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Original Article

# Lung transplantation in cystic fibrosis normalizes essential fatty acid profiles

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**Abstract**

*Background:* Cystic fibrosis (CF) can be a devastating disease. Disorders in essential fatty acid state are increasingly reported and various supplementation trials have been performed in an attempt to improve outcomes. However, the mechanisms leading to these disturbances remain elusive.

We wanted to investigate the role of the diseased CF lung on fatty acid profiles.

*Methods:* We compared fatty acid profiles in patients with CF after lung transplantation (n=11) to age-matched healthy controls and homozygous F508del patients (n=22 each).

*Results:* Compared to healthy controls, in patients with CF, there are decreased levels of docosahexaenoic, linoleic and arachidonic acid and increased levels of mead acid. In patients that underwent a lung transplantation, levels of docosahexaenoic, linoleic and arachidonic acid were normal. Mead acid did not decrease significantly.

*Conclusions:* The diseased CFTR deficient lung is a major determinant in the disturbed fatty acid profile in CF.


*Keywords:* Cystic fibrosis; Lung transplantation; Essential fatty acid; Poly-unsaturated fatty acid

## 1. Introduction

Cystic fibrosis (CF) is the most frequent lethal genetic disorder within the Caucasian race, with an incidence of 1 in 2500. Due to the current medical treatment, the median survival of these patients rose to 30–35 years. Pulmonary problems are the main cause of death. In an effort to further improve care, more and more attention is paid to metabolic disturbances, as for instance diabetes and also essential fatty acid (EFA) deficiency [1].

Abnormal FA profile has been reported in children with CF since many years [2]. Renewed interest in this subject emerges from the ability to influence FA profiles by supplements, thereby adding to the dietary strategies to obtain an optimal nutritional state [1]. Moreover, disturbances in fatty acid profiles have been implicated in the predisposition to lung disease [3]. Also, in a CF mouse model, supplementation of one of the FA (docosahexaenoic acid (DHA, C22:6n−3) was beneficial to the *Pseudomonas* endotoxin-enhanced lung inflammation, CF pancreatic, ileal and liver disease [4,5]. Nevertheless, randomized controlled trials are needed to confirm or deny beneficial effects of EFA supplementation in patients with CF [1].

The major EFA defect in patients with CF seems to be an increased release of arachidonic acid (AA, C20:4n−6), the most important metabolic product of linoleic acid (LA) [6]. In serum as well as in nasal and rectal biopsy samples, a high ratio of AA to DHA is seen [7]. In addition there is a decrease in plasma levels of linoleic acid (LA, C18:2n−6) and DHA) and a compensatory increase in levels of eicosatrienoic acid (C20:3n−9) (also known as mead acid (MA)) [1,8–10].

The aetiology of this abnormal fatty acid profile remains obscure. Initially it was thought to result from fat malabsorption linked to the exocrine pancreatic insufficiency. However, since it

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occurs in well-nourished young patients with CF [11], this hypothesis is less probable. It has been suggested that there is a disturbance in essential fatty acid metabolism [12]. This disturbance is related to the CF-genotype and minor changes in the essential fatty patterns are even seen in CF-heterozygotes [7,13].

Indisputably, lung disease is the most important aspect of CF. There have been no trials (including a placebo group) showing improvement or stabilization of lung function after improvement of fatty acid state. Although lung function (measurement of the forced expiratory volume in one second, $FEV_1$) has been related to CF fatty acid abnormalities [14] and as already stated, is implicated in the predisposition of CF lung disease [3], its precise relation is not clear. Moreover, interdependency of variables (e.g. genotype, pancreatic state and lung function) poses a problem to independently evaluate its relationship. Therefore, instead of studying the influence of FA profile on lung disease, we decided to study the reverse: the influence of removal of a diseased CF lung on FA profile. We do this by comparing fatty acid status in patients with CF and patients with CF that have undergone a lung transplantation at least one year previously.

## 2. Patients and methods

### 2.1. Patients

Plasma levels of fatty acids were determined in a group of 11 DF508/DF508 patients with CF who underwent a lung transplantation at least one year before the onset of the study (CF-transplant group). All patients were followed at the CF clinic of the university hospital Gasthuisberg, (Leuven, Belgium). Ten patients underwent a double lung transplantation, one patient a single lung transplantation. This group was age-matched with two DF508/DF508 patients with CF drawn from our cohort of 225 patients with CF (in an age-sorted patient list the preceding and the following patient were selected, CF group). Additionally we collected two healthy control samples per CF lung transplant (again one older and one younger) (Control group). The plasma fatty acid levels of these three groups were compared.

The study was approved by the local ethics committee and (parental) informed consent was obtained for all patients.

At the time of the plasma fatty acid measurements, or as close to as possible, following clinical information was retrieved from the patients clinical files: age; age at diagnosis; gender; lung function (FVC, $FEV_1$ expressed as % predicted for sex, height and age according to Knudson, within 3 months of the EFA measurement); chronic *Pseudomonas aeruginosa* colonization status (defined as repeated isolation of *Pseudomonas aeruginosa* (at least 3 positive sputum samples, with at least 1 month interval over at least a 6 month period [15]); weight, height, BMI and their respective z-scores (using recent Flemish reference values); serum vitamin E and cholesterol levels, dietary intake (daily average for total caloric intake and fat intake calculated from prospective 6 day written diary and fish intake during the last 2 months; number of fish meals/week); history of meconium ileus. Pancreatic insufficiency was defined by the need for pancreatic enzyme supplements for the treatment of clinically overt steatorrhea.

This study was approved by the local ethics committee and (patient and/ or parental) informed consent was obtained for all patients.

### 2.2. Plasma fatty acid determination

FA levels of the patients and controls were determined as previously reported [16]. Hence, blood samples were drawn at random times. Briefly, blood samples (5 mL on EDTA) were centrifuged at 1000 *g* for 15 min at 4 °C. The plasma was collected and stored at −80 °C for subsequent analysis. In glass tubes containing 100 μL plasma, we added 20 μL of the internal standard solution (providing 50 mg C19:0/L) and 2 mL of a methanol/toluene (4/1, vol/vol) mixture. Samples were vortex-mixed for 30 s, and we carefully added 200 μL acetyl chloride dropwise while swirling the tubes. Tubes were capped under nitrogen and transferred to a heating oven at 103 °C for 90 min. After cooling, the tubes were uncapped, and samples were treated by addition of 5 mL 6% $K_2CO_3$ and of 3 mL hexane. Recapped tubes were vortex-mixed for 1 min and centrifuged for 7 min at 500 g. We collected the upper organic phase and repeated the extraction procedure on the lower phase by adding 1 mL hexane. The two upper combined phases were washed with 1 mL distilled water, followed by a new centrifugation. The final organic phase was collected and evaporated under nitrogen to dryness at 40 °C. The dry residue was then redissolved in 200 μL hexane and transferred to capped vials for further analysis.

Fatty acid methyl esters were separated by use of a gas chromatograph (HP6890; Hewlett-Packard-Agilent) equipped with a capillary column (DB-FFAP), 30 m by 0.25 mm ID by 0.25 μm (J and W Scientific, Agilent Technologies) and identified and quantified by mass spectrometry (MS 5973; Agilent Technologies). The temperature program was as follows: initial at 130 °C with a 1-min hold; ramp: 4 °C/min to 178 °C, 1 °C/min to 210 °C, and 40 °C/min to 245 °C with a 7-min hold. Helium was used as carrier gas, under a flow rate of 2.5 mL/min. We performed selected ion monitoring and identified fatty acid methyl esters both by their respective retention times and by comparison with spectra of pure reference substances. Intra- and interassay CVs were $<3\%$ and $<5.7\%$, respectively (except for gamma-linolenate methyl ester, with 9.3%).

### 2.3. Statistical analysis

SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Demographic variables are expressed as mean ± standard deviatiation. As fatty acid levels were not normally distributed, they are expressed as median (interquartile range). For differences between two continuous variables in two groups the Mann–Whitney U test (with exact correction if groups were too small) was used. To compare categorical variables Fisher's exact test (2-sided) was used. For correlations within a group the Spearman Rank Correlation coefficient was used. A p-value $<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Demographic variables (see Table 1)

All patients with CF were DF508 homozygous and had pancreatic insufficiency. There were no differences between the patients with CF with and without a lung transplantation regarding age, sex, age at diagnosis, lung function, biometric variables (height, weight and BMI z-score), fish intake or history of meconium ileus. However transplanted patients had decreased caloric intake (also decreased fat intake), higher levels of serum cholesterol and vitamin E. All had been chronically colonized with *Pseudomonas auruginosa* (contrary to the non-transplanted group (14/22, see Table 1). Patients who underwent a lung-transplantation did so 4.32±2.08 year before the plasma FA determination.

### 3.2. Analysis of fatty acids (see Table 2)

Total plasma fatty acid concentration was significantly higher in lung transplanted patients.

Table 1
Baseline characteristics.
Demographic variables are expressed as mean±standard deviation.

| | | CF transplant group (n=11) | P-value (TXvsCF) | CF group (n=22) |
|---|---|---|---|---|
| Gender M/F | | 4/7 | 0.282 | 13/9 |
| Age(y) | | 27.48±7.75 | 0.417 | 25.91±6.84 |
| Age at diagnosis (y) | | 2.326±2.702 | 0.069 | 3.312±7.815 |
| Age at transplantation (y) | | 23.16±7.05 | - | Not performed |
| Lung function | FVC1 (%pred) | .80±.13 | 0.955 | .79±.19 |
| | FEV1 (%pred) | .74±.20 | 0.105 | .60±.23 |
| Chronic *Ps aer* | (n) | 11 (pretransplant) | **0.031** | 14 |
| Biometrics | Height (z-score) | −1.22±0.93 | 0.316 | −0.87±1.00 |
| | Weight (z-score) | −1.15±1.00 | 0.806 | −1.02±1.27 |
| | BMI (z-score) | −0.52±0.70 | 0.720 | −0.56±1.07 |
| Dietary intake | Kcal/d | 2.381.82± 360.37 | **0.0003** | 2.985.68± 464.90 |
| | Fat (Kcal/d) | 890.18± 204.57 | **0.0006** | 1.195.82± 205.63 |
| | Fish intake (times/week) | 0.95±0.61 | 0.509 | 0.77±0.67 |
| History of meconium ileus | (n) | 1 | 0.378 | 6 |
| Serum cholesterol | mg/dL | 155.73±44.00 | **0.032** | 125.18±35.52 |
| Serum vitamin E | mg/dL | 11.99±4.52 | **0.003** | 7.57±2.94 |

Bold = statistical significant.

#### 3.2.1. Saturated fatty acids

There were no differences between the patients with CF who underwent a lung transplantation and the healthy controls, except for Lignocerate (c24:0). These were decreased, however, not to the extent as in the non-transplanted CF group. In the CF group, levels of various unsatured fatty acids are significantly decreased both compared to the control group as well as compared to the CF transplant group (see Table 2).

#### 3.2.2. Mono-unsaturated fatty acids

There were few differences between the groups. Only Vaccenate (c18:1n−7) was higher in the CF transplant group compared to the control group (not to the CF group) and Palmitoleate (c16:1) was higher in the CF group compared to the control group (not to the CF transplant group) (see Table 2).

#### 3.2.3. Poly-unsaturated fatty acids (PUFA)

In the CF groups there was a significant decrease in DHA, LA, AA compared to both the control group and the CF transplant group. On the contrary these fatty acids were not different between the CF transplant group and the control group. MA was significantly increased in the CF group and also in the CF transplant group compared to the control group. MA was the only PUFA that was different between the control and the CF transplant group (see Fig. 1.).

### 3.3. Relation between PUFA and other clinical variables

Within the CF group there was no difference with regard to one of the four significantly changed PUFA levels (DHA, LA, AA, MA) and *Pseudomonas aeruginosa* colonization ($p \geq 0.05$ for all), caloric intake, fat intake or vitamin E levels ($p \geq 0.05$ for all). However cholesterol levels were positively correlated with absolute levels (expressed as μM) of DHA (r=.531), LA(r=.528) and AA (r=.630).

Within the transplanted group there was the same significant correlation between cholesterol and PUFA levels (DHA r=.838, LA r=.752, AA r=.866), but also with the decreased fat intake (AA r=−.656 and MA r=−.715). Vitamin E (which minimizes DHA peroxidation [5]) also correlated positively with DHA level (r=.636) ($p<0.05$ for all correlations).

### 3.4. Ratios between PUFAs (Table 3)

There was evidence for an essential fatty acid deficiency (documented by an elevated ratio of MA to AA [11,14]) in patients with CF (above the 0.02 cut-off) that was not present in the CF transplant group. AA level multiplied by DHA level (suggested as diagnostic marker for CF [16]) was decreased in patients with CF, but not in CF lung transplanted patients. The ratio of AA to DHA (a marker of inflammatory state and a reflection of the n−6 to n−3 composition [7]) was not different between the 3 groups although there was a trend to increased values in patients with CF. The ratio of AA to dihomo-gamma linoleic acid (DHGLA) (an indicator of Δ5-desaturase activity [1]) was decreased in patients with CF, but not in the CF transplant group (results in Table 3).

Table 2
Fatty acid levels of controls, patients with CF and CF lung transplanted patients.
(TX) Transplant
As fatty acid levels were not normally distributed, they are expressed as median (interquartile range).

| Fatty acids (μM) | Control | TX | CF | Control versus TX (p) | CF versus TX (p) | CO versus CF (p) |
|---|---|---|---|---|---|---|
| Lauric acid (c12:0) | 6.92 (13.93) | 14.58 (30.93) | 3.93 (12.85) | 0.26 | 0.23 | 0.59 |
| Myristic acid (c14:0) | 120.05 (103.98) | 146.37 (311.63) | 103.3 (106.58) | 0.06 | 0.08 | 0.81 |
| Hexadecanal dimethyl acetate (c16:0) | 24.29 (10.14) | 24.36 (15.64) | 13.61 (12.01) | 0.87 | **0.003** | **<0.001** |
| Palmitic acid (c16:0) | 1852.99 (1274.63) | 2671.18 (2454.21) | 1723.83 (832.58) | 0.13 | **0.048** | 0.30 |
| Stearic acid (c18:0) | 617.19 (267.82) | 618.76 (393.57) | 488.98 (135.64) | 0.90 | 0.06 | **0.008** |
| Arachidic acid (c20:0) | 18.56 (8.47) | 16.29 (12.13) | 10.38 (3.67) | 0.38 | **0.024** | **<0.001** |
| Behenic acid (c22:0) | 49.72 (15.85) | 45.01 (26.62) | 28.82 (8.18) | 0.09 | **0.040** | **<0.001** |
| Lignoceric acid (c24:0) | 39.27 (14.55) | 34.08 (19.53) | 20.1 (6.49) | **0.04** | **0.012** | **<0.001** |
| Myristoleic acid (c14:1) | 2.58 (31.85) | 2.00 (21.67) | 2.38 (14.58) | 0.81 | 0.90 | 0.65 |
| Palmitoleic acid (c16:1) | 153.22 (141.19) | 316.58 (312.71) | 277.24 (221.21) | 0.17 | 0.96 | **0.024** |
| Oleic acid (c18:1n−9) | 1672.74 (1226.72) | 2176.53 (2457.35) | 1558.23 (661.81) | 0.07 | 0.05 | 0.94 |
| Vaccenic acid (c18:1n−7) | 136.87 (65.91) | 198.66 (179.77) | 151.74 (95.87) | **0.011** | 0.08 | 0.39 |
| Eicosenoic acid (c20:1n−9) | 6.1 0 (7.87) | 10.66 (25.14) | 6.96 (3.77) | 0.13 | 0.18 | 0.82 |
| Erucic acid (c22:1n−9) | 0.00 (0.00) | 0.00 (0.17) | 0.00 (0.00) | 0.84 | 0.99 | 0.52 |
| Nervonic acid (c24:1n−9) | 77.68 (39.79) | 76.84 (28.16) | 73.82 (19.93) | 0.96 | 0.32 | 0.12 |
| Alpha-Linolenic acid (c18:3n−3) | 48.07 (49.29) | 61.61 (71.12) | 32.48 (28.5) | 0.49 | 0.08 | 0.06 |
| Eicosatrienoic acid n−3 (c20:3n−3) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.69 | 0.69 | 1.00 |
| Eicosapentaenoid acid (c20:5n−3) | 54 (40.52) | 47.97 (89.29) | 55.77 (32.78) | 0.87 | 0.81 | 1.00 |
| Docosatrienoic acid (c22:3n−3) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 1.00 | 1.00 | 1.00 |
| Docosapentaenoic acid n−3 (c22:5n−3) | 28.91 (16.95) | 33.32 (38.6) | 29.52 (25.54) | 0.34 | 0.34 | 0.85 |
| Docosahexaenoic acid (c22:6n−3) | 135.67 (60.13) | 165.66 (113.87) | 89.22 (63.4) | 0.34 | **0.021** | **0.003** |
| Linoleic acid (c18:2n−6) | 2398.51 (918.4) | 2172.24 (930.35) | 1478.86 (633.75) | 0.61 | **<0.001** | **<0.001** |
| Gamma-linolenic acid (c18:3n−6) | 26.19 (24.22) | 28.38 (35.9) | 27.18 (30.69) | 0.72 | 0.82 | 0.34 |
| Dihomo-gamma-linolenic acid (c20:3n−6) | 121.66 (95.53) | 150.75 (109.13) | 119.63 (70.38) | 0.38 | 0.26 | 0.77 |
| Arachidonic acid (c20:4n−6) | 561.84 (322.29) | 682.6 (276.12) | 444.38 (185.39) | 0.67 | **0.015** | **0.021** |
| Docosatetraenoic acid (c22:4n−6) | 11.01 (8.99) | 12.02 (15.72) | 11.04 (8.68) | 0.13 | 0.23 | 0.59 |
| Docosapentaenoic acid n−6 (c22:5n−6) | 11.41 (7.95) | 11.96 (32.86) | 13.32 (8.37) | 0.20 | 0.64 | 0.22 |
| Mead acid/eicosatrienoic acid n−9 (c20:3n−9) | 8.41 (17.66) | 23.56 (41.19) | 29.91 (43.45) | **0.014** | 0.32 | **<0.001** |
| Total | 8327.04 (4981.21) | 10046.33 (9056.46) | 7009.9 (3081.33) | 0.26 | 0.02 | 0.06 |

Bold = statistical significant.

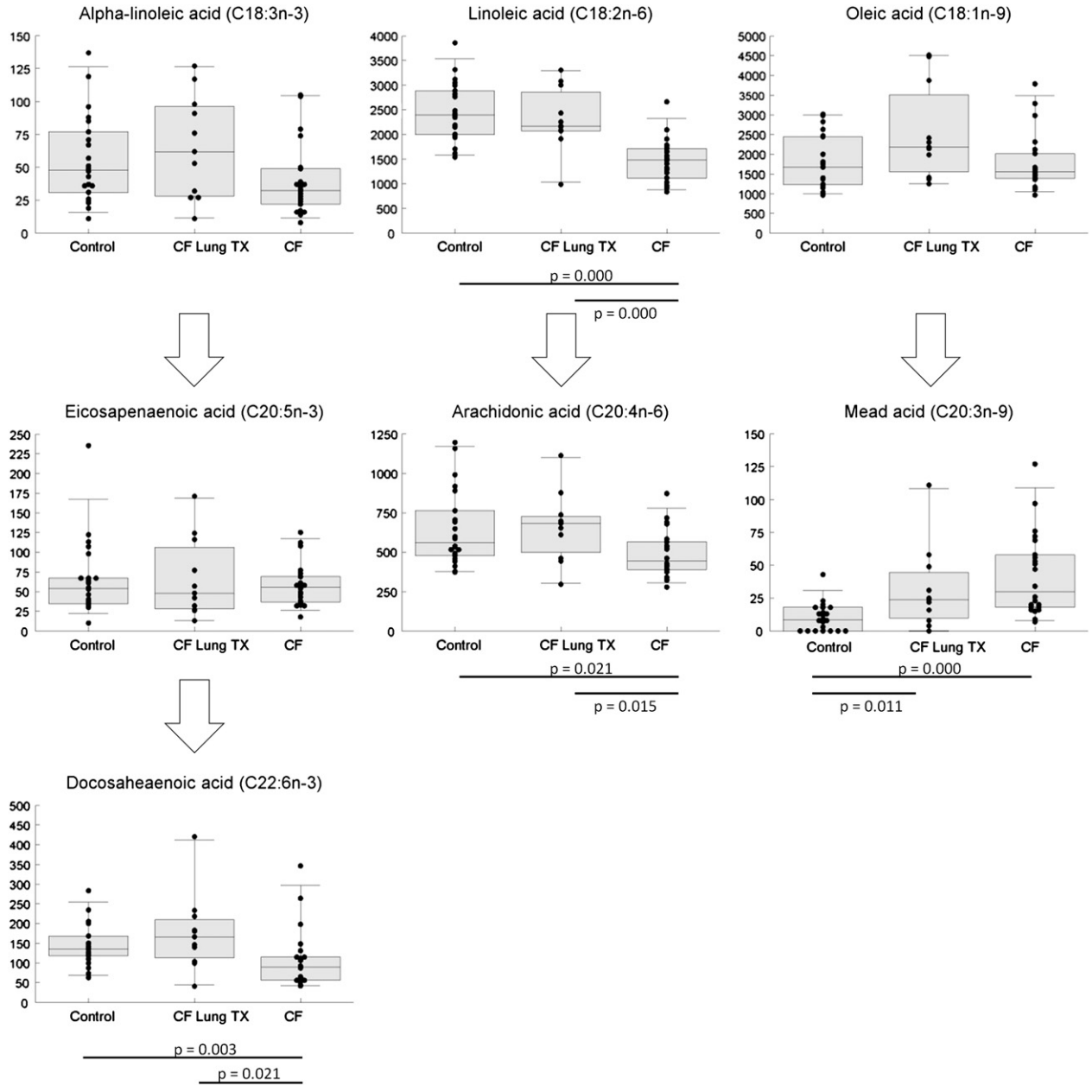


Fig. 1. Boxplots showing PUFA state in the three groups. Control group (left), CF group (middle), CF transplant group (right). Each dot represents an individual measurement. Boxplot indicates the 25th, 50th and 75th centiles. The whiskers represent 1.5 and 3 times the interquartile range.

## 4. Discussion

In our small cohort of patients with CF we corroborate the frequently observed changes in serum fatty acid profile in patients with CF [1,8–14] confirming decreased levels of LA, AA, DHA and increased levels of MA. The exact etiology of these changes in plasma PUFA state in patients with CF remains unknown. In this study, we compared a group of 11 lung transplanted patients with CF with an age-matched, genotype matched (all pancreatic insufficient) group of 22 patients with CF with comparable biometric data and comparable fish intake and a healthy control group. We demonstrated for the first time that after lung transplantation there is a more normal fatty acid profile (especially the poly-unsaturated fatty acids). Indeed, in all 11 patients with CF (all DF 508) that underwent a lung transplantation (longer than one year ago), the plasma levels of LA, AA and DHA became comparable to healthy controls and MA levels decreased toward more normal values.

The mechanisms leading to the abnormal PUFA profiles are elusive. Numerous not mutually excluding hypotheses exist. In the setup we used, we can partially break the interdepency of pulmonary disease, pancreatic insufficiency and nutritional state with regard to genotype. All patients were pancreatic insufficient and remained so after lung transplant. So, decreased uptake seems unlikely to be an explanation, which is in accordance with previous findings in well-nourished patients with CF [11]. In fact, the dietary intake was lower in the transplanted group despite the better fatty acid profile.

Table 3
Ratios between selected fatty acids.
(TX) Transplant
As fatty acid levels were not normally distributed, they are expressed as median (interquartile range).

| Median (IQR) | Control | TX | CF | Control versus TX (p-value) | CF versus TX (p-value) | Control versus CF (p-value) |
|---|---|---|---|---|---|---|
| AA/DHA | 4.57 (0.98) | 4.02 (1.81) | 6 (2.53) | 0.218 | 0.063 | 0.057 |
| AA × DHA | 73446 (73553) | 109886 (104449) | 45697 (52034) | 0.486 | **0.009** | **0.004** |
| MA/AA | 0.02 (0.02) | 0.04 (0.03) | 0.06 (0.09) | **0.004** | **0.019** | **<0.001** |
| AA/DHGLA | 4.81 (2.65) | 4.52 (0.88) | 3.73 (1.31) | 0.375 | 0.123 | **0.024** |

Bold = statistical significant.

Other proposed mechanisms are: decreased delta-5-desaturase activity in CF [16] (which fits with our finding of lower AA/DHGLA in non-transplanted patients with CF compared to controls but could also be a sign of increased LA to AA conversion [17]), increased use of fatty acids as a source of energy [18] (which fits with the increased energy intake in patients with CF compared to the transplanted patients although there was no difference in nutritional state expressed as BMI z-score), increased lipid turnover in membranes [19] and production of eicosanoids, possibly related to an exacerbated inflammatory state [20].

Lung disease is the number one cause of death in CF and is clinically the most important for the patient's well-being. Therefore, we hypothesized that they could also play a central role in the development of abnormal fatty acid profiles. Moreover, there are some clues in this direction. Bronchoalveolar lavage fluid of patients with CF contains more AA as compared to three control groups (of healthy, chronic bronchitis patients, *P. aeruginosa* infected patients without CF) [21]. Furthermore, CF-EFA abnormalities have been related to $FEV_1$ [14] and implicated in the predisposition of CF lung disease [3]. Finally, in CF knockout mice lipid imbalances were detected in CF regulated organs as for instance in the lungs with decreased DHA levels and increased AA levels [5]. DHA supplementation could also block *Pseudomonas* endotoxin-enhanced lung inflammation [5].

By performing a lung transplantation, and thereby normalizing PUFA state, we show that CF lungs are a major contributor to abnormal fatty acid state. However, when performing a lung transplantation, we induce three major changes: firstly, there is possibly a dramatic change in inflammatory profile due to the removal of a heavily infected and inflamed CF lung. This is reflected by the observed decrease in AA/DHA ratio (Table 3). Secondly, there is a functional CFTR present in the lung(s) of the transplanted patients. Therefore, the CFTR status changes partially, as 100% of the right cardiac output (in case of a double lung transplantation) is exposed to the non-CF tissue (transplanted lung) in the pulmonary circulation. Finally, all the secondary effects of the diseased CF lung on various organs (e.g. mediated by hypoxia), and of a lung transplantation (e.g. immunosuppression) could also play a role. Unfortunately, in this observational study, no distinction between these mechanisms can be made.

Several lines of evidence indicate a role for CFTR itself, in the fatty acid metabolism. Firstly, the degree of PUFA impairment is independently associated with the genotype group (mutation class) [12]. Secondly, in some studies heterozygotes with almost no inflammation have intermediate values between patients with CF and healthy controls (7;13 and our own unpublished data). Thirdly, other diseases with inflammation as upper respiratory tract disease or asthma do not present with changes in the same magnitude as in CF [7]. This was also the case in bronchoalveolar lavage fluid on non-CF *P. aeruginosa* infected patients [21]. Fourthly, similar PUFA changes in CF affected organs (lung, pancreas, ileum) have been demonstrated in CFTR knock-out mice [5]. Finally, in cell culture models, it was shown that CFTR dysfunction itself leads to defective fatty acid composition (showing an increased conversion form LA tot AA) [17]. Nevertheless, inflammation is capable of changing PUFA composition [7], so it will probably also play a role in this complex metabolic alteration.

In this article, we chose to report our data in the form of μM rather than mole%. This is because we believe any biological effect modulated by the plasma is dependent on the concentration rather than the relation to the total fatty acid level. Moreover, this makes it more realistic, rather than just selecting fatty acids to calculate the composition [22]. Of course, ratios between selected fatty acids (Table 3) remain important and are unchanged by this. The within group comparisons between cholesterol level or vitamin E and DHA, LA, AA should however be interpreted with caution as there is significant correlation of total FA and cholesterol and vitamin E.

A limitation to our study is the CF lung transplant patients are not just patients with CF with healthy lungs. They all take immunosuppressive medication which could have an effect on fatty acid state. Nevertheless, that this effect would lead to 'normalization', apart form their effect on inflammation, seems unlikely. Another limitation is that we chose to measure only total plasma fatty acid levels. However, these have shown to have a good relationship to lipid profiles in cell membranes of CF affected tissue and those obtained in red blood cells [1,5,11]. Finally we did not measure EFA profile before and after transplantation, so we can not really state that lung transplantation reverses abnormalities in EFA profile. However, the existence of a selection bias with patients with a better EFA profile undergoing a lung transplantation seems unlikely.

To summarize, in this study we showed that the CF lung is an important player in the development of abnormal fatty acid profile. Rather than studying the effect of fatty acids on lung

function (as in EFA supplementation trial) we did the reverse by documenting fatty acid profiles in a subset of patients with CF who underwent lung transplantation. We showed that post-transplant an evolution toward normal fatty acid profiles is to be expected.

## Conflict of interest

PW is an aspirant researcher for the FWO Vlaanderen. DC is a fundamental-clinical researcher for the FWO Vlaanderen.

There is no conflict of interest.

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