ZINC AND ESSENTIAL FATTY ACID STATUS AND SUPPLEMENTATION IN CYSTIC FIBROSIS PATIENTS

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4.2.2 Cystic fibrosis related liver disease and essential fatty acid status

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"La science crée plus de mystères qu’elle n’en éclairet."
Gustave Le Bon
Chapter 1: Introduction

1.1 Introduction

Although respiratory failure remains the main cause of death, gastro-enterological and nutritional issues play an important role in cystic fibrosis care. It is well documented that improved nutritional status contributes to improved outcome and survival of children with cystic fibrosis [1–3]. Normal body weight is associated with better lung function and a slower decline in forced expiratory volume in 1 second (FEV1) in all age groups [2], and it is an independent predictor of mortality and survival in cystic fibrosis [3].

Treatment of pancreatic insufficiency, leading to severe malnutrition, became possible with the availability of exocrine pancreatic enzyme replacement therapy. Despite the improvement of enzyme preparations there are still patients suffering from malabsorption [4].

The ultimate aims of improving nutritional status in children are the achievement of normal weight gain and growth, prevention of pubertal delay, and slowing-down the rate of clinical decline with dietary advice and therapy, optimisation of the deficient essential fatty acid, vitamin and mineral status. Dietary recommendations are based on the need to compensate for the increased energy needs of infection and the increased energy cost of breathing. Guidelines state that individuals with cystic fibrosis should achieve 120% to 150% of the age adapted recommended daily allowances for energy (40% of which should be derived from fat) [5].

For the prevention of fat soluble vitamin deficiencies, it is generally accepted to use supplements [5]. Other micronutrients such as zinc and essential fatty acid are also influenced by malabsorption, but no consensus on supplementation exists currently. The debate on the necessity of other supplements continues, and thus these studies were performed to increase the understanding of the factors influencing the zinc and fatty acid status, and the possible effects of supplementation on the clinical evolution of cystic fibrosis.
1.2 Cystic Fibrosis

1.2.1 Genetics

Cystic fibrosis is an autosomal recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator gene localised on the long arm of chromosome 7. Until now more than 1300 disease associated mutations have been registered by the cystic fibrosis genetic analysis Consortium database (www.genet.sickkids.on.ca/cftr/). The mutations of the cystic fibrosis transmembrane conductance regulator gene are classified in relation to the properties of the cystic fibrosis transmembrane conductance regulator protein (fig. 1) [6]. The effect on the cystic fibrosis transmembrane conductance regulator protein of some of the mutations remains unknown.

*Class I* mutations produce premature transcription termination signals resulting in unstable, truncated or no protein expression.

*Class II* mutations, usually mis-sense mutations including ΔF508, cause the protein to misfold leading to premature degradation and failure to reach the apical cell membrane.

*Class III* mutations are primarily located in the two nuclear-binding domains and result in an unactivable cystic fibrosis transmembrane conductance regulator protein.

*Class IV* mutations are primarily located in the membrane spanning domains that form the chloride channel and result in reduced chloride conductance.

*Class V* mutations result in reduced amounts of functional protein due to abnormal or alternative splicing.

![Fig.1: The cystic fibrosis transmembrane conductance regulator genotypes have been divided into five broad classes based on the impact of the cystic fibrosis transmembrane conductance regulator transporter molecule [6].](image-url)
The cystic fibrosis transmembrane conductance regulator protein after proper folding, glycosylation and insertion into the membrane consists of different transmembrane segments, nucleotide binding folds and regulatory domains (fig. 2).

**Fig 2:** Predicted structure of the cystic fibrosis transmembrane conductance regulator protein once integrated in the cell membrane

The cystic fibrosis transmembrane conductance regulator protein is not only responsible for chloride transport but also for efflux of glutathione and glutathione adducts as glutathione thiocyanate [7-9]. It regulates other Cl⁻ channel proteins [10], is responsible for acidification of intracellular Golgi and vesicles, vesicle trafficking [11] and membrane phospholipid composition [12]. Although the gene locus is known since 1989, the search for a genotype phenotype correlation has not been very successful [13]. It has been possible to predict the severity of the organ-level phenotype from the genotype with respect to the sweat test, pancreatic function and reproductive system. However, predicting the severity of the lung disease is still not possible.

Cystic fibrosis is more common in Caucasian populations [14]. A survey of the newborn screening for cystic fibrosis in Europe reveals 1 affected infant in 4000 screened infants [15]. The estimated carrier frequency is near to 5%.

### 1.2.2 Clinical presentation

CF is a multi-organ disease with a highly variable involvement of the lung, pancreas and other organs. The presenting symptoms are acute or persistent respiratory symptoms (51%), failure to thrive or malnutrition with steatorrhoea (43%). Meconium ileus is the presenting symptom in 19% of the children with cystic fibrosis.

**Respiratory tract:** The mucus lining the airways consists of high weight mucins, whose properties change according to the water content, ion concentrations and pH. Cystic fibrosis transmembrane conductance regulator protein regulates Cl⁻ transport across fluid transporting epithelial cells. This leads to altered secretions blocking the gland ducts and reducing the non-inflammatory defence of the respiratory tract. This results in infection, inflammation and subsequently lung damage.
Cough is one of the most constant symptoms. It can vary from a dry to a productive cough. The expectorated mucus is usually purulent and viscous.

Patients with cystic fibrosis are particularly sensitive for pseudomonas aeruginosa colonisation. This micro-organism forms biofilms leading to persistent infection of the adherent mucus. In these plaques the bacteria are relatively protected from host defence mechanisms [16]. The biofilm will probably also deplete the infected mucus from oxygen resulting in changed antibiotic sensitivities of the bacteria [17].

The chronic inflammation of the lung will further disrupt its structure. The results are cor pulmonale, respiratory failure and finally death. Since intensive pulmonary treatment with antibiotics, inhalation therapy and physiotherapy was installed, life expectancy dramatically increased.

Sinusitis, rhinorrhea and nasal polyposis are also often observed.

Although respiratory failure is the final cause of death these patients have many gastro-intestinal symptoms.

**Pancreatic Disease:**

More than 90% of the patients suffer from **exocrine pancreatic insufficiency**. These patients have an insufficient excretion of pancreatic enzyme and bicarbonate leading to maldigestion. This results in bulky, shiny stools due to the nutrient loss. Although some children compensate their losses with a voracious appetite, it usually results in failure to thrive. As a result of the fat-soluble vitamin losses, deficiency syndromes can also occur. The distinction between sufficient and insufficient pancreatic function is important for treatment [18].

The few patients with pancreatic sufficiency can suffer from recurrent episodes of acute pancreatitis [19]. The older cystic fibrosis patients are at risk of developing **cystic fibrosis related diabetes mellitus**. The aetiology is not yet completely understood but it is associated with a significantly increased mortality [20]. Since respiratory function and nutritional status deteriorate 2 years before the cystic fibrosis related diabetes mellitus becomes overt, many cystic fibrosis centres started to perform systematic oral glucose tolerance tests. Early diagnosis and management of this problem should improve the outcome.

**Gastrointestinal tract:**

Cystic fibrosis patients often suffer from **gastro-esophageal reflux** symptoms and many develop reflux esophagitis [21, 22]. This multifactorial problem is linked to the severity of the lung disease. However, lung physiotherapy and drugs relaxing the lower oesophageal sphincter are further contributing factors. Delayed gastric emptying and inappropriate lower oesophageal sphincter relaxation will also aggravate the reflux [23]. In some patients, gastro-oesophageal reflux results in anorexia, vomiting and promotion of failure to thrive.

**Meconium ileus**, a condition where neonates fail to pass meconium, is the earliest clinical sign of cystic fibrosis in 10 to 19%. It causes neonatal gastro-intestinal obstruction and sometimes leads to intestinal perforation.

The aetiology of **distal intestinal obstruction syndrome** later in life remains unclear. The syndrome is characterised by impacting stools in the terminal ileum, caecum and proximal colon. It results in a partial or complete intestinal obstruction. Whether nutrition is of importance in this syndrome has not been proved [24].

**Rectal prolapse** is often a presenting symptom in CF. It is attributed to the passage of large bulky, frequent stools, malnutrition and increased intra-abdominal pressure during cough [25]. It improves generally after the initiation of pancreatic enzyme replacement therapy.
Malnutrition:
Cystic fibrosis patients are at constant risk of developing malnutrition due to malabsorption, decreased appetite at pulmonary exacerbations and increased losses due to vomiting associated with cough bouts and gastroesophageal reflux. However, there is also increasing energy expenditure while pulmonary function declines (fig. 3) [26]. Malnutrition dramatically impairs the prognosis in cystic fibrosis [27, 28].

**Pathogenesis of energy imbalance in CF**

Fig 3: Interdependent factors that may give rise to progressive energy deficit and weight loss as pulmonary function deteriorates. From Durie and Pencharz [26].

In pancreatic insufficiency fat soluble vitamins can be deficient due to the fat malabsorption. The evaluation of the importance of biochemical deficiencies is difficult since for most patients there are no overt vitamin deficiency symptoms. Vitamin levels are often difficult to correct despite vitamin supplements and pancreatic enzyme replacement therapy [29]. Disturbances in vitamin metabolism and increased needs due to enhanced oxidative stress in cystic fibrosis patients can also contribute to the biochemical deficiencies [5]. Vitamin A is generally known for its retinal function. However, it plays also important roles in maintenance of epithelial cell integrity. The serum concentrations in cystic fibrosis are often low regardless of age or pancreatic function [29]. There is a poor correlation between biochemical and clinical findings and the presence of low serum concentrations in patients without steatorrhoea suggest a disturbed vitamin A metabolism. However, five out of ten cystic fibrosis patients with low serum vitamin A concentrations were found to have disturbed dark adaptation, responding to increased vitamin A supplements and sometimes even xerosis is reported [30, 31]. A significant association between pulmonary function and vitamin A has been demonstrated. [32]. Vitamin E plays a crucial role as antioxidant. It is reported to be low in many cystic fibrosis patients and is also difficult to correct
Although there are no clinical symptoms of deficiency, vitamin E status is also associated to pulmonary function [34]. A recent study demonstrated a relation between the number of pulmonary exacerbations and the serum vitamin A and E levels even when serum values were considered to be normal, regardless of pancreatic function [35]. 25 OH-cholecalciferol concentrations are decreased in infants with cystic fibrosis diagnosed by screening due to decreased vitamin D-binding protein [33, 36]. This finding suggests that the hypovitaminosis D in cystic fibrosis might be mainly a transport problem which should be treated with sufficient sunlight exposure. Vitamin K acts not only as a cofactor in the coagulation but is also important in bone formation. Deficiencies are common in unsupplemented pancreatic insufficient patients, but also in the presence of liver disease or intestinal resections [37].

The water soluble vitamins are not appreciated as a problem. Vitamin C is related to age and inflammation [38] but its antioxidative role needs further investigation. Only the cystic fibrosis patients with extensive resection of the terminal ileum will need parenteral vitamin B12.

From the minerals, sodium and chloride loss can cause hypochloremic alkalosis. Basic needs in cystic fibrosis patients are higher since they have an increased loss through sweat production. Decompensations occur during increased sweat loss or gastroenteritis. Calcium is important for bone mineralization and its absorption in cystic fibrosis is impaired due to vitamin D deficiency and fat malabsorption. Plasma calcium levels and urinary excretion are reported to be normal [39]. On the other hand, many older cystic fibrosis patients have decreased bone mineral density and bone content and even suffer from spontaneous fractures [40]. The data on the trace elements in cystic fibrosis are rather scarce. Iron deficiency can be present in cystic fibrosis due to inadequate intake, malabsorption, chronic infection and increased losses. There is insufficient data about copper in cystic fibrosis to make any comment. Selenium is an essential part in the anti-oxidant defence. Data on the selenium status in cystic fibrosis patients are also scarce. The enzyme preparations contain sufficient selenium to influence the glutathione peroxidase activity [41]. Zinc needs are 5-10 mg/day in children and up to 15 mg/day for adults. A subclinical zinc deficiency is suspected to be wide spread in cystic fibrosis due to the formation of complexes with the unabsorbed fat. Pancreatic enzymes replacement therapy improves the zinc absorption. This thesis gives extra data on the zinc status in cystic fibrosis patients.

Patients with cystic fibrosis have an increased risk for essential fatty acid deficiency [42 – 46]. They tend to have low parent fatty acids linoleic acid and α-linolenic acid. Most studies reveal reduced levels of omega-3 long chain polyunsaturated fatty acids but the results for the omega-6 long chain polyunsaturated fatty acids the results differ. As it is the case for the vitamins, it is a biochemical deficiency of which the importance needs further research. Some extra data will be provided in this thesis.

Liver:

In the liver, cystic fibrosis transmembrane conductance regulator is expressed primarily on the apical surface of the biliary epithelium. The location and the characteristic pathology suggest that altered ductular secretion results in concentrated viscous bile with subsequent plugging and inflammation. The resulting lesion is called focal biliary cirrhosis [47]. The factors that initiate, accentuate, and perpetuate the development of liver disease have not been identified. Cirrhosis complicates cystic fibrosis in 1.4% of patients, with a peak frequency of 2.7% in the age group 16 to 20 years [48]. Liver disease is silent until portal hypertension and its complications occur. The virtually unique
focal nature of the fibrosis in cystic fibrosis related liver disease may explain the divergence in timing between presentation with variceal hemorrhage and decompensation of hepatocellular function. Only a few cystic fibrosis related liver disease patients develop liver failure.

**Sweat glands:** Excessive salt loss in the sweat predisposes young children to salt depletion during episodes of warm weather or gastroenteritis. This results in a hypochloremic alkalosis [49].

**Genitourinary tract:** Sexual development is often delayed, by an average of 2 years. Almost all males with cystic fibrosis are azoospermic because of congenital bilateral absence of the vas deferens [50]. It seems that congenital bilateral absence of the vas deferens develops with time because of inspissation of secretions and secondary atresia. In cystic fibrosis fetuses, the vas deferens appears similar to that of normal controls but with secretions filling the mid vas [51]. Women can experience secondary amenorrhea in situations of malnutrition or pulmonary exacerbation.

**Bone disease:** There is an increased frequency of spontaneous fractures with age due to osteoporosis. This is probably the result of combined problems as delayed puberty, malabsorption of vitamins and calcium, malnutrition, chronic inflammatory state and the steroid therapy, sometimes necessary for treatment of lung disease [52].

### 1.2.3 Diagnosis

The diagnosis is based on a clinical suspicion and confirmed by a sweat test. However, since the knowledge on the genetic defect, there are also patients with an atypical clinical picture diagnosed as cystic fibrosis. The use of a diagnostic flow chart becomes necessary (fig 4) [53].

**Sweat testing** using the pilocarpine iontophoresis to collect sweat and chemical analysis of its Cl⁻ content is still the golden standard for the diagnosis of cystic fibrosis. For a reliable test at least 100 mg sweat should be collected. Cl⁻ content above 60 mmol/L confirms the diagnosis of cystic fibrosis. A repeated Cl⁻ content between 30 and 60 mmol/L needs further confirmation by other methods [53].

**Genotype:** First screening is based upon commercial available assay kits which screen for about 30 mutations. (INNO-LiPA CFTR19 ®, INNO-LiPA CFTR17+Tn Update ® kit, Innogenetics N.V.) Their majority is associated with classical cystic fibrosis presentations. With borderline sweat test results extensive screening of both cystic fibrosis transmembrane conductance regulator genes can be necessary. This can be done by other assay kits or by DNA sequencing. The latter is the only test which will approach a sensitivity of 100%.

**Nasal potential difference:** The potential difference is measured between a fluid filled exploring bridge on the nasal mucosa and a reference bridge on the skin of the forearm. The basal potential difference, the effect of amiloride, Cl⁻ free solution and isoproterenol are measured. The nasal potential difference of a patient with cystic fibrosis is much higher, the amiloride response is exaggerated and there is very little or no response to Cl⁻ free and isoproterenol solutions [53].
Cystic fibrosis transmembrane conductance regulator bioassays: They measure the epithelial ion fluxes or their resulting voltage potential at the mucosal surface. These assays provide a direct view of the physiology at the cellular and the ion channel level. The tests can be done on respiratory or intestinal epithelium [53].

Fig. 4: Algorithm for the diagnosis of cystic fibrosis starting with the sweat test. (According to the diagnostic working group) [53].

Neonatal screening is based on the immunoreactive trypsinogen assay, which is relatively inexpensive and adaptable to large numbers [54]. Increased immunoreactive trypsinogen concentrations at birth are characteristic of newborns affected by cystic fibrosis, but there are many false positive results. However, in cystic fibrosis, immunoreactive trypsinogen values tend to remain raised for several months. To improve the specificity of neonatal screening, a second blood sample is obtained in neonates with raised levels of immunoreactive trypsinogen at birth, and only infants with persistently raised immunoreactive trypsinogen values progress to a sweat test.

Nowadays, the second immunoreactive trypsinogen test is often replaced by analysis of a panel of cystic fibrosis causing mutations in the neonatal blood sample [55]. Homozygotes and compound heterozygotes have cystic fibrosis (a confirmatory sweat test is anyway desirable). In babies carrying only one mutation a sweat test is performed in order to distinguish affected individuals from carriers.
There is up to now no systematic screening program in Belgium.

1.2.4 Gastrointestinal and nutritional therapy

Over the last decades, nutritional problems gained a high priority in the management of cystic fibrosis since the nutritional status is associated with pulmonary function [1, 2, 27, 28]. The objective of the therapy is to obtain normal growth and to prevent pubertal delay. To achieve these goals cystic fibrosis patients are advised to take a high-fat, high-energy diet aiming at a caloric intake of 120% - 150% of the recommended dietary allowances [5]. To improve the nutritional absorption, pancreatic enzyme replacement therapy is added in pancreatic insufficient patients. Dietary supervision and pancreatic enzyme replacement therapy are the corner stones of nutritional therapy [5]. Most of the sodium losses can be compensated by increased dietary sodium intake. Only in case of increased losses supplements might be needed.

Despite correct pancreatic enzyme replacement therapy some patients struggle with malnutrition due to insufficient caloric intake, persisting losses or increased needs [26]. The vicious circle of malnutrition can sometimes be stopped by the use of overnight tube feeding [56].

Most centres prescribe larger monitored doses of fat-soluble vitamins to all pancreatic insufficient patients [5]. The advised supplements are 4000 to 10000 IU vitamin A, 400 to 2000 IU vitamin D and up to 400 IU vitamin E a day and 5mg vitamin K a week. The effect of supplementation should be monitored.

For other systematic supplements there are up to now no undeniable evidences. It is advised to give a zinc supplement in case of continuing fat malabsorption [5]. The sufficient intake of anti-oxidantia are recommended [57].

Gastro-oesophageal reflux needs treatment with histamine2-receptor antagonists or proton pump inhibitors. These substances will, besides decreasing the acid reflux, also increase the duodenal pH. A duodenal pH above 5 is necessary to dissolve the pancreatic enzyme replacement therapy coating allowing the enzymes to initiate digestion. In this way they contribute to digestive processes [58].

In distal intestinal obstruction syndrome episodes, surgery should be avoided and the obstruction should be treated with polyethylene glycol solutions, gastrografin ® or N-acetylcystein [59]. It is not yet clear how to prevent reoccurrence of this problem. Increased water intake or lower doses polyethylene glycol solutions have been suggested [60].

No specific therapies are known for cystic fibrosis related liver disease. The presumed underlying hyperviscous secretions suggest a rationale for an attempt of decreasing bile viscosity or also to replace hepatotoxic bile acids. Ursodeoxycholic acid showed promising results in cystic fibrosis related liver disease [61]. However, its long-term benefit, particularly in preventing cirrhosis, remains to be determined. Since portal hypertension is by far, the most common significant manifestation of cystic fibrosis related liver disease, treatments focusing on its complications are commonly used [62]. Liver transplantation has also a role in the management of end-stage liver disease of cystic fibrosis. However, the data presented by Gooding et al. suggest that it should be limited to the patients with evidence of true hepatocellular dysfunction and failure [63].
1.3 Studied micronutrients: zinc and essential fatty acids

1.3.1 Zinc

Zinc is special among individual nutrients since it has been designated as a “problem” nutrient of which adequate intake is difficult to obtain from complementary foods without fortification [64]. Of all the oligoelements zinc is second only to iron in total body content. The adult body contains about 2 g zinc [64]. It is located evenly throughout the body. Zinc is a component of hundreds of zinc metalloproteins.

The functions of zinc can be classified into 3 categories: catalytic, structural and regulatory [65]. It plays important roles in enzymes, transcription factors, hormonal receptor sites and biological membranes. It has numerous roles in DNA and RNA metabolism [66] and therefore plays an important role in cell proliferation, differentiation, growth and apoptosis [67, 68]. Zinc finger motifs, a reoccurring pattern of amino acids with conserved residues of cysteine and histidine where zinc binds, have been discovered in over 3 % of all identified human genes [69]. In animals zinc deficiency has shown to affect the skin, the gastrointestinal, immune, respiratory, skeletal and reproductive systems [70] (Fig 5).

**Fig 5: Different functions of zinc in humans (according to Salguiero et al.)[71]**

Dietary zinc is mainly found in meat (Fig 6). The presence of animal protein will, besides increasing the absolute amount of zinc, also promote its absorption [72]. However, absorption will be decreased by high dietary content of phytic acid and fiber [72], present in varying amounts in vegetables and grains. Unabsorbed fatty acids chelate zinc ions, making its absorption impossible [73]. The interactions between zinc and other minerals remain another concern. There is probably a negative interaction with iron and also calcium [74].

Zinc interferes with the absorption of copper [75]. In future zinc supplementation studies, attention should be paid to the influence of zinc supplementation on the copper status.
The exact absorption mechanisms have not yet been completely characterised in humans. Since it has such important physiologic functions, the zinc levels are highly regulated (Fig 7) \[77\]. Different zinc transporting transmembrane proteins have been identified. The transmembrane zinc transporters of the Zip-family increase the intracellular concentrations by promoting zinc influx into the cells or release from the intracellular vesicles \[78\], while the cation diffusion facilitator- family (zincT transporters) mobilises zinc in the opposite direction \[78\].
The zinc balance is primarily regulated in the intestine [74, 79] by excretion and reabsorption of endogenous zinc. Urinary zinc loss is low [80]. It has been suggested that the absorption of zinc is mediated by a pancreatic ligand, enhancing jejunal zinc uptake [81, 82]. ZIP4, localised in the apical membrane of the enterocyte, is the main importer of dietary zinc [83]. ZIP1 may function as a back-up system in the dietary zinc uptake [78]. ZincT-1 is the only member of the zincT family localised in the basolateral membrane of enterocytes and renal tubular cells, indicating the zinc efflux responsibility of the protein [84]. Potential sources of endogenous zinc are the pancreatic, biliary and gastrointestinal secretions and the transepithelial flux or sloughing of mucosal cells. The excretion is directly related to the total amount of absorbed zinc.

The only known inborn error of zinc metabolism is Acrodermatitis Enteropathica. It is an autosomal recessive disease, caused by a mutation in the SLC39A4 gene located on 8Q24 [85]. This gene encodes for the ZIP4 transporter. Patients have difficulties with the intestinal zinc absorption resulting in zinc deficiency [86].

The evaluation of the zinc status is frustrating, due to a paucity of adequate biomarkers. Small decrements in tissue zinc are associated with zinc-deficient morbidity and impaired development. Furthermore the homeostatic mechanisms are very effective in maintaining tissue and circulating serum zinc concentrations. Finally, many biological aspects including gene expression and different aspects of cellular growth and replication depend on zinc. This makes the interpretation of the zinc status difficult. Most of the body zinc is located in the fat-free intracellular mass and only 0.2% circulates in the plasma, associated with albumin and α2-macroglobulin [87, 88].

Serum zinc concentration is the most widely used and accepted marker despite its poor sensitivity and imperfect specificity [88]. They are maintained within strict limits even if the zinc intake differs dramatically. Decrease of serum zinc will therefore only be seen if the zinc depletion is prolonged and severe [89, 90]. Attention to a number of factors can reduce the difficulties with interpretation [88]. Diurnal serum zinc concentrations cumulatively decrease after meals [91]. Zinc concentrations are age related and gender differences are observed in some studies [92]. Infection and inflammation cause a decrease of serum zinc [93].

Although the interpretation of serum zinc has its limitations, it remains a useful indicator of a population zinc status. A population has an elevated risk of zinc deficiency when a higher than expected percentage of the population has low serum zinc concentrations [94]. The Belgian children can be at risk for the development of zinc deficiency since 13% of the population [95] has a serum zinc below the -2 standard deviation of the American NHANES II study [92].

The activity of zinc metalloenzymes were expected to be a possible surrogate marker of the zinc status. Although there is some evidence for the utility of enzyme essays such as alkaline phosphatase, copper-zinc superoxide dismutase and lymphocyte 5’-nucleotidase, the attempts to confirm the results often failed [88].

The most reliable data on the epidemiology of human zinc deficiency has been derived from double blind placebo controlled supplementation studies [88].
1.3.2 Essential fatty acids

Fatty acids are divided in saturated, monounsaturated and polyunsaturated fatty acids depending on the number of double bonds in the carbon chain. Long chain fatty acids contain at least 16 carbon atoms. De novo synthesis of fatty acids in the human liver produces mainly palmitate (16:0) and minor amounts of stearate (18:0). Many cells have the capacity for 2-carbon chain elongation of fatty acids which takes place in the endoplasmatic reticulum.

All eukaryotic organisms contain polyunsaturated fatty acids in their membrane lipids. Many tissues can modify the acyl chain composition by introducing double bonds by means of desaturases (Δ5, Δ6, Δ9). The Δ9-desaturase is the predominant, if not exclusive, desaturation enzyme for saturated fatty acids. The desaturation of stearate (18:0) by Δ9-desaturase is the rate limiting step in the formation of oleic acid (18:1n-9). Δ9-desaturases respond quickly to dietary alterations. Dietary polyunsaturated fatty acids, particularly linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), selectively inhibit monoene formation, more rapidly than they influence de novo fatty acid synthesis.

In 1930 the essentiality of certain polyunsaturated fatty acid became clear. Animals fed a rigid fat free diet developed growth retardation, dermatitis and infertility. Supplementation with small amounts of polyunsaturated fatty acid induced growth promotion and prevented dermatitis [96].

The essential fatty acids are devided in 2 families the n-6 and n-3 series depending on the place of the first double bond. They are essential since both families are needed for normal development and biological function and cannot be synthesised de novo by humans [97]. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are referred as the parent fatty acids since they can be elongated and desaturated to form longer-chain, more unsaturated fatty acids (fig 8). A number of data from in vivo studies indicate the essentiality of long chain polyunsaturated fatty acid in early life [97]. The fatty acid of the n-6 and n-3 series compete for the same enzymes of elongation and desaturation, thereby decreasing the polyunsaturated fatty acid production of the competing fatty acid.

![Fig 8: Metabolic pathways converting 18-carbon unsaturated fatty acids into long chain polyunsaturated fatty acids.](image-url)
The source of linoleic acid (18:2n-6) is mainly green vegetables, eggs and cereals. α-linolenic acid (18:3n-3) is obtained from some plant oils as flaxseed, canola and nuts but also from herbs and purslane. The long chain polyunsaturated fatty acids can also be absorbed from nutrition. Docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3) are found in fish oils and arachidonic acid (20:4n-6) is found in meat. (Fig 9)

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![Fig 9: fatty acid composition according to prof. Olivier Pradier.](image)

Fatty acids have **multiple functions**. Depending on the type of fatty acid they will preferably be used for one or other function.

Fatty acids as component of triglycerides or phospholipids are the major source of energy production and storage. The stepwise degradation of fatty acid is called the β-oxidation. During zinc or energy deficit normal accumulation of essential fatty acid can be impaired due to increased β-oxidation [98].

They also become part of **biomembranes** by esterification to complex lipids. The fatty acid tails of the phospholipids are responsible for the apolar nature of membrane bilayers. Fatty acid as arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are important structural fatty acids in neural tissue as the brain and retina. The amount of polyunsaturated fatty acid in the membrane determines membrane fluidity. 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 [99]. They contribute to the control processes of nuclear transcription via special receptors and response elements [100, 101]. Polyunsaturated fatty acids, released by agonist stimulated phospholipase A₂, are involved in signal transduction [102].

Some polyunsaturated fatty acids are precursors of biologically active molecules such as **eicosanoids** and **docosanoids**. After release from the membrane by phospholipase A₂, they enter the pathway of the cyclooxygenases resulting in prostaglandins and thromboxanes or in the pathway of the lipoxygenases resulting in leukotrienes and lipoxines (Fig 10). These are important regulating elements in inflammation.
prostaglandin2 series, derived from arachidonic acid (20:4n-6), induce inflammation and muscle contraction while the prostaglandin3 series, derived from n-3 fatty acids, will have an anti-inflammatory effect [103]. Different fatty acids compete with each other for the cyclooxygenases and lipoxygenases. The balance between the different polyunsaturated fatty acids in the cell membranes will therefore influence the severity of the inflammation.

**Fig. 10:** Arachidonic acid is released by the action of phospholipase A2 on membrane phospholipids. Phospholipase A2 is activated by multiple cell signals, bradykinin, angiotensin II. Some derivatives of alpha-linolenic acid inhibit the release of arachidonate by phospholipase A2 and result in antagonistic actions. The three main directions of the metabolism of arachidonic acid are cyclooxygenation, lipoxygenation and epoxygenation. The cyclooxygenase products of arachidonic acid generate prostaglandins, prostacyclin and thromboxanes of the 2 series. Lipoxygenase products of arachidonic acid 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, N-terminal HETEs and epoxyeicosatrienoic acids (EETs).

For the assessment of the **essential fatty acid status** of an individual, the total amount of essential fatty acid in blood plasma is a useful indicator [104]. Phospholipids and cholesteryl esters have a slow turnover in contrast to free fatty acids and triglycerides that are very dependent on recent nutrition. Furthermore, phospholipids are the major structural components of membranes and the fatty acid pattern of serum phospholipid reflects that of tissue phospholipid [105]. The essential fatty acid profile of serum phospholipids or red blood cell phospholipids is considered suitable to document the overall essential fatty acid status of a given individual [106].

When insufficient essential fatty acids are available to meet the requirements of the body, the human organism starts to synthesise fatty acids, which are hardly present under normal conditions. Under these conditions oleic acid (18:1n-9) will be desaturated and elongated to form Mead acid (20:3n-9) and di-homo-Mead acid (22:3n-9). The presence of Mead acid (20:3n-9) is an indicator of a generalised shortage of the parent essential fatty acid and their derived long-chain homologues. The trienoic/tetraenoic ratio (20:3n-9/20:4n-6) was proposed as an index of essential fatty acid deficiency [107]. In essential fatty acid deficiency the carbon recycling of linoleic acid (18:2n-6) for de novo lipogenesis remains quantitatively important [98]. On the other hand, essential fatty
acid deficiency will induce an increased activity of the Δ5- and Δ6-desaturases resulting in an increased production of arachidonic acid (20:4n-6) [108]. It seems that maintaining adequate arachidonic acid tissue levels is more important than restoring its linoleic acid (18:2n-6) levels [98].
1.3.3 Interactions between zinc and essential fatty acids

The symptoms of zinc deficiency and essential fatty acid deficiency, which include growth retardation, delayed sexual maturation, infertility, dermal lesions, alopecia and decreased rate of wound healing, show remarkable similarities. This evoked the possibility of a mutual interaction between zinc and essential fatty acid [109].

The presence of fatty acid abnormalities in acrodermatitis enteropathica, an inherited zinc absorption disease, and in transient zinc deficiencies sustains this theory [110-112]. In these patients the increased serum phospholipid linoleic acid (18:2n-6) and decreased serum phospholipid arachidonic acid (20:4n-6) argues for a role of zinc in the linoleic acid (18:2n-6) metabolism.

Hamilton reports on the relation between serum zinc and essential fatty acids in cystic fibrosis [113]. He observed a positive association between serum zinc and arachidonic acid (20:4n-6), 20:3n-6 and the arachidonic acid (20:4n-6)/linoleic acid (18:2n-6) ratio [113].

The specific function of zinc in fatty acid metabolism is still not fully understood. It is probably the result of both direct modulation of the desaturase activities involved in the fatty acid metabolism and an indirect effect by influencing absorption, oxidation and incorporation of the fatty acids [109].

The major problem in animal studies is the complete loss of appetite in zinc deficiency which further negatively influences the results [114, 115].

Cunnane postulated that zinc had an influence on the desaturation of LA (18:2n-6), based on increased linoleic acid (18:2n-6) and reduced arachidonic acid (20:4n-6) concentrations in various tissues of the zinc-deficient rats [115-117]. This hypothesis is sustained by decreased Δ-5 and Δ-6 desaturase activity in various tissues of zinc-deficient rats [118, 119]. Others disagreed, however [111, 120]. Eder et al. observed that the other metabolites of desaturases such as eicosapentaenoic acid (EPA) (20:5n-3), 22:4n-6 and 22:5n-6 were not significantly different, or sometimes increased [121]. This could argue against a direct influence on desaturase activity.

In various tissues the effect of zinc deficiency was completely different. In contrast to the general decrease of Δ-6 desaturation in zinc deficiency, an increased Δ-6 desaturation was described in mammary tissue of zinc deficient rats [122]. The Δ-9 desatrases of liver and testes react oppositely on hormonal stimulation [123]. This could reflect the specific tissue function differences.

To complicate the picture even more, at least 2 different Δ-9 desaturases have been described, with a tissue specific expression [123]. In transfected rodent hepatic cells at least two different Δ-6 desaturases with different kinetic properties were demonstrated [124]. Until now these findings have not been confirmed in human tissues (Cunnane, personal communication 17/9/07).

Others have postulated that zinc would influence the incorporation of fatty acid into the phospholipids, explaining the differences observed in zinc-deficient rats depending on diet [125].

Zinc deficient rats also have an increased β-oxidation of LA (18:2 n-6), resulting in decreased amounts of linoleic acid (18:2n-6) for metabolisation into arachidonic acid (20:4n-6) [126]. Finally, fatty acid and zinc in the diet of rat pups mutually influence the intestinal absorption [127]. Addition of linoleic acid (18:2n-6) to the diet of zinc deficient pups improves the absorption of zinc [127].
Chapter 2: Research objectives

Our goals are to achieve normal growth, weight gain and prevention of pubertal delay with dietary counselling, administration of nutritional supplements, enteral and parenteral nutrition. A further goal is to assess the effects of direct nutritional intervention on the fundamental mechanisms of cystic fibrosis pathology.

The first part of this research focuses on the possible presence of Zinc deficiency. However, the assessment of zinc deficiency is tedious. We determined serum zinc concentration, a generally accepted approach in these kinds of studies. We looked for possible interference of zinc deficiency on some zinc dependant enzyme systems and finally looked at the influence on clinical condition of zinc supplementation. We approached these problems in 4 different chapters. The main questions raised are:

1. What is the normal serum zinc concentration in children? To be able to compare serum zinc in cystic fibrosis to healthy controls a reference frame of normal local serum zinc values in children is needed.
2. Is the Serum zinc concentration in cystic fibrosis different from controls? Is it influenced by age or treatment? Serum zinc concentrations were evaluated at diagnosis, after one year of treatment and at an older age.
3. Is the presence of zinc deficiency reflected in the activity of zinc dependant enzymes in cystic fibrosis patients?

The second part deals with the unexplained abnormalities of essential fatty acid composition in cystic fibrosis. Beside transgenic animal experiments very little is known about the influencing factors and clinical effect of essential fatty acid supplementation in cystic fibrosis.

1. Factors influencing the essential fatty acid distribution in cystic fibrosis: nutritional status, pancreatic function, liver disease, genotype.
2. Effect of essential fatty acid supplementation on serum fatty acid composition, pulmonary function, infections, parameters of inflammation
Chapter 3: Zinc

3.1 Importance of zinc for cystic fibrosis patients

Importance of Zinc in childhood applied to Cystic Fibrosis patients.
Stephanie Van Biervliet, Jean-Pierre Van Biervliet, Eddy Robberecht
Cystic Fibrosis Centre, Ghent university hospital

Abbreviations
Zn zinc       AA arachidonic acid
CF cystic fibrosis       ALP Alkaline phosphatase
CFTR cystic fibrosis transmembrane conductance regulator
PERT pancreatic enzyme replacement therapy
IGF-1 insulin-like growth factor-1
EFA essential fatty acid
FA fatty acid

Key words
Zinc, cystic fibrosis, fatty acids, growth, immunity, oxidative stress

Abstract
Zinc (Zn) is a multipurpose trace element, which is difficult to assess. The factors interfering with Zn absorption are discussed below. Zn deficiency leads to taste disturbances, decreased appetite and impaired growth. Due to interference in different aspects of the fatty acid handling, Zn deficiency may lead to disturbed fatty acid profiles. Zn plays an important role in immunity, inflammation and oxidative stress defence, being extremely important for the lung.

All these aspects are discussed below and applied to what is known in cystic fibrosis.

Introduction
Zinc is notable for being designated as the “problem” nutrient, since its adequate intake is difficult to obtain (1). From all oligoelements, Zn is second to iron in total body content and is located evenly throughout the body. It is a component of hundreds of Zn metalloproteins or Zn-binding proteins. The functions of Zn are organised into 3 categories: catalytic, structural and regulatory (2,3). It plays important roles in enzymes, transcription factors, hormonal receptor sites and biological membranes. Numerous roles of Zn in DNA and RNA metabolism are known (3). Therefore, it plays an important role in cell proliferation, differentiation, growth and apoptosis (4,5). Zn finger motifs, a reoccurring pattern of amino acids where Zn binds, have been discovered in over 3% of all identified human genes (6).

Because so many enzyme systems and tissues are affected, symptoms of Zn deficiency are general. They include anorexia, diminished sense of taste, growth retardation, immune dysfunction, infertility, impaired wound healing and other skin changes.

Cystic Fibrosis (CF), an autosomal recessive disease is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR primarily functions as a chloride channel in the apical membrane of the respiratory and gastrointestinal tracts. Other CFTR functions are: regulation of distinct chloride channel proteins (7,8), Golgi acidification (9) and control of vesicle trafficking (10). It has also been suggested that the CFTR may regulate membrane phospholipids composition (11). Further on, the efflux of glutathione and glutathione adducts as glutathione thiocyanate is regulated by CFTR (12,13). CF is characterised by chronic pulmonary inflammation and infection, often associated with pancreatic insufficiency. Some patients also suffer from anorexia, failure to thrive and stunting. Although in CF, other plausible explanations can be found for the symptoms, there are symptoms overlapping with Zn deficiency.

This review focuses on the data regarding Zn and applied to CF patients to evaluate the potential benefits of Zn supplementation.

Zinc Source, Absorption and Homeostasis
Dietary Zn is mainly found in meat. Animal protein increases the absolute amount of Zn and promotes its absorption (14). A high dietary phytic acid and fiber content (14), present in varying amounts in vegetables and grains, will decrease the Zn absorption. Fat malabsorption will also increase the Zn losses (15). The interactions with other dietary minerals remain an unsolved concern with a probable negative interaction with iron and a possible negative interaction with calcium (16).
The exact absorption mechanisms have not yet been completely characterised in humans. Since it has important physiological functions, the Zn concentrations are highly regulated (17). Different Zn transporting transmembrane proteins have been identified. The Zip-family transporters increase the intracellular concentrations by promoting Zn influx into the cells or release from the intracellular vesicles (18), while the cation diffusion facilitator-family (ZnT transporters) mobilises Zn in the opposite direction (18).

The Zn balance is primarily regulated in the intestine (15, 19) by the excretion and reabsorption of endogenous Zn. However, urinary Zn loss is low (20). It has been suggested that the absorption of Zn is mediated by a pancreatic ligand, enhancing jejunal Zn uptake (21, 22). ZIP4, localised in the apical membrane of the enterocyte, is the main importer of dietary Zn (23). ZIP1 may function as a back-up system in the dietary Zn uptake (18). ZnT-1 is the only member of the ZnT family localised in the basolateral membrane of enterocytes and renal tubular cells indicating the Zn efflux responsibility of the protein (24). Potential sources of endogenous Zn are pancreatic, biliary and gastrointestinal secretions besides transepithelial flux or sloughing of mucosal cells. The excretion is directly related to the total amount of Zn absorbed.

Acrodermatitis enteropathica is an autosomal recessive disease, caused by a mutation in the SLC39A4 gene located on 8Q24 (25). This gene encodes the ZIP4 transporter. Patients will have difficulties with the intestinal Zn absorption resulting in Zn deficiency (26).

The pancreatic insufficiency, frequently present in CF, causes a major problem for Zn absorption. Krebs et al. demonstrated a decreased fractional absorption and no ability to minimise the endogenous Zn loss in CF infants (15). Untreated pancreatic insufficiency also increases Zn losses (15, 27). The fractional Zn absorption is improved by pancreatic enzyme replacement therapy (PERT) (27). However, some CF patients continue to have faecal fat losses despite correct PERT (28) and might therefore be at risk for developing Zn deficiency.

**Evaluation of the Zinc Status**

Most of the body Zn is located in the fat-free intracellular mass and only 0.2% circulates in the plasma associated with albumin and α2-macroglobulin (29, 30). Serum Zn concentrations are maintained within strict values even if Zn intake differs dramatically. Decrease of serum Zn will therefore only be seen if the Zn depletion is prolonged and severe (31, 32). Serum Zn concentrations cumulatively decrease after meals (33). Age related and gender differences have been observed in some studies (34). Infection and inflammation cause a decrease of serum Zn (35). Although serum Zn has its limitations, it remains a useful indicator of a population Zn status. A population shows an elevated risk of Zn deficiency when a higher than expected percentage of the population has low serum Zn concentrations (36). The Belgian children can be at risk for the development of Zn deficiency, since 13% of the population (37) has a serum Zn below the -2 standard deviation of the American NHANES II study (34).

An acrodermatitis enteropathica-like eruption is reported as the presenting symptom of CF (38-40). Literature on serum Zn status in CF is conflicting. Most of the studies do not find a significant difference with the control population (41-45). Young CF patients, detected by screening, have very low serum Zn values (46). We confirmed these results in young newly diagnosed CF patients. However, no significant difference was found when they were compared to a local age matched control group (42).

**Importance of Zinc in Nutritional Aspects**

**Zinc in Relation to Caloric Intake, Growth and Nutritional Status**

Within one week, anorexia and decreasing growth velocity emerge in rats fed a low Zn diet (47). The growth reduction is correlated with decreased cell proliferation and DNA synthesis. In cell cultures, depletion of intracellular Zn with chelators, suppresses DNA synthesis and supplementation with Zn promotes it again (48). Zn deficient cell cultures produce less growth hormone (GH) m-RNA and insulin-like growth factor-1 (IGF-1) m-RNA (49). Further, Zn deficient mice seem to be GH insensitive (49). With Zn supplements, they display an enhanced expression of IGF-1 mRNA but not of GH or GH m-RNA (49).

A meta-analysis concluded that Zn supplementation should be considered for children at risk of Zn deficiency, as it improved growth (50). The effects were most prominent for children being under weight and of restricted height for age.

Animals on a Zn reduced diet are anorectic (47). Krebs et al. confirmed an improved energy intake in children during Zn supplementation (51).

**CF patients** are advised to increase energy intake aiming at an intake of 120% of recommended daily allowances (28). This is advised to compensate for their persistent malabsorption (52) and increased energy expenditure (53). The abnormal activity of mitochondrial enzymes in CF (54) could be responsible for the increased resting energy expenditure. These enzyme complexes are inhibited by Zn (55).

Many CF patients have a suboptimal nutritional status despite the nutritional advice and PERT. Up to now, no studies were able to demonstrate a relation between serum Zn and growth parameters (44, 45). However, a decreased IGF-1 correlating to the nutritional status has been described in CF patients (56). Zn supplements could theoretically have beneficial effects on energy expenditure, appetite, nutritional status as well as growth. Up to now, only one retrospective study clearly demonstrated an improved caloric intake in patients with Zn supplements (57).
Zinc in Relation to Essential Fatty Acid (EFA) Status

The deficiency symptoms of Zn and EFA, which include growth retardation, delayed sexual maturation, infertility, dermal lesions, alopecia and decreased rate of wound healing, show remarkable similarities. This evoked the possibility of a mutual interaction between Zn and EFA. The presence of fatty acid (FA) abnormalities in acrodermatitis enteropathica, an inherited Zn absorption disease, and in transient Zn deficiencies, sustains this theory (58-60). An increased linoleic acid (LA) (18:2n-6) and decreased arachidonic acid (AA) (20:4n-6) argue for a role of Zn in the LA (18:2n-6) metabolism. The specific function of Zn in FA metabolism is still not fully understood. It is probably the result of a direct modulation of the desaturase activities involved in the FA metabolism and an indirect effect by influencing absorption, oxidation and incorporation of the FAs (61).

Cunnane postulated a function for Zn in the desaturation of LA (18:2n-6), based on increased LA (18:2n-6) and reduced AA (20:4n-6) concentrations in various tissues of the Zn-deficient rats (62-64). This hypothesis is sustained by decreased Δ-5 and Δ-6 desaturase activity in various tissues of Zn-deficient rats (65, 66), which was not confirmed by others (67, 68). Eder et al. observed no change or even an increase of the other desaturase metabolites as eicosapentaenoic acid (20:5n-3), 22:4n-6 and 22:5n-6 (69). This could argue against a direct influence on desaturase activity. In different tissues, the effect of Zn deficiency was opposite. An increase in the Δ-6 desaturation in the mammary tissue of Zn deficient rats was described (70). The Δ-9 desaturases of liver and testes react oppositely on hormonal stimulation (71). This could reflect the specific tissue function differences.

To complicate the picture even more, at least 2 different Δ-9 desaturases have been described, with a tissue specific expression (71). In transfected rodent hepatic cells, at least two different Δ-6 desaturases with different reactions to inhibitors were demonstrated (72). Others have postulated that Zn would influence the incorporation of FA into the phospholipids, explaining the differences observed in Zn-deficient rats depending on diet (69, 73). Zn deficient rats also have an increased β-oxidation of LA (18:2 n-6), resulting in decreased amounts of LA (18:2n-6) for metabolism into AA (20:4n-6) (74). Finally, FA and Zn in the diet of rat pups mutually influence the intestinal absorption (61, 75). Addition of LA (18:2n-6) to the diet of Zn deficient pups improves the absorption of labelled Zn (75).

In CF patients, shifts in polyunsaturated FA composition have been described (76). They are attributed to an abnormal functioning phospholipase A2 (77-79) and are related to the genotype (80). Hamilton reports on the improvement of labelled Zn (75).

Shifts in polyunsaturated FA composition have been described (76). They are attributed to an abnormal functioning phospholipase A2 (77-79) and are related to the genotype (80). Hamilton reports on the improvement of labelled Zn (75).
middle binding affinity, such as thymulin, may result as Zn-unbound because of Zn shifting to proteins with higher binding affinity as α2-macroglobulin (101).

In several patient groups immune reactions improved with Zn supplements (102-104). In elderly, Zn supplementation decreases upper respiratory infections (102). Infants and preschool children are reported to have less low respiratory infections during Zn supplementation (103). In the developing world, Zn supplements reduce diarrhoea episodes (104).

In CF, a significant decrement of T-helper, T-suppressor and NK cell functions has been observed in advanced stages of the disease (105-107). Although the results of serum Zn in CF are conflicting (42-46, 96-98), an increase in α2-macroglobulin has been demonstrated in several studies (107, 111). A shift of Zn can therefore be present in CF, documented by its increased unsaturated thymulin (107). Further, a down-regulation of IL-10 production has been described in CF patients leading to an inability to stop inflammation (112).

Although there are several potential positive effects of Zn supplementation, we should also be aware of its possible risk since the pathogens also need Zn for proliferation. The decreased plasma Zn during infection could be a defence mechanism (113). Extremely high Zn concentrations (>30µmol/L) may also decrease T-cell function (90).

Zinc and Anti-Oxidative Properties

Zn forms together with copper and selenium a triad of trace elements involved in cytosolic antioxidant defence. A number of in vivo and in vitro studies have shown increased oxidative stress in lung and airway following Zn deprivation (114, 115). Zn acts as a protective agent for thios in enzymes and proteins and protects their function. Zn deficiency is known to increase lipid peroxidation (116). Long term Zn deprivation renders an organism more susceptible to injury induced by a variety of oxidative stresses.

Wood et al. demonstrated repeatedly increased oxidative stress in CF patients (117, 118). The observed alterations in the FA composition of CF patients could therefore also be induced by increased peroxidation. However, Wood et al. improved the antioxidant status in CF, with high doses of vitamin E, C, A, β-carotene and selenium, without a decrease in oxidative stress or improvement of serum FA composition (118).

Zinc and Chloride Transport

Zn, applied to the mucosal surface, is able to restore airway epithelium chloride excretion both in vitro and in vivo (119). Via P2X purinergic receptor channels, Zn triggers a sustained increase in cytosolic calcium. The activation of calcium-dependent chloride secretory response followed. This finding could lead to new therapeutic options. The best way to deliver Zn to the airway epithelium surface needs to be sorted out.

Conclusion

As pointed out in this review, CF patients may theoretically benefit from Zn supplements in different aspects of their disease. A retrospective analysis of clinical data suggests an improved appetite, caloric intake, nutritional status as well as pulmonary function with Zn supplements (57). The only small and short lasting - double blind cross over study showed no clinical changes (120). Larger double blind studies are needed to answer the question whether all or only a part of CF patients benefit from Zn supplements. Different aspects as FA status, immune function and oxidative stress resistance should be followed during the study.

References

4. MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000; 130: 1500-85

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Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation.

30. Hambidge M. Biomarkers of trace mineral intake and status. J Nutr 2003; 133: 948-55
46. Krebs NF, Sontag M, Accurso FJ, Hambidge MK. Low plasma zinc concentrations in young infants with cystic fibrosis. J ped 1998; 133: 761-4
54. Shapiro BL. Evidence for a mitochondrial lesion in cystic fibrosis. Life Sci 1989; 44: 1327-34
55. Link TA, Von Jagow G. Zinc ions inhibit the QP center of bovine heart mitochondrial bc1 complex by blocking a protonatable group. J Biol Chem 1995; 270: 25001-6
70. Cunnane SC, Wahle KW. Zinc deficiency increases the rate of delta 6 desaturation of linoleic acid in rat mammary tissue. Lipids 1981; 16: 771-4
71. Saether T, Tran TN, Rootwelt H, Christophersen BO, Haugen TB. Expression and regulation of Δ5-Desaturase, Δ6-Desaturase, Stearoyl-Coenzyme A (CoA) Desaturase 1, and Stearoyl-CoA Desaturase 2 in Rat Testis. Biol Reprod 2003; 69: 117-24
81. Hamilton RM, Gillespie CT, Cook HW. Relationships between levels of essential fatty acids and zinc in plasma of cystic fibrosis patients. Lipids 1981; 16: 374-9

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Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation. | Stephanie Van Biervliet
102. Langkamp-Henken B, Bender BS, Gardner EM, et al. Nutritional formula enhanced immune function and reduced days of symptoms of upper respiratory tract infection in seniors. JAGS 2004; 52: 3-12
106. Knutsen AP, Muller KR. T-Cell cytotoxicity in cystic fibrosis relation to pulmonary status. Int Arch Allergy Appl Immunol 1990; 93: 54-8
117. Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Increased plasma fatty acid concentrations after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis patients. Am J Clin Nutr 2002; 75: 668-75
118. Wood LG, Fitzgerald DA, Lee AK, Garg ML. Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. Am J Clin Nutr 2003; 77: 150-9
3.2 Serum Zinc in healthy controls

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Serum Zinc in Healthy Belgian Children

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ABSTRACT

Many reports mention marginal zinc status in childhood. Information on serum zinc (Zn) in Belgian children since the last reports are old and feeding habits are changing. Four hundred fifty-seven healthy children (0–14 yr; 262 boys) had a venipuncture after an overnight fast during a vaccination campaign. Serum Zn, α-tocopherol (α-T), cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein B (Apo B), Apo A, and malondialdehyde (MDA) were determined. The median Zn value is lower in infants than in older children (respectively 11.6 μmol/L vs 12.8 μmol/L). The type of infant feeding does not influence the serum Zn concentrations (breast-feeding, adapted, hypoallergenic, soy, or thickened). No children had increased serum MDA concentrations and the value is not influenced by the Zn concentration. Children presenting higher serum Zn values also have significantly higher serum α-T levels. In infants, there is a significant positive correlation between serum Zn and cholesterol, LDL-C, and Apo B. In this apparently healthy population, no signs of abnormal in vivo peroxidation of fatty acids are observed, even in the children with low serum Zn. More sensitive methods for the detection of peroxidation are necessary for determination of in vivo effects of marginal trace element status.

Index Entries: Serum zinc concentrations; infant feeding; healthy children.

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Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation. | Stephanie Van Biervliet
INTRODUCTION

Although plasma or serum zinc (Zn) values are not considered as a perfect index of body Zn, it is the only readily available method for larger epidemiological surveys. Deficiencies of trace elements in humans can be the result of inadequate intake of the mineral in the diet or impaired absorption in presence of adequate intakes. Factors influencing decreased absorption include dietary consitutents as phytate, fibers, or drugs. Different chemicals can share a common pathway for absorption, thus resulting in competition for uptake in the mucosal cells (1). In this study, we make a cross-sectional analysis of the serum Zn status of Belgian children.

SUBJECTS AND METHODS

The study was performed on 457 Caucasian children (195 girls, 262 boys) during the vaccination campaign for hepatitis B in West Flanders, Belgium. All are in apparently good health and free of malformation or disease. Only children with anthropometric values for height and weight within percentile (P)10 and P90 of our population were recruited. After informed consent of the accompanying parent, venipuncture was done.

Results are analyzed for three age groups: 0–1 yr (n=126), from 1 to 4 yr (n=157), and from 4 to 14 yr (n=174). A brief recall of dietary habits was made. Only 13% were breast-fed. Adapted formulas, originating from different European manufacturers, are given to 84%, and 3% are fed homemade formula. Vitamins are added to all industrial infant formulas in Belgium. Vitamin A–D supplements are provided to 54% of the studied infants. Beyond the age of 1 yr and sometimes earlier, children decrease milk and fat consumption in Belgium. Of the studied toddlers, 31% still get vitamin supplements. Over age 4, vitamin supplements are provided in 12% of the studied children. In all age categories, vitamin C intake either from formula, beikost, or other dietary sources is largely sufficient.

After an overnight fast, 2 mL of blood was drawn by venipuncture by the Multify system in Monovette Sarstedt D-5223 vials, before the vaccination procedure. After clotting and centrifugation, the serum was stored at -60°C until examination. Serum α-tocopherol (α-T) and retinol were analyzed by isocratic high-pressure liquid chromatography (HPLC) (2). Total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and apolipoproteins (Apos) were measured as described by Van Biervliet et al. (see ref. 3). Zn was determined on a Perkin-Elmer 2380 flame atomic spectrophotometer, as described by Carter (4), adapted according to Dubrowski (5). In 70 children there was enough serum to determine thiobarbituric acid-reactive substances as malondialdehyde (MDA) by HPLC (6).

A nonparametric statistic method (Mann–Whitney U-test) is used with Stat-View 5.01 (SAS Institute, Abacus Concepts, Berkeley, CA) for
Macintosh. Results are given as medians and interquartile ranges (IQRs), the significance of the differences between groups as tied p-values. For some data, mean and standard deviations (SD) and linear regression were calculated by the standard methods. The study protocol was approved by the Ethics Committee on Research involving human subjects of West Flanders (no. 356).

RESULTS

_**Median and IQR of Serum Zn**_

The median serum Zn in the 457 children is 12.24 μmol/L and the IQR is 3.82 μmol/L (12.22 ± 2.81; mean ± SD). The perceptual distribution of serum Zn is quite symmetric and mesokurtic (skewness, 0.2; kurtosis, 0.02) (see Fig. 1). A value of 10.4 μmol/L is generally considered low.

_**Age, Gender, Infant Feeding, and Serum Zn**_

Figure 2 shows the analysis of the age cohorts. A significantly lower serum Zn is observed in the first year of life (<1 yr: 11.6 [3.8] μmol/L; 1–4 yr: 12.8 [3.7] μmol/L, p=0.025). From the age of 4, there is no significant change in serum Zn concentration. There is no difference of median Zn
levels analyzed for gender. The type of infant feeding does not influence the serum Zn level (breast-feeding, adapted, soy, hydrolyzed formulas).

**Serum Zn and Peroxidative Lipid Damage**

The serum MDA concentrations are normal (1.15 [0.5] μmol/L). MDA is age and gender independent. There is no correlation between Zn and the MDA serum concentrations. No difference of serum MDA between the lowest (n=12) and the highest Zn quartiles (n=14) is observed.

**Serum Zn and Cholesterol**

Infants with a serum Zn concentration below the P25 (10.4 μmol/L) have significantly lower cholesterol, LDL-C, and Apo B than those
Table 1
Infants (<1 yr): Median Serum Values in the Lowest and Highest Zinc Quartiles and Significances of the Differences in the Median Values (Calculated as Tied p-values, Mann–Whitney U-Test).

<table>
<thead>
<tr>
<th>Infants &lt;1Y</th>
<th>Serum Zn ≤ 10.4 μmol/L</th>
<th>Serum Zn &gt; 10.4 μmol/L</th>
<th>Significance</th>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>N</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.13</td>
<td>0.96</td>
<td>26</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>1.49</td>
<td>0.7</td>
<td>26</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.43</td>
<td>0.12</td>
<td>20</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.01</td>
<td>0.44</td>
<td>26</td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>0.93</td>
<td>0.33</td>
<td>20</td>
</tr>
<tr>
<td>Apo A-I/ B</td>
<td>2.61</td>
<td>1.35</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: The median, IQR, and number of pairs in the quartiles are shown. For the total population, the p25 for serum Zn is 10.4 μmol/L and the p75 is 14.23 μmol/L.

above the P75 (14.23 μmol/L) (see Table 1). However, there is no such association observed with HDL-C or Apo-A or in the older children (data not shown).

**Serum Zn and Retinol Concentration**

In view of the metabolic effects of Zn on the retinol metabolism, we analyzed the serum Zn in the small group of children (n=10) with serum retinol values of ≤ 0.7 μmol/L, with those presenting higher values (n=365). The serum Zn is significantly lower in the children with a low serum retinol (12.39 [3.67] vs 8.72 [4.9] μmol/L; p<0.0005).

**DISCUSSION**

Among all oligoelements, Zn is second to iron in total-body content. It functions with more than 300 enzyme systems. Zn is involved in a variety of general cellular functions, many biochemical processes that support life, including cellular respiration, protein synthesis, DNA, RNA, and carbohydrate metabolism, utilization of nitrogen and sulfur, cell division and growth, pituitary and adrenal gland function, wound healing, membrane integrity, immune system efficiency, sensory responses, and bone
metabolism (7-11). Because so many enzyme systems and tissues are affected, symptoms of Zn deficiency are general. They include loss of appetite, diminished sense of taste, growth retardation, impaired wound healing, and other skin changes. Severe deficiency leads to dwarfism, hypogonadism, acrodermatitis enteropathica, immunodeficiencies, and malabsorption. Secondary Zn deficiency occurs in diabetes mellitus, malabsorption syndromes, and liver and kidney diseases.

The normal Zn concentration in serum is 10.7–15.3 μmol/L (12). Different laboratories report different normal values. Such differences appear to arise largely from methodological considerations. We report on serum Zn levels of healthy Belgian children and adolescents. In our population, the median serum Zn values are slightly lower than in the literature. Twenty-five percent of our population is within the low range of ≤ 10.4 μmol/L. It is generally accepted that Zn deficiency is a widespread public health problem.

Serum Zn levels is lower in infants than in older children. Hambidge et al. (13) reported on the influence of infant diet on serum Zn levels and showed that Zn levels were higher in breast-fed infants. We cannot confirm these findings; unfortunately, breast-feeding is rather exceptional in our population. As observed by most reports (14,15), serum Zn levels are not influenced by gender.

Many reports stress the influence of Zn on the retinol metabolism. We observe higher retinol values in the upper Zn quartile. Inversely, the few infants with a very low retinol have significantly lower Zn values. These data confirm the observation of Koslowski et al. (16). Smith et al. (17) observed that supplementing Zn increased the vitamin A level in children. It has been stated that Zn increased vitamin A absorption and utilization (18). The α-T level in infants is very high and decreases after infancy (19). These high levels are induced by the addition of α-T to infant formulas. During this exogenous supply of α-T in infants, no differences of serum α-T between the high and low Zn quartiles are seen. Afterward, when formula is replaced by, low-fat, homemade formulation, the intake of α-T decreases. Higher serum Zn levels are then associated with higher α-T values. All these observations could point to a better nutritional condition of children with the higher Zn quartiles.

A hypocholesterolemic effect of Zn deficiency was shown in animals and humans (20). In contrast to LDL-C, the HDL-C is uninfluenced by the levels of serum Zn in healthy infants. This contrasts with the in vitro experiments of Wu et al., who showed that the Apo A-I gene expression is regulated by cellular Zn status (21). The observed association of higher serum Zn levels to higher concentrations of total cholesterol, LDL-C, Apo B, and the Apo A-I/B ratio could suggest an effect of Zn on the synthesis or catabolism of LDL particles in infancy.

Zinc is an essential element in the protection against peroxidation. Decreased activity of the copper-zinc dismutase (22), a key enzyme in protection against peroxidative damage, was shown at the serum Zn levels comparable to our lowest Zn quartile. Apparently, these Zn levels...
are insufficiently decreased to result in measurable increase of MDA. Protection against lipid peroxidation is a complex mechanism, involving many different metabolic pathways. In order to measure the direct impact of low Zn status, more sensitive tests will be necessary. The profound influence of Zn on different metabolic pathways stresses the importance of an optimal Zn support in infancy and childhood.

REFERENCES

7. B. L. Carson, H. V. Ellis, and J. L. McCann, Toxicology and Biological Monitoring of Metal in Humans, Lewis, Chelsea, MI, pp. 159 and 290–291 (1987).
3.3 Serum Zinc at different ages in cystic fibrosis patients

Serum Zinc in Patients with Cystic Fibrosis at Diagnosis and After One Year of Therapy

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ABSTRACT

There is no consensus whether zinc (Zn) supplementation is necessary in cystic fibrosis (CF). For assessment of the Zn status, serum Zn concentration is the only easy available method. It is, however, age dependent. We compare the serum Zn levels of CF patients with earlier reported normal values. Serum Zn was determined in all new diagnosed CF patients and a second time 1 yr later. Data concerning fat-soluble vitamin status, cholesterol, albumin, pancreatic insufficiency, and genotype were collected. Thirty-two patients, median age of 1.21 yr, were included. Four were pancreatic sufficient. The median Zn concentration at diagnosis was 10.7 μmol/L (5–21.4), with a significant increase 1 yr later (median: 12.1 μmol/L [7.803–16.1]). An association of serum Zn with vitamin A ($p < 0.03$) and with vitamin E ($p < 0.02$) was observed. Compared to age-matched healthy controls, there is no significant difference in serum Zn concentration either at diagnosis or 1 yr later. Although it was demonstrated that steatorrhea causes Zn loss, the serum Zn concentration in CF is not significantly different from healthy controls. The relation with vitamin A and E points to the increased losses by steatorrhea. Therefore, Zn supplementation is advised in persisting steatorrhea.

INTRODUCTION

Although plasma or serum zinc (Zn) values are not considered a perfect index of body Zn, it is the only easily available method for larger epi-
demiological surveys (1). Deficiencies of trace elements in humans can be the result of inadequate dietary intake or of impaired absorption in presence of adequate intake. Fat malabsorption is proven to be associated with increased Zn loss (2). Although there are case reports concerning clinical Zn deficiencies in children with cystic fibrosis (CF) (3–5), Zn supplements are only advised in CF patients with ongoing steatorrhoea and poor weight gain despite their correct pancreatic enzyme replacement therapy (PERT) (6). Previous reports have demonstrated an increase in plasma Zn concentration after the start of PERT (7). However, these results were not controlled with large local healthy control groups. In this study, all new diagnosed CF patients were compared to a large local normal age-matched control group described elsewhere (8).

METHODS

Study Design

This is a prospective study of all new patients with CF, diagnosed between 1998 and 2003 in our center. At diagnosis, 0.5 mL of serum was taken for the determination of Zn in a Zn-free plastic tube, after informed parental consent. A second aliquot of 0.5 mL of serum was taken at the control examinations performed 1 yr after diagnosis. The results were compared to the corresponding age cohort from earlier published normal values of a cross-sectional study (8). The serum results of total cholesterol, vitamins A, E, and D, and albumin were collected. Because of hemolysis in the blood sample or insufficient serum, two Zn determinations at diagnosis and four after 1 yr of therapy were not performed.

Laboratory Analysis

After clotting and centrifugation, serum was stored at −60°C until examination. Zn was determined on a Perkin-Elmer 2380 flame atomic spectrophotometer, as described by Carter (9), adapted according to Dubrowski (10). Serum μ-tocopherol and retinol were analyzed by isocratic high-pressure liquid chromatography (11). A radioimmunoassay was used to determine the 1–25α(OH)2 vitamin D (DiaSorin Inc., Stillwater, MN, USA). Total cholesterol was measured using an enzymatic colorimetric analysis as described by Allain, et al., (12).

Statistics

A nonparametric statistic methods (Mann–Witney U-test Wilcoxon rank test, and Spearman rank correlation test) were used with Stat-View 5.1 (Abacus Concepts, Inc., Berkeley, CA) for Windows. Results are given as median, maximum, and minimum and the significance of the differences between groups as tied p-values. The study protocol was approved.
Zinc in Cystic Fibrosis

Fig. 1. Correlation between serum Zn and vitamin A using the Spearman rank correlation.

by the Ethics Committee on Research involving human subjects of West-Flanders (no. 356).

RESULTS

Patients

Thirty-two new diagnoses of CF were made in the study period (20 boys). The median age at diagnosis was 1.21 yr (0–12.5 yr). Seven patients were older than 1 yr at diagnosis, of which three were pancreatic insufficient (fecal elastase 1 [FE-1] < 15 μg/g feces). Four patients had an FE-1 above 200 μg/g and two were between 100 and 200 μg/g. All patients with an FE-1 below 200 μg/g were started on PERT. Fourteen patients were ΔF508 homozygous, 15 were ΔF508 heterozygous, and 3 were non-ΔF508.

The median weight for height at diagnosis was 96% (80–109%) and 97% (89–115%) after 1 yr of therapy (using the Flemish growth chart, 2004).

Serum Zn Concentration in CF Patients

The median Zn concentration at diagnosis was 10.7 μmol/L (5–21.4), with a significant increase 1 yr later (median; 12.1 μmol/L [7.803–16.1]) (p = 0.01). Thirteen patients (40%) presented Zn concentrations below 10.4 μmol/L at diagnosis vs 12.5% after 1 yr of therapy.
Fig. 2. Correlation between serum Zn and vitamin E using the Spearman rank correlation.

Fig. 3. Serum Zn of the CF patients diagnosed before the age of 1 at diagnosis (n = 25) and after 1 yr of therapy (n = 22) compared to age cohorts <1 yr and 1–2 yr of the healthy population. There were no significant differences using the Mann–Whitney U-test.
Zinc in Cystic Fibrosis

There was no difference in Zn concentration depending on gender, genotype, or pancreatic function. There was no association of serum Zn with serum albumin, vitamin D, or cholesterol in the CF patients. However, a statistical significant correlation was observed between serum Zn and vitamin A ($p < 0.03$) (Fig. 1) as well as vitamin E ($p < 0.02$) (Fig. 2).

**Serum Zn Compared to the Age-Matched Healthy Control Group**

Compared to age-matched healthy controls, there is no significant difference in serum Zn concentration either at diagnosis or 1 yr later (Fig. 3). Eleven (44%) of the CF patients diagnosed before the age of 1 yr had a serum Zn within the low range (10.4 μmol/L) compared to 33% of healthy controls below the age of 1 yr and after 1 yr of therapy (12.5% vs 19.8% of the controls). Of the patients with a late diagnosis, two had a low serum Zn at diagnosis, but because of to hemolysis, two values are missing at diagnosis in this group. They were both pancreatic insufficient, aged 5 and 2 yr. Unfortunately, the control value after 1 yr of therapy of one of the patients is missing; the youngest normalized his serum Zn after 1 yr of therapy.

**DISCUSSION**

Zinc has the second highest prevalence of all oligoelements in the total human body. More than 300 enzyme systems rely on its presence (13,14). Because so many enzyme systems and tissues are affected, Zn deficiency symptoms are nonspecific. They include loss of appetite, diminished sense of taste, growth retardation, impaired wound healing, disturbed immune function, and other skin changes (15–17). Patients with CF can have similar symptoms. Serum Zn remains the most widely used index of Zn status, although it lacks optimal sensitivity and specificity (1,18,19). Zinc is located in the cell and only a small portion is found in the circulation bound to plasma protein. Even human depletion studies have not identified a reliable marker for marginal chronic Zn deficiency (20,21). The assessment of marginal Zn status remains problematic because there is no universally accepted method. In CF, there are regular reports of clinical Zn deficiency (3–5). However, the data on prevalence of Zn deficiency are inconsistent (7,22–25). We report on serum Zn levels in CF patients at diagnosis and 1 yr after the start of therapy compared to the age-matched healthy control group. Although serum Zn concentrations are very low in newly diagnosed CF patients, they do not differ from the healthy controls in our population, as earlier described (8). Serum Zn levels are lower in infants than in older children (8).

A serum Zn of 10.4 μmol/L, is generally considered low. The percentage of CF patients (below the age of 1 yr) with low serum Zn concentrations is 44% at diagnosis, whereas only 33% of our healthy population...
below the age of 1 yr are in this range. The frequency of low serum Zn in
our young healthy population (<1 yr) is comparable to the 30% low
plasma Zn concentration in young CF patients detected by screening (26).
One year later, only 12.5% of treated CF patients are still in this range, com-
pared to 19.8% of the healthy controls. This observation is consistent with
the published data of Krebs et al. (7). They described a lower fractional
absorption of Zn when PERT is withheld (2). They also described the same
increase before and after PERT (7). Because patients with CF are closely
monitored and receive regularly dietary counseling, they tend to improve
more than the control population.

Although vitamin A is a Zn-dependent vitamin, the observed correla-
tion between vitamin A and Zn is probably attributable to the steatorrhea
because there is also a correlation with vitamin E. It has been demon-
strated that there is a correlation between the losses of endogenous Zn and
steatorrhea as well as between the fractional absorption of Zn and the per-
cent of fat malabsorption (26). This underlines the importance of close
monitoring of the persistence of steatorrhea in order to detect deficiencies
early.

CONCLUSION

Serum Zn as a marker of human Zn status is far from ideal, but it
remains the only available method. Although as a group CF patients do
not differ in Zn concentration from age-related healthy controls, in some
patients with ongoing steatorrhea and poor growth supplementation
might be considered. The clinical response to this treatment remains the
best evaluation of Zn status.

REFERENCES

(2000).
eruption as the presenting sign of cystic fibrosis: case report and review of the litera-
4. C. Mazzochi, J. L. Michel, V. Chalencon, G. Teysier, I. Rayet, and F. Cambazard,
5. G. L. Darmstadt, J. McGuire, and V. A. Ziboh, Malnutrition associated rash of cystic
7. N. F. Krebs, M. Sontag, F. J. Accurso, and K. M. Hambidge, Low plasma zinc concen-
Zinc in Cystic Fibrosis


Serum Zinc Concentrations in Cystic Fibrosis Patients Aged Above 4 Years: A Cross-sectional Evaluation

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Abstract

Aim Assess the risk of zinc (Zn) deficiency in the older cystic fibrosis (CF) population.
Method Cross-sectional investigation of all CF patients above the age of 4 followed at the Ghent University center between 2002 and 2003. Data on age, weight, height z-score, pancreatic and pulmonary functions, chronic Pseudomonas infection, and CF transmembrane conductance regulator (CFTR) mutations were collected. Serum Zn, vitamins (vit) A and E, retinol-binding protein (RBP), albumin, sedimentation rate, total IgG, and cholesterol were determined. Serum Zn was compared with a local healthy control group (Van Biervliet et al., Biol Trace Elem Res 94:33–40, 2003) and with literature data (Hotz C, et al. Am J Clin Nutr 78:756–764, 2003).
Results 101 patients (median age 16 years) were included. There was no difference in serum Zn concentration between CF patients and controls. In CF patients no difference in serum Zn concentration between pancreatic-sufficient or pancreatic-insufficient patients was seen. Serum Zn was not associated to nutritional status or height z-score. A significant association serum Zn to serum albumin (p<0.0005) and to vit A (p<0.01) was seen. No associations of serum Zn to serum vit E, RBP, cholesterol, or CFTR were present, but there is a significant association serum Zn to forced vital capacity (p<0.01). Serum Zn was not associated to inflammatory parameters or chronic Pseudomonas infection.
Conclusion Comparison of CF patients with local controls revealed no significant differences. However, because persisting steatorrhea increases Zn loss (Easley et al., J Pediatr Gastroenterol Nutr 26:136–139, 1998) and 12.6% of our population has a serum Zn below the p value of 2.5 of the NHANES II study (Hotz C, et al. Am J Clin Nutr 78:756–764, 2003), there could remain an increased risk of Zn deficiency in some CF patients. Furthermore, the association with pulmonary function needs more investigation.

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Keywords Zinc concentrations · Cystic Fibrosis · CF transmembrane conductance regulator · *Pseudomonas* infection. Pulmonary function

Abbreviations
- Zn: Zinc
- PERT: pancreatic enzyme replacement therapy
- CF: cystic fibrosis
- W/H %: percentage of ideal weight for height
- Vit: vitamin
- FEV1%: forced expiratory volume in 1 s
- FVC%: forced vital capacity
- CFTR: Cystic fibrosis transmembrane conductance regulator
- Lz-score: length z-score
- RBP: retinol-binding protein
- PS: pancreatic-sufficient
- PI: pancreatic-insufficient

Introduction

Assessment of marginal zinc (Zn) status in humans is problematic. The serum Zn is far from the ideal method because it lacks sensitivity and specificity to evaluate the Zn status. It is, however, a useful biomarker of a population’s risk of Zn deficiency and can be used to determine whether interventions on the Zn status are needed [1]. Zinc deficiency was originally described in 1961 by Prasad et al. [2]. The symptoms of Zn deficiency are stunted growth, delayed sexual maturation, disturbed immunity, poor appetite, and diarrhea each of which are frequently present in patients with cystic fibrosis (CF) [3–5].

In a previous contribution, newly diagnosed, untreated CF patients were compared to healthy age-matched controls and no differences of serum Zn concentrations were observed in CF as well as at diagnosis and after 1 year of CF therapy [6]. Because many of the older CF patients still present symptoms also commonly seen in Zn deficiency, a cross-sectional study of the serum Zn concentration of treated CF patients was performed here.

Subjects and Methods

This is a cross-sectional study of all CF patients over the age of 4 followed at a CF center in Ghent during the period of 2002–2003 (n=101, female=48). From the age of 4 serum Zn is leveling in previously described normal controls [7]. Therefore, CF patients from the age of 4 are compared to 174 local controls and reference values [7, 8].

All were treated during at least 1 year. None of the patients took Zn supplements. Pancreatic-insufficient (PI) patients (n=90) received pancreatic exocrine replacement therapy and fat soluble vitamin (vit) supplements targeted to the serum values.

In patients above the age of 6 (n=90) the pulmonary function was measured every 3 months on a Jaeger Masterscreen Body, a half year before and after the Zn determination. For evaluation of lung function the mean value of the five measurements was considered.
Laboratory Analysis

Blood was drawn by venupuncture after an overnight fasting of 8 h and collected in a Zn-free tube. After clotting and centrifugation, the serum was stored at -60°C until examination. Zinc was determined on a PerkinElmer 2380 flame atomic spectrophotometer, as described by Carter [9], adapted according to Dubrowski [10]. Serum α-tocopherol and retinol were analyzed by isocratic high-pressure liquid chromatography [11]. Total cholesterol was measured using an enzymatic colorimetric analysis as described by Allain et al. [12]. Serum albumin was determined by the Bromcresol green method of COBAS 6000® (Roche Diagnostics). The serum retinol-binding protein (RBP) concentration was measured on a Behring Nephelometer Analyzer II.

Genotypes were determined with the INNO-LiPA CFTR19® and INNO-LiPA CFTR17+ Tn Update® kit (Innogenetics N.V.) or sequencing of the CF transmembrane conductance regulator (CFTR) genome. CFTR gene mutations were classified as proposed by Welsh and Smith [13] using the CF mutation database http://www.genet.sickkids.on.ca). Group A included patients with type I, II, or III mutations resulting in no functional CFTR protein. Group B included patients with at least one type IV or V mutation resulting in a partially functional CFTR protein or those with unknown effect on the CFTR protein (Table 1).

Statistics

Nonparametric statistical methods (Mann–Whitney U test, Wilcoxon rank, Kruskal–Wallis, and Spearman rank correlation) were used with StatView 5.1 (Abacus Concepts, Inc. Berkeley, CA, USA) for Windows. Results are given as median and interquartile range (IQR) the significance of the differences between groups as tied p values. The study protocol was approved by the Ethics Committee of Ghent University Hospital, 2005/411 (date 6/1/2006).

Results

Patients

There were 101 patients (48 females) included. Their median age was 16 years (IQR 11.2). The median age for gender was not different. Eleven patients were pancreatic-sufficient (PS). The percentage of ideal weight for height (W/H %) was 98% (IQR 14.95). There was no significant difference in W/H % according to gender or pancreatic function. The height z-score (H z-score) was -1.3 (IQR 1.2). Girls were significantly more stunted than the boys with a median H z-score of -1.6 (IQR 1.3) vs -1.1 (IQR 1.03) (p<0.03). None of the patients was vegetarian. The median forced expiratory volume in 1 s (FEV1%) was 82.8% (IQR 39.1) and the forced vital capacity (FVC%) was 91.7% (IQR 27.25). There were 39 (IQR 38.5) patients colonized with Pseudomonas aeruginosa. They had a significantly worse pulmonary function (p<0.03). FVC% was 96.6% (IQR 25) for Pseudomonas-negative and 87.3% (IQR 27.2) for the colonized patients.

The CFTR mutation subgroups are described in Table 1. Group A includes mutations where no CFTR function is expected (n=68). Group B includes mutations with decreased function or unknown effect on CFTR function (n=33). All PS patients were in group B.
Table 1  Genotype of the 101 CF Patients: Details of the CF Mutations and Classification into Two Groups

<table>
<thead>
<tr>
<th>Genotype Groups</th>
<th>Genotype</th>
<th>No of Patients</th>
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<tr>
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There were no significant differences in the clinical parameters between the genotype groups except for the H z-score (p<0.02). Group B (H z-score -1.2, IQR 1.375) was significantly taller than group A (H z-score -1.4, IQR 1.43) (p<0.02). Group B had higher serum cholesterol (145 mg/dl, IQR 48.75) than group A (132 mg/dl, IQR 50) (p<0.02). The other laboratory data did not differ between the genotype groups.

Serum Zinc Concentration

The median serum Zn concentration was 82 μg/dl (IQR 20). There was no difference between the CF patients and the healthy local controls (84 μg/dl, IQR 24). However, the age range in the control group was significantly lower with a median age of 6.5 year (4–13.8 year). Considering a fully age-matched CF patients (n=42), similar results were obtained.

Of our CF population and healthy controls, 16.8 and 12.6%, respectively, had a serum Zn below the lower cut-off (2.5 percentile=65 μg/dl) of the NHANES II [8].
There was no difference according to gender or age. Dividing the CF patients in the age classes of NHANES II (<18, 18–25, and >25 years), no differences were found.

Serum Zn is not associated with nutritional status expressed as WH % or H z-score. Even between patients with high or low (<65 μg/dl) serum Zn no differences in growth and nutritional status could be observed.

There was no statistical evidence of differences in serum Zn between PS (n=11) (82.9 μg/dl, IQR 21) and PI patients (n=90) (81.5 μg/dl, IQR 16.25). However, none of the PS CF patients had a serum Zn below 65 μg/dl.

Comparing serum Zn of the genotype classes, no differences were seen. However, in group A 17.6% (n=12) and in group B 15% (n=5) had serum Zn below the lower cut-off (P 2.5=65 μg/dl) of NHANES II [8].

Serum Zinc and other Serum Values in Cystic Fibrosis Patients

A significant association between serum Zn and albumin (p<0.0005) and Zn-serum vit A (p<0.01) were obvious. There was no association of serum Zn with cholesterol, RBP, vit E, or with markers of inflammation (erythrocyte sedimentation rate, total IgG).

Serum Zinc and Pulmonary Function

There was no difference in serum Zn between patients with or without Pseudomonas colonization. The pulmonary function in colonized patients (FVC% 87.3%, IQR 27.3) was, however, significantly lower (p<0.004). There was no association between Zn and FEV1% but a significant association was found with FVC% (p<0.01) (Fig. 1).

Discussion

Zinc has the second highest prevalence of all oligoelements in the human body [14]. One could distinguish three main categories of Zn functions: catalytic, structural, and regulatory. Zinc is an essential component of the catalytic site of hundreds of different metalloenzymes [15]. Furthermore, it is on an important structural element of gene regulatory proteins [16]. The structure of these proteins is dependent on Zn chelation centers. At these sites Zn
facilitates appropriate protein folding. These “Zn finger” proteins play a key role in formation and maintenance of all tissues [17]. The last function of Zn is regulatory. It acts as an ionic signal in cells through gated membrane channels [18]. More than 300 enzyme systems rely on its presence [19, 20].

Therefore, Zn deficiency symptoms are nonspecific. They include loss of appetite, diminished sense of taste, growth retardation, disturbed immune functions, impaired wound healing, and other skin changes [3–5, 21]. Patients with CF can have similar symptoms.

Serum Zn remains the easiest and most widely used index of Zn status for population research. For determination of an individual Zn status, serum Zn lacks optimal sensitivity and specificity [1]. Zinc is located in the cell and only a small portion is found in the circulation bound to plasma protein [14]. As about 70% of the plasma Zn is bound to albumin [14], the correlation ($p<0.0005$) found between serum Zn and albumin is not surprising.

Multiple aspects of the vit A status (absorption, metabolism, release, transport, and utilization) may be influenced by Zn [22]. A significant association of Zn with vit A has been described in multiple other populations [23, 24] and is confirmed in this study despite the treatment with high doses of vit A. Zinc is required for hepatic synthesis of RBP, implying a regulatory role for Zn in mobilizing vit A within cells and from the liver [25]. As observed by others [26], there was, however, no association of serum Zn and RBP in this CF population.

Although the influence of Zn on vit A absorption [22] opens interesting theories concerning the possibilities of improving the vit A status of CF patients by Zn supplementation, they were not confirmed in therapeutic trials [27].

Acrodermatitis enteropathica-like eruption is regularly described as a presenting picture of CF [28–30]. Because in our healthy controls [7] 12.6% has a serum Zn concentration below the lower cut-off of the NHANES II study [8], they could be considered as a population at risk for Zn deficiency. Therefore it is interesting to have a closer look at populations with diseases known to cause increased Zn losses such as CF [31, 32], especially because the data on prevalence of Zn deficiency in the CF population are inconsistent [32–36]. This study confirms the absence of a difference in serum Zn between treated CF patients and controls [33, 35]. Because of the small study size, the age-related fluctuations described in NHANES II study are not consistent [8].

In general the severity of malabsorption is less pronounced in PS CF patients. In this population it is reflected by their higher H z-score and serum cholesterol values. The group of Hambidge [31] showed a decreased fractional absorption of Zn in PI CF and its increase by pancreatic enzyme supplementation. The fecal Zn loss correlated with fecal fat loss [32]. From our CF population 16.8% had Zn values below the NHANES II cut-off. This was, however, exclusively seen in PI CF.

As observed by other groups [35, 37], the absence of association Zn nutritional status in this study population is not surprising because malnutrition in CF is a complex multifactorial problem. It is influenced a.o. by nutritional intake, absorption, and energy expenditure.

The relation between serum Zn and pulmonary function merits further investigation. Inflammatory conditions are known to reduce serum Zn concentrations [38]. There was, however, no association between serum Zn and red blood cell sedimentation rate or total IgG, which are both parameters of inflammation. Although patients colonized by *P. aeruginosa* had a significantly lower pulmonary function, there was no difference in serum Zn according to the colonization status. Because Zn plays an important role in immunity
the decreased serum Zn in patients with decreased pulmonary function could enhance infection, leading to a vicious circle. Zinc supplementation reduces pulmonary infections in many different conditions and it can be interesting to investigate such in CF, especially in those patients with low serum Zn values. The only double-blind Zn supplementation study in CF did not select the population according the initial serum Zn value. They were not able to observe differences in nutritional status or pulmonary function after the 8-week short duration of the Zn supplementation. Probably the effects of Zn supplementation could be more pronounced in the initially low Zn group.

Conclusion

A subgroup of CF patients shows marginal serum Zn and could be at risk of its major metabolic consequences. In this condition Zn supplementation needs to be thoroughly examined. Furthermore, the decreased long function of the low Zn group needs more attention.

References

3.4 Intestinal alkaline phosphatase: a zinc dependent enzyme, in cystic fibrosis

3.4.1 Maldigestion, malabsorption in cystic fibrosis

In cystic fibrosis the malabsorption is thought to be a multifactorial process. The pancreatic insufficiency, being the most important cause, is treated with pancreatic enzyme replacement therapy. However, there is little to no correlation between the coefficient of fat malabsorption and the amount of enzymes used. Moreover, no correlation could be found between the enzyme dose and growth or gastrointestinal symptoms. The asynchrony demonstrated between the gastric emptying of the enzymes and the food particles might in part explain this observation [128]. On the other hand, an intestinal acidification is present in cystic fibrosis due to absent pancreatic bicarbonate and decreased duodenal bicarbonate excretion [129]. This causes a deficient or delayed dissolution of the enteric coating of the pancreatic enzyme replacement therapy but also an impaired activity of lipase and co-lipase.

The digestion of lipids proceeds at the surface of multilaminar emulsion particles through the action of gastric or pancreatic lipases. The digested lipids organise into intestinal micelles by mixing with bile salts. In animal cystic fibrosis models as well as in humans deranged bile salt pharmacokinetics have been demonstrated [130]. If the intralumenal content is acid, the fatty acids will be present in a protonated form, which is not incorporated in micelles and remain in the emulsion phase. The micelles then diffuse through the unstirred water layer. As they approach the intestinal membrane, an acidic microclimate destabilizes the micelle and facilitates translocation of fatty acids. The exact way of entry into the intestinal cell is not yet completely understood. In CF mice the acidic, weakly sulphated surface of the intestinal mucus is thought to alter the functional properties and nutrient diffusion [131]. There is some evidence that long chain monomeric fats are malabsorbed in cystic fibrosis [4]. Inside the cells the fatty acids are re-esterified and packed into lipoprotein particles for distribution through the body.

In cystic fibrosis patients as well as in the cystic fibrosis mice models a low grade intestinal inflammation is present [132, 133]. This might be secondary to deficiencies in host defence or due to microbial interactions. Cystic fibrosis patients might be at risk for bacterial overgrowth [134] since in mice models the mucus obstruction of the crypts interferes with the innate defence mechanisms of the Paneth cells [135]. Further on the decreased flushing of the intestine by pancreatic, biliary and intestinal secretions as well as the mucus accumulation and altered biochemical properties of intestinal mucins might predispose cystic fibrosis patients for bacterial overgrowth. Finally, the therapeutic suppression of the gastric acid production might increase the risk for bacterial colonisation. Bacterial overgrowth will accentuate malnutrition by bacterial competition for the ingested nutrients, deconjugation of bile salts and increased intestinal inflammation.

Although intestinal biopsies are not routinely taken at diagnosis of cystic fibrosis, we performed the studies to evaluate the contribution of the intestinal brush border in the malabsorption present in cystic fibrosis. The data in literature were limited and contradictory [136-138].

3.4.2 Intestinal alkaline phosphatase

Alkaline phosphatase is a known metalloprotein, containing zinc or magnesium necessary for the catalytic function [139]. Intestinal alkaline phosphatase is one of three isoenzymes: Intestinal, placental and germ-cell alkaline phosphatase. The enzymes differ in posttranslational modifications. Intestinal alkaline phosphatase gene
expression is activated by a zinc binding protein [140]. Intestinal alkaline phosphatase is deeply buried in the lipid bilayer of the brush border membrane via glycosylphosphatidylinositol [141]. Due to this anchoring the enzyme is more readily released from the brush border towards the intestinal lumen and plasma [142]. The release is facilitated during lipid absorption [143]. Recently Goldberg et al. demonstrated that intestinal alkaline phosphatase detoxifies the bacterial lipopolysaccharides and prevents bacterial invasion [144].

The enzyme has shown to be very sensitive for oxygen free radicals [145]. Zinc is an essential part of the copper-zinc dismutase, an important defence mechanism against oxygen free radicals.

Zinc deficiency will therefore result in a decreased intestinal alkaline phosphatase activity due to absence of zinc in its catalytic site and increased inactivation as a result of increased presence of oxygen free radicals but also in a decreased concentration as a consequence of the decreased transcription.

In cystic fibrosis mice De Lisle et al observed a decreased intestinal alkaline phosphatase gene expression and they are currently looking at protein concentration and enzyme activity to confirm this (personal communication 11/03/08). The observed decrease in intestinal alkaline phosphatase activity in cystic fibrosis patients could therefore contribute to the increased risk for bacterial overgrowth in cystic fibrosis patients. On the other hand could the increased permeability, decreased intestinal alkaline phosphatase activity and the small bowel bacterial overgrowth lead to an increase of active bacterial liposaccharides in the portal circulation. This might contribute to the development of cystic fibrosis liver disease [146].
3.4.2 Intestinal alkaline phosphatase activity in cystic fibrosis

Small intestinal brush border enzymes in cystic fibrosis

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Abstract

The study concerns the role of normal and decreased intestinal alkaline phosphatase activity in small intestinal brush border specimens from 61 consecutive admitted, untreated, Caucasian cystic fibrosis patients. A group of 219 age matched controls admitted during the same time period for unrelated gastrointestinal or nutritional disorders acted as the controls.

In order to eliminate morphological damage as a confounding factor, the enzyme activity was studied in small intestinal biopsy specimens having both normal microscopic and histological features. It was shown that neither milder nor severe form activity was different in the two groups, in contrast to lactase and alkaline phosphatase activity, which was significantly lower in cystic fibrosis patients. The differences could not be explained by the nutritional status as judged by the body mass index.

Lactase activity is known to be easily affected by numerous enteropathogens. At the information on alkaline phosphatase activity is limited, the low activity is discussed in more detail. Taking into account the literature data, the low alkaline phosphatase activity is tentatively attributed either to enhanced release from the brush border or to the faulty handling of alkaline phosphatase protein in the post-gastric compartments secondary to the accumulation of incorrectly glycosylated CFTR in the same cell structures (Acta gastroenterol., belg., 1999, 62, 267-271).

Key words: cystic fibrosis, intestinal alkaline phosphatase, small intestinal brush border enzymes.

Up to now, the information on the small intestinal enzyme activities in cystic fibrosis (CF) is limited and controversial. Antonowicz found increased disaccharidase activity and speculated that the decreased output of pancreatic enzymes in CF caused a decreased degradation of the small intestinal brush border enzymes (1,2). On the contrary, isolated lactase deficiency, as well as partial enzyme depletion of glycosidases and alkaline phosphatases, but not of enterokinase, have been observed in CF (3,4,5). These data prompted us to study the enzymatic activities in small intestinal biopsy specimens in CF.

Patients

61 Untreated Caucasian CF patients entered the study at the time of initial diagnostic work up. There were 26 boys and 35 girls with a median age of 16 months, ranging from 1 month to 14 years. The diagnosis of CF was made on clinical signs and symptoms and 3 positive sweat tests (Gibson-Cooke method), defined by a chloride concentration of > 60 mEq/L. The genotype was defined in 24 patients.

As intestinal biopsy sampling in normal control subjects is critically not allowed, 319 age matched subjects served as the concurrent control group. The control subjects underwent an intestinal biopsy because of failure to thrive or unspecific gastrointestinal complaints. In none of the controls a diagnosis of well defined digestive or respiratory disorder was made.

Informed consent was obtained from the parents of patients and controls.

Methods

At the time of diagnosis, duodenal juice with alkaline pH was obtained by use of the open paediatric Crosby capsule placed at the angle of Treitz. At the end of the collection a biopsy specimen was aspirated from the intestinal mucosa.

After collection of the specimen, stereomicroscopic examination was performed, allowing classification of the mucosal biopsies into 3 types according to Stenning (6): type-I: normal mucosa, type-II: partial mucosal atrophy and type-III: total atrophy.

A fragment was taken for classical histology and the remaining tissue was homogenised, after wet weighing, for determination of enzymatic activities. The glycosidases were determined according to Eggermont (7), and the intestinal alkaline phosphatases according to Garten and Levinthal (8). Luminol chemiluminescence was determined by the Boehringer Mannheim Monotest Chemiluminescin (9) and amylase by the Bio-Merieux α-Amylase PNP-kit (10). Activities were respectively expressed in international units as μMoles of substrate hydrolysed per minute and μMoles of substrate hydrolysed per minute and μMole of juice.

For each patient or control subject, the quotient of the body mass index (BMI) to the 50th centile value of the BMI of a normal population, matched for gender and age, was used to compare the nutritional status of CF patients and control subjects (11).

Computer analysis of the data was performed with a Macintosh Computer using Excell 4.0β, Microsoft and Statview IV β, Abacus. A standard non-parametric statistical method was used: the Mann-Whitney U test, to know whether the studied groups have been drawn.
from the same or a different population and the Spearman rank correlation coefficient.

**Results**

**Nutritional status.** The height or length of CF patients and control subjects had a normal distribution around the 50th centile value of age- and gender-matched normal children. Their weight distributions, however, were shifted towards the 25th centile values of the normal population (data not shown).

As shown in fig. 1 the quotients of BMI values of CF as well as control subjects to the BMI of a normal population matched for gender and age were below 1 and not significantly different from each other.

![Graph showing BMI values](image)

**Fig. 1.** — Box plots of the quotient of body mass index (BMI) in cystic fibrosis patients (CF) and control subjects by the 50th centile value of BMI in normal children matched for age and gender.

**Genotypes.** Of the 24 CF patients in whom the genotype was determined, 16 (66%) were homozygous for the Δ F-508 mutation. A compound heterozygosity was noticed in the 8 (33%) others. In this small group we were unable to detect genotype-phenotype relations for the clinical condition or the enzyme activities, studied.

**Pancreatic function.** Although basal pancreatic enzyme activities in the duodenal juice are not an unequivocal indicator of pancreatic function, the chymotrypsin activity was significantly lower in the CF patients compared to the controls (p < 0.0001). The median and the (25–75%) values (IU/mL) in the CF and the control subjects being 0.6 (0.1-1.9) and 15 (8.4-27) respectively. In agreement with the known low basal luminal amylase activity in normal infants aged less than 6 months (12), a significantly lower basal luminal amylase activity (p < 0.01) was only found in CF children aged 6 months or more (fig. 2).

**Mucosal morphology.** On stereomicroscopic examination, 89% (n = 42) of the CF patients displayed a normal mucosa described as typical by Shmerling (6). 29% (n = 18) the Shmerling type II and 16% (n = 7) the Shmerling type III. In the controls 64.5% (n = 205) had the Shmerling type I and 35.5% (n = 114) the Shmerling type II mucosa. On histological examination, all patients with the Shmerling type I mucosa presented normal microscopic findings. The patients with the type II mucosa had microscopic abnormalities from aspecific changes to partially flattened villi. In the patient with the type III mucosa, the microscopic examination displayed total villous atrophy. As we were interested in the effects of CFTR-mutation proteins on the alkaline phosphatase and glycosidase activity, the enzyme determinations presented in this study, both on control and CF samples, are made on stereomicroscopically normal (type I) and histologically normal mucosae. The age distribution of the subjects with type II mucosa is given in table 1.

![Graph showing enzyme activity](image)

**Fig. 2.** — Duodenal amylase activity shown by age categories in months. From the age of 6 months on, significant differences are observed between cystic fibrosis patients and controls (M-W U-test, tied p values).

**Table 1.** — Age distribution in CF-patients and controls with normal mucosa.

<table>
<thead>
<tr>
<th>Age distribution</th>
<th>CF-patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2 Years</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2 - 5 Years</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>&gt; 5 Years</td>
<td>5</td>
<td>24</td>
</tr>
</tbody>
</table>

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Small intestinal enzyme activities: Morphological damage of the small intestinal mucosa is known to result in decreased activities of the brush border enzymes (13). As we were interested in the effect of CF mutations on the brush border enzyme activities, we only report on the data obtained on structurally normal small intestinal mucosa. In the CF population with normal villi, the small intestinal maltase and sucrase activity does not differ from the controls (table II). The lactase activity on the contrary is significantly lower in the CF group (p = 0.009). This decrease persists if we analyse the children below the age of 2 years (p = 0.02). The lactase activity did not differ in CF patients having basal luminal activities of amylase and chymotrypsin either below or above the 10th centile value of age matched controls (data not shown). Also a correlation between the nutritional status and the disaccharidase activities (maltase, sucrase and lactase) could not be demonstrated (data not shown).

As seen in fig. 3, the small intestinal alkaline phosphatase activity (IAP) is significantly decreased in the CF patient group (p = 0.003). The IAP activity is not influenced by the nutritional status (data not shown). In CF patients having a basal luminal activity of amylase and chymotrypsin either below or above the 10th centile activity of age matched controls, the IAP activity was not different (data not shown).

Discussion

Up to now, the information on the small intestinal brush border enzymes in CF is scarce and the data are even contradictory (14). In the present study, we deal only with untreated CF patients at the time of diagnosis. Genetic studies of the cystic fibrosis transmembrane conductance regulator (CFTR) reveal the same distribution of AF508 as found in other studies (15,16). As a genotype-phenotype relationship for neither the clinical condition, mucosal morphology, nor the enzyme activities studied could be detected, the data on the CF patients are presented for the whole group. Sampling small intestinal biopsy specimens from normal healthy infants and children is ethically not allowed. The control group, therefore, is made of a group of patients referred for ill-defined nutritional or gastrointestinal complaints for which no diagnosis could be found. The selection of control subjects explains their suboptimal nutritional status (fig. 1).

In the CF patients group 42 out of 51 (82%) have normal histological and histochemical features of the small intestinal mucosa. In the control group, taking into account the same criteria, 65% have normal small intestinal morphology. Autonomically, using different histological typing methods, found a normal small intestinal morphology in 86% of their CF patients (12). Cox et al. could show that proximal small intestinal injury in CF patients correlates with increased basal acid output (17). The low pancreatic bicarbonate output in CF further impedes the neutralisation of the acidic duodenal luminal content (18,19) resulting in postprandial duodenal pH values as low as 5.

Pancreatic insufficiency occurs in about 50% of the CF patients (20). Ingomar and Terslev, studying the enzyme content of duodenal juice from infants and children with chronic diarrhoea, concluded that neither the fasting condition nor the oral stimulation by a test meal proved to be superior for the diagnosis of pancreatic insufficiency (21). For practical reasons, we measured the pancreatic enzyme activities only in basal conditions in the duodenal juice obtained at the time of the intestinal biopsy sampling (12,13). As can be seen in table II and fig. 2, pancreatic function is significantly lower in the CF group. For amylase, because of the low normal values during the first months of life, the difference was seen from the third month of life only (fig. 2).

Table II. — Basal luminal amylase and chymotrypsin activities (UI/ml duodenal juice) and small intestinal enzyme activities (UI/g wet mucosa) of cystic fibrosis patients and controls with normal mucosa. Figures are median values with p25 and p75 values between parentheses

<table>
<thead>
<tr>
<th></th>
<th>Cystic fibrosis (n = 42)</th>
<th>Controls (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>6.8 (6.01 - 19)</td>
<td>13 (6.4 - 29)</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>23.5 (15.8 - 28)</td>
<td>24.7 (17.7 - 37.5)</td>
</tr>
<tr>
<td>Mucosal enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltase</td>
<td>7.1 (4.3 - 9)</td>
<td>7.8 (4.2 - 11)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>2.8 (1.4 - 4.7)</td>
<td>3.9 (2.3 - 6.7)</td>
</tr>
</tbody>
</table>

Fig. 3. — Small intestinal alkaline phosphatase activities (IAP) in controls and cystic fibrosis patients (CF).
Small intestinal mucosal maltase and sucrase activities do not differ in the CF patients from the controls (table 1). These findings are contradictory with the data of Antonovics showing increased disaccharidase activities in the CF mucosa (1,2). She attributed the higher disaccharidase activities in the CF mucosa to the absence of pancreatic enzymes, normally involved in the turnover of brush border enzymes. On the other hand, in agreement with previous studies by several authors (4,5,22,23), the lactase activity is significantly decreased (Table 1). The lower lactase activity in structurally normal intestinal mucosa from CF patients, can be seen as a molecular defect of the lactase enzyme. According to Allerå, Kretchmer and Lehenhan, the finding of depressed or absent lactase activity in the presence of normal alpha-glucosidases with normal intestinal morphology is strongly suggestive of primary lactase deficiency (24). Usually the decline of lactase activity begins between 2 and 3 years and is almost complete by the age of 5 to 10 years. Both in animal and human studies, the nutritional decline in lactase activity has been attributed to transcriptional and posttranscriptional mechanisms (25). Even when only the children below the age of 2 years were evaluated, the CF patients had still a significantly lower lactase activity. It is tentative to speculate that the reduced lactase activity in our CF patients is due to an interaction of the inefficiently processed CFTR protein, along its way between endoplasmatic reticulum and Golgi apparatus (26).

In contrast to the maltase and sucrase small intestinal mucosal activity but in line with the lactase activity, the intestinal alkaline phosphatase (IAP) activity is significantly lower in the CF compared to the control population (fig. 3). The difference of about 22% is due to a shift to the left of the IAP activity in the CF patients (fig. 4).

IAP is one of three isoenzymes : intestinal, placental and germ-cell alkaline phosphatase, arisen by a series of gene duplications from the ancestral tissue-nonspecific alkaline phosphatase. The genes for intestinal, placental and germ-cell alkaline phosphatase are clustered near the top of the long arm of chromosome 2 (2q34-37) and the corresponding enzymes only differ in posttranslational modifications involving carbohydrate residues (27). IAP is deeply buried in the lipid bilayer of the brush border membrane and bound to it via glycosylphosphatidylinositol (28,29). On the contrary, the sucrase-isomaltase, the maltase-glucosamylase and the lactase-phlorizinhydrolase are lipidop-like proteins anchored via a hydrophobic segment crossing the brush border membrane once (28). In addition to the alternative anchoring of IAP, the enzyme is more easily released from the brush border, both into the intestinal lumen and into the plasma (30). The release of IAP is facilitated in bloodstream seconers and during lipid absorption but no information is available about the release in CF (31).

Fig. 4. — The IAP activity is significantly lower in the CF population due to a relatively higher number of CF patients with a low IAP activity.

IAP is more resistant than the disaccharidases to the action of processes (32) but the opposite has been shown for the action by oxygen free radicals (33). Although certain CF patients have been found to be more susceptible to oxidative damage (34), there are no published data on lowered IAP in CF patients.

Finally, both the lowered IAP activity and the low lactase activity in CF mucosa could be related to the basic defect of CFTR. In a recent scanning and transmission electron study, it has been shown that in CF the absorbing cells of the villi are well preserved but that the mucous-containing ducts protrude from the apical membrane of the goblet cells (35). In contrast, evident ultrastructural lesions were found in the upper portion of the crypts. In about 60% of the CF crypts examined, both secretory and immature absorbing cells show degenerative features ranging from accumulation of lysosomes in the apical portion of the cell to cytoplasmic swelling and vacuolization. About 70% of the known CF gene results in the absence of mature CFTR at the correct cellular location and the accumulation of incompletely glycosylated protein (26,36,37).
Therefore, it is possible that the disturbed sorting-out of matured CFTR could influence the normal cellular processing of IAP and laxase in the enteroctye. The absence of CFTR proteins from the brush border membrane could also negatively influence both activities. Indeed, a protein-protein interaction between CFTR and the amiloride sensitive Na+ channel has been documented (38).

In conclusion, the small intestinal mucosa of CF patients shows normal stereoscopic and histological features in about two thirds of the patients. Enzyme studies on morphologically normal mucosa from CF patients show normal maltase and saccharase activities but significantly decreased lactase and IAP activities. In various enterocytes, low lactase activity is a common finding. The decrease of IAP activity, however, is more surprising and commented in more detail. An increased release of IAP and lactase from the brush border membrane and, or a disturbing sorting-out at the Golgi-apparatus, secondary to the abnormal CFTR-protein handling, seem to be the most likely hypotheses for the lowered enzyme activities in CF patients.

References

COMBINED IMPACT OF MUCOSAL DAMAGE AND OF CYSTIC FIBROSIS ON THE SMALL INTESTINAL BRUSH BORDER ENZYME ACTIVITIES


Key-words: Cystic fibrosis, small intestinal mucosa damage, chymotrypsin, amylase, sucrase, maltase, lactase, intestinal alkaline phosphatase.

ABSTRACT

In 61 cystic fibrosis (CF) patients, the small intestinal mucosa was studied at the time of diagnosis before starting therapy. In 19 out of 61 patients, partial villous atrophy on light microscopy and shortened villi on stereomicroscopic examination were seen. On the bi-

opsy specimens, maltase, sucrase, lactase and alkaline phosphatase activities were studied. Comparison of the enzymatic activities in CF patients having damaged mucosa and a group of patients having similar mucosal lesions of unspecified origin (UTID), reveals a significantly more pronounced decrease of the alkaline phosphatase activity (p<0.005) in the CF patients. This is in agreement with previous reported results in CF patients with normal mucosa.

The abnormal mucosal findings could be due to the decreased neutralization of the gastric content delivered into the duodenum, the early inflammatory reaction present in the CF mucosa and/or to the impaired synthesis of membrane glycoproteins and enzymes secondary to the CFTR mutation.

INTRODUCTION

In a previous study, we examined the impact of CF on the activity of some brush border enzymes in patients with normal morphology of the duodenal mucosa (1). It was shown that the lactase and intestinal alkaline phosphatase (LAP) activities were significantly reduced in the CF small intestinal mucosa. We wondered if we could confirm these results in the CF patients with damaged mucosa.

The present study reports on the maltase, sucrase, lactase and LAP activity in small intestinal biopsy specimens from 19 CF patients and 144 subjects with active small intestinal damage by unspecified temporary disorders (UTID). Both CF and UTID small intestinal bi-

opsy specimens had type II stereomicroscopic lesions as described by Sinnerling (2) and partial villous atro-
phy on histological examination (3). For comparable structural damage, the IAP activity is significantly lower in the CF specimens. This confirms the previous reported results in undamaged CF mucosa (1).

SUBJECTS AND METHODS

Subjects

61 untreated Caucasian CF patients were studied at the time of initial diagnostic workup. The findings on the 42 CF patients, having normal stereo microscopic (type I of Shneerling) and normal histological small intestinal biopsy features have been published elsewhere (1). The present study compares the 19 CF patients, having partial villus atrophy by light microscopy and type II features of the mucosal surface on stereomicroscopy (2, 3), and a group of 144 age matched subjects with alike small intestinal lesions (UTID). The UTID patients were referred because of failure to thrive in whom an intestinal biopsy was justified for the exclusion of primary small intestinal disease. In none of these children, the final diagnosis pointed to a primary intestinal disease. The diagnosis retained, after thorough search for the cause of their failure to thrive, was inadequate feeding, insufficient caloric intake or loose stools of unspecified origin. They are referred as the unspecified temporary intestinal disorder (UTID) group since one cannot call a subject with a partial villus atrophy a normal control patient. Oral informed consent was obtained from the parents of both CF and control subjects.

Methods

The biopsy specimen was obtained by use of the open paediatric Crosby capsule placed at the angle of Treitz. The specimens were classified according to Shneerling

TABLE I: Effects of mucosal damage and of cystic fibrosis on enzyme activities (IU/g wet weight mucosa): Medians and interquartile ranges. Percentile decrease of enzyme activity when control type I activity is regarded as 100%.

<table>
<thead>
<tr>
<th>Type I mucosa</th>
<th>Type II mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/g Wet Weight mucosa</td>
<td>Controls (n = 205)</td>
</tr>
<tr>
<td>Multase</td>
<td></td>
</tr>
<tr>
<td>24.7 (±)</td>
<td>23.5 (±)</td>
</tr>
<tr>
<td>(17.7-37.5)</td>
<td>(15.8-28)</td>
</tr>
<tr>
<td>100 %</td>
<td>95 %</td>
</tr>
<tr>
<td>Sacrase</td>
<td></td>
</tr>
<tr>
<td>7.5 (±)</td>
<td>7.2 (±)</td>
</tr>
<tr>
<td>(5.2-11)</td>
<td>(4.3-9)</td>
</tr>
<tr>
<td>100 %</td>
<td>96 %</td>
</tr>
<tr>
<td>Lactase</td>
<td></td>
</tr>
<tr>
<td>3.9 (±)</td>
<td>2.8 (±)</td>
</tr>
<tr>
<td>(2.2-6.7)</td>
<td>(1.4-4.7)</td>
</tr>
<tr>
<td>100 %</td>
<td>72 %</td>
</tr>
<tr>
<td>IAP</td>
<td></td>
</tr>
<tr>
<td>29.8 (±)</td>
<td>23.2 (±)</td>
</tr>
<tr>
<td>(20-38.1)</td>
<td>(13.2-32)</td>
</tr>
<tr>
<td>100 %</td>
<td>78 %</td>
</tr>
</tbody>
</table>

*p<0.001 | ** Significant disease related differences (Mann-Whitney U test) |
| *p<0.005 | * Significant intertype differences (Mann-Whitney U test) |
| *p<0.001 | UTID = unspecified temporary intestinal disorder |

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RESULTS

Clinical data

The median age of the CF children with partially damaged type II small intestinal mucosa was 0.25 year. The age matched UTID group of 144 children, with alike small intestinal mucosa appearance, had a median age of 0.33 year (n.s.). The type II CF and UTID patients tend to be slightly more malnourished than the type I CF and control patients respectively (Fig 1); the difference however, does not reach statistical significance. The median quotient of the BMI of the CF patients type II and of the UTID patients type II to the 50th centile value of the BMI in normal children, matched for age and gender, minus 1 was respectively -0.115 and -0.118 (n.s.).

Brush border enzyme activities

Table 1 shows the enzyme activities found in the UTID and CF patients with partially damaged type II small intestinal mucosa. For comparison, the data from CF patients and all the controls, having normal type I mucosa and previously published, are also presented (1).

The data on morphologically normal type I mucosa show that CF significantly affects the lactase (p<0.01) and the IAP activity (p<0.05). On the other hand, neither maltase nor sucrase activity is significantly affected by CF.

Comparing the control subjects, with type I small intestinal mucosa and the UTID subjects with type II mucosa, the mucosal damage significantly reduces all enzymatic activities to about 60-75 %. The maltase activity is the least and the sucrase activity the most affected.

In patients with damaged type II mucosa, CF additionally decreases the residual activity of all enzyme activities to about 40-60 %. The effect of CF, however, is only significant for IAP. There is no correlation between the IAP activity and the nutritional status in any of the examined groups (data not shown).

Chymotrypsin activity in the duodenal content

As both CF (8) and small intestinal mucosa damage (9) may lower the pancreatic enzyme activities in the duodenal content, the chymotrypsin activity in the basal duodenal content was measured in the control and in the three groups of patients (Fig. 2). In both groups of
COMBINED IMPACT OF MUCOSAL DAMAGE AND OF CYSTIC FIBROSIS ON THE SMALL INTESTINAL BRUSH BORDER ENZYME ACTIVITIES

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DISCUSSION

At the time of diagnosis, the small intestinal mucosa was morphologically impaired in 19 out of 61, about 30 %, of the CF patients. Using histological criteria only, a comparable figure of 42 % has been published (10). In CF, the cause of small intestinal injury has been attributed to the increased basal acid output of the stomach (11, 12) and the lower pancreatic bicarbonate secretion (12), both resulting in a lower pH of the upper intestinal tract. Subclinical mucosal inflammation, not by the presence of bacterial colonization but by the action of cytokines as IL-1 and IL-8, could be an alternative explanation (14). There is also evidence of an increased permeability of the CF intestinal mucosa. Ca²⁺-EDTA and disaccharides of more than 0.3 nm² are taken up, to a greater extent via the para-cellular route, across the tight junctions between the enterocytes (15). Furthermore, an intestinal losing of plasma-derived proteins such as albumin, immunoglobulin G and alpha-1-antitrypsin has also been shown (14). On the other hand, metabolically inert monosaccharides such as xylose, manitol or rhamnose with a molecular volume of 0.2 nm³ are equally well taken up by transcellular diffusion in normal and in CF subjects (16).

Table 1 shows the effects of CF on the brush border enzyme activities maltase, sucrase, lactase and alkaline phosphatase. In CF patients with normal mucosa, the maltase and sucrase activities are almost not affected as evidenced by the residual activities of nearly 95 %; in contrast, CF affects lactase and IAP activities resulting in residual activities of 72 and 78 % respectively. On the other hand, the residual enzyme activities of the two patient groups with type II mucosa are much lower than of the control group: 60 to 75 % and 40 to 65 % in the UTID and the CF patients respectively. Comparing the two mucosa type II patient groups only the IAP activity is significantly different, 20.7 IU/g mucosa in the UTID group and 12 IU/g mucosa in the CF patients (P<0.005).

The multiple enzyme deficiency in CF and UTID patients could be explained by the mucosal damage. It is also possible that the multiple brush border enzyme deficiency is caused by the lack of shared, pleiotropic regulatory factors for the synthesis of the brush border enzymes (17). The defective acidification of the trans-Golgi/trans-Golgi network, of prelysosomes and endosomes, as a result of the diminished chloride conductance in CF, could indeed impair the final processing of the brush border membrane and its enzymes (8,18). The mutant CFTR-protein may also fail to reach the brush border and accumulate in the endoplasmic reticulum, an anomaly known as endoplasmatic reticulum storage disease (19).

The more pronounced decrease of the IAP activity might be caused by the greater sensitivity of the enzyme to free radicals (20). There is also evidence of increased oxidative stress in CF patients (21). The subclinical zinc deficiency in some CF patients could further contribute to the decrease of IAP activity as zinc is capable of protecting IAP from oxidative stress (22).

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Although pancreatic insufficiency is the main culprit of fecal fat losses in CF, it is known that the losses cannot always be corrected by higher intakes of pancreatic enzymes (23). Recent studies, using 14C-labelled triglycerides and fatty acids, give direct evidence that the defective absorption of long-chain fatty acids by the small intestinal mucosa is higher than previously supposed (24, 25). Whenever the fecal fat losses cannot be corrected by the usual dosages of pancreatic enzyme preparations, attention should be paid to the absorption of the lipolytic end products and their handling by the enterocytes (26). The variable absorptive capacity of the intestinal mucosa may also explain the inter subject differences in plasma levels of the fat soluble vitamins A and E (26). Those vitamins are not only absorbed by but also metabolized within the enterocytes (27, 28).

REFERENCES

5. Garen A, Levin MD, A f u t u r i t r i c o n d t y k e n c t a r p o y c e m e n . E. coli. J. Gastroenterol. 1968; 38: 420-33.
4.1 Importance of essential fatty acids in cystic fibrosis

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

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Chapter 4: Essential Fatty Acids

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-dependent 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-nesteroid 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.

<table>
<thead>
<tr>
<th>Δ6-Desaturation</th>
<th>6ω3</th>
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<th>6ω9</th>
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<tr>
<td>6ω3</td>
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<tr>
<td>Δ6-Desaturation</td>
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<tr>
<td>Octadecatrienoic acid (18:3ω3)</td>
<td>Δ6-Desaturation</td>
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<td>Δ5-Desaturation</td>
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<td>Docosapentaenoic acid (20:5ω3)</td>
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<td>Δ6-Desaturation</td>
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Fig. 1. Fatty acid notation: number of carbon atoms: number of double bonds followed by biochemical series.
In the cerebral cortex, retina, testes and muscle the most abundant ω3 acyl chains are eicosapentaenoic acid (EPA, 20:5ω3) and DHA. The alternating sequence of desaturation and elongation is also the primary pathway of DHA synthesis. The ω3 and ω6 long chain fatty acids compete for the same elongases and desaturases.

4. Phospholipase A2

PLA2 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 localization and calcium dependency [7,8]. These types of PLA2 contain different isoenzymes making the understanding of the FA metabolism even more complicated [8].

The intracellular calcium-independent phospholipases A2 (iPLA2) is a membrane-associated enzyme that is implicated in membrane phospholipid remodelling and signal transduction [9]. The type IIa extracellular secretory PLA2 (sPLA2) 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-dependent cytosolic PLA2 (cPLA2) and the sPLA2 are linked to the cyclooxygenase (COX)-2 enzymes responsible for the production of prostaglandins and thromboxanes [13].

PLA2 is activated by multiple agonists and cell specific signals [14,15], resulting in the release of PUFAs, precursors of inflammatory mediators, eicosanoids [12]. The best-studied eicosanoid system is that of AA.

Some derivatives of ω-linolenic acid, the parent EFA of the ω3 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). Cyclooxygenase (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.

Fig. 2. Arachidonic acid is released by the action of phospholipase A2 on membrane phospholipids. Phospholipase A2 is activated by multiple cell signals, bradykinin, angiotensin II. Some derivatives of α-linolenic acid, the essential fatty acid of the ω3 series, inhibit the release of arachidonic by phospholipase A2, and result in antagonistic actions. The three main directions of the metabolism of arachidonic are cyclooxygenation, lipoxygenation and epoxygenation. The cyclooxygenase products of arachidonic generate prostaglandins, prostacyclins and thromboxanes of the 2 series. Lipoxygenase products of arachidonic generate the pro-inflammatory leukotrienes, but also some biologically very active hydroxyeicosatetraenoic acids (HETEs) and the anti-inflammatory lipoxins. The epoxygenation pathways result, by action of cytochrome P450, in formation of 3 categories of biologically very active products: midchain HETEs, ω-terminal HETEs and epoxyeicosatrienoic acids (EETs).
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 α6 FAs stimulates the production of pro-inflammatory PGs (PG2 series) derived from AA, while consumption of α3 FA stimulates the production of anti-inflammatory PGs (PG3 series). PGs have a wide variety of actions but most of them cause muscle 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 hydroperoxyicosatetraenoates 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 transcription of lipoxin analogues in a CF mouse model resulted in decreased lung inflammation and infection [28].

Eicosanogenesis pathways result by action of different cytochromes P450, in formation of hydroxyeicosatetraenoate and epoxieicosatrienoic 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.

Dihomogamma-linolenic 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].

Eicosapentenoic 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 eicosanoid. They are induced by aspirin via the acetylation of COX-2. These derivatives might contribute to the frequently reported beneficial responses obtained by α3 supplementation [38].

6. Fatty acids in cystic fibrosis

6.1. Essential fatty acid status in cystic fibrosis

Freedman and Alvarens 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 ideal 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 α3 and α6 PUFA concentrations [41–44]. Although attention was not drawn to the imbalance of α3 and α6 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.
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Freedman et al found the same lipid imbalance in nasal and rectal biopsies of CF patients as they 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. Aetiology of EFA deficiency in CF

The initial hypothesis was that the deficiency was secondary to malnutrition, dietary fat 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 EFA 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 dexanmethason. 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 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 vitamin E, vitamin C, vitamin A, β carotene and selenium. However, the 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 Shea, 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 vascular constrictions [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-lipoxigenase 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 transspatial 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 α6 PUFAs. Moreover, long chain α6 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 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

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4.2 Factors influencing the essential fatty acid status in cystic fibrosis

4.2.1 Essential fatty acid status in relation to genotype

Key Words
Fatty acids · Phospholipids · Cystic fibrosis · Genotype · Clinical condition

Abstract
Objective: To evaluate the relation of clinical parameters and genotype with the serum phospholipid fatty acid (FA) composition in cystic fibrosis (CF) patients. Methods: A blood sample was taken from CF patients with stable pulmonary disease for the determination of phospholipid FA composition and vitamin E concentration who had been followed for at least 6 months at our Cystic Fibrosis Centre. Genotype, age, pancreatic function, nutritional status, caloric intake, pulmonary function and presence of Pseudomonas colonization, liver disease or diabetes mellitus were recorded. Patients were divided into two groups according to their genotype (group A: mutation class I, II, or III, group B: mutation class IV, V). Results: CF patients (group A and B together) have significantly lower docosahexaenoic acid (DHA) (p < 0.007) and linoleic acid (LA) (p < 0.0001) and higher dihomogamma-linolenic acid (DGLA) (p < 0.0001), oleic acid (OA) (p < 0.0001) and Mead acid (MA) (p < 0.0001), resulting in an increased ratio of arachidonic acid (AA)/DHA (p < 0.004), MA/AA (p < 0.0001) and OA/LA (p < 0.0001). Compared to group B, group A had a lower LA (p < 0.002) and a higher DGLA (p < 0.002), 22:4ω-6 (p < 0.03), 22:5ω-6 (p < 0.03) and 20:3ω-9 (p < 0.04). There was however no significant difference between the groups for age, pulmonary function, nutritional status and vitamin E concentration. There was no relation of serum FA composition with nutritional status, caloric intake, pancreatic function, gender, pulmonary function, Pseudomonas colonization or diabetes mellitus. In CF with liver disease the DHA was lower than in the patients of the same genotype. Conclusion: FA disturbances are more pronounced in the severe CF genotypes and the presence of CF-related liver disease. Future studies on supplementation should take these parameters into account.

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CFTR is not only a cAMP-dependent chloride (Cl⁻) channel in the apical membrane of the respiratory and gastrointestinal tract [1], but it also regulates other Cl⁻ channel proteins [2], acidification of intracellular Golgi and vesicles [3] and vesicle trafficking [4]. Although the gene locus has been known since 1989 and more than 1,300 mutations have been identified, the search for a genotype-phenotype relationship has not been very successful [5].
Different old reports highlight the presence of essential fatty acid (EFA) abnormalities in CF [6–10] of which the clinical relevance and influencing factors are not yet well understood. Renewed interest in this aspect of CF arose when Freedman et al. [11] described a mouse model of CF in which important abnormalities of the fatty acid (FA) metabolism were shown. The biochemical aberrations as well as morphological abnormalities were corrected by pharmacological doses of docosahexaenoic acid (DHA; 22:6ω-3). However, different CF mice models do not display the same FA abnormalities [12, 13]. This could be a result of differences in functional CFTR protein, since Strandvik et al. [14] described a relation between some severe mutations and FA abnormalities. Many different factors can influence FA composition in CF patients and extensive studies are needed to clarify the relation between FA composition and CFTR as well as clinical parameters [15].

This study goes into the influence of clinical parameters as nutritional status, liver disease, pulmonary function, diabetes mellitus and genotype on the FA status of the current CF patients. Only the FA of the ω-3, ω-6 and ω-9 series are considered since in animal studies changes in the ratio of these FAs can influence outcome [11].

Table 1. Clinical characteristics of the different subgroups expressed as median and interquartile range (IQR) in parentheses, significant differences between the subgroups are calculated with a Mann-Whitney U test or χ² test, relevance as tied p value

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>Group A (n = 79)</th>
<th>Group B (n = 25)</th>
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<td>W/H%</td>
<td>97.8 (12.85)</td>
<td>95.5 (9.9)</td>
<td>97.5 (12.45)</td>
</tr>
<tr>
<td>Cal. intake</td>
<td>129 (31)</td>
<td>116 (32)*</td>
<td>126 (30.25)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.9 (0.4)</td>
<td>0.9 (0.42)</td>
<td>0.9 (0.4)</td>
</tr>
<tr>
<td>FEV, %</td>
<td>80.4 (40.35)</td>
<td>75 (41.35)</td>
<td>80.4 (39.53)</td>
</tr>
<tr>
<td>FVC%</td>
<td>89.7 (21.3)</td>
<td>86.6 (21.28)</td>
<td>89.1 (21.2)</td>
</tr>
<tr>
<td>Pseudomonas col.</td>
<td>36/79</td>
<td>7/25</td>
<td>43/104</td>
</tr>
<tr>
<td>Pancreatic suff.</td>
<td>0/79</td>
<td>8/25**</td>
<td>8/104</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9/79</td>
<td>4/21</td>
<td>13/104</td>
</tr>
<tr>
<td>Liver disease</td>
<td>13/79</td>
<td>2/25</td>
<td>15/104</td>
</tr>
</tbody>
</table>

W/H% = Weight as percentage of ideal weight for height; Pancreatic suff. = pancreatic-sufficient patients with a fecal elastase 1 >200 IU/g feces; FEV, % = forced expiratory volume as percentage of predicted; FVC% = functional vital capacity as percentage of predicted; Pseudomonas col. = Pseudomonas colonization; Cal. intake = as percentage of RDA for matched healthy individuals.

* p < 0.05, ** p < 0.01.

Methods and Patients

Patients

Patients attending the Cystic Fibrosis Centre of Ghent University Hospital were asked to participate in the study (n = 104). All had a positive sweat test (chloride >60 mU). Patients with a pulmonary exacerbation defined as increased production of sputa, worsened pulmonary function or the start of antibiotics were excluded. After informed consent, overnight fasting blood samples were drawn. Height and weight were recorded and percentage of ideal weight for height was calculated (W/H%). The caloric intake using a 3-day diary is calculated as a percentage of the recommended daily allowances (RDA) (revised guidelines 2006; www.health.fgov.be/CSH_HGR) for age and gender. Patients are instructed to aim at an intake of 130% of the RDA [16]. The mean seafood intake expressed as grams per day was also calculated using the 3-day intake diary. Pancreatic function is based on the fecal elastase-1 (FE-1) concentration. FE-1 concentration of >200 IU/g feces is considered as pancreatic-sufficient [17]. The presence of CF-related diabetes mellitus and genotype were considered. The diagnosis of CF-related liver disease was made on ultrasound aspects of the liver [18]. When the ultrasound score was >6 together with a splenomegaly, the diagnosis of CF-related liver disease was made [18]. None of the patients took polyunsaturated fatty acid (PUFA) supplements or drank PUFA-enriched milk. The patients did not receive parenteral lipid supplements for at least 4 weeks before the study. All pancreatic-insufficient patients receive a supplement of fat-soluble vitamins containing cholecalciferol 1,000 IU, α-tocopherolacetate 190 mg, phytonemadin 1 mg, retinolacetate 10,000 IU and pancreatic enzyme replacement therapy. In patients >6 years (n = 88) the pulmonary function was measured by a Jaeger MasterScreen body plethysmograph and expressed as percentage of predicted for height.

Cystic CFTR mutations were classified as proposed by Welsh and Smith [19] using the CF mutation database (www.genet.sickkids.on.ca). Group A included patients with type I, II or III mutations resulting in no functional CFTR protein (n = 79). Group B included patients with at least one type IV or V mutation resulting in a partially functional CFTR protein (n = 25) or those with unknown effect on the CFTR protein (table 1).

Controls

The control values are obtained from 44 healthy controls aged 1–47 years (median age: 18). All were well nourished; their W/H% was between 90 and 110%. They did not take PUFA supplementation or PUFA-enriched milk and did not carry one of the mutations as determined by the INNO-LiPA CFTR19® (Innogenetics NV, Ghent, Belgium).

Laboratory

Serum was prepared and stored at −20°C until analysis. Lipids were extracted [20], phospholipids separated by thin-layer chromatography [20] and converted into methyl esters [21]. The methyl esters were extracted and separated by temperature-programmed capillary gas chromatography [22] with a Varian model 3500 gas chromatograph equipped with a 30 m × 0.25 mm × 0.2 μm 10% cyanopropylphenyl–90% bis-cyanopropyl polysiloxane column (RIX®-2390, Restek, USA). The injector and detector
were set at 285°C. The column temperature was programmed from 150 to 240°C at a heating rate of 2°C/min. N₂ was used as carrier gas, with a flow rate of 1 ml/min. The FA methyl esters were identified by comparison with retention times of known standards (Sigma). Peak integration and calculation of the percent composition was performed by computer using make of Star GC Workstation software, Version 5.3.1. A review of the response factors were determined by the use of standard methyl ester mixtures (NuCheck and Applied Science). The results were expressed as weight %. No internal standard was used. The intraday variance for the determination of the fraction of each FA depended on relative abundance with variation coefficients varying from 5% for small peaks (≤0.5% of total) to about 0.5% for large peaks (about 25% of total).

Serum α-tocopherol was analyzed by isocratic high-pressure liquid chromatography [23]. Genotypes were determined with the INNO-LIPA CFTR1® and INNO-LIPA CFTR17+Tn Update® kit (Innogenetics NV) or sequencing of the CFTR genome. FE-1 was determined with a commercial ELISA kit (ScheBo® Biotech AG, Giessen, Germany).

**Statistics**

Non-parametric statistical methods (Mann-Whitney, Wilcoxon rank, Kruskal-Wallis and Spearman rank correlation) were used with StatView 5.1 (Abacus Concepts, Inc., Berkeley, Calif., USA) for Windows. Results are given as median and interquartile range, the significance of the differences between groups as tied p values, the p value of the Spearman rank correlation is given when significant. Differences are considered significant when the p value is <0.05. The study protocol was approved by the Ethics Committee of Ghent University Hospital (2005/282).

**Results**

**Patients**

104 patients were included of which 50 were females. They had all been treated for at least 6 months. The median age was 15 years (range 1–41). The patient characteristics are summarized in Table 1. The mean seafood intake was 25 g/day (SD 46) which is comparable with the average seafood intake (27 g/day) in this region [24]. Using a stepwise multivariate regression, pulmonary function was determined by age and nutritional status.

The genotype of the patients and classification into two groups is given in Table 2. The patient characteristics of the different genotype groups are also presented in Table 1. There were no significant clinical differences between the groups using a Mann-Whitney U test or a χ² test between the two genotype groups except for pancreatic function (p<0.01), pancreatic sufficiency only being present in group B.

**Fatty Acid Disturbances of Total Group (table 3)**

In the α-3 series, the parent FA ALA (18:3n–3) is significantly higher in CF compared to controls (p<0.006). Its metabolic end product of the FA cascade DHA (22:6n–3) is significantly lower (p<0.007).

In the α–6 series, the parent FA linoleic acid (LA) (18:2n–6) is significantly lower (p<0.0001) and dihomo-gammalinolenic acid (DHGLA, 20:3n–6) significantly higher (p<0.0001). Arachidonic acid (AA, 20:4n–6) is normal but its elongation and desaturation product docosapentaenoic acid (22:5n–6) is significantly higher in CF patients (p<0.0001) (fig. 1).

In the ω–9 series, oleic acid (OA) (18:1n–9) and its elongation/desaturation product Oleo acid (MA, 20:3n–9) are significantly higher in CF (p<0.0001). The ratio of MA (20:3n–9)/AA (20:4n–6) is significantly higher in CF
Fig. 1. Elongation and desaturation steps of the EFAs. The FAs in bold letters are significantly different from controls with the arrow indicating the direction of change compared to controls.

Table 3. Median % weight and interquartile range of phospholipid EFAs in different CF genotype groups compared to controls.

<table>
<thead>
<tr>
<th></th>
<th>CF total</th>
<th>Group A</th>
<th>Group B</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA (18:3n-3)*</td>
<td>0.18 (0.06)*</td>
<td>0.18 (0.07)*</td>
<td>0.18 (0.05)</td>
<td>0.16 (0.06–0.51)</td>
</tr>
<tr>
<td>DHA (22:6n-3)*</td>
<td>2.72 (1.36)*</td>
<td>2.68 (1.9)*</td>
<td>2.83 (0.76)*</td>
<td>3.39 (2.21–5.39)</td>
</tr>
<tr>
<td>LA (18:2n-6)*</td>
<td>18.51 (5.97)*</td>
<td>17.41 (4.25)*</td>
<td>21.35 (5.52)*</td>
<td>20.99 (4.2)</td>
</tr>
<tr>
<td>DHGLA (20:3n-6)*</td>
<td>3.55 (1.39)*</td>
<td>3.66 (1.22)*</td>
<td>2.67 (1.37)*</td>
<td>1.67 (0.51)</td>
</tr>
<tr>
<td>AA (20:4n-6)</td>
<td>8.92 (2.51)</td>
<td>9.0 (2.69)</td>
<td>8.45 (1.63)</td>
<td>9.44 (1.78)</td>
</tr>
<tr>
<td>(22:5n-6)*</td>
<td>0.325 (0.2)*</td>
<td>0.34 (0.19)*</td>
<td>0.24 (0.15)*</td>
<td>0.14 (0.08)</td>
</tr>
<tr>
<td>OA (18:1n-9)*</td>
<td>9.65 (2.3)*</td>
<td>9.83 (2.31)*</td>
<td>9.19 (1.59)*</td>
<td>8.54 (1.22)</td>
</tr>
<tr>
<td>MA (20:3n-9)*</td>
<td>0.24 (0.25)*</td>
<td>0.27 (0.27)*</td>
<td>0.14 (0.15)*</td>
<td>0.15 (0.08)</td>
</tr>
<tr>
<td>OA (18:1n-9)/LA (18:2n-6)c</td>
<td>0.51 (0.21)c</td>
<td>0.53 (0.22)c</td>
<td>0.4 (0.15)c</td>
<td>0.40 (0.31–0.61)</td>
</tr>
<tr>
<td>AA(20:4n-6)/DHA(22:6n-3)c</td>
<td>3.41 (1.66)c</td>
<td>3.52 (1.75)c</td>
<td>3.28 (1.1)</td>
<td>2.67 (1.64–4.57)</td>
</tr>
</tbody>
</table>

ALA = α-Linolenic acid; DHA = docosahexaenoic acid; LA = linoleic acid; DHGLA = dihomogammalino- lenic acid; AA = arachidonic acid; OA = oleic acid; MA = Mead acid.

Differences using a Kruskal-Wallis test according to Group A, Group B and Control: * p < 0.05, † p < 0.01, ‡ p < 0.001.

Differences between the subgroups using a Mann-Whitney-U test: CF total vs. Control: * p < 0.01, †p < 0.001, ‡ p < 0.0001; Group B vs. Group A: * p < 0.05, † †p < 0.01; Group A or Group B vs. Control: † † p < 0.01, † † † p < 0.0001.

than in controls (p < 0.0001). This ratio is a measure for EFA deficiency.

The ratio of AA/DHA (20:4n-6/22:6n-3), an important indicator of the inflammatory state, is significantly higher in CF (p < 0.004). **FAs in Relation to Clinical Parameters**

Correlations were calculated between biochemical, clinical and nutritional parameters on one hand and FA concentrations of ω-3, ω-6 and ω-9 FAs on the other. There were no differences in FAs according to Pseudomonas colonization, gender or diabetes mellitus using a
Mann-Whitney U test. There was no association calculated with a Spearman rank correlation between FA and caloric intake, forced vital capacity (FVC%) or forced expiratory volume in 1 s (FEV1%).

In the CF group, LA (18:2n–6) decreases with age (p = −0.231, p < 0.02). There was an association between W/ H% and DGLA (20:3n–6) (p = 0.231, p < 0.02). A lower serum vitamin E is associated with a lower DHA (22:6n–3) (p = 0.23, p < 0.03) and a higher 22:5n–6 (p = −0.258, p < 0.009).

Patients with liver disease (n = 13 in A; n = 2 in B) display the same FA pattern as group A but have an even lower DHA (22:6n–3) concentration as the patients of group A without liver disease (p < 0.02). They did however not differ in the other clinical parameters.

**Genotype Subgroups (table 3)**

There are no significant differences between the genotype groups for age, nutritional status, pulmonary function, serum vitamin E concentration and diabetes mellitus (n = 15) (table 1). Group A had a slightly higher caloric intake expressed as percent of RDA compared to group B (p < 0.05). Pancreatic sufficiency (n = 8) was only present in group B. They display, however, the same FA pattern as patients of group B with pancreatic insufficiency. Significant differences in FA composition between the subgroups and the control group were observed using a Kruskal-Wallis test (table 3; fig. 2). The LA (18:2n–6) concentration is significantly lower in group A compared to group B. DHGLA (20:3n–6) and 22:5n–6 decrease stepwise going from group A to group B and controls. OA (18:1n–9) and ALA (18:3n–3) are equally higher in group A and B compared to controls and DHA (22:6n–3) equally lower. The ratio MA (20:3n–9)/AA (20:4n–6) is significantly higher in group A compared to group B (p < 0.002) and the controls (p < 0.0001).

**Discussion**

The frequently reported FA abnormalities in CF were confirmed in this study [6–10, 25]. Although the laboratory data suggest EFA deficiency (decreased LA (18:2n–6), increased MA/AA ratio), clinical symptoms of EFA deficiency were not found in this patient group, which is in accordance with earlier publications where symptoms have only been reported in untreated patients at diagnosis [26–28]. The EFA deficiency is not only a consequence of intestinal fat malabsorption since the abnormalities were described in the first weeks of life [29] and were also present in well-nourished CF patients [7, 14]. This study confirms the lack of relation between nutritional status and EFA deficiency. Other mechanisms can be related to FA abnormalities as there are oxidation of FA for energy purposes [30], increased use for eicosanoid production [31, 32], higher lipid turnover in the cell membranes [33], impaired FA metabolism, desaturase dysfunction [10, 34] and lipid peroxidation [35].

The higher ALA (18:3n–3) concentration of our patient group compared to controls is probably due to the current dietary advice for CF patients which stimulates the use of ω–3–rich oils as canola oil. The seafood intake in our CF population was comparable to the intake of a local control group [24].

Although the parent FA of DHA (22:6n–3) was significantly higher in concentration than in controls, this did not result in an increase of DHA (22:6n–3). This could be a result of the competition of ALA (18:3n–3) with 24:5n–3, which is an intermediate in the conversion of 22:5n–3 into DHA (22:6n–3) for the Δ6-desaturase [36] or of a malfunctioning Δ6-desaturase in CF patients. It would also be interesting to look at factors influencing the desaturase activity such as trans-FA in the high fat diet of the CF patient [37].

As the condition of the patients and their genotype might have an influence on FA [15], we wanted to investigate the relation of the FAs with clinical parameters and genotype. There was no association of the FA status with gender, caloric intake, *Pseudomonas* colonization, FVC% or FEV1%. Until now, only one old and one recent study described a relation of LA with FEV1% [9, 38], which was not confirmed in this study. Even if we looked at the same age subgroup as Maqbool et al. [38], there was no correlation between the pulmonary function and FA status. In this group of patients, pulmonary function was only determined by age and nutritional status as described in many other reports [39, 40]. It remains therefore questionable whether influencing the FA composition will influence clinical outcome of the CF patients which is merely dependent on pulmonary function.

The high sensitivity of DHA (22:6n–3) for peroxidation [41] might be reflected by the positive association of DHA concentration with the serum vitamin E concentration. The negative association of vitamin E and 22:5n–6 could be secondary to DHA deficiency. Indeed, low levels of DHA, possibly a result of low levels of vitamin E, lead to increased 22:5n–6 [42]. This hypothesis is sustained by a strong negative correlation between 22:5n–6 and DHA (p < 0.0001).
Fig. 2. Box plots according to their genotype of LA (18:2n−6), DGLA (20:3n−6), 22:5n−6, ALA (18:3n−3) and DHA (22:6n−3). The box plots of DHA (22:6n−3) are subdivided into the presence of liver disease. The number of patients in each subgroup are indicated for DHA (22:6n−3) on the box. The significant differences calculated by Mann-Whitney U tests are indicated with an arrow.

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We observed a negative association of LA (18:2n-6) with age in CF patients. It could be hypothesized that older CF patients have worse pulmonary functions leading to an increased oxidative stress [43] and energy need [44] leading to increased oxidation of LA (18:2n-6). If so it remains strange that only LA (18:2n-6) displays this association. This hypothesis is, however, not sustained by an association between the FAs and pulmonary function. In contrast to this finding in CF patients, a decreased Δ6-desaturase activity with aging, leading to increased LA concentrations in the general population, has been described [45].

DHA (22:6n-3) is significantly lower in patients with liver disease when they are compared to the other patients of the same genotype group. This could be the result of decreasing hepatocellular function. However, in CF, liver disease is mostly a problem of fibrosis and portal hypertension [46, 47]. None of the patients had signs of functional liver failure. EFA deficiency itself can also cause liver abnormalities and liver steatosis being most frequently described [48]. Although liver steatosis can be present in CF in case of malnutrition [49], the patients we describe have portal hypertension. Although it is unclear whether the lower DHA (22:6n-3) concentration is primary or secondary to the CF liver disease, it remains a matter of concern. In CF mice models, treatment with DHA prevents the development of liver disease [50, 51]. If we want to confirm this in humans, large studies will be needed to evaluate the effect of DHA on the development of CF-related liver disease since only 10% of the patients will develop it [52] and up until now, risk factors for its development are unknown. Despite the treatment of all our CF liver disease patients with ursodeoxycholic acid, the disturbances in the FA profile are still observed. The amelioration of FA composition due to ursodeoxycholic acid, described by Lepage et al. [53], was not able to restore the FA composition of our CF patients with liver disease.

In most reports, genotype is not taken into account when describing the FA abnormalities observed. Not all CF animal models display the FA abnormalities however [11–13]. Strandvik et al. [14] described an association of genotype severity with FA disturbances in CF patients. We classified our patients according to the expected severity of the mutation as described by Welsh and Smith [19]. Although there were no significant differences in clinical parameters between the genotype groups except for pancreatic sufficiency which was only present in group B and a higher caloric intake in group A, it remains difficult to know how much of the observed differences between the genetic subgroups can be accounted for by the genotype. Indeed, the small number of subjects in group B and the substantial difference in pancreatic deficiency by themselves could account for the observed differences in phospholipid FAs. The LA (18:2n-6) concentration is significantly lower in the patients with severe mutations, DGLA (20:3n-6) concentration as well as the MA (20:3n-9)/AA (20:4n-6) ratio is higher in the patient group with more severe mutations. Since the genotype subgroups did not differ in clinical status, increased energy needs are unlikely to be the cause of the observed differences. Furthermore, as the pancreatic-sufficient patients display the same pattern as their pancreatic-insufficient genotype controls, it is unlikely that fat malabsorption could be the main reason of the observed FA imbalance [15]. Our results are in concordance with the hypothesis that the CFTR mutation itself results in a different FA metabolism in CF [33, 54, 55], since more severe mutations result in more pronounced FA abnormalities.

Conclusion

FA disturbances are more pronounced in the severe CF genotypes, suggesting a relation with the basic defect. Patients with CF liver disease have the lowest DHA concentration of all CF patients. Future studies on FA in CF should describe as precisely as possible the influencing factors.

References


Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation. | Stephanie Van Biervliet

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Vani Bierbliet et al.
4.2.2 Influences of Cystic Fibrosis related liver disease on essential fatty acid status

Fatty acid status in Cystic Fibrosis patients with CF related Liver Disease
S. Van Bievliet MD, JP. Van Bievliet MD PhD, E. Robberecht MD PhD, A. Christophe PhD
Submitted to J Cyst Fibros

Abstract:
In cystic fibrosis (CF), the genotype is known to influence the fatty acid (FA) composition. Diabetes mellitus and liver disease can influence FA status. Until now, there are no data on the influence of CF related diabetes mellitus and CF related liver disease (CFRLD) on the FA status of CF patients. The aim of this study was to evaluate whether the presence of CFRLD influences the FA status. Method: A fasting blood sample for the determination of serum vitamin E and phospholipid FA composition was withdrawn in 79 CF patients, with stable pulmonary disease, under regular control in our CF centre. Patients with CFRLD (n = 13) were compared to CF patients with the same severe genotype (n = 66). Results: The CF patients with CFRLD have lower DHA (22:6 n-3) and increased docosatetraenoic acid (22:4 n-6). There were no significant differences in the precursors of these FAs. Conclusion: DHA concentration in patients with CFRLD is more profoundly decreased than in their genotype controls. The presence of CFRLD should be taken into account in future FA studies in CF patients.

Introduction:
Cystic fibrosis (CF) is the most common lethal genetic disease in Caucasians. The CF gene codes for an integral membrane protein, the CF transmembrane conductance regulator (CFTR). The mutations of the CFTR gene are classified in relation to the properties of the CFTR protein as proposed by Welsh (1). Several reports on CF patients describe blood and tissue fatty acid (FA) imbalances (2-9). They are characterised by low linoleic acid (LA, 18:2n-6) and docosahexaenoic acid (DHA, 22:6n-3), increased dihomogamma linolenic acid (DHGLA, 20:3n-6) and normal or increased arachidonic acid (AA; 20:4n-6) as compared to controls (2-9). The aetiology of the FA imbalance is still a matter of debate. Malabsorption secondary to pancreatic insufficiency is a possible cause (3, 6, 11). However, the imbalances were also described in well nourished and pancreatic sufficient patients (2, 5, 10). Other groups describe an abnormal FA metabolism (12-14).
The FA composition is known to be influenced by the genotype in both CF mouse models (15-17) and human CF (10, 18). The FA imbalance is more pronounced in the severe CF genotypes resulting in non functional CFTR protein (10, 18). Little is known about the influence of accompanying CF problems on the FA composition.
FA abnormalities have been described in cholestasis (19, 20) and cirrhosis (21, 22). This study goes into the differences in PL FA composition of CF patients with a severe genotype with or without CF related liver disease (CFRLD).

Methods
Patients
Patients attending the CF centre of the Ghent university hospital were asked to participate in a study on the relation in CF patients between their genotype and fatty acid composition (n= 104). The results were published elsewhere (18). Of these patients 79 had a severe CFTR mutation, resulting in non functional CFTR protein (1) (table 1). All had a positive sweat test (chloride > 60 mmol/L). Height and weight were recorded and percentage of ideal weight for height was calculated (W/H%). The mean seafood intake expressed as g per day was also calculated using the 3-day intake diary. None of the patients took polyunsaturated fatty acid (PUFA) supplements or drank PUFA enriched milk. The patients did not receive parenteral lipid supplements for at least 4 weeks before the study.

In patients above the age of 6y (n=66/79) the pulmonary function was measured by a “Jaeger Masterscreen Bodyplethysmograph” and expressed as percentage of predicted for height. Patients with a pulmonary exacerbation defined as increased production of sputa, worsened pulmonary function or the start of antibiotic treatment were excluded.
All patients were pancreatic insufficient, defined as a faecal elastase-1 (FE-1) concentration < 200 IU/g (23). All patients receive supplements of fat soluble vitamins containing cholecalciferol 1000 IU, α-tocopherol acetate 100mg, phytomenadion 1mg, retinol acetate 10000 IU and pancreatic enzyme replacement therapy.

The diagnosis of CFRLD was made on ultrasound aspects of the liver (24). According to Williams et al. CFRLD is present when the ultrasound score is above 6 together with a splenomegaly (24). All patients received an ultrasound maximum 1 month prior to the blood sampling.

13 patients had CFRLD diagnosed on ultrasound with a cirrhotic liver and splenomegaly. In 5, liver biopsy demonstrated a multilobular biliary fibrosis and cholangitis. Eight patients had endoscopically proven varices with repeated variceal bleeding in 5. Patients with variceal bleeding were treated with transjugular intrahepatic portosystemic stent shunt in 1 and splenectomy with a shunt between the splenic vein and the renal vein in 4 (25). The liver enzymes were normal in all patients. Liver function evaluated by serum albumin concentration...
(median 4.2 g/dL (interquartile range (IQR) 0.45 g/dL)) and prothrombin time (79% (IQR 24.5%)) were normal. The median white blood cell count of the CFRLD patients was 6960/µL (IQR 4967/µL) and thrombocyte count was 272000/µL (IQR 299750/µL). All patients with CFRLD received ursodeoxycholic acid (UDCA) (3 X 10 mg/kg a day with a maximum of 3 X 300mg).

After informed consent, overnight fasting blood samples were drawn. The study protocol was approved by the Ethics Committee of Ghent University Hospital (#2005/282).

**Laboratory**

Serum was prepared and stored at -20°C until analysis. Lipids were extracted (26), PL separated by thin layer chromatography (27) and converted into methyl esters (27). The methyl esters were extracted and separated by temperature programmed capillary gas chromatography (28) with a Varian model 3500 gas chromatograph equipped with a 30 m x0.25 mm x 0.2µm 10% cyanopropylphenyl-90%biscyanopropyl polysiloxane column (RTX®- 2330, Restek, USA). The injector and detector were set at 285°C. The column temperature was programmed from 150°C to 240°C at a heating rate of 2°C per minute. N2 was used as carrier gas, with a flow rate of 1 mL per minute. The FA methyl esters were identified by comparison with retention times of known standards (Sigma). Peak integration and calculation of the percent composition was performed by computer making use of Star GC Workstation software, version 5.5 (Varian, USA). The results were expressed as weight %.

Serum α-tocopherol was analysed by isocratic high-pressure liquid chromatography (29).

Genotypes were determined with the INNO-LiPA CFTR19® and INNO-LiPA CFTR17+Tn Update® kit (Innogenetics N.V.) or sequencing of the CFTR genome.

FE-1 was determined with a commercial ELISA kit (SheBo.Tech®, Giessen, Germany).

**Statistics**

Nonparametric statistical methods (Mann-Witney U, Kruskal-Wallis) were used with Stat-View 5.1 (Abacus Concepts, Inc. Berkeley,CA®) for Windows. Median results are given and interquartile range (IQR) between brackets, the significance of the differences between groups as tied P-values. Differences are considered significant when P value is <0.05.

**Results**

**Patients**

79/104 patients, with severe mutations, were included of which 39 females. They had all been treated for at least 6 months. The median age of the CF patients was 15 years (IQR 13.75 years). The mean seafood intake was 26 g/day (s.d. 43) which is comparable with the average seafood intake (27 g/day) in this region (30).

The clinical and pulmonary function data of the CF patients with or without CFRLD are presented in table 2. The patients with liver disease had a significantly better weight for height percentage (W/H%) (P<0.03). No other differences between the groups were detected (Table 2).

**Fatty acid disturbances**

The CF patient subgroup suffering from CFRLD is compared to those CF patients of the same severe genotype group without liver disease. The patients with CFRLD have a significantly lower docosahexaenoic acid (DHA, 22:6n-3) (P<0.02), increased 22:4 n-6 (P<0.03), but comparable arachidonic acid (AA, 20:4n-6). Their ratio AA/DHA is significantly increased (P<0.05) (table 3).

**Discussion**

It is known for long that CF patients have deviating FA profiles (2-9). Compared to healthy age matched controls, described elsewhere (18) CF patients with severe CFTR mutations had an increased α-linolenic acid (ALA; 18:3 n-3), dihomogammalinolenic acid (DHGLA; 20:3 n-6), 22:5 n-3, oleic acid (OA; 18:1 n-9) and Mead acid (MA; 20:3 n-9) and decreased linoleic acid (LA; 18:2 n-6) and docosahexaenoic acid (DHA; 22:6 n-3).

However, many studies do not specify which types of patients were examined. Nevertheless it has been demonstrated that FA profiles are more disturbed in patients with severe genotypes resulting in non functional CFTR (10, 18).

CFRLD is mainly a problem of portal hypertension and rarely evolves to liver failure (25, 31, 32). The studied patients with CFRLD had no signs of liver failure but all had an ultrasound image of liver cirrhosis and an important splenomegaly due to their portal hypertension.

In order to evaluate the impact of CFRLD on the PL-FA profile we compared the PL-FA profiles of CFRLD patients to these of CF patients of the same genotype class without liver disease. In cholestasis and liver failure plasma FA disturbances have been described (19-22). However, cholestasis causes a decrease in LA (18:2 n-6) and its elongation and desaturation products. In contrast the n-3 FA family shows in cholestasis only a decreased 22:5 n-3 (19, 20). The CF patients with liver disease displayed a fully different FA profile. They had a significantly lower DHA (22:6 n-3) as well as AA/DHA ratio and an increased 22:4 n-6, the elongation product of AA (20:4 n-6). Watanabe et al report a decreased DHA (22:6n-3)
concentration in liver cirrhosis being more profound in patients with decompensated liver disease (22). However, their patients had a significantly lower intake of DHA (22:6n-3) and liver function failed. The patients described in this study have a comparable seafood consumption (the main source of DHA (22:6n-3)) as the general local population (30) and their liver function was preserved. Other studies did not confirm the results from Wanatabe in liver cirrhosis (21).

Biliary excretion and therefore intralumenal solubilisation of fat could be a problem in CFRLD (32), the exclusive decrease of DHA argues against this as main cause of the decreased DHA. For the same reason malabsorption of the long chain FA, as stated by Kalivianakis et al., is less likely the cause of an isolated DHA deficiency (11). Despite the treatment of all our CF liver disease patients with ursodeoxycholic acid, the disturbances in the FA profile are still observed. The amelioration of FA composition due to ursodeoxycholic acid, described by Lepage et al., was not reproduced in our CF patients with liver disease treated with 3 X 10 mg/kg a day (maximum of 3 X 300 mg) (33).

DHA (22:6 n-3) is very sensitive for oxidation (34). A decreased anti-oxidant defence in the patients with liver disease could therefore be the cause of the decreased DHA (22:6 n-3). Only the serum vitamin E concentrations were analysed and there was no difference between the groups. Essential FA deficiency itself can also cause liver abnormalities and liver steatosis being most frequently described (35). The importance of linoleic acid deficiency relates with the degree of liver steatosis in CF as described by Lindblad (36) and Strandvik described less disease progression in CF patients treated with intravenous essential fatty acid supplements (37). However, our patients with CFLD had the same degree of LA deficiency as their genotype controls. Although liver steatosis can be present in CF in case of malnutrition (38), the patients described in this study displayed multilobular biliary fibrosis on ultrasound which was confirmed by biopsy in 1/3 of the patients.

The present findings do not denote any causative relation between liver disease and DHA deficiency nevertheless it remains a matter of concern. CFTR -/- mice models display the same FA disturbances as CF patients (15). DHA supplementation improved the clinical outcome of the the Cfr/-/- mice (15), although others nevertheless it remains a matter of concern. CFTR -/- mice models display the same FA disturbances as CF patients (15). DHA supplementation improved the clinical outcome of the the Cfr/-/- mice (15), although others

References

33. Harper TB, Chase HP, Henson J, Henson PM. Essential fatty acid deficiency in the rabbit as a model of nutritional impairment in cystic fibrosis: in vitro and in vivo effects on lung defence mechanisms. Am Rev Respir Dis 1982; 126: 540-547
Table 1 Genotypes of the studied patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n°patients</th>
<th>n° CFRLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508/ΔF508</td>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>ΔF508/G542X</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>ΔF508/1717-1G</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ΔF508/Y1092X</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ΔF508/394delTT</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ΔF508/E60X</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ΔF508/R553X</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>394delTT/394delTT</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ΔF508/N1303K</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>394delTT/Y913C</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ΔF508/ΔI507</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ΔF508/R785X</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>N1303K/N1303K</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2184insA exon13/2184insA exon13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>R553X/1717-1G&gt;A</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Median and interquartile ranges of CF patients with and without CF related liver disease (CFRLD).

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>CF with normal liver (n=66)</th>
<th>CFRLD (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>15 (14.0)</td>
<td>11 (12.8)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>32/34</td>
<td>8/5</td>
</tr>
<tr>
<td>W/H %</td>
<td>97 (11.7)</td>
<td>102.6 (10.0) **</td>
</tr>
<tr>
<td>Length s.d.</td>
<td>-1.3 (1.4)</td>
<td>-1.2 (1)</td>
</tr>
<tr>
<td>Vitamin E (normal 0.6 -1 mg/dL)</td>
<td>0.9 (0.4)</td>
<td>0.9 (0.4)</td>
</tr>
<tr>
<td>FEV1%</td>
<td>80.4 (42.0)</td>
<td>84.6 (36.0)</td>
</tr>
<tr>
<td>FVC%</td>
<td>88.5 (20.9)</td>
<td>94.9 (27.4)</td>
</tr>
</tbody>
</table>

Significance of the differences calculated with Mann Whitney-U test, expressed as tied P value. ** = P< 0.03. W/H% weight as percentage of ideal weight for height, FEV1% forced expiratory volume in 1 second as percentage of normal value for height, FVC% forced vital capacity as percentage of normal value for height.
Table 3: Median weight % (and IQR) of serum phospholipids fatty acids in different CF phenotype groups compared to controls.

<table>
<thead>
<tr>
<th>Fatty Acid Description</th>
<th>no liver disease (n=66)</th>
<th>Liver disease (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA (18:3n-3)</td>
<td>0.180 (0.070)</td>
<td>0.200 (0.053)</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>2.745 (1.780)</td>
<td>2.16 (0.703)**</td>
</tr>
<tr>
<td>LA (18:2n-6)</td>
<td>17.120 (4.170)</td>
<td>18.640 (5.330)</td>
</tr>
<tr>
<td>DHGLA (20:3n-6)</td>
<td>3.610 (1.200)</td>
<td>3.950 (1.305)</td>
</tr>
<tr>
<td>AA (20:4n-6)</td>
<td>9.035 (2.690)</td>
<td>8.940 (1.923)</td>
</tr>
<tr>
<td>(22:4n-6)</td>
<td>0.380 (0.178)</td>
<td>0.450 (0.160)**</td>
</tr>
<tr>
<td>(22:5n-6)</td>
<td>0.335 (0.20)</td>
<td>0.400 (0.160)</td>
</tr>
<tr>
<td>OA (18:1n-9)</td>
<td>9.685 (2.340)</td>
<td>10.780 (2.333)</td>
</tr>
<tr>
<td>MA (20:3n-9)</td>
<td>0.230 (0.260)</td>
<td>0.325 (0.200)</td>
</tr>
<tr>
<td>OA(18:1n-9)/LA(18:2n-6)</td>
<td>0.523 (0.226)</td>
<td>0.596 (0.145)</td>
</tr>
<tr>
<td>AA(20:4n-6)/DHA(22:6n-3)</td>
<td>3.285 (1.955)</td>
<td>3.898 (1.291)*</td>
</tr>
<tr>
<td>MA (20:3n-9)/AA (20:4n-9)</td>
<td>0.024 (0.024)</td>
<td>0.034 (0.014)*</td>
</tr>
</tbody>
</table>

Differences between the subgroups on the basis of presence or absence of CF related liver disease (CFRLD) using a Mann-Witney-U test: * = P<0.05, ** = P<0.03, *** = P<0.02 (FA with significant difference due to liver disease are marked in bold). ALA= α-linolenic acid, DHA= docosahexaenoic acid, LA= linoleic acid, DHGLA= dihomogammalinolenic acid, AA= arachidonic acid, OA= oleic acid, MA= Mead acid.
4.3 DHA supplementation and clinical evolution of cystic fibrosis patients

Oral DHA supplementation in ΔF508 homozygous cystic fibrosis patients

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Received 20 September 2007; revised in revised form 3 December 2007; accepted 24 December 2007

Abstract

Aim: The aim of this study was to evaluate whether the previously observed changes in the fatty acid profile, as a result of DHA supplementation, could be maintained during longer study trials and to observe its effect on the clinical outcome of cystic fibrosis (CF) patients.

Method: A year-long double-blind placebo-controlled study was performed in ΔF508 homozygous CF patients above the age of 6. Clinical data, including pulmonary function and number of infections, were collected. Blood for the determination of the fatty acid (FA) composition of serum phospholipid, vitamin E, liver enzymes, immunoglobulins, erythrocyte sedimentation rate and complement was drawn at the beginning and then every 6 months after the start of the study.

Results: Seventeen patients were included; one dropped out. The treatment group was supplemented with an algal DHA-rich oil and the control group with sunflower seed oil. There was no difference between the control and treatment groups for W/H, calorie intake, FEV1%, and PCCs at the start of the study and after 1 year of supplements. The phospholipid FA composition did not change in the control group. The treatment group had a significant increase in DHA and docosapentaenoic acid (EPA) concentration. A concurrent decrease of dioleoylphosphatidylethanolamine, arachidonic acid, 22:5 n-6 and Mead acid was observed. The laboratory results showed no changes in vitamin E level, liver enzymes, albumin, erythrocyte sedimentation rate and IgG concentration in either the placebo or intervention group.

Conclusion: Although DHA-rich oil shifted the serum phospholipid FAs to a less pro-inflammatory profile, no conclusive clinical improvement could be observed so far.

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1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disease in Caucasians. The CF gene codes for an integral membrane protein that is the CF transmembrane conductance regulator (CFTR). The mutations of the CFTR gene are classified in relation to the properties of the CFTR protein as proposed by Welsh [1]. Several reports on the CF patients describe blood and tissue fatty acid (FA) imbalances [2-9]. They are characterized by low linoleic acid (LA, 18:2 n-6) and docosahexaenoic acid (DHA, 22:6 n-3), increased dihomogamma linolenic acid (DHGLA, 20:3n-6) and normal or increased arachidonic acid (AA, 20:4n-6), as compared to controls [2-9]. This imbalance can be influenced by malabsorption secondary to pancreatic insufficiency [6]. However, the imbalances were also described in well-nourished and pancreatic-sufficient patients [2,5,10]. Other groups describe an abnormal FA metabolism [11-13]. The FA imbalance is more pronounced in the severe CF genotypes, resulting in non-functional CFTR protein [10,14].

The intrinsic imbalance in the ratio of membrane FA found in the CF-affected tissues was suggested to promote inflammation [9,15]. Different medications have been used to influence the inflammation such as antibiotics, steroids and non-steroidal anti-inflammatory drugs (NSAIDs).
Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation.

Stephanie Van Biervliet

2. Methods

2.1. Study design

A double-blind placebo-controlled prospective study on the long-term effects of DHA (22:6n-3) supplementation was performed. The study period was 1 year. The 34 ΔF508 homozygous CF patients above the age of 6 were asked to take 3 × 2 identical capsules a day together with one extra pancreatic enzyme capsule (Creon®) for every two oil capsules. The study was explained to the first 34 ΔF508 homozygous CF patients visiting the CF centre. Seventeen accepted the invitation to participate and were randomly distributed into a DHA treatment group (n = 9) and a placebo group (n = 8) according to the date of entry in the study. The major objectives in participation in the study were its duration and the daily intake of supplementary pills. The oil capsules contained either 500 mg algal triacylglycerol oil with 40% of DHA (22:6n-3) (DHASCO oil®, Martek Biosciences Corp., USA) or sunflower seed oil. Their FA compositions are given in Table 1. The capsules looked identical. Patients received standard CF care including pancreatic enzyme replacement therapy and a supplement of the fat-soluble vitamins containing cholecalciferol 1000 IU, α-tocopherol / ascorbic acid (100 mg, 100 mg, respectively) and retinol acetate 10000 IU.

At the start of the study and every 3 months thereafter, the patients were clinically evaluated and their pulmonary function tested. The pulmonary function was measured on a "Jougon Masterscreen Bodyplethysmograph". Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were expressed as a percentage of the normal expected value for height, weight and height were measured and nutritional status was expressed as a percentage of ideal weight for height. Decisions on the start of antibiotic treatment were always taken in deliberation with a member of the CF centre.

At the start and every 6 months of the study, blood was drawn for blood count, coagulation, liver enzymes, erythrocyte sedimentation rate, total immunoglobulin G (IgG) and serum phospholipid (PL) FA status. At the start and at the end of the study, energy intake was calculated, based on a 3-day diary, and expressed as a percentage of the advised energy intake. The advised energy intake is 30% above the recommended daily allowance for age, weight and gender of healthy children (Revised guidelines 2006; http://www.health.gov.au/fgov/Consumer/Healthассistance/health-assistance 中文印刷).

Exclusion criterion was the presence of severe CF-related liver disease. This diagnosis was made as described by Williams et al. (21) on ultrasound aspects of the liver with a score above six and concomitant splenomegaly. This was the case in two of the eligible 34 patients.

2.2. Controls

The control values are obtained from 44 healthy controls, aged 1-47 yr (median age: 18). All were well nourished; their W/H% was between 90% and 110%. They did not take PUFA supplementation or PUFA-enriched milk and did not carry one of the mutations as determined by the INNO-LIPA CFTR1® (Innogenetics N.V.).

2.3. Laboratory

Serum was prepared and stored at −20°C until analysis. Lipids were extracted [22], phospholipids...
separated by thin-layer chromatography [23] and converted into methyl esters [23]. The methyl esters were extracted and separated by temperature-programmed capillary gas chromatography [24] with a Varian model 3500 gas chromatograph equipped with a 50 m × 0.25 mm × 0.25 μm 10% cyanopropylphenyl-90% bis cyclohexyl polysiloxane column (RTX® 2330, Restek, USA). The injector and detector were set at 285 °C. The column temperature was programmed from 150 to 240 °C at a heating rate of 2 °C/min. N₂ was used as the carrier gas with a flow rate of 1 ml/min. The FA methyl esters were identified by comparison with retention times of known standards (Sigma). Peak integration and calculation of the percent composition were performed by a computer, making use of Star GC Workstation software, version 5.5 (Varian, USA). The results were expressed as weight percent. The intra-run variance for the determination of the fraction of each fatty acid depended on relative abundance with variation coefficients varying from 5% for small peaks (less than 0.5% of total) to about 0.5% for large peaks (about 25% of total). For the determination of the FA composition of the oils, the same procedure was followed, omitting the extraction and thin-layer chromatography step.

Genotypes were determined with the INNO-LiPA CFTR®.

Standard clinical chemistry methods were used for the determination of vitamin E, liver enzymes, albumin, erythrocyte sedimentation rate and IgG concentration.

2.4. Statistics

Nonparametric statistical methods (Mann-Whitney U and Wilcoxon rank) were used with Stat-View 5.1 (Abacus Concepts, Inc. Berkeley, CA®) for Windows.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>DHA-supplemented group (n = 8)</th>
<th>Placebo group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At start</td>
<td>After 1 year</td>
</tr>
<tr>
<td>Age</td>
<td>12.2 (11.0)</td>
<td>11.4 (11.1)</td>
</tr>
<tr>
<td>W/H %</td>
<td>100 (9)</td>
<td>99.6 (10)</td>
</tr>
<tr>
<td>Energy intake %</td>
<td>100 (51)</td>
<td>109.5 (56)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1.60 (0.67)</td>
<td>0.85 (0.40)</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>65 (10)</td>
<td>67 (11)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>132 (3.2)</td>
<td>131 (3.1)</td>
</tr>
<tr>
<td>FEV1%</td>
<td>74.7 (33.7)</td>
<td>76.1 (37.3)</td>
</tr>
<tr>
<td>FVC%</td>
<td>96.3 (22.2)</td>
<td>97.8 (26.6)</td>
</tr>
<tr>
<td>No. infections</td>
<td>4 (3.5)</td>
<td>2 (1.5)</td>
</tr>
</tbody>
</table>

There were no significant differences observed using a Mann-Whitney U test or a Wilcoxon rank test. W/H %: weight as a percentage of ideal weight for height, energy intake as a percentage of RDA ≥ 30% for enriched healthy individuals. FEV1%: forced expiratory volume as a percentage of predicted. FVC%: functional vital capacity as a percentage of predicted. No. infections = number of infections treated with antibiotics per year preceding or during supplementation.
3.3. Evolution of the serum phospholipid fatty acids

Data presented in Table 3 and Fig. 1 show the phospholipid FA composition at the start of the study and after 1 year of supplementation. Before the intervention started, DHA (22:6n-3) and LA (18:2n-6) were lower in both the CF groups, compared to healthy controls, whereas DHGLA (20:3n-6), docosapentaenoic acid n-6 (DPA, 22:5n-6) and oleic acid (OA, 18:1n-9) were higher. Mead acid (MA, 20:3n-9) was only higher in the treatment group. Such differences between CF patients and healthy controls have often been described [2-9]. At the start of the study there were no significant differences between the DHA treatment group and the sunflower treatment control group. In the control group there were no significant changes in the FA profile at the end of the study. In contrast, the DHA treatment group had a significant increase of DHA (22:6n-3) and EPA (20:5n-3) in serum phospholipids at the end of the study. A concomitant decrease of DHGLA (20:3n-6), AA (20:4n-6), DPA (22:5n-6) and MA (20:3n-9) was found. This resulted in a significant decrease in the AA/DHA ratio. Further, the laboratory signs of PUFA deficiency decreased as MA (20:3n-9) decreased. The decrease of DPA (22:5n-6) indicates a resolution of DHA (22:6n-3) deficiency during the study period. These differences were already present after six months of supplementation (data not shown). There was no significant change between the FA profiles at 6 months and 1 year (data not shown).

Table 3

<table>
<thead>
<tr>
<th>FA (%)</th>
<th>DHA supplemented group</th>
<th>Placebo Group</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>After 1 year</td>
<td>Start</td>
</tr>
<tr>
<td>ALA (18:3n-3)</td>
<td>0.145 (0.05)</td>
<td>0.180 (0.15)</td>
<td>0.130 (0.15)</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
<td>0.685 (0.46)</td>
<td>1.122 (0.34)</td>
<td>0.760 (0.33)</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>2.315 (0.43)</td>
<td>3.070 (4.83)**</td>
<td>2.700 (1.56)**</td>
</tr>
<tr>
<td>DHGLA (20:3n-6)</td>
<td>3.970 (1.09)</td>
<td>2.635 (1.20)**</td>
<td>3.660 (1.60)</td>
</tr>
<tr>
<td>AA (20:4n-6)</td>
<td>0.376 (0.17)</td>
<td>0.376 (0.24)</td>
<td>0.345 (0.16)**</td>
</tr>
<tr>
<td>DPA (22:5n-6)</td>
<td>0.640 (0.60)**</td>
<td>0.670 (0.93)**</td>
<td>0.615 (0.72)**</td>
</tr>
<tr>
<td>OA (18:1n-9)</td>
<td>10.393 (1.39)**</td>
<td>9.680 (1.32)</td>
<td>11.085 (2.73)**</td>
</tr>
<tr>
<td>MA (20:3n-9)</td>
<td>0.255 (0.25)</td>
<td>0.065 (0.15)</td>
<td>0.065 (0.15)</td>
</tr>
<tr>
<td>AA(DHGLA) (22:6n-3)</td>
<td>5.959 (2.05)**</td>
<td>6.670 (1.72)**</td>
<td>3.950 (1.19)**</td>
</tr>
</tbody>
</table>

ALA = α-linolenic acid, DHA = docosahexaenoic acid, LA = linoleic acid, DHGLA = dihomo-octadecenoic acid, AA = arachidonic acid, OA = oleic acid, MA = mead acid.

*p < 0.05, **p < 0.01.

3.4. Clinical evolution

There was no difference between the controls or the treated patients for W/H%, caloric intake, FEV1%, FVC%, number of infections at the start and after 1 year of supplements. The vitamin E level did not change during the study period. Liver enzymes and albumin remained normal throughout the study. There was no significant change in the inflammatory parameters measured with erythrocyte sedimentation rate and IgG concentration. There was no significant difference between the two groups in the pulmonary function or in the number of infections at any time during the study (Table 1). The only side effects reported were burps with a fishy taste in two patients.

4. Discussion

Ever since FA distribution abnormalities have been described, interventions were set up to shift the FA composition towards a less pro-inflammatory profile using different types of oil [25,26]. Studies with fish oil (containing EPA (20:5n-3) and DHA (22:6n-3)) demonstrated an enhanced production of S-lipoxxygenase products, with a reduced leukotriene B4 production and chemokinesis to leukotriene B4 [27-30]. Pulmonary function improvement during fish oil supplementation was observed in two studies, one being of very short duration (6 weeks) and the other being uncontrolled.
The other studies did not reveal a clinical improvement [29,30].

Based on the initial positive results of the supplementation in CFTR−/− mice [19], an algal DHA-rich triacylglycerol was chosen for this study. However, other CF mice models did not reveal the same FA disturbances [32,33], and even in the same model, the effect was not observed after long-term supplementation [18]. This study confirmed the absorption of algal DHA as described by others [19,20]. The supplements were well tolerated and no side effects on liver function or coagulation were observed. Although in most other studies the genotype is not specified, this study only included ΔF508 homozygous patients, since FA imbalance is more important in patients with severe genotypes [10,14]. Patients with CF-related liver disease were excluded since they have an even more pronounced decrease of DHA compared to their genotype controls [14].

The patients have, as described in other studies, a significant decreased DHA (22:6n-3) with a normal AA (20:4n-6) [2;10,14-26]. Although there was no clinical evidence for EFA deficiency in the patients studied, the high levels of DPA (22:5n-6) and MA (20:3n-9) are suggestive of such deficiency [34].
Although the supplement did not contain EPA (20:5n-3) a significant increase of this FA was observed in the group on the DHA supplement. An increase of EPA (20:5n-3) after DHA (22:6n-3) supplementation has been shown to be due to retroconversion of the latter FA [5]. The decrease in DHGLA (20:3n-6) and AA (20:4n-6) can be attributed to the inhibitory effects of DHA (22:6n-3) on the delta 6 desaturase and delta 5 desaturase activity, as demonstrated in rat liver cells [36] and competition of long-chain polyunsaturated FAs for incorporating into PLs [37]. The parameters of EPA deficiency, DPA (22:5n-6) and MA (20:3n-9) dropped significantly during DHA (22:6n-3) supplementation.

Administration of DHA (22:6n-3) results in decreased AA (20:4n-6) concentration in the phospholipids [35, 37]. The imbalance between AA (20:4n-6) and DHA (22:6n-3) is thought to contribute to the inflammatory characteristics in CF patients [38]. A shift in this balance is therefore supposed to induce a decrease in the inflammation, and secondary to it, an improved pulmonary function. To our knowledge, this study is only 1 year-long double-blind intervention study with DHA (22:6n-3) supplementation in CF patients. The serum FA composition proved patient compliance. However, there was no significant clinical improvement and no change in the inflammation as measured by erythrocyte sedimentation rate and IgG. Detecting clinical differences in studies with small populations is difficult. Before a change in the clinical practice can be advised, more data are needed [39].

5. Conclusion

Although oral DHA (22:6n-3)-rich algal triacylglycerol oil supplementation shifted the FA composition of serum phospholipids to a less pro-inflammatory profile, no conclusive clinical improvement could be observed thus far.

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References

Chapter 5: Conclusions & Future research

Question 1: What is the zinc status in the cystic fibrosis population?
At diagnosis as well as during cystic fibrosis treatment (without zinc supplements) the serum zinc concentration is not significantly different from a local age-matched control group. However, in comparison to the NHANES II study 44% of our cystic fibrosis infants at diagnosis, 12.5% of the cystic fibrosis patients treated for 1 year and 16.8 % of the older treated cystic fibrosis patients have serum zinc below the -2.5 s.d.

Question 2: Is the presence of zinc deficiency reflected in the activity of zinc dependent enzymes in cystic fibrosis patients?
In 30% of the patients mucosal damage was present in the intestinal biopsies of newly diagnosed cystic fibrosis patients. A decreased intestinal alkaline phosphatase activity was present in the brush border normal as well as in damaged mucosa.

Question 3: Fatty acid status of cystic fibrosis patients?
The essential fatty acid distribution in phospholipids of cystic fibrosis patients is significantly different from controls. They had significantly lower docosahexaenoic acid (22:6n-3) and linoleic acid (18:2n-6) and higher dihomogammalinolenic acid (18:3n-6), oleic acid (18:1n-9) and Mead acid (20:3n-9), the latter being a sign of essential fatty acid deficiency. This resulted in increased ratios of arachidonic acid/ docosahexaenoic acid, Mead acid /arachidonic acid and oleic acid / linoleic acid.

Question 4: Which factors influence the fatty acid distribution?
There was no association of serum fatty acid composition with nutritional status, caloric intake, pancreatic function, gender, pulmonary function, pseudomonas colonisation or presence of diabetes mellitus. The abnormalities, described above, were more pronounced in the “severe” genotypes.
In presence of cystic fibrosis related liver disease there is a more pronounced decrease of docosahexaenoic acid (22:6n-3) and increase of docosatetraenoic acid (22:4n-6) compared to genotype controls without liver disease.

Question 5: Is there a clinical effect of docosahexaenoic acid supplementation in cystic fibrosis?
A double blind randomised control trial demonstrated a significant increase in docosahexaenoic acid and eicosapentaenoic acid concentrations in the treatment group. A concomitant decrease of dihomogammalinolenic acid, arachidonic acid, 22:5n-6 and Mead acid was observed.
There was no difference between controls (n=8) and treatment group (n=8) for weight for height %, caloric intake, FEV1%, FVC% at the start and after 1 year of supplemention. No changes of serum vitamin E level, liver enzymes, serum albumin and IgG concentration and erythrocyte sedimentation rate were observed.
The docosahexaenoic acid supplementation shifted the serum phospholipid fatty acids to a less pro-inflammatory profile. No conclusive clinical improvement could be observed over the 1 year study period.

Future questions to be answered:
- Do all or only a subgroup of cystic fibrosis patients benefit from zinc supplementation?
- Can the zinc status be improved by improving fat absorption through for instance proton pump inhibitors?
- Is there a nutritional explanation for the observed differences in serum zinc between different populations?
- Is there an association between serum zinc status and essential fatty acid disturbances in cystic fibrosis?
Do zinc supplements influence essential fatty acid distributions?

Do DHA supplements challenge the anti-oxidant defence?

Is there a clinical effect of docosahexaenoic acid supplementation if more patients over prolonged periods would be included?

Would the study of docosahexaenoic acid metabolites as resolvins... contribute to the insight of inflammatory processes in cystic fibrosis?

Would a combined supplementation of zinc and essential fatty acids improve the clinical outcome?
Chapter 6: Summary

Cystic fibrosis is a multi-organ disease in which maintaining a good nutritional status is crucial for its outcome. An overview of the disease is given in chapter 1. In cystic fibrosis, the maldigestion is a factor not only influencing the nutrient but also the micronutrient absorption. The obvious deficiencies in fat soluble vitamins led to a standard monitored supplement for pancreatic insufficient patients. There are other micronutrients which could theoretically influence the course of the disease, where the benefit for the patient is not yet known.

Zinc and essential fatty acids mutually influence each other and are important in inflammation and immunity. Chapter 2 summarises the research objectives.

In chapter 3 we focus on the zinc status, first by means of the serum zinc concentration. The normal serum zinc concentration in our region is significantly lower than reported in literature (chapter 3.2). This makes our population more sensible for zinc deficiency, especially when malabsorption syndromes are present. Further on, an age dependency was observed with significantly lower serum zinc concentrations in infants. Serum zinc concentrations did not significantly change after the age of 4 years. There was no influence of the type of infant feeding. There is an association of zinc with vitamin A.

Since in literature a zinc deficiency was observed in cystic fibrosis infants detected with neonatal cystic fibrosis screening, we performed a study where serum zinc was evaluated at diagnosis and one year later (chapter 3.3). The serum zinc concentration at diagnosis was very low indeed and increased significantly without zinc supplements after 1 year of standard cystic fibrosis treatment. However, there were no significant differences when compared to age matched healthy controls. There was a significant association of zinc with vitamin A and E.

In older cystic fibrosis children the same low serum zinc concentrations were observed as in the healthy local control group. There is an association of serum zinc with serum albumin and serum vitamin A. The association with albumin is not surprising since 70% of serum zinc is bound to albumin. Further, multiple aspects of the vitamin A status as absorption, metabolism, release, transport and utilisation are influenced by zinc. There was no relation with nutritional status, growth, inflammation parameters or colonisation with Pseudomonas Aeruginosa. However, a positive association with the forced vital capacity was observed.

Serum zinc lacks sensitivity and specificity for the evaluation of the zinc status since only 0.2% circulates in plasma associated with albumin and α-2 macroglobulin. It is kept within a narrow range and only prolonged zinc deficiency will reflect in the serum zinc concentration. Another more complex way to evaluate zinc-deficiency is to look at zinc dependent enzymes. However, there is no consensus in literature on which enzymes are the best in reflecting the zinc status. The earlier studies we performed on intestinal biopsies of newly diagnosed cystic fibrosis patients showed a decreased intestinal alkaline phosphatase activity (chapter 3.4). This was the case in the normal and the damaged mucosa. Alkaline phosphatase is a known metalloprotein, containing zinc or magnesium necessary for the catalytic function. Furthermore intestinal alkaline phosphatase gene expression is activated by a zinc binding protein. Finally, the enzyme has shown to be very sensitive for oxygen free radicals. Zinc deficiency will therefore result in a decreased intestinal alkaline phosphatase activity due to absence of zinc in its catalytic site and increased inactivation as a result of increased presence of oxygen free radicals but also in a decreased concentration as a consequence of the decreased transcription.
Chapter 4 deals with the essential fatty acid status of cystic fibrosis patients. The disturbances of the phospholipid essential fatty acid distribution described in literature were also present in the patients we studied. They have significantly lower docosahexaenoic acid (22:6n-3) and linoleic acid (18:2n-6) and higher dihomogammalinolenic acid (18:3n-6), oleic acid (18:1n-9) and Mead acid (20:3n-9), the latter being a sign of essential fatty acid deficiency. This resulted in increased ratios of arachidonic acid/docosahexaenoic acid, Mead acid/arachidonic acid and oleic acid/linoleic acid. There was no relation of serum fatty acid composition with nutritional status, caloric intake, pancreatic function, gender, pulmonary function, pseudomonas colonisation or the presence of diabetes mellitus (chapter 4.2).

When patients were divided into 2 groups according to genotype (group A: mutation class I, II, or III, group B: mutation class IV, V) the abnormalities were more pronounced in the “severe” genotypes. However, there was no significant difference between the groups for age, pulmonary function, nutritional status and vitamin E concentration.

Cystic fibrosis patients can have some concomitant problems known to influence fatty acid composition such as diabetes mellitus and liver disease. Therefore a study was performed to evaluate whether the presence of cystic fibrosis related liver disease influenced the fatty acid status. In the presence of cystic fibrosis related liver disease there is an even more pronounced decrease in docosahexaenoic acid (22:6n-3) and an increase of docosatetraenoic acid (22:4n-6) compared to genotype controls without cystic fibrosis related liver disease.

Since in cystic fibrosis transmembrane conductance regulator-/- mice docosahexaenoic acid was capable of reversing the cystic fibrosis symptoms, we performed a double blind placebo controlled study to evaluate the effect of docosahexaenoic acid in humans (chapter 4.3). To eliminate the disturbances due to genotype and liver disease we included only ΔF508 homozygous patients without liver disease. The treatment group had a significant increase in docosahexaenoic acid and eicosapentaenoic acid concentrations. A concomitant decrease of dihomogammalinolenic acid, arachidonic acid, 22:5n-6 and Mead acid was observed. There was no difference between controls and treatment group for weight for height %, caloric intake, forced expiratory volume in 1 second1%, forced vital capacity % at the start and after 1 year of supplements. The laboratory results showed no changes in vitamin E level, liver enzymes, albumin, sedimentation and IgG concentration. Although docosahexaenoic acid -rich oil shifted the serum phospholipid fatty acids to a less pro-inflammatory profile no conclusive clinical improvement could be observed so far.
Samenvatting

“Cystic fibrosis” of mucoviscidose is een multi-orgaan aandoening waarbij het zeer belangrijk is voor zijn beloop om een goede nutritionele status te bewaren. Een overzicht van de ziekte wordt gegeven in Hoofdstuk 1. Bij mucoviscidosepatiënten zal de maldigestie niet alleen de voedselopname maar ook de opname van micronutriënten beïnvloeden. Het voorkomen van klinisch waarnembare vetoplosbare vitamine deficiënties heeft geleid tot algemene suppletie van deze vitamines bij patiënten die pancreas insufficiëntie vertonen. De concentraties ervan worden ook minstens jaarlijks gevolgd en indien nodig aangepast. Er zijn andere micronutriënten die theoretisch een belangrijke rol zouden kunnen spelen in het verloop van de ziekte. Hiervan is nog niet bekend of supplementen een voordeel zouden bieden voor de patiënt.

Zink en essentiële vetzuren beïnvloeden elkaar en spelen een belangrijke rol bij de controle van ontstekingsreacties en immuniteit. Verder worden de theoretische achtergronden en hun belang bij mucoviscidose toegelicht.

Hoofdstuk 2 behandelt de onderzoeksvragen die gesteld werden voor dit werk.

In hoofdstuk 3 gaan we dieper in op de zinkstatus, onderzocht door middel van de serum zinkconcentratie. Zowel serum selenium- en zinkconcentratie zijn in onze regio significant lager dan wat er in de literatuur wordt gerapporteerd (Hoofdstuk 3.2). Dit zorgt ervoor dat onze populatie gevoeliger kan zijn voor tekorten vooral bij ziekte. Bij malabsorptie zal er een groter deel van het ingenomen zink niet opgenomen worden. Bij de gezonde controlepopulatie was er een duidelijke evolutie van de serum zinkconcentratie in functie van de leeftijd. Zuigelingen beneden het jaar hadden een significant lagere zinkconcentratie. Vanaf de leeftijd van 4 jaar was er geen duidelijke toename meer. Er werd tevens een associatie van de zink concentratie met de vitamine A concentratie gevonden. Bij de zuigeling had het type zuigelingenvoeding geen invloed op zijn serum zinkconcentratie.

Omdat in de litteratuur al zeer lage zink concentraties beschreven werden bij mucoviscidose (Hoofdstuk 3.3), gedetecteerd door neonatale screening, voerden we een studie uit, waarbij bij diagnose en een jaar later, de serum zinkconcentraties werd bepaald. Dit bevestigde dat de serum zinkconcentratie bij diagnose laag was en dat deze op significante wijze steg ook zonder zink supplementen, na een jaar behandeling voor mucoviscidose. Er was echter geen significant verschil in de serum zinkconcentraties wanneer deze vergeleken werden met de gezonde locale leeftijdsgenoten. Wel stelden wij in deze studie een significant verband tussen serum zink en serum vitamine A en E vast.

Bij de oudere kinderen met mucoviscidose werden dezelfde lage zink concentraties gevonden als bij de gezonde controle groep. In deze studie werd weernom een verband tussen serum zink en serum vitamine A vastgesteld, ondanks vitamine A supplementen bij alle pancreasinsufficiënte patiënten. Verder was er eveneens een associatie met serum albumine gehalte. Het verband met serum albumine is niet verrassend gezien 70% van het serum zink gebonden is aan albumine. Tenslotte, zijn er multiple aspecten van de vitamine A status die door zink beïnvloed worden zoals zijn absorptie, metabolisatie, vrijlating, transport en gebruik. De serum zinkgehaltes vertoonden geen associatie met de voedingstoestand, groei, ontstekingsparameters of kolonisatie met Pseudomonas Aeruginosa. Er werd echter wel een positieve associatie van de serum zinkconcentratie met de “forced vital capacity” vastgesteld.
Een van de majeure problemen van serum zink als parameter van zijn status is zijn geringe gevoeligheid. Slechts 0,2% van het totale lichaamszink circuleert in het plasma. Zink wordt in het lichaam binnen strikte grenzen gehouden, zodat enkel langdurig zinktekort zal leiden tot een serum zink concentratiedaling. Andere manieren van evaluatie van de zinkstatus zijn het bepalen van de activiteit of concentratie van zink afhankelijke enzymen. Er is echter in de literatuur geen consensus over welke enzymen de zinkstatus het best zouden weergeven. In onze eerste studies, werden darmbiotopen genomen bij nieuw gediagnosticeerde mucoviscidose patiënten, om borstelzoomenzymen te bepalen (Hoofdstuk 3.4). Deze toonden een verlaagde activiteit van intestinaal alkalisch fosfatase, zowel in de beschadigde als in de morfologisch normale duurarmucosa. Intestinaal alkalisch fosfatase is een gekend metalloproteïne, dat zink of magnesium nodig heeft voor zijn katalytische functie. Verder wordt de genexpressie van intestinaal alkalisch fosfatase geactiveerd door een zinkbindend eiwit. Tenslotte is het enzym zeer gevoelig voor vrije-zuurstofradicalen. Zinktekort kan niet alleen een vermindere activiteit van het enzym uitlokken maar ook verhoogde inactivatie door vrije-zuurstofradicalen en verder nog lagere enzymconcentratie door verminderde gentranscriptie ervan.

**Hoofdstuk 4** behandelt de vetzuurstatus van mucoviscidose patiënten. De, in de literatuur vermelde, afwijkingen van de essentiële vetzuren, werden ook in onze patiëntpopulatie waargenomen. De distributie van docosahexaenzuur en linolzuur in de serum fosfolipiden is significant lager en deze van dihomogammalinoleenzuur, oleïne zuur en Mead zuur is hoger. Verhoging in de distributie van oleïne zuur en van Mead zuur is tekens van tekort aan essentiële vetzuren. Door dit alles ontstaan verhoogde verhoudingen van arachidonzuur/docosahexaenzuur, Mead zuur/arachidonzuur en oleïne zuur/linolzuur. Er is geen relatie aantoonbaar tussen de serum fosfolipiden vetzuursamenstelling en de voedingstoestand, calorie inname, pancreasfunctie, geslacht, longfunctie, pseudomonas aeruginosa kolonisatie of aanwezigheid van suikerziekte (Hoofdstuk 4.2).

Wanneer patiënten werden ingedeeld naar ernst van hun mutatie, waren bovengenoemde afwijkingen meer uitgesproken bij patiënten met de “ernstigste” mutaties. Op klinische gronden waren echter geen verschillen tussen de groepen waarneembaar. Dit moet gezien worden in het licht van de, ondanks alles, nog relatief kleine groepen, de grote spreiding in leeftijd en talloze andere factoren.

Mucoviscidose patiënten kunnen verder ook lijden aan aandoeningen waarvan geweten is dat ze de vetzuursamenstelling kunnen beïnvloeden zoals suikerziekte of leverlijden. Daarom gebeurde er onderzoek om na te gaan of het leverlijden van mucoviscidose op zichzelf eveneens afwijkingen van het vetzuurspectrum uitlok. Bij leverlijden was er een grotere daling van distributie van het docosahexaenzuur en een stijging van het docosatetraeenuzuur te zien, in vergelijking met met patiënten van dezelfde genotype klassering zonder leverlijden.

In een mucoviscidose muizenmodel, de cystic fibrosis transmembrane conductance regulator-/- muis, konden Freedman et al. de symptomen van mucoviscidose doen keren door orale toediening van zeer grote hoeveelheden docosahexaenzuur. Om dat effect bij de mens te evalueren, gebeurde een dubbelblind gerandomiseerd onderzoek (Hoofdstuk 4.3). Om geen verwarring te krijgen door genotypeverschillen werden enkel ΔF508 homozygote patiënten ingesloten, zonder tekens van leverlijden. De behandelde groep had een duidelijke toename van het docosahexaenzuur en eicosapentaëenzuur, verder daalde hun dihomogammalinoleenzuur, arachidonzuur en Mead zuur. Er was echter geen verschil in de klinische toestand van de behandelde patiënten t.o.v. de controles. Zowel hun gewicht naar lengte, de calorische inname, als hun “forced expiratory volume in
1 second” %, “forced vital capacity”% bij start en na 1 jaar behandeling waren gelijk. De laboratoriumresultaten toonden dat er geen veranderingen ontstonden in vitamine E concentratie, leverenzyme activiteit, albumine, bloedstolling, sedimentatie van de rode bloedcellen en serum IgG concentratie. Onze preliminaire studie kon niet tot het besluit leiden dat docosahexaenzuur een klinisch verschil betekent voor de patiënten met mucoviscidose. Toekomstige studies zouden nog langerduriger moeten zijn, gevoeliger klinische en biochemische parameters hanteren, alsook grotere aantallen patiënten moeten insluiten. Hoewel we in het serum van de patiënten een duidelijk verschuiven kregen naar minder pro-inflammatoire vetzuurprofielen werd er tot op heden geen duidelijk herkenbare klinische respons vastgesteld.
Résumé

La mucoviscidose est une maladie atteignant de multiples organes. Le suivi de la condition nutritionnelle est essentiel pour le patient atteint de mucoviscidose. Dans le chapitre 1, un résumé de la physiopathologie de la maladie est décrit. Dans la mucoviscidose la mal-digestion interfère avec la résorption des nutriments mais tout aussi bien avec celle des micronutrients. La carence en vitamines liposolubles a été bien définie et les recommandations nutritionnelles classiques prévoient leur supplémentation. Un suivi du taux de ces vitamines et leur supplémentation titrée est essentiel. Certaines composantes nutritionnelles pourraient jouer un rôle important dans l’évolution de la maladie. Jusqu’à présent leur supplémentation est sujet à discussion. Parmi ces éléments, le zinc et les acides gras essentiels, s’influencant mutuellement, pourraient jouer un rôle essentiel dans les processus inflammatoires et dans l’immunité en général.

Plusieures questions qui se sont posées au cours de nos travaux sont exposées au deuxième chapitre. Dans le troisième chapitre nous explorons la possibilité d’un déficit en zinc dans les patients atteint de mucoviscidose. Avant d’aborder ces problèmes nous avons exploré les concentrations sanguines du zinc dans un groupe de contrôle. Les taux sanguins de sélénium et de zinc chez l’enfant normal sont significativement plus bas dans nos contrées que ceux de la littérature (Chapitre 3.2). On pourrait en déduire que notre population pourrait être plus sensible au développement d’une carence en zinc en cas de maladie chronique. Les maladies malabsorptives causent une perte importante de zinc dans les selles. Chez l’enfant sain le taux de zinc dépend de son âge. Les nourrissons ont une concentration plus basse. A partir de l’âge de 4 ans on n’observe plus de différence avec une population plus âgée. Il y a une association entre les taux sanguins de zinc et de la vitamine A. Chez le nourrisson le type de lait de nourrisson n’influence pas les résultats.

Dans la littérature on décrit des taux de zinc très bas chez l’enfant atteint de mucoviscidose, découverts par dépistage néonatal. S’avancant sur ces données, nous avons déterminé le taux sérique de zinc lors du diagnostic de la mucoviscidose et on l’a répété après 1 an de thérapie (Chapitre 3.3). Nous confirmions le taux de zinc bas au moment du diagnostic et leur augmentation significative après le traitement. Néanmoins il faut se rendre compte que la population normale évolue dans le même sens. Dans cette étude, on démontre une association entre les taux sériques de zinc et de vitamine A et de vitamine E.

Dans les patients plus âgés, (Chapitre 3.3), déjà traités pour mucoviscidose, on retrouve les mêmes concentrations (plutôt basses) que la population témoin. A nouveau une association entre les taux sanguins de zinc et de vitamine A est présente, malgré les suppléments de vitamines chez les patients souffrant d’une insuffisance pancréatique. Une association significative se retrouve également entre les taux sériques d’albumine et de zinc, ce qui n’est pas surprenant, car 70% du zinc sérique est transporté sur l’albumine. On doit se rendre compte que le zinc intervient à plusieurs niveaux dans le cycle et le métabolisme de la vitamine A, entre autres dans l’absorption, sa métabolisation, le transport et son usage. On n’a pas retrouvé d’associations entre les taux de zinc et la condition nutritionnelle du patient, la croissance, les paramètres inflammatoires ou la colonisation par pseudomonas aeroginosa. Un taux sérique de zinc bas est cependant associé à une "forced vital capacity" plus basse.

Les concentrations sériques de zinc ne sont pas très représentatives de la réserve corporelle de zinc car seulement 0.2% du zinc corporel se trouve dans le sérum. Le corps essaye de maintenir les taux de zinc dans des limites...
strictes. Ce n’est qu’après une carence prolongée qu’on observera une diminution de ces taux. Une autre façon d’évaluer les effets biologiques du zinc est le contrôle de l’activité et des taux d’enzymes ou le zinc est un cofacteur de leur activité. Dans la littérature on ne retrouve pas de consensus sur l’enzyme qui refléterait au mieux les taux corporels de zinc. Une de ces enzymes est la phosphatase alkaline intestinale. A cet effet nous avons pratiqué des biopsies lors du diagnostic de mucoviscidose afin d’analyser l’activité enzymatique dans la muqueuse intestinale (Chapitre 3.4). Ces travaux ont démontré une activité diminuée de la phosphatase alkaline intestinale. Cette anomalie était présente tout aussi bien dans la muqueuse normale que dans l’atrophie villueuse. Le zinc ou le magnésium font parti de la fonction enzymatique de la phosphatase alkaline intestinale. L’expression du gène de la phosphatase alkaline intestinale est dépendante d’une protéine liant le zinc. Finalement la phosphatase alkaline intestinale est très sensible à peroxydation, un problème courant dans la mucoviscidose. Un déficit en zinc va diminuer l’activité de cette enzyme, augmenter son inactivation mais tout aussi bien diminuer sa quantité protéique.

Dans le quatrième chapitre on traite les acides gras dans la mucoviscidose. On confirme les anomalies décrites dans la littérature. La distribution proportionnelle de l’acide docosahexaenoïque et de l’acide linoléique dans les phospholipides est abaissée de façon significative et celle de l’acide dihomogammalinoléique, oléique et l’acide Mead est augmentée (Chapitre 4.2). On n’a pas retrouvé d’associations entre la distribution des acides gras et la condition nutritionnelle, l’apport calorique, fonction pancréatique, le sexe, la fonction pulmonaire, la colonisation par le pseudomonas aérginosa ou la présence d’un diabète sucré. Groupeant les patients par son sévérité de leur mutation du cystic fibrosis transmembrane conductance regulator, on voit que les anomalies sont plus prononcées chez les patients souffrant de ces mutations sévères. Malgré tout on n’a pas découvert des différences cliniques entre ces deux groupes. Le phénomène est probablement dû au nombre trop peu élevé de sujets étudiés. Tout comme dans le diabète sucré et les maladies du foie, les patients souffrant de mucoviscidose présentent des affections perturbant le métabolisme des acides gras. Comparant les patients d’un même génotype avec ou sans atteinte hépatique, on voit chez les premiers une distribution des acides gras présentant un taux de l’acide docosahexaenoïque fortement diminué.

Freedman et ses collaborateurs ont observé dans la souris cystic fibrosis transmembrane conductance regulator-/-, une normalisation des symptômes de la mucoviscidose en donnant des doses pharmacologiques de l’acide docosahexaenoïque par voie orale.

Nous avons appliqué ce même type de traitement chez l’enfant souffrant de mucoviscidose, homozygote pour la mutation ΔF508 sans maladie hépatique (Chapitre 4.3). Ceci évite toute confusion due aux effets du génotype. On a administré en double aveugle durant une année soit le l’acide docosahexaenoïque, soit l’huile de tournesol. Dans le groupe traité on observe une augmentation significative de l’acide docosahexaenoïque et de l’acide eicosapentaenoïque, tandis que l’acide dihomogammalinoléique, arachidonique et l’acide Mead s’abaissent. Par contre les effets sont nettement moins spectaculaires que ceux observés dans le modèle animal. L’index poids-taille, l’apport calorique, la fonction pulmonaire ne diffèrent pas entre les deux groupes. Les taux sanguins de la vitamine E, des enzymes hépatiques, de l’albumine, de la coagulation, de la vitesse de séditionement des érythrocytes et la concentration d’IgG ne changent pas au cours de l’observation. L’évolution pulmonaire est différent si on inclus les résultats de l’année précédente. Ces résultats ne permettent pas de conclure que l’acide docosahexaenoïque induit une évolution clinique différente. Des études futures devraient inclure un nombre plus
élevé de patients, envisager l’emploi de paramètres plus sensibles et une plus grande durée de traitement. Malgré l’évolution des acides gras vers un profile moins inflammatoire on n’a jusqu’à présent pas pu démontrer une différence clinique.
References

Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation. | Stephanie Van Biervliet


42. Robinson PG. Essential fatty acids in cystic fibrosis. Lancet 1975; 8: 919


52. Boyle MP. Update on maintaining bone health in cystic fibrosis. Curr Opin Pediat 2006; 12: 453-8


56. MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000; 130: 1500-8S


63. MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000; 130: 1500-8S


70. Krebs NF. Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr 2000; 130: 1374S-1377S


Zinc & Essential Fatty Acid status in Cystic Fibrosis, Clinical Effects of Supplementation.  

Stephanie Van Biervliet


143. Domar U, Karpe F, Hamsten A, Stigband T, Olive-Crorna T. Human intestinal alkaline phosphatase release to the blood is linked to lipid absorption, but removal from the blood is not linked to lipoprotein clearance. Eur J Clin Invest 1993; 23: 753-60


Curriculum vitae

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List of international publications


List of national publications

Van Biervliet S. Selenium bij Belgische kinderen. Percentiel 2001; 5: 154


Abstracts


CD-ROM


**RPGN award 2000:** Spoorrelementen bij kinderen.
Dankwoord

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