Oil supplementation with a special combination of n-3 and n-6 long-chain polyunsaturated fatty acids does not protect for exercise induced asthma. A double-blind placebo-controlled trial.

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Exercise-induced asthma, exercise challenge, Polyunsaturated fatty acids, PUFA, exhaled nitric oxide
Abstract

Background Many patients suffering from exercise-induced asthma (EIA) have normal lung function at rest and show symptoms and a decline in FEV 1 when they do sports or during exercise-challenge. It has been described that long-chain polyunsaturated fatty acids (LCPUFA) could exert a protective effect on EIA.

Methods In this study the protective effect of supplementation with a special combination of n-3 and n-6 LCPUFA (sc-LCPUFA) (total 1.19 g/ day) were investigated in an EIA cold air provocation model. Primary outcome measure: Decrease in FEV 1 after exercise challenge and secondary outcome measure: anti-inflammatory effects monitored by exhaled NO (eNO) before and after sc-LCPUFA supplementation versus placebo.

Results 99 patients with exercise-induced symptoms aged 10 to 45 were screened by a standardized exercise challenge in a cold air chamber at 4 °C. 73 patients fulfilled the inclusion criteria of a FEV 1 decrease >15% and were treated double-blind placebo-controlled for four weeks either with sc-LCPUFA or placebo. 32 patients in each group completed the study. Mean FEV 1 decrease after cold air exercise challenge and eNO were unchanged after four weeks sc-LCPUFA supplementation.

Conclusion Supplementation with sc-LCPUFA at a dose of 1.19 g/d did not have any broncho-protective and anti-inflammatory effects on EIA.

Introduction

Bronchial asthma is one of the most common chronic diseases and has considerable economic and health relevance (1). Asthma triggers include respiratory infections, allergens, airborne pollutants and physical stress. Many patients suffering from exercise induced asthma (EIA) have normal lung function at rest but show symptoms and a decline in FEV 1 of at least 10-15 % during exercise-challenge. EIA is particularly prevalent among children and adolescents.

Exhaled NO (eNO) as marker of eosinophil airway inflammation has been described as a good predictor of EIA, as several studies have shown higher eNO levels among subjects with EIA (2 – 7).

Although pharmacological treatment is well established, alternative treatment options get more and
more important for the patients in order to reduce their daily amount of pharmacologic medication. The first step of non-pharmacologic treatment of EIA involves advising the patient to warm slowly and to avoid exercising in cold weather or on high-pollen days (8). In addition, long-chain polyunsaturated fatty acids (LCPUFA) have been suggested as a possible complementary / alternative therapy for EIA. Especially, n-3 long-chain PUFA (n-3 LCPUFA) exert well known anti-inflammatory effects which are in part associated with a change in cell membrane composition (8, 9).

In line with this concept many studies demonstrated that supplementation of n-3 LCPUFA have beneficial effects on EIA (10–15). In contrast, there are many studies which failed to show clinical improvement of EIA by n-3 LCPUFA supplementation (16–22). The diverging study results on n-3 LCPUFA supplementation may be attributable to many factors, of which the poor design of earlier studies, i.e. small patient cohorts and different concentration and duration of n-3 LCPUFA supplementation are likely affecting the results. Therefore, we designed a synergistic combination of n-3 and n-6 LCPUFA (sc-LCPUFA) containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and echium seed oil containing stearidonic (SDA) and gamma-linolenic acid (GLA) as recently described in our review (23). The idea of this study was to test possible synergistic effects of a combination of four different n3- and n6-PUFA-species (SDA, GLA, EPA and DHA). SDA, GLA, EPA and DHA influence several immunologic parameters, connected to clinical aspects of the asthmatic inflammation addressing distinctively different metabolic and regulatory pathways (23). This specific sc-LCPUFA is a promising approach to reduce eosinophilic inflammation and improve lung function in patients with asthma by restoring fatty acid homeostasis and increasing EPA and DHA levels as recently shown in an asthma mouse model (23, 24). The given oil-dosis of 1190 mg PUFA/d allows comparisons with other trials addressing PUFA-treatments of bronchial inflammation merely with EPA or DHA.

Taken together, the aim of this double-blind placebo-controlled study was to evaluate the effect of a sc-LCPUFA combination on the bronchoconstriction response during exercise provocation and on airway inflammation in a large sample size of patients suffering from EIA.

Material And Methods
2.1 Study design

The double-blind placebo-controlled trial took place from April 2015 until January 2016 and consisted of 3 visits in a time frame of 4 to 8 weeks. Participation in the study was voluntary. Prior to the commencement of the first visit (Screening visit, V1), written informed consent was required from each subject or from the parents of children under the age of 18 years.

At V1, we confirmed the participants’ inclusion and exclusion criteria, administered a lung function test using the MasterScreen spirometer, performed a skin prick test (SPT), measured eNO and provided an asthma control questionnaire (ACQ) to participants.

On the second (V2, Randomization) and the third visit (V3, End of study) we conducted a lung function test, measured eNO, administered an exercise challenge in a cold chamber (ECC) and collected blood.

If the patient fulfilled the inclusion criteria `FEV₁ decrease ≥ 15% in the ECC` at V2, they were randomised to one of the two study arms (interventional group with sc-LCPUFA supplementation or placebo group). V3 was performed after 4 weeks of supplementation. The study flow chart with assessments is shown in Fig.1. The study was approved by the ethics committee of Goethe University (reference number 360/14) Clinical trials registration number: NCT02410096.

2.2 Subjects

We recruited 99 subjects aged 10 to 45 years with asthmatic symptoms while exercising from our outpatient clinic for allergology and pulmonology by a public posting. Subjects with chronic asthma and regular use of inhaled corticosteroids (ICSs) or leukotriene receptor antagonists were excluded.

Further exclusion criteria were: subjects with FVC < 75%, subjects > 18 years/ < 18 years with FEV₁ < 60/ < 70%, oral corticosteroids, other known chronic disease or infection, pregnancy, documented alcohol, medication or drug abuse and inability to perform and understand all study procedures.

2.3 sc-LCPUFA supplementation and Placebo

The total amount of functional LCPUFA in the investigational product came to 1190 mg/day (710 mg
EPA, 161 mg DHA, 175 mg GLA and 144 mg SDA). Therefore, the patients had to take 4 different capsules each morning combined with their breakfast:

1 capsule PlusEPA (containing EPA, Minami Nutrition, Aartselaar, Belgium); 1 capsule EPA/DHA/GLA (containing EPA/DHA/GLA, Peak Performance Products S.A., Grevenmacher, Luxemburg); 2 capsules Echiomega (containing SDA/GLA, Igennus Healthcare Nutrition, Cambridge, UK). The placebo compound (Allcura, naturheilmittel GmbH) was composed of 500 mg olive oil.

### 2.4 Pulmonary function test

Baseline pulmonary function tests were performed using the MasterScreen spirometer (CareFusion, Germany). The following parameters were recorded: forced vital capacity (FVC), FEV$_1$ and FEV$_1$/FVC.

### 2.5 Measurement of exhaled nitric oxide

Measurements of eNO were conducted using the NIOX1 gas analyzer (Aerocrine, Sweden). NIOX1 measures eNO in exhaled air according to ATS guidelines (25).

### 2.6 Exercise challenge in a cold chamber

The exercise challenge was performed according to the ATS guidelines for EIA (26) as recently described (7, 27). The subjects ran in a cold chamber (Ilkazell Isoliertechnik, Germany) cooled to 2–4 °C (microprocessor-based controller, Dixell, Emerson Climate Technologies, United Kingdom) on a treadmill (Schiller Intertrack 8100T Med, Germany) with an incline of 10% for 6 minutes (≤ 12 years) or 8 minutes (> 12 years). At 5, 10, 15 and 30 minutes after running, spirometry were performed. A positive reaction was defined as a decline in FEV$_1$ ≥ 15% from the baseline value at 2 time points after exercise.

### 2.7 Asthma control questionnaire (ACQ)

The ACQ has strong evaluative and discriminative properties and can be used with confidence to measure asthma control (28). The ACQ assesses seven items, which include asking patients to recall
their experiences in the previous week and to respond to questions about nighttime waking, symptoms on waking, activity limitations, shortness of breath, wheezing, required use of short-acting β₂-agonists for rescue, and FEV₁ percent predicted before bronchodilator on a 7-point scale (28).

The items were equally weighted and the ACQ score was the mean of the 7 items and therefore between 0 (totally controlled) and 6 (severely uncontrolled). Thus, patients with an ACQ < 0.75 have a good asthma control, patients with ACQ > 1.5 have a not controlled asthma, in between the asthma is partially controlled (29, 30). The ACQ is valid for use in children from the age of 6 (31).

2.8 Verification of PUFA incorporation by gas-chromatography

The fatty acid profile of blood plasma and blood cells were separately determined by fatty acid methyl esters (FAME) analysis as previously described (24).

The FAMEs of plasma and blood cells were analyzed by capillary gas chromatography (CGC) (Trace1300, Thermo Scientific, Dreieich, Germany) equipped with an autosampler AS1310 (Thermo Fisher Scientific, Dreieich, Germany). Fatty acid separation was achieved by using a capillary pre-column GuardGOLD, length: 2 m, i.d.: 0.25 mm (Thermo Fisher Scientific, Milan, Italy) and downstream a capillary column TRACE TR-FAME (70% cyanopropyl polysilphenylene siloxane), length: 60 m, i.d.: 0.25 mm, film thickness: 0.25 μm (Thermo Fisher Scientific, Dreieich, Germany).

The gas chromatographic conditions were the following: injector (SSL): 250°C, splitless and carrier gas: helium (purification 99%) at a flow of 20 mL x min⁻¹. Compounds were monitored by flame-ionization detector (FID) at 250°C. Fatty acids were identified by comparison of their retention times with those of external standard (Supelco 37-Component FAME Mix, Sigma-Aldrich, St. Louis, Missouri, United States). The column oven temperature was maintained at 60 °C for 0.5 min after injection and then programmed at 40 min to 180 °C (held for 2 min), then at 2 min-1 to 210°C (held for 3 min) and finally at 3 min-1 to 240°C (held for 10 minutes). The total run-time was 44 min.

2.9 Statistical analyses

GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses.
Data are presented as median and range or as mean and standard deviation (SD). The primary outcome measure was the decrease in FEV$_1$ after exercise challenge in cold air as measured by spirometry (FEV$_1$, percent predicted). The secondary outcomes were the anti-inflammatory effects monitored by exhaled NO (eNO) before and after PUFA supplementation versus placebo, the incorporation of the different components of the sc-LCPUFA supplementation and the side effects of the sc-LCPUFA supplementation. Intra-group and inter-group comparisons were calculated either by One-Way ANOVA, paired t-test or Wilcoxon test and unpaired t-test or Mann-Whitney test, respectively, according to the Kolmogorov-Smirnov test for normal distribution. $P < 0.05$ was considered as statistically significant. Power Calculation: For the sample size calculation we used the results of a previous study: “Predictors and reproducibility of exercise-induced bronchoconstriction in cold air” (27). Assuming an improvement of the maximal FEV$_1$ decrease after ECC of 35% in the interventional group and a test power of 80% (corresponding to a probability of beta-error of $\beta = 0.2$) 30 patients in the interventional and placebo group for an effect with an alpha coefficient of 0.05 had to be investigated.

Results

3.1 Patient characteristics

99 patients with exercise-induced symptoms aged 10 to 45 were recruited for V1, 73 of them fulfilled all inclusion criteria and were randomized for the study and treated placebo-controlled for 4 weeks. With 9 drop-outs between V2 and V3, 64 patients (32 patients in each group) completed the study and entered the statistical calculations. 50 subjects (78.1%) showed a positive SPT and 32 (50.0%) suffered from symptoms of allergic rhinoconjunctivitis and were challenged beyond the pollen season. 24 subjects (37.5%) suffered from cough during the pollen season, 48 (75.0%) had doctor’s diagnosed bronchial asthma and 26 of them (40.6%) used short-acting $\beta_2$-agonists on demand. 33 subjects (51.6%) showed an ACQ < 0.75, 18 (28.1%) an ACQ 0.75-1.5 and 13 (20.3%) an ACQ > 1.5. For the FEV$_1$ and FVC measures, the
ATS/European Respiratory Society test criteria for acceptability and repeatability were met (32). The characteristics of the subjects are summarized in Table 1. There was no significant difference between the subgroups interventional and placebo for age, sex, BMI, FEV₁, eNO and ACQ.

3.2 Decrease of FEV₁ after ECC and eNO before and after 4 weeks off sc-LCPUFA supplementation

The maximal decrease of FEV₁ after ECC showed no significant difference between the interventional and the placebo group before (31.7% ± 12.0 interventional; 31.1% ± 12.3 placebo; p = 0.90) and after 4 weeks of sc-LCPUFA or placebo supplementation (26.4% ± 17.7 interventional; 27.0% ±16.1 placebo; p = 0.86). The same results emerge in the subgroups for adult and children (Fig 2 a-c).

The eNO before (20.0 ppb (5.0 – 249.0) interventional; 29.0 ppb (6.0 – 88.0) placebo; p = 0.69) and after 4 weeks of PUFA or placebo supplementation (18.5 ppb (6.0 – 209.0) interventional; 28.5 ppb (5.0 – 99.0) placebo; p = 0.61). The same results emerge in the subgroups for adult and children (Fig. 2 d-f).

We analyzed the subgroups with low eNO < 25 ppb and high eNO >30 ppb separately to revise the hypothesis if the type of EIA with high eNO associated to more eosinophilic inflammation shows better response to sc-LCPUFA supplementation. However, there were no significant differences in FEV₁-decrease and eNO before and after treatment for both subgroups (data not shown).

In addition, we analysed the effect of the sc-LCPUFA supplementation in patients with an increase of EPA in the blood cells of at least 0.25% and 0.5% as evidence for good compliance and good incorporation of EPA in the cell membrane. However, there were no significant differences in FEV₁-decrease and eNO before and after treatment for both subgroups 0.25% and 0.5% respectively (data not shown).

3.3 Side effects

The data of 58 patients (29 placebo group (90.6%), 29 interventional group (90.6%)) who completed their symptom diary on a regular basis were used for the calculations of side effects.
Patients in the interventional group complained significantly more often about “belching” [7 (0 – 50) vs. 0 (0 – 20) (p = 0.0048)]. Both, the gastrointestinal side effects “bloating” [0 (0 – 57) vs. 0 (0 – 41) (p = 0.18)] and “diarrhea” [0 (0 – 55) vs. 0 (0 – 25) (p = 0.8)] and the pulmonal complaints “use of salbutamol” [0 (0 – 35) vs. 0 (0 – 36) (p = 0.31)] or “dyspnoe” [2.5 (0 – 31) vs. 0.5 (0 – 50) (p = 0.28)] were not significantly different between interventional and placebo group (Fig. 3).

Furthermore, we analysed the side effects of two subgroups of the interventional group: those with an increase of EPA in the blood cells of at least 0.5% and those below. There were no significant differences in salbutamol use, dyspnoe, bloating, belching and diarrhea for both subgroups (data not shown).

3.4 Incorporation of sc-LCPUFA supplementation

Gas chromatographic analysis of plasma and blood cells of patients showed a significant increase of EPA at V3 compared to V2 in the investigational (p < 0.001), but not in the placebo group. However, PUFA supplementation did not increase DHA in plasma and blood cells of EIA patients (Fig. 4 a-c). In comparison, placebo lowered DHA amounts in plasma of patients at V3 compared to V2 and patients who received the sc-LCPUFA.

Reduction of AA in plasma at V3 in both groups is probably due to a supplementation effect. However, there were no differences between the groups regarding the amount of AA. Results were the same in adults and children except for DHA which was significantly higher in in plasma and blood cells in children (P<0.05) of the investigational group at V3 compared to V2 (data not shown).

Discussion
The aim of of this double-blind placebo-controlled study was to evaluate the effect of a sc-LCPUFA on EIA and airway inflammation in a large sample size of patients.

We found that the maximal decrease of FEV₁ and eNO after the supplementation phase showed no significant difference between the interventional and the placebo group. Moreover, even a subgroup analysis in patients with EIA and high eNO were negative. PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have shown to decrease the production of pro-inflammatory
eicosanoids, such as (cysteinyl)-leukotrienes, prostaglandins as well as pro-inflammatory cytokines by competitive inhibition of the arachidonic acid metabolism at cyclooxygenase-2 and 5- lipoxygenase enzymes (23, 24). Moreover, EPA and DHA generate via several enzymatic reactions specialized pro-resolving mediators (SPMs) like protectins, resolvins, and maresins to counter-regulate airway eosinophilic inflammation and promote the resolution of inflammation in asthma (34,35).

Thus, the question arised, why our study demonstrated no beneficial effect regarding lung function and eNO in our study. Different reasons have to be discussed like the daily dose of LCPUFA, the proportion of EPA and DHA in the mixture, the provocation model used to induce an inflammatory response and of course the statistical power of the trial design.

Many studies showed a positive effect of n-3 LCPUFA supplementation in EIA with significantly higher doses > 1 g/d (10, 11, 13, 14). A daily dose of 5.4 g n-3 PUFAs (3.2 g EPA and 2.0 g DHA) showed a significant reduction of eNO and FEV$_1$ decrease in three different studies dealing with EIA (10, 11, 14). In another study a dose of 3.1 g/d n-3 PUFAs and of 6.2 g/d n-3 PUFAs showed an equal reduction in eNO and FEV$_1$ (13).

In contrast, there were many reports which found no clinical improvement after supplementation of high doses n-3 LCPUFA (16–20). In a recent study supplementation with 3.0 g/d EPA and 3.0 g/d DHA did not show an effect on FEV$_1$ decrease after eucapnic voluntary hyperpnea (19).

With increasing amount of n-3 LCPUFA supplementation, costs and gastrointestinal side effects increase, while compliance declines (13). Therefore, it is interesting that other studies in EIA and asthmatic patients with low dose n-3 LCPUFA supplementation equivalent to our study again showed positive (12, 15) and negative (21, 22) results.

Supplementation with lipids of the New-Zealand green-lipped mussel (576 mg/d EPA, 384 mg/d DHA) for 3 weeks showed significant reduction of eNO and FEV$_1$ decrease in EIA patients after dry air exercise challenge (12).

Summarized, there are positive and negative studies for both - high and low dose n-3 LCPUFA supplementation. Consequently, the total dose by itself could not be the reason that no effect of sc-
LCPUFA-supplementation could be demonstrated in our study. In addition, the compliance of our patients was well controlled by keeping a diary, and determination of the LCPUFA levels in plasma and blood cells. The latter is a well-established tool for dietary supplementation studies (24, 33). However, as shown by Fig. 2 only EPA was significantly increased after supplementation, whereas DHA levels were unchanged. Thus, it is tempting to speculate that the DHA content of the sc-LCPUFA was maybe too low.

Current data suggest that EPA and DHA have distinct anti-inflammatory effects. EPA is endogenously converted into specialized pro-resolving mediators i.e. E-series resolvins, whereas DHA is converted into D-series resolvins, protectins and maresins (34). Thus, it is important to well-balance a LCPUFA mixture, especially with a significant content of DHA (23, 24). In mice DHA and Resolvin (Rv) D1 induce a phenotypic switch in macrophages from a pro-inflammatory towards an anti-inflammatory activation profile (35). DHA was shown to be superior to EPA in respect of asthma prevention (36). Furthermore, high concentrations of DHA were suggested to have a protective effect on lung function (37). However, there is evidence that EPA and DHA in combination exhibit their highest beneficial potential on chronic inflammation (10–15, 24).

In conclusion, studies investigating a therapeutically effect of n-3 LCPUFA on bronchial asthma do not show consistent effects. The diverging study results may arise among others attributable by small study populations, different subtypes of asthma, different provocational methods and the different composition of the LCPUFA supplementation and the different duration of the supplementation period. In a review on this topic it was stated that negative results could be caused by methodological and statistical limitations (38). In contrast to many other studies, we used an ATS guidelines conform exercise challenge. Thus the number of our patient cohort was well powered by previous results and we tested mild and moderate asthmatics as recommended as well as our subjects were well balanced (half adults, half children, mixed male and female). Consequently, the negative results of our trial cannot be attributed to methodological and statistical limitations.

There are some clinical limitations of our study. First, we did not gathered data on the normal dietary habits of our patients (amount of weekly fish, meat, oils etc.) and did not make any regulations for the
diet during study participation. In addition, we did not measure eNO 24 hours after exercise to detect a possible late effect of our sc-LCPUFA supplementation.

Conclusion
In conclusion, our sc-LCPUFA supplementation had no positive effect on EIA. Both, the maximal decrease of FEV\textsubscript{1} and eNO as inflammatory marker were unchanged.

List Of Abbreviations
| Abbreviation | Term/Definition |
|--------------|----------------|
| ACQ          | Asthma control questionnaire |
| APS          | Aerosol provocation system |
| ATS          | American Thoracic Society |
| BHR          | Bronchial hyper-responsiveness |
| d            | Day |
| DHA          | Docosahexaenoic acid |
| ECC          | Exercise challenge in a cold chamber |
| EIA          | Exercise-induced asthma |
| EPA          | Eicosapentaenoic acid |
| eNO          | Exhaled nitric oxide |
| f            | Female |
| FA           | Fatty acid |
| FEV₁         | Forced expiratory volume in 1 second |
| FVC          | Forced vital capacity |
| GLA          | Gamma-Linolenic acid |
| ICS          | Inhaled corticosteroid |
| LCPUFA       | Long-chain poly unsaturated fatty acid |
| m            | Male |
| MCFA         | Middle-chain fatty acids |
| mg           | Milligrams |
| n            | Number or Omega |
| PUFA         | Poly unsaturated fatty acid |
| ppb          | Parts per billion |
| Sc-LCPUFA    | special combination long-chain poly unsaturated fatty acid |
| SDA          | Stearidonic acid |
| SPT          | Skin prick test |
| SD            | Standard deviation |
| SEM          | Standard error of mean |
| V1/2/3       | Visit 1/2/3 |
Statements

**Ethical Approval and Consent to participate**

Participation in the study was voluntary. Prior to the commencement of the first visit, written informed consent was required from each subject or from the parents of children under the age of 18 years. The study was approved by the ethics committee of Goethe University (reference number 360/14) and registered at ClinicalTrials.gov, registration number: NCT02410096.

**Availability of data and material**

The data from this study are available on request from the Department for Children and Adolescents, Division of Allergology, Pulmonology and Cystic fibrosis, Goethe University, Frankfurt (please contact melanie.dressler@kgu.de)

**Declaration of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors alone are responsible for the content and writing of the paper.

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**Authors' contributions**

MD contributed to the conception and design, participated in the data acquisition, contributed to the analysis and interpretation of the data and drafted the article.

DF contributed to lab analyses, drafted labor parts of the article and critically revised the article.

LB contributed to the data acquisition and critical revision of the article.

EH contributed to the sample size calculation and critical revision of the article.
NB contributed to the data acquisition, the lab analyses and critical revision of the article.

MT contributed to the lab analyses.

JS contributed to the conception and design, participated in the data acquisition, analysis and interpretation and critically revised the article.

RS contributed to the conception and design participated in the data acquisition, analysis and interpretation, contributed to lab analyses and critically revised the article.

CB assembled the investigational product, contributed to lab analyses and critically revised the article.

SZ contributed to the conception and design, participated in the data acquisition, analysis and interpretation and critically revised the article.

All authors approved the final version of the manuscript.

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Table 1 Supplement: Max. FEV\textsubscript{1} decrease and eNO before and after sc-LCPUFA or placebo supplementation

% pred, % predicted; ppb, parts per billion.

Table 2 Supplement: Fatty acid measurements in plasma and blood cells before and after sc-LCPUFA or placebo supplementation

Exact values for significant p-values:

EPA Plasma: sc-LCPUFA pre – sc-LCPUFA post: < 0.0001, sc-LCPUFA post – Placebo post: > 0.0001

EPA Blood cells: sc-LCPUFA pre – sc-LCPUFA post: < 0.0001, sc-LCPUFA post – Placebo post: > 0.0001

DHA Plasma: sc-PUFA post – Placebo post: 0.004, Placebo pre – Placebo post: 0.0003

AA Plasma: sc-LCPUFA pre – sc-LCPUFA post: 0.037, Placebo pre – Placebo post: 0.014

Table

Table 1: Patient characteristics
| Subjects                  | [n] | Total | Adult | Children | Placebo |
|--------------------------|-----|-------|-------|----------|---------|
| Female/Male              | [n] |       |       |          |         |
| Age                      | [years] | 19.0 ± 5.5 | 23.5 ± 3.0 | 13.8 ± 2.2 | 18.4 |
| Weight                   | [kg] | 61.0 ± 14.0 | 67.6 ± 12.4 | 53.6 ± 11.9 | 60.1 |
| Height                   | [m]  | 1.7 ± 0.1 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.7 |
| FVC                      | [% pred] | 96.6 | 94.0 | 98.8 | 94.0 |
|                          |      | 70.2 – 140.1 | 70.2 – 117.3 | 77.7 – 140.1 | 70.2 |
| FEV₁                     | [% pred] | 94.9 | 91.8 | 97.3 | 94.9 |
|                          |      | 64.4 – 118.4 | 64.4 – 115.2 | 69.6 – 118.4 | 64.4 |
| eNO                      | [ppb] | 27.5 | 27.5 | 28.5 | 27.5 |
|                          |      | (5.0 – 197.0) | (5.0 – 197.0) | (7.0 – 88.0) | (6.0) |
| ACQ                      |      | 0.7 | 0.7 | 0.8 | 0.7 |
|                          |      | (0.0 – 2.9) | (0.0 – 2.9) | (0.0 – 2.0) | (0.0) |

Normally distributed data mean ± SD; not normally distributed data and % values median and min/max; SD, standard deviation; n, number; yrs, years; m, meter; kg, kilogram; % pred, % predicted; ppb, parts per billion

Figures
Figure 1

Flowchart of study design with assessments
Max. FEV1 decrease and exhaled NO before and after sc-LCPUFA or placebo supplementation. Data FEV1 decrease a) and Total n = 64; b) Adult n = 34; c) Children n = 30) and eNO d;e;f; were presented as median, 25%/75% percentile and min/max. The p-values were calculated with the Mann Whitney test. Results were considered as statistically significant when p < 0.05. None of the results were significant. The exact values for median and 25%/75% percentile are presented separately in Table 1 see supplement.
EPA, DHA and AA in plasma and blood cells before and after sc-LCPUFA or placebo supplementation. Data ($n = 64$) were presented as mean ± SD. The exact values for mean ± SD are presented separately in Table 2 in the Supplement. Intra-group and inter-group comparisons were calculated by One-Way ANOVA with post-hoc Bonferroni analysis. Results were considered as statistically significant when $p < 0.05$ (**$p < 0.01$, *$p < 0.05$).
Frequency of the side effects “during the sc-LCPUFA or placebo supplementation The frequency of the side effects “salbutamol use”, “dyspnoe”, “bloating”, “belching” and “diarrhea” during the sc-LCPUFA or placebo supplementation are shown. Data were presented as median, 25%/75% percentile and min/max. The p-value was calculated with Mann Whitney test. Results were considered as statistically significant when p < 0.05. The frequency of the side effect “belching” showed a significant difference (p = 0.005) between the two groups, all the other side effects showed no significant difference between the interventional and placebo group.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Table 1 Supplement.docx
Table 2 Supplement.docx