Studies on the metabolic fate of n-3 polyunsaturated fatty acids

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Abstract Several different processes involved in the metabolic fate of docosahexaenoic acid (DHA, C22:6n-3) and its precursor in the biosynthesis route, C24:6n-3, were studied. In cultured skin fibroblasts, the oxidation rate of [1-^{14}C] 24:6n-3 was 2.7 times higher than for [1-^{14}C]22:6n-3, whereas [1-^{14}C]22:6n-3 was incorporated 7 times faster into different lipid classes than was [1-^{14}C]24:6n-3. When determining the peroxisomal acyl-CoA oxidase activity, similar specific activities for C22:6(n-3)-CoA and C24:6(n-3)-CoA were found in mouse kidney peroxisomes. Thioesterase activity was measured for both substrates in mouse kidney peroxisomes as well as mitochondria, and C22:6(n-3)-CoA was hydrolyzed 1.7 times faster than C24:6(n-3)-CoA. These results imply that the preferred metabolic fate of C24:6(n-3)-CoA, after its synthesis in the endoplasmic reticulum (ER), is to move to the peroxisome, where it is β-oxidized, producing C22:6(n-3)-CoA. This DHA-CoA then preferentially moves back, probably as free fatty acid, to the ER, where it is incorporated into membrane lipids.—Ferdinandusse, S., S. Denis, G. Dacremont, and R. J. A. Wanders. **Studies on the metabolic fate of n-3 polyunsaturated fatty acids. *J. Lipid Res.* 2003.** 44: **1992–1997.**

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N-3 polyunsaturated fatty acids (PUFAs) are involved in diverse physiological processes, such as visual and cognitive functions and neuronal development. N-3 PUFAs are important components of phospholipids in membranes and thereby influence the structure and functioning of membranes. In addition, n-3 PUFAs are substrates for eicosanoid synthesis, but they also influence eicosanoid signaling and affect gene expression (1). Docosahexaenoic acid (DHA) (C22:6n-3) is the most important n-3 PUFA and is the major PUFA in adult mammalian brain and retina. A deficiency of DHA can lead to memory loss, learning disabilities, and impaired visual acuity (1).

It was originally thought that formation of DHA from dietary linolenic acid (C18:3n-3) occurred exclusively in the endoplasmic reticulum (ER) via a series of elongation and desaturation reactions. This pathway required that C22:5n-3 become desaturated at position 4 by a microsomal acyl-CoA-dependent Δ4-desaturase to form C22:6n-3. Several studies in the past few years, however, have indicated that such a Δ4-desaturase does not exist (2–4). Instead, it was found that a 24-carbon n-3 fatty acid is synthesized, which is desaturated at position 6 to produce C24:6n-3, followed by one round of β-oxidation in the peroxisome, with C22:6n-3 as the final product (5–7).

The involvement of two different organelles in the biosynthesis of DHA implies that intracellular movement of fatty acids occurs between the ER and the peroxisome in this biosynthesis route (8). The direct precursor of DHA, C24:6n-3, after synthesis in the ER, has to move to the peroxisome to be β-oxidized to form DHA. Because DHA is the most abundant n-3 PUFA in most tissues and is found at the *sn*-2 position of phospholipids, DHA has to move back to the ER to be acylated into phospholipids instead of being further β-oxidized in the peroxisome (see **Fig. 1**). This means that the biosynthesis of DHA is compartmentalized and must be strictly regulated. It has been hypothesized that competition between peroxisomal β-oxidation and ER-associated acylation reactions plays a role in this regulation and therefore also in regulating membrane lipid fatty acid composition (8, 9).

In this study, we have investigated several different processes involved in the metabolic fate of C24:6n-3 and C22:6n-3. We have measured β-oxidation with both substrates in fibroblasts from patients with different peroxisomal and mitochondrial fatty acid oxidation defects and, for comparison, we performed studies of incorporation into different lipid classes with these substrates. In addition, we studied acyl-CoA oxidase activities in isolated peroxisomes for C24:6n-3 and C22:6n-3, because the acyl-CoA oxidases are

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Abbreviations: CACT, carnitine acyl-carnitine translocator; D-BP, D-bifunctional protein; DHA, docosahexaenoic acid; MTP, mitochondrial trifunctional protein; PBD, peroxisome biogenesis disorder; TE, thioesterase.

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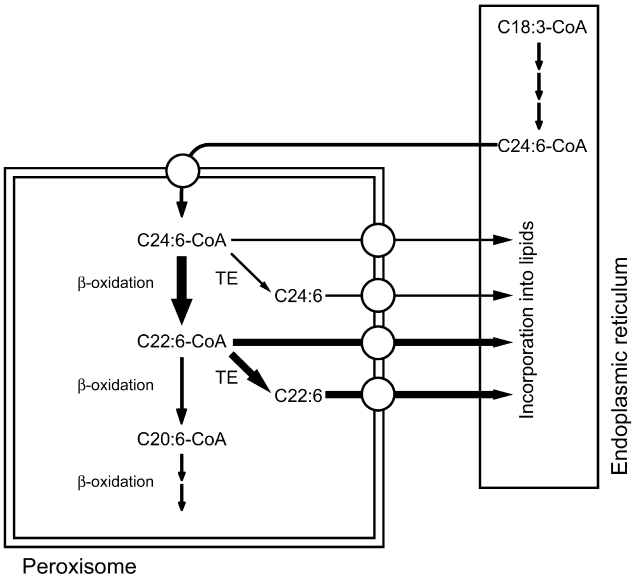

Fig. 1. The intracellular movement of n-3 PUFAs and their metabolism. TE, thioesterase.

considered to be the rate-limiting enzymes of the β-oxidation system. Finally, because it is not known whether C24:6n-3 and C22:6n-3 are transported from the ER to the peroxisome and vice versa as CoA-esters or as free fatty acids, we also studied thioesterase (TE) activities for both substrates.

MATERIALS AND METHODS

Materials

Radiolabeled [1-^{14}C]22:6n-3 was purchased from New England Nuclear (DuPont, Boston, MA).

Synthesis of 6,9,12,15,18,21-C24: 6n-3, its CoA-ester, and [1-^{14}C]6,9,12,15,18,21-C24:6n-3

6,9,12,15,18,21-C24:6n-3 was prepared from 4,7,10,13,16,19-C22:6n-3 (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) by two sequential one-carbon elongation reactions. C22:6n-3 was first converted to its corresponding methylester in hexane-methanol (3:1; v/v) by adding 2 equivalents of (trimethylsilyl)diazomethane. After removal of the solvent, the crude methylester was dissolved in ether and refluxed for 2 h with 3 equivalents of lithium aluminium hydride to yield docosa-4,7,10,13,16,19-hexaen-ol. After destruction of the excess lithium aluminium hydride with ammonia, the alcohol was extracted with hexane and taken to dryness. The alcohol was dissolved in dichloromethane and converted to its mesylate by stirring with 1.25 equivalents of methane sulphonyl chloride for 16 h at room temperature. After purification of the mesylate by silica gel column chromatography with dichloromethane as eluent, the mesylate was converted into its corresponding nitrile with 1.2 equivalents of potassium cyanide in dimethylsulphoxide at 70° for 16 h. The nitrile was extracted with hexane, taken to dryness, and hydrolyzed in ethanol-10% aqueous sodium hydroxide (2:1; w/v) at 80° for 16 h to yield 5,8,11,14,17,20-C23:6n-3, which was subsequently purified by silica gel column chromatography with hexane-ether-acetic acid (60:40:1; v/v/v) as eluent. This intermediate was then converted to C24:6n-3 following the same sequence of reactions involving esterification, reduction, mesylation, displacement with potassium cyanide, hydrolysis, and final purification. Gas liquid chromatography-mass spectrometry (GC-MS) analysis of the methylester of the purified C24:6n-3 showed one homogeneous peak with a molecular ion at *m/z* 356 (M^+ 4.7%). The CoA thioesters of C22:6n-3 and C24:6n-3 were chemically synthesized by the method described by Rasmussen, Borchers, and Knudsen (10).

[1-^{14}C]6,9,12,15,18,21-C24:6n-3 was prepared from the methane sulphonate of 5,8,11,14,17,20-C23:6n-3 obtained after the esterification, reduction, and mesylation of C23:6n-3. This sulphonate (9.5 mg) was reacted with [^{14}C]KCN (1 mCi, 55 mCi/mmol) in dimethylsulphoxide at 70° for 32 h, and the resulting 1-^{14}C-labeled nitrile was extracted with hexane. After hydrolysis of the nitrile to its corresponding carboxylic acid, [1-^{14}C]24:6n-3 was purified on a small silica gel column with hexane-ether-acetic acid (60:40:1; v/v/v) as eluent. Thin-layer chromatography of the purified acid showed only one labeled band, and GC-MS analysis of the methyl ester showed one single peak with a molecular ion at *m/z* 367 ([C^{14}]M^+ 4.5%) and 365 ([C^{12}]M^+ 0.5%). The synthesized [1-^{14}C]24:6n-3 had a specific activity of 50 mCi/mmol.

Patient cell lines

Cell lines were used from several patients suffering from various peroxisomal and mitochondrial fatty acid β-oxidation disorders. Fibroblasts were used from seven patients with a peroxisome biogenesis disorder (PBD), four patients with a deficiency of peroxisomal D-bifunctional protein (D-BP), eight patients with a mitochondrial carnitine acyl-carnitine translocator (CACT) deficiency, and four patients with a deficiency of mitochondrial trifunctional protein (MTP). The patients with a PBD all had the clinical and biochemical abnormalities described for patients with a PBD, including deficient C26:0, pristanic acid β-oxidation, phytanic acid α-oxidation, and the absence of peroxisomes, as assessed by immunofluorescence microscopy analysis using antibodies against catalase (11). The D-BP-deficient patients all had mutations in the encoding gene, and no enzyme activity could be measured in the fibroblasts of these patients (12–14). The fibroblasts from patients with a mitochondrial β-oxidation disorder used in this study were from patients with a confirmed deficiency of CACT or MTP due to mutations in the encoding genes [see (15, 16) for review]. These mutations result in a deficiency of mitochondrial fatty acid oxidation, as established by individual enzyme activity measurements in cultured skin fibroblasts.

All patient cell lines used in this study were taken from the cell repository of the Laboratory for Genetic Metabolic Diseases, University of Amsterdam, Academic Medical Center, and were derived from patients diagnosed in this center. Informed consent was obtained from parents or guardians of the patients whose fibroblasts were studied in this paper.

Fatty acid oxidation in cultured skin fibroblasts

C22:6n-3 and C24:6n-3 β-oxidation was measured as described previously (17).

Subcellular fractionation of mouse kidney

Kidneys from 10 male C57BL/6J mice were homogenized in 250 mM sucrose, 5 mM MOPS, and 0.1 mM EGTA (final pH, 7.4). A postnuclear supernatant was produced by centrifugation of the homogenate at 600 *g* for 10 min at 4°C and was subjected to differential centrifugation as described previously (18). The light mitochondrial fraction, enriched in peroxisomes and lysosomes, was subfractionated by equilibrium density gradient centrifugation in a linear Nycodenz gradient as described previously (19). The following enzymes were used as marker enzymes for the different subcellular compartments and were measured as described: catalase for peroxisomes (20); glutamate dehydrogenase for mito-

chondria (21); esterase for microsomes (18); β-hexosaminidase for lysosomes (18); and phosphoglucose isomerase for cytosol (22).

Acyl-CoA oxidase activity measurements

Acyl-CoA oxidase activity measurements were performed in the peroxisomal fraction (fraction 3; see **Fig. 2**) of the density gradient essentially as described previously (23). H_2O_2 production by the action of the acyl-CoA oxidases was measured by fluorometric quantitation of H_2O_2 using homovanillic acid and horseradish peroxidase. Measurements were carried out in the following incubation mixture: 50 mM MOPS-NaOH (pH 7.6), 1 mM homovanillic acid, 18 U/ml horseradish peroxidase, 0.1 mM NaN_3, 0.01 mM flavin adenine dinucleotide, and 5 μM BSA. The reactions were started by addition of 20 μM C22:6(n-3)-CoA or C24:6(n-3)-CoA. Fluorescence was followed at 30 s intervals for 10 min using a Cobas Bio Centrifugal Analyzer (excitation wavelength: 327 nm, emission filter: 410–490 nm; Hoffmann-La Roche, Basel, Switzerland).

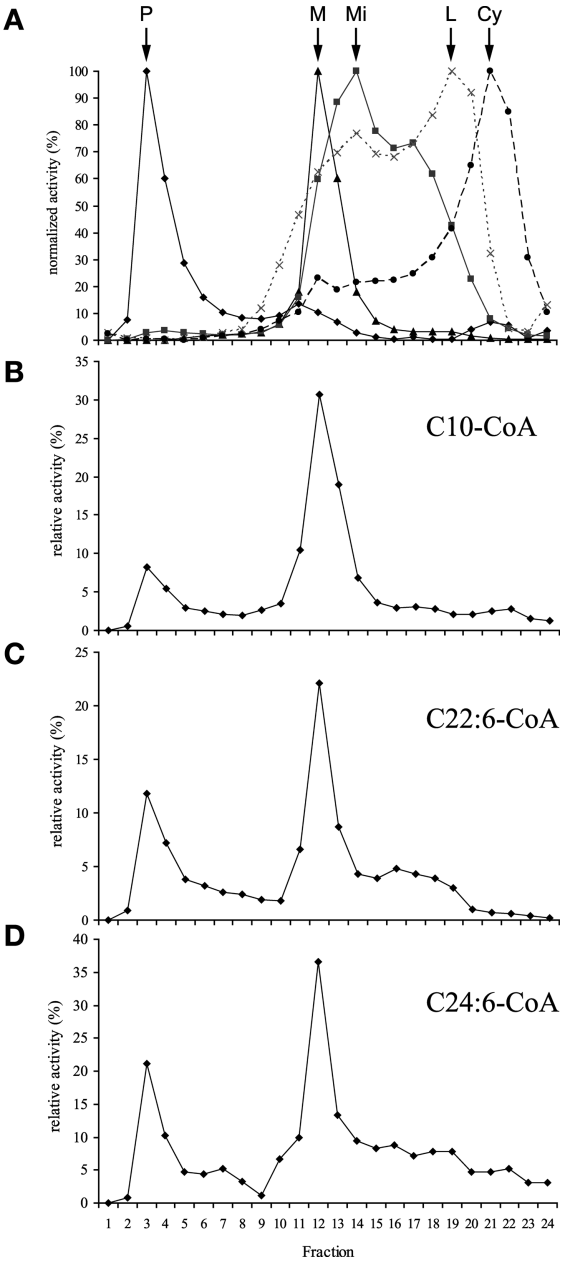

Fig. 2. A light mitochondrial fraction prepared from mouse kidney was subjected to equilibrium density gradient centrifugation as described in the Materials and Methods section. Fractions were analyzed for the activity of the peroxisomal (P) marker enzyme catalase (black solid diamonds), the mitochondrial (M) marker enzyme glutamate dehydrogenase (black solid triangles), the microsomal (Mi) marker esterase (gray solid squares), the lysosomal (L) marker β-hexosaminidase (gray crosses) and the cytosolic (Cy) marker phosphoglucose isomerase (black solid circles) (A) and for TE activity measured with C10-CoA (B), C22:6(n-3)-CoA (C), C24:6(n-3)-CoA (D) as substrate. Relative activities are expressed as a percentage of total gradient activity present in each fraction.

Incorporation of [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 into lipids in cultured skin fibroblasts

Incorporation of [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 into different lipid classes was studied in cultures of fibroblasts grown in tissue culture flasks (25 cm^2). Incubations were carried out in F10 (Ham) supplemented with penicillin/streptomycin, and containing 10% fetal calf serum (fatty acid-free) and 20 mM HEPES. After the addition of 7 μM of ^{14}C-labeled fatty acid, the fibroblasts were kept in an incubator at 37°C for 2 h. Cellular lipids were isolated as described previously (5). The incubation medium was removed, and the fibroblasts were scraped into 1 ml of methanol and transferred to a glass vial. The tissue culture flask was washed with 1 ml of methanol, which was also transferred to the glass vial. One volume of chloroform was added, and after vortexing and adding 1 vol of 0.88% KCl, each sample was centrifuged at 1,300 *g* for 10 min to separate the phases. After the aqueous phase was washed with 1 vol of chloroform, the two chloroform layers were combined, dried under N_2, and resuspended in 300 μl chloroform. The lipids in these lipid extracts were separated into neutral lipids, fatty acids, neutral phospholipids, and acidic phospholipids using aminopropyl disposable columns (J. T. Baker, Deventer, The Netherlands), essentially as described (24, 25). The column was equilibrated by rinsing twice with 2 ml of hexane, and then 250 μl of lipid extract dissolved in chloroform was loaded onto the column. The neutral lipids were eluted with 4 ml chloroform-2-propanol (2:1; v/v). Then 4 ml of ether containing 2% acetic acid followed by 4 ml of methanol was applied to elute free fatty acids and neutral phospholipids, respectively. Finally, acidic lipids were eluted using 4 ml of a mixture of hexane-2-propanol-ethanol-0.1 M ammonium acetate in water-formic acid (420:350:100:50:0.5; v/v/v/v/v) containing 5% phosphoric acid. Radioactivity was determined in the different fractions by liquid scintillation counting.

TE activity measurements

TE activity was measured in the different fractions of the density gradient in a standard medium containing 50 mM HEPES, pH 7.4, 50 mM KCl, 10 μM BSA, and 75 μM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB). The formed DTNB-CoA complex was measured spectrophotometrically at 412 nm every 10 s using a Cobas Bio Centrifugal Analyzer. During the first minute of the measurements, only protein was present in the incubation mixture, to correct for a specific complex formation of DTNB, and after 1 min, the reaction was started by adding 50 μM of C22:6(n-3)-CoA or C24:6 (n-3)-CoA. The increase in absorbance at 412 nm was measured and, by using the molar extinction coefficient of the DTNB-CoA complex (13,600/M cm), the specific TE activity was calculated.

RESULTS

Oxidation of [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 in cultured skin fibroblasts

Both [1-^{14}C]22:6n-3 and its direct precursor in the biosynthesis pathway, [1-^{14}C]24:6n-3, were β-oxidized in cul-

TABLE 1. β-Oxidation of [1-^{14}C]24:6n-3 and [1-^{14}C]22:6n-3 in human skin fibroblasts

	n	Specific Activity C24:6n-3 (10 μM)	Specific Activity C22:6n-3 (10 μM)	Ratio C24:6/C22:6
		pmol/h·mg		
Controls	11	1,311 ± 316[a]	481 ± 141	2.7
PBD[b]	7	136 ± 77	78 ± 44	1.7
D-BP[c]	4	155 ± 53	82 ± 22	1.9
CACT[d]	8	952 ± 177	334 ± 88	2.9
MTP[e]	4	1,334 ± 131	501 ± 127	2.7

n = number of different cell lines; all incubations were performed in duplicate.
[a] Mean value ± standard deviation.
[b] PBD, patients with a peroxisome biogenesis disorder.
[c] D-BP, patients with a deficiency of peroxisomal D-bifunctional protein.
[d] CACT, patients with a carnitine acyl-carnitine translocator deficiency.
[e] MTP, patients with a deficiency of mitochondrial trifunctional protein.

tured skin fibroblasts (**Table 1**). The specific activity for the oxidation of C24:6n-3 was 2.7 times higher in fibroblasts of control subjects than was the oxidation of C22:6n-3. Fibroblasts from patients with a mitochondrial fatty acid oxidation defect (at the level of either the mitochondrial CACT or of the MTP) revealed normal β-oxidation of both C22:6n-3 and C24:6n-3, compared with the oxidation in control fibroblasts. In contrast, fibroblasts from patients with a peroxisomal fatty acid oxidation defect (patients with a PBD and patients with a D-BP deficiency) showed strongly reduced oxidation activities for both substrates (Table 1). The ratio for the oxidation of C24:6n-3 over C22:6n-3 was slightly lower in these patients (1.7 and 1.9 in PBD and D-BP fibroblasts, respectively), compared with the ratio in fibroblasts from control subjects and from patients with a mitochondrial fatty acid oxidation defect.

Incorporation of [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 into different lipid classes in cultured skin fibroblasts

Incorporation of C22:6n-3 and C24:6n-3 into different lipid classes was studied by incubation of five different control fibroblast cell lines with [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 for 2 h, followed by extraction of the lipids and separation into the different lipid classes using aminopropyl disposable columns. This method allows separation of neutral lipids, free fatty acids, neutral phospholipids, and acidic phospholipids. The specific activity for the incorporation of [1-^{14}C]22:6n-3 into neutral lipids, as well as neutral and acidic phospholipids, was higher than for the acylation of [1-^{14}C]24:6n-3 into these lipid classes (**Table 2**). The ratio of acylation of C22:6n-3 over C24:6n-3 into phospholipids (neutral plus acidic) was 6.2. Neither [1-^{14}C]22:6n-3 nor [1-^{14}C]24:6n-3 was recovered in the free fatty acid fraction.

Acyl-CoA oxidase activity for C22:6(n-3)-CoA and C24:6(n-3)-CoA in mouse kidney peroxisomes

Because no purified human peroxisomes were available, acyl-CoA oxidase activity, the first enzyme of the peroxisomal β-oxidation system, was measured in purified mouse kidney peroxisomes with C22:6(n-3)-CoA and C24:6(n-3)-CoA as substrates. The mean specific activity (±SD) of three independent experiments measured with a substrate concentration of 20 μM was 17.6 (±5.9) pmol/min·mg for C22:6(n-3)-CoA, while the mean specific activity for C24:6(n-3)-CoA was 19.5 (±10.5) pmol/min·mg, resulting in a ratio for the oxidation of C24:6(n-3)-CoA over C22:6(n-3)-CoA of 0.9.

TE activity for C22:6(n-3)-CoA and C24:6(n-3)-CoA in subcellular fractions of a mouse kidney density gradient

TE activity was measured with C22:6(n-3)-CoA, C24:6(n-3)-CoA, and C10-CoA as substrates in different fractions of mouse kidney obtained by equilibrium density gradient centrifugation of a light mitochondrial fraction, which is enriched in peroxisomes and lysosomes. The distinct activity patterns for the marker enzymes demonstrated a good separation between the various subcellular organelles (Fig. 2). When TE activity was measured, the activity was associated with both the mitochondrial and the peroxisomal fractions for all substrates. The activity with C10-CoA was mainly mitochondrial, with a ratio of the activity in the highest mitochondrial fraction (number 12) over the highest peroxisomal fraction (number 3) of 5.7. In contrast, this ratio was 1.9 and 1.7 for C22:6(n-3)-CoA and C24:6(n-3)-CoA, respectively, indicating that relatively more TE activity was localized in peroxisomes for these substrates compared with C10-CoA. The mean specific ac-

TABLE 2. Incorporation of [1-^{14}C]22:6n-3 and [1-^{14}C]24:6n-3 into different lipid classes in cultured skin fibroblasts

	Specific Activity Neutral Lipids[a]	Neutral Phospholipids[b]	Acidic Phospholipids[c]	Neutral + Acidic Phospholipids
		pmol/h·mg		
[1-^{14}C]22:6n-3 (n = 5)	3,321 ± 529[d]	1,607 ± 469	257 ± 72	1,864
[1-^{14}C]24:6n-3 (n = 5)	433 ± 249	234 ± 68	66 ± 17	300

n = number of different cell lines; all incubations were performed in duplicate.
[a] Neutral lipids include cholesterol, cholesterol esters, and triglycerides.
[b] Neutral phospholipids include phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin.
[c] Acidic phospholipids include phophatidylinositol, phosphatidylserine, and phosphatidic acid.
[d] Mean value ± standard deviation.

tivity (±SD) of three independent experiments measured with a substrate concentration of 50 μM in the peroxisomal fraction 3 was 60.0 (±11.9) nmol/min·mg for C22:6(n-3)-CoA, whereas the mean specific activity for C24:6(n-3)-CoA in the same fraction was 36.2 (±7.6) nmol/min·mg, resulting in a ratio of 1.7 for the activity of C22:6(n-3)-CoA over C24:6(n-3)-CoA.

DISCUSSION

It is now firmly established that the last step in the biosynthesis of DHA consists of one peroxisomal β-oxidation cycle of its precursor C24:6(n-3)-CoA, which is synthesized in the ER. C24:6(n-3)-CoA therefore has to move from the ER to the peroxisome. Because DHA is the most abundant n-3 PUFA in neuronal and retinal membranes, C22:6(n-3)-CoA has to move out of the peroxisome to the ER to be incorporated into (phospho)lipids after its synthesis. C22:6(n-3)-CoA, however, is also a substrate for the β-oxidation system in the peroxisome, where it is degraded (Fig. 1). This implies that the processes involved in DHA synthesis, incorporation into membrane lipids, and degradation are tightly regulated. To obtain a better insight into this regulation, we have investigated several processes involved in the determination of the metabolic fate of C22:6n-3 and its precursor C24:6n-3, with particular emphasis on the β-oxidation of the two compounds and their incorporation into various lipids.

In cultured human skin fibroblasts, we found that C24:6n-3 was β-oxidized 2.7 times faster than C22:6n-3, while C22:6n-3 was incorporated 7 times faster into different lipid classes than C24:6n-3. These results are in good agreement with the results of Luthria, Mohammed, and Sprecher (9), who compared acylation of these substrates into 1-acyl-*sn*-glycero-3-phosphocholine by rat liver microsomes with β-oxidation by purified rat liver peroxisomes. β-Oxidation of both C22:6n-3 and C24:6n-3 takes place mainly in the peroxisome, because β-oxidation activity was strongly reduced in patients with a peroxisomal fatty acid oxidation defect, whereas the activity was normal in patients with a mitochondrial fatty acid oxidation defect. The residual activity for C22:6n-3 was somewhat higher than for C24:6n-3 in fibroblasts of PBD patients and patients with D-BP deficiency, suggesting that mitochondria can take over the degradation of C22:6n-3 somewhat better than C24:6n-3.

Because the acyl-CoA oxidases are considered to be the rate-limiting enzymes of the peroxisomal β-oxidation system, and because they play an important role in determining the substrate specificity of this β-oxidation system, we investigated whether the acyl-CoA oxidases in the peroxisome are involved in determining the β-oxidation rates of C22:6n-3 and C24:6n-3. In mouse kidney peroxisomes, we found similar specific activities for C22:6(n-3)-CoA and C24:6(n-3)-CoA, suggesting that the substrate specificities of the acyl-CoA oxidases are not involved in determining the different β-oxidation rates of these substrates.

These results imply that other processes must be involved in determining the metabolic fate of C22:6n-3 and C24:6n-3. Sprecher (26) has postulated the hypothesis that the β-oxidation of C22:6n-3 is a relatively slow process, because auxiliary enzymes are required. First, an NADPH-dependent 2,4-dienoyl-CoA reductase has to reduce 2-*trans*-4-*cis*-7,10,13,16,19-C22:7(n-3)-CoA to a 3-*trans*-enoyl-CoA, which has to be converted into a 2-*trans*-enoyl-CoA by the Δ^3,Δ^2-enoyl-CoA isomerase, after which it can reenter the β-oxidation spiral (27). In contrast, for β-oxidation of C24:6n-3, only the enzymes of the β-oxidation system are required. Sprecher concluded that the reaction catalyzed by the 2,4-dienoyl-CoA reductase is a slow or perhaps even rate-limiting step, because when C22:6n-3 or C24:6n-3 were incubated with peroxisomes, 2-*trans*-4,7,10,13,16,19-C22:7, the substrate for 2,4-dienoyl-CoA reductase, accumulated (9). In contrast, De Nys et al. (28) concluded that the reductase does not seem to constitute a rate-limiting step in the peroxisomal degradation of C22:6n-3, because in vitro, the human recombinant enzyme was active toward 2,4,7,10,13,16,19-C22:7-CoA (with a K_m of 102 μM), which also has been shown for the mouse recombinant enzyme (K_m 155 μM) (29). This result, however, does not exclude the possibility that in vivo, the required reductase activity is the rate-limiting process in the β-oxidation of C22:6n-3.

It is likely that a combination of processes is involved in determining the metabolic fate of C22:6n-3 and C24:6n-3. We measured TE activity with both substrates in peroxisomes and mitochondria. In isolated peroxisomes, the CoA-ester of C22:6n-3, which is the substrate for the β-oxidation system, was hydrolyzed to a free fatty acid 1.7 times as fast as C24:6(n-3)-CoA. This suggests that DHA could be transported to the ER as a free fatty acid (see Fig. 1).

In summary, the results presented in this paper show that the preferred metabolic fate of C24:6(n-3)-CoA, after its synthesis in the ER, is to move to the peroxisome, where it is β-oxidized, producing C22:6(n-3)-CoA. This DHA-CoA then preferentially moves back, perhaps as free fatty acid, to the ER, where it is incorporated into membrane lipids after reactivation to DHA-CoA, presumably by the long-chain acyl-CoA synthetase localized in the ER membrane. We have found that there is an inverse relationship between rates of peroxisomal β-oxidation versus incorporation into different lipid classes. This also has been shown by Luthria, Mohammed, and Sprecher (9). They performed their experiments, however, with isolated organelles and measured acylation into synthetic 1-acyl-*sn*-glycero-3-phosphocholine, whereas we measured these processes in whole cells, further supporting their results.

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