A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity

Teruyo Nakatani,* Hyoun-Ju Kim,* Yasushi Kaburagi,† Kazuki Yasuda,§ and Osamu Ezaki[1],*

Division of Clinical Nutrition,* National Institute of Health and Nutrition, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8636, Japan; Division of Community Health† and Department of Metabolic Disorder,§ Research Institute, International Medical Center of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

Abstract **Rodents fed fish oil showed less obesity with a reduction of triglyceride synthesis in liver, relative to other dietary oils, along with a decrease of mature form of sterol regulatory element binding protein-1 (SREBP-1) and activation of peroxisome proliferator-activated receptor α (PPARα). Decrease of mature SREBP-1 protein by fish oil feeding was due to either inhibition of SREBP-1 proteolytic cascade or to decrease of its mRNA. To clarify its mechanism and relation to antiobesity effect, mice were fed fish oil in a range from 10 to 60 energy percent (en%). Fish oil feeding decreased body weight and fat mass in a dose-dependent manner, in parallel with PPARα activation and a decrease of SREBP-1 mRNA. However, compared with 0 en% fish oil feeding, 10 en% fish oil feeding decreased mature SREBP-1 protein by 50% with concomitant decreases of lipogenic genes, while precursor SREBP-1 protein rather increased by 1.3-fold. These data suggest that physiological doses of fish oil feeding effectively decrease expression of liver lipogenic enzymes by inhibiting SREBP-1 proteolytic cascade, while substantial decrease of SREBP-1 expression is observed in its pharmacological doses, and that activation of PPARα rather than SREBP-1 decrease might be related to the antiobesity effect of fish oil feeding.**—Nakatani, T., H-J. Kim, Y. Kaburagi, K. Yasuda, and O. Ezaki. **A low fish oil feeding inhibits SREBP-1 proteolytic cascade, while a high fish oil feeding decreases SREBP-1 mRNA in mice liver: its relationship to anti-obesity effect.** ***J. Lipid Res.*** **2003.** 44: **369–379.**

Supplementary key words n-3 fatty acids • dual-energy X-ray absorptiometry • stearoyl-CoA desaturase • fatty acid synthase • acetyl-CoA carboxylase • acyl-CoA oxidase • medium-chain acyl-CoA dehydrogenase • uncoupling protein • lipoprotein lipase • sterol regulatory element binding protein-1

In rodents, fish oil feeding prevents lipid accumulation in white adipose tissue (WAT) compared with other types of dietary oils (1–4). It is known that triglyceride stored in fat cells is largely derived from circulation triglyceride, especially high-fat feeding. It is speculated that fish oil feeding limits triglyceride supply to adipose tissues by decreased VLDL synthesis in liver. Thus, increased fatty acid oxidation and inhibition of triglyceride synthesis in liver may play an important role in fish oil-induced body weight (BW) decrease (5). It has been shown that n-3 fatty acids, which are abundant in fish oil in vivo or in cell culture inhibited the transcription of genes coding for lipogenesis enzymes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD), and S14 protein with decrease of mature form of sterol regulatory element binding (SREBP-1) protein (6–11). On the other hand, n-3 fatty acids increased the transcription of the regulatory enzymes of fatty acid oxidation, such as acyl-CoA oxidase (ACO), medium-chain acyl-CoA dehydrogenase (MCAD), lipoprotein lipase (LPL), fatty acid binding protein, acyl-CoA synthetase (ACS), uncoupling protein-2 (UCP2), and carnitine palmitoyltransferase-1, with activation of peroxisome proliferator-activated receptor (PPAR)α (12–16).

SREBPs are master transcription factors that regulate fatty acid and cholesterol metabolism in liver (17). In sterol depletion, SREBPs are cleaved and become mature forms to bind sterol regulatory elements (SREs) (18, 19)

Manuscript received 23 July 2002 and in revised form 15 October 2002.

Published, JLR Papers in Press, November 16, 2002.
DOI 10.1194/jlr.M200289-JLR200

Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; DEXA, dual-energy X-ray absorptiometry; DHA, docosahexaenoic acid; en%, energy percent; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; MCAD, medium-chain acyl-CoA dehydrogenase; PLSD, protected least significant difference; PPAR, peroxisome proliferator-activated receptor; PSL, phosphostimulated luminescence; S1P, Site-1 protease; SCAP, SREBP cleavage-activating protein; SCD, stearoyl-CoA desaturase; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein; UCP, uncoupling protein; WAT, white adipose tissue.

[1] To whom correspondence should be addressed.
e-mail: ezaki@nih.go.jp

and/or E-box sequences (20) and then activate the target gene expression. SREBP cleavage-activating protein (SCAP) escorts the SREBPs to the Golgi complex, where they are cleaved sequentially by two membrane-bound proteases designated Site-1 protease (S1P) and Site-2 protease (S2P), thereby liberating the NH2-terminal domain so that it can enter the nucleus (21). 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; SREBP-1c plays a crucial role in the dietary regulation of the most hepatic lipogenic genes, whereas SREBP-2 is actively involved in the transcription of cholesterol biosynthetic genes (22–24).

Consistent with the notion that SREBP-1c is a dominant regulator for lipogenesis, several reports have been published demonstrating that administration of polyunsaturated fatty acid (PUFA) such as n-6 and n-3 fatty acids suppresses mature SREBP-1 protein and/or its mRNA in cultured cells and rodent liver tissues (6–11). However, the mechanisms of decrease of mature SREBP-1 protein were diverse; in culture cells, PUFA addition inhibited the SREBP-1 proteolytic cascade in CV-1 and human embryonic kidney (HEK)-293 cells and down-regulated SREBP-1 mRNA in HEK-239 cells (6, 11). In primary culture of rat hepatocytes, PUFA suppressed SREBP-1 mRNA by accelerating transcript decay (25). In rodent studies, 25 energy percent (en%) menhaden fish oil, 60 en% tuna fish oil, and 25 en% fish oil feeding decreased SREBP-1 mRNA by 60, 86, and 80%, respectively, compared with their appropriate controls (7–9), while 40 en% sardine or tuna fish oil decreased mature SREBP-1 protein but not its mRNA (10).

In this study, to examine the mechanism of decrease of mature SREBP-1 by fish oil feeding in greater detail, mice were given a different amount of fish oils in place of safflower oil and then premature and mature SREBP-1 protein conten and SREBP-1 mRNA levels in liver were measured. In addition, to examine the correlation between body fat and alteration of activities of SREBP-1 and PPARα, body composition was assessed by dual-energy X-ray absorptiometry (DEXA) and expression levels of target genes of SREBP-1 and PPARα were examined.

EXPERIMENTAL PROCEDURES

Animals

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 12-h light/12-h dark cycle and maintained at a constant temperature of 22°C.

Diet

Mice were divided into seven groups (n = 5–6 in each group). All groups of mice were fed a high-fat diet containing 14 en% carbohydrate, 60 en% dietary oil, and 26 en% protein. The dietary fats are a mixture of safflower oil and fish oil. The amount of fish oil increased from 0 to 60 en% with concomitant decrease of safflower oil from 60 to 0 en%, maintaining the total amount of fat constant at 60 en%. Detailed composition of the experimental diets is described in **Table 1**. Fatty acid compositions of dietary oils were measured by gas-liquid chromatography. Safflower oil (high-oleic type) contained 46% oleic acid (18:1n-9) and 45% linoleic acid (18:2n-6) from total fatty acids; fish oil contained 7% eicosapentaenoic acid (EPA, 20:5n-3) and 24% docosahexaenoic acid (DHA, 22:6n-3). The materials and methods of diet preparation were the same as those used in our previous studies (8, 16, 26). Fish oil was provided by NOF (Tokyo, Japan). Food consumption was measured for three consecutive days. The mean food intake per day was estimated by subtracting the food weight of the day from the initial food weight of the previous day and dividing by the number of mice housed in the cage. Thus, standard error for food intake shown in **Fig. 1A** was variation of daily intake, but not from the individual mouse. Mice were fed each diet for 1 or 13 weeks and were anesthetized at about 10 AM by intraperitoneal injection of pentobarbital sodium (0.08 mg/g BW, Nembutal, Abbot, North Chicago, IL). Liver, gastrocnemius, and parametrial WAT were isolated immediately, weighed, and homogenized in guanidine-thiocyanate, and RNA was prepared by the method described by Chirgwin et al. (27). A part of liver of each mouse was immediately homogenized to obtain membrane fractions and nuclear extracts (28).

Immunoblotting

Pooled liver membranes and nuclear extracts from five mice of each group were prepared by the method described by Sheng et al. (28). The same amount of protein from each fraction was applied to 8% SDS-PAGE transferred to Hybond-P membranes

TABLE 1. Composition of the experimental diets

Experimental Diet	0 en%[a] Fish Oil	10 en% Fish Oil	20 en% Fish Oil	30 en% Fish Oil	40 en% Fish Oil	50 en% Fish Oil	60 en% Fish Oil
Fish oil (%[b])	0	5.5	11	16.5	22	27.5	33.5
Safflower oil (%)	33.5	28	22.5	17	11.5	6	0
Casein (%)	29	29	29	29	29	29	29
Sucrose (%)	23.29	23.29	23.29	23.29	23.29	23.29	23.29
Vitamin mix (%)	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Mineral mix (%)	5.08	5.08	5.08	5.08	5.08	5.08	5.08
Cellulose powder (%)	7.25	7.25	7.25	7.25	7.25	7.25	7.25
L-Cystein (%)	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Fat energy (en%)	60.5	60.9	60.9	60.7	60.7	60.6	60.6
DHA (en%)	0	2.4	4.8	7.2	9.6	12	14.4
EPA (en%)	0	0.7	1.4	2.1	2.8	3.5	4.2

DHA, docosahexaenoic; EPA, eicosapentaenoic acid.
[a] Percent of energy from total energy intake.
[b] Percent of weight from total food weight.

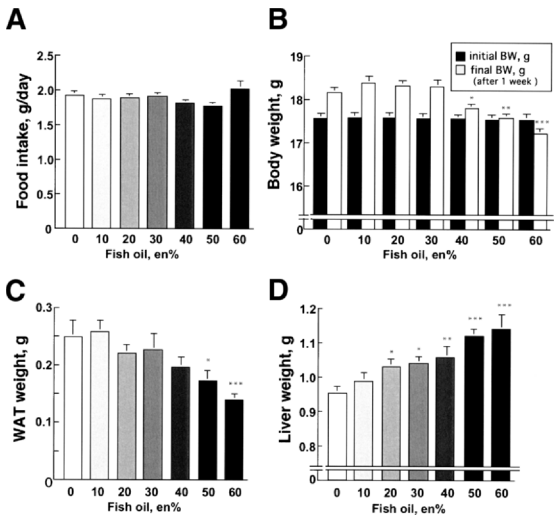

Fig. 1. Food intake, body weight (BW), parametrial white adipose tissue (WAT), and liver weight in mice fed 0, 10, 20, 30, 40, 50, and 60 energy percent (en%) fish oil for 1 week. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. A: Food intakes were measured for 3 days and expressed as the mean ± SE intake per day (n = 12). Food intake was not significant by ANOVA. Mice were killed at 1 week feeding, and final BW (B), WAT weight (C), and wet liver weight (D) were measured. Initial BW is BW at the beginning of diet experiments (B). In panel B–D, each data point represents mean ± SE of 14–44 mice from 3–9 independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, different doses of fish oil compared with 0 en% fish oil feeding by Fisher's protected least significant difference (PLSD) test.

(Amersham Pharmacia Biotech, Tokyo, Japan). Immunoblot analysis was performed by using Enhanced Chemiluminescence Western Blotting Detection System kit (Amersham Pharmacia Biotech). Membrane sheets were first incubated with antibody against SREBP-1 for 1 h at 22°C, then washed 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 Canon IX-4015 (Canon Inc., Tokyo, Japan). Monoclonal antibodies to SREBP-1 (IgG-2A4) were purified by protein A sepharose (Amersham Pharmacia Biotech) from the supernatant of hybridoma cell lines CRL 2121. These cell lines were purchased from American Tissue Culture Collection (Rockville, MD).

Preparation of cDNA probe for Northern blot

The cDNA fragments for mouse SREBP-1, UCP2, ACO, and MCAD were obtained by PCR as described in our previous studies (8, 29, 30). A part of cDNAs of mouse SCAP and S1P were obtained by PCR from first strand DNA using mouse liver total RNA. First strand cDNA was prepared using a Ready-To-Go T-Primed First Strand kit (Amersham Pharmacia Biotech). The PCR primers used were as follows: SCAP (XM033294), 5′ primer, 5′-TGTGAAGGATTACTTCGCC-3′ and 3′ primer, 5′-CCAGTCATTCTGCCAGAAGT-3′; S1P (NM019709), 5′ primer, 5′-CAACTGTGGTGGAGTACGAA-3′ and 3′ primer, 5′-CGACCTGGCGAGGAA-3′. PCR was performed with a Taq DNA polymerase (Takara, Shiga, Japan). The cDNA probes for rat ACS was kindly provided by Dr. T. Yamamoto at Tohoku University, mouse SCD-1 by Dr. Daniel M. Lane at Johns Hopkins University, and rat ACC and rat FAS by Dr. N. Iritani at Tezukayama Gakuin College. These cDNA were used as probes for Northern blotting.

Body composition analysis

At 13 weeks of feeding, mice were anesthetized by pentobarbital sodium (0.08 mg/g BW, Nembutal) and scanned with a Lunar PIXI mus2 densitometer (31) (Lunar Corporation, Madison, WI).

Northern blotting

Aliquots of total RNA (15 μg) were denatured with glyoxal and dimethyl sulfoxide, subjected to electrophoresis in a 1% agarose gel, and transferred to nylon membranes (NEN Life Science Products, Boston, MA). After transfer and UV cross-linking, RNA blots were stained with methylene blue to locate 28*S* and 18*S* rRNAs and to ascertain the amount of loaded RNAs (32). The membranes were hybridized with each cDNA probe labeled with [α-^{32}P]dCTP (NEN) by a Megaprime DNA labeling kit (Amersham Pharmacia Biotech). The membranes were hybridized overnight at 42°C in hybridization buffer subsequently washed two times for 20 min at 50°C with 1XSSC, 0.1% SDS and one time for 20 min at 65°C. The membranes were exposed to Kodak XAR-5 film at −80°C with intensifying screens. Quantitative analysis was performed with an image analyzer (BAS 2000, Fuji Film, Tokyo, Japan) and expressed as the intensity of phosphostimulated luminescence (PSL).

Statistical analysis

Comparisons of data from multiple groups were made by one-way ANOVA. When they were significant, each group was compared with the others by Fisher's protected least significant difference (PLSD) test (Statview 4.0, Abacus Concepts). Statistical significance is defined as $P < 0.05$. Values are mean ± SE.

RESULTS

BW, parametrial WAT, and liver weight in mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week

According to our previous data (4, 8, 26), compared with high-carbohydrate-fed mice, 60 en% safflower oil-fed mice showed increases of BW and WAT weight, while 60 en% fish oil feeding increased energy expenditure and did not develop obesity. To examine whether the antiobesity effect by 60 en% fish oil feeding is also observed in lower fish oil feeding, mice were fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week under 60 en% high-fat diet feeding conditions. Although there were no significant differences in the average energy intake of mice fed each diet (Fig. 1A), even in a relatively short term feeding period there were significant decreases of BW in 40–60 en% fish oil feeding compared with 0 en% fish oil feeding (Fig. 1B). Corresponding with BW decreases, wet weight of parametrial WAT in fish-oil-fed mice decreased in a dose-dependent manner and reached significance in more than 50 en% feeding (Fig. 1C). Liver weight from fish-oil-fed mice also increased in a dose-dependent manner and reached significance in more than 20 en% feeding (Fig. 1D). This might be due to the well-known effects of fish oil on peroxisomal proliferation (33), and also suggested that a dose-dependent intake of fish oils might have occurred in this experiment. The increase of liver weight was not accompanied by liver damage, since there were no increases

of transaminases in 60 en% fish oil feeding (data not shown).

SREBP-1 protein in membrane fractions (precursor form) and nuclear extracts (mature form) from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week

In our previous study, 60 en% fish oil feeding decreased mature SREBP-1c with decrease of its mRNA (8). To examine whether a lower dose of fish oil feeding also decreases mature SREBP-1c protein, the precursor and mature SREBP-1 protein in liver of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week were measured by immunoblotting (**Fig. 2A, B**). Since antibody to SREBP-1 reacted to both SREBP-1a and -1c forms, we could not distinguish these two forms and thus used the noncommittal term SREBP-1. In mouse liver, the ratio of SREBP-1c to 1a transcripts is 9 to 1 (34), and thus 1c form accounted for most of SREBP-1 observed on the immunoblots. In preliminary experiments, to confirm that 125 kDa and 68 kDa proteins we observed are really the precursor and mature SREBP-1, fasting and refeeding experiments were conducted (data not shown).

Compared with 0 en% fish oil feeding, 10–30 en% fish oil feeding decreased mature SREBP-1 by about 50%, but rather increased precursor SREBP-1 protein. Doses of 40–60 en% fish oil feeding decreased both precursor and mature SREBP-1 proteins. To incorporate the observed decrease in mature and precursor SREBP-1 into a single variable, the ratio of the mature SREBP-1 to its precursor was plotted (Fig. 2C). Since 10 en% fish oil feeding increased premature SREBP-1 by 1.3-fold (Fig. 2A), a 60% decrease of this ratio was observed in 10 en% fish oil feeding. A reciprocal increase of precursor SREBP-1 protein with a reduction of mature SREBP-1 by lower fish oil feeding suggests the inhibition of SREBP-1 proteolytic cascade. It is unlikely that fish oil feeding degraded the mature form of SREBP-1 preferentially, since fatty acids did not reduce SRE-mediated gene expression in mutant cells overexpressing the mature SREBP protein (6). In higher doses of 40–60 en% fish oil feeding, about 40% decreases of the ratio were also observed. These data indicated that fish oil feeding decreased mature SREBP-1 protein by two mechanisms: at lower 10–30 en% fish oil feeding, inhibition of SREBP-1 proteolytic cascade; while at 40–60 en% fish oil feeding, decrease of precursor SREBP-1 and inhibition of SREBP-1 proteolytic cascade.

The 80-kDa protein, which appeared just above mature SREBP-1 protein and increased in a dose-dependent manner (Fig. 2B), was identified as peroxisomal bifunctional enzyme (J02748) by amino acid sequencing (unpublished observation; T. Nakatani et al.). This protein might be the previously reported hepatic peroxisome-proliferation-associated polypeptide located in the peroxisome, referred to as PPA-80 (35, 36). Since this protein was markedly noticed by Coomassie blue-stained gel (data not shown), we assumed that this protein was extremely abundant and the nuclear rich fraction was somewhat contaminated with the peroxisome and reacted with the antibody to SREBP-1 non-specifically.

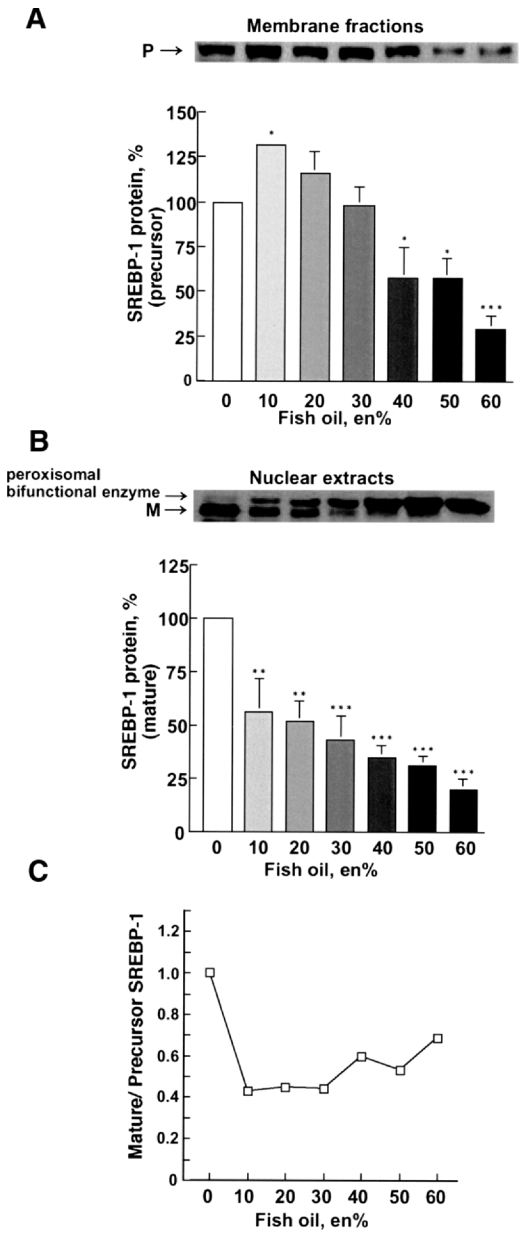

Fig. 2. Immunoblot analysis of SREBP-1 in membrane fractions (A) and nuclear extracts (B) and its ratio (C) from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Mice were killed at 1 week feeding. For each group, livers from mice (n = 5) were pooled, and aliquots of membrane fractions (90 μg protein) and nuclear extracts (30 μg protein) were subjected to 8% SDS-PAGE and electrophoretically transferred to Hybond-P membranes. The membranes were incubated with 5 μg/ml of mouse monoclonal antibody IgG 2A4 against amino acid 301–407 of human SREBP-1. Immunoblot analysis was carried out by the enhanced chemiluminescence system. Filters were exposed to film for 60 s. The bands were quantified by scanning with Canon IX-4015 (Canon Inc., Tokyo, Japan). A typical autoradiogram of SREBP-1 from membrane fractions (A) and nuclear extracts (B) and their relative levels are shown. P and M denote the precursor and mature forms of SREBP-1. Each value represents mean ± SE of three independent experiments. In panel C, the ratio of the mature SREBP-1 to its precursor is plotted. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, various doses of fish oil feeding compared with 0 en% fish oil feeding by Fisher's PLSD test.

Decreases of lipogenic enzymes mRNA occurred in parallel with decreases of mature SREBP-1 protein

To examine whether decrease of mature SREBP-1 protein affected mRNAs of its target genes, mRNAs of lipogenic enzymes, ACC, FAS, and SCD-1 were measured by Northern blotting (**Fig. 3**). Compared with 0 en% fish oil feeding, 10 en% fish oil feeding decreased ACC, FAS and SCD-1 by 30% ($P < 0.01$), 50% ($P < 0.1$), and 50% ($P < 0.001$), respectively. At doses 10–40 en%, dose-dependent decreases of these enzymes mRNAs were observed. However, further dose-dependent decreases of ACC and FAS mRNAs were not manifested in more than 40 en% fish oil feeding. Thus, corresponding with the decrease of SREBP-1 mature form, fish oil feeding downregulated mRNA levels of lipogenic enzymes. Since marked reductions of body and WAT weight were not observed in 10 en% fish oil feeding, decrease of liver mature SREBP-1 protein and expression levels of its target genes might not be causes of decreases of body and WAT weight.

More than 30 en% fish oil feeding decreases SREBP-1 mRNA

To examine whether the decrease of precursor form of SREBP-1 was due to the decrease of its transcript, SREBP-1 mRNA levels were measured (**Fig. 4**). The decrease of SREBP-1 mRNA was dose-dependent; significant decreases of SREBP-1 mRNA were observed in more than 30 en% fish oil feeding. Decreases of SREBP-1 mRNA observed in different doses of fish oil feeding correlated well with decreases of precursor SREBP-1 protein, although slight increases of premature protein in lower fish oil feeding were observed (Fig. 2A).

Fish oil feeding did not decrease SCAP and S1P mRNA

SREBPs are synthesized as precursors that are bound to the endoplasmic reticulum. There they form complexes with SCAP (37). SCAP escorts the SREBPs to the Golgi complex, where they are cleaved sequentially by two membrane bound proteases designated S1P and S2P, thereby liberating the NH2-terminal domain so that it can enter the nucleus (21). Conditional SCAP or S1P-deficient liver showed decrease of mature SREBP-1 protein with decrease of its mRNA (38, 39). Thus, decreases SCAP or S1P mRNAs inhibited SREBP-1 proteolytic cascade and might lead to decrease of SREBP-1 mRNA and its target gene expression. To examine this possibility, Northern blotting of SCAP and S1P was made (**Fig. 5**). Fish oil feeding did not decrease SCAP and S1P expression in all doses but rather increased their expression levels, suggesting that SCAP and S1P expression may not be the cause for fish oil feeding-mediated decreases of SREBP-1 protein and its mRNA. Other mechanisms for inhibition of SREBP-1 processing might be involved in a decrease of mature SREBP-1 protein.

Decrease of BW correlated well with up-regulation of PPARα target genes, ACO, MCAD, ACS, LPL, and UCP2 in liver

Since a good correlation was absent between lipogenic gene expression controlled by SREBP-1 protein and reduction of obesity, we have examined expression levels of PPARα target genes such as ACO (a marker of peroxisomal β-oxidation), MCAD (a marker of mitochondrial β-oxidation), ACS, LPL, and UCP2 in liver (**Fig. 6**). Expression of these genes upregulated mostly in a dose-dependent manner, corresponding with increase of liver weight and decrease of body and WAT weight (Fig. 1). Thus, decrease of WAT weight is well correlated with liver PPARα activation.

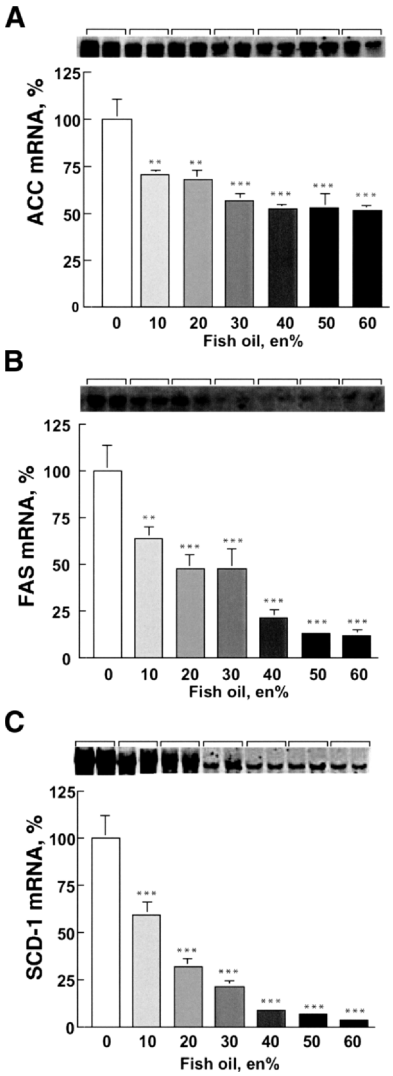

Fig. 3. Northern blotting for target genes of SREBP-1c from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Total RNA was isolated from livers of each group at 1 week feeding. Fifteen-microgram aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membrane filters were hybridized with the indicated ^{32}P-labeled probe of SREBP-1 target genes, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and SCD-1. The radioactivity in each band was quantified using an image analyzer. The data for each band are shown as relative value to the mRNA level of 0 en% fish oil mice. Each value represents the mean ± SE of 3–9 mice. $^{**}P < 0.01$, $^{***}P < 0.001$, various doses of fish oil feeding compared with 0 en% fish oil feeding by Fisher's PLSD test.

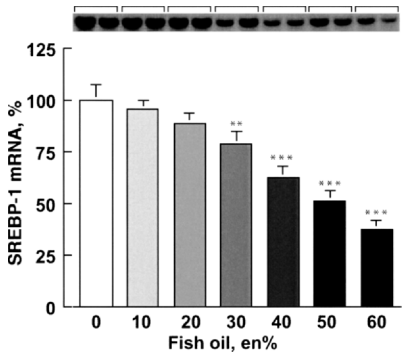

Fig. 4. Northern blotting for SREBP-1 from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Total RNA was isolated from livers of each group at 1 week feeding. Fifteen microgram aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membranes were hybridized with ^{32}P-labeled probe for SREBP-1. In autoradiogram, each line represents a sample from an individual mouse. The radioactivity in each band was quantified using an image analyzer. The data for each band are shown as relative value to the mRNA level of 0% fish-oil-fed mice. Each value represents mean ± SE of 8–9 mice. $^{**}P < 0.01$, $^{***}P < 0.001$, various doses of fish oil feeding compared with 0 en% fish oil feeding by Fisher's PLSD test.

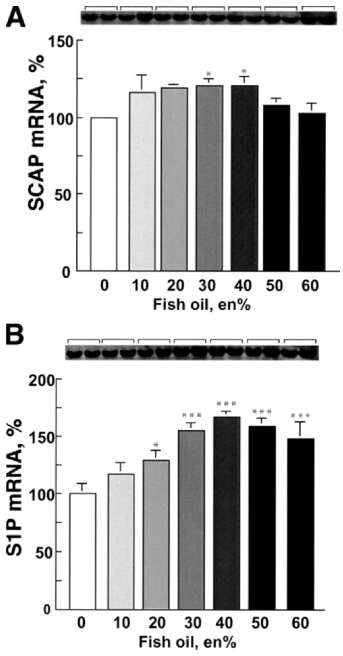

Fig. 5. Northern blotting for SREBP cleavage-activating protein (SCAP) and Site-1 protease (S1P) from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Total RNA was isolated from livers of each group at 1 week feeding. Fifteen-microgram aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membranes were hybridized with ^{32}P-labeled probe for SCAP (A) and S1P (B). In autoradiogram, each line represents a sample from an individual mouse. The radioactivity in each band was quantified using an image analyzer. The data for each band are shown as relative value to the mRNA level of 0% fish-oil-fed mice. Each value represents mean ± SE of 3–4 mice. $^{*}P < 0.05$, $^{***}P < 0.001$, various doses of fish oil feeding compared with 0 en% fish oil feeding by Fisher's PLSD test.

Expression of PPARα target genes, ACO, and MCAD in skeletal muscle and WAT

To examine whether fish oil feeding also activated PPARα target genes in skeletal muscles and WAT, expression levels ACO and MCAD in these tissues were compared with those in liver in 0, 30, and 60 en% fish-oil-fed mice (**Fig. 7**). Expression level of ACO in gastrocnemius and WAT was very low compared with those in liver, and was not up-regulated by fish oil feeding. This suggested that substantial peroxisomal proliferation might not occur in these tissues. As for mitochondrial β-oxidation, fish oil feeding upregulated MCAD expression in gastrocnemius ($P < 0.05$) and WAT ($P < 0.1$), as well as in liver by about 1.4–1.5-folds. Although the increase of MCAD mRNA in gastrocnemius and WAT was small and not a dose-dependent, activation PPARα in these tissues might contribute to antiobesity effects of fish oil feeding.

Effects of long-term fish oil feeding on body fat and expression levels of SREBP-1 and PPARα target genes in liver

Next, to examine whether there is also a good correlation between reduction of obesity and PPARα activation in long-term fish oil feeding, mice were fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 13 weeks and BW, fat mass assessed by DEXA, and expressions of target genes of SREBP-1 and PPARα in liver were measured (**Fig. 8**). Similar to the 1-week feeding, in 13-week feeding, almost linear decreases of BW and fat masses were observed with increase of fish oil feeding. Expression of SREBP-1 target genes such as FAS and SCD-1 decreased markedly by 30% ($P < 0.01$) and 50% ($P < 0.001$), respectively, in 10 en% fish oil feeding, while ACS, a PPARα target gene, increased linearly with the increase of fish oil dose. Corresponding to downregulation of SREBP-1 target genes, 10 en% fish oil feeding decreased mature SREBP-1 by about 50% (data not shown). Thus, even in a long-term fish oil feeding, a good correlation was observed between reduction of obesity and PPARα activation.

DISCUSSION

The two major findings of this study are that *1*) fish oil feeding reduced the amount of mature, active SREBP-1 protein by two mechanisms: at low fish oil feeding, by an inhibition of SREBP-1 proteolytic cascade, while at high fish oil feeding, by both an inhibition of SREBP-1 proteolytic cascade and a decrease of SREBP-1 mRNA; and *2*) PPARα activation but not decrease of mature SREBP-1 protein might be related to the antiobesity effects of fish oil feeding.

In our experiments, the mice are fed a very-high-fat diet and the effects observed are due to the replacement of safflower oil (mainly oleic acid and linoleic acid) with fish oil. Thus, the effects of fish oil we observed are relative to those of safflower oil. It is unlikely that safflower

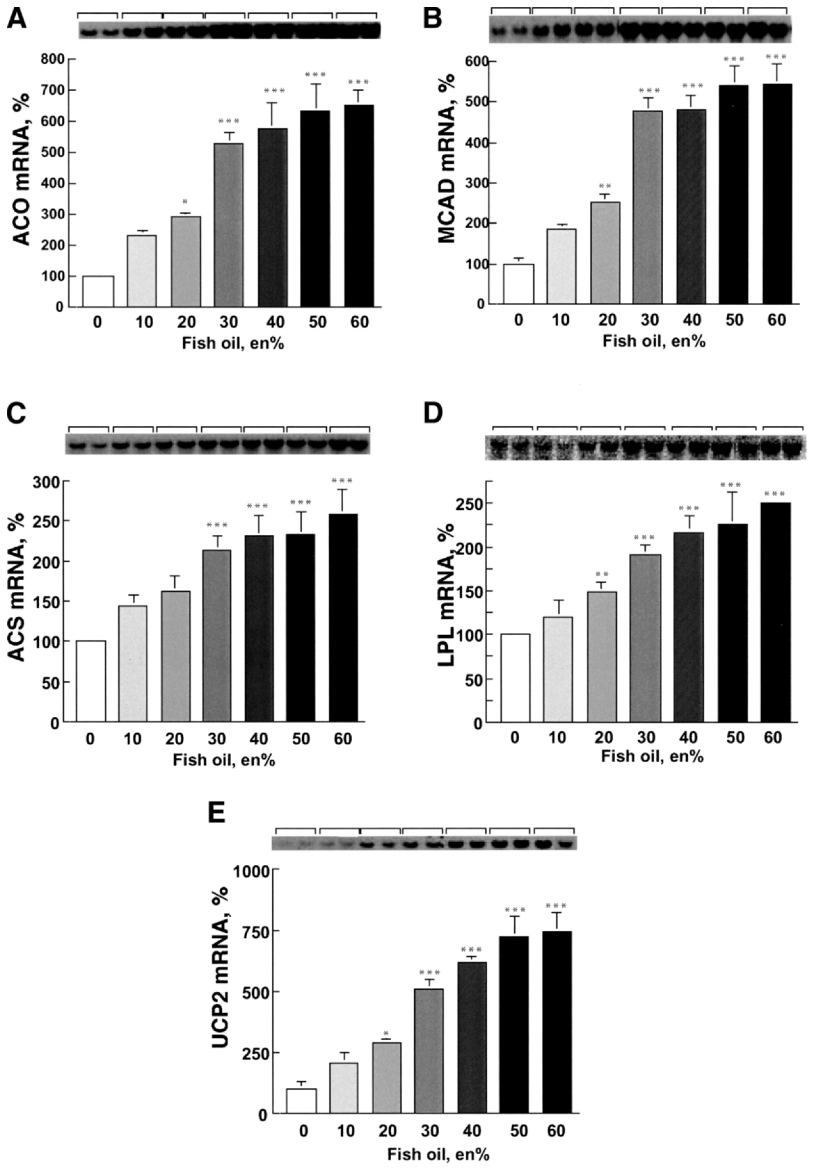

Fig. 6. Northern blotting for target genes of peroxisome proliferator-activated receptor (PPAR)α from livers of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 1 week. Mice were fed on 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Total RNA was isolated from livers of each group at 1 week feeding. Fifteen-microgram aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membrane filters were hybridized with the indicated ^{32}P-labeled probe of PPARα target genes of acyl-CoA oxidase (ACO) (A), medium-chain acyl-CoA dehydrogenase (MCAD) (B), acyl-CoA synthetase (ACS) (C), LPL (D), and uncoupling protein-2 (UCP2) (E). In autoradiogram, each line represents a sample from an individual mouse. The radioactivity in each band was quantified using an image analyzer. The data for each band are shown as relative value to the mRNA level of 0 en% fish-oil-diet mice. Each value represents the mean ± SE of 3–9 mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, various doses of fish oil feeding compared with 0 en% fish oil feeding by Fisher's PLSD test.

oil in the fish oil rich diet affected fish oil-induced downregulation of lipogenic gene expression, since in an experiment where the fixed amount of fish oil was given to mice in different doses of safflower oil, we observed the similar effects of fish oil feeding on the downregulation of lipogenic genes (unpublished observation; T. Nakatani et al.). Tuna fish oil used in this study contained 7% EPA, 24% DHA, and other highly polyunsaturated fatty acids; it is unclear which fatty acid, or their combination or metabolites, caused these various fish oil effects we observed.

In humans, in the United States, the average intake of n-3 fatty acid is about 0.7 en% (40), while in Eskimos, the average intake of n-3 fatty acid, mostly from fish oils, is about 5 en% (41). Thus, 10 en% fish oil feeding, which contains 3.1 en% DHA plus EPA (Table 1), in our mice study corresponds to the average fish oil intake in Eskimos. At this physiological dose of fish oil feeding, by inhi-

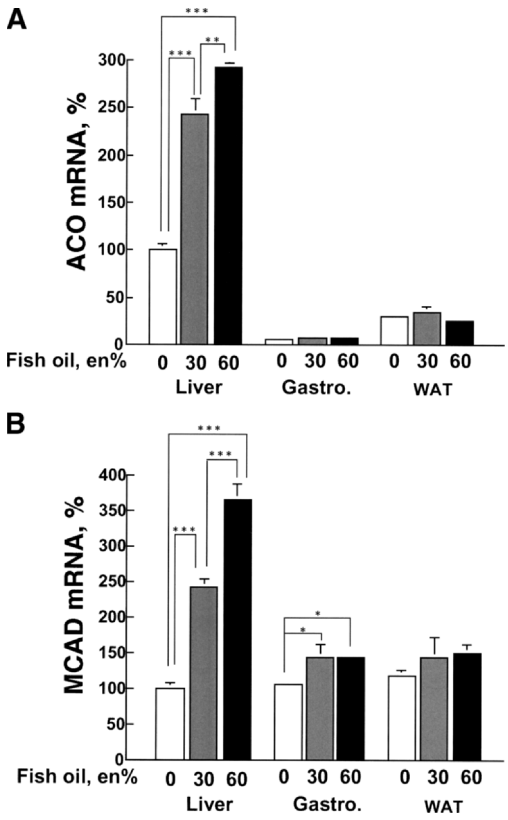

Fig. 7. Northern blotting for ACO (a marker of peroxisomal β-oxidation) and MCAD (a marker of mitochondrial β-oxidation) from liver, gastrocnemius, and parametrial WAT of mice fed 0, 30 and 60 en% fish oil for 1 week. Mice were fed on 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Total RNA was isolated from livers, gastrocnemius, WAT, and brown adipose tissue of each group at 1 week feeding. Fifteen-microgram aliquots of total RNA were subjected to electrophoresis and transferred to nylon membranes. The membrane filters were hybridized with the indicated ^{32}P-labeled probe of PPARα target genes of ACO (A) and MCAD (B). The radioactivity in each band was quantified using an image analyzer. The data for each band are shown as relative value to the liver mRNA level of 0 en% fish-oil-diet mice. Each value represents the mean ± SE of five mice. Statistical differences are shown as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, by Fisher's PLSD test.

bition of SREBP-1 proteolytic cascade, fish oil feeding downregulated active, mature form of SREBP-1 by 50% and then down-regulated expressions of its target genes related to lipid synthesis.

Since the enhancer regions of SREBP-1c have SREs that are required for high-level transcription of this gene (42), it is hypothesized that when SREBP processing is blocked, nuclear SREBPs decline and feed-forward stimulation of SREBP gene transcription is disrupted, leading to a fall in SREBP mRNAs (38). In contrast, our data did not support this hypothesis; the evidence that at 10 en% fish oil feeding, mature SREBP-1 decreased but its mRNA level remained unchanged suggests that decrease of mature SREBP-1 may not lead to decrease of its mRNA. It is also conceivable that about a 50% decrease of mature SREBP-1 observed in 10 en% fish oil feeding was insufficient to lead to the downregulation of SREBP-1 mRNA. The mechanism for inhibition of SREBP-1 proteolytic cascade by fish oil is not clear at present. Neither SCAP nor S1P mRNAs was responsible for fish oil feeding-mediated decrease of mature SREBP-1 protein. Intracellular cholesterol and/or ceramide that were recently considered as the regulators of SREBPs processing might be involved in these fish oil effects (43). Further studies of SREBP-1 processing, including identification of its proteolytic enzymes, might be required to understand the mechanism(s) of lipid-lowering effects by fish oil feeding.

In addition, dose-dependent studies demonstrated that PPARα activation rather than reduction of mature SREBP-1 protein in liver was well correlated with a fat mass decrease in fish oil feeding. This finding is in an agreement with alteration of fat mass in knockout and overexpressed mice of PPARα or SREBP-1. Two studies of PPARα-null mice, although using mice having different genetic backgrounds, showed increased gonadal adipose stores (44, 45). In addition, fenofibrate (=PPARα activator) treatment prevented high-fat diet-induced increase of BW and adipose tissue mass in mice and rats (46, 47). Ablation of SREBP-1 gene did not decrease the amount of WAT in hybrids between C57BL/6J and 129Sv/Ev mice strains (48) and in ob/ob mice (49). In addition, the animal model in which both SREBP-1 and SREBP-2 were ablated was produced by a conditional deficiency of SCAP expression (38). These mice showed marked reductions in hepatic lipogenesis, but gained weight normally up to 3 months after pIpC injection/Cre expression. Transient SREBP-1 overexpression study in liver resulted in increase of triglyceride synthesis in liver, but did not increase adipose tissue weight (23). These data are in a good agreement with our conclusion that PPARα activation but not decrease of the SREBP-1 mature form might be the primary cause of antiobesity effects of fish oil feeding. Recently, ablation of SCD-1 in ob/ob mice showed a significant reduction of obesity with significantly reduced liver triglyceride storage and an increased whole-body energy expenditure (50). Since SCD-1 is one of the SREBP-1c target genes in liver, this result was not in a good agreement with the hypothesis that a reduction of liver triglyceride synthesis by a decrease of a mature SREBP-1c did not contribute to the development of obesity. It is conceivable that SCD-1 expressed in another tissue such as brain, in which SREBP-1c might not regulate SCD-1 expression, could be responsible for increased energy expenditure and reduction of body fat.

Although a marked PPARα activation was not observed in skeletal muscles and WAT (Fig. 7B), these organs might contribute to fish oil-mediated antiobesity effects. It is also conceivable that other metabolic effects unrelated to PPARα activation may also contribute to antiobesity effects; an increased expression of UCP3 in skeletal muscles might contribute to fish oil mediated antiobesity effects (26), or AMP-kinase in skeletal muscles might be up-regulated and increase energy expenditure (51). If we assumed that PPARα activation was a primary cause of antiobesity effects of fish oil feeding, since fish oil-mediated

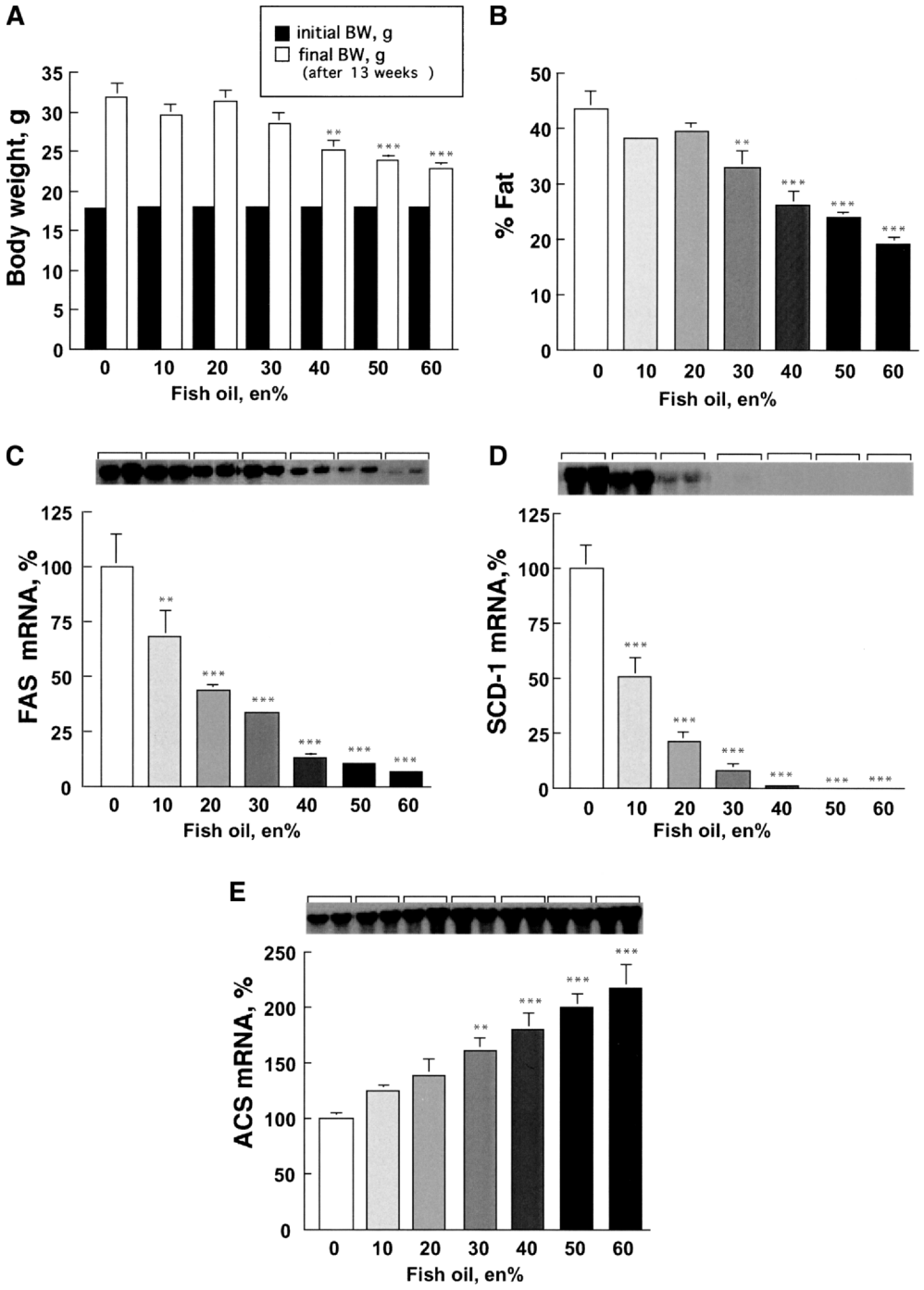

Fig. 8. BW (A), fat mass (B), and expression of FAS (C), SCD-1 (D), and ACS (E) mRNAs from liver of mice fed 0, 10, 20, 30, 40, 50, and 60 en% fish oil for 13 weeks. Mice were fed on a 60 en% high-fat diet with various doses of fish oil in place of safflower oil. Mice were killed at 13 week feeding, and final BW (A), and fat mass (B) by dual-energy X-ray absorptiometry (DEXA), expressions of target genes of SREBP-1 such as FAS (C) and SCD-1 (D), and target gene of PPARα, ACS (E) in liver were measured by Northern blotting. Initial BW is BW at the beginning of diet experiments (A). In autoradiogram, each line represents a sample from an individual mouse. Each data point represents mean ± SE of five mice. $^{**}P < 0.01$, $^{***}P < 0.001$, different doses of fish oil compared with 0 en% fish oil feeding by Fisher's PLSD test.

liver PPARα activation is dose-dependent, a lower, physiological dose of fish oil may not markedly decrease body fat. However, it is not ruled out that a continued, slight activation of PPARα observed in a low dose of fish oil feeding may contribute to a small decrease of body fat. Human studies to examine the long-term effects of fish oil feeding on fat mass are required to resolve this question.

The authors thank Dr. Y. Kamei, Dr. N. Tsuboyama-Kasaoka, and Dr. M. Takahashi for helpful suggestions in the initial phases of the study. We are grateful to Dr. Daniel M. Lane for the supply of mouse SCD-1 cDNA; Dr. N. Iritani for supply of rat FAS and rat ACC cDNAs; and Dr. T. Yamamoto for supply of rat ACS cDNA. This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Tokyo); by research grants from the Japanese Ministry of Health, Labor and Welfare (Tokyo); and by a grant from the Promotion of Fundamental Studies in Health Sciences from the Organization for Pharmaceutical Safety and Research (OPSR).

REFERENCES

1. Cunnane, S. C., K. R. McAdoo, and D. F. Horrobin. 1986. N-3 essential fatty acids decrease weight gain in genetically obese mice. *Br. J. Nutr.* **56:** 87–95.
2. Belzung, F., T. Raclot, and R. Groscolas. 1993. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. *Am. J. Physiol.* **264:** R1111–R1118.
3. Hill, J. O., J. C. Peters, D. Lin, F. Yakubu, H. Greene, and L. Swift. 1993. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. *Int. J. Obes. Relat. Metab. Disord.* **17:** 223–236.
4. Ikemoto, S., M. Takahashi, N. Tsunoda, K. Maruyama, H. Itakura, and O. Ezaki. 1996. High-fat diet-induced hyperglycemia and obesity in mice. Differential effects of dietary oils. *Metabolism.* **45:** 1539–1546.
5. Couet, C., J. Delarue, P. Ritz, J-M. Antoine, and F. Lamisse. 1997. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int. J. Obes.* **21:** 637–643.
6. Worgall, T. S., S. L. Sturley, T. Seo, T. F. Osborne, and R. J. Deckelbaum. 1998. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J. Biol. Chem.* **273:** 25537–25540.
7. Xu, J., M. T. Nakamura, H. P. Cho, and S. D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J. Biol. Chem.* **274:** 23577–23583.
8. Kim, H-J., M. Takahashi, and O. Ezaki. 1999. 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. *J. Biol. Chem.* **274:** 25892–25898.
9. Mater, M. K., A. P. Thelen, D. A. Pan, and D. B. Jump. 1999. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. *J. Biol. Chem.* **274:** 32725–32732.
10. Yahagi, N., H. Shimano, A. H. Hasty, M. Amemiya-Kudo, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamada. 1999. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J. Biol. Chem.* **274:** 35840–35844.
11. Hannah, V. C., J. Ou, A. Luong, J. L. Goldstein, and M. S. Brown. 2001. Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. *J. Biol. Chem.* **276:** 4365–4372.
12. Reddy, J. K., and G. P. Mannaerts. 1994. Peroxisomal lipid metabolism. *Annu. Rev. Nutr.* **14:** 343–370.
13. Schoonjans, K., B. Staels, and J. Auwerx. 1996. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim. Biophys. Acta.* **1302:** 93–109.
14. Krey, G., O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M. G. Parker, and W. Wahli. 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11:** 779–791.
15. Latruffe, N., and J. Vamecq. 1997. Peroxisome proliferators and peroxisome proliferator activated receptors (PPARs) as regulators of lipid metabolism. *Biochimie.* **79:** 81–94.
16. Nakatani, T., N. Tsuboyama-Kasaoka, M. Takahashi, S. Miura, and O. Ezaki. 2002. Mechanism for peroxisome proliferator-activated receptor-alpha activator-induced up-regulation of UCP2 mRNA in rodent hepatocytes. *J. Biol. Chem.* **277:** 9562–9569.
17. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109:** 1125–1131.
18. Briggs, M. R., C. Yokoyama, X. Wang, M. S. Brown, and J. L. Goldstein. 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. *J. Biol. Chem.* **268:** 14490–14496.
19. Wang, X., M. R. Briggs, X. Hua, C. Yokoyama, J. L. Goldstein, and M. S. Brown. 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. Purification and characterization. *J. Biol. Chem.* **268:** 14497–14504.
20. Kim, J. B., G. D. Spotts, Y. D. Halvorsen, H. M. Shih, T. Ellenberger, H. C. Towle, and B. M. Spiegelman. 1995. Dual DNA binding specificity of ADD1/SREBP1 controlled by a single amino acid in the basic helix-loop-helix domain. *Mol. Cell. Biol.* **15:** 2582–2588.
21. Brown, M. S., and J. L. Goldstein. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl. Acad. Sci. USA.* **96:** 11041–11048.
22. Shimano, H., J. D. Horton, R. E. Hammer, I. Shimomura, M. S. Brown, and J. L. Goldstein. 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J. Clin. Invest.* **98:** 1575–1584.
23. Shimano, H., J. D. Horton, I. Shimomura, R. E. Hammer, M. S. Brown, and J. L. Goldstein. 1997. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J. Clin. Invest.* **99:** 846–854.
24. Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein, and H. Shimano. 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J. Clin. Invest.* **101:** 2331–2339.
25. Xu, J., M. Teran-Garcia, J. H. Y. Park, M. T. Nakamura, and S. D. Clarke. 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. *J. Biol. Chem.* **276:** 9800–9807.
26. Tsuboyama-Kasaoka, N., M. Takahashi, H. Kim, and O. Ezaki. 1999. Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice. *Biochem. Biophys. Res. Commun.* **257:** 879–885.
27. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
28. Sheng, Z., H. Otani, M. S. Brown, and J. L. Goldstein. 1995. Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc. Natl. Acad. Sci. USA.* **92:** 935–938.
29. Tsuboyama-Kasaoka, N., N. Tsunoda, K. Maruyama, M. Takahashi, H-J. Kim, S. Ikemoto, and O. Ezaki. 1998. Up-regulation of uncoupling protein 3 (UCP3) mRNA by exercise training and down-regulation of UCP3 by denervation in skeletal muscles. *Biochem. Biophys. Res. Commun.* **247:** 498–503.
30. Ikeda, S., H. Miyazaki, T. Nakatani, Y. Kai, Y. Kamei, S. Miura, N. Tsuboyama-Kasaoka, and O. Ezaki. 2002. Up-regulation of SREBP-1c and lipogenic genes in skeletal muscles after exercise training. *Biochem. Biophys. Res. Commun.* **296:** 395–400.
31. Nagy, T. R., and A-L. Clair. 2000. Precision and accuracy of dual-energy x-ray absorptiometry for determining in vivo body composition of mice. *Obes. Res.* **8:** 392–398.
32. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 206.
33. Reddy, J. K., and R. Chu. 1996. Peroxisome proliferator-induced pleiotropic responses. Pursuit of a phenomenon. *Ann. N. Y. Acad. Sci.* **804:** 176–201.
34. Shimomura, I., H. Shimano, J. D. Horton, J. L. Goldstein, and M. S. Brown. 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J. Clin. Invest.* **99:** 838–845.

35. Reddy, M. K., S. A. Qureshi, P. F. Hollenberg, and J. K. Reddy. 1981. Immunochemical identity of peroxisomal enoyl-CoA hydratase with the peroxisome-proliferation-associated 80,000 mol wt polypeptide in rat liver. *J. Cell Biol.* **89:** 406–417.
36. Watanabe, T., N. D. Lalwani, and J. K. Reddy. 1985. Specific changes in the protein composition of rat liver in response to the peroxisome proliferators ciprofibrate, Wy-14,643 and di-(2-ethylhexyl) phthalate. *Biochem. J.* **227:** 767–775.
37. Hua, X., A. Nohturfft, J. L. Goldstein, and M. S. Brown. 1996. Sterol resistance in CHO cells tranced to point mutation in SREBP cleavage-activating protein. *Cell.* **87:** 415–426.
38. Matsuda, M., B. S. Korn, R. E. Hammer, Y-A. Moon, R. Komuro, J. D. Horton, J. L. Goldstein, M. S. Brown, and I. Shimomura. 2001. SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. *Genes Dev.* **15:** 1206–1216.
39. Yang, J., J. L. Goldstein, R. E. Hammer, Y-A. Moon, M. S. Brown, and J. D. Horton. 2001. Decreased lipid synthesis in livers of mice with disrupted site-1 protease gene. *Proc. Natl. Acad. Sci. USA.* **98:** 13607–13612.
40. Kris-Etherton, P. M., D. S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R. L. Hargrove, G. Zhao, and T. D. Etherton. 2000. Polyunsaturated fatty acids in the food chain in the United States. *Am. J. Clin. Nutr.* **71:** 179S–188S.
41. Feskens, E. J., and D, Kromhout. 1993. Epidemiologic studies on eskimos and fish intake. *Ann. N. Y. Acad. Sci.* **683:** 9–15.
42. Amemiya-Kudo, M., H. Shimano, T. Yoshikawa, N. Yahagi, A. H. Hasty, H. Okazaki, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Sato, S. Kimura, S. Ishibashi, and N. Yamada. 2000. Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. *J. Biol. Chem.* **275:** 31078–31085.
43. Worgall, T. S., R. A. Johnson, T. Seo, H. Gierens, and R. J. Deckelbaum. 2002. Unsaturated fatty acid-mediated decreases in sterol regulatory element-mediated gene transcription are linked to cellular sphingolipid metabolism. *J. Biol. Chem.* **277:** 3878–3885.
44. Costet, P., C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau. 1998. Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273:** 29577–29585.
45. Akiyama, T. E., C. J. Nicol, C. Fievet, B. Staels, J. M. Ward, J. Auwerx, S. S. T. Lee, F. J. Gonzalez, and J. M. Peters. 2001. Peroxisome proliferator-activated receptor-α regulates lipid homeostasis, but is not associated with obesity. *J. Biol. Chem.* **276:** 39088–39093.
46. Guerre-Millo, M., P. Gervois, E. Raspé, L. Madsen, P. Poulain, B. Derudas, J-M. Herbert, D. A. Winegar, T. M. Willson, J-C. Fruchart, R. K. Berge, and B. Staels. 2000. Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity. *J. Biol. Chem.* **275:** 16638–16642.
47. Mancini, F. P., A. Lanni, L. Sabatino, M. Moreno, A. Giannino, F. Contaldo, V. Colantuoni, and F. Goglia. 2001. Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats. *FEBS Lett.* **491:** 154–158.
48. Shimano, H., I. Shimomura, R. E. Hammer, J. Herz, J. L. Goldstein, M. S. Brown, and J. D. Horton. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J. Clin. Invest.* **100:** 2115–2124.
49. Yahagi, N., H. Shimano, A. H. Hasty, T. Matsuzaka, T. Ide, T. Yoshikawa, M. Amemiya-Kudo, S. Tomita, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamada. 2002. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in *Lep^{ob}*/Lep^{ob} mice. *J. Biol. Chem.* **277:** 19353–19357.
50. Cohen, P., M. Miyazaki, N. D. Socci, A. Hagge-Greenberg, W. Liedtke, A. A. Soukas, R. Sharma, L. C. Hudgins, J. M. Ntambi, and J. M. Friedman. 2002. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science.* **297:** 240–243.
51. Winder, W. W. 2001. Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J. Appl. Physiol.* **91:** 1017–1028.