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Effects of fish oil and n-3 fatty acids on the regulation of Δ^9-fatty acid desaturase mRNA and -activity in rat liver

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This study was designed to study the effects of treatment with dietary fish oil, or with long-chain n-3 polyunsaturated fatty acids, on Δ^9-desaturase in rat liver. The desaturase activity was measured in isolated microsomes in parallel with measurements of liver content of Δ^9-desaturase mRNA. Fish oil-diet [20% (w/w) of dietary fat] led to an 85% decrease in Δ^9-desaturase activity, whereas the decrease in Δ^9-desaturase mRNA was about 90%. In comparison, soybean oil diet [20% (w/w)] decreased Δ^9-desaturase activity by 50%, and the hepatic mRNA content by about 60%. Treatment with docosahexaenoic acid per os decreased the Δ^9 desaturase mRNA by 70%, whereas treatment with eicosapentaenoic acid decreased the mRNA level by 15%. Clofibrate treatment, in contrast, caused about a six fold stimulation of Δ^9-desaturase activity, and about two fold increase in mRNA concentration. At the same time the amounts of acyl-CoA oxidase- and acyl-CoA dehydrogenase mRNA did not vary significantly with high-fat diets or long-chain n-3 fatty acids, but increased two and two and-a-half-fold, respectively, with clofibrate. The experiments show that fish oil has a strong decreasing effect on the amount of Δ^9-desaturase mRNA, and that docosahexaenoic acid in the oil may be one potent component in this respect. (J. Nutr. Biochem. 8:408–413, 1997) © *Elsevier Science Inc. 1997*

Keywords: Δ^9-desaturase; mRNA; fish oil; docosahexaenoic acid; clofibrate; liver

Introduction

Regulation of enzymatic activity by control of transcriptional activity is well established as a general tool of metabolic regulation. The hepatic Δ^9-desaturase is subjected to a complex hormonal control. Its activity is enhanced by insulin[1,2] and by treatment with clofibrate,[3,4] whereas diabetes[1,5,6] and some high fat diets, especially fish oil[7,8] are known to inhibit its activity. We therefore wished to investigate the transcriptional regulation of Δ^9-desaturase by a diet as fish oil and by treatment with clofibrate. Fatty acyl-CoA Δ^9-desaturase (EC 1.14.99.5) is the most active of desaturases in mammals, producing palmitoleate and oleate acid from palmitate and stearate, respectively. It is therefore of considerable physiological importance.

Desaturation of fatty acids occurs primarily in the endoplasmic reticulum. The reaction involves several enzymes to facilitate the electron-transfer from NADH to O_2 necessary for generation of the Δ^9-double bond. The process requires flavine-dependent cytochrome b_5-reductase, as well as cytochrome b_5, which supplies electrons directly to the desaturase. The transcriptional regulation of this enzyme has been reviewed recently by Ntambi.[9] Our approach has been to measure hepatic Δ^9-desaturase activity and the

Presented at the XV International Congress of Nutrition in 1993; Ormstad, H.K., Brodal, B.P., Christiansen, E.N., and Osmundsen, H. Dietary regulation of Δ^9-desaturase in rat liver. *Abstract Book* **II**, 692. Adelaide, Australia.

Supported by the Novo Nordisk Foundation, Bagsvaerd, Denmark and by the Research Society of the Norwegian Edible Fat Producers, Fredrikstad, Norway. Eicosapentaenoic- and docosahexaenoic acids were kindly supported from Pronova Biocare A/S, Lysaker, Norway.

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Received October 8, 1996; accepted February 27, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00049-1

amount of Δ^9-desaturase mRNA after treatment of rats either with selected high-fat diets, with eicosapentaenoic or docosahexaenoic acids, or with clofibrate.

We found that the Δ^9-desaturase activity is regulated in parallel to mRNA formation by both fish oil and clofibrate, which may suggest that the enzyme is regulated at the level of gene transcription by these compounds. Alternatively the treatments may affect factors regulating the stability of mRNA. The data do not exclude, however, the possibility that activity may also be regulated through post-translational mechanisms.

Methods and materials

Materials

[1-^{14}C]stearic acid was purchased from the Amersham Plc, UK. Dithiothreitol, NADH, CoA (Na-salt), HEPES, EGTA, EDTA, human placental ribonuclease-inhibitor, sodium dodecylsulphate, and defatted bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO USA. All reagents used during isolation of RNA were of molecular biology grade, excepting guanidinium isothiocyanate (analytical grade) purchased from E. Merck, Darmstadt, FRG. All other reagents were of analytical grade. [1-^{14}C]stearoyl-CoA was synthesised, and solutions of [1-^{14}C]stearoyl-CoA were assayed, as described elsewhere.[10] Ethyl-eicosapentaenoate (95%) and ethyl-docosahexaenoate (90%) were gifts from Pronova Biocare A/S, Lysaker, Norway. The fish oil (Japanese) and soybean oil were gifts from DeNoFa and Lilleborg Fabriker A/S, Fredrikstad, Norway. Oligonucleotides (30 mer), which had been purified by HPLC, were purchased from the Biotechnology Centre of University of Oslo, Blindern, Oslo. The Oligo-program (National Biosciences, Plymouth, MN USA) was used to select probes with minimal ability to form dimers or hairpin loops using cDNA sequences for Δ^9-desaturase,[11] acyl-CoA dehydrogenase,[12] acyl-CoA oxidase,[13] and rRNA.[14] All cDNA sequences were retrieved from the EMBL-gene library.

Experimental animals

Male Wistar rats (200 to 250 gm body wt.) were kept on a rat chow (Ewos, Södertälje, Sweden), and were allowed fodder and water ad libitum. The fish oil and soybean oil diets (both 20% w/w) were based on a semisynthetic diet described elsewhere.[7] The composition of the fish oil was (in %): 14:0; 8.1, 16:0; 21.3, 16:1; 8.6, 18:0; 2.7, 18:1 n-9; 9.5, 18:1 n-7; 3.7, 18:2; 4.3, 18:3 n-3; 3.7, 20:5 n-3; 14.2, 22:1, 1.5, 22:5 n-3; 1.4, 22:6 n-3; 7.7. The rats were not fasted before they were killed. Rats were given eicosapentaenoic, docosahexaenoic, and palmitic acids by gastric tube delivery; 1.0 mg/gm weight of rats/day for 10 days and the rats were killed the day after. The fatty acids (10% w/v) were disolved in 0.5% (w/v) carboxymethyl cellulose, including 0.5% (w/v) α-tocopherol. Clofibrate was added as a mixture with the chow (0.5% w/w). The rats were allowed food and water ad libitum. The clofibrate rats were fasted overnight and they were killed the following morning.

The animals were killed by decapitation between 9 and 10 a.m. The abdomen was opened, and a piece of a liver lobe (0.5 gm) was transferred quickly to liquid N_2. The remaining liver was homogenized with two strokes in a Potter-Elvehjem glass homogenizer equipped with a Teflon^R piston, in 10 volumes (w/w) ice-cold mannitol-medium (containing 0.3 mol/L mannitol, 10 mmol/L HEPES, and 0.1 mmol/L EGTA, pH adjusted to 7.2 with KOH).

No significant differences were found as regarding body weights or relative liver weights at the end of the experiment.

Isolation of microsomes

The homogenate was centrifuged at 4°C for 1 min at 1.000 × g, and the resulting supernatant for 15 min at 27000 × g. The pellet was discarded, and the supernatant was centrifuged at 180000 × g for 60 min (Centrikon T-2070, equipped with the TFT.65. 13 rotor). The microsomal pellet, was solubilized in a minimal volume of a medium containing 30 mmol/L HEPES, pH 7.4 and 1 mmol/L dithiothreitol (DTT). This suspension was used for desaturase assays.

Determination of Δ^9-desaturase activity

Fatty acyl Δ^9-desaturase activity was measured using microsomal fractions isolated from rat livers as described, with [1-^{14}C]stearoyl-CoA (100 μmol/L, of specific radioactivity of about 18 kBq/nmol) as substrate. The desaturase reaction was performed in 1.0 mL of a medium containing 30 mmol/L HEPES buffer, pH 7.4 at 37°C, with 1 mmol/L NADH, 1 mg/mL serum albumin, 1 mmol/L DTT and 0.1 mmol/L $MgCl_2$. The incubation also contained 4 mg of microsomal protein, which was mixed thoroughly with the incubation medium. All incubations were performed at 37°C.

The reaction was started by addition of substrate, and stopped after 20 min with 1.0 mL 5 mol/L KOH. The mixture was heated to 90°C for 30 min, and subsequently cooled on ice. To the cooled hydrolysates were added 2.0 mL of 5 mol/L HCl, and the acidified reaction mixture was extracted two times with approximately 8 mL of diethylether. The etherphases were pooled and blown to dryness with a stream of N_2. The residue was redissolved in 160 μL of methanol. Usually 50 μL were injected into the radio-HPLC.

Radio-HPLC analysis was performed using Perkin Elmer 410 chromatograph fitted with a UV and radioactivity detector (Raytest Ramona LS) coupled in series. Separation of [1-^{14}C]stearic acid from reaction product [1-^{14}C]oleic acid was achieved using a Spheri-5-RP-18 column, 100 × 4.6 mm (Applied Biosystems, San Jose, CA, USA), and eluted using water: methanol (15:85) at start, changing nonlinearly to 100% methanol after 12 min, using a flow-rate of 2.0 mL/min.

Assay of protein

The protein concentrations were determined by BCA Protein Assay Reagent (Pierce, Rockford, IL. USA), with bovine serum albumin as standard.

Isolation of mRNA

Total RNA was extracted from 0.5 gm of rat liver that had been homogenized in a medium containing 5.5 mol/L guanidinium isothiocyanate, 2.2 mol/L mercaptoethanol, 25 mmol/L Na_3 citrate, 0.5% (w/v) *N*-lauroyl sarcosinate, 25 mmol/L Na_2 EDTA, and 0.5 mL Antifoam A/100 mL.[15] Samples (2 mL) of the final homogenate were centrifuged in a self-generated CsTFA gradient (SW 56 rotor, 20 hr at 130,000 × g and 15°C) as described.[15] After centrifugation, the upper layer was removed, and the RNA pellet washed with ethanol. The pellet was solubilized in 10 mmol/L Tris-HCl-buffer, containing 1 mmol/L EDTA and 0.1% Na-dodecylsulphate, pH 7.5. The RNA was subsequently precipitated using ice-cold ethanol, containing 3 mol/L Na-acetate. The pellet was dried in a vacuum centrifuge. The pellet was redissolved in 100 μL sterile distilled water, and the concentration of RNA in each sample was based on absorbance at 260 nm. One mmol/L DTT and five units RNAase inhibitor were added to the samples, and they were stored at −70°C.

Analysis of mRNA by hybridization with oligonucleotide probes

RNA-samples were analyzed by submarine agarose electrophoresis, using formaldehyde to maintain denaturing conditions. Dot blots and Northern blots were performed as described,[16] using Hybond N membranes (Amersham PLC, UK). The membranes were hybridized with specific digoxigenin (DIG)-labeled oligonucleotide probes (30 mers),[17] chosen from the corresponding base-sequence of the corresponding cDNA. Hybridization signals were detected as CSPD-dependent chemiluminescence (Boehringer Mannheim, Germany). Hybridization of Northern blots showed clear mRNA bands when hybridized with 30-mer oligonucleotide-probes (e.g., Δ^9-desaturase, acyl-CoA oxidase, or acyl-CoA dehydrogenase). The recorded signals were quantitated by using the Millipore BioImage scanning system. Corrections for variation in amounts of RNA applied to dot blots was performed by rehybridizing every membrane with a rRNA 30-mer probe. All integrated signals were subsequently corrected using the rRNA integral as the reference value. The signals from treated rats were calculated in percent of controls (100%).

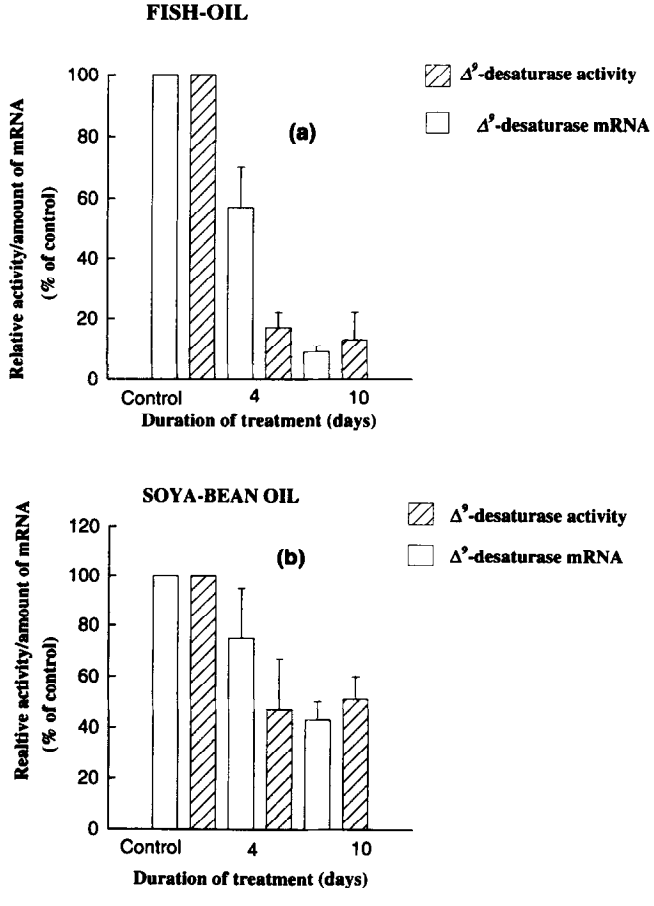

Figure 1 Effects of fish- and soybean oil-diets on hepatic Δ^9-desaturase activity and amount of mRNA in % of control values. Rats were fed on high fat-diets containing 20% (wt) fish oil (a), or soybean oil (b) for 10 days. At various times of treatment as shown in the figures, the rats were killed and the livers removed. The amounts of Δ^9-desaturase mRNA (in %) were measured and activities of Δ^9-desaturase were assayed as described in Methods and materials. Control value of Δ^9-desaturase activity was 0.15 ± 0.02 nmol mg/min (100% activity). The values represent duplicate measurements each from four rats.

Table 1 Effects of fish- and soybean oil diets on hepatic acyl-CoA oxidase- and acyl-CoA dehydrogenase mRNA (n = 4)

Time of treatment (days)	Acyl-CoA oxidase mRNA (%)		Acyl-CoA dehydrogenase mRNA (%)	
	Fish oil	Soybean oil	Fish oil	Soybean oil
0	100	100	100	100
4	91 ± 10	98 ± 5	98 ± 6	92 ± 9
10	92 ± 12	105 ± 4	96 ± 12	92 ± 7

Untreated rats were used as control values (and have been set to 100%). Rats were fed on high fat-diets containing soybean or fish oil (20% by weight). At the times of treatment shown, the rats were killed and livers removed. The amounts of mRNA were measured as described in Methods and materials. The tabulated values represent means derived from measurements with preparations from four rats, with SD indicated. Two measurements were performed per rat. No significant differences between population means were found.

Results

Effects of fish or soybean oil

After 10 days of treatment with high-fat diets marked differences between rats fed on fish- or soybean oil-diets were apparent. Fish oil gave about 85% decrease (*Figure 1a*), whereas the soybean oil diet gave a smaller decrease (about 50%) in Δ^9-desaturase activity (*Figure 1b*). The amount of Δ^9-desaturase mRNA decreased about 90% with fish oil and about 60% with soybean oil diets. After 4 days of treatment with both high-fat diets, there was also a considerable decrease in Δ^9-desaturase activity and amount of -mRNA (*Figure 1a and b*), but the Δ^9-desaturase activity decreased more rapidly than the amount of Δ^9-desaturase mRNA.

In comparison to amounts of Δ^9-desaturase mRNA, no significant effects of 10 days of treatment with these high fat diets on amounts of mRNAs for acyl-CoA oxidase and acyl-CoA dehydrogenase were found (*Table 1*).

Effects of treatment per os with ethyl-docosahexaenoate or ethyl-eicosapentaenoate

Treatment with docosahexaenoate for 10 days resulted in a 70% decrease of Δ^9-desaturase mRNA compared with controls (*Figure 2a*). The decrease after treatment with eicosapentaenoate was lower (50%), whereas palmitate caused a small change in the amount of mRNA. Acyl-CoA oxidase- and acyl-CoA dehydrogenase mRNA's were not affected significantly by the fatty acid treatments (*Figure 2b*), however, a slight increase in the amount of acyl-CoA oxidase mRNA was demonstrated.

Effects of treatment with clofibrate

The activity of Δ^9-desaturase was about 6 times higher in livers from rats treated with clofibrate for 10 days as compared with controls. During this period the amount of mRNA increased steadily to about two-fold (*Figure 3a*). In similar experiments performed in our laboratory up to a five-fold amount of Δ^9-desaturase mRNA was obtained (not shown).

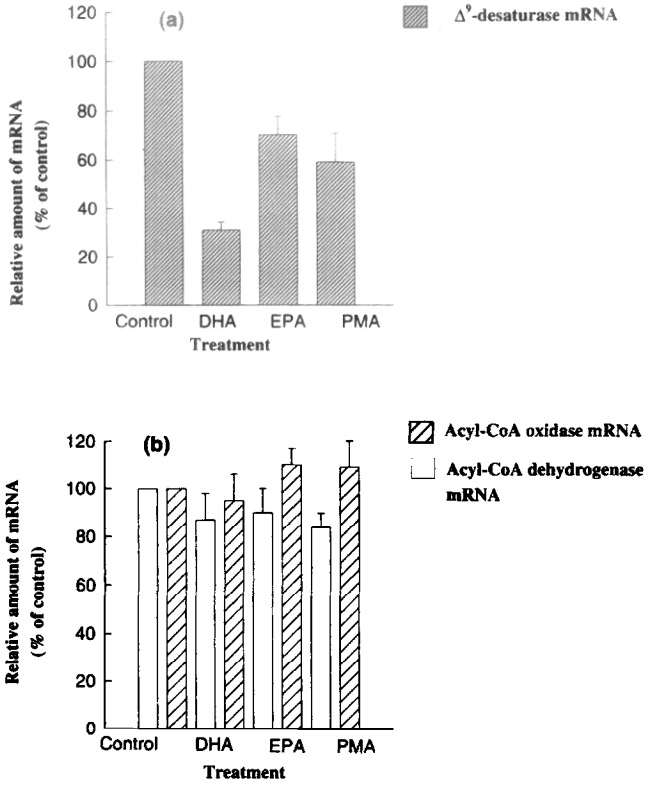

Figure 2 Effects of ethyl-docosahexaenoate (DHA), ethyl-eicosapentaenoate (EPA) and palmitic acid (PMA) on amount of hepatic Δ^9-desaturase mRNA in percent of control values *(a)*, and amounts of hepatic acyl-CoA oxidase and acyl-CoA dehydrogenase mRNA in % of control values *(b)*. Rats were given the fatty acid per os for 10 days as described in Methods and materials. The experimental details are given in Methods and materials. The values represent duplicate measurements each from four to eight rats.

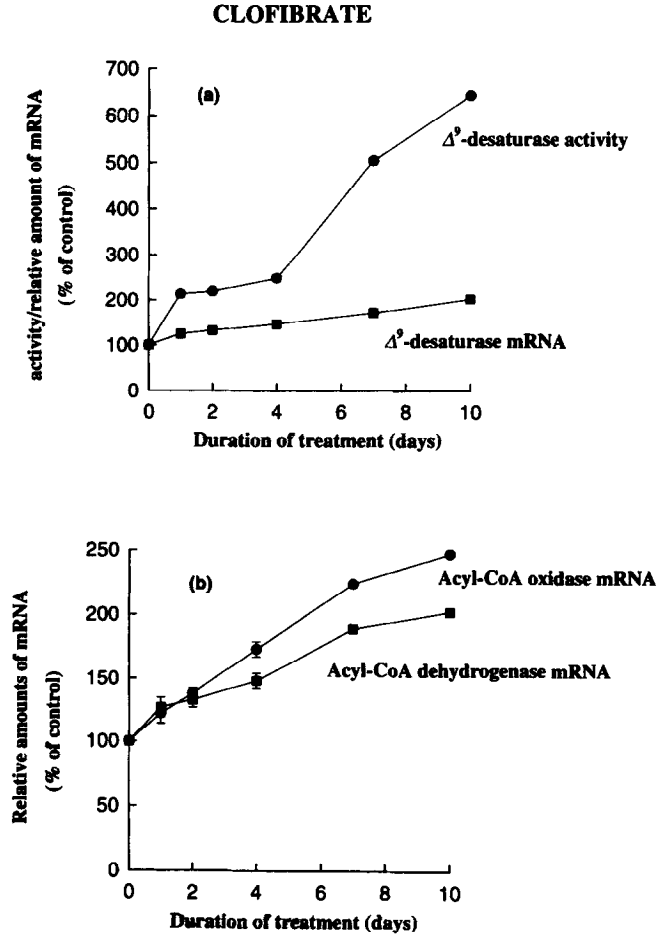

Figure 3 Effects of treatment with clofibrate on hepatic Δ^9-desaturase activity and amount of mRNA *(a)*, and amounts of hepatic acyl-CoA oxidase- and acyl-CoA dehydrogenase mRNA in percent of control values *(b)*. After different times of treatment (shown in the figure) the rats were killed, and hepatic amounts of Δ^9-desaturase mRNA were measured (in percent of controls). After 10 days of treatment the activity of the Δ^9-desaturase was also assayed. The experimental details are given in Methods and materials. Control value of Δ^9-desaturase activity was 0.28 ± 0.03 nmol/mg/min (100% activity). The values represent duplicate measurements each from two rats.

In the same period also the amounts of acyl-CoA oxidase and dehydrogenase mRNA increased considerably; two-and-a-half and two-fold, respectively (*Figure 3b*).

Discussion

This study confirms that hepatic Δ^9-desaturase activity is regulated powerfully at the level of Δ^9-desaturase mRNA. Both Δ^9-desaturase activity and the amount of mRNA have been shown to change during a short timespan, suggesting that the hepatic concentration of this mRNA species is subjected to regulation by high-fat diets and clofibrate.

Our results demonstrate that the hepatic level of Δ^9-desaturase mRNA is influenced by the fatty acid composition of high-fat diets. A potent downregulation was obtained with fish oil, where about 22% of the fatty acids are very long-chain n-3 fatty acids (20:5 and 22:6). In rats given this diet the enzymatic activity was decreased by 85%, and the concentration of Δ^9-desaturase mRNA by 90%. In soybean oil, where about 56% of the fatty acids are 18:2 n-6 and 8% 18:3 n-3, the decrease in enzymatic activity was about 50%, and the amount of mRNA was 60% (*Figure 1*).

The cause of the decreased activity of Δ^9-desaturase by high-fat diets is unclear. Altered physicochemical properties of the microsomal membrane surrounding the Δ^9-desaturase, mediated by changed fatty acid composition have been proposed.[8]

There is strong evidence that regulation by gene expression may explain some of our effects by dietary fats. This has been elaborated in a review by Ntambi,[9] where he describes two different genes for Δ^9-desaturase (stearoyl-CoA desaturase): SCD1 and SCD2. SCD1 is expressed mainly in liver and adipocytes, while SCD2 is not expressed in the liver but in brain and kidney. Long-chain PUFAs and diets containing these fatty acids were shown to inhibit the SCD1 gene. Landschulz[18] studied the SCD1 gene expression in mice with different high-fat diets; cocoa butter and olive oil containing saturated and monounsaturated fatty acids gave high levels of mRNA, whereas safflower oil (rich in linoleic acid) suppressed the gene expression considerably. Trilinolein also suppressed the gene expression considerably and dietary ethyl arachidonate most effectively. They also tested arachidonate and eicosapentaenoate on

hepatocytes in culture and found a considerable suppression of the gene expression with both fatty acids. These data give an explanation to our results with fish oil.

Our results (*Figure 2a*) with respect to eicosapentaenoate are in accordance with the results by Landschulz et al.,[18] and with respect to palmitic acid with the results by Ntambi.[19] Dietary docosahexaenoate, however, has not been studied before, and in our hands seems to be the most suppresive of the PUFAs.

The mechanism for regulation of Δ^9-desaturase is complicated, and for the regulation of expression by fatty acids insulin has also been shown to be necessary.[20,21]

However, we observed a higher extent of inhibition of enzymatic activity as compared with the decline in concentration of Δ^9-desaturase mRNA. This may be taken as evidence suggesting that other regulatory mechanisms are operating, e.g., at the level of translation or mediated by altered membrane fluidity.

Although both dietary fats used in this study were found to have an inhibitory effect on Δ^9-desaturase activity, this does not occur for other desaturases. A high fat-diet, rich in 18:2(n-6) or 18:3(n-3), has been reported to stimulate the activities of Δ^5- and Δ^6-desaturases, whereas a fish oil-diet was inhibitory.[7,22] The study of Δ^6- and Δ^5-desaturases on transcriptional level has not been possible, because the enzyme has still not been purified to a satisfying level,[23] however Brenner suggested in some of his early publications that a feedback inhibition occurred using arachidonic or docosahexaenoic acids[24–26] in enzyme kinetic experiments with the respective desaturase substrates and purified microsomes. Also, Garg et al. suggested a feedback inhibition by fatty acids in fish oil on Δ^6- and Δ^5-desaturase activities.[22]

A clue to gene-regulation of Δ^6- and Δ^5-desaturases has been given by Kawashima et al.,[27] who studied their regulation by clofibric acid. They found a strong stimulation on both desaturases, and inhibition of the stimulatory effect by cycloheximide, a potent inhibitor of protein synthesis. Clofibrate is an agent that generally regulates enzymatic activities on the transcriptional level through a receptor mediated mechanism.[28] Thus these experiments may give an indication to that Δ^6- and Δ^5-desaturases are regulated at the transcriptional level by clofibrate, as well as by high-fat diets as fish oil. In former experiments by Christiansen et al.[7] fish oil was found to have a strong inhibitory effect on Δ^6- and Δ^5-desaturases. Oils rich in linoleic acid (sunflowerseed oil) and α-linolenic acid (linseed oil) had, however, a stimulatory effect on the Δ^6- and Δ^5-desaturase activities.

Contrary to treatment with fish oil, clofibrate was shown to have a strong stimulatory effect both on Δ^9-desaturase activity and on the concentration of Δ^9-desaturase mRNA (*Figure 3a*). Clofibrate is furthermore well known as a peroxisomal proliferator,[32] and does also stimulate several microsomal enzymes, for review; see [4]. Clofibrate dependent stimulation of Δ^9-desaturase activity has been demonstrated previously,[4] and recently its effect on the level of mRNA has been demonstrated.[33] The stimulatory effect on the concentration of mRNA is, however, lower than that on the enzymatic activity. This may again suggest the existence of additional regulatory mechanisms not directly related to factors controlling the level of mRNA, as e.g. stability of membranes.[34]

Diszfalusi et al.[31] have studied Δ^9-desaturase in liver from mice treated with clofibrate using tetradecylthioacetic acid as substrate. In accordance with our results with clofibrate there was a stimulation on the level of synthesis of mRNA, and a stronger effect on desaturase activity. Our responses, however, where stronger both for mRNA and activity which could be an effect of a oligonucleotide-probe used in our experiments, while Diszfalusi et al. used a cDNA-probe. However, Ntambi[33] demonstrated a 14-fold increase in mRNA after 18 h in mouse liver experiments stimulated with clofibrate after a dietary regime including feeding, fasting and refeeding. For the measurement of mRNA he used a RNA probe from SCD 1. The discrepancy in the degree of stimulation may have a number of explanations of which may be mentioned: Cook & Spence[35] demonstrated that the activity of Δ^9-desaturase in rat liver is superinduced after a fasting and subsequent refeeding dietary regime (50-fold above fasted state), diurnal variation may be another explanation, as well as strain of mice, species difference between mice and rats, and amount of clofibrate. In our studies, however, the intention was simply to demonstrate that clofibrate caused an increase in Δ^9-desaturase mRNA. We did not attempt to maximize this response.

Treatment with high-fat diets and clofibrate are known to increase many enzyme activities involved in lipid metabolism (for reviews see [32, 34]). Clofibrate has been shown to increase the levels of mRNA for acyl-CoA oxidase[36,37] in agreement with our data (*Figure 3b*). Treatment with fish oil (*Table 1*) or fatty acids as eicosapentaenoic (EPA) or docosahexaenoic (DHA) acids (*Figure 2b*) did not give any significant increase in mRNA levels for acyl-CoA oxidase and acyl-CoA dehydrogenase. To our knowledge this has not been reported before. An increase in acyl-CoA oxidase activity, however, has been reported with fish oil feeding[38] and after administration of EPA and DHA to rats.[39,40] Partially hydrogenated fish oil, on the other hand, gives a strong stimulation of acyl-CoA oxidase activity as well as amount of mRNA.[41]

In conclusion we have found that in rats dietary fish oil strongly decrease the amount of Δ^9-desaturase-mRNA, in parallell to an almost total loss of Δ^9-desaturase activity. This strong correlation between amount of mRNA and enzyme activity suggests that regulation of activity is exerted either at the level of transcription, or at the level of control of mRNA stability. The most active component in fish oil seems in this respect to be docosahexaenoic acid. Further experiments on the regulatory effects of polyunsaturated fatty acids on the regulation of Δ^9-desaturase are in progress.

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