Omega-3 polyunsaturated fatty acid supplementation improves postabsorptive and prandial protein metabolism in patients with Chronic Obstructive Pulmonary Disease: a Randomized Clinical Trial

Mariëlle P.K.J. Engelen[1], Renate Jonker[1], Hooriya Sulaiman[1], Helena L. Fisk[2], Philip C. Calder[2], and Nicolaas E.P. Deutz[1]

[1]Center for Translational Research in Aging & Longevity, Dept of Health and Kinesiology, Texas A&M University, College Station, TX, USA

[2]School of Human Development and Health, Faculty of Medicine, University of Southampton and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, United Kingdom

Corresponding author: Mariëlle PKJ Engelen, PhD, Center for Translational Research in Aging & Longevity. Dept. of Health and Kinesiology, Texas A&M University, College Station, TX. E-mail: mpkj.engelen@ctral.org

Short running head: PUFA supplementation and protein kinetics in COPD

Conflicts of Interest: The authors declare no conflicts of interest.

Sources of Support: Research reported in this publication was supported by the National Institutes of Health under grant numbers R01HL095903 and R56HL141744. “The content is

solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health."

Clinical Trial Registry: https://clinicaltrials.gov/ct2/show/NCT01624792

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval of the principal investigator.

LIST OF ABBREVIATIONS

AA: Amino acids

BCAA: Branched-chain amino acids

BMI: Body mass index

COPD: Chronic obstructive pulmonary disease

CRP: C-reactive protein

DHA: Docosahexaenoic acid

EAA: Essential amino acids

EPA: Eicosapentaenoic acid

FEV1: Forced expiratory volume in 1 second

FeNO: Exhaled nitric oxide

FFM: Fat-free mass

FVC: Forced vital capacity

HDL: High density lipoprotein

LC-MS/MS: liquid chromatography mass spectrometry

LDL: Low density lipoprotein

NEAA: Non-essential amino acids

PHE: Phenylalanine

PB: Protein breakdown

PS: Protein synthesis

PUFA: Polyunsaturated fatty acid

Ra: Rate of appearance

TTR: Tracer tracee ratio

TYR: Tyrosine

VLDL: Very low-density lipoprotein

WBP: Whole body production rate

ABSTRACT

Background: Disturbances in protein metabolism and impaired muscle health have been observed in Chronic Obstructive Pulmonary Disease (COPD). The omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known for their anti-inflammatory and muscle health enhancing properties.

Objectives: We examined whether daily EPA+DHA supplementation can improve daily protein homeostasis in patients with COPD by reducing postabsorptive whole-body protein breakdown (PB) and enhancing the anabolic response to feeding in a dose-dependent way.

Methods: Normal-weight subjects with moderate to severe COPD (n=32) received daily for 4-weeks, according to a randomized double-blind placebo controlled three-group design, a high dose (3.5 g, n=10), low dose (2.0 g, n=10) of EPA+DHA, or placebo (olive oil, n=12) via gel capsules. At pre- and post-intervention, stable isotope tracers were infused to assess postabsorptive netPB (postabsorptive PB- protein synthesis (PS)) and the anabolic response (prandial netPS=prandial PS-PB) to a protein meal. In addition, muscle mass and function were measured.

Results: Plasma phosphatidylcholine EPA and DHA concentrations were higher after 4 weeks of supplementation in both EPA+DHA groups ($p<0.004$), and there was a trend towards higher values for plasma EPA after the high versus low dose of EPA+DHA ($p=0.065$). Postabsorptive PB was lower after 4 weeks of the high dose of EPA+DHA, whereas netPB was lower independent of the dose of EPA+DHA (low dose ($p=0.037$), high dose ($p=0.026$)). Prandial

netPS was increased only after the high dose of EPA+DHA ($p=0.03$). Extremity lean mass but not muscle function was increased, independent of the EPA+DHA dose ($p<0.05$).

Conclusions: Daily n-3 PUFA supplementation for 4 weeks induces a shift towards a positive daily protein homeostasis in patients with COPD in part in a dose-dependent way. Daily doses up to 3.5 g of EPA and DHA are still well tolerated and lead to protein gain in these patients.

Key words: COPD, stable isotopes, PUFA, intervention, RCT, protein metabolism

Introduction

Early and effective nutritional intervention as part of the treatment program of patients with Chronic Obstructive Pulmonary Disease (COPD) is of clinical importance as impaired muscle health is associated with physical inactivity, cognitive decline, and increased hospital (re)admissions (1-6). We previously observed alterations in whole body protein and amino acid kinetics in patients with COPD which were linked to poor muscle health outcomes (1, 7-11)}(12). Systemic inflammation is recognized as an underlying factor contributing to muscle wasting and weakness in COPD (13). Approaches to reduce the systemic inflammatory response are therefore needed to restore protein homeostasis and improve muscle health outcomes in patients with COPD.

The omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), known for their anti-inflammatory properties (14), are of interest in COPD as the plasma EPA and DHA concentrations were found to be reduced (15). In normal weight COPD, daily intake of 1-2 g of n-3 PUFAs (as part of oral nutritional support (ONS)) for 5-12 weeks resulted in reduced symptoms after a 6-minute walk test (16) and enhanced exercise capacity (17) but inflammatory markers, muscle mass or function were not improved. In malnourished COPD, 1.2 g/day of n-3 PUFAs for 12 weeks (alongside low intensity exercise) resulted in increased muscle mass, strength, and functional performance (18) but no changes were found after 4 months of daily intake of 766 mg EPA+DHA in COPD (15). The conflicting data might be related to the variability in PUFA dose used. The dose of n-3 PUFAs required for effects on muscle health has been suggested to be around 2 g/day in chronic conditions (19), although the optimal daily dose and period of intervention remain unclear. Four weeks of 3.2 g/day of EPA+DHA resulted in an increase in EPA incorporation into white blood

cell phospholipids to levels of 2.5% of total lipids (20), and an elevation in incorporation was already been observed after 1 week of intake (21). Incorporation of EPA and DHA into the phospholipid membrane of skeletal muscle cells has been linked to an upregulated activity of cell signaling pathways known to control the remodeling of muscle tissue (22, 23) via protein synthesis (PS) and breakdown (PB). N-3 PUFA supplementation was able to increase postabsorptive muscle PS (24) and enhance the anabolic response to feeding in healthy older adults (25). Also, in cancer, 3.2 g of EPA +DHA/day as part of ONS resulted in some normalization of the metabolic response in both the fasted and fed states (26). (27, 28).

In the present study, we examined whether 4 weeks of daily EPA+DHA supplementation was able to improve daily protein homeostasis (27, 28), in a dose-dependent way in patients with COPD. We tested the hypothesis that daily intake of 2 g EPA+DHA for 4 weeks in patients with COPD increases the anabolic response to a high-quality protein meal (primary outcome), reduces postabsorptive whole body protein breakdown, and improves muscle health (mass and function as secondary outcomes), and that 3.5 g/day EPA+DHA improves whole body protein metabolism even further. This information is critical to further refine nutritional supplementation in COPD to enhance protein gain and ultimately restore progressive muscle wasting and dysfunction in these patients.

Subjects and methods

Study population

In the present study, 45 clinically stable patients with a diagnosis of COPD (grade II-IV) (29) were enrolled and 32 of them completed it (see **Figure 1** (Cohort diagram), **Table 1**). Recruitment took place through pulmonologist referral and *via* advertisements in the local community. We assessed medical history and medication use as part of the screening process and measured transcutaneous oxygen saturation using pulse oximetry. Exclusion criteria were pre-existent untreated metabolic or renal disease, malignancy, recent surgery, daily use of supplements containing > 1000 mg EPA+DHA in the 3 months prior to the first test day, use of protein or amino acid containing nutritional supplements within 5 days of first test day, indications related to interaction with study products, known allergy to milk or milk products or hypersensitivity to fish and/or shellfish, and use of long-term oral corticosteroids or a short course of oral corticosteroids 4 weeks preceding the first test day. Written informed consent was obtained, and the study was approved by the local Institutional Review Boards at University of Arkansas Medical Sciences and Texas A&M University. Period of recruitment and follow-up was October 2011-June 2016.

Study design

All subjects were screened by the study nurse or other trained research staff during the screening visit. Informed consent was obtained before any study related procedures were performed. All subjects were subsequently studied at the Clinical Research Unit of the Center for Translational Research in Aging and Longevity, Department of Geriatrics, University of Arkansas for Medical

Sciences, or Department of Health and Kinesiology, Texas A&M University. The study involved 2 test days (~ 9 hours per test day) and a 4-week intervention period in between.

Nutritional intervention

The 4-week nutritional intervention was performed according to a double-blind, randomized, placebo-controlled 3-group design and consisted of a) 3.5 g EPA+DHA per day (7 capsules of 1000 mg of Swanson EFAs superior essential fatty acids – Super EPA, consisting of 300 mg EPA, 200 mg DHA, 50 mg other omega-3 PUFAs per capsule) or b) 2 g EPA+DHA (4 capsules) + 3 g of olive oil per day (3 capsules of 1000 mg Swanson EFAs Certified Organic Extra Virgin Olive oil consisting of 9% palmitic acid, 66% oleic acid, 4.2% linoleic acid), or c) 7 g placebo per day (7 capsules of 1000 mg of olive oil). The total energy content provided was equal among all groups (10 cal/capsule). Two g of EPA+DHA daily was used as this is the commonly used dose in previous studies (30). The higher dose of 3.5 g of EPA+DHA daily has been approved by the Food and Drug Administration for lowering plasma triglyceride concentrations in hypertriglyceridemic subjects and has therefore previously been shown to be physiologically relevant in human subjects. As we previously observed in a pilot study that ~ 3-4 hours is needed to reach the highest plasma EPA and DHA concentrations after n-3 PUFA intake, we decided to provide 3 capsules together with breakfast and 4 capsules with lunch to increase the possibility to obtain an enhanced anabolic response to lunch and dinner, respectively. In the case of the low EPA+DHA group,1 olive oil and 2 EPA+DHA capsules were provided at breakfast and 2 olive oil and 2 EPA+DHA capsules at lunch. Capsules were provided in foil bag pouches. The pouches were sealed and labeled with “day nr (1-7) morning” or “day nr (1-7) afternoon” and week nr. Permuted block (6) randomization was done using randomizer.org. The randomization

sequence was generated by an independent researcher who had no further involvement in the conduct of the study and who closely monitored the intervention allocations to ensure protocols were being adhered to and balance was maintained. All details of the randomization were unknown to the investigators, collaborators, and study staff, except for the independent researcher and the supplies manager who needed to be unblinded to label the study products. The packaging of the test and control products was identical in appearance.

Pre- and post-intervention study visits

Anthropometrics, body composition, and lung function

Both study visits were identical and started in the postabsorptive state with body weight and height measurements by a digital beam scale and stadiometer, respectively. Whole body and extremity fat mass and lean mass were obtained by dual-energy X-ray absorptiometry (Hologic QDR 4500/ Version 12.7.3.1 (Bedford, MA)). Forced expiratory volume in 1 second (FEV1) and Forced Vital Capacity (FVC) were assessed with the highest value from ≥3 technically acceptable maneuvers being used (31). Peripheral arterial oxygen saturation was measured using a finger pulse oximeter while at rest.

Muscle function and Questionnaires

Respiratory muscle function (inspiratory pressure) was assessed by a hand-held mouth pressure device (Micro Respiratory Pressure Meter (RPM), MD spiro, Lewiston, ME). Peak handgrip force (Vernier dynamometry (Vernier software and Technology, Beaverton, OR) was used as a marker of muscle strength (32). Habitual physical activity level was assessed using the Physical Activity Scale for the Elderly questionnaire (PASE) (33). The modified Medical Research

Council dyspnea scale (mMRC) was used to assess the level of dyspnea, and Charlson index (34) for assessment of associated comorbidities. Quality of life was assessed by Saint George Respiratory Questionnaire (SGRQ-C) (35) and presented as symptoms, activity, impact and total scores.

Protein metabolism in the postabsorptive and prandial state

After an overnight fast **(Figure 2)**, one catheter was inserted in a peripheral vein of the lower arm for stable isotope tracer (Cambridge Isotope Laboratories (Woburn, MA, USA) primed constant infusion of phenylalanine (PHE-[ring-$^{2}H_{5}$] 0.61mg/kg/h, prime 0.37 mg/kg) and tyrosine (TYR-[3,5-$^{2}H_{2}$] or TYR- [U-$^{13}C_{9}$, ^{15}N]: 0.02 mg/kg/h, prime 0.02 mg/kg), and enteral TYR-[ring-$^{2}H_{4}$] prime was simultaneously provided (0.063 mg/kg). In addition, one line was placed in a superficial dorsal vein of the contralateral arm for blood sampling. The hand was placed in a thermostatically controlled hot box (internal temperature: 60°C), a technique to mimic direct arterial sampling (36). After baseline venous sampling at t=0 min (for analysis of baseline enrichment, concentrations of glucose, lipid profile, and the inflammatory parameter hs-CRP), followed by intake of a full dose of capsules according to the assigned group, arterialized-venous blood was drawn at 200, 220, 240 min (fasted state) and 380, 400, 420 min (fed state) for analysis of tracer enrichments and concentrations of amino acids. Three hours after the start of the infusion, all subjects received every 20 minutes for 3 hours a mixture of 0.06 g/kg ffm high quality hydrolyzed casein protein as 1 ml sips (obtained from a batch consisting of 240 ml water, 23.1 g hydrolyzed casein (CE90STL, DMV international, Veghel, The Netherlands) and 20.0 g of polycose (37, 38).

Biochemical analysis and calculations of metabolic parameters

Arterialized-venous blood was put in Li-heparinized or EDTA tubes, immediately put on ice, and centrifuged (4°C, 3120 x *g* for 5 min) to obtain plasma. Plasma was aliquoted with either 0.1 vol of 33% (w/w) trichloroacetic acid or its residue after evaporation of 0.17 vol of 33% (w/w) 5-sulfosalicylic dihydrate, and stored at -80°C. Tracer enrichments [tracer:tracee ratio (TTR)] and amino acid concentrations were analyzed batch-wise by LC-MS/MS by isotope dilution (39).

The rates of appearance (Ra) of PHE and TYR were calculated to measure whole body production (WBP) in the postabsorptive state from the last hour of the primed constant infusion period before the feeding period started, as Ra = tracer infusion rate/median TTR, and the interconversion as a marker of net protein breakdown (40). The conversion of phenylalanine into tyrosine was calculated by using Ra of the product amino acid and the ratio between the TTR at plateau. In the fed state, whole body protein breakdown, synthesis, net protein synthesis (netPS, protein synthesis - protein breakdown) were calculated from the plasma isotope enrichment from the last hour of the prandial state, using previously described equations (41). We determined plasma hs-CRP and glucose concentrations using a COBAS c111 semi-automatic analyzer (Gluc2 Kit; Roche Diagnostics®).

To determine the plasma phosphatidylcholine fatty acid profile, total lipid was first extracted into chloroform-methanol (2:1 v/v). Then, phosphatidylcholine was isolated from the lipid extract by solid phase extraction on aminopropyl silica SPE cartridges (42). Fatty acid methyl esters were formed by incubation of the isolated phosphatidylcholine with methanol containing 2% (v/v) sulfuric acid at 50ºC for 2 hours. The fatty acid methyl esters were extracted into hexane and then resolved by gas chromatography on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 fused silica capillary column. Run conditions were as

184 described elsewhere (42). Fatty acid methyl esters were detected by a flame ionization detector.
185 Each fatty acid was expressed as a % of total fatty acids measured in the sample. Plasma
186 concentrations of high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and
187 low-density lipoprotein (LDL) cholesterol, and triglycerides were analyzed by Labcorp.

188 **Statistical analysis**

189 Calculations of protein kinetics were done for each subject using a horizontal regression line
190 robust fitting procedure using measurements taken at 200, 220, and 240 min for the
191 postabsorptive phase, and 380, 400, and 420 min for the prandial phase.
192 Analysis of covariance (ANCOVA), using multiple linear regression with if needed weighing to
193 obtain homoscedasticity and log transformation to obtain a normal distribution of the residuals
194 (43), was applied to compare the 3 groups on each dependent variable, including the primary
195 outcome (post-intervention anabolic response to the meal) and the secondary outcomes (post-
196 intervention whole body production rates (in the postabsorptive and prandial state), body
197 composition, lab clinical characteristics, muscle function, physical activity, quality of life, and
198 plasma amino acid concentrations. Independent variables included intake of 2.0 g EPA+DHA
199 (0=no, 1=yes) and 3.5 g EPA+DHA (0=no, 1=yes) as dummy variables, baseline value of the
200 dependent variable, and BMI (not in case of body composition measurements), The level of
201 significance was set at $P<0.05$ as compared to placebo or low (2.0 g) vs high (3.5 g) dose of
202 EPA+DHA, and Graphpad Prism (version 9.3) was used for data analysis.
203 As we used ANCOVA to estimate the effect of EPA+DHA supplementation on the
204 primary outcome (anabolic response to a protein meal), we adjusted the power calculation by
205 using a previously calculated correlation coefficient of 0.8 between pre- and post measurements
206 (44). Our previous study in COPD showed an anabolic response to a protein meal (net protein

207 synthesis) of 226 (SD: 42) μmol/kg ffm/min (45). An anticipated 10% increase in net protein
208 synthesis was used to calculate the estimated sample size per group (independent samples,
209 power: 0.80, α: 0.05 (46)). We calculated a sample size of 11.1 subjects per group.
210

Results

Population characteristics

45 patients with COPD (24 m/21 f) were enrolled in the study **(Figure 1)**. The 5 screening failures and 8 dropouts (17%) were equally distributed among the groups. The dropouts were due to factors predominantly unrelated to PUFA intake: 2 subjects were no longer meeting the inclusion criteria, 3 were early discontinuation (2: difficulty with IV catheter, 1: deteriorating health (kidney stones)), 1 was wrongfully enrolled (using fish oil at home), and 2 were voluntary withdrawal/changed their mind or no specific reason given.

The 32 patients who completed the study were characterized by moderate to very severe airflow obstruction and dyspnea **(Table 1)**. Their average exacerbation rate in the preceding year was 0.84 and hospitalization rate was 0.26. They were on average normal to overweight (BMI: 27.6 (2.10) kg/m^2) and characterized by slightly elevated fasting glucose levels (6.0 (0.5)) mmol/L) and low-grade systemic inflammation (CRP: 4.5 (3.5) mg/L).

Effects of 4 weeks of EPA+DHA supplementation

A few side effects were present in 25% of the study group: n = 3 in the high EPA+DHA group (gas, fishy taste, diarrhea), n = 3 in the low EPA+DHA group (dry mouth, diarrhea, indigestion), and n = 2 in the placebo group (upset stomach, diarrhea). 18% of patients missed 1 or 2 doses over the course of the study.

Analysis of the plasma phosphatidylcholine fatty acid profile **(Table 2)** revealed a 4.5-fold increase for EPA and 1.7-fold increase for DHA ($p=0.0001$) after low EPA+DHA supplementation, and a 5.8-fold increase for EPA and 1.9-fold increase for DHA ($p<0.0001$) after high EPA+DHA supplementation. This increase in n-3 PUFAs was accompanied by a

decrease in the n-6 PUFAs dihomo-gamma-linolenic acid (20:3n-6). Linoleic acid (18:2n-6) and Arachidonic acid (20:4n-6) decreased only after intake of the high EPA+DHA dose ($p<0.05$). The placebo supplementation had no significant effect on the plasma phosphatidylcholine fatty acids. The single dose of capsules at the start of both study days (t=0) did not change the plasma phosphatidylcholine fatty acid concentration of EPA and DHA after 4 and 8 hours of intake for any of the 3 groups (**Supplementary Figure 1**), suggesting no acute response to the EPA+DHA intake.

Clinical characteristics, body composition

No significant differences were observed after low or high EPA+DHA intervention as compared to placebo for exhaled NO, blood pressure (systolic and diastolic), plasma hs-CRP, and plasma glucose and total cholesterol concentrations (**Table 3**). The plasma triglycerides and VLDL cholesterol (Table 3) were decreased in the low EPA+DHA group ($p<0.003$), while other plasma lipid measurements were not significantly affected. Lean mass in the extremities was significantly increased after 4 weeks of EPA+DHA, independent of the dose (Table 3). Total lean mass was also increased after the high dose of EPA+DHA intake as compared to the placebo ($p=0.030$). Extremity lean mass was increased after 4 weeks of EPA+DHA independent of the EPA+DHA dose (<0.03).

Muscle function, physical activity, quality of life, and habitual dietary intake

Respiratory and handgrip strength were not changed by the EPA+DHA intervention. Quality of life (impact score) was improved in the low EPA+DHA group ($p=0.033$) as compared to the placebo group (**Table 4**).

255 ***Plasma amino acid concentrations in the postabsorptive state***

256 The plasma amino acid profile was relatively unaffected after 4 weeks of EPA+DHA
257 supplementation as only a few changes were observed (**Table 5**). For instance, plasma glutamate
258 was higher and hydroxyproline lower after the high EPA+DHA intervention (p=0.02) as
259 compared to the low EPA+DHA. Tau methylhistidine concentration was higher after the high
260 EPA+DHA dose as compared to the low dose of EPA+DHA and the placebo (p=0.012). No
261 significant differences were found in sum of BCAA, EAA, NEAA, or AA plasma
262 concentrations.

263 ***Whole body protein metabolism in the postabsorptive and prandial states***

264 Postabsorptive phenylalanine production (marker of protein turnover) was lower after the high
265 EPA+DHA as compared to placebo supplementation (p=0.026) (**Table 6**). Moreover, the
266 phenylalanine to tyrosine conversion (marker of postabsorptive net protein breakdown) (**Figure**
267 **3, 4, Supplementary Figure 1**) was 10% (p=0.037) and 13% (p=0.026) lower after 4 weeks of
268 low and high EPA+DHA, respectively, with no difference between the high vs. low dose of
269 EPA+DHA groups. Prandial netPS was 15% (p=0.031) higher after 4 weeks of high dose
270 EPA+DHA but not different after the low dose of EPA+DHA.

271

272

Discussion

In the present RCT, the daily low (2.0 g) and high (3.5 g) doses of n-3 fatty acids for 4 weeks were able to reduce postabsorptive net protein breakdown, whereas enhanced meal-induced net protein anabolism was found after the high dose, indicating a positive shift in daily protein homeostasis in patients with COPD. The studied group was normal to overweight, and characterized by preserved muscle mass, reduced glucose tolerance, low-grade systemic inflammation, and muscle weakness (1). A high compliance in intake of the supplements was observed (82% of all doses were taken), as also previously observed in COPD (16). In line with this, plasma concentration of phosphatidylcholine EPA increased 4.5 to 5.8 times and DHA 1.7 to 1.9 times after low and high EPA+DHA supplementation, respectively. Still no differences were observed in exhaled NO, blood pressure, plasma hs-CRP, glucose, and total cholesterol concentrations after either low or high EPA+DHA intervention as compared to placebo.

We measured fatty acids in plasma phosphatidylcholine, the main circulating phospholipid. Mean baseline values for EPA in the three groups were 0.75, 0.75 and 0.79% and for DHA they were 2.25, 2.66 and 2.80%. Hodson et al. (47) combined data for EPA and DHA from multiple studies, mainly in healthy subjects. For total plasma (combination of triglycerides, phospholipids, cholesteryl esters and non-esterified fatty acids) they identified average values from 9 studies for EPA and DHA of 1.4 and 2.4%, respectively. For plasma phospholipids they identified average values from 16 studies for EPA and DHA of 1.0 and 3.3% respectively. Previously in COPD, EPA and DHA in total plasma were around 1.2 and 2.3%, respectively (16). The current results are in general accordance with these previous reports for EPA and DHA. The ideal choice of placebo oil for studies of n-3 PUFAs is unclear and other studies have usually used olive oil, maize oil, sunflower oil or medium-chain triglycerides. We used an olive

oil which contained 66% oleic acid. At an oil dose of 7 g/day this would provide 4.62 g of oleic acid daily. Oleic acid is very prevalent in the US diet, being a common constituent of many vegetable oils (olive oil, high-oleic sunflower oil, canola oil), and also many animal fats. Data from NHANES 2007-2014 indicates an average daily intake of oleic acid amongst US adults of 27 g (48). Thus, the amount of oleic acid provided in the placebo is about 17% of typical daily intake which we consider to be modest. Our choice of olive oil as placebo is supported by the fact that there were no changes in fatty acid composition seen in the placebo group.

Reduced postabsorptive protein breakdown and increased anabolic response to feeding after EPA+DHA supplementation. The low and high doses of EPA+DHA were able to reduce postabsorptive net protein breakdown in COPD. The plasma amino acid profile remained relatively unchanged as only plasma concentration of hydroxyproline was lower, and glutamate and tau methylhistidine higher after intake of the high versus the low EPA+DHA dose. No differences were observed in plasma BCAA, EAA, or NEAA. We observed an enhancing effect on meal-induced protein anabolism by the high dose (3.5 g) of EPA+DHA only. Our results seem to be in accordance to previous studies in which eight weeks of n-3 PUFA supplementation (1.86 g EPA and 1.50 g DHA daily) was able to increase muscle protein synthesis rates during a hyperaminoacidemic-hyperinsulinemic clamp procedure in young, middle-aged, and older adults (25, 49). Basal response of muscle protein synthesis was not modulated by n-3 PUFAs but the anabolic response was enhanced by 30–60% following n-3 PUFA supplementation. The proposed working mechanism was an increased phosphorylation status of the intramuscular cell signaling proteins, known to upregulate muscle protein synthesis, (e.g., mTORC1-p70S6k1 pathway) (25). In vitro studies showed that EPA, rather than DHA, is the active n-3 PUFA in upregulating muscle protein synthesis in response to an anabolic (leucine) stimulus (50).

Reducing inflammation using non-steroidal anti-inflammatory drugs was able to improve postprandial protein synthesis (51)(18) No significant changes were observed in hs-CRP after 4 weeks of EPA+DHA intervention in the currently study, in line with previous studies in COPD (16) and healthy older adults (24) as no changes were observed in hs-CRP, IL-6, IL-8, or TNF (25, 52). However, at the muscle level, there was downregulation of inflammation-related genes (24), suggesting that n-3 PUFAs may modulate local tissue inflammation without influencing circulating inflammatory markers, and that the anabolic action of n-3 PUFAs is mediated via an indirect mechanism rather than exerting a direct anabolic effect. Whether the whole body anabolic response is a good representative of the muscle anabolic response after n-3 PUFA supplementation, and if EPA is mainly responsible for the enhanced anabolic effects to protein / amino acid feeding deserves further study. Recent studies (24, 53) examining the anabolic effects of 8 weeks of fish oil derived n-3 PUFAs (3.5 g/d EPA) to 30 g of whey protein, failed to show a change in muscle protein synthesis in trained young men (53). Our hypothesis is that when anabolism is already maximally stimulated, n-3 PUFA intake will not be able to further increase the anabolic response. N-3 PUFA supplementation therefore may benefit chronic wasting diseases by improving protein anabolism particularly when habitual dietary protein intake is low.

Systemic health effects after EPA+DHA supplementation. Previous experimental research has shown that n-3 PUFAs are able to improve muscle maintenance by modulating systemic inflammation and NF-kB (54). We observed that the positive effect of EPA+DHA supplementation on overall protein balance was associated with an increase in muscle mass in the extremities, independent of the dose, but no change in fat mass. Previously, n-3 PUFA supplementation was able to increase fatty acid availability and oxidation in COPD which could imply a stimulating effect on muscle oxidative metabolism of fat (17). However, we were not

342 able to study the potential positive effects on muscle endurance or exercise capacity. No
343 differences were observed in handgrip strength, but our study was not adequately powered to
344 detect a statistical difference in muscle strength, and the intervention period of 4 weeks might be
345 too short to see such an effect (55). To improve functional performance, incorporation of
346 exercise training is likely needed during n-3 PUFA supplementation as an enhanced anabolic
347 response to exercise was observed in older adult women (56). In line with this, following 16
348 weeks of 3.9 g/day of n-3 PUFA supplementation in healthy subjects (24), postabsorptive mixed
349 muscle protein synthesis rates were increased in older adults, and mitochondrial and myofibrillar
350 protein synthesis were elevated (15-18 hr) following a bout of resistance exercise. The enhanced
351 anabolic response to exercise following n-3 PUFAs was attributed to transcriptional changes and
352 changes to the activation of anabolic signaling proteins in skeletal muscle as lower levels of
353 postexercise myostatin, a negative regulator of muscle growth and differentiation, and altered
354 phosphorylation of signaling transduction proteins in muscle such as mTOR and p70s6k (25)
355 have been found. Further research is required to what extent n-3 PUFAs are also able to improve
356 the anabolic responsiveness to exercise in COPD, and to characterize the COPD subjects that are
357 not able to increase their anabolic response to feeding after n-3 PUFA supplementation (57)(24).
358 Future studies are also needed in COPD to determine if the observed adaptations translate into
359 meaningful improvements in physical function when n-3 PUFAs are given over longer periods of
360 time or in combination with an exercise training program.
361 **Limitations.** The number of subjects per group was small and the results might not be
362 applicable to the general COPD population as the participants were normal to overweight with
363 preserved muscle mass, and the group who might benefit the most from n-3 PUFA support,
364 COPD patients with impaired muscle health and exercise performance, were not specifically

included. Data on the anabolic response to a high-quality protein was provided so it remains unclear whether the enhanced anabolic response would also be found with lower quality proteins. Furthermore, 71% of the randomized subjects completed the intervention and were included in the analysis, which was mainly due to factors unrelated to PUFA intake or disease changes. We realize that missing data for outcomes as well as associated prognostic covariates may create the potential for bias due to the analysis being based on completers rather than intent-to-treat.

Conclusion. Daily oral intake of n-3 PUFAs (EPA+DHA) for 4 weeks is able to improve postabsorptive protein metabolism and enhance the anabolic response to feeding in patients with COPD in part in a dose-dependent way, indicating a more positive daily protein homeostasis. High (up to 3.5 g daily) EPA+DHA levels are well tolerated and lead to protein gain in patients with COPD even after a short period (4 weeks) of supplementation. This information is critical to further refine nutritional supplementation in COPD to improve muscle health and daily functioning particularly in those at risk for reduced protein intake.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We thank the patients for their willingness to participate in this research study and who have made this work possible. Furthermore, we thank the CTRAL research personnel for assisting in the data collection.

386 **Author Contributions**

387 MPKJE, RJ, PCC, and NEPD had full access to all data in the study and took responsibility for
388 the integrity of the data and the accuracy of the data analysis. MPKJE, RJ, and NEPD designed
389 the research and were involved in the recruitment of subjects and conduct of the study. MPKJE,
390 RJ and NEPD were involved in the data analysis, sample analysis, and writing of the manuscript.
391 HLF and PCC were involved in sample analysis, writing, and reviewing of the manuscript. All
392 authors have read and approved the final version of the manuscript.

References

1. Cruthirds CL, van der Meij BS, Wierzchowska-McNew A, Deutz NEP, Engelen M. Presence or Absence of Skeletal Muscle Dysfunction in Chronic Obstructive Pulmonary Disease is Associated With Distinct Phenotypes. Arch Bronconeumol (Engl Ed). 2021;57(4):264-72.
2. Demeyer H, Burtin C, Hornikx M, Camillo CA, Van Remoortel H, Langer D, et al. The Minimal Important Difference in Physical Activity in Patients with COPD. PLoS One. 2016;11(4):e0154587.
3. Waschki B, Kirsten AM, Holz O, Mueller KC, Schaper M, Sack AL, et al. Disease Progression and Changes in Physical Activity in Patients with Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2015;192(3):295-306.
4. Katajisto M, Koskela J, Lindqvist A, Kilpelainen M, Laitinen T. Physical activity in COPD patients decreases short-acting bronchodilator use and the number of exacerbations. Respiratory medicine. 2015;109(10):1320-5.
5. Meshe OF, Claydon LS, Bungay H, Andrew S. The relationship between physical activity and health status in patients with chronic obstructive pulmonary disease following pulmonary rehabilitation. Disabil Rehabil. 2017;39(8):746-56.
6. Deutz NE, Ziegler TR, Matheson EM, Matarese LE, Tappenden KA, Baggs GE, et al. Reduced mortality risk in malnourished hospitalized older adult patients with COPD treated with a specialized oral nutritional supplement: Sub-group analysis of the NOURISH study. Clin Nutr. 2021;40(3):1388-95.
7. Engelen MP, Wouters EF, Deutz NE, Menheere PP, Schols AM. Factors contributing to alterations in skeletal muscle and plasma amino acid profiles in patients with chronic obstructive pulmonary disease. Am J Clin Nutr. 2000;72(6):1480-7.
8. Engelen MP, Deutz NE, Wouters EF, Schols AM. Enhanced levels of whole-body protein turnover in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2000;162(4 Pt 1):1488-92.

9. Engelen MP, Deutz NE, Mostert R, Wouters EF, Schols AM. Response of whole-body protein and urea turnover to exercise differs between patients with chronic obstructive pulmonary disease with and without emphysema. Am J Clin Nutr. 2003;77(4):868-74.

10. Rutten EP, Franssen FM, Engelen MP, Wouters EF, Deutz NE, Schols AM. Greater whole-body myofibrillar protein breakdown in cachectic patients with chronic obstructive pulmonary disease. Am J Clin Nutr. 2006;83(4):829-34.

11. Jonker R, Deutz NE, Erbland ML, Anderson PJ, Engelen MP. Alterations in whole-body arginine metabolism in chronic obstructive pulmonary disease. Am J Clin Nutr. 2016;103(6):1458-64.

12. Engelen M, Jonker R, Thaden JJ, Ten Have GAM, Jeon MS, Dasarathy S, et al. Comprehensive metabolic flux analysis to explain skeletal muscle weakness in COPD. Clin Nutr. 2020;39(10):3056-65.

13. Dalle S, Koppo K. Is inflammatory signaling involved in disease-related muscle wasting? Evidence from osteoarthritis, chronic obstructive pulmonary disease and type II diabetes. Experimental gerontology. 2020;137:110964.

14. Calder PC. n-3 PUFA and inflammation: from membrane to nucleus and from bench to bedside. The Proceedings of the Nutrition Society. 2020:1-13.

15. van de Bool C, Rutten EPA, van Helvoort A, Franssen FME, Wouters EFM, Schols A. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. Journal of cachexia, sarcopenia and muscle. 2017;8(5):748-58.

16. Calder PC, Laviano A, Lonnqvist F, Muscaritoli M, Ohlander M, Schols A. Targeted medical nutrition for cachexia in chronic obstructive pulmonary disease: a randomized, controlled trial. Journal of cachexia, sarcopenia and muscle. 2018;9(1):28-40.

17. Broekhuizen R, Wouters EF, Creutzberg EC, Weling-Scheepers CA, Schols AM. Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. Thorax. 2005;60(5):376-82.

18. Sugawara K, Takahashi H, Kasai C, Kiyokawa N, Watanabe T, Fujii S, et al. Effects of nutritional supplementation combined with low-intensity exercise in malnourished patients with COPD. Respiratory medicine. 2010;104(12):1883-9.

19. de van der Schueren MAE, Laviano A, Blanchard H, Jourdan M, Arends J, Baracos VE. Systematic review and meta-analysis of the evidence for oral nutritional intervention on nutritional and clinical outcomes during chemo(radio)therapy: current evidence and guidance for design of future trials. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2018;29(5):1141-53.

20. Yaqoob P, Pala HS, Cortina-Borja M, Newsholme EA, Calder PC. Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. Eur J Clin Invest. 2000;30(3):260-74.

21. Faber J, Berkhout M, Fiedler U, Avlar M, Witteman BJ, Vos AP, et al. Rapid EPA and DHA incorporation and reduced PGE2 levels after one week intervention with a medical food in cancer patients receiving radiotherapy, a randomized trial. Clin Nutr. 2013;32(3):338-45.

22. Gerling CJ, Mukai K, Chabowski A, Heigenhauser GJF, Holloway GP, Spriet LL, et al. Incorporation of Omega-3 Fatty Acids Into Human Skeletal Muscle Sarcolemmal and Mitochondrial Membranes Following 12 Weeks of Fish Oil Supplementation. Front Physiol. 2019;10:348.

23. McGlory C, Galloway SD, Hamilton DL, McClintock C, Breen L, Dick JR, et al. Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. Prostaglandins Leukot Essent Fatty Acids. 2014;90(6):199-206.

24. Lalia AZ, Dasari S, Robinson MM, Abid H, Morse DM, Klaus KA, et al. Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. Aging. 2017;9(4):1096-129.

25. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr. 2011;93(2):402-12.

26. Barber MD, McMillan DC, Preston T, Ross JA, Fearon KC. Metabolic response to feeding in weight-losing pancreatic cancer patients and its modulation by a fish-oil-enriched nutritional supplement. Clin Sci (Lond). 2000;98(4):389-99.

27. Millward DJ, Price GM, Pacy PJH, Quevedo RM, Halliday D. The Nutritional Sensitivity of the Diurnal Cycling of Body Protein Enables Protein Deposition to Be Measured in Subjects at Nitrogen Equilibrium. Clinical Nutrition. 1991;10(5):239-44.

28. Millward DJ, Pacy PJ. Postprandial protein utilization and protein quality assessment in man. Clin Sci (Lond). 1995;88(6):597-606.

29. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary. Am J Respir Crit Care Med. 2017;195(5):557-82.

30. van der Meij BS, Mazurak VC. Fish oil supplementation and maintaining muscle mass in chronic disease: state of the evidence. Curr Opin Clin Nutr Metab Care. 2020;23(3):164-73.

31. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med. 1999;159(1):179-87.

32. Engelen M, Klimberg VS, Allasia A, Deutz NE. Presence of early stage cancer does not impair the early protein metabolic response to major surgery. Journal of cachexia, sarcopenia and muscle. 2017;8(3):447-56.

33. Washburn RA, Smith KW, Jette AM, Janney CA. The Physical Activity Scale for the Elderly (PASE): development and evaluation. Journal of clinical epidemiology. 1993;46(2):153-62.

34. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of clinical epidemiology. 1994;47(11):1245-51.

35. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. The American review of respiratory disease. 1992;145(6):1321-7.

36. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metabolism: clinical and experimental. 1981;30(9):936-40.

37. Jonker R, Deutz NEP, Schols A, Veley EA, Harrykissoon R, Zachria AJ, et al. Whole body protein anabolism in COPD patients and healthy older adults is not enhanced by adding either carbohydrates or leucine to a serving of protein. Clin Nutr. 2019;38(4):1684-91.

38. Jonker R, Deutz NE, Erbland ML, Anderson PJ, Engelen MP. Hydrolyzed casein and whey protein meals comparably stimulate net whole-body protein synthesis in COPD patients with nutritional depletion without an additional effect of leucine co-ingestion. Clin Nutr. 2014;33(2):211-20.

39. Deutz NEP, Thaden JJ, Ten Have GAM, Walker DK, Engelen M. Metabolic phenotyping using kinetic measurements in young and older healthy adults. Metabolism: clinical and experimental. 2018;78:167-78.

40. Engelen MP, Rutten EP, De Castro CL, Wouters EF, Schols AM, Deutz NE. Supplementation of soy protein with branched-chain amino acids alters protein metabolism in healthy elderly and even more in patients with chronic obstructive pulmonary disease. Am J Clin Nutr. 2007;85(2):431-9.

41. Jonker R, Deutz NEP, Ligthart-Melis GC, Zachria AJ, Veley EA, Harrykissoon R, et al. Preserved anabolic threshold and capacity as estimated by a novel stable tracer approach suggests no anabolic resistance or increased requirements in weight stable COPD patients. Clin Nutr. 2019;38(4):1833-43.

42. Fisk HL, West AL, Childs CE, Burdge GC, Calder PC. The use of gas chromatography to analyze compositional changes of fatty acids in rat liver tissue during pregnancy. JoVE (Journal of Visualized Experiments). 2014(85):e51445.

43. Rosopa PJ, Schaffer MM, Schroeder AN. Managing heteroscedasticity in general linear models. Psychol Methods. 2013;18(3):335-51.

44. Borm GF, Fransen J, Lemmens WA. A simple sample size formula for analysis of covariance in randomized clinical trials. Journal of clinical epidemiology. 2007;60(12):1234-8.

45. Engelen MP, Rutten EP, De Castro CL, Wouters EF, Schols AM, Deutz NE. Altered interorgan response to feeding in patients with chronic obstructive pulmonary disease. Am J Clin Nutr. 2005;82(2):366-72.

46. Lenth RV. Java Applets for Power and Sample Size [Computer software]. Retrieved April 1st 2009 from http://www.stat.uiowa.edu/~rlenth/Power. May 2007 ed2009.

47. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Progress in lipid research. 2008;47(5):348-80.

48. Raatz SK, Conrad Z, Jahns L, Belury MA, Picklo MJ. Modeled replacement of traditional soybean and canola oil with high-oleic varieties increases monounsaturated fatty acid and reduces both saturated fatty acid and polyunsaturated fatty acid intake in the US adult population. Am J Clin Nutr. 2018;108(3):594-602.

49. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. Clin Sci (Lond). 2011;121(6):267-78.

50. Kamolrat T, Gray SR. The effect of eicosapentaenoic and docosahexaenoic acid on protein synthesis and breakdown in murine C2C12 myotubes. Biochem Biophys Res Commun. 2013;432(4):593-8.

51. Rieu I, Magne H, Savary-Auzeloux I, Averous J, Bos C, Peyron MA, et al. Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats. The Journal of physiology. 2009;587(Pt 22):5483-92.

52. Da Boit M, Sibson R, Sivasubramaniam S, Meakin JR, Greig CA, Aspden RM, et al. Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial. Am J Clin Nutr. 2017;105(1):151-8.

53. McGlory C, Wardle SL, Macnaughton LS, Witard OC, Scott F, Dick J, et al. Fish oil supplementation suppresses resistance exercise and feeding-induced increases in anabolic signaling without affecting myofibrillar protein synthesis in young men. Physiol Rep. 2016;4(6):e12715.

54. Komatsu W, Ishihara K, Murata M, Saito H, Shinohara K. Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-gamma plus lipopolysaccharide-

stimulated murine macrophages by inhibiting the oxidative stress. Free Radic Biol Med. 2003;34(8):1006-16.

55. Smith GI, Julliand S, Reeds DN, Sinacore DR, Klein S, Mittendorfer B. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. Am J Clin Nutr. 2015;102(1):115-22.

56. Rodacki CL, Rodacki AL, Pereira G, Naliwaiko K, Coelho I, Pequito D, et al. Fish-oil supplementation enhances the effects of strength training in elderly women. Am J Clin Nutr. 2012;95(2):428-36.

57. Lalia AZ, Johnson ML, Jensen MD, Hames KC, Port JD, Lanza IR. Effects of Dietary n-3 Fatty Acids on Hepatic and Peripheral Insulin Sensitivity in Insulin-Resistant Humans. Diabetes care. 2015;38(7):1228-37.

Legends

FIGURE 1: Participant flow through the study

FIGURE 2: Overview of study design to measure protein metabolism in the postabsorptive and prandial states

FIGURE 3: Predicted % difference from placebo (with 95% CI) (n=10) for lean mass extremities, postabsorptive rates of appearances (Ra) of phenylalanine and tyrosine, net protein breakdown, prandial rate of appearance of phenylalanine and net protein synthesis at 4 weeks of supplementation of 2.0 g/day eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (circles, n=10) versus 3.5 g/day EPA+DHA (squares, n=12). Closed circles or squares means statistical significance ($p<0.05$) was obtained.

FIGURE 4: Predicted means with 95% CI of postabsorptive net protein breakdown (left panel) and net protein synthesis during feeding (protein anabolism; right panel) after 4 weeks of intake of Placebo (dark gray bar, n=10), 2.0 g/day eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (light gray bar, n=10) and 3.5 g/day EPA+DHA (white bar, n=12). Analysis of covariance, using multiple linear regression, was applied to compare the 3 groups with intake of low EPA+DHA dose (0=no, 1=yes) and high EPA+DHA dose (0=no, 1=yes) as dummy variables, and baseline value of the dependent variable and BMI as covariates.

Table 1 - Disease characteristics of the COPD groups

	Total COPD group (n=32)	**COPD Placebo (n=10)**	**COPD low EPA+DHA (n=10)**	**COPD high EPA+DHA (n=12)**
Age (years)	66.79 (4.35)	62.10 (11.09)	70.70 (7.85)	67.58 (7.48)
Sex (female/male)	14/18	3/7	5/5	6/6
BMI (kg/m^2)	27.58 (2.10)	25.31 (5.30)	27.95 (4.30)	29.47 (6.76)
GOLD stage	2.88 (0.04)	2.90 (0.88)	2.90 (0.99)	2.83 (0.58)
FEV1 (%pred)	42.27 (1.66)	41.20 (16.52)	41.40 (16.62)	44.17 (12.09)
FEV1/FVC (%)	49.18 (6.94)	41.17 (11.57)	53.29 (14.17)	53.10 (11.03)
Transcutaneous O2 saturation (%)	94.93 (0.51)	94.80 (3.12)	95.50 (2.51)	94.50 (1.78)
Waist circumference (cm)	97.67 (5.62)	91.50 (14.39)	99.00 (10.00)	102.5 (10.61)
COPD symptoms (yr)	11.30 (1.08)	10.40 (5.86)	12.50 (7.63)	11.00 (6.81)
COPD exacerbations past year (nr)	0.84 (0.25)	0.60 (0.52)	1.10 (1.29)	0.83 (0.94)
COPD hospitalization for exacerbation past year (nr)	0.26 (0.21)	0.10 (0.32)	0.50 (0.71)	0.17 (0.39)

Dyspnea scale (score)	2.25 (0.43)	2.67 (0.82)	2.29 (0.76)	1.80 (0.79)
Plasma glucose (mmol/L)	5.98 (0.52)	5.61 (0.77)	5.74 (0.76)	6.57 (1.18)
Plasma C-reactive protein (mg/L)	4.50 (3.51)	1.98 (1.59)	3.00 (1.60)	5.51 (4.65)

Values are mean (SD) except for Sex (number of subjects).

Table 2 - Plasma phosphatidylcholine fatty acids of the COPD groups before and at the end of 4 weeks of low vs high EPA+DHA supplementation as compared to placebo

Fatty Acid	**COPD Placebo (n=10)**			**COPD low EPA+DHA (n=10)**			**COPD high EPA+DHA (n=12)**		
	Baseline	4 weeks	p	Baseline	4 weeks	p	Baseline	4 weeks	p
16:1n-7 (Palmitoleic acid)	0.514 [0.341, 0.687]	0.464 [0.402, 0.526]	0.759	0.611 [0.452, 0.770]	0.444 [0.314, 0.574]	**0.048**	0.553 [0.355, 0.740]	0.540 [0.324, 0.755]	0.838
18:1n-7	1.300 [1.115, 1.485]	1.314 [1.151, 1.478]	0.995	1.342 [1.174, 1.510]	1.289 [1.150, 1.428]	0.966	1.373 [1.223, 1.523]	1.266 [1.150, 1.383]	0.309
18:1n-9 (Oleic acid)	9.678 [8.853, 10.502]	9.068 [8.396, 9.740]	0.486	9.640 [8.554, 10.725]	8.747 [8.169, 9.324]	0.176	9.489 [8.305, 10.673]	8.223 [7.282, 9.164]	**0.028**
18:2n-6 (Linoleic acid)	23.889 [21.273, 26.505]	23.954 [21.226, 26.682]	>0.999	22.870 [19.791, 25.950]	21.801 [19.407, 24.195]	0.633	22.179 [19.529, 24.830]	19.590 [17.646, 21.534]	**0.045**
18:3n-3 (α-Linolenic acid (ALA))	0.276 [0.203, 0.350]	0.232 [0.184, 0.281]	0.567	0.232 [0.161, 0.303]	0.230 [0.174, 0.286]	0.999	0.253 0.173, 0.333]	0.225 [0.161, 0.289]	0.754

18:3n-6	0.117 [0.064, 0.169]	0.112 [0.076, 0.149]	0.998	0.112 [0.076, 0.149]	0.074 [0.039, 0.108]	0.119	0.111 [0.088, 0.134]	0.078 [0.057, 0.057]	0.191
20:1n-9	0.273 [0.175, 0.371]	0.247 [0.194, 0.300]	0.876	0.237 [0.197, 0.278]	0.224 [0.181, 0.267]	0.989	0.276 [0.204, 0.347]	0.218 [0.169, 0.268]	0.326
20:2n-6	0.437 [0.327, 0.547]	0.371 [0.338, 0.404]	0.167	0.404 [0.359, 0.450]	0.349 [0.313, 0.385]	0.307	0.398 [0.362, 0.431]	0.344 [0.303, 0.385]	0.286
20:3n-6	3.504 [2.805, 4.203]	3.258 [2.518, 3.997]	0.474	3.340 [2.872, 3.809]	2.636 [2.149, 3.123]	**0.003**	3.428 [3.046, 3.810]	2.512 [2.075, 2.949]	**<0.0001**
20:4n-3 Eicosatetraenoic acid (ETA)	0.361 [0.165, 0.556]	0.289 [0.257, 0.322]	0.606	0.243 [0.161, 0.325]	0.215 [0.161, 0.269]	0.965	0.304 [0.226, 0.382]	0.229 [0.155, 0.304]	0.536
20:4n-6 (Arachidonic acid)	12.097 [10.435, 13.759]	11.240 [9.638, 12.843]	0.510	12.227 [10.089, 14.366]	10.363 [8.840, 11.887]	0.074	12.265 [9.910, 14.821]	10.119 [9.239, 10.998]	**0.034**
20:5n-3 (Eicosapentaenoic acid (EPA))	0.75 [0.45, 1.04]	1.23 [0.25, 2.22]	0.304	0.75 [0.54, 0.95]	3.38 [2.41, 4.35]	**0.0002**	0.79 [0.48, 1.10]	4.56 [3.56, 5.57]	**0.0001**

22:5n-3 (Docosapentaenoic acid (DPA))	1.012 [0.699, 1.324]	1.079 [0.773, 1.385]	0.906	0.933 [0.815, 1.051]	1.285 [1.060, 1.457]	**0.0214**	0.845 [0.765, 0.923]	1.349 [1.136, 1.561]	**0.0002**
22:6n-3 (Docosahexaenoic acid (DHA))	2.351 [1.927, 2.774]	2.729 [1.667, 3.790]	0.472	2.657 [2.126, 3.188]	4.574 [4.12, 5.032]	**<0.0001**	2.801 [2.210, 3.393]	5.180 [4.621, 5.738]	**<0.0001**

Data are mean with 95% CI as percentage of sum of all measured fatty acids. Mixed effect analysis was applied to compare the 4 weeks intervention and baseline values for the 3 groups with daily intake of 2.0 g eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) and 3.5 g EPA+DHA. **Bold** is $p<0.05$ as compared to placebo or low vs high dose of EPA+DHA.

Table 3 - Clinical characteristics and body composition of the COPD groups at the end of 4 weeks intervention in response to the low vs high EPA+DHA supplementation as compared to placebo

	COPD Placebo n=10	**COPD low EPA+DHA n=10**			**COPD high EPA+DHA n=12**				
	Pred. Mean	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Δ Estimate High vs low EPA+DHA**	**p**
Clinical characteristics									
FeNO fasted	18.34 [12.67, 23.92]	20.68 [15.62, 25.74]	2.338 [-1.922, 6.597]	0.2603	15.66 [11.59, 19.73]	-2.679 [-9.077, 3.718]	0.3862	-5.017 [-11.13, 1.099]	0.1008
FeNO fed	18.35 [8.081, 28.63]	18.45 [9.06, 27.85]	0.0996 [-9.424, 9.623]	*0.2686*	16.33 [7.06, 25.60]	-2.028 [-8.108, 4.052]	*0.0531*	-2.127 [-11.19, 6.933]	*0.3059*
Systolic blood pressure (mm Hg)	141.1 [129.5, 152.7]	135.1 [123.8, 146.5]	-6.006 [-22.00, 9.984]	0.4464	133.9 [123.5, 144.3]	-7.237 [-22.49, 8.019]	0.3379	-1.231 [-16.56, 14.10]	0.8700
Diastolic blood	80.57 [75.96,	74.52 [70.33,	-6.043 [-12.37,	0.0603	77.41 [73.18,	-3.158 [-9.420,	0.3089	2.885 [-3.063,	0.3273

pressure (mm Hg)	85.17]	78.71]	0.2824]		81.63]	3.104]		8.833]	
CRP (mg/L)	4.410 [1.483, 7.337]	7.094 [4.091, 10.096]	1.405 [-2.974, 5.784]	0.157 2	5815 [2.829, 8.801]	1.405 [-2.974, 5.784]	0.6141	-1.279 [-5.634, 3.076]	0.366 5
Glucose (mmol/L)	5.689 [5.396, 5.982]	5.876 [5.559, 6.193]	0.1879 [-0.2003, 0.5739]	0.329 8	5.902 [5.582, 6.221]	0.2124 [-0.2251, 0.6500	0.3269	0.0256 [-0.4275, 0.4788]	0.908 2
Cholesterol total (mg/dL)	163.0 [147.7, 178.2]	178.8 [157.0, 200.5]	15.78 [-9.935, 41.49]	0.217 0	166.9 [151.2, 182.6]	3.876 [-17.21, 24.97]	0.7073	-11.0 [-38.40, 14.59]	0.362 4
Triglycerides (mg/dL)	104.1 [87.61, 120.6]	84.18 [68.86, 99.50]	-19.96 [-32.12, -7.794]	**0.002 5**	100.9 [84.53, 117.3]	-3.204 [-24.24, 17.84]	0.7556	16.75 [-3.283, 36.79]	0.071
HDL cholesterol (mg/dL)	62.11 [56.35, 67.88]	59.67 [53.83, 65.50]	-2.446 [-8.885, 3.993]	0.439 2	63.67 [58.37, 68.98]	1.560 [-4.858, 7.978]	0.6192	4.006 [-2.337, 10.35]	0.203 8
VLDL cholesterol (mg/dL)	20.82 [17.46, 24.19]	16.82 [13.71, 19.93]	-4.001 [-6.486, -1.515]	**0.002 9**	20.19 [16.82, 23.56]	-0.6318 [-4.954, 3.690]	0.7651	3.369 [-0.7477, 7.486]	0.104 0
LDL cholesterol	77.41 [66.74,	95.22 [77.75,	17.81 [-1.238,	0.065 5	88.63 [75.24,	11.22 [-5.298,	0.1733	-6.583 [-28.14,	0.533 8

(mg/dL)	88.08]	112.7]	36.85]		102.0]	27.74]		14.98]	
LDL/HDL ratio	1.395 [1.139, 1.651]	1.549 [1.261, 1.838]	0.1542 [-0.1085, 0.4169]	0.2363	1.571 [1.287, 1.856]	0.1763 [-0.1385, 0.4912]	0.2579]	0.0221 [-0.3278, 0.3721]	0.8968
Body Composition									
Body weight (kg)	77.17 [76.48, 77.86]	77.92 [77.22, 78.61]	0.7454 [-0.0887, 1.580]	0.0776	77.67 [76.96, 78.38]	0.4972 [-0.3244, 1.319]	0.2241	-0.2482 [-1.160, 0.6633]	0.5799
BMI (kg/m^2)	27.15 [26.89, 27.42]	27.41 [27.13, 27.68]	0.2520 [-0.0893, 0.5932]	0.1408	27.31 [27.04, 27.58]	0.1547 [-0.1824, 0.4919]	0.3536	-0.0972 [-0.4664, 0.2719]	0.5923
Lean mass (g)	47616 [46981, 48250]	48096 [47463, 48730]	480.4 [-249.7, 1210]	0.1875	48495 [47843, 49146]	878.9 [91.31, 1666]	**0.0302**	398.5 [-416.3, 1213]	0.3235
Lean mass extremities (g)	19113 [18741, 19485]	19745 [19369, 20121]	631.6 [241.0, 1022]	**0.0027**	19653 [19267, 20040]	540.0 [86.57, 993.4]	**0.0215**	-91.61 [-561.3, 378.0]	0.6913
Fat mass (g)	27192 [26238, 28146]	27914 [26903, 28925]	722.0 [-484.7, 1929]	0.2293	27526 [26550, 28502]	333.8 [-326.4, 994.1]	0.3077	-388.2 [-1630, 853.3]	0.5255
Fat mass extremities	11957 [11534,	11766 [11365,	-190.3 [-626.0,	*0.4470*	11917 [11474,	-39.98 [-345.6,	*0.2121*	150.4 [-309.6,	*0.5933*

(g)	12379]	12167]	245.3]		12360]	265.7]		610.4]	

Data are expressed as post intervention predicted mean and difference between groups with 95% CI. Analysis of covariance, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (0=no, 1=yes) and 3.5 g EPA+DHA (0=no, 1=yes) as dummy variables, and baseline value of the dependent variable as covariate.

m/f: number of males / females. FeNO: exhaled nitric oxide, HDL: high density lipoprotein, VLDL: very low density lipoprotein, LDL: low density lipoprotein, CRP: C-reactive protein. **Bold** is $p<0.05$ as compared to placebo or low vs high dose of EPA+DHA, and p value written in *italics* obtained after log transformation.

Table 4 - Muscle function, physical activity, quality of life of the COPD groups at the end of 4 weeks intervention in response to the low vs high EPA+DHA supplementation as compared to placebo

	COPD Placebo (n=10)	**COPD low EPA+DHA (n=10)**			**COPD high EPA+DHA (n=12)**				
	Pred. Mean	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Δ Estimate High vs low EPA+DHA**	**p**
Handgrip strength (N)	235.5 [218.1, 252.9]	229.8 [214.8, 244.7]	-5.792 [-22.57, 10.99]	0.4824	242.7 [226.1, 259.2]	7.111 [-15.32, 29.54]	0.5185	12.90 [-8.037, 33.84]	0.2151
Maximal inspiratory pressure (cm H_2O)	75.95 [69.85, 82.05]	71.24 [65.51, 76.97]	-4.706 [-10.53, 1.113]	0.1076	66.28 [60.36, 72.20]	-9.667 [-18.39, -0.9396]	0.0715	-4.961 [-13.50, 3.575]	0.2409
Physical activity (score)	94.06 [67.42, 120.7]	90.55 [69.47, 111.6]	-3.504 [-37.15, 30.14]	0.8281	86.30 [68.36, 104.2]	-7.755 [-39.81, 24.30]	0.6150	-4.251 [-29.45, 30.95]	0.7253
Quality of life - symptoms	62.64 [54.89,	64.21 [54.75,	1.568 [-10.52,	0.7909	59.03 [51.96,	-3.616 [-12.85,	0.4261	-5.184 [-16.46,	0.3517

(score)	70.41]	73.64]	13.66]		66.10]	5.615]		6.096]	
Quality of life - activity (score)	72.73 [66.02, 79.43]	76.99 [70.01, 83.97]	4.263 [-5.341, 13.87]	0.3680	76.78 [69.76, 83.80]	4.055 [-5.437, 13.55]	0.3860	-0.2080 [-9.008, 8.592]	0.9614
Quality of life - impact (score)	45.41 [38.30, 52.52]	35.70 [30.96, 40.43]	-9.710 [-16.28, -3.141]	***0.0328***	41.88 [35.21, 48.56]	-3.530 [-8.494, 1.435]	0.3694	6.181 [0.4238, 11.94]	0.1782
Quality of life - total (score)	55.85 [51.15, 60.56]	50.98 [46.87, 55.10]	-4.870 [-10.61, 0.8740	*0.3088*	55.18 [50.87, 59.48]	-0.6781 [-5.625, 4.269]	*0.8201*	4.192 [-0.9953, 9.380]	*0.2082*

Data are expressed as post intervention predicted mean and difference between groups with 95% CI. Analysis of covariance, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (0=no, 1=yes) and 3.5 g EPA+DHA (0=no, 1=yes) as dummy variables, and baseline value of the dependent variable as covariate.

FeNO: exhaled nitric oxide, HDL: high density lipoprotein, VLDL: very low density lipoprotein, LDL: low density lipoprotein. **Bold** is $p<0.05$ as compared to placebo or low vs high dose of EPA+DHA, and p value written in *italics* obtained after log transformation.

Table 5 - Postabsorptive plasma amino acid concentration of the COPD groups at the end of 4 weeks intervention in response to the low vs high EPA+DHA supplementation as compared to placebo

	COPD Placebo (n=10)	**COPD low EPA+DHA (n=10)**			**COPD high EPA+DHA (n=12)**				
	Pred. Mean	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Δ Estimate High vs low EPA+DHA**	**p**
Aspartate	1.538 [1.150, 1.925]	1.509 [1.119, 1.899]	-0.029 [-0.3934, 0.3356]	*0.7435*	2.037 [1.423, 3.191]	0.7691 [-0.1720, 1.710]	*0.0722*	0.7980 [-0.1409, 1.737]	*0.1496*
Glutamate	51.73 [45.22, 58.23]	46.03 [40.34, 51.73]	-5.694 [-10.91, -0.4780]	*0.4069*	61.93 [52.45, 71.40]	10.20 [-0.5164, 20.91]	*0.1213*	15.89 [5.549, 26.23]	***0.0234***
Hydroxy proline	19.89 [16.18, 23.61]	19.98 [16.26, 23.69]	0.0827 [-4.455, 4.620]	0.9704	14.95 [12.47, 17.43]	-4.946 [-9.012, -0.8794]	**0.0192**	-5.028 [-9.204, -0.8522]	**0.0202**
Asparagine	53.19 [47.96,	51.08 [45.52,	-2.114 [-9.884,	*0.8738*	52.97 [47.70,	-0.2280 [-7.362,	*0.6706*	1.886 [-5.935,	*0.5761*

	58.43]	56.64]	5.626]		58.23]	6.906]		9.707]	
Glutamine	549.1 [522.9, 575.2]	544.5 [516.2, 572.7]	-4.606 [-43.21, 34.00]	0.8079	535.6 [509.5, 561.6]	-13.51 [-49.84, 22.82]	0.4509	-8.906 [-48.41, 30.60]	0.6464
Citrulline	37.06 [31.29, 42.83]	34.44 [29.48, 39.39]	-2.630 [-9.576, 4.317]	*0.4199*	38.82 [33.02, 44.62]	1.757 [-5.615, 9.128]	*0.8590*	4.386 [-2.614, 11.39]	*0.3253*
Serine	87.00 [80.78, 93.22]	83.07 [77.26, 88.88]	-3.929 [-12.42, 4.556]	0.3494	89.59 [83.32, 95.86]	2.691 [-6.210, 11.39]	0.5498	6.520 [-1.103, 14.14]	0.0904
Glycine	223.9 [205.4, 242.5]	234.9 [214.4, 255.5]	10.99 [-14.24, 36.22]	0.3784	244.7 [220.3, 269.1]	20.76 [-5.644, 47.15]	0.1179	9.969 [-15.07, 34.61]	0.4257
Arginine	74.07 [65.17, 82.98]	69.69 [61.26, 78.11]	-4.385 [-15.68, 6.910]	*0.5273*	71.30 [63.51, 79.10]	-2.766 [-13.88, 8.346]	*0.7788*	1.919 [-7.607, 10.84]	*0.7221*
Threonine	120.43 [105.66, 135.2]	111.44 [97.12, 125.8]	-8.997 [-28.80, 10.81]	0.3584	120.3 [104.5, 136.1]	-0.1218 [-19.35, 19.10]	0.9897	8.878 [-10.26, 28.01]	0.3485
Alanine	254.0 [220.23, 287.7]	238.5 [203.2, 273.7]	-15.51 [-54.42, 23.40]	0.4194	246.8 [214.6, 279.0]	-7.189 [-48.93, 34.55]	0.7258	8.323 [-32.22, 48.86]	0.6760

Taurine	34.31 [28.98, 39.65]	36.70 [31.01, 42.40]	2.389 [-5.541, 10.23]	*0.3725*	37.94 [32.66, 43.22]	3.627 [-3.878, 11.13]	*0.3319*	1.238 [-6.525, 9.001]	*0.9752*
Proline	189.2 [169.9, 208.4]	160.4 [141.6, 179.2]	-28.76 [-48.57, -8.62]	*0.1677*	173.7 [156.4, 191.1]	-15.42 [-38.58, 7.729]	*0.5358*	13.34 [-7.508, 34.19]	*0.4165*
Tau-methyl-histidine	4.813 [4.444, 5.182]	4.334 [3.999, 4.669]	-0.4789 [-0.8251, -0.1327]	**0.0087**	5.523 [5.047, 5.998]	0.7098 [0.1695, 1.250]	**0.0121**	1.189 [0.6580, 1.719]	**<0.0001**
Valine	168.9 [149.6, 188.2]	156.0 [137.3, 174.6]	-12.92 [-39.83, 14.00]	*0.4258*	175.9 [156.5, 195.4]	7.066 [-17.32, 31.46]	*0.2674*	19.98 [-6.965, 46.93]	*0.0731*
Methionine	17.67 [15.89, 19.44]	16.80 [15.10, 18.51]	-0.8624 [-3.200, 1.475]	*0.5772*	17.34 [15.63, 19.05]	-0.3243 [-2.645, 1.996]	*0.9998*	0.5381 [-1.666, 2.743]	*0.5787*
Isoleucine	53.96 [48.22, 59.70]	50.32 [44.88, 55.75]	-3.643 [-11.15, 3.868]	*0.5592*	54.40 [48.60, 60.21]	0.4441 [-6.725, 7.613]	*0.6130*	4.087 [-3.460, 11.63]	*0.2908*
Leucine	91.72 [81.40, 102.0]	89.70 [79.08, 100.3]	-2.022 [0.5795, 1.361]	*0.5542*	96.02 [84.76, 107.3]	4.305 [-9.107, 17.72]	*0.4105*	6.327 [-8.281, 20.93]	*0.1686*
Tryptophan	30.66 [27.22,	29.94 [26.38,	-0.7200 [-5.545,	0.7611	31.33 [27.73,	0.6718 [-4.103,	0.7744	1.392 [-3.554,	0.5674

	34.10]	33.50]	4.105]		34.93]	5.446]		6.338]	
Phenyl alanine	48.49 [45.61, 51.38]	48.58 [45.46, 51.70]	0.0870 [-4.038, 4.202]	0.9657	49.00 [46.08, 51.92]	0.5041 [-3.466, 4.475]	0.7958	0.4171 [-3.766, 4.600]	0.8389
Ornithine	62.20 [52.81, 71.58]	54.90 [47.66, 62.14]	-7.295 [-18.69, 4.100]	*0.3150*	61.55 [52.74, 70.36]	-0.6488 [-12.85, 11.55]	*0.7811*	6.646 [-1.464, 14.76]	*0.2144*
Histidine	64.63 [59.02, 70.24]	61.52 [55.72, 67.33]	-3.107 [-11.18, 4.963]	0.4353	61.41 [56.24, 66.58]	-3.216 [-10.65, 4.221]	0.3816	-0.1099 [-7.867, 7.647]	0.9770
Lysine	162.7 [146.1, 179.4]	161.6 [144.1, 179.1]	-1.182 [0.7547, 1.306]	*0.7005*	172.4 [154.5, 190.3]	9.966 [-11.66, 30.97]	*0.2647*	10.84 [-10.53, 32.21]	*0.1547*
Tyrosine	47.56 [43.00, 52.12]	44.71 [40.44, 48.97]	-2.854 [-8.383, 2.675]	0.2979	46.25 [41.83, 50.66]	-1.311 [-7.440, 4.818]	0.6633	1.543 [-4.398, 7.483]	0.5975
BCAA	316.0 [282.7, 349.4]	294.7 [262.4, 327.1]	-21.27 [-66.45, 23.91]	*0.4227*	325.3 [291.2, 359.5]	9.304 [-32.95, 51.56]	*0.3321*	30.57 [-15.61, 76.75]	*0.0913*
EAA	762.7 [689.2, 836.1]	713.9 [641.3, 786.6]	-48.73 [-150.4, 52.95]	*0.4682*	773.9 [698.5, 849.4]	11.27 [-86.81, 109.3]	*0.3654*	59.99 [-42.43, 162.4]	*0.1277*

NEAA	1531 [1449, 1613]	1461 [1381, 1542]	-69.94 [-181.4, 41.54]	*0.5019*	1542 [1435, 1595]	-16.29 [-125.5, 92.96]	*0.8895*	53.65 [-53.74, 161.0]	*0.5987*
SumAA	2288 2145, 2431]	2170 [2029, 2310]	-118.3 [-315.2, 78.61]	*0.4716*	2294 [2151, 2438]	6.299 [-188.1, 200.7]	*0.6942*	124.6 [-69.51, 318.8]	*0.2886*

Data (μM) are expressed as post intervention predicted mean and difference between groups with 95% CI. Analysis of covariance, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (0=no, 1=yes) and 3.5 g EPA+DHA (0=no, 1=yes) as dummy variables, and baseline value of the dependent variable as covariate.

BCAA: sum of branched-chain amino acids, EAA: sum of essential amino acids, NEAA: sum of non-essential amino acids, SumAA: sum of all measured amino acids. **Bold** is $p<0.05$ as compared to placebo or low vs high dose of EPA+DHA, and p value written in *italics* obtained after log transformation.

Table 6 - Whole Body Production in postabsorptive vs. prandial state of the COPD groups at the end of 4 weeks intervention in response to the low vs high EPA+DHA supplementation as compared to placebo

	COPD Placebo (n=10)	**COPD low EPA+DHA (n=10)**			**COPD high EPA+DHA (n=12)**				
	Pred. Mean	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Pred. Mean**	**Δ Estimate vs placebo**	**p**	**Δ Estimate High vs low EPA+DHA**	**p**
Phenylalanine (PB) – fasted	3470 [3285, 3655]	3339 [3156, 3523]	-130.4 [-392.4, 131.7]	0.3147	3160 [2979, 3340]	-309.7 [-578.6, -40.78]	**0.0258**	-179.4 [-436.8, 78.06]	0.1633
Tyrosine - fasted	2544 [2370, 2718]	2486 [2326, 2645]	-58.16 [-264.9, 148.6]	0.5670	2388 [2238, 2539]	-155.3 [-378.5, 67.95]	0.1640	-97.11 [-303.6, 109.4]	0.3415
Phenylalanine - fed	3793 [3455, 4131]	3740 [3421, 4059]	-52.66 [-459.4, 354.1]	0.7916	3637 [3331, 3943]	-155.6 [-591.7, 280.5]	0.4686	-102.9 [-520.7, 314.9]	0.6158

Data are expressed in μmol/hour as post intervention predicted mean and difference between groups with 95% CI. Analysis of covariance, using multiple linear regression, was applied to compare the 3 groups with intake of

2.0 g eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) (0=no, 1=yes) and 3.5 g EPA+DHA (0=no, 1=yes) as dummy variables, and baseline value of the dependent variable and BMI as covariate to correct for difference in body size. PB=protein breakdown. **Bold** is $p<0.05$ as compared to placebo or low vs high dose of EPA+DHA.