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Metabolism Clinical and Experimental 55 (2006) 381–390

Metabolism
Clinical and Experimental

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Dietary sesamin and docosahexaenoic and eicosapentaenoic acids synergistically increase the gene expression of enzymes involved in hepatic peroxisomal fatty acid oxidation in rats

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Received 4 June 2005; accepted 19 September 2005

Abstract

The interaction of sesamin, one of the most abundant lignans in sesame seed, and highly purified docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) in the form of ethyl ester in affecting hepatic fatty acid oxidation was examined in rats. In the first experiment, 3 groups of rats were fed with purified experimental diets free of n-3 fatty acid ethyl ester and containing 0%, 0.2%, and 0.4% sesamin (1:1 mixture of sesamin and episesamin), and 2 groups of animals were fed with a 2% DHA ethyl ester diet containing either 0% or 0.2% sesamin. In the second trial, 4 groups of rats were fed with either a 0% or a 2% EPA ethyl ester diet containing 0% or 0.2% sesamin. After 15 days of feeding, DHA and EPA ethyl esters added to a sesamin-free diet little affected the activity and messenger RNA (mRNA) levels of various enzymes involved in fatty acid oxidation. Sesamin increased the activity levels of various hepatic enzymes involved in fatty acid oxidation irrespective of the presence or absence of n-3 fatty acid ethyl ester in diets. However, the diet containing sesamin and DHA or EPA ethyl ester in combination increased many of these parameters synergistically. In particular, the peroxisomal palmitoyl-coenzyme A oxidation rate and acyl-coenzyme A oxidase activity level were much higher in rats fed with sesamin and DHA or EPA in combination than in animals fed with a diet free of n-3 fatty acid ethyl ester and containing sesamin. Analyses of mRNA levels revealed that a diet simultaneously containing sesamin and n-3 fatty acid ethyl ester increased the gene expression of various enzymes involved in peroxisomal fatty acid oxidation in a synergistic manner. However, the combination of sesamin and n-3 fatty acid ethyl esters was ineffective in causing a synergistic increase in mRNA levels of enzymes of mitochondrial fatty acid oxidation, microsomal cytochrome P-450 IV A1, and cytosolic liver-type fatty acid–binding protein. It was concluded that sesamin and DHA or EPA ethyl ester synergistically increased hepatic fatty acid oxidation primarily through up-regulation of the gene expression of peroxisomal fatty acid oxidation enzymes. The results essentially reproduced those observed in our previous study with a diet containing both fish oil and sesamin despite the fact that DHA and EPA ethyl esters were much less effective than fish oil in increasing hepatic fatty acid oxidation.

1. Introduction

Sesamin is one of the most abundant lignans in sesame seed. Our previous studies demonstrated that sesamin strongly induces hepatic fatty acid oxidation in rats, presumably through the activation of peroxisome proliferator–activated receptor α (PPARα) [1-3]. It is well demonstrated that fish oil rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) also increases hepatic fatty acid oxidation despite the fact that the magnitude of the increase is much weaker with fish oil than with sesamin [1-7]. Sesamin at a 0.2% dietary level causes a 4- to 5-fold and 2- to 2.5-fold increase in the peroxisomal and mitochondrial fatty acid oxidation rate [1-3], respectively, whereas more than a 10% dietary level is required for fish oil to cause similar changes in these parameters [3-7]. We previously demonstrated that a diet simultaneously containing sesamin and fish oil at levels of 0.2% and 8%, respectively, increased the activity of many enzymes involved in hepatic fatty acid oxidation in a synergistic manner [3]. Analysis of the messenger RNA (mRNA) levels of various hepatic enzymes involved in fatty acid oxidation strongly indicated that the up-regulation of the gene expression of peroxisomal enzymes is responsible for this effect. We hypothesized that fish oil not only

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doi:10.1016/j.metabol.2005.09.013

increases hepatic fatty acid oxidation but also acts as a coactivator of sesamin to enhance peroxisomal fatty acid oxidation. It is most plausible that DHA and/or EPA abundant in fish oil causes a synergistic increase in peroxisomal fatty acid oxidation with sesamin as a counterpart. However, there is still the possibility that some unknown compound or fatty acid other than DHA and/or EPA in fish oil is responsible for this. We therefore currently examined the interaction of sesamin and highly purified DHA and EPA in the form of ethyl esters in affecting hepatic fatty acid oxidation in rats.

2. Materials and methods

2.1. Animals and diets

Male Sprague-Dawley rats obtained from Charles River Japan (Kanagawa, Japan) were housed individually in animal cages in a room with controlled temperature (20°C-22°C), humidity (55%-65%), and lighting (lights on from 7:00 AM to 7:00 PM), and fed with a commercial nonpurified diet (Type NMF, Oriental Yeast, Tokyo, Japan). After 7 days of acclimatization, rats were fed with purified experimental diets for 15 days. In the first experiment (experiment 1), 3 groups of rats were fed with diets supplemented with 0%, 0.2%, or 0.4% sesamin (1:1 mixture of sesamin and episesamin; Takemoto Oil, Aichi, Japan), and containing 10% palm oil. Another 2 groups of animals were fed with diets supplemented with 0% or 0.2% sesamin, and containing 8% palm oil and 2% DHA ethyl ester as dietary lipids. In the second experiment (experiment 2), 2 groups of rats were fed with diets supplemented with 0% or 0.2% sesamin, and containing 10% palm oil. Another 2 groups of animals received diets supplemented with 0% or 0.2% sesamin, and containing 8% palm oil and 2% EPA ethyl ester. Docosahexaenoic acid and EPA ethyl esters were donated by Harima Chemicals (Tsukuba, Japan) and Kewpie (Tokyo, Japan), respectively. Analysis of the fatty acid composition of the DHA ethyl ester preparation revealed it to contain 94.5% DHA and 4.8% EPA as main components. The purity of the EPA ethyl ester preparation exceeded 99%. The fatty acid composition of palm oil was 14:0, 0.87; 16:0, 44.1; 16:1, 0.03; 18:0, 4.19; 18:1, 41.3; 18:2 (n-6), 9.19, 18:3 (n-3), and 0.31. The basal composition of the purified experimental diets was the same as described previously [3]. As DHA and EPA are susceptible to oxidation, the diets were put into plastic bags in small portions (170 g), flushed with nitrogen, and stored at −30°C before use. New diets were served to animals in the morning (9:00-10:30 AM) on each day of the experimental period, and old diets were discarded. Animals had free access to the diets and water during the experimental period. This study was approved by the review board of animal ethics of our institute, and we followed the institute's guidelines in the care and use of laboratory animals.

2.2. Enzyme assays

Upon termination of the experimental period, animals were anesthetized using diethyl ether and killed by bleeding from the abdominal aorta, after which livers were excised immediately. About 2 g of each liver was homogenized with 15 mL of 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris-HCl (pH 7.2). The KCN-insensitive palmitoyl-coenzyme A (CoA) oxidation (peroxisomal palmitoyl-CoA oxidation) rate was measured radiochemically using the whole-liver homogenate as an enzyme source and [1-^{14}C]palmitoyl-CoA as a substrate [1-6]. Activity levels of various enzymes involved in hepatic fatty acid oxidation were measured spectrophotometrically using the whole-liver homogenate as an enzyme source as detailed previously [1-6]. Acyl-CoA oxidase and carnitine palmitoyltransferase activities were measured using palmitoyl-CoA as a substrate. We used crotonyl-CoA, acetoacetyl-CoA, and 3-ketooctanoyl-CoA as substrates in assaying enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-keotacyl-CoA thiolase activities, respectively.

2.3. RNA analysis

Hepatic RNA was extracted, and the mRNA abundance was analyzed by slot-blot hybridization using specific complementary DNA (cDNA) probes as detailed previously [1-6]. The values were corrected using those of a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase). We also carried out Northern blot hybridization for typical samples to confirm the specificity of our cDNA probes and the results obtained with slot-blot hybridization.

2.4. Statistical analysis

StatView for Macintosh (SAS Institute, Cary, NC) was used for statistical analysis. The data for the first and second experiments were analyzed with a 1-way and a 2-way analysis of variance (ANOVA), respectively. These tests were followed by a Tukey-Kramer post hoc analysis to detect significant differences of the means at the level of $P < .05$.

3. Results

3.1. Activity of enzymes involved in hepatic fatty acid oxidation

No significant differences in food intake (18.4-19.1 and 17.7-18.8 g/d for experiments 1 and 2, respectively) and growth (110-120 and 121-128 g/15 d, respectively) were seen among the groups of rats fed with the various experimental diets.

In the absence of sesamin in diets, DHA only slightly increased peroxisomal palmitoyl-CoA oxidation rates and activity levels of various enzymes for fatty acid oxidation (experiment 1, Fig. 1). Only a significant difference was seen in 3-hydroxyacyl-CoA dehydrogenase activity. Among

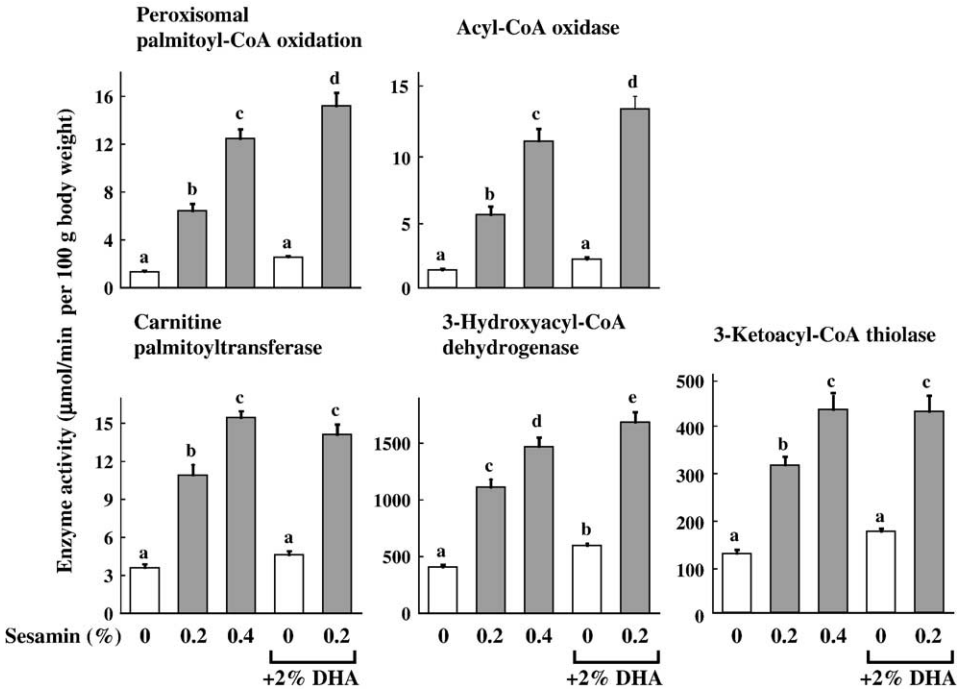

Fig. 1. Interaction of sesamin and DHA in affecting the activity of hepatic fatty acid oxidation enzymes. The activity of the enzymes was analyzed using total homogenate as an enzyme source. Values represent means ± SEM for 7 to 8 rats. Values with different superscript letters (a, b, c, d, e) differ significantly at $P < .05$.

rats fed with DHA-free diets, sesamin increased the activity of enzymes involved in fatty acid oxidation in a dose-dependent manner. Despite the fact that DHA was rather irrelevant in increasing the activity levels of the enzymes, the activity levels were much higher in rats fed with a diet simultaneously containing DHA and 0.2% sesamin than in the animals fed with 0.2% sesamin alone, and comparable to those in the animals fed with a 0.4% sesamin diet. Therefore, DHA and sesamin synergistically increased the activity of enzymes involved in hepatic fatty acid oxidation.

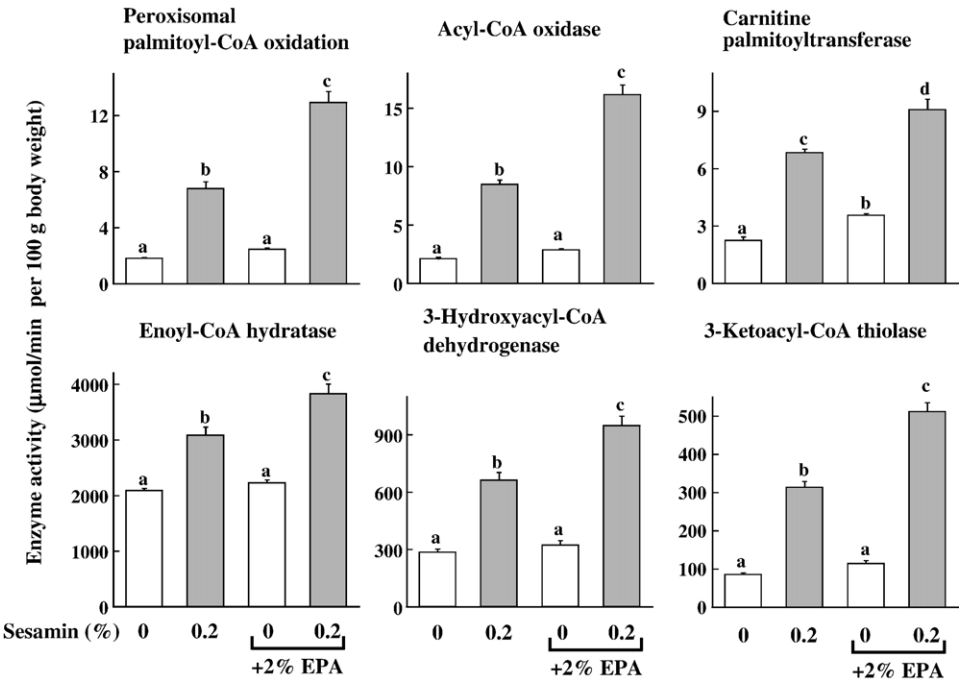

Fig. 2. Interaction of sesamin and EPA in affecting the activity of hepatic fatty acid oxidation enzymes. The activity of the enzymes was analyzed using total homogenate as an enzyme source. Values represent means ± SEM for 7 to 8 rats. Values with different superscript letters (a, b, c, d) differ significantly at $P < .05$.

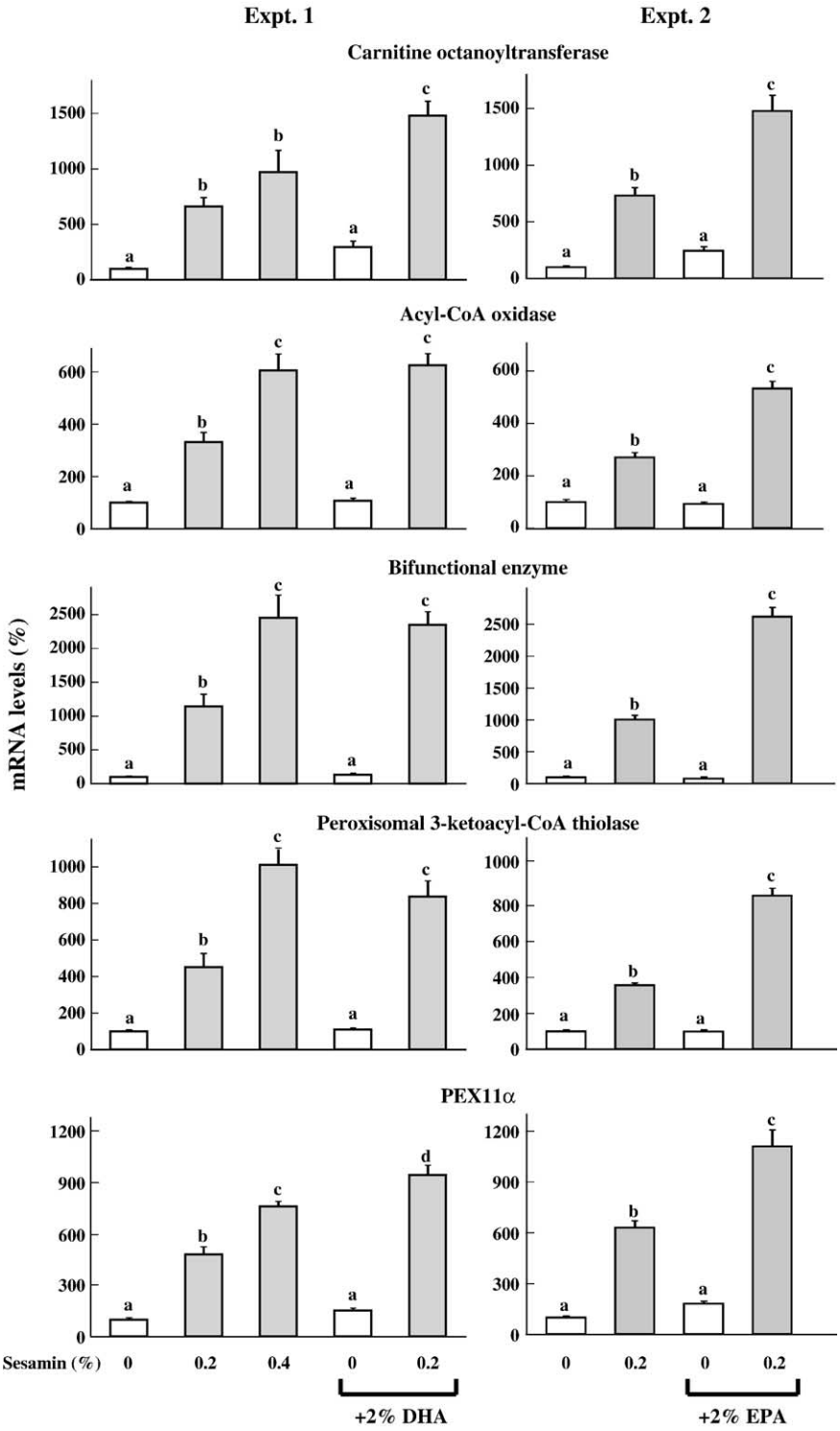

Fig. 3. Interaction of sesamin, and DHA (experiment 1) and EPA (experiment 2) in affecting mRNA levels of hepatic peroxisomal proteins. Hepatic RNA was extracted and the mRNA abundance was analyzed by slot-blot hybridization using specific cDNA probes. The values were expressed as percentages, assigning the value in animals fed with a diet free of $n-3$ fatty acid and sesamin as 100. Values represent means ± SEM for 7 to 8 rats. Values with different superscript letters (a, b, c, d) differ significantly at $P < .05$.

In experiment 2, we examined the interaction of EPA and sesamin in affecting hepatic fatty acid oxidation (Fig. 2). In this experiment, in addition to parameters analyzed in experiment 1, we measured the activity of enoyl-CoA hydrates. As in the case of DHA, EPA added to a sesamin-free diet only slightly increased the activity levels of enzymes involved in hepatic fatty acid oxidation. A significant EPA-dependent increase in activity was noted

Table 1
Interaction of sesamin with DHA or EPA in affecting mRNA levels of enzymes involved in hepatic mitochondrial fatty acid oxidation, microsomal cytochrome P-450 IV A1, and cytosolic liver-type fatty acid–binding protein

mRNA level (%)	Groups								
	Experiment 1					Experiment 2			
	0% Sesamin	0.2% Sesamin	0.4% Sesamin	0% Sesamin + 2% DHA	0.2% Sesamin + 2% DHA	0% Sesamin	0.2% Sesamin	0% Sesamin + 2% EPA	0.2% Sesamin + 2% EPA
Carnitine palmitoyltransferase II	100 ± 8^{a}	208 ± 16^{b}	347 ± 30^{c}	99.8 ± 9.9^{a}	206 ± 8^{b}	100 ± 9^{a}	216 ± 11^{b}	90.6 ± 5.1^{a}	219 ± 10^{b}
Medium-chain acyl-CoA dehydrogenase	100 ± 4^{a}	171 ± 12^{b}	247 ± 27^{c}	85.9 ± 5.8^{a}	159 ± 12^{b}	100 ± 2^{a}	194 ± 5^{b}	122 ± 7^{a}	229 ± 16^{c}
Trifunctional enzyme subunit α	100 ± 5^{a}	215 ± 11^{b}	328 ± 22^{c}	98.2 ± 8.0^{a}	220 ± 12^{b}	100 ± 5^{a}	213 ± 10^{b}	121 ± 5^{a}	252 ± 9^{c}
Trifunctional enzyme subunit β	100 ± 4^{a}	232 ± 15^{b}	342 ± 47^{c}	110 ± 12^{a}	250 ± 17^{b}	100 ± 7^{a}	235 ± 15^{b}	118 ± 6^{a}	269 ± 17^{b}
Mitochondrial 3-ketoacyl-CoA thiolase	100 ± 3^{a}	264 ± 22^{b}	394 ± 30^{c}	129 ± 13^{a}	261 ± 26^{b}	100 ± 9^{a}	220 ± 7^{b}	122 ± 6^{a}	234 ± 11^{b}
Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase	100 ± 6^{a}	175 ± 11^{c}	291 ± 10^{e}	136 ± 7^{b}	218 ± 19^{d}	100 ± 3^{a}	209 ± 7^{c}	123 ± 6^{b}	252 ± 12^{d}
Cytochrome P-450 IV A1	100 ± 6^{a}	175 ± 11^{b}	291 ± 10^{d}	136 ± 7^{a}	218 ± 19^{c}	100 ± 5^{a}	408 ± 32^{b}	115 ± 6^{a}	492 ± 62^{b}
Liver-type fatty acid–binding protein	ND	ND	ND	ND	ND	100 ± 6^{a}	201 ± 5^{b}	117 ± 3^{a}	218 ± 17^{b}

Values represent means ± SE for 7 to 8 rats. Hepatic RNA was extracted, and the mRNA abundance was analyzed by slot-blot hybridization using specific cDNA probes. The values were expressed as percentages, assigning the value in animals fed with a diet free of sesamin and DHA (experiment 1) or EPA (experiment 2) as 100. Values with different superscript letters (a, b, c, d, e) differ significantly at $P < .05$. ND indicates not determined.

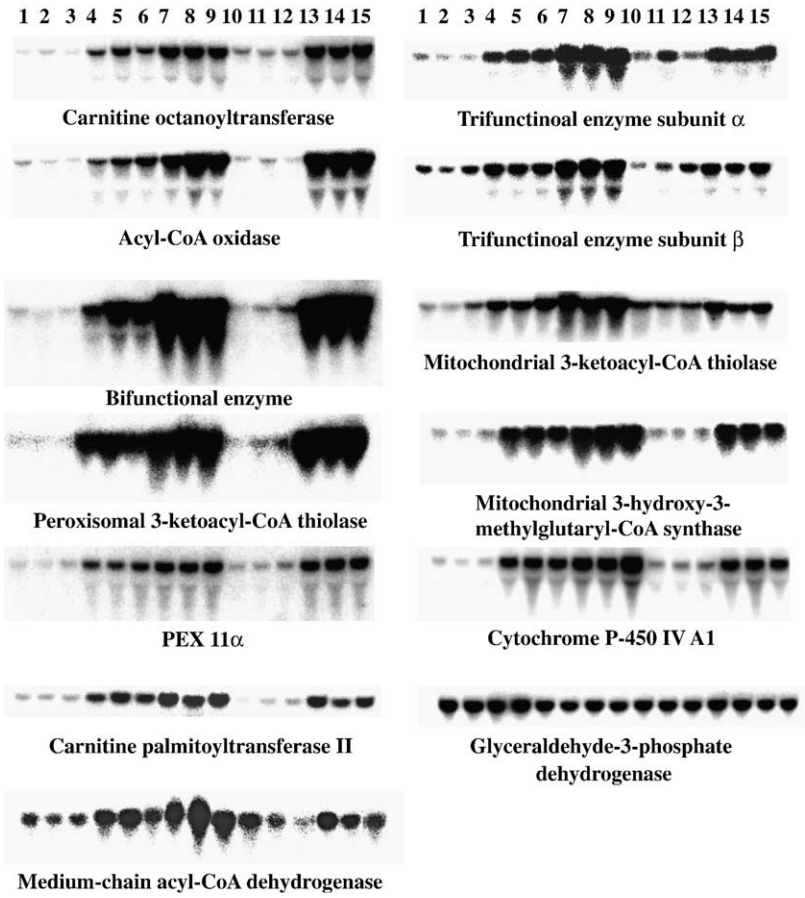

Fig. 4. Northern blot analysis of mRNAs of enzymes involved in fatty acid oxidation, PEX11α, cytochrome P-450 IV A1, and glyceraldehyde-3-phosphate dehydrogenase in the liver of rats fed with DHA-free and 2% DHA diets containing different amounts (0%, 0.2%, or 0.4%) of sesamin (experiment 1). RNA samples (30 μg) were denatured and subjected to electrophoresis on a 1.1% agarose gel containing 0.66 mol/L formaldehyde, then transferred to a nylon membrane and fixed with UV irradiation. The RNA on the nylon membrane was hybridized with radiolabeled cDNA probes specific for mRNAs of respective proteins. Lanes 1 to 3 indicate rats fed with a diet free of DHA and sesamin; 4 to 6, a diet free of DHA and containing 0.2% sesamin; 7 to 9, a diet free of DHA and containing 0.4% sesamin; 10 to 12, a diet containing 2% DHA and free of sesamin; 13 to 15, a diet containing 2% DHA and 0.2% sesamin.

for carnitine palmitoyltransferase, but not for the other parameters between the 2 groups of rats fed with sesamin-free diets. However, sesamin added to EPA-free and EPA-containing diets caused significant increases in all parameters observed. A diet simultaneously containing sesamin and EPA caused greater increases in many of these parameters than to be expected from the physiological activity of individual compounds. In fact, a 2-way ANOVA revealed significant interaction ($P < .01$) between 2 dietary factors, that is, EPA and sesamin, in affecting the activities of various enzymes involved in fatty acid oxidation.

3.2. Messenger RNA levels of enzymes involved in hepatic fatty acid oxidation

Great diversity is characteristic of the β-oxidation pathway. Several enzyme species differing in substrate specificity located either in peroxisomes or in mitochondria are involved at each step of the β-oxidation cycle. The fatty acid oxidation enzyme activity measured in cell-free enzyme preparations therefore represents the sum of the activity of various enzymes under given conditions of the enzyme assay. A cDNA probe specific to each enzyme's mRNA, however, can discriminate the gene expression of individual enzymes.

The analyses of the activities of hepatic fatty acid oxidation enzymes indicated that sesamin and n-3 fatty acids synergistically increase fatty acid oxidation. The observation that synergistic increases are most prominent for the peroxisomal fatty acid oxidation rate and acyl-CoA oxidase activity emphasizes that alterations in the expression of peroxisomal β-oxidation enzymes are responsible for this change. Therefore, mRNA levels of various hepatic peroxisomal β-oxidation enzymes as well as peroxin (PEX)11α, a peroxisomal membrane protein known to be induced by peroxisome proliferators [8,9], were analyzed and are shown in Fig. 3. Messenger RNA levels are expressed as percentages, assigning the value in the animals fed with a diet free of sesamin and n-3 fatty acid ethyl ester as 100. In experiment 1, comparing the 2 groups of rats fed with sesamin-free diets, expression levels of mRNAs for acyl-CoA oxidase, bifunctional enzyme possessing enoyl-CoA hydratase and 3-hydoxyacylCoA dehydrogenase activi-

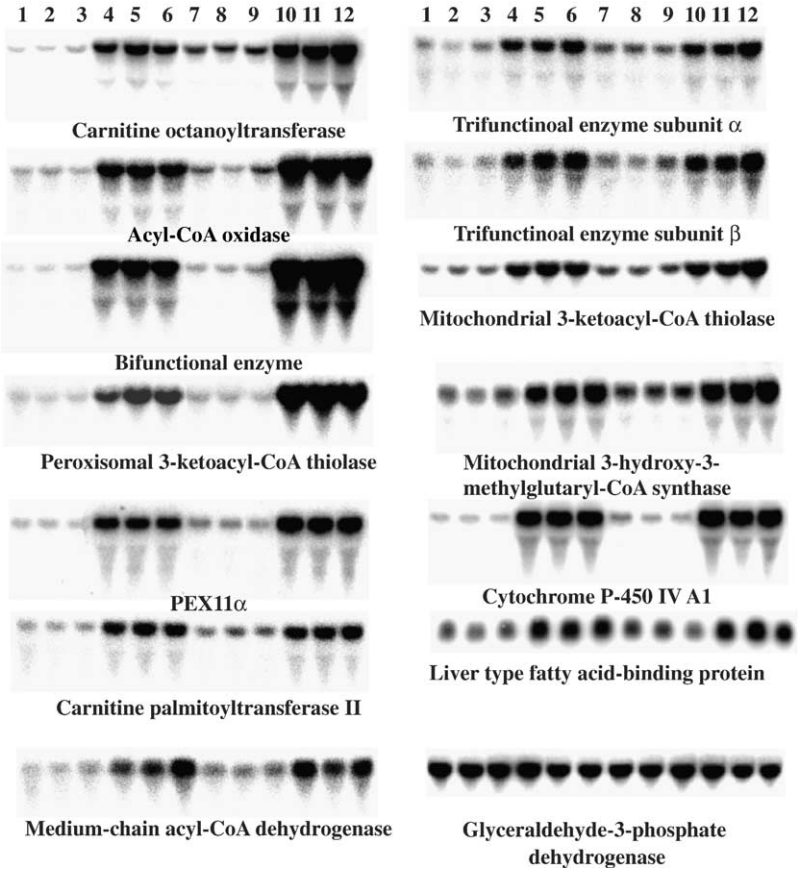

Fig. 5. Northern blot analysis of mRNAs of enzymes involved in fatty acid oxidation, PEX11α, cytochrome P-450 IV A1, liver-type fatty acid–binding protein, and glyceraldehyde-3-phosphate dehydrogenase in the liver of rats fed with EPA-free and 2% EPA diets containing 0% or 0.2% sesamin (experiment 2). RNA samples (30 μg) were treated as described in the legend to Fig. 4. Lanes 1 to 3 indicate rats fed with a diet free of EPA and sesamin; 4 to 6, a diet free of EPA and containing 0.2% sesamin; 7 to 9, a diet containing 2% EPA and free of sesamin; 10 to 12, a diet containing 2% EPA and 0.2% sesamin.

ties [10], and 3-ketoacyl-CoA thiolase were only slightly and insignificantly higher (7%-32%) in the animals fed with DHA than in those fed with a DHA-free diet. Docosahexaenoic acid added to a sesamin-free diet caused a considerable increase in the mRNA levels of carnitine octanoyltransferase (3.0-fold) and PEX11α (1.5-fold), but the differences were not significant. Among rats fed with DHA-free diets, sesamin dose dependently increased all the mRNA levels. Messenger RNA levels were much higher in rats fed with a diet simultaneously containing 0.2% sesamin and DHA (6.3- to 23.5-fold increase) than in the animals fed with a 0.2% sesamin diet free of DHA (3.3- to 11.4-fold increase) despite the fact that DHA at this dietary level only marginally increased these parameters in rats fed with a sesamin-free diet. These values were comparable to or even higher than those observed in rats fed with a 0.4% sesamin diet (6.1- to 24.5-fold increase). Therefore, it is apparent that sesamin and DHA synergistically increased the mRNA levels of these proteins. A similar synergism in affecting the mRNA expression of peroxisomal proteins was confirmed in rats fed with EPA and sesamin in combination (experiment 2). Eicosapentaenoic acid added to a sesamin-free diet was ineffective in increasing the mRNA levels of acyl-CoA oxidase, bifunctional enzyme, and peroxisomal 3-ketoacyl-CoA thiolase, but it increased the mRNA levels of carnitine octanoyltransferase 2.5-fold and PEX11α 1.8-fold. However, the increases were again not significant. The simultaneous consumption of sesamin and EPA greatly increased the mRNA levels of these peroxisomal proteins to levels much higher than those observed with a diet containing sesamin alone. Two-way ANOVA proved the significant interaction ($P < .01$) of EPA with sesamin in affecting the mRNA levels of all these peroxisomal proteins.

The mRNA levels of hepatic enzymes involved in mitochondrial β-oxidation and ketogenesis, and microsomal cytochrome P-450 IV A1 involved in ω-oxidation of fatty acids [11], were also analyzed (Table 1). Mitochondrial trifunctional enzyme is composed of 2 subunits (α and β), and the α subunit possesses enoyl-CoA hydratase activity and the β subunit possesses both 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase activities [12]. In experiment 1, sesamin dose dependently increased these parameters among rats fed with a DHA-free diet. Docosahexaenoic acid added to a sesamin-free diet was

rather irrelevant in modulating mRNA levels, and a significant increase was noted for the mRNA level of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase involved in ketogenesis but not in the other parameters. In contrast to the mRNA levels of peroxisomal proteins, mRNA levels of mitochondrial enzymes and cytochrome P-450 IV A1 in rats fed with sesamin and DHA in combination were comparable to those observed in the animals fed with a diet containing 0.2% sesamin alone. In experiment 2, in addition to the parameters analyzed in experiment 1, we measured the mRNA levels of liver-type fatty acid–binding protein. Results obtained were similar to those observed in the first experiment. Eicosapentaenoic acid added to a sesamin-free diet significantly increased the mRNA levels of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, but did not affect other parameters. Sesamin added to EPA-free and EPA-containing diets increased the mRNA levels of mitochondrial enzymes, cytochrome P-450 IV A1, and liver-type fatty acid–binding protein. However, mRNA levels in rats fed with a diet containing both sesamin and EPA were only slightly higher than or comparable to those observed in rats fed with a diet containing 0.2% sesamin alone. Analysis by 2-way ANOVA indicated no interaction between 2 dietary factors, sesamin and EPA, in affecting these parameters.

Figs. 4 and 5 show typical results for Northern blotting of mRNAs of peroxisomal and mitochondrial proteins, microsomal cytochrome P-450 IV A1, cytosolic liver-type fatty acid–binding protein, and glyceraldehyde-3-phosphate dehydrogenase observed in experiments 1 and 2, respectively. The results confirmed those obtained by slot-blot hybridization.

4. Discussion

Sesamin is a potent inducer of hepatic fatty acid oxidation in rats [1-3]. Fish oil as a natural product also increases hepatic fatty acid oxidation. In our previous study [3], we examined the interaction of various dietary fats (8% in diets), including palm oil (a saturated fat), safflower oil rich in linoleic acid, and fish oil rich in EPA and DHA, and sesamin (0.2% in diet) in affecting hepatic fatty acid oxidation. We not only confirmed previous findings [4-7] that fish oil, among various dietary fats, gave the highest activity levels of enzymes involved in hepatic fatty acid oxidation, but also unexpectedly found that a diet containing fish oil and sesamin in combination increased the activity of hepatic fatty acid oxidation enzymes to levels much higher than those expected from the physiological activity of the individual ingredients. Although sesamin and fish oil added individually to experimental diets increased the mRNA levels of both mitochondrial and peroxisomal enzymes in the liver, a synergistic increase in mRNA levels caused by a diet simultaneously containing fish oil and sesamin was noted in peroxisomal but not in mitochondrial enzymes. Therefore, it was suggested that the synergistic increase in the activity levels of enzymes involved in hepatic fatty acid oxidation caused by the diet containing both fish oil and sesamin was primarily due to the up-regulation of enzymes involved in peroxisomal fatty acid oxidation.

There is a general consensus that the physiological activity of fish oil is ascribable to EPA and DHA. However, there is still the possibility that some unknown compound and fatty acid other than EPA and DHA that are included in the fish oil preparation used in our previous study caused this synergism. Our current study using highly purified EPA and DHA ethyl esters in place of fish oil as counterparts with sesamin supports the idea that EPA and/or DHA is the compound causing this synergism. It has been demonstrated that EPA and DHA ethyl esters can increase hepatic fatty acid oxidation [13,14]. However, we previously found that diets containing highly purified EPA and DHA as ethyl esters compared with a diet containing equivalent amounts of these n-3 fatty acids as fish oil were much less effective in increasing the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation in rats [5] and apolipoprotein E–deficient mice [6]. Studies [15,16] have indicated that EPA and DHA are less absorbable in the small intestine in the form of ethyl ester than in the form of triacylglycerol. This may account for the divergent effects of EPA and DHA ethyl esters, and fish oil on fatty acid oxidation. Consistent with our previous findings [5,6], the present study showed that sesamin-free diets containing 2% DHA and EPA ethyl esters only moderately increased the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation, but they caused a synergistic up-regulation of the hepatic activity levels of enzymes involved in fatty acid oxidation and the mRNA expression of peroxisomal proteins when they were served in combination with sesamin. Therefore, it is considered that n-3 fatty acids in combination with sesamin can cause a synergistic increase in hepatic fatty acid oxidation independent of their role in increasing this metabolic activity.

In relation to this notion, we examined the impact of low levels (1.5% and 3% in diets) of fish oil rich in EPA (10%) and DHA (33%) added to 0.2% sesamin diets in affecting hepatic peroxisomal fatty acid oxidation (Ide et al, unpublished observation). In the absence of sesamin in diets, 3% fish oil diet compared with a fish oil–free diet caused a significant 2.4-fold increase in the peroxisomal fatty acid oxidation rate. However, a diet containing 1.5% fish oil caused only a moderate and insignificant 1.2-fold increase in this value. In the absence of fish oil in the diet, sesamin caused a 4.6-fold increase in the peroxisomal fatty acid oxidation rate, and diets containing 1.5% and 3% fish oil, and sesamin in combination relative to a diet free of these components caused a huge 9.8- and 12-fold increase, respectively, in this parameter. To clarify the dose-dependent effect of fish oil on this synergism, sesamin-dependent increases in the enzyme activity were calculated by subtracting the value in the rats fed with a sesamin-free

diet containing 0% or 1.5% and 3% fish oil from the corresponding value in the animals fed with 0.2% sesamin diets containing varying amounts of fish oil. They were more than 2-fold higher in rats fed with diets containing sesamin and 1.5% and 3% fish oil in combination (9.01 ± 0.46 and 9.75 ± 0.88 μmol/min per 100 g body weight, respectively) than in the animals fed with a fish oil–free sesamin-containing diet (3.72 ± 0.35 μmol/min per 100 g body weight). Apparently, however, these values were comparable between 2 groups of rats fed with 0.2% sesamin diets containing different amounts of fish oil. Similar changes were observed on the activity of acyl-CoA oxidase and mRNA levels of various peroxisomal proteins. In addition, it should be stated that the magnitude of the changes appeared comparable with that observed in rats fed with a 0.2% sesamin diet simultaneously containing 8% fish oil [3], or 2% DHA (Fig. 1) and 2% EPA (Fig. 2) in the form of ethyl esters. Therefore, it is apparent that fish oil at a dietary level as low as 1.5% (this diet contained 0.65% n-3 fatty acids as EPA and DHA in the form of triacylglycerol) causes maximal synergistic changes in peroxisomal fatty acid oxidation in rats fed with a 0.2% sesamin diet. The observations also imply that the changes induced by the combination of n-3 fatty acids and sesamin are saturable, and it is currently unknown if n-3 fatty acids also cause a synergistic increase in peroxisomal fatty acid oxidation in rats fed with sesamin at dietary levels higher than 0.2%.

Although the DHA ethyl ester preparation used in this study contained small amounts of EPA, the present observations suggested that the ability to cause a synergistic increase in peroxisomal β-oxidation with sesamin as a counterpart is similar between DHA and EPA.

The mechanism by which n-3 fatty acids and sesamin cause the synergistic increase in the gene expression of peroxisomal enzymes is not clear at present. Sesamin is not an antioxidant in vitro, but is metabolized to compounds having the propensity for antioxidation [17]. Therefore, there is the possibility that sesamin when given to animals in combination with n-3 fatty acids increases the retention in tissue of n-3 polyunsaturated fatty acids through the prevention of oxidative degradation. This consequence may cause a synergistic increase in hepatic fatty acid oxidation. In fact, in the present study, sesamin added to diets containing n-3 fatty acids increased the hepatic levels of n-3 fatty acids in both experiments 1 and 2 (data not shown). However, changes (32% and 23% increases for experiments 1 and 2, respectively) to account for the synergistic increase in hepatic fatty acid oxidation are moderate. Alternatively, there is the possibility that n-3 fatty acids modulate the metabolism of sesamin and cause changes in hepatic fatty acid oxidation. In this context, we analyzed the hepatic levels of lignan in rats fed with sesamin-containing diets in experiment 2 where EPA ethyl ester was used as the dietary n-3 fatty acid (data not shown). As the sesamin preparation used in this study is a 1:1 mixture of sesamin and episesamin, these compounds are detected in liver. However, EPA added to sesamin-containing diets did not affect the hepatic levels of these compounds. There is the possibility that some metabolite(s) of sesamin or episesamin rather than the original compound is the actual inducer of hepatic fatty acid oxidation. Therefore, the possibility cannot be disregarded that n-3 fatty acids increase the conversion of lignan to generate a compound capable of causing a huge increase in hepatic fatty acid oxidation.

Peroxisome proliferator–activated receptor α is a member of the nuclear receptor superfamily highly expressed in liver and is activated by peroxisome proliferators and up-regulates the expression of a number of genes involved in hepatic fatty acid metabolism [18,19]. Genes of peroxisomal acyl-CoA oxidase and bifunctional enzymes involved in fatty acid oxidation were first identified as targets to be activated by the PPAR [18-21]. Later studies [18,22-28], however, showed that the PPAR up-regulates the gene expression not only of peroxisomal proteins, but also of proteins associated with organelles other than peroxisomes. In this study, synergistic increases in mRNA expression in the animals on diets containing both sesamin and n-3 fatty acids were noted for the enzymes involved in fatty acid oxidation and a membrane protein (PEX11α) associated with peroxisomes, but not for enzymes associated with mitochondria, and microsomal cytochrome P-450 IV A1 and cytosolic liver-type fatty acid–binding protein. It should be stated that among the various proteins associated with organelles other than peroxisomes, at least medium-chain acyl-CoA dehydrogenase [23], mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase [22], cytochrome P-450 IV A1 [24], and liver-type fatty acid–binding protein [25] are proven to be the downstream targets to be activated by PPAR. Therefore, our current observation strongly indicates the existence of a mechanism independent of PPAR to specifically induce the gene expression of peroxisomal proteins.

In conclusion, we showed that sesamin and purified DHA or EPA in the form of ethyl ester synergistically increased hepatic fatty acid oxidation. A detailed examination of the impact of the combination of these dietary factors affecting the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation indicated that the dietary treatment specifically induced the gene expression of peroxisomal enzymes. These results essentially reproduced those obtained in a previous study [3] using a diet containing sesamin and fish oil in combination despite the fact that DHA and EPA in the form of ethyl ester are much less effective than fish oil in inducing hepatic fatty acid oxidation [5,6]. Therefore, it is considered that the physiological activity of n-3 fatty acids in the form of either fish oil or ethyl ester to cause the synergistic increase in hepatic fatty acid oxidation with sesamin as a counterpart is independent of their role in increasing hepatic fatty acid oxidation.

Acknowledgment

This study was supported in part by a grant from the Ministry of Agriculture, Forestry and Fisheries Food Research Project "Integrated Research on Safety and Physiological Function of Food."

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