Minireview

Secondary carnitine deficiency and impaired docosahexaenoic (22:6*n*-3) acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and β-oxidation

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Abstract A critical analysis of the literature of mitochondrial disorders reveals that genetic diseases of oxidative phosphorylation are often associated with impaired β-oxidation, and vice versa, and preferentially affect brain, retina, heart and skeletal muscle, tissues which depend on docosahexaenoic (22:6*n*-3)-containing phospholipids for functionality. Evidence suggests that an increased NADH/NAD^+ ratio generated by reduced flux through the respiratory chain inhibits β-oxidation, producing secondary carnitine deficiency while increasing reactive oxygen species and depleting α-tocopherol (α-TOC). These events result in impairment of the recently elucidated mitochondrial pathway for synthesis of 22:6*n*-3-containing phospholipids, since carnitine and α-TOC are involved in their biosynthesis. Therapeutic supplementation with 22:6*n*-3 and α-TOC is suggested.

Key words: Acyl-CoA dehydrogenase; Acylcarnitine; Cardiomyopathy; Adenine nucleotide translocator; Neuronal ceroid lipofuscinosis (Batten disease); Retinopathy

1. Introduction

Disorders of oxidative phosphorylation and β-oxidation are known to preferentially affect excitatory tissues such as brain, retina, heart and skeletal muscle [1–3]. These findings cannot be explained by depletion of ATP since recent data show that ATP homeostasis is not significantly altered in mitochondrial myopathies [4]; therefore, impairment of other mitochondrial functions is likely to be responsible for the preferential pathophysiology of excitatory systems. A common denominator for the nervous and muscular systems is their high content of docosahexaenoic (22:6*n*-3)-containing phospholipids [5,6]. A variety of data indicate that these phospholipid species act as specific structural or conformational cofactors in the functional assembly and integration into organelle membranes of enzymes, ion pumps (such as the SR Ca^{2+}-ATPase) and receptor proteins, which are particularly active in excitatory tissues [5,7]. In addition, mitochondria, where the oxidative phosphorylation machinery is located (recently reviewed in [8]), have a substantial concentration of 22:6*n*-3-containing phospholipids [9,10], suggesting that these are essential for the functional assembly of the respiratory chain complexes. Recent evidence suggests that 22:6*n*-3 is synthesized in mitochondria via a carnitine- and α-tocopherol (α-TOC)-dependent enzymatic pathway [11] (see Fig. 1). Since mitochondrial disorders induce secondary carnitine deficiency [12,13], and also lead to depletion of α-TOC [14] due to an increase in reactive oxygen species (ROS) [1], one would predict impaired synthesis of 22:6*n*-3 in these diseases, which should subsequently impair functioning of tissues dependent upon phospholipid species containing this fatty acid. Reviewed below are data indicating that diseases of oxidative phosphorylation produce impairment in mitochondrial β-oxidation, and vice versa, and that these disorders result in impaired 22:6*n*-3 synthesis as a consequence of secondary carnitine and α-TOC deficiency.

2. Mitochondrial pathways of fatty acid desaturation–elongation

As recently reviewed [7,11,15], there are two independent desaturation–elongation pathways: a channeled carnitine- and α-TOC-dependent mitochondrial fatty acid desaturation–elongation system which synthesizes 22:6*n*-3 (and 22:5*n*-6) by *n*-3- and *n*-6-specific multifunctional enzymes or enzyme complexes (Fig. 1), and the open microsomal system which operates via separate desaturases and elongation enzymes and is able to synthesize only up to 22:5*n*-3 (as well as being able to synthesize 22:5*n*-6). We have previously discussed evidence indicating that impaired mitochondrial synthesis of 22:6*n*-3 can be compensated for by up-regulation of the microsomal synthesis of 22:5*n*-3 and 22:5*n*-6 [5,11]. The net result of the interplay of the mitochondrial and microsomal systems is decreased 22:6*n*-3 and increased 22:5*n*-3, thus showing an apparent impairment of Δ4 desaturation of 22:5*n*-3. The existence of these two systems explains the fact that microsomes appear to be devoid of Δ4-desaturase for *n*-3 fatty acids even though intact cells can synthesize 22:6*n*-3 [15]. The involvement of the mitochondrial and microsomal fatty acid desaturation–elongation systems in mitochondrial disorders is explored below.

3. Disorders of oxidative phosphorylation

Defects in the oxidative phosphorylation machinery manifest clinically by hypotonia, hypoglycemia, non-ketotic lactic acidosis, myopathy, cardiomyopathy, retinopathy and encephalopathy [1–3]. Histologically, these disorders produce fatty

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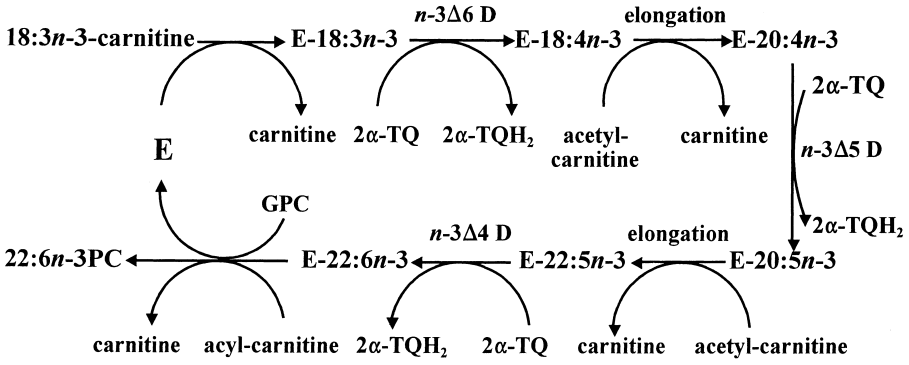

Fig. 1. Enzymatic mechanism for the proposed mitochondrial carnitine- and α-tocopherolquinone (α-TQ)-dependent channeled synthesis of 22:6*n*-3-containing phospholipids with phosphatidylcholine (PC) as an example. The reaction starts with 18:3*n*-3-carnitine which is transported across the outer mitochondrial membrane followed by binding of 18:3*n*-3 to the multifunctional enzyme or enzyme complex (E). A series of enzyme-bound alternating desaturations and elongations follows. Each of the three desaturation steps (Δ6, Δ5 and Δ4 *n*-3 desaturases) requires two molecules of the α-TOC metabolite α-TQ as an electron withdrawing cofactor (along with one molecule of NADH, which has been omitted from the figure for clarity, to allow for full reduction of the two α-TQ), which results in the formation of two reduced α-tocopherolhydroquinones (α-TQH_2) which eventually transfer their four hydrogens to molecular oxygen forming two molecules of water; for further details on the mechanism of α-TQ involvement in polyunsaturated fatty acid desaturation, see [11]. An intramitochondrial source of acetyl units, such as acetyl-carnitine, is the acetyl donor for the elongation reactions (discussed in [11]). The final synthesis of 22:6*n*-3-containing phospholipids requires the transfer of this fatty acid to a glycerophosphodiester acceptor, such as GPC, along with acylation by a second acyl moiety; these two acylations could either be concerted or occur consecutively via a lyso-PC intermediate (see [59] for details of glycerophosphodiester involvement in phospholipid synthesis).

infiltration of liver and skeletal muscle, and subsarcolemmal proliferation of abnormal mitochondria (ragged-red fibers) [1–3]. Biochemically, defects of the respiratory chain or of the adenine nucleotide translocator (ANT) are known to result in an increased $NADH/NAD^+$ ratio [16] which inhibits the Krebs cycle and pyruvate dehydrogenase [17]; the accumulated pyruvate is reduced to lactate, leading to lactic acidosis. An increased $NADH/NAD^+$ ratio inhibits β-oxidation at the level of the 3-hydroxyacyl-CoA dehydrogenases; defects downstream of complexes I and II will also inhibit β-oxidation due to decreased electron transfer from the acyl-CoA dehydrogenases to ubiquinone (which occurs via electron transfer flavoprotein (ETF) and ETF:ubiquinone oxidoreductase) [18–20]. One consequence of impaired β-oxidation is the accumulation of acyl-CoA β-oxidation intermediates, which are released from mitochondria as carnitine esters (in an attempt to regenerate free CoASH), subsequently transported to the plasma and finally excreted in the urine; these metabolic changes result in secondary carnitine deficiency [12,13,19]. Indeed, a number of patients harboring oxidative phosphorylation defects show low levels of free carnitine in muscle and plasma, and have impaired β-oxidation [20–24]. Carnitine deficiency would further impair β-oxidation, leading to a vicious cycle. Increased $NADH/NAD^+$ ratios would also be expected to inhibit carnitine synthesis, since the γ-trimethylaminobutyraldehyde dehydrogenase step in this pathway is catalyzed by an NAD^+-driven reaction [13]. In addition, generalized mitochondrial cytopathy may further impair carnitine synthesis at the mitochondrial ε-*N*-trimethyllysine hydroxylase step.

A second consequence of impaired oxidative phosphorylation is depletion of α-TOC, due to increased ROS generated through accumulation of free radical intermediates [1,14]. Therefore, defects in the oxidative phosphorylation machinery are predicted to result in impaired carnitine- and α-TOC-dependent 22:6*n*-3 synthesis. If 22:6*n*-3-containing phospholipids are required for assembly of the respiratory chain complexes and operation of the SR Ca^{2+}-ATPase pump [5], depletion of these phospholipid species would further impair oxidative phosphorylation and muscle contractility, and could account for the hypotonia and myopathy observed in these diseases. Consistent with this contention is the fact that vitamin E deficiency impairs 22:6*n*-3 synthesis [11], leads to decreased activity of some of the respiratory chain complexes [25] and produces myopathy in man and experimental animals [26,27]. Low levels of red blood cell α-TOC have been found in a number of mitochondrial defects [14]. Supplemental α-TOC given to a patient with decreased ANT resulted in a surprising therapeutic response: the patient went from being wheelchair-bound to being able to walk and play unassisted [28]. The increased oxidative stress in ANT is likely to lead to reduced levels of α-TOC; supplementation with α-TOC might have restored the synthesis of 22:6*n*-3-containing phospholipids. If the electron transport complexes were more tightly coupled as a result of an increased supply of 22:6*n*-3-containing phospholipids, this could have improved the residual ANT activity and decreased ROS production. Consistent with the role of these phospholipids in electron transport coupling and decreased ROS is the observation that 22:6*n*-3 supplementation to aged rats decreased ROS levels in the brain [29].

4. Diseases of impaired β-oxidation

Defects in the mitochondrial fatty acid β-oxidation machinery have recently been reviewed [30–32]. The clinical manifestations of these diseases bear a great similarity to those of disorders of oxidative phosphorylation. Clinically, they are characterized by myopathy, cardiomyopathy, pigmentary retinopathy and tissue steatosis, with fasting producing non-ketotic lactic acid acidosis and hypoglycemia [12,13,30–34]; the neonatal forms also manifest hypotonia [3,31]. Histologically, they induce proliferation of aberrant mitochondria [35]; biochemically, they produce a striking elevation of tissue and plasma carnitine esters in an effort to regenerate free CoASH from the accumulated acyl-CoAs, with a concomitant decrease in free carnitine [2,3,12,13]. Lower levels of free carnitine would be expected to impair the carnitine-dependent mi-

tochondrial 22:6*n*-3 synthesis. Significantly, recent reports indicate that defects in long chain 3-hydroxyacyl-CoA dehydrogenase, which induce secondary carnitine deficiency, are indeed associated with low levels of plasma 22:6*n*-3, and supplementation with this fatty acid greatly improves the clinical condition [36–38]. Interestingly, this decrease in 22:6*n*-3 is accompanied by normal concentrations of 22:5*n*-3 [37], which is consistent with a compensatory up-regulation of the microsomal desaturation–elongation pathway. We would also predict increased microsomal synthesis of 22:5*n*-6, as has been observed in other disorders associated with impaired 22:6*n*-3 synthesis [7,11].

As discussed above, decreased 22:6*n*-3 synthesis induced by deficiency of free carnitine may impair the functional assembly of the respiratory chain complexes of oxidative phosphorylation, in addition to decreasing SR Ca^{2+}-ATPase activity. Indeed, β-oxidation defects are associated with impaired oxidative phosphorylation [24,39]. The accumulation of acyl-CoAs and/or acylcarnitines could also inhibit oxidative phosphorylation and desaturases via the known surfactant effects of these metabolites on membrane-bound enzymes [39]; acyl-CoAs are also strong inhibitors of the ANT [40] which can lead to impaired oxidative phosphorylation.

5. Other conditions associated with decreased carnitine and 22:6*n*-3 levels

Neuronal ceroid lipofuscinosis (NCL, Batten disease), a severe neurodegenerative disorder, was long thought to be a disease of increased lipid peroxidation; however, since ROS metabolizing enzymes show near-normal activities [41] and blood levels of α-TOC are actually increased in NCL [42], this is not the case. Recent evidence convincingly shows that an impairment of the carnitine synthesis pathway at the step of lysosomal proteolysis of a mitochondrial trimethyllysine-containing protein is the primary biochemical lesion in this disease [43]. Since NCL patients show both carnitine deficiency and abnormally low levels of 22:6*n*-3, and higher levels of 20:4*n*-6 [43–46], an impairment of the carnitine-dependent pathway of 22:6*n*-3 synthesis appears to be a major cause of its pathophysiology; this is consistent with data showing that supplementation with 22:6*n*-3 ameliorates its pathology [47,48]. Other conditions associated with carnitine deficiency, such as treatment with certain pharmacologic agents, vegan diets, and excess dietary fat intake, also show decreased levels of 22:6*n*-3 (but normal 22:5*n*-3 levels) and higher levels of 20:4*n*-6 and 22:5*n*-6 (when measured) [7,13,49–54]. These observations indicate an impairment of the carnitine-dependent pathway of 22:6*n*-3 synthesis and compensatory up-regulation of the microsomal desaturases. In the absence of 18:3*n*-3 deficiency, a decreased ratio of 22:6*n*-3/22:5*n*-6 would be a useful diagnostic index of impaired α-TOC- and carnitine-dependent mitochondrial synthesis of 22:6*n*-3 in various disorders or physiological conditions.

6. Proposed therapies for disorders of 22:6*n*-3 synthesis

Dietary supplementation with appropriate levels of 22:6*n*-3, i.e. 10–15 mg/kg body wt/day [55], should be included in the treatment of disorders of oxidative phosphorylation and β-oxidation. However, since these conditions also encompass a more generalized mitochondrial pathology, and the synthesis of 22:6*n*-3-containing phospholipids probably requires the concomitant synthesis of its glycerophosphodiester acyl-acceptors such as glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), which are also synthesized in mitochondria [56,57], supplementation with commercial soybean lecithin (5.2–7.8 g, equivalent to 250–375 mg choline/day as required for children [58]) as a source of GPC, GPE and other glycerophosphodiesters would be advisable to tap into the extramitochondrial glycerophosphodiester recycling pathways [59], since dietary phospholipids are readily hydrolyzed to their glycerophosphodiesters by intestinal phospho- and lysophospholipases [60,61]. Alternatively, supplementation with 22:6*n*-3-containing phospholipids, such as from 22:6*n*-3-rich algal lecithin [62] or fish oil lecithin, would provide both the needed 22:6*n*-3 and glycerophosphodiesters; 22:6*n*-3 from algal lecithin is more readily incorporated into cell phospholipids than it is from triglycerides [62]. Supplementation with 22:6*n*-3 has already proven beneficial in improving retinal and muscle function in other disorders characterized by retinopathy and hypotonia and decreased 22:6*n*-3 levels, such as Zellweger syndrome and neonatal adrenoleukodystrophy [63,64]. In addition, in diseases of long chain fatty acid β-oxidation, supplementation with medium chain triglycerides (which is part of the conventional dietary treatment) should be decreased since dicarboxylic aciduria remains high when these triglycerides are included at relatively high levels [65]; dicarboxylic fatty acids are produced by microsomal–peroxisomal ω-oxidation of excess monocarboxylic fatty acids. This is not surprising since, contrary to the conventional opinion, recent evidence suggests that exogenous medium chain fatty acids enter mitochondria as carnitine esters [66,67], and thus would be poorly β-oxidized in the face of carnitine deficiency. Supplementation with carnitine (100–150 mg/kg body wt/day) may also be beneficial [13,68]. The known anti-arrhythmic effects of 22:6*n*-3 [69] may counteract the possible arrhythmic effects of excess acylcarnitines.

For mothers who are known heterozygotes for mitochondrial disorders, prenatal supplementation with the above nutrients is also indicated. Although the mother transfers 22:6*n*-3 to the fetus via the placenta [70,71], neonatal hypotonia is often present in homozygous offspring [3,31]; if this hypotonia is due to low levels of muscle 22:6*n*-3-containing phospholipids, transfer of 22:6*n*-3 is either inadequate or is not being incorporated into the proper muscle acyl-specific phospholipids. This would be the case if these fetuses have a concomitant defect in GPC and GPE synthesis, which also occurs in mitochondria [56,57]; this would not be surprising since generalized mitochondrial cytopathy has been described in these diseases [1,35]. A similar prenatal supplementation might also be beneficial in other diseases which involve mitochondrial cytopathy such as Zellweger syndrome [72], as these patients are known to have both low levels of 22:6*n*-3 and hypotonia at birth [73]; supplementation with 22:6*n*-3 resolves this condition [63].

7. Experimental animal models

There are three experimental animal models which would be useful to test the proposed mechanisms of impaired 22:6*n*-3 synthesis by defects in oxidative phosphorylation, mitochondrial β-oxidation and carnitine deficiency, as well as the effects of the above proposed dietary modifications. The knock-out

mouse ($Ant1^{PGKneo(-/-)}$) for the heart/muscle isoform of the ANT (ANT1), which uses the electrochemical gradient across the inner mitochondrial membrane to drive the exchange of ATP for ADP, is a valuable model for mutations in oxidative phosphorylation, since defects in this translocator will inhibit the electron flux in the respiratory chain and ATP synthesis [16]. These mice develop the expected myopathy with ragged-red fibers (i.e. subsarcolemmal accumulations of abnormal mitochondria) and cardiomyopathy [16]. Although carnitine levels in these mice have not been determined, in one human case with a 4-fold decrease in the muscle-specific ANT, lower levels of total carnitine were reported in muscle [74], which is consistent with secondary impairment of β-oxidation; unfortunately, levels of free carnitine and 22:6*n*-3 were not determined. This mouse model also displays increased oxidative stress [16], suggesting that it has lower levels of α-TOC. The $Ant1^{PGKneo(-/-)}$ mouse would be a valuable tool to determine if defective ANT1 produces the expected decreases in free carnitine, α-TOC and 22:6*n*-3, and impaired β-oxidation.

A second model of interest is the knock-out mouse for long chain acyl-CoA dehydrogenase (LCAD) which reproduces much of the pathophysiology of the human disease having the same enzymatic defect [75], and would also be a good model for other mitochondrial disorders of β-oxidation such as of the long chain 3-hydroxyacyl dehydrogenase. The LCAD(−/−) genotype shows the expected increased levels of acylcarnitines [75]; however, the free carnitine status of these mice was not reported. We would predict a decrease in free carnitine and a resultant impaired synthesis of 22:6*n*-3 accompanied by compensatory increases of 22:5*n*-3 and 22:5*n*-6. This LCAD-deficient mouse would be a valuable tool to test the prediction that defects in β-oxidation lead to impaired oxidative phosphorylation via the mechanisms discussed above.

The two knock-out mouse genotypes for NCL, which have recently been produced by targeted disruption of the mouse ortholog of the CLN3 gene (CL*n*3) [76,77], provide a third valuable model to study the role of carnitine in 22:6*n*-3 synthesis; these mice would be expected to have impaired synthesis of carnitine and 22:6*n*-3.

The three mouse models above would constitute valuable tools for determining the effects of dietary and prenatal supplementation with carnitine, α-TOC, 22:6*n*-3 (at levels that do not produce peroxisomal proliferation, i.e. 0.2% of diet by weight [55]) and lecithin (3.3% of diet by weight, equivalent to 0.16% dietary choline [78]) on the pathophysiology of these diseases. However, for these experiments, it would be essential to provide controls with defined diets without pre-formed 22:6*n*-3, but with adequate 18:3*n*-3 and 18:2*n*-6, i.e. 1% and 5% of calories, respectively [79]. Most standard laboratory diets for rodents contain significant amounts of 22:6*n*-3 from the fish meal component and this may significantly contribute to the milder pathophysiology observed in mouse models for mitochondrial diseases and NCL, in addition to the capacity of mice to up-regulate microsomal synthesis of 22:5*n*-3 and 22:5*n*-6.

8. Conclusion

A variety of apparently unrelated disorders share common pathophysiological features; these include diseases of oxidative phosphorylation and β-oxidation, in addition to NCL, Zellweger syndrome, experimental vitamin E deficiency, and carnitine deficiency syndromes. Several of these disorders are associated with decreased levels of 22:6*n*-3, and this fatty acid has been used with therapeutic success in three of the above diseases. A common impairment of the carnitine- and α-TOC-dependent mitochondrial pathway of 22:6*n*-3 synthesis would explain some of the similarities, as each disorder shows low levels of either carnitine or α-TOC, or has mitochondrial defects.

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