Nuclear receptors and hepatic lipidogenic enzyme response to a dyslipidemic sucrose-rich diet and its reversal by fish oil n-3 polyunsaturated fatty acids

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A sucrose-rich diet (SRD), compared with a starch diet, induces time-dependent metabolic disorders and insulin resistance with hypertriglyceridemia, similar to type 2 diabetes. In this study, we examined the effect of SRD, after 8 mo, on nuclear receptors peroxisome proliferator-activated receptor-α (PPARα), and liver X receptor-α (LXRα), stearoyl-CoA desaturase-1 (SCD-1), and Δ6 and Δ5 desaturases mRNA and activity, hepatic enzymes involved in lipid metabolism, and fatty acid (FA) composition as well as the reversal produced by cod liver oil. SRD induced triglyceride increase in plasma and liver, increasing the anabolic FA synthase, malic enzyme, and glucose-6-phosphate dehydrogenase, but not the prooxidative enzymes FA oxidase and carnitine palmitoyltransferase I, and correspondingly decreased PPARα and increased LXRα expressions. Results suggest a contribution of both nuclear receptors’ interaction on these enzymatic activities. SRD depressed SCD-1 without altering oleic acid proportion and increased Δ6 and Δ5 desaturases and the proportion of n-6 arachidonic acid. Therefore, the data do not support that SRD hypertriglyceridemia is produced by increased SCD-1-dependent oleic acid biosynthesis. The administration of 7% cod liver oil for 2 mo depressed LXRα, enhancing PPARα in control and SRD-fed rats, reversing the activity of the hepatic enzymes involved in lipid metabolism and therefore the hyperlipidemia produced by the SRD. Fish oil increased n-3 PUFA and depressed n-6 PUFA of liver lipids without altering the 18:1/18:0 ratio, suggesting that its effects were produced mainly by competition of dietary n-6 and n-3 FA and not through desaturase activity modification.

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long-term SRD feeding evokes changes in the mRNA expression and activity of hepatic SCD-1 and Δ6 and Δ5 desaturases that lead to a modification of the proportion of PUFA in liver (7, 40). Therefore, it was important to investigate the extent to which dietary fish oil n-3 FAs’ beneficial effect could be related to changes in desaturase activity. Thus, the present study was conducted in male rats fed a SRD or a starch-rich diet for 8 mo. In the final 2 mo, the source of fat provided by corn oil rich in n-6 FAs was replaced in one-half of the animals of each group by cod liver oil, rich in n-3 FAs. The comparative effect produced was investigated, measuring plasma and liver parameters, mRNA and expression of SCD-1 and Δ6 and Δ5 hepatic desaturases, and FA composition of total liver and endoplasmic reticulum, as well as PPARα and LXRα protein levels, along with the expression of target metabolic enzymes. The study of these combined nuclear receptors’ control is relevant in view of the fact that both are expressed mainly in the liver, but whereas PPARα produces catabolic effects on the FAs, LXRα produces lipidogenic effects, in contrast to LXRβ, which controls cholesterol metabolism.

MATERIALS AND METHODS

Materials. [1-14C]Palmitic acid (57.5 mCi/mmol, 98% radiochemically pure) and [1-14C]linoleic acid (56.0 mCi/mmol, 98% radiochemically pure) were purchased from PerkinElmer (Boston, MA), [1-14C]eicosa-8,11,14-trienoic acid (55 mCi/mmol, 99% radiochemically pure) was provided by American Radiolabeled Chemicals (St. Louis, MO). Unlabeled FAs were provided by Doosan Serdary Research Laboratories (Toronto, ON, Canada), Cofactors were obtained from Sigma Chemical (St. Louis, MO), and solvents for HPLC were purchased from Carlo Erba (Milan, Italy).

Rat cDNAs of SCD-1 (isoform of the Δ9 desaturase), Δ6 desaturase, and Δ5 desaturase were kind gifts from Dr. Juris Ozols (Dept. of Biochemistry, Univ. of Connecticut, Central Health, Farmington, CT), Dr. Tsunehiro Aki (Dept. of Molecular Biotechnology, Hiroshima University, Higashi-Hiroshima, Japan), and Drs. Reza Zolfaghari and A. Catharine Ross (Dept. of Nutritional Sciences, Penn State University, University Park, PA), respectively.

Animal model and diets. Two-month-old male Wistar rats weighing 180–200 g were maintained under controlled temperature (22 ± 1°C) with a fixed 12:12-h light-dark cycle. Animal care followed international rules for experimentation. After 1 wk of acclimation, the rats were randomly distributed into two groups, experimental and control. The experimental group received a purified SRD containing, by weight, 62.5% sucrose, 17% vitamin-free casein, 8% corn oil, 7.5% cellulose, 3.5% salt mixture (AIN-93M-X), 1% vitamin mixture (AIN-93-VX), 0.2% choline, and 0.3% methionine. The control group received the same semisynthetic diet, but sucrose was replaced by starch. The FA composition of both diets (% by weight) was as follows: 10.4% 16:0, 2.2% 18:0, 32.1% 18:1n-9, 51.5% 18:2n-6, 0.4% 18:3n-3, 0.4% 20:0, and 1.6% 20:1n-9.

The experimental group received this diet for 6 mo, after which the rats were distributed into two subgroups. The rats of the first subgroup, sucrose control (Su.c) continued with the SRD diet up to 8 mo. The second subgroup, sucrose fish (Su.f) received the SRD in which the source of fat (corn oil 8/100 g) had been replaced by cod liver oil (7/100 g; ICN Biochemicals, Costa Mesa, CA) plus 1/100 g of corn oil during the last 2 mo on the diet. On the other hand, half of the rats of the starch control group (St.c) continued receiving this diet for 8 mo while the other half, starch fish (St.f), changed the source of dietary fat after 6 mo, during the last 2 mo, replacing the 8/100 g corn oil with 7/100 g cod liver oil plus 1/100 g corn oil.

The FA composition (% by weight) of the diet, containing 1/100 g corn oil and 7/100 g fish oil was 4.1% 14:0, 12.8% 16:0, 9.5% 16:1, 2.8% 18:0, 26.0% 18:1n-9, 9.0% 18:2n-6, 0.9% 20:0, 12.0% 20:1n-9, 3.1% 20:4n-3, 9.0% 20:5n-3, 0.5% 22:5n-3, and 8.1% 22:6n-3.

Diets were isoenergetic, providing ~16.3 kJ/g of food and were available ad libitum. They were freshly prepared every day. Fish oil was kept under nitrogen atmosphere during storage.

After the periods indicated, fed or overnight-fasted animals were killed by decapitation without anesthesia, at the same time, in the morning, to avoid circadian rhythm effects.

Analytic methods. Plasma glucose, cholesterol, and triglycerides were determined as described elsewhere (7, 39). Liver triglyceride content was determined as already indicated (47). Free FAs were measured in the same tissue homogenate following the procedure of Rodgers et al. (51) using a Wako kit (53).

Liver organelle fractionation. After the rats were killed, livers were excised and homogenized in a solution (1:3 wt/vol) composed of 0.25 M sucrose, 0.15M KCl, 9.1 mM EDTA, 1.14 mM Na-acytelycysteine, 5 mM MgCl2, and 62 mM phosphate buffer (pH 7.4). Samples were centrifuged at 10,000 g for 30 min, and postmitochondrial supernatant was used for fatty acyl-CoA oxidase assay. Microsomes were separated by differential ultracentrifugation at 100,000 g (Beckman Ultracentrifuge) as usual. Protein concentration was measured according to the procedure of Lowry et al. (35).

Lipid analysis. Lipids were extracted from liver homogenate and microsomes according to the procedure of Folch et al. (15). Samples were esterified with 1:1B at 64°C for 3 h, and the FA composition of total lipids was determined by gas liquid chromatography of their methyl esters as described previously (39).

Microsomal phosphatidylcholine (PC) was separated from other lipids by high-performance liquid chromatography (HPLC) as described previously (5). The separation of PC molecular species was done using the method of Bowers et al. (8), as already described (5).

Enzymatic activity assays. SCD-1 activity was measured in hepatic microsomes immediately after separation to avoid deactivation through proteolytic hydrolysis. It was estimated using as substrate 30 μM [1-14C]palmitic acid. Δ6 desaturase was measured using 33 μM [1-14C]linoleic acid and Δ5 desaturase by incubation of 40 μM [1-14C]eicosa-8,11,14-trienoic acid. They were incubated with 2.5 mg of microsomal protein respectively in a final volume of 1.5 ml at 36°C for 15 min. The reaction mixture consisted of 0.25 M sucrose, 0.15 M KCl, 1.41 mM Na-acytelycysteine, 40 mM NaF, 60 mM CoA (sodium salt), 1.3 mM ATP, 0.87 mM NADH, 5 mM MgCl2, and potassium phosphate buffer (pH 7.4). The desaturation reaction was stopped with 10% (wt/vol) KOH in ethanol, followed by saponification. The extracted FFA were dissolved in methanol-water-acetic acid (85:15.0:2.0 by vol) and fractionated by RP-HPLC on an Econosil C18 10-mm particle size, reversed-phase column (250 × 4.6 mm; Alltech Associates, Deerfield, IL) coupled to a guard column (10.4 mm) filled with pellicular C18. The mobile phase consisted of methanol-water-acetic acid (90:10:0.2 by vol). The column eluate was monitored by a UV spectrometer at 205 nm for FA identification on the basis of their retention times. The effluent was mixed with Ultima Flo-M scintillation cocktail (Packard Instruments, Downers Grove, IL) at a 1:3 ratio, and the radioactivity was measured by passing the mixture through an on-line Radiomatic Instruments Flo-One-β radioactivity detector (Tampa, FL) fitted with a 0.5-m1 cell. Peroxosomal fatty acid oxidase (FAO) was determined in liver by the procedure reported by Vamecq et al. (58) with the addition of 0.1 g/liter Brij 58 to the reaction mixture according to Yu et al. (68). FA synthase was assayed by the method of Halestrap (18), malic enzyme according to Hsu et al. (21), and carnitine palmitoyltransferase I (CPT I) spectrophotometrically following Karlic et al. (26).

Isolation and analysis of desaturase mRNAs. Total RNA was isolated from liver by use of TRIzol reagent (Invitrogen, Carlsbad, CA). The isolated RNA from the livers of 5 rats in each group (1.5 μg) was separated on 1% agarose-1 M formaldehyde gel electrophoresis to assess the RNA quality. Real-time quantitative PCR analysis was performed using an MX3000 apparatus (Stratagene, La Jolla, CA). The isolated RNA from the livers of 5 rats in each group (1.5 μg) was separated on 1% agarose-1 M formaldehyde gel electrophoresis to assess the RNA quality. Real-time quantitative PCR analysis was performed using an MX3000 apparatus (Stratagene, La Jolla, CA).
Jolla, CA). Total RNA (1 µg) from individual rats was used to generate cDNA by use of the Affinity Script QPCR cDNA kit (Stratagene). Equal amounts of cDNA were used and amplified with Brilliant SYBR Green QPCR Master Mix (Stratagene). Levels of various mRNAs were normalized to those of β-actin. The real-time quantitative PCR primers were as follows: SC-1, 5′-ACATTCATACCTCGGAAGAACA-3′ (forward) and 5′-CCATCGAGTCATGAGAACG-3′ (reverse); ΔΔ desaturase, 5′-TGTCACAAGTGGTACATTGG-3′ (forward) and 5′-ACGTGGCTTCTTTGAAC-3′ (reverse); β-actin, 5′-TGGAGCAGTGCTGAGCGGAGGG-3′ (forward) and 5′-ACTATACGCGCAGCGC-3′ (reverse).

Western blot analysis of liver PPARα. Frozen liver powder (100 mg) was homogenized in a buffer containing 20 mM Tris · HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1% aprotonin, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM benzamidine for 120 min at 4°C (14). The tissue lysates were centrifuged at 10,000 g for 2 h to remove insoluble materials. For Western blot analysis, each sample (50 µg protein/lane) was denatured by boiling for 3 min in Laemmli sample buffer and resolved on 12% SDS-PAGE gels. After electrotransfer (constant 50 V) onto PVDF membranes by electroblotting overnight in a buffer containing 0.1% SDS and 20% methanol, blots were blocked with Tris-buffered saline containing 0.1% Tween-20 and 5% dry milk for 8 h at 4°C. For immunoblotting, the membranes were probed overnight at 4°C with 1:400 specified polyclonal rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The blots were then incubated with horseradish peroxidase-linked secondary antibody (1:2,000) for 2 h at room temperature followed by chemiluminescence detection according to the manufacturer’s instruction (SuperSignal West Pico Chemiluminescent Substrate, Pierce Biotechnology, Rockford, IL). The intensity of the bands was quantified by the National Institutes of Health (Bethesda, MD) imaging software. Preliminary studies showed linearity of Western blot assay from 25 to 100 µg of protein. The correlation coefficient between the amount of protein and the enhanced chemiluminescence image intensity was 0.97 for the PPARα fraction. The relationship between the amount of sample subjected to immunoblotting and the signal intensity observed was linear under the conditions described above. Kidney tissue extract was employed as a PPARα-positive control. Protein concentrations were quantified with the Bio-Rad Protein Assay (14, 41).

LXRα western blot analysis. Total protein samples (70 µg) from liver were analyzed by SDS-PAGE and blotted onto Hybond-ECL nitrocellulose membrane (Amersham Bioscience, Little Chalfont, UK). Membrane was probed with an anti-LXRα antibody (1:200 dilution; Santa Cruz Biotechnology) and then with horseradish peroxidase-conjugated anti-rabbit antibody (1:5,000 dilution; Sigma Chemical, St. Louis, MO) using PBS-T buffer containing 1% and 5% nonfat dry milk for saturation and incubation with antibodies, respectively. Finally, peroxidase activity was revealed using Pierce ELC substrate and quantified using Kodak Digital Science software (Kodak, Rochester, NY).

Statistical analyses. Results are expressed as means ± SD. Data were subjected to ANOVA (Instat v. 2; GraphPad Software, San Diego, CA). The Tukey-Kramer multiple comparison test was used. Differences were considered significant at P < 0.05.

RESULTS

Effect of SRD and fish oil on plasma and hepatic triglyceride levels. In agreement with previous publications (33, 40), plasma triacylglycerols, NEFA, cholesterol, and glucose levels were higher in nonfasted rats fed an SRD (Su.c) for 8 mo compared with age-matched controls fed a starch diet (St.c) (Tables 1 and 2). This was accompanied by a significant increase of liver triglyceride content without changes in plasma insulin levels. When cod liver oil replaced corn oil as dietary source of fat in the SRD-fed rats, a significant reversal of all parameters was observed, reaching values similar to those of the control St.c. The new data show that none of these parameters was decreased in rats fed starch plus fish oil (St.f).

A similar picture with milder changes emerges from overnight fasted Su.c animals fed a SRD (Table 2). However, plasma NEFA were higher than in nonfasted rats. Besides, a significant increase of liver free FAs was observed. The addition of dietary fish oil to the SRD-fed animals normalized both lipids and glucose levels in plasma as well as liver lipid content compared with St.c rats. The behavior of St.f was similar to that observed in the fed state.

Therefore, current results confirm and expand on our previous reports (30, 32) demonstrating that cod liver oil leads to a reversal of the dyslipidemic syndrome found in SRD-fed rats, depressing plasma and hepatic triacylglycerol levels.

Because lipid metabolism may be modulated by a change of the biological activities of PPARα, LXRa, and SREBP-1c, and this change may be produced via the interaction of agonists like free specific PUFA (25, 42, 44), we examined the plasma NEFA composition. We found (data not shown) that, in both control St.c and Su.c fed rats, arachidonic acid was the most abundant (~50%) followed by linoleic acid (~20%). The administration of cod liver oil similarly modified the NEFA composition, decreasing 20:4n-6 and 18:2n-6 to nearly one-half. On the other hand, it enhanced n-3 FAs 20:5n-3 and 22:6n-3 from slightly detectable amounts to ~20 and 2% for EPA and DHA, respectively. Therefore, the change of NEFA from n-6 to n-3 structure could be one of the factors that produced the hypolipidemic effect.

Effects of SRD and fish oil on hepatic enzymes related with lipid metabolism and PPARα and LXRa. The results of these studies are outlined in Table 3 and Figs. 1 and 2. They show in the

<table>
<thead>
<tr>
<th>Table 1. Effect of SRD and fish oil on plasma and hepatic parameters in nonfasted rats</th>
<th>St.c (a)</th>
<th>St.f (b)</th>
<th>Su.c (c)</th>
<th>Su.f (d)</th>
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<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
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<tr>
<td>Insulin, µU/ml</td>
<td>56.1 ± 8.3</td>
<td>35.8 ± 9.4</td>
<td>63.7 ± 10.9 b‡</td>
<td>54.4 ± 4.7 c‡</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>118.6 ± 7.6</td>
<td>113.4 ± 11.6</td>
<td>146.0 ± 6.3 a/b‡</td>
<td>118.4 ± 8.5 c‡</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>84.40 ± 4.81</td>
<td>75.00 ± 7.47</td>
<td>134.70 ± 1.90 a/b‡</td>
<td>80.00 ± 10.82 c‡</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>0.98 ± 0.16</td>
<td>0.73 ± 0.02</td>
<td>2.23 ± 0.22 a/b‡</td>
<td>1.04 ± 0.25 b/c‡</td>
</tr>
<tr>
<td>NEFA, µM</td>
<td>270 ± 25</td>
<td>260 ± 42</td>
<td>728 ± 72 a/b*</td>
<td>307 ± 94 c‡</td>
</tr>
<tr>
<td>Liver triglycerides, µmol/g wet tissue</td>
<td>15.33 ± 0.49</td>
<td>14.86 ± 3.90</td>
<td>24.83 ± 3.88 a/b‡</td>
<td>16.06 ± 0.76 c‡</td>
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</table>

Values are expressed as means ± SD; n = 5. St.c, control starch diet with 8% corn oil (8 mo); St.f, control starch diet with 1% corn oil and 7% fish oil (2 last months); Su.c, sucrose-rich diet (SRD) with 8% corn oil (8 mo); Su.f, sucrose-rich diet with 1% corn oil and 7% fish oil (2 last months). *P < 0.05, †P < 0.01, ‡P < 0.001
Table 2. Effect of SRD and fish oil on plasma and hepatic parameters in fasted rats

<table>
<thead>
<tr>
<th></th>
<th>St.c (a)</th>
<th>St.f (b)</th>
<th>Su.c (c)</th>
<th>Su.f (d)</th>
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<tr>
<td><strong>Plasma</strong></td>
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<tr>
<td>Glucose, mg/dl</td>
<td>91.80 ± 13.97</td>
<td>94.30 ± 9.91</td>
<td>112.00 ± 13.01</td>
<td>85.60 ± 10.51</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>72.40 ± 16.97</td>
<td>64.69 ± 6.51</td>
<td>78.78 ± 7.51</td>
<td>66.93 ± 10.06c</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>0.77 ± 0.07</td>
<td>0.64 ± 0.07</td>
<td>1.34 ± 0.25a<em>b</em></td>
<td>0.87 ± 0.18b<em>c</em></td>
</tr>
<tr>
<td>NEFA, μM</td>
<td>696 ± 130</td>
<td>688 ± 136</td>
<td>882 ± 80 a<em>b</em></td>
<td>634 ± 89 c†</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
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<tr>
<td>Triglycerides, μmol/g wet tissue</td>
<td>13.80 ± 3.76</td>
<td>14.30 ± 1.23</td>
<td>19.70 ± 1.23a</td>
<td>b</td>
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<tr>
<td>Free fatty acid, mg/100 g wet tissue</td>
<td>466.2 ± 34.2</td>
<td>448.2 ± 14.5</td>
<td>532.0 ± 38.2 a</td>
<td>j</td>
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</table>

Values are expressed as means ± SD; n = 5. *P < 0.05, †P < 0.01, ‡P < 0.001.

Table 3. Hepatic enzymatic activities involved in lipid metabolism

<table>
<thead>
<tr>
<th></th>
<th>St.c (a)</th>
<th>St.f (b)</th>
<th>Su.c (c)</th>
<th>Su.f (d)</th>
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<tbody>
<tr>
<td>FA synthase</td>
<td>7.09 ± 0.42</td>
<td>7.80 ± 0.64</td>
<td>18.14 ± 2.25a</td>
<td>7.48 ± 0.58c‡</td>
</tr>
<tr>
<td>Malic enzyme</td>
<td>6.90 ± 0.64</td>
<td>7.60 ± 1.52</td>
<td>12.74 ± 2.23a</td>
<td>8.64 ± 1.10c†</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>22.57 ± 4.53</td>
<td>10.90 ± 3.00a</td>
<td>36.50 ± 3.43a</td>
<td>23.10 ± 4.78b‡</td>
</tr>
<tr>
<td>FA oxidase</td>
<td>2.40 ± 0.42</td>
<td>3.87 ± 0.83a</td>
<td>2.45 ± 0.32</td>
<td>5.33 ± 0.86b*c‡</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase I</td>
<td>1.11 ± 0.27</td>
<td>2.11 ± 0.27a‡</td>
<td>0.69 ± 0.05a‡</td>
<td>1.94 ± 0.27c‡</td>
</tr>
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</table>

Values are means ± SD in mU/mg protein; n = 6. *P < 0.05, †P < 0.01, ‡P < 0.001.
Effect of SRD on the FA composition of liver lipids. As summarized in Tables 5 and 6 the SRD modified the FA composition of total liver lipids and also of the microsomal phospholipids. In the compositions of both total liver and microsomal lipids, palmitic acid proportion was slightly decreased despite fact that the increases of FA synthase, malic enzyme, and glucose-6-phosphate dehydrogenase (Table 3) would suggest an opposite effect. On the other hand, the proportions of monoenoic acids 16:1 and 18:1 were not modified despite the SCD-1 transcription being depressed, as shown before (Fig. 3 and Table 4). Therefore, these results demonstrate that the alteration of the aforementioned enzymatic activity must not be the only factor that can modify the proportion of saturated and monoenoic acids, and the SRD diet would induce compensatory mechanisms that counteract them.

One of these possible mechanisms might be the competition with PUFA for the lipid molecular building. In this respect, the SRD provoked significant increases of 20:4n-6, 22:4n-6, and 22:5n-6 acids at the expense of dietary 18:2n-6 acid, in agreement with the significant increases of Δ6 and Δ5 desaturase mRNA expression and Δ6 desaturase activity (Fig. 3 and Table 4).

However, the proportion of the n-3 FAs 20:5n-3, 22:5n-3, and 22:6n-3 was depressed by the SRD diet. In this particular case, it is important to note that the proportion of the precursor n-6 and n-3 PUFA linoleic acid and α-linolenic, respectively, in the diet was very dissimilar. It was very high for linoleic acid (51.5%) and very low for α-linolenic acid (0.4%). In consequence, the competition of both acids for the Δ6 desaturase would be in favor of linoleic acid desaturation and n-6 PUFA biosynthesis.

Effect of fish oil on FA composition of liver lipids. Incorporation of cod liver oil into the diets produced remarkable changes in the composition of the liver lipids n-6 and n-3 PUFA in both starch- and sucrose-fed rat (Tables 5 and 6).

The fish oil, as expected, increased the proportion of all the n-3 PUFA, 20:5n-3, 22:5n-3, and 22:6n-3 in liver tissue. However, although the main components of cod liver oil, 20:5n-3 (9.0%) and 22:6n-3 (8.1%), were equivalently provided by the diet, the highest percentage increase (~7-fold) for the starch-fed and more than 60-fold for the SRD-fed rats was found in the eicosapentaenoic acid (20:5n-3). On the other hand, only ~2-fold and ~4-fold increases were found for the 22:5n-3 and 22:6n-3 acids, respectively. Nevertheless, the amount of 22:6n-3 acid incorporated into either starch-fed or sucrose-fed rats approximately doubled the 20:5n-3 incorporation in the lipids.

On the other hand, the proportion of n-6 high PUFA was strongly depressed in the liver lipids of both sucrose- and starch-fed animals by the administration of fish oil. Arachidonic acid, the main n-6 FA of liver lipids, was depressed to approximately one-half in the SRD-fed rats, whereas the starch-fed animals showed a lesser depression. The minor n-6 PUFA components 22:4n-6 and 22:5n-6 were also reduced in quite similar proportions.

In view of that the fact that these n-6 PUFA are not directly supplied by the diet and their presence in liver lipids is due to

**Fig. 1.** Liver protein mass expression of peroxisome proliferator-activated receptor-α (PPARα). St.c, control starch diet with 8% corn oil (8 mo); St.f, control starch diet with 1% corn oil and 7% fish oil (2 last mo); Su.c, sucrose-rich diet with 8% corn oil (8 mo); Su.f, sucrose-rich diet with 1% corn oil and 7% fish oil (2 last mo). Top: immunoblots of PPARα. Molecular marker is shown on the right. Lane 1, kidney tissue as a positive control; lane 2, St.c; lane 3, St.f; lane 4, Su.c; lane 5, Su.f. Bottom: densitometric immunoblot analysis of PPARα protein mass in liver tissue of starch-fed control (St.c), starch-fed + fish oil (St.f), sucrose-fed control (Su.c), and sucrose-fed + fish oil (Su.f) rats. Values are means ± SD (n = 5) expressed relative to control diet. *P < 0.05, **P < 0.01.

**Fig. 2.** Relative hepatic liver X receptor-α (LXRα) expression (arbitrary units) determined by Western blot analysis. Legends as in Table 1. Results are means ± SD (n = 3). *P < 0.05, **P < 0.01.
a biosynthetic process from dietary linoleic acid, the decrease found must be ascribed in first place to a lesser linoleic acid provision by the diet. This effect is clearly proved by comparing the linoleic acid proportion in the food of animals fed and not fed fish oil (9.0 and 51.5%, respectively). On the other hand, the contribution of a depression of Δ6 and Δ5 desaturase activities, which might evoke a similar decrease, is not supported by the current data, because neither the Δ6 nor the Δ5 desaturase activity was depressed by the fish oil administration to the starch- and sucrose-fed animals (Table 3), although the Δ6 and Δ5 desaturase mRNAs were lowered in the Su.f animals.

The displacement of n-6 PUFA by n-3 PUFA through a mechanism of direct competition is, on the other hand, highly supported. In this respect, Table 7 shows the effect produced by fish oil feeding on the microsomal PC molecular species composition and distribution. The main microsomal PC molecular species in St.c and Su.c rats were the 16:0/20:4 and 18:0/20:4 species, and these species were significantly reduced by the fish oil administration. On the other hand, the 16:0/22:6 species was highly enhanced, showing a competitive displacement of the 20:4n-6 by the 22:6n-3 acid in the corresponding phospholipid molecules having palmitic acid bound to carbon-1. Therefore, the main mechanism by which fish oil administration modified the PUFA composition of liver lipids in either starch or sucrose fed rats is the relative proportion in the diet and the corresponding competition between n-6 and n-3 PUFAs.

The fish oil diet, in addition to the relevant modification provoked on the PUFA n-6/n-3 ratio in liver lipids, also evoked a small increase in the proportion of 16:0 FA in the lipids of either starch-fed or sucrose-fed rats (Tables 5 and 6). This specific increase of palmitic acid in the fish oil-fed rats is difficult to explain in that its proportions in the corn oil diet or fish oil diet were very similar (~11%). Moreover, the enzymes involved in de novo FA synthesis, FA synthase, malic enzyme, and glucose-6-phosphate dehydrogenase were depressed by the fish oil addition (Table 3). A possible explanation might be less β-oxidation due to its incorporation in new 22:6n-3/16:0 PC molecular species (Table 7) provoked by the increase of 22:6n-3 provision. This molecular species would endow membranes with new and special properties (59).

Table 4. Effect on liver microsomal desaturase activities

<table>
<thead>
<tr>
<th></th>
<th>St.c (a)</th>
<th>St.f (b)</th>
<th>Su.c (c)</th>
<th>Su.f (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD-1</td>
<td>0.223 ± 0.038</td>
<td>0.081 ± 0.018 a‡</td>
<td>0.158 ± 0.038</td>
<td>0.172 ± 0.023 b†</td>
</tr>
<tr>
<td>Δ6 desaturase</td>
<td>0.085 ± 0.023</td>
<td>0.128 ± 0.023</td>
<td>0.221 ± 0.038 a‡</td>
<td>0.171 ± 0.022</td>
</tr>
<tr>
<td>Δ5 desaturase</td>
<td>0.097 ± 0.013</td>
<td>0.080 ± 0.014</td>
<td>0.118 ± 0.020</td>
<td>0.115 ± 0.012 b*</td>
</tr>
</tbody>
</table>

Results are means ± SD in nmol/min·mg protein; n = 4 or 5 animals. *P < 0.05, †P < 0.01, ‡P < 0.001.
Effect of SRD diet. Current data as well as earlier publications (7, 17, 40) clearly demonstrate that long-term SRD administration leads rats to a stable hypertriglyceridemia, high NEFA levels, normoinsulinemia, and insulin resistance (9, 56, 47, 31) together with increases of triglycerides and FFAs in liver. The plasma NEFA were increased in both fasted and nonfasted rats (Tables 1 and 2). Therefore, this increase cannot be produced exclusively by an enhanced adipose tissue lipolysis provoked by fasting but additionally by other mechanisms.

In addition (7), FFAs and PPARs are both involved in insulin resistance, whereas insulin, glucocorticoids, and NEFA regulate PPAR expression (12). Besides, data clearly prove that SRD produces an activation of hepatic anabolic enzymes involved in FA biosynthesis like FA synthase, malic enzyme, and glucose-6-phosphate dehydrogenase, a decrease of the mitochondrial catalytic enzyme CPT I, and no modification of the peroxisomal FAO favoring the lipidogenic state.

It is well known also that, in opposition to the PPARγ isotype, which is highly expressed in adipose tissue but not in liver (40) and directs the expression of genes involved in adipocyte differentiation and in fat and glucose storage (3), PPARα is well expressed in liver and targets genes involved mainly in mitochondrial, peroxisomal, and microsomal FA oxidation (11, 12). Therefore, since the present results demonstrate that SRD produces a significant decrease of PPARα protein level, depression of CPT I, and no activation of FAO, being both target enzymes of PPARα with identified PPAR response elements (12), these results suggest that the SRD-dependent lipidogenesis is at least partially associated with a PPARα decline.

Consistent with these results, Nagai et al. (41) found that a fructose-rich diet also produced in the rat a decline of PPARα protein and activity, reducing FA oxidation. This effect was reversed by fenofibrate, which is known to be a xenobiotic agonist of this nuclear receptor. Similarly, Qu et al. (48) showed that in fructose-fed hamsters fenofibrate-PPARα activation also antagonized forhead box 01 (Fox01) in hepatic apolipoprotein CIII expression, which led to augmented triglyceride metabolism ameliorating the hypertriglyceridemia.

However, SRD increased both plasma and liver NEFA in fasted and fed rats (55), and these NEFA showed a high proportion on unsaturated n-6 FAs. Therefore, since these PUFAs particularly bind to the ligand-dependent activation domain (LBD) of PPARα (12, 42), it would be expected to provoke its activation, which was not found here. Nevertheless, this dilemma could be explained by considering new findings by Sanderson et al. (52). Taking advantage of a transgenic mouse model displaying a gradient in plasma FFAs, in combination with a PPARα−/− mouse model, they revealed that 1) PPARα really does not mediate the effect of plasma FFAs on hepatic gene expression during fasting; 2) induction of classical PPARα target genes during fasting would be likely related to upregulation of the PPARγ coactivator PGC1α; 3) dietary FAs, unlike plasma endogenous FFAs, were able to ligand-activate PPARα in liver. Therefore, those authors proposed that in liver FFAs are differently activated according to the origin of the pool of lipids. That is, the effect of agonists would be dependent on and could be modified by a compartmentalization effect. Besides, not only the type of unsaturated FA or fatty acyl-CoA but their binding to the liver FA-binding
protein (54, 61) L-FABP, their entry to the nucleus and corresponding channeling to the PPARα could also determine their effect.

Moreover, we found that LXR expression is enhanced in vivo by the SRD, corresponding to an increase of NEFA. But it was shown by Paward et al. (45) in an in vitro study using HEK 293-E and HEK 293-L cells that the addition of unsaturated FAs depressed the oxysterol-dependent upregulation of LXRα activity. Therefore, the SRD-dependent increase of NEFA at first instance, considering the results of Paward et al., would lead to a decrease of LXRs activity and not to an enhancement as found by us. Anyway, Figure 2 shows an SRD-dependent enhancement of LXRα protein that correlates with a FA synthase increase.

Therefore, the same paradigm is shown to exist for LXRα as for PPARα response to the endogenous NEFA produced by the SRD. However, other factors are altered by the SRD that might exert an increase of LXRα expression. For example, Mitro et al. (36) found that glucose and D-glucose-6-phosphate are direct agonists of hepatic LXRα with an efficacy similar to that of oxysterol.

However, the increase of lipogenic LXRα expression and decrease of PPARα evoked simultaneously by the SRD might also be attributed to the heterodimerization with RXR (22, 23). Moreover, they may even form LXRs-PPARα heterodimers (4, 37, 62), which may bind to overlapping sequences in enzyme genes, changing the activity of these enzymes. On the other hand, Joseph et al. (24) proved that activation of LXRs in vivo induced the expression of target genes of FA synthase, directly or indirectly, through the SREBP-1c pathway, evoking a lipogenic effect similar to that shown in the present work.

In addition to these results, we have also found that SRD depresses the hepatic expression of SCD-1 mRNA and activity (Fig. 3, Table 4, and Ref. 40). This enzyme is the rate-limiting enzyme of hepatic desaturation of palmitic and stearic acids to palmitoleic and oleic acids, respectively. Therefore, it may modulate the 18:1/18:0 ratio in the cells and specially the fluidity of membranes. Moreover, Dobrzens and Ntambi have just forwarded, based on experimental data (13), that many of the factors that elevate triglyceride levels and provoke lipogenesis do so by converging upon the up regulation of SCD activity and oleic acid biosynthesis. Besides, they consider that this effect is not produced by dietary oleic acid, and Legrand et al. (30) had already shown that the inhibition of Δ9 desaturase activity impairs triglyceride secretion in cultured chicken hepatocytes. In this respect, Miyazaki et al. (38) formally proposed that a high sucrose (55%) lipogenic diet requires an endogenous oleic acid biosynthesis increase, through the SCD-1 enzyme, to induce the triglycerides’ synthesis and their increase. However, in spite of that, our current and previous results (40) show that SRD provoked an up regulated hypertriglyceridemia and hepatic steatotare, correlated to a decreased SCD-1 activity and endogenous oleic acid biosynthesis.

Therefore, in the present experimental conditions, the hypertriglyceridemia is produced by a mechanism independent of SCD-1 activation and different from that described by Ntambi’s group. Thus, we consider that this important problem is not yet settled and it still needs careful analysis, and study in which the interactions of diets, hormones, nuclear receptors, genes, and enzymatic reactions involved in lipid metabolism must be taken into account.

While the SRD, in general, depressed monoenoic FA biosynthesis, it enhanced PUFA biosynthesis by upregulation of one or both Δ6 and Δ5 desaturases, as shown in present and previous results (40). In this way, all the n-6 PUFA of 20 and 22 carbons, 20:4 and 22:5, were enhanced in total liver and microsomal membrane lipids, and these increases were produced mainly in the 16:0/20:4n-6 and 18:0/20:4n-6 PC molecular species that selectively incorporate arachidonic acid.

Hepatic Δ6 and Δ5 desaturases are known to be upregulated by the effects of SREBP-1c, PPARα, and LXRα in the presence or absence of insulin (4, 39). But in contrast, the present findings show that PPARα amount and activity were found to be depressed by SRD, whereas LXRα was increased despite the fact that both of them are activators of Δ6 and Δ5 desaturating enzymes (40). The possible explanation for these anomalous results could be, as discussed before, the existence of the recognized competition between PPARα and LXRα in their heterodimerization with RXR and activation that would produce compensatory effects (22).

Effect of fish oil. The replacement of dietary n-6 linoleic acid by n-3 PUFA from fish oil in the SRD, which has been shown to reduce dyslipidemia and improve insulin action (32, 33), is herein corroborated. Therefore, since SRD evokes a similar dyslipidemic syndrome to that found in type 2 diabetes, the administration of fish oil, rich in n-3 PUFA, is a promising natural treatment for the reversal of those symptoms of the illness. These effects and reversal of hypertriglyceridemia and high intrahepatic lipid content are coincident and would be related to the reported normalization of the hyperactivated lipogenic enzymes and the activation of CPT I, the major rate-limiting enzyme in mitochondrial FA oxidation. Moreover, fish oil also activated the peroxisomal FAO, indicating an increase of the hepatic PPARα activity since it was shown (49) that fish oil-dependent activation of the FAO is not operative in PPARα knockout mice. This increase of PPARα activity by n-3 PUFA, also shown by others (42), would be, in consequence, the cause of the activation of CPT I and FAO.

On the other hand, FA synthase can be downregulated by fish oil through a PPARα-independent mechanism (49). But fish oil feeding also decreased mature SREBP-1 by downregu-

### Table 7. Main molecular species of phosphatidylcholine microsomes

<table>
<thead>
<tr>
<th>Molecular Species</th>
<th>Stc (a)</th>
<th>Stf (b)</th>
<th>Su.c (c)</th>
<th>Su.f (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0/22:6</td>
<td>8.20 ± 0.68</td>
<td>17.36 ± 2.78</td>
<td>50.67 ± 5.48</td>
<td>15.05 ± 2.01</td>
</tr>
<tr>
<td>16:0/20:4</td>
<td>37.38 ± 1.93</td>
<td>28.46 ± 3.02</td>
<td>19.59 ± 3.38</td>
<td>14.25 ± 8.93</td>
</tr>
<tr>
<td>16:0/18:2</td>
<td>13.80 ± 3.66</td>
<td>21.92 ± 5.64</td>
<td>26.40 ± 2.43</td>
<td>26.40 ± 2.43</td>
</tr>
<tr>
<td>18:0/20:4</td>
<td>24.97 ± 8.47</td>
<td>18.49 ± 5.14</td>
<td>12.73 ± 2.32</td>
<td>12.73 ± 2.32</td>
</tr>
<tr>
<td>18:0/18:2</td>
<td>9.79 ± 3.64</td>
<td>13.76 ± 2.73</td>
<td>12.39 ± 1.61</td>
<td>12.39 ± 1.61</td>
</tr>
</tbody>
</table>

Results are means of 4 or 5 animals ± SD in wt%. Main molecular species only considered. *P < 0.05, †P < 0.01, ‡P < 0.001.
loration of SREBP-1c mRNA (28), thus downregulating the liver SRE-dependent expression of FA synthase shown in Table 3. Moreover, Ou et al. (44) found that unsaturated FA-dependent depression of SREBP-1c mRNA level is produced because these FAs competitively block the activation of LXR, which stimulates per se SREBP-1c expression. Currently, we have shown in Fig. 2 that the addition of fish oil to the diet of sucrose-fed rats but not of starch-fed animals depressed LXRα protein and that this depression could produce the decrease of FA synthase through the SREBP-1c pathway. Correspondingly, we have proved (39) that T090137, a xenobiotic agonist of LXRα, stimulates the expression of SREBP-1 and enhances nSREBP-1 level independently or linked to an insulin-dependent mechanism.

The results reported in Fig. 3 and Table 4 also imply that the n-3 PUFA of fish oil did not exert a widespread effect on the desaturase expression and activity of SRD-fed rats; even the decrease of the the mRNA expression of SCD-1 was not followed by a modification of oleic acid proportion nor of 18:1/18:0 ratio in liver lipids. In the starch-fed animals, nevertheless, depression of mRNA and activity of SCD-1 was shown; however, it was unable to modify the oleic acid proportion and 18:1/18:0 ratio in liver lipids.

Therefore, the remarkable depression of all n-6 PUFA induced by fish oil in both starch- and sucrose-fed rat liver lipids is apparently a consequence of the lower linoleic acid provision in their diets and, therefore, less conversion to high n-6 PUFA. This conclusion is convergent with results of Christiansen et al. (10), who measured the activities of hepatic desaturases after feeding the rats for a short period of time with diets containing different PUFAs including n-3 FA.

Dietary fish oil, as expected, produced relevant increases of 20:5n-3 and 22:6n-3 FAs in both starch- and sucrose-fed rats, modifying PC molecular species in liver membrane. These changes imply modifications in membrane structure and functions (59). It also depressed prostaglandin synthesis and effects counteracting arachidonic acid-derived prostanoid biosynthesis started by cyclooxygenases. Therefore, it depressed trobomaxane levels, antagonizing inflammatory and atheromathous reactions, respectively, as well as leukotriene formation. The relevance of this mechanism to fructose-induced hypertriglyceridermia was revealed by Kelley et al. (27), showing that lipoxigenase/cyclooxygenase inhibitor administration overcomes the high-fructose feeding-dependent depression of PPARα expression and restores the hepatic PPARα RNA level, normalizing the hypertriglyceridermia.

Conclusions

This research demonstrates the importance of SRD-fed rats as an experimental model that mimics the type 2 diabetes dyslipidemic syndrome and is helpful to elucidate the mechanism, causes, and development of this physiological alteration as well as possible ways to reverse it. The importance of PPARα and LXRα activity modification evoked by the SRD on the lipidogenic effects produced in liver by the corresponding targeted enzymes with identified response elements is well established. However, the discovery of novel putative target genes of PPARα involved in lipid metabolism (49), in addition to the classic ones that modulate mouse or human FA activation, oxidation, binding, and desaturation, as well as the recognition that dietary vs. endogenous FAs are differently sensed by PPARα (52), determines the necessity of further research. Moreover, the existence of compartmentalized pathways as suggested by Sanderson et al. (52) might explain the apparent inconsistency of some results coming from different authors and experimental conditions.

Specifically, the role played by SRD in the increase of plasma and hepatic triacylglycerol levels, attributed by Dobrzyn and Ntambi (13) to an upregulated SCD-1 that increased endogenous oleic acid, is not confirmed in the present work, demonstrating again the necessity to look for other causes, here suggested, but also to find out the reason for the discrepancy. Fish oil on the other hand, due to its high content of n-3 20:5 and 22:6 FAs (1) and the combined effect of n-6/n-3 PUFA ratio depression, is demonstrated to be a promising natural treatment to reverse the dyslipidemic syndrome found in type 2 diabetes and its pathophysiological effects.

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GRANTS

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REFERENCES


