs. The data presented here suggest that hypolipidemic effect of fish oil feeding is produced by PPARγ for supply of rat LDL receptor and rat ACS cDNAs. To Dr. N. Iritani for supply of rat FAS and rat ACC cDNAs, and to Dr. T. Suzuki for advice in preparation of liver nuclear extract. We are also including SREBP-1c promoter analysis and search for a cell dependent (Fig. 5). To verify this hypothesis, further studies, SREBP-1c mRNA down-regulation in liver, and they are inde-

Acknowledgments—We thank Dr. Hitoshi Shimano at Tokyo University for advice in preparation of liver nuclear extract. We are also grateful to Dr. Daniel M Lane for supply of mouse SCD-1 cDNA, to Dr. S. Nemoto for supply of rat FAS and rat ACC cDNAs, and to Dr. N. Iritani for supply of rat LDL receptor and rat ACS cDNAs.

REFERENCES

1. Nestel, P. J. (1990) Annu. Rev. Nutr. 10, 149–167
2. Rustan, A. C., Christiansen, E. N., and Drevon, C. A. (1988) J. Nutr. 118, 53–58
3. Solé, A., Monari, A., and Ezaki, O. (1996) Metabolism 45, 683–689
4. Clarke, S. D., and Jump, D. B. (1994) J. Nutr. 124, 533–538
5. Eggers, J. P., and Blüher, M. N. (1996) J. Nutr. 126, 319–324
6. Reddy, J. K., and Chu, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8997–8998
7. Deeb, S., Staels, B., and Auwerx, J. (1996) Annu. N. Y. Acad. Sci. 79, 153–163
8. Simons, G., and Ploegh, H. L. (1996) Annu. Rev. Biochem. 65, 531–552
9. Deeb, S., Staels, B., and Auwerx, J. (1996) Biochim. Biophys. Acta 1290, 93–109
10. Brune, D., and Pfeifer, M. (1996) Biochem. Biophys. Res. Commun. 222, 467–472
11. Fiehn, O., and Weckhuysen, B. M. (1997) FEBS Lett. 404, 39–44
12. Ezaki, O., and Nishida, S. (1997) Metabolism 46, 1021–1028
13. Deeb, S., and Staels, B. (1997) J. Lipid Res. 38, 1499–1507
14. Kim, J. B., Spotts, G. D., Halvorsen, Y. D., Shih, H. M., Ellenberger, T., Towle, M. G., and Wahli, W. (1997) EMBO J. 16, 967–975
15. Katsurada, A., Iritani, N., Fukuda, H., Matsumura, Y., Nishimoto, N., and Nakai, K. (1997) J. Clin. Invest. 100, 1490–1497