Review article

Docosahexaenoic acid trials in cystic fibrosis: A review of the rationale behind the clinical trials

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1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disease in Caucasians. The cystic fibrosis gene codes for an integral membrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR). It is a cAMP-dependant chloride channel with the capacity of not only excreting organic anions as glutathione and cytochrome P450 metabolites conjugated to glutathione but has also other regulatory functions [1]. Some of the most important symptoms of CF are not directly the consequence of impaired chloride transport. The pulmonary inflammation and infection resulting in pulmonary failure is the major cause of death. Different medications have been used to...
influence the inflammation as antibiotics, steroids and non-steroidal anti-phlogistics, antioxidants... resulting in an important gain of life expectancy.

Recent studies give however new insights in the possible pathophysiology of the increased sensitivity for inflammation of CF patients. Especially the role of the metabolism of fatty acids (FAs) is highlighted by recent findings in CFTR /-/- mice. Freedman et al. described a mouse model of CF in which important abnormalities of the FA metabolism were shown [2]. The biochemical aberrations were corrected by oral supplementation of pharmacological doses of docosahexaenoic acid (DHA, 22:6ω3). This results in new therapeutic perspectives. The first clinical DHA supplementation trials have already started. This review goes into the possible rationale behind these trials. A brief overview of the metabolism of the essential fatty acids (EFAs) is given.

2. Essential fatty acids and their metabolism

FAs as triglycerides and phospholipids are the main components of energy production and storage. After digestion, absorption and biosynthetic transformations, acyl chains are not only used for triglyceride synthesis but also become part of biomembranes after esterification to complex lipids. FAs account for more than 50% of the molecular mass of phospholipids. The fatty acid tails of the phospholipids are responsible for the apolar nature of membrane bilayers. The phospholipid membrane components influence many membrane functions as ion channelling and transport, endo- and exocytosis and the functions of membrane-associated receptors and enzymes [3].

Polyunsaturated fatty acids (PUFAs), originating from EFAs by elongation and desaturation, are precursors of biologically active molecules, the eicosanoids and docosanoids. PUFAs contribute to the control processes of nuclear transcription, via special receptors and response elements [4,5]. PUFAs released by agonist stimulated phospholipase A2 (PLA2), are involved in signal transduction [6]. Moreover they are involved in activation or modulation of protein kinase C, in direct stimulation of membrane receptors and in interaction with guanylate cyclases. They also participate in translocation processes of biosynthetic key-enzymes. A diversity of acyl chains may be required to fulfil so many different tasks [4–6].

3. Interconversion of long-chain PUFAs

De novo synthesis of FAs produces mainly palmitate (C16:0), with minor amounts of stearate (C18:0). Many cells have the capacity for 2-carbon chain elongation of FAs that takes place mostly in the endoplasmatic reticulum. It is the main source of acyl chains greater than 16 carbon atoms in membrane phospholipids.

All eukaryotic organisms contain polyenoic fatty acyl chains in their membrane lipids. Most tissues can modify acyl chain composition by introducing double bonds by means of desaturases (Δ5, Δ6, Δ9). Linoleic acid (LA, 18:2ω6) and α-linolenic acid (18:3ω3) are EFAs. These acyl chains are converted into other FAs containing 3 to 6 double bonds (Fig. 1). Arachidonic acid (AA, 20:4ω6), an ω6 FA found in most tissues, can be formed from LA by alternating sequence of Δ6 desaturation, chain elongation of the 18:3ω6 intermediate thus formed and Δ5 desaturation of 20:3ω6 (Fig. 1). AA is a component of phospholipids contributing to structural integrity of membranes and is the primary precursor of several classes of oxygenated derivatives.

![Fig. 1. Fatty acid notation: number of carbon atoms: number of double bonds followed by biochemical series.](image_url)
In the cerebral cortex, retina, testes and muscle the most abundant $\omega_3$ acyl chains are eicosapentaenoic acid (EPA, 20:5$\omega_3$) and DHA. The alternating sequence of desaturation and elongation is also the primary pathway of DHA synthesis. The $\omega_3$ and $\omega_6$ long chain fatty acids compete for the same elongases and desaturases.

4. Phospholipase A$_2$

PLA$_2$ represent a family of enzymes catalysing the hydrolysis of glycerophospholipids at the sn-2 position, thereby liberating free FAs. They are classified according to their localisation and calcium dependency [7,8]. These types of PLA$_2$ contain different isoenzymes making the understanding of the FA metabolism even more complicated [8].

The intracellular calcium-independent phospholipases A$_2$ (iPLA$_2$) is a membrane-associated enzyme that is implicated in membrane phospholipid remodelling and signal transduction [9]. The type IIA extracellular secretory PLA$_2$ (sPLA$_2$) has been isolated from inflammatory fluids and cells [8,10]. This isoenzyme is induced by pro-inflammatory stimuli and is therefore thought to play a role in inflammatory responses [11,12]. Finally it has been demonstrated that the calcium-depant cytosolic PLA$_2$ (cPLA$_2$) and the sPLA$_2$ are linked to the cyclooxygenase (COX)-2 enzymes responsible for the production of prostaglandins and thromboxanes [13].

PLA$_2$ is activated by multiple agonists and cell specific signals [14,15], resulting in the release of PUFA, precursors of inflammatory mediators, eicosanoids [12]. The best-studied eicosanoid system is that of AA.

Some derivatives of $\alpha$-linolenic acid, the parent EFA of the $\omega_3$ series, inhibit the release of AA by phospholipase A2 and results in antagonistic effects.

5. The eicosanoid production from polyunsaturated fatty acids

5.1. Main directions of metabolism of AA

The three main directions of metabolism of AA are cyclooxygenation, lipoxygenation and epoxygenation (Fig. 2).

Cyclooxygenases (COXs) generate intermediates that can be converted into prostaglandins, prostacyclins and thromboxanes. Blocking these enzymes by ibuprofen reduced in CF the production of COXs metabolites and resulted in a reduced decline of pulmonary function, weight and chest radiographic scores [16]. This type of treatment, however has a very narrow therapeutic window.

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![Fig. 2. Arachidonic acid is released by the action of phospholipase A$_2$ on membrane phospholipids. Phospholipase A$_2$ is activated by multiple cell signals, bradykinin, angiotensin II. Some derivatives of alpha-linolenic acid, the essential fatty acid of the $\omega_3$ series, inhibit the release of arachidonate by phospholipase A$_2$, and result in antagonistic actions. The three main directions of the metabolism of arachidonate are cyclooxygenation, lipoxygenation and epoxygenation. The cyclooxygenase products of arachidonate generate prostaglandins, prostacyclins and thromboxanes. Lipoxygenase products of arachidonate generate the pro-inflammatory leukotrienes, but also some biologically very active hydroperoxyeicosatetraenoates (HETEs) and the anti-inflammatory lipoxins. The epoxygenation pathways result, by action of cytochromes P450, in formation of 3 categories of biologically very active products: midchain HETEs, $\omega$-terminal HETEs and epoxyeicosatrienoic acids (EETs).](image-url)
**Prostaglandins** (PGs) are local autocrine and or paracrine hormones. They are found in virtually all tissues and organs. Their activities are mediated by G-protein mediated PG receptors. The same PGs have different effects on different tissues. Consumption of omega-6 FAs stimulates the production of pro-inflammatory PGs (PG2 series) derived from AA, while consumption of omega-3 FA stimulates the production of anti-inflammatory PGs (PG3 series). PGs have a wide variety of actions but most of them cause muscaric constriction and mediate inflammation. Other effects are calcium movement, hormone regulation and cell growth control [17,18].

**Thromboxanes** (TXs) whose principal pro-inflammatory mediator is the highly unstable TXA2, a potent aggregator of platelets and vasoconstrictor. TXs are the physiologic antagonists of prostacyclin. An imbalance in favour of TX will lead to initiation of platelet aggregation and an acute inflammatory response [19].

In CF patients increased PGE2 and TXB2, a stable transformation product of TXA2, as well as their metabolites was demonstrated at all ages [20]. They also have a hyperaggregability due to the increased TXA2 release [21].

**Prostacyclin** (PGI2) is mainly produced in the endothelial cells and is antagonistic to TX. It inhibits platelet aggregation as well as the activation of leukocytes. PGI2 plays an important role in vascular function because it inhibits platelet adhesion to the vascular endothelium and is a powerful vasodilator [22].

**Lipoxigenase** (LOX) products of AA generate via the 5-LOX the pro-inflammatory leukotrienes, via the 12-LOX some very active hydroperoxyeicosatetraenoates and finally via the 15-LOX the anti-inflammatory lipoxins.

**Leukotrienes** (LTs) are potent mediators of inflammation stimulating chemotaxis, chemokinesis, aggregation, enhancement of lysosomal enzyme release, superoxide anion production and ion fluxes in the leukocyte. They promote together with the PGs endothelial permeability. Thereby they promote the oedema formation during inflammation [23,24].

Increased LTs are discovered in sputa of CF patients and treatment with LT antagonists yields promising results [25,26].

**Lipoxins** are endogenously generated small chemical mediators, playing a key role in inflammation control and resolution. These trihydroxytetraene-containing eicosanoids are generated by tight cell to cell interactions by transcellular biosynthesis [27]. Although the cellular regulation of 15-LOX activity is not fully elucidated, an important defect was discovered in CF lungs. The administration of lipoxin analogues in a CF mouse model resulted in decreased lung inflammation and infection [28].

**Epoxygenation** pathways result by action of different cytochromes P450, in formation of hydroxyeicosatetraenoate and epoxyeicosatrienoic acids. These products modulate renal, pulmonary and cardiac function, regulate vascular tone and are involved in the metabolism of many other tissues [29–32].

### 5.2. Eicosanoids from other long chain PUFAs

Besides AA several other FAs as EPA, DHA and dihomogamma linolenic acid (20:3ω6) are also substrates for COXs, LOXs and epoxygenases. Many of these metabolites have still unknown structure and function.

**Dihomogammalinolenic acid** is the precursor of anti-inflammatory eicosanoids. It exerts however also a modulatory effect on cytokine production since it reduces interleukin-10 and tumour necrosis factor alpha but leaves interleukin-6 unaffected [33].

Christophe et al. reported increased vital capacity in CF patients supplemented with an oil rich in gamma-linolenic acid [34].

**Eicosapentaenoic acid** is a substrate for COXs, LOXs and the epoxygenase activities resulting in products with potent anti-inflammatory activity [35]. Many of the end products have still unknown structure and function.

### 5.3. Docosahexaenoic acid derivatives

A novel series of DHA derivatives are the docosatrienes and resolvins, present in blood, leukocytes, brain and glial cells. They are biosynthesised via the epoxide-containing intermediates and decrease leukocyte infiltration and glial cell cytokine production [36]. Resolvins are produced in the resolution phase of acute inflammation and stop neutrophil entry and reduce exsudate [37]. They are induced by aspirin via the acetylation of COX-2. These derivatives might contribute to the frequently reported beneficial responses obtained by ω3 supplementation [38].

### 6. Fatty acids in cystic fibrosis

#### 6.1. Essential fatty acid status in cystic fibrosis

Freedman and Alvares demonstrated a membrane lipid imbalance in mice with a targeted deletion of the CFTR gene (CFTR −/−) characterized by an increased phospholipid-bound AA and a decreased phospholipid-bound DHA. This imbalance was present in the CFTR expressing organs as lung, pancreas and ileum. By supplementing these CFTR −/− mice with high doses of DHA the membrane lipid imbalance was reversed. The ileal hypertrophy, pathological changes in the pancreas and the pulmonary inflammation disappeared [2]. This observation renewed the interest in old data.

It was known for long that CF patients often have fatty acid deficiencies [39,40]. They display an increase of the monounsaturated fatty acids and a decrease of the ω3 and ω6 PUFA concentrations [41–44]. Although attention was not drawn to the imbalance of ω3 and ω6 PUFA at the time, it is present in the results of Lloyd-Still [45]. Clinical symptoms of EFA deficiency, however, are rare except for the skin manifestations in patients with severe deficiencies.
Studies demonstrated however a lack of correlation between nutritional state, pancreatic function and the fatty acid profile in CF patients as described in the CF mouse model [47].

Strandvik et al. demonstrated a correlation between the genotype and the observed decrease in linoleic acid and DHA [48]. Patients with the more severe mutations had more important lipid imbalances. This is in concordance with the observation that the FA imbalance as described by Freedman [2] is not observed in CF mice models where modified CFTR proteins reached the membrane [49].

It has been demonstrated that supplementation with specific oils can influence the fatty acid profiles in CF patients [34,50,51]. DHA fed as algal triacylglycerol is readily absorbed, incorporated in blood and tissues and well tolerated as proven by recent studies [52]. The clinical relevance however remained obscure until the observations of Freedman [2,47].

6.1.1. Aetiology of EF A deficiency in CF

The initial hypothesis was that the deficiency was secondary to malnutrition, diet and pancreatic insufficiency [39]. Studies demonstrated however a lack of correlation between the nutritional state, pancreatic function and the fatty acid deficiency [41,48,53]. A correlation between genotype and EFA deficiency has been described by Strandvik et al. [48].

As discussed above PUFAs are also used for production of eicosanoids. In contrast to normal patients with EFA deficiency, CF patients develop EFA deficiency in parallel with increased eicosanoid production [20]. This abnormal turnover of EFAs was reported by several groups [54–57]. Carlstedt-Duke et al. hypothesised an increased turnover of AA [56]. They showed defective AA regulation in CF since the release of AA by PLA2 was not blocked by dexamethason. They and other investigators concluded to an increased AA release induced by defective regulation of PLA2 [56–59].

These data along with the observations in CFTR −/− mice led to the speculation that the abnormalities of AA and DHA may be primary in CF [46,56].

However, it has to be considered that these PUFAs are very susceptible for peroxidation and the susceptibility increases with the number of double bonds [60]. Wood et al. demonstrated repeatedly that oxidative stress is increased in CF and there is a strong correlation with plasma fatty acid levels and the consumption of and high fat diets [61,62]. The clinical relevance is not yet clear since high fat diets have proved clinical benefit despite the associated increased peroxidation [63]. Wood et al. were able to improve antioxidant status by supplementation with a high dose of vitamin E, vitamin C, vitamin A, β carotene and selenium but there was no corresponding decrease in oxidative stress or improvement of plasma fatty acid composition [62].

6.2. Phospholipase A2 and CFTR

Pathological regulation of AA release has been suggested by many different research groups [56–59]. It was demonstrated that in different CF cell lines the basal cPLA2 activity was increased but the amount of immunoreactive cPLA2 was identical to the control cell lines. In response to bradykinin only the ΔF508 homozygous CF cells displayed an increase of cPLA2 activity and immunoreactive cPLA2 [64]. By cooling down the cells, which restores the delivery of the CFTR protein to the membrane, the overstimulation by bradykinin of PLA2 disappeared in the ΔF508 homozygous CF cells. This difference between cells with or without CFTR protein was also observed earlier by Levistre et al. [58]. This could at least in part explain the heterogeneity observed between the different genotypes.

6.3. Relation between EFA deficiency and CF symptoms

In animals EFA deficiency is known to cause liver steatosis, increased caloric needs, increased bacterial colonisation and decreased immune response [65]. Symptoms that are very similar to some of the symptoms observed in CF. Recently, a study on EFA status in pre-adolescent children demonstrated a positive correlation between serum LA levels and FEV1 as well as growth status. 20 3o9, a marker for essential fatty acid deficiency, is inversely correlated with growth [66].

As mentioned earlier AA is the major substrate for the eicosanoid synthesis and is the precursor of very potent pro-inflammatory mediators. Multiple studies were able to demonstrate increased prostaglandins [67,68], thromboxanes [20], leukotrienes [69–71] and hydroxyeicosatetraenoic acids derived from AA in CF. They were increased in blood [67], breath condensate [65], saliva [69], sputa [70] and broncho-alveolar lavage fluid [71]. These observations are directly related to the CF symptoms since these pro-inflammatory products are responsible for increased mucus release, neutrophil influx and activation, resulting in additional inflammation. They cause also broncho- and vaso-constriction [17–19,23,24].

Inflammation is present very early in the course of the disease and in absence of bacterial infection [72–74]. Increased inflammation is not only limited to the lungs but can also be demonstrated in the duodenum [75]. This leads to the conclusion that CF patients have a predisposition for inflammation and the defective CFTR contributes directly to the inflammation.

The excretion of prostanoid metabolites in urine is also increased [20] and this is not correlated with colonisation, pulmonary function or genotype. However, a negative correlation with the phospholipid levels of essential fatty acids has been demonstrated [20]. This observation supports the hypothesis concerning the pathological regulation of AA release in CF since this is the rate-limiting step in the prostanoid production.

This knowledge leads to supplementation trials with omega-3 fatty acids resulting in a decrease of the AA derived 5-lipoxygenase products [76].
At last it is known that fatty acids influence electrolyte transports through membranes. AA inhibits the chloride channel currents in CF and normal airway epithelium cells when applied on the cytosolic site of the membrane [77]. It also reduces the surface expression of sodium channels and thereby induces a time dependant inhibition of sodium transport [78].

New knowledge on the role of isoprostanes, formed by free radicals or reactive oxygen species action on free or membrane bound PUFAs [79,80] is gathered. CF patients have increased plasma levels of isoprostanes [81]. They are not only markers of oxidative stress but have physiological effects. 8-iso-prostaglandin E2, a peroxidation product of AA stimulates a transepithelial anion transport mediated by the CFTR Cl⁻ channel [82]. Cowley suggests a direct role for the isoprostanes in pulmonary host defence which can be absent in CF due to the CFTR mutation [83].

6.4. Effects of DHA

DHA supplementation inhibits the delta 6-desaturase and the delta 5-desaturase activity as demonstrated in rat liver cells [84] and by this way result in a decreased production of ω6 PUFAs. Moreover, long chain ω3 FA will compete with AA for incorporation in phospholipids. Administration of DHA will result not only in decreased AA concentration in the phospholipids [84] but also cause a shift in the eicosanoid production to less potent pro-inflammatory or anti-inflammatory products, as explained above. However, there remains controversy in different studies. DHA inhibits in vitro prostaglandin but not leukotriene synthesis [85]. Other in vivo data were able to demonstrate a decreased leukotriene production [76].

DHA also selectively augments muscarinic stimulation of epithelial Cl⁻ secretion [86]. The stimulation was more closely related to the free DHA than to the membrane bound DHA. Freedman et al. also suggested the existence of a block in the biosynthesis of DHA leading to a DHA deficiency in CFTR regulated organs [2], since the supplementation of a DHA precursor resulted in a DHA decrease. This resulted in the conclusion that supplementation should be done with DHA.

Up to now supplementation studies were done with combined EPA and DHA supplements. An 8 month treatment study demonstrated decreased inflammation measured by immunoglobulins, a positive effect on pulmonary function and a decreased antibiotic use [87]. The expected decrease of AA and increase of LA in membrane phospholipids was also present [87].

7. Conclusion

The disturbance of the fatty acid metabolism can explain the intrinsic incapacity of the CF patient to control an environmental challenge and the symptoms as a consequence of this. Although according to the results of Freedman et al. [2] combined EPA and DHA supplementation could be less valuable, positive effects on inflammation and pulmonary function are described [87]. Perhaps supplementation with DHA dominant oils can improve the results? Question remains to what extend one needs to supplement [88] and which patients will have the best benefits of such a supplementation since genotype does seem to play a role [48]. Although there were no side effects observed in the supplementation studies up to now and some clinical improvement has been seen in patients with CF [51,89], there is still insufficient evidence to recommend the routine use of omega-3 fatty acids in CF [90]. New studies using DHA dominant oils with different doses and in different genotypes have to be performed to answer these questions.

References


