Double-blind randomized controlled study of linoleic acid supplementation for 1 year in patients with cystic fibrosis.

–Influence on clinical status and metabolism.

-NETwork study of LA supplementation in CF patients (NETLACF)

The study is registered at ClinTrial.gov ID #NCT04531410

Funding: European Society of Pediatric Gastroenterology and Nutrition; Swedish CF Association; Heart-Lung Foundation; Erica Lederhausen´s Foundation.

The supplement was generously provided by AAK Ltd, Karlshamn/Copenhagen, Sweden/Denmark.

Six center study

1. *Sweden, Stockholm*
   Birgitta Strandvik[1] MD, PhD, Project coordinator, Senior advisor
   Craig Wheelock[2], PhD, Biochemist

2. *Poland, Poznan*
   Jaroslaw Walkowiak[3] MD, PhD Principal investigator
   Sławomira Drzymała-Czyż[3] RD, PhD

3. *Italy, Milan*
   Carla Colombo[4] MD PhD Principal investigator
   Gianfranco Alicandro[4] MSc
   Nadia Faelli[4] MD
   Arianna Biffi[4] RN Local coordinator
   Anna Bulfamante[4] RD, Dietician

4. *Norway, Oslo.*
   Egil Bakkeheim[5] MD, Principal investigator
   Christien Sachs-Olsen[5], Local coordinator
   Magnhild Kolsgaard[5], RD, PhD, Dietician

Department of Biosciences and Nutrition[1] and Department of Medical Biochemistry and Biophysic[2], Karolinska Institutet, Stockholm, .
Department of Pediatric Gastroenterology and Metabolic Diseases[3], Poznan University of Medical Sciences, Poznan, Poland.
Cystic Fibrosis Center University of Milan[4], Fondazione IRCCS Ca´Granda Opsedale Maggiore Policlinico, Milan Ital.,
Norwegian Center for Cystic Fibrosis[5], Oslo University, Oslo, Norway.

Summary:

Undernutrition is a common problem in patients with cystic fibrosis (CF) despite international consensus that the patients shall be given 120-200% of energy recommendations. Studies imply that one problem might be that the patients are not compensated for the essential fatty acid deficiency (linoleic acid, LA), which is well known in these patients. This deficiency is shown not to be due to fat malabsorption, but associated to an increased turnover of archidonic acid, a transformation product of LA. This abnormality is related to mutations associated with a more severe clinical phenotype. The most common and typical symptom of LA deficiency is poor growth. Studies in animals have further indicated that many of the symptoms in CF are related to the deficiency. A series of recent prospective studies from Wisconsin corroborate the importance of LA for growth. In Sweden LA has been supplemented to most patients since the late 70´, and the condition of patients have been among the leading in the world regarding growth, pulmonary function and survival. Short-term studies have shown better effect of LA supplementation compared to similar supply of energy without including extra LA. There are few long-term studies, performed before the gene was identified, giving very heterogeneous patient groups in regard to genotype, but some positive results on growth and physiology. It´s of interest that modern personalized extremely expensive therapy with correctors and potentiators for Cystic Fibrosis Transmembrane Conductance Regulator may influence lipid metabolism. LA might thus tentatively be a cheap adjuvant to this modern therapy, but this has to be specially studied.

The benefit for the patients would be great if the expected positive effect can be proved in the planned study. ***The treatment will be cheap and without adverse effects.*** From socioeconomic point of view is would be a great advantage.

AIM

To investigate the impact of long-term supplementation of fatty acids on clinical status and metabolism in patients with cystic fibrosis (CF).

DESIGN

A double-blind controlled randomized study of supplementation of linoleic acid (LA) compared to oleic acid (OA), and with long-chain omega 3 fatty acid (DHA) for one year to patients with CF.

BACKGROUND

CF is a systemic disease with major clinical symptoms from the gastrointestinal tract, predominantly pancreatic insuffficency and hepatobiliary disease, and from the lungs. Although survival has increased during the latest decades it is still a fatal disease with a high therapeutic burden and high percentage of impaired growth despite recommendations of very high energy intake, also in early infancy (Shoff et al 2006; Munck et al. 2018). Underweight is common both in children and adults with CF (NACFF Patient Register, ECFS Patient Registry).

It has been known for more than 50 years that patients with CF have low levels of the essential fatty acid linoleic acid (18:2w6, LA) and also that the long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (22:6w3, DHA) may be decreased in plasma and tissues (Kuo et al.1962, Underwood et al.1972, Freedman et al 2004). After 1975, when a newborn temporarily recovered in pancreatic

function after Intralipid® administration (Elliott et al.1975), some short-term trials with LA or high energy diets including LA have been performed showing a positive association to growth (van Egmont et al 1996, Steinkamp et al 2000) and pulmonary function (Chase et al 1979, Mischler et al 1986, Walkowiak J 2007, Maqbool et al 2008, Hjelte et al 2008, Lai et al. 2009). A few long-term studies have indicated beneficial effect of intravenous linoleic acid supplementation (Chase et al 1979, Kusoffsky 1983, Strandvik et al 1989, Strandvik et al 1994). In comparison, exclusive high energy intake does not have the same effect (van Egmont et al 1996, Steinkamp et al 2000, Aldámeiz-Echevarria et al 2013, Munck et al 2018). One study combining omega-6 and omega-3 fatty acids showed marked improvement in several parameters, compared to supplementation with only omega-3 fatty acids, which rarely gives any clinical benefit (Oliveira et al. 2010, Oliver et al. 2011, Alicandro G et al. 2013). In a small long-term controlled supplementation study performed before the gene was identified, improvement was seen in renal, liver and bile acid parameters (Strandvik et al.1989, Strandvik et al. 1994). Long-term treatment increased LA in many organs (Mischler et al 1986). Very long-term treatment with omega-6 rich oils and Intralipid• have resulted in exceptionally good clinical conditions regarding growth, bone mineral density, pulmonary function, reduction of pulmonary exacerbations and low clinical infectious status in a relatively large patient cohort, i.e. most of the Swedish patients with CF, compared with other international centers (Strandvik 1988; Strandvik 2006, Gronowitz et al.2004). The role of LA in these observations are supported by studies finding better growth and lung function in patients with higher LA concentrations in serum

(Maqbool et al 2008). However, there is a lack of long-term randomized controlled studies. Other treatment modalities, such as increased physical activity from early age, also introduced in the Swedish patients in the late 80´s (Blomqvist et al.1988), have probably also a large impact on the good clinical status of the Swedish patients.

It was shown more than 30 years ago that the LA deficiency was not due to malabsorption, but probably to an increased release of arachidonic acid (20:4w6, AA), causing an increased metabolism of LA, resulting in LA deficiency (Carlstedt-Duke et al. 1986, Strandvik et al 1988) and increase of eicosanoid metabolites (Strandvik Seybert 1996, Jabr et al 2013). Many studies in animals and cells have confirmed the hypothesis of high turnover (for review see Strandvik 2010). A hypothesis with an algorithm was formed to explain the mechanisms for the different symptoms (Strandvik 1989, Strandvik 1992) supported in later studies. From studies of newborns diagnosed by neonatal screening, it has been shown that 80% of patients with meconium ileus have markers of LA deficiency, high Mead acid and EFA index (T/T ratio), and similar markers were increased in around 50 % of patients without meconium ileus (Lai et al 2000). A relative improvement occurred during infancy, but again the markers deteriorate in late toddler age to prepuberty (Lai et al 2000), which coincides with the dip in growth in the patients as illustrated in both the European and North American CF patient registers. The Wisconsin CF Neonatal Screening Group have followed their patients in a series of papers and showed that intake of LA up to 2 years of age resulted in higher plasma LA concentrations with an OR of 7.46 for maintaining high LA if intake was > 5% of total

energy intake and both high energy and LA concentration increased the possibility to recover in weight to 2 years of age (OR 30.6, p=0.002). They conclude that "maintaining normal plasma LA for a prolonged period in addition to sustaining a high energy intake may be important in facilitating adequate weight gain" (Shoff et al 2006). These early "responders" also required lower energy intake to maintain adequate growth (Lai et al 2009), which is similar to our observation that patient who received regular LA supplementation had normal weight without increasing the energy intake (Kindstedt-Arfwidson et al 1988). In a follow up study the Wisconsin group found that the early response in growth was associated with better growth and lung function up to 12 years of age and in this paper they also included patients with meconium ileus, who initially had a more severe LA deficiency and they were also later much worse off (Sanders et al. 2018). They summarize that "Further studies are needed to identify optimal CF interventions that can maximize the chance for achieving early growth recovery" (Sanders et al 2018).

People with CF have low IGF-1 concentrations (Switzer et al 2009; Rogan et al 2010; Bessich et al 2013), and the low IGF-I concentrations correlates with lean body mass (Sermet-Gaudelus et al 2003), which usually is low in patients with CF, except in the LA supplied Swedish population (Gronowitz et al. 2008). In transgenic CF rats low IGF-1 levels correlated with impaired growth and bone content (Stalvey et al 2017). Studies have shown that low LA concentrations are associated with low IGF-1 levels in infants (Kjellberg et al 2018) and in animals (Korotkova et al 2004 and 2005). Other factors for poor growth might be an increased caloric demand, and it has been shown that severe CF phenotype is associated with an increased basic

metabolic turn-over (O´Rawe et al 1992). In rats with essential fatty acid deficiency an increased basal respiration has been reported (Rafael et al 1984). Furthermore, the increased intestinal permeability, which relates to membrane phospholipid layers, is correlated to the clinically more severe phenotypes in patients with CF (Hallberg et al 1997).

The cause of the low DHA is not clear, but the improvement of DHA by LA supplementation suggests that it might not be of primary importance (Farrell et al 1985, Strandvik et al 2001) and maybe associated with a high phospholipid turn-over (Bhura-Bandali et al. 2000, Strandvik 2010, Rogiers et al1984). However, long-term studies in CF mice and a recent paper suggest that the ratio between AA/DHA in blood cell tissues is related to the liver disease in CF, suggesting that the concentrations of polyunsaturated fatty acids (PUFA) might contribute to the unexplained liver affection in many of the patients (Lindblad et al. 1999; Beharry et al. 2007; van Biervliet et al 2010; Jörgensen et al. 2012, Drzymala-Cryz et al, 2017). Because one study of transgenic CF mice showed low DHA associated with morphological changes in ileum and pancrease (Freedman et al 1999), although later not confirmed in a long-time study (Beharry et al 2007), many clinical trials with supplementation of omega-3 LCPUFA started, but mainly failed to show clinical improvement despite marked increase of omega-3 fatty acids in plasma (van Biervliet et al. 2008, Oliver et al. 2011, Alicandro et al. 2013). One study with regular intravenous supplementation of omega-3 for 3 months even showed negative impact on the patients (Durieu et al. 2007). As mentioned above one study showed

marked clinical improvement by a combination of omega-3 and omega-6 supplementation (Oliveira et al. 2010).

It is well known that low LA concentrations are associated with high AA concentration, while supplementation with LA decreases the AA concentration (Liou et al. 2007, Friesen et al 2010, Bjermo H et al 2012, Wheelock & Strandvik 2020). In healthy individuals no increase of inflammatory markers have been reported with omega-6 supplementation (Bjermo et al 2012, Johnson GH & Fritsche K 2012). This is of importance since the release of AA is the rate-limiting step for eicosanoid production, which is increased in CF (Strandvik a Seybert 1996, Chase & Dupont 1978, Jabr et al 2013). In one study of 10 months supplementation of LA in CF patients prostaglandin F2α poduction decreased (Chase & Dupont 1978). Ivacaftor did not influence LA concentration but decreased AA concentration in plasma and decreased PGE-M in urine in the GOAL study for patients with G551D mutations (O´Connor & Siegmiller 2016). These studies show that both LA and CFTR modulators might influence lipid metabolism and decrease inflammatory mediators. Despite this fact, the lack of good randomized clinical studies have made many centers hesitate to supply LA because it's a substrate for AA and thereby also for the pro-inflammatory lipid mediators. High AA concentrations have been found in bronchial secretions in CF (Gilljam et al 1986) and was confirmed in a recent study of sputum from CF patients (Yang J, et al 2012)

In a recent double-blind randomized trial high AA concentration in plasma was associated with low NO in the airways of CF patients (Keen et al. 2010). Low NO can contribute to pseudomonas colonization, which is considered a threat for

progression of the pulmonary damage. Furthermore many studies have shown that OA increases compensatory when LA decreases (Christophe et al 1994), and it was shown in Toronto, (contributing to their persistent use of high fat diet when rest of the world prescribed low fat diet to CF) that high OA stimulated the growth of *Staphylococcus aureus*, the first colonizer of the airways in patients with CF (Campbell et al.1983). Similar imbalance of LA and OA was also found to impair the oxygen pressure influencing the hemoglobin oxygenation which would be a factor increasing the airway pathology in CF (Campbell et al 1976). Thus, it seems important to increase LA concentration and reduce AA concentration in the patients with CF also from reported pulmonary associations (Wheelock & Strandvik 2020). Many studies have shown that the pulmonary symptoms are the most affected persistent problem in the neonatally screened patients despite their high caloric diet, and improved early growth is associated with better pulmonary function up to 12 years of age (Sanders et al 2018).

It is also important that the hyperactivity of the epithelial sodium channel (ENaC), which is shown to contribute to the pulmonary symptoms, seem to be normalized by LA supplementation, as notified in sweat glands and kidneys (Strandvik 2021), and which might be an effect of the protein-lipid interaction. In recent studies the interaction of modulators and phospholipids have been shown (Liessi et al.2020; Kopp et al.2018 .

The recent discovery of the importance of the long-chain PUFA of the omega-3 series for the normal inflammatory response by transformation to prosolving specialized markers (SPM), the anti- inflammatory lipoxines (Rogeiero et al. 2012)

may indicate that supply of omega-3 fatty acids might be important as a general treatment for CF patients with their chronic airway inflammation. Further improvement might therefore be expected with a combined supplementation, especially since the long-chain omega-3 fatty acids inhibit delta-6 desaturase and thereby may further reduce the transformation of LA to AA (Hagve et al 1988).

WORKING PLAN

Patients and Eligibility.

Patients with CF, carrying 2 severe mutations referred to Class I and II, i.e. mutations associated with more severe LA deficiency and more severe clinical symptoms, (like delF508, 394delTT) are included after informed consent. The reason for including only those with clinically more severe phenotype is that those have pancreatic insufficiency and also other symptoms more expressively related to genotype (see above). Exclusion criteria will be pregnancy and transplantation and age above 15 and below 5 years (due to pulmonary function tests) and severe liver disease (cirrhosis, portal hypertension). The patients shall not have taken any lipid supplements for 2 months before inclusion and not be treated with any modulators (like Orkambi, Trikafta).

The patients will keep to their ordinary treatment of PERT and vitamins and that for the pulmonary symptoms and infections. No special dietary recommendations will be given, but PERT might be increased, when the supplement is given and that shall be registered. Number of pulmonary exacerbations and type of treatment, including antibiotics, are registered and also compared with the year before the study.

Sample size

A sample of 84 patients (42 per group) will ensure that a two-sided test with α =0.05 has 80% power to detect a 0.5 mean difference in 1-year change in weight-

for-age Z-score, assuming a pooled standard deviation of 0.8. This last figure has been obtained from a previous intervention study on cystic fibrosis patients (Alicandro et al. 2013). Since we expect a withdrawal rate of close to 10%, we planned to enroll 90 patients (45 per groups). The patients and caregivers will be informed by oral and written information and included after informed consent. The study is approved by local Ethic Committées and by the one at Karolinska Institutet and the study is performed according to the Helsinki Declaration.

Design

The study is designed as a double-blind randomized clinical trial

The patients will be randomized to either

- treatment A: linoleic acid (12 g for children 5-15 years) + 600 mg algeal DHA
- treatment B:, oleic acid (12 g for children 5-15 years) + 600 mg algeal DHA

The fatty acids will be given daily for 12 months, in vials containing an emulsion of totally 20 ml (provided by AKK Company, Karlshamn, Sweden and Copenhagen Denmark, packed by WinterMedic, Odense, Denmark), which can be mixed with milk, juice, sour cream, porridge or any similar feeding at breakfast time. Some extra enzymes may be added.

The treatment will last 12 months, starting and ending at time of the yearly check up of the patients. Half the number of patients will be randomly allocated to receive either treatment A or B. An independent researcher (not involved in the study) will generate the allocation sequence at each center. This will reduce small differences in local treatment policy. The vials with supplementation will only be coded (black or white capsula) so neither patient nor caregivers or staff in charge will be informed by the type of supplementation.

Primary Outcome

- BMI Z-score, weight-Z-score

Secondary outcomes

- Height Z-score
- FEV1 (% of predicted) and oxygen saturation
- Exacerbations and infectious parameters compared to year before.
- Plasma phospholipid fatty acid profile.
- Plasma concentration of IGF-I

Explanatory outcomes

- Bone mineral density
- Lean body mass and fat mass.
- Liver function tests
- Lipid mediators (like eicosanoids and docosanoids)
- Cytokines
- 8-iso-prostane
- Sweat sodium and chloride concentrations
- Renal sodium excretion
- Resting energy expenditure
- Intestinal function (fecal calprotectin and fat absorption coefficient)
- Oral glucose tolerance with insulin determination.

METHODS

Patients will be screened for enrollment and will be allocated to one of the treatment at baseline visit. Follow-up visits will be scheduled at 6 and 12 months after treatment allocation. Some measures, like fat absorption coefficient and calprotectin and resting energy expenditure, exhaled NO and ergometer test will not be performed in all patients due to limited capacity at some centres. The study procedures are summarized in table 1.

Table 1. Outline of study procedures

	Baseline visit: Screening and allocation	+6 months	+12 months
Anthropometry	X	X	X
DXA	X		X
FFQ	X	X	X
24-h dietary recall	X	X	X
Pulmonary function	X		X
Blood sample	X	X	X
F-Calprotectin	X		X
Urine sample	X		X
Ergometer test with oxygen saturation	X		X
Oral glucose tolerance	X		X
Indirect calorimetry	X		X
Sweat test	X		X
Compliance to treatment	X	X	X
CF Questionnaire	X	X	X

1. *Anthropometry*.

Height and weight are controlled with the same equipment for each patient at respective centre and at each scheduled visit (baseline, 6 and 12 months after allocation). Z-scores of weight, height and BMI will be computed using WHO as growth standard. DXA is performed at start and after 12 months period for determination of bone mineral content and density, lean body mass and fat mass.

1. *Food registration*.
    - Food frequency questionnaire (FFQ) will be performed at startand each scheduled visit, mainly used for calculation of energy intake and intake of fat and fatty acids (validated questionnaire Eriksson S et al. 2010). Since the products are different in different countries the FFQ form can only suggest ***type*** of products to be evaluated, complemented by the local dietician. The reason to include these registrations is to individually make a comparison with intake at start and end of study possible, checking that not major changes have occurred in diet, which can influence the results.
    - 24-hour recall will be performed at start, 6 months and 12 months after allocation as a complement to FFQ for portion size, type of products etc.

3. *Pulmonary function.*

    - Routine pulmonary function (FEV1) at each clinical visit
    - Full spirometry at start and end of treatment
    - Exhaled NO (if available)
    - Ergometer test at start and end
    - Oxygen saturation (ear-tip).

4. *Biochemistry.*

    • Routine blood biochemistry (fasting) at start and end of treatment period, including Hb, platelets, white blood cell count and differential count, ASAT, ALAT, alkaline phosphatase, GGT, PK, total and conjugated bilirubin, bile acids, cholesterol, lipoproteins, triglycerides, urea, creatinine, glomerulus filtration rate. Ig-G. CRP, sedimentation rate.

- 1 ml serum/plasma for later analyses (to freezer divided in 4 tubes for later analyses of lipid mediators at KI).
- 0,5 ml for IGF-I analysis (to freezer for later analysis in Sweden)
- 0.5 ml plasma for cytokines (to freezer for sending to Stockholm for analyses (OLINK, Uppsala, Sweden).
- 0.5 ml plasma for analyses of phospholipid fatty acids (to free8zer for later analysis in Poznan or Stockholm).
- Urine sample for routine tests, incl sodium and creatinine concentrations.

5. *Intestinal function*

- F-Calprotectin at start and after12 months (in freezer for later analyses at one center)
- Fat absorption at start and 12 months. Three day dietary recording and feces

Sampling. Mixture in local laboratory with freezing of an aliquote for later transport to Poznan

6. *Metabolic tests*

- Urine sample for 8-isoprostane and lipid mediators as eicosanoids, docosanoids and linoleic acid metabolites (directly to freezer -80 each sample in 2 x 8 ml and in 4 x 2 ml) for later analyses in Stockholm.
- Indirect calorimetry at start and after 12 months (if available in the hospital).
- Sweat test at start and after 12 months. **Both sodium** and chloride analyses.
- Oral glucose tolerance test.

7.*Quality of life.*

Quality of life will be assessed through the CF Questionnaire Revised (CFQ), a disease-specific tool that measures health-related quality of life in patients with

CF. There are different versions of the questionnarie: two for children over 6 years, one to be completed by the child and one to be completed by parent (CFQ Child and CFQ-Parent, respectively).

8. *Compliance with the treatment*

Compliance with the treatment will be evaluated by monthly contact with the study coordinator of respective centre, when not used vials will be registered and new supplied. The patients are asked to make a dairy over the daily intake also reported monthly to the coordinator. Extent of physical activity shall be notified.

9. *Statistics*

All data will be collected in identical Excel files and analysed in one center at end study.

The main analysis will be carried out on the intention-to-treat population defined as all randomized patients. We will also conduct a secondary analysis on the per-protocol population excluding patients not receiving the allocated treatment for more than 2 months.

Between-treatment differences in the primary outcome across time points will be evaluated by fitting random intercept linear mixed-effects models. Mixed effects model can account for intra-correlated repeated measures on the same subjects and accommodate missing data due to dropouts. The models will include the z-score of BMI as response variable, treatment, time and time x treatment interaction as independent variables. The model coefficients for the time x treatment interaction will be considered an estimate of the treatment effect on changes in the study outcome. Time will be included in the model as categorical variable with the following categories: baseline (pre-treatment), +6 months and +12 months from treatment allocation.

Seondary and explanatory outcomes will be compared across treatment groups using the paired t-test for normally distributed variables, otherwise the non-parametric Wilcoxon signed-rank test for dependent samples will be used.

A stratified analysis by sex will be performed.

RISK-BENEFIT

There will be no adverse effects to expect of this treatment , since the control group will have the same treatment as offered generally to patients, i.e. extra calories. The extra LA will not exceed the general intake in US (Choque et al 2014). Both groups get similar amount of omega-3 fatty acids; dose in agreement with general recommendations. The extra burden of investigations is small since the patients always have more extensive investigations at the yearly checkup.

IMPORTANCE

If clinical status is improved the patients in both groups will be asked for the possibility to continue with Group A therapy for one further year (requires new ethical applications). Furthermore, good result would indicate a multicenter study for treatment from diagnosis in countries with neonatal screening, which would be extremely important since many of the symptoms start early and especially the supply of high caloric diet gives many problems in patients and parents, and unsatisfying results, It can also have the potential to be a cheap complement to modern expensive drug treatment.

A special benefit for individuals and society would be if this simple, cheap and non hazardous treatment can reduce the enormous costs of the modern drug treatment, which is further not always available.

References:

Aldámiz-Echevarría L, Prieto JA, Andrade F, et al. Persistence of essential fatty acid deficiency in cystic fibrosis despite nutritional therapy. Pediatr Res 2009; 66: 585-9.

Alicandro G, Faelli N, Gagliardini R et al. A randomized placebo-controlled study on high-dose oral algal docosahexaenoic acid supplementation in children with cystic fibrosis. Prostagland Leurkotr Ess Fatty Acids 2013; 88: 163-9.

Beharry S, Ackerley C, Corey M, Kent G, Heng YM, Christensen H, Luk C, Yantiss RK, Nasser IA, Zaman M, Freedman SD, Durie PR. Long-term docosahexaenoic acid therapy in a congenic murine model of cystic fibrosis. Am J Physiol Gastrointest Liver Physiol 2007; 292: G839-48.

Bessich JL, Nymon AB, Moulton LA, Dorman D, Ashare A. Low levels of Insulin-like Growth Factor-1 contribute to alveolar macrophage dysfunction in cystic fibrosis. J Immunol 2018;

Bhura-Bandali FN, Suh M, Man SFP, Clandinin MT. The Δ%508 mutation in the cystic fibrosis transmembrane conductance reuglator alters control of essential fatty acid utili<zation in epithelial cells. J Nutr 2000; 130: 2870-5.

Bjermo H, Iggman D, Kullberg J et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled study. Am J Clin Nutr 2012; 95: 1003-12

Blomquist M., Freyschuss U., Wiman L.G., och Strandvik B.: Physical activity and self treatment in cystic fibrosis. Arch Dis Child. 61:362-367, 1986.

Campbell IM, Douglas N, Crozier DN, Caton RB. Abnormal fatty acid composition and impaired oxygen supply in patients with cystic fibrosis. Pediatrics 1976;57: 480-6.

Campbell IM, Douglas N, Crozier DN, Pawagi AB, Buivids IA. In vitro response of Staphylococcus aureus from cystic fibrosis patients to combinations of linoleic and oleic acids added to nutrient medium. J Clin Microbiol 1983; 18: 408-415

Carlstedt-Duke J, Brönnegård M, and Strandvik B: Pathological regulation of arachidonic acid release in cystic fibrosis: The putative basic defect. Proc Natl Sci USA 83: 9202-9206, 1986

Chase HP, cotton EK, Elliott RB. Intravenous linoleic acid supplementatuion in children with cystic fibrosis. Pediatrics 1979; 64: 207-13

Chase HP, Dupont J. Abnormal levels of prostaglandins and fatty acids in blood of children with cystic fibrosis. Lancet 1978; 2(8083): 235-8.

Choque B, Catheline D, Rioux V, Legrand P. Linoleic acid: Between doubts and certainties. Biochimie 2014;96:14-21.

Christophe AB, Warwick WJ, Holman RT. Serum fatty acid profiles in cystic fibrosis patients and their parents. Lipids 1994; 29: 569-75.

Drzymała-Czyż S, Krzyżanowska P,Koletzko B et al. Determinants of serum glycerophospholipid fatty acids in cystic fibrosis. Int J Mol Sci.2017;18: E185.

Drzymała-Czyż S, Szczepanik M, Krzyżanowska P et al. Serum phospholipid fatty acid composition in cystic fibrosis patients with and without liver cirrhosis. Ann Nutr Metab 2017; 71: 91-98.

Durieu I, Vericel E, Guichardant D et al. Fatty acids platelets and oxidative markers following intravenous n-3 fatty acid administration in cystic fibrosis patients: an open pilot observational study. J Cyst Fibr 2007;6:320-6.

Elliott RB, Robinson PG. Unusual clinical course in a child with cystic fibrosis treated with fat emulsion. Arch Dis Child.1975 ;50: 76-8.

Eriksson S, Strandvik B. Food choice is reflected in serum markers and anthropometric measures in healthy 8-yr-olds. Eur e-J Clin Nutr Metabol 2010;5: e117-e124.

Farrell PM, Mischler EH, Engle MJ, et al. Fatty acid abnormalities in cystic fibrosis. Pediatr Res 1985;19:104-9.

Freedman SD, Katz MH, Parker EM, Laposata M, Urman MY, Alvarez JG. A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in cftr(-/-) mice.Proc Natl Acad Sci U S A. 1999;96:13995-4000.

Freedman SD,, Blanco PG, Zaman MM, et al. Associationn of cstic fibrosis with abnormalities in fatty acid metabolism. New Engl J Med 2004;350:560-69.

Friesen RW, Innis SM. Linoleic acid is associated with lower long-chain n-6 and n-3 fatty acids in red blood cell lipids of Canadian pregnant women. Am J Clin Nutr. 2010; 91 23-31.

Gilljam H, Strandvik B, Ellin A, Wiman LG. Increased mole fraction of arachidonic acid in bronchial phospholipids in patients with cystic fibrosis. Scand J Clin Lab Invest. 1986; 46: 511-8.

Griese M., S. Costa, R. W. Linnemann, M. A. Mall, E. F. McKone, D. Polineni, et al. Safety and Efficacy of Elexacaftor/Tezacaftor/Ivacaftor for 24 Weeks or Longer in People with Cystic Fibrosis and One or More F508del Alleles: Interim Results of an Open-Label Phase 3 Clinical Trial Am J Respir Crit Care Med 2021 ;203 :381-385

Gronowitz E, Lorentzon M, Ohlsson C, Mellström D, Strandvik B. Docosahexaenoic acid is associated with endosteal circumference in long bones in young males with cystic fibrosis. Br J Nutr. 2008; 99: 160-7.

Gronowitz E, Mellström D, Strandvik B. Normal annual increase of bone mineral density during two years in patients with cystic fibrosis Pediatrics 2004;114:435–42

Hagve TA, Christensen E, Grønn M, Christophersen BO. Regulation of the metabolism of polyunsaturated fatty acids. Scand J Clin Lab Invest 1988; 191 (Suppl): 33-46.

Hallberg K, Grzegorczyk A, Larson G, Strandvik B. Intestinal permeability in cystic fibrosis in relation to genotype. J Pediatr Gastroenterol Nutr 1997; 25: 290-5.

Hjelte L, Nilsson K, Moen IE, et al. Linoleic acid but not EPA and DHA correlates to prognostic markers in Scandinavian CF patients. *J Cyst Fibr* 2008; **7**(Suppl 2): S93.

Jabr s, Gartner S, Milne GL et al. Quantification of major urinary metabolites of PGE2 and PGD2 in cystic fibrosis. Correlation with disease severity. Prostagland Leukotr Ess Fatty Acids 2013;89:121-26.

Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: A systematic review of randomized controlled trials. J Acad Nutr Diet 2012; 112: 1029-41.

Jørgensen MH, Ott P, Lanng S, et al. Long-chain PUFA in granulocytes, mononuclear cells, and RBC in patients with cystic fibrosis: relation to liver disease. J Pediatr Gastroenterol Nutr 2012;55:76-81

Keen C, Olin A-C, Eriksson S, Ekman A, Lindblad A, Basu S, Beermann C, Strandvik B Supplementation with fatty acids influences the airway nitric and inflammatory markers in patients with cystic fibrosis. J Pediatr Gastroeneterol Nutr 2010; 50: 537-44.

Kindstedt-Arfwidson K, Strandvik B. Food intake in patients with cystic fibrosis on an ordinary diet. Scand J Gastroenterol. 1988; 23 (suppl 143): 160-162.

Kjellberg E, Roswall J, Bergman S, et al. Serum n-6 and n-9 fatty acids correlate with serum IGF-1 and growth up to 4 months of age in healthy infants. J Pediatr Gastroenterol Nutr 2018; 66: 141-146.

Kopp BT, McCulloch, Shrestha CL et al. Metabolomic responses to lumacaftor/ivacaftor in cystic fibrosis. Pediatr Pulmonol.2018;53:583-591.

Korotkova M, Ohlsson C, Gabrielsson B, Hanson LA, Strandvik B. Perinatal essential fatty acid deficiency influences body weight and bone parameters in adult male rats.Biochim Biophys Acta 2005; 1686: 248– 254

Korotkova M, Ohlsson C, Hanson LA, Strandvik B. Dietary n-6:n-3 fatty acid ratio in the perinatal period affects bone parameters in adult female rats. Brit J Nutr 2004; 92: 643–648.

Kuo PT, Huang NN, Bassett DR. The fatty acid composition of the serum chylomicrons and adipose tissue of children with cystic fibrosis of the pancreas. J Pediatr 1962;60:394-403.

Kusoffsky E, Strandvik B, Troell S. Prospective study of fatty acid supplementation over 3 years in patients with cystic fibrosis J Pediatr Gastroenterol Nutr 1983; 2: 434-8.

Lai HC, Kosorok MR, Laxova A, Davis LA, FitzSimmon SC, Farrell PM. Nutritional status of patients with cystic fibrosis with meconium ileus: a comparison with patients without meconium ileus and diagnosed early through neonatal screening. Pediatrics 2000;105:53-61.

Lai HJ, Shoff SM, Farrell PM; Wisconsin Cystic Fibrosis Neonatal Screening Group. Recovery of birth weight z score within 2 years of diagnosis is positively associated with pulmonary status at 6 years of age in children with cystic fibrosis. Pediatrics. 2009 ;123:714-22.

Liessi N, Pesce E, Braccia C, et al. Distinctive lipid signatures of bronchial epithelial cells associated with cystic fibrosis drugs, including Trikafta. JCI Insight 2020; 5: e138722.

Lindblad A, Glaumann H, Strandvik B: Natural history of liver disease in cystic fibrosis. Hepatology 1999: 30: 1151-1158.

Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr. 2007; 137: 945-52.

Lloyd-Still JD, Bibus DM, Powers CA, et al. Essential fatty acid deficiency and predisposition to lung disease in cystic fibrosis. Acta Pediatr 1996; 85: 1426-32

Maqbool A, Schall JI, Garcia-Espana JF et al. Serum linoleic acid status as a clinical indicator of essential fatty acid status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2008; 47: 635-44

Mischler EH, Parrell SW, Farrell PM, Raynor WJ, Lemen RJ. Correction of linoleic acid deficiency in cystic fibrosis. Pediatr Res 1986;20:36-41..

Munck A, Boulkedid R, Weiss L, et al. Nutritional status in the first 2 years of life in cystic fibrosis diagnosed by newborn screening. J Pediatr Gastroenterol Nutr. 2018 ;67:123-130.

O´Connor MG, Seegmiller A. The effects of ivacaftor on CF fatty acid metabolism An analysis from the GOAL study. J Cyst Fibr 2017; 16:132-8.

Oliver C, Jahnke N. Omega-3 fatty acids for cystic fibrosis. Cochrane Database Syst Rev 2011; 8 : CD002201.

Oliveira G, Olveira C, Soriguer F, et al. Fatty acid supplementation improves respiratory, inflammatory and nutritional parameters in adults with cystic fibrosis. Arch Bronconeum. 2010; 46: 70-77

O'Rawe A, McIntosh I, Dodge JA, Brock DJ, Redmond AO, Ward R, Macpherson AJ. Increased energy expenditure in cystic fibrosis is associated with specific mutations. Clin Sci (Lond) 1992; 82: 71-6.

Rafael J, Patzelt J, Schäfer H, Elmadfa I. The effect of essential fatty acid deficiency on basal respiration and function of liver mitochondria in rats. J Nutr.1984; 114: 255-62.

Rogan MP, Reznikov LR, Pezzulo AA, et al. Pigs and humans with cystic fibrosis have reduced insulin-like growth factor 1 (IFG1) levels at birth. Proc North Am Sci 2010; 107: 20571-5.

Rogerio AP, Haworth O, Croze R, Oh SF, Uddin M, Carlo T, Pfeffer MA, Priluck R, Serhan CN, Levy BD. Resolvin d1 and aspirin-triggered resolvin d1 promote resolution of allergic airways responses. J Immunol 2012; 189: 1983-91.

Rogiers V, Dab I Michotte Y, Vercruysse A, Crokaert R, Vis HL, Abnormal fatty acid turnover in the phospholipids of the red blood cell membranes of cystic fibrosis patients, *in vitro* study. Pediatr Res 1984;18:704-09

Sanders DB, Zhang Z, Farrell PM et al Early life growth patterns persist for 12 years and impact pulmonary outcomes in cystic fibrosis. J Cyst Fibr 2018; 17: 528-35.

Sermet-Gaudelus I, Souberbielle JC, Azhar I et al. Insulin-like growth factor I correlates with lean body mass in cystic fibrosis patients. Arch Dis Child 2003;88: 956-61.

Shoff SM, Ahn H-Y, Davis L et al Temporal associations among energy intake, plasma linoleic acid, and growth improvement in responst to treatment initiation after diagnosis of cystic fibrosis. Pediatrics 2006; 117: 391-400.

Stalvey MS, Havasi V, Tuggle KL et al Reduced bone length, growth plate thickness, bone contents, and IGF-I as a model for poor growth in the CFTR-deficient rat. PLoS One 2017; 12: e188497.

Steinkamp G, Demmelmair H, Koletzko B, et al. Energy supplements rich in linoleic acid improve body weight and essential fatty acid status of cystic fibrosis patients. J Pediatr Gastroenterol Nutr 2000; 34: 418-23

Strandvik B.: Antibiotic therapy of pulmonary infections in cystic fibrosis. Dosage and dose schedules. Chest 1988; 94 (Suppl): 146S-149S.

Strandvik B.: Relation between essential fatty acid metabolism and gastrointestinal symptoms in cystic fibrosis. Acta Paediatr Scand 1989; 363: 58-65.

Strandvik B.: Long chain fatty acid metabolism and essential fatty acid deficiency with special emphasis on cystic fibrosis. In: Polyunsaturated Fatty Acid in Human Nutrition. (Ed.U. Bracco & R. Deckelbaum) Raven Press, New York. Nestlé Nutr Workshop Ser 1992; 28: 159- 167.

Strandvik B. Care of patients with cystic fibrosis. Treatment, Screening and Clinical outcome. Ann Nestlé 2006; 64: 131-40.

Strandvik B. Fatty acid metabolism in cystic fibrosis. Prostagland Leukotr Ess Fatty Acids 2010;83:121-9.

Strandvik B. Is the ENaC dysregulation in CF an effect of protein-lipid interaction in the membranes? Int J Mol Sci 2021. 22, 2739. https://doi.org/10.3390/ijms 22052739.

Strandvik B, Brönnegård M, Gilljam H, Carlstedt-Duke J. Relation between defective regulation of arachidonic acid release and symptoms in cystic fibrosis. Scand J Gastroenterol 1988;23 Suppl 143: 1-4.

Strandvik B, Gronowitz E, Enlund F, Martinsson T, Wahlström J.: Essential fatty acid deficiency in to genotype in patients with cystic fibrosis. J Pediatr 2001;139: 650-5.

Strandvik B, Berg U, Kallner A, Kusoffsky E. Effect on renal function of essential fatty acid supplementation in cystic fibrosis. J Pediatr 1989; 115:2 42-50.

Strandvik B, Hultcrantz R. Liver function and morphology during long-term fatty acid supplementation in cystic fibrosis. Liver 1994; 14: 32-6.

Strandvik B, Svensson E, Seyberth HW. Prostanoid biosynthesis in patients with cystic fibrosis Prostagland Leukot Essent Fatty Acids. 1996; 55: 419-25.

Switzer M, Rice J, Rice M, Hardin DS. Insulin-like growth factor-I levels predict weight, height and protein catabolism in children and adolescents with cystic fibrosis. J Pediatr Endocrinol Metab 2009; 22: 417-24.

Underwood BA, Denning CR, Navab M. Polyunsaturated fatty acids and tocopherol levels in patients with cystic fibrosis. Ann NY Acad Sci 1972;203:237-47

Walkowiak J, Lisowska A, Blaszcynki M, Przyslawski J. Polyunsturated fatty acids in cystic fibrosis are relatd to nutrtion and clinical expression of the disease J Pediatr Gastroenterol Nutr 2007; 45: 488-90.

Van Biervliet S, Devos M, Delhaye T, Van Biervliet JP, Robberecht E, Christophe A. Oral DHA supplementation in dF508 homozygous cystic fibrosis patients. Prostagland Leukotr Ess Fatty Acids 2008; 78: 109-115.

Van Biervliet S, Van Biervliet JP, Robberecht E, Christophe A. Fatty acid composition of serum phospholipids in cystic fibrosis (CF) patients with or without CF related liver disease. Clin Chem Lab Med. 2010; 48: 1751-5

van Egmond AW, Kosorok MR, Koscik R, Laxova A, Farrell PM. Effect of linoleic acid intake on growth of infants with cystic fibrosis. Am J Clin Nutr. 1996; 63: 746-52

Wheelock CE, Strandvik B. Abnormal n-6 fatty acid metabolism in cystic fibrosis contributes to pulmonary symptoms. Prostagland Leukotr Ess Fatty Acids 2020; 160: 102156

Yang J, Eiserich JP. Cross CE, Morrissey BM, Hammock BD. Metabolomic profiling of regulatory lipid mediators in sputum from adult cystic fibrosis patients. *Free Radic Biol Med* 2012; **53**: 160-71.