Minireview

Analysis of the putative role of 24-carbon polyunsaturated fatty acids in the biosynthesis of docosapentaenoic (22:5*n*-6) and docosahexaenoic (22:6*n*-3) acids

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Abstract The recent literature on the putative involvement of a single cycle of peroxisomal β-oxidation of 24:5*n*-6 and 24:6*n*-3 polyunsaturated fatty acids in the biosynthesis of the respective docosapentaenoic (22:5*n*-6) and docosahexaenoic (22:6*n*-3) fatty acids is critically reviewed. Present evidence suggests that in vitro data in support of the above proposition is an artifact of a low 2,4-dienoyl-CoA reductase activity due to depletion of NADPH resulting from incubation conditions. Kinetic studies with radiolabeled precursors in cell cultures have shown lower initial rates of labeling of 24:6*n*-3 than that of 22:6*n*-3, indicating that 24:6*n*-3 is an elongation product of 22:6*n*-3 rather than its precursor. Analysis of other literature data supports the proposal that 22:5*n*-6 and 22:6*n*-3 are synthesized in mitochondria via channeled carnitine-dependent pathways involving separate *n*-6- and *n*-3-specific desaturases. It is proposed that impaired peroxisomal function in some peroxisomal disorders is a secondary consequence of defective mitochondrial synthesis of 22:6*n*-3; moreover, some disorders of peroxisomal β-oxidation show normal or increased 22:5*n*-6 concentrations, indicating that 22:5*n*-6 is synthesized by independent desaturases without peroxisomal involvement.

Key words: Zellweger syndrome; Adrenoleukodystrophy; Sperm; Motility; Bacterium; Alga

1. Introduction

More than 30 years ago Holman and Hofstetter [1] found 24:4*n*-6 to be a major constituent of neutral lipids in bovine testes. A few years later, Carpenter [2] and Bridges and Coniglio [3] confirmed the presence of 24:4*n*-6 in rat testes and also described the presence of 24:5*n*-6 in this tissue, which was also found to accumulate in neutral lipids [2]. In addition to the more likely possibility that 24:5*n*-6 is formed by a two-carbon elongation of 22:5*n*-6, Bridges and Coniglio [3] proposed that 24:5*n*-6 might also arise via a Δ^6-desaturation of 24:4*n*-6. Voss et al. [4] have revived and expanded this proposal more than two decades later in an attempt to explain the difficulties in demonstrating in vitro microsomal Δ^4-desaturation for the synthesis of 22:5*n*-6 and 22:6*n*-3. In their putative pathway, the intermediates 22:4*n*-6 and 22:5*n*-3 would be biosynthesized by the conventionally accepted microsomal scheme involving the alternative Δ^6- and Δ^5-desaturation-elongation reactions, with the same desaturases presumably acting on both the *n*-3 and *n*-6 fatty acid series. However, instead of a Δ^4-desaturation, microsomes would elongate the above docosapolyenoic fatty acid intermediates by a two-carbon unit to 24:4*n*-6 and 24:5*n*-3, respectively, which would then undergo a second Δ^6-desaturation to form 24:5*n*-6 or 24:6*n*-3. At this stage, these 24-carbon fatty acids would be transported to peroxisomes to undergo a putative single cycle of peroxisomal β-oxidation to produce the final respective 22:5*n*-6 and 22:6*n*-3 fatty acids.

2. Experiments with peroxisomal incubations

There are a number of problems with the above proposal. Peroxisomes are known to readily β-oxidize 22:6*n*-3 to 20:5*n*-3 [5–7], and diets rich in 22:6*n*-3 are known to produce peroxisomal proliferation [8,9]; it is also known that 22:5*n*-6 is β-oxidized to 20:4*n*-6 [10–13], presumably by the same β-oxidation machinery. Therefore, for the involvement of peroxisomal β-oxidation in the synthesis of 22:5*n*-6 from 24:5*n*-6 to be a viable mechanism, it would be necessary to demonstrate that the β-oxidation of 22:5*n*-6 is slow compared to that of 24:5*n*-6. In an in vitro cell-free system containing mostly microsomes and peroxisomes, Mohammed et al. [14] claim to demonstrate that 24:5*n*-6 primarily undergoes a single cycle of β-oxidation in peroxisomes, to preferentially produce 22:5*n*-6. The explanation offered for this finding is that the NADPH-dependent 2,4-dienoyl-CoA reductase, an auxiliary peroxisomal enzyme needed to allow the β-oxidation of polyunsaturated fatty acids in which the first double bond at position 4 (such as 22:5*n*-6 or 22:6*n*-3) is rate-limiting; thus, when 24:5*n*-6 undergoes the first β-oxidation cycle to produce a fatty acid with a double bond at the 4th carbon, i.e. 22:5*n*-6, a larger molar fraction of this fatty acid would move out of the peroxisomes relative to the fraction that is further β-oxidized. Indeed, these authors did find a limited activity of the NADPH-dependent 2,4-dienoyl-CoA reductase in their in vitro system. Although their incubation system contained NADPH, a closer examination of their experimental conditions reveals that their system also included pyruvate and lactate dehydrogenase; these reactants are added to maintain a low steady concentration of NADH, and as an NAD^+ regenerating device to drive the peroxisomal β-oxidation of saturated fatty acids in in vitro systems [15]. Although it is conventionally assumed that lactate dehydrogenase is specific for NADH, this specificity is only relative and some isozymes appear to prefer NADPH as the electron donor [16–19]. Therefore, it is likely that most of the added NADPH was actually oxidized to $NADP^+$ under the above assay conditions, and the apparent low NADPH-dependent 2,4-dienoyl-

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CoA reductase activity was an artifactual consequence of the resulting low NADPH concentration; Mohammed et al. [14] did not provide any data to show that the concentration of NADPH was not affected by the addition of exogenous lactate dehydrogenase and pyruvate. There is clear evidence from other sources that peroxisomes contain a very active 2,4-dienoyl-CoA reductase which is involved in the β-peroxidation of 22:6*n*-3 and 22:5*n*-6; when peroxisomes are incubated with 0.1 mM NADPH (half of the concentration that Mohammed et al. [14] used) or exogenous $NADP^+$-dependent isocitrate dehydrogenase (to regenerate NADPH using endogenous isocitrate) the β-oxidation of 22:6*n*-3 is increased by 300% [20], demonstrating that the 2,4-dienoyl-CoA reductase is not rate-limiting when it has access to normal concentrations of NADPH [5,6]. Therefore, we would predict that if Mohammed et al. [14] were to add exogenous isocitrate and $NADP^+$-specific isocitrate dehydrogenase to their incubation medium they would observe a rapid β-oxidation of 22:5*n*-6.

Mohammed et al. [14] also provide other data that cast further doubt on the role of 24-carbon fatty acids in the synthesis of 22:5*n*-6; the putative Δ^6-desaturase substrate, i.e. 24:4*n*-6, is rapidly β-oxidized by peroxisomes under their conditions. This loss of 24:4*n*-6 must necessarily greatly diminish its desaturation to 24:5*n*-6, and if this destruction of the Δ^6-desaturase substrate occurs in vivo, the putative microsomal-peroxisomal pathway would be an inefficient pathway of 22:5*n*-6 synthesis indeed. The artifactual production of fatty acid synthesis from manipulation of in vitro conditions of β-oxidation enzymes would not be new; back in the late 1950's when it was proposed that fatty acid synthesis occurred by reversal of β-oxidation, Seubert et al. [21] were able to manipulate in vitro NAD^+/NADH and $NADP^+$/NADPH concentrations by exogenous dehydrogenases to show fatty acid synthesis with some of the enzymes of the β-oxidation pathway, even though it was later shown that fatty acid synthesis in vivo occurs via an entirely different and efficient pathway now known as the multifunctional fatty acid synthetase.

3. Biosynthetic studies with cell cultures

Kinetic experiments with radiolabeled precursors in cell cultures have also produced data that are not compatible with the hypothesis that 24:5*n*-6 and 24:6*n*-3 are direct precursors of 22:5*n*-6 and 22:6*n*-6, respectively. When human skin fibroblasts are incubated with [1-^{14}C]20:5*n*-3, 22:6*n*-3 is labeled 48 h before any labeled 24:6*n*-3 is detected [22]. Similarly, when Y79 retinoblastoma cells are incubated with [1-^{14}C]20:4*n*-6, 22:5*n*-6 is labeled 24 h before any labeled 24:5*n*-6 can be detected [22]. Additional experiments with Y79 retinoblastoma cells clearly confirm these findings. Pulse-chase experiments with [1-^{14}C]20:5*n*-3 show a rapid labeling of 22:5*n*-3, followed by 22:6*n*-3 which gained label at the same rate as label loss from 22:5*n*-3; labeling of 24:6*n*-3 was much lower and decreased very slowly over the entire chase period, while 24:5*n*-3 showed the least labeling and this remained essentially steady over the course of the chase period [23]. The slower labeling of the 24-carbon fatty acids at all times strongly supports the contention that 24:5*n*-3 and 24:6*n*-3 are the elongation products of 22:5*n*-3 and 22:6*n*-3, respectively, rather than their precursors. Moreover, the lesser rate of labeling of 24:5*n*-3 than 24:6*n*-3, concomitantly with no loss of label from 24:5*n*-3, is not compatible with 24:5*n*-3 being the precursor of 24:6*n*-3 as required by the putative microsomal Δ^6-desaturation of 24:5*n*-3; however, this metabolic inactivity would be expected for dead-end elongation products. Additional experiments with fish primary hepatocytes incubated with [1-^{14}C]18:3*n*-3 also show a precursor-product cross-over between 22:5*n*-3 and 22:6*n*-3, with no label incorporation into 24:5*n*-3, while labeling of 24:6*n*-3 lags far behind that of 22:6*n*-3 at all time points [24]; these data indicate that the 24-carbon fatty acids are not precursors of 22:6*n*-3. Other evidence not consistent with the proposal that 24:6*n*-3 is the direct precursor of 22:6*n*-3 has been obtained in kinetic experiments with retinal tissue. When retinal pigment epithelium is incubated with [3-^{14}C]22:5*n*-3, labeled 22:6*n*-3 is formed faster than 24:6*n*-3 during the first hours of incubation [25]. These data, again, are not consistent with these 24-carbon fatty acids being precursors of 22:6*n*-3, but the other way around. Other evidence inconsistent with the operation of the putative microsomal-peroxisomal pathway has previously been reviewed [26].

4. Evidence from peroxisomal disorders

Zellweger syndrome and several other peroxisomal disorders are associated with very low concentrations of 22:6*n*-3 in brain and other tissues [27–29], and these data have been cited as evidence in support of the putative involvement of peroxisomal β-oxidation in the synthesis of 22:6*n*-3 [4,30]. However, the original cytological description of Zellweger syndrome explicitly detailed a profound impairment of both mitochondrial and peroxisomal functions [31]; indeed, mitochondrial dysfunction was later found to be a characteristic of these diseases [32–35]. These observations led Goldfischer [36] to suggest that peroxisomal dysfunction may be secondary to a primary mitochondrial lesion in Zellweger syndrome. Patients with other disorders of peroxisomal β-oxidation, such as pseudo-Zellweger syndrome and neonatal adrenoleukodystrophy [37], provide further inconsistencies with the proposed peroxisomal involvement in synthesis of docosapolyenoic fatty acids; these patients have normal or increased brain concentrations of 22:5*n*-6 while showing very low levels of 22:6*n*-3 [28,29]. Contrary to the conventional view, these data clearly indicate that 22:6*n*-3 and 22:5*n*-6 must be biosynthesized by respective separate *n*-3- and *n*-6-specific desaturases (which are probably transcriptionally regulated), as previously proposed [26,38], and clearly show that peroxisomes are not involved in the biosynthesis of 22:5*n*-6, at least. Moreover, tissues from Zellweger syndrome patients show no accumulation of the putative direct precursors of 22:5*n*-6 and 22:6*n*-3, i.e. 24:5*n*-6 and 24:6*n*-3 respectively [39], which violates one of the predictions of the proposed microsomal-peroxisomal scheme. Further, when these 24-carbon fatty acids are synthesized in normal tissues they accumulate in the neutral lipid fraction [1,2,6,40]. Since known dead-end elongation products, such as 20:3*n*-3, 22:3*n*-3, and 24:3*n*-3, are also known to accumulate in this fraction [40] rather than in a metabolically active phospholipid pool, these data further suggest that 24:5*n*-6 and 24:6*n*-3 are primarily dead-end elongation products of 22:5*n*-6 and 22:6*n*-3, respectively, rather than their precursors. Additional evidence inconsistent with peroxisomal involvement in polyenoic fatty acid metabolism is the finding that patients with Zellweger syndrome or peroxisomal bifunctional enzyme deficiency (another peroxisomal disorder) show

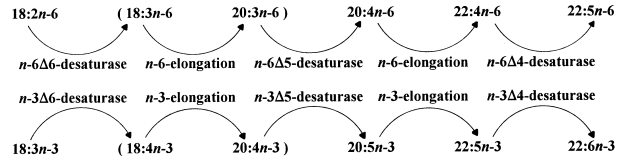

Fig. 1. Fatty acid desaturation and elongation reactions of the proposed mitochondrial pathways for the synthesis of *n*-6 and *n*-3 long-chain polyunsaturated fatty acids. The essential features of these pathways are: (i) acetate and long-chain fatty acid substrates are transported into mitochondria as carnitine esters; (ii) separate desaturases and elongation enzymes are required for the *n*-6 and *n*-3 fatty acid series; and (iii) the Δ^6 desaturation and elongation products are channeled. The channeled intermediates are indicated in parentheses.

decreases in plasma 20:4*n*-6 in addition to decreased 22:6*n*-3 [41,42]; since peroxisomes are not implicated in 20:4*n*-6 synthesis, decreases in this fatty acid cannot be explained by peroxisomal β-oxidation defects.

Based on the hypothesis that much of the clinical pathology observed in peroxisomal disorders could be explained by a deficiency of 22:6*n*-3 [27,30], Martinez and collaborators pioneered the treatment of infants with peroxisomal disorders using dietary administration of this fatty acid [43–45]. In addition to a striking clinical improvement after supplemental 22:6*n*-3, these patients showed a decrease in the erythrocyte concentrations of 24:1*n*-9 and 26:1*n*-9 (which characteristically accumulate in disorders of impaired peroxisomal β-oxidation), and an increase in plasmalogen synthesis [43–45]. The observation that the peroxisomal functions of β-oxidation and plasmalogen synthesis [46,47] improve after the administration of 22:6*n*-3 clearly indicates that such impaired peroxisomal activities in these patients are secondary consequences of defective 22:6*n*-3 synthesis, and not the other way around. These findings have, therefore, led to the proposal that 22:6*n*-3-containing phospholipids are required as specific structural or conformational cofactors for the functional assembly and integration of enzymes and receptor proteins into organelle membranes, including peroxisomes [26].

5. Mitochondrial involvement in 22:5*n*-6 and 22:6*n*-3 synthesis

If microsomes lack significant Δ^4-desaturation activity, and the putative peroxisomal-dependent system is an artifact of in vitro assay conditions, the puzzle remains how to account for the active synthesis of 22:5*n*-6 and 22:6*n*-3 demonstrated in intact cell systems [22–24]. One attractive alternative that explains the above inconsistencies is the postulation that the synthesis of these fatty acids occurs in the outer mitochondrial membrane via a channeled carnitine-dependent pathway involving separate *n*-3- and *n*-6-specific Δ^4-desaturases (Fig. 1) [26]. This mitochondrial system would be the primary synthetic site for 22:5*n*-6, 22:6*n*-3, and 20:4*n*-6, while the microsomal pathways would act as compensatory-redundant systems synthesizing 20:4*n*-6, 22:5*n*-6 and 20:5*n*-3 (but not 22:6*n*-3) [26].

Jakobsson et al. [48] have obtained some of the best recent evidence for mitochondrial synthesis of 22:6*n*-3; when primary rat hepatocytes were incubated with [1-^{14}C]18:3*n*-3, and the kinetics of label incorporation into 22:6*n*-3-containing phospholipids was followed, the initial rate of labeling of mitochondrial 22:6*n*-3-containing phosphatidylcholine (PC) was about 30 times that of microsomal PC. These striking results cannot be explained by peroxisomal or microsomal contamination of mitochondria since their carefully isolated mitochondria showed only a 3% contamination with microsomes and 7% with peroxisomes, while the purified microsomes only had 0.5% and 2% contamination with mitochondria and peroxisomes, respectively. Studies in retinal photoreceptors with labeled 22:6*n*-3 precursors have also shown a precursor-product relationship between mitochondria and the outer segments which are rich in 22:6*n*-3-containing phospholipids [49,50]. Other lines of evidence in support of mitochondrial synthesis of 22:6*n*-3 have been discussed previously [26].

Other data consistent with a mitochondrial synthesis of docosapolyenoic fatty acids can be found in experiments with testicular tissue. In rat testes, sexual maturation produces a pronounced increase in its characteristic 22:5*n*-6 containing phospholipids [51,52]; however, this accumulation of 22:5*n*-6 is accompanied by striking decreases in microsomal Δ^6- and Δ^5-desaturases [53–55] along with the expected disappearance of their products, i.e. 18:3*n*-6 and 20:3*n*-6 [2]. Such increases in testicular 22:5*n*-6 have been demonstrated to occur from in situ synthesis [56] and not by liver export, since it is well known that the liver does not synthesize significant concentrations of 22:5*n*-6, except under conditions of *n*-3 fatty acid deficiency as a compensatory response to decreased 22:6*n*-3 concentrations [57]. These observations suggest that liver biosynthesis of 22:5*n*-6 is catalyzed by transcription of an *n*-6-specific Δ^4-desaturase that is normally repressed by 22:6*n*-3 or its upstream *n*-3 precursors; its mechanism of transcriptional repression may be similar to the one described for the Δ^9-desaturase gene by *n*-6 fatty acids [58–60], whose expression is known to be increased by *n*-6 fatty acid deficiency [61]. The above data clearly indicate that 22:5*n*-6 in rat testes must be biosynthesized from 18:2*n*-6 via a pathway that does not involve the participation of microsomes. In addition, since the conventional microsomal desaturation intermediates are not observed during active synthesis of testicular 22:5*n*-6, we would propose that this fatty acid is made via the channeled mitochondrial pathway discussed above, which would include an androgen-controlled, tissue- and *n*-6-specific Δ^4-desaturase isozyme whose transcription is not repressed by 22:6*n*-3 or its precursors; evidence consistent with this postulation is the fact that testicular synthesis of 22:5*n*-6 in rats is not inhibited by a diet containing 22:6*n*-3 and 20:5*n*-3 [2]. Vitamin E has been implicated in fatty acid desaturation [38]; because vitamin E deficiency is associated with infertility and decreased concentrations of 22:5*n*-6 in rat testes (reviewed in [38]), it is likely that D-α-tocopherol or its quinone metabolites are specific cofactors for the mitochondrial fatty acid desaturation-elongation pathway located in the outer membrane. Consistent with this postulation is the finding that the mitochondrion is the organelle that contains the highest concentration D-α-tocopherol [62,63], with its outer membrane fraction containing most of this vitamin [64].

Further evidence consistent with a mitochondrial synthesis of 22:5*n*-6 in the testes is the observation that Sertoli cells show a much lower incorporation of exogenous labeled acetate than labeled 18:2*n*-6 into 22:5*n*-6 [65]; these results are corroborated by other data showing low incorporation of exogenous acetate into 22-carbon polyunsaturated fatty acids in other tissues [66–68], while intramitochondrial acetate is readily incorporated into these fatty acids (reviewed in [26]).

Similarly, lactate, as a source of intramitochondrial acetate (via lactate and pyruvate dehydrogenases), greatly increases the incorporation of [^{14}C]18:3*n*-3 into 22:6*n*-3 in cultured hepatocytes [69]; under these conditions, 20:5*n*-3 is labeled at a higher rate than its upstream conventional microsomal desaturation-elongation intermediates 18:4*n*-3 and 20:4*n*-3, indicating that the proposed mitochondrial pathway operates as a channeled system in which these intermediates remain enzyme bound and are therefore not readily available to the phospholipid acyltransferases (see Fig. 1). However, addition of D-decanoylcarnitine (an acylcarnitine antagonist) alters this labeling pattern such that 18:4*n*-3 and 20:4*n*-3 are labeled before 20:5*n*-3, and the total label incorporation into 22:6*n*-3 is decreased [69]; this alteration in labeling kinetics indicates that, in the presence of D-decanoylcarnitine, the channeled mitochondrial carnitine-dependent pathway is inhibited while the conventional microsomal pathway remains operative.

The carnitine dependency of the mitochondrial pathway of 22:6*n*-3 synthesis may explain the decreased 22:6*n*-3 and increased 22:5*n*-6 concentrations observed in tissues from patients with pseudo-Zellweger syndrome and neonatal adrenoleukodystrophy, as indicated in Section 4. These patients are affected by mutations in some of the enzymes of the peroxisomal β-oxidation pathway and display a smaller peroxisomal population (reviewed in [26]). Since the accumulated fatty acids and β-oxidation intermediates are known to be excreted as carnitine esters [70–72], it is likely that the resulting secondary carnitine deficiency is the cause of impaired mitochondrial 22:6*n*-3 synthesis, which would be partially compensated for by an increased expression of the carnitine-independent microsomal pathway for 22:5*n*-6 synthesis in these two disorders; defective 22:6*n*-3 synthesis would secondarily impair peroxisomal assembly. The decrease in 22:6*n*-6 in Zellweger patients might be due either to a primary defect in the mitochondrial desaturation-elongation pathway, or to a secondary carnitine deficiency resulting indirectly from defects in some of the genes governing the functional integration of peroxisomal proteins leading to an accumulation of acyl-CoA's; since tissues from these Zellweger patients also show decreased levels of 22:5*n*-6, they may additionally lack the genetic control elements necessary for a compensatory increase in microsomal 22:5*n*-6. Recent metabolic studies performed with mice with a disrupted acyl-CoA oxidase gene support the hypothesis of secondary carnitine deficiency being implicated in peroxisomal disorders. The acyl-CoA oxidase (ACOX) deficient mouse has been developed as an animal model for neonatal adrenoleukodystrophy; ACOX deficient homozygotes show a strikingly reduced population of peroxisomes during the first 3–4 months of age coupled with severe microvesicular steatosis and sterility; however, after 6–7 months of age these mice demonstrate a spontaneous peroxisomal proliferation with a concomitant disappearance of hepatic steatosis [73]. The initial steatosis and defective peroxisomal assembly observed in the ACOX deficient mice are consistent with a resulting secondary carnitine deficiency, which would cause both impaired mitochondrial β-oxidation and 22:6*n*-3 synthesis and could consequently produce defective integration of peroxisomal proteins. However, this physiological condition might trigger an up-regulation of the carnitine synthetic pathway with increased expression of mitochondrial acyl-carnitine transferases, which would eventually restore both mitochondrial β-oxidation and 22:6*n*-3 synthesis; this metabolic adaptation might, in turn, resolve the hepatic steatosis and allow the functional assembly of peroxisomes observed in the older animals. Although the ACOX deficient mouse shares a common enzymatic defect with human neonatal adrenoleukodystrophy, these mice show markedly less clinical pathology [73]; this may well be due to the proposed compensatory systems discussed above; however, these metabolic adaptations based upon genetic control elements appear to be lacking or greatly reduced in the human disorder. As discussed in Section 4, patients with Zellweger syndrome or peroxisomal bifunctional enzyme defect show decreased serum concentrations of both 22:6*n*-3 and 20:4*n*-6; however, the reduction in 20:4*n*-6 is less than that of 22:6*n*-3 [41,42]. This apparent discrepancy would be a predictable consequence of an impaired mitochondrial synthesis of both 22:6*n*-3 and 20:4*n*-6, accompanied by a concomitant partial compensation of up-regulated microsomal synthesis of 20:4*n*-6. If this hypothesis proves to be correct, administration of carnitine as well as of 22:6*n*-3 and 20:4*n*-6 might be of therapeutic value in peroxisomal disorders.

Biosynthesis of 22:6*n*-3 by some microorganisms provides additional evidence that peroxisomes are not involved in its synthesis. Recent data indicate that certain motile marine bacterial species are able to synthesize 22:6*n*-3; this biosynthetic pathway appears to be channeled since their fatty acid composition reveals very low concentrations of 22:6*n*-3 precursors [74,75]. Because prokaryotes do not contain cell organelles, these bacteria must biosynthesize 22:6*n*-3 via a peroxisome-independent pathway. One could contend that these marine prokaryotes have a hitherto unknown pathway for the synthesis of this fatty acid, different from eukaryotes. However, since mitochondria appear to have evolved from endosymbiotic bacteria [76–78], mitochondria and bacteria may share the same pathway of 22:6*n*-3 synthesis.

Some marine eukaryotic microalgae are also known to synthesize significant concentrations of 22:6*n*-3-containing lipids [79], and provide further evidence that mitochondria, rather than peroxisomes, are responsible for this synthesis. Among these unicellular eukaryotes, the dinoflagellate *Crypthecodinium cohnii* is known to contain a large concentration of 22:6*n*-3, particularly esterified to phospholipids [80]. This algal species is able to synthesize 22:6*n*-3 from acetate by a channeled pathway since labeled acetate is not significantly incorporated into any of the upstream intermediates, and labeled exogenous intermediates are similarly not significantly incorporated into 22:6*n*-3 [81]. Consistent with mitochondrial synthesis of 22:6*n*-3 is the finding that this algal species has large numbers of mitochondria; however, it lacks morphologically detectable peroxisomes [82], and it does not β-oxidize fatty acids longer than octanoate [81,83]. These data indicate that *Crypthecodinium cohnii* lacks long-chain fatty acid β-oxidation (either mitochondrial or peroxisomal), and therefore could not utilize peroxisomal β-oxidation in 22:6*n*-3 synthesis. Interestingly, as with the above bacteria, these phytoplankton species which synthesize 22:6*n*-3 are also motile [84,85], even though most plant species do not make this fatty acid, suggesting that phospholipids containing 22:6*n*-3 may play an essential role in motility. Significantly, defects in human sperm motility (asthenospermia) are associated with low levels of 22:6*n*-3-containing phospholipids [86,87]; conceivably, the sterility of the 3–4 month old ACOX deficient mice, described above, may also be due to 22:6*n*-3 deficiency, since, unlike

rats, mice and human testes contain significant amounts of 22:6*n*-3 [56]. The recurrent association between 22:6*n*-3 and motility is consistent with the previous postulation that 22:6*n*-3-containing phospholipids are necessary structural or conformational cofactors for fast-kinetics receptor-activated systems, such as ion pumps and channels, which are the hallmarks of excitatory and contractile cells [26,88]. Given the important functions of 22:5*n*-6 and 22:6*n*-3, it is unlikely that nature could not have evolved a more reliable and direct biosynthetic pathway for these essential fatty acids than the inefficient and roundabout microsomal-peroxisomal scheme proposed by Voss et al. [4].

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