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ω-3 polyunsaturated fatty acid supplementation improves postabsorptive and prandial protein metabolism in patients with chronic obstructive pulmonary disease: a randomized clinical trial

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ABSTRACT

Background: Disturbances in protein metabolism and impaired muscle health have been observed in chronic obstructive pulmonary disease (COPD). The ω-3 (n–3) PUFAs EPA and 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 participants with moderate to severe COPD ($n = 32$) received daily for 4 wk, according to a randomized double-blind placebo controlled 3-group design, a high dose (3.5 g, $n = 10$) of EPA + DHA, a low dose (2.0 g, $n = 10$) of EPA + DHA, or placebo (olive oil, $n = 12$) via gel capsules. At pre- and postintervention, 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 wk of supplementation in both EPA + DHA groups ($P < 0.004$), and there was a trend toward higher values for plasma EPA after the high compared with the low dose of EPA + DHA ($P = 0.065$). Postabsorptive PB was lower after 4 wk 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 wk induces a shift toward a positive daily protein homeostasis in patients with COPD in part in a dose-dependent way. Daily doses up to 3.5 g EPA and DHA are still well tolerated and lead to protein gain in these patients. This trial was registered at clinicaltrials.gov as NCT01624792. *Am J Clin Nutr* 2022;116:686–698.

Keywords: COPD, stable isotopes, PUFA, intervention, randomized controlled trial, 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 that were linked to poor muscle health outcomes (1, 7–12). Systemic inflammation is recognized as an underlying factor contributing to muscle wasting and

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Supplemental Figure 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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

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Abbreviations used: BCAA, branched-chain amino acid; COPD, chronic obstructive pulmonary disease; EAA, essential amino acid; FEV1, forced expiratory volume in 1 s; hs-CRP, high-sensitivity C-reactive protein; NEAA, nonessential amino acid; NO, nitric oxide; ONS, oral nutritional support; PHE, phenylalanine; PB, protein breakdown; PS, protein synthesis; Ra, rate of appearance; TTR, tracer/tracee ratio; TYR, tyrosine; WBP, whole-body production rate.

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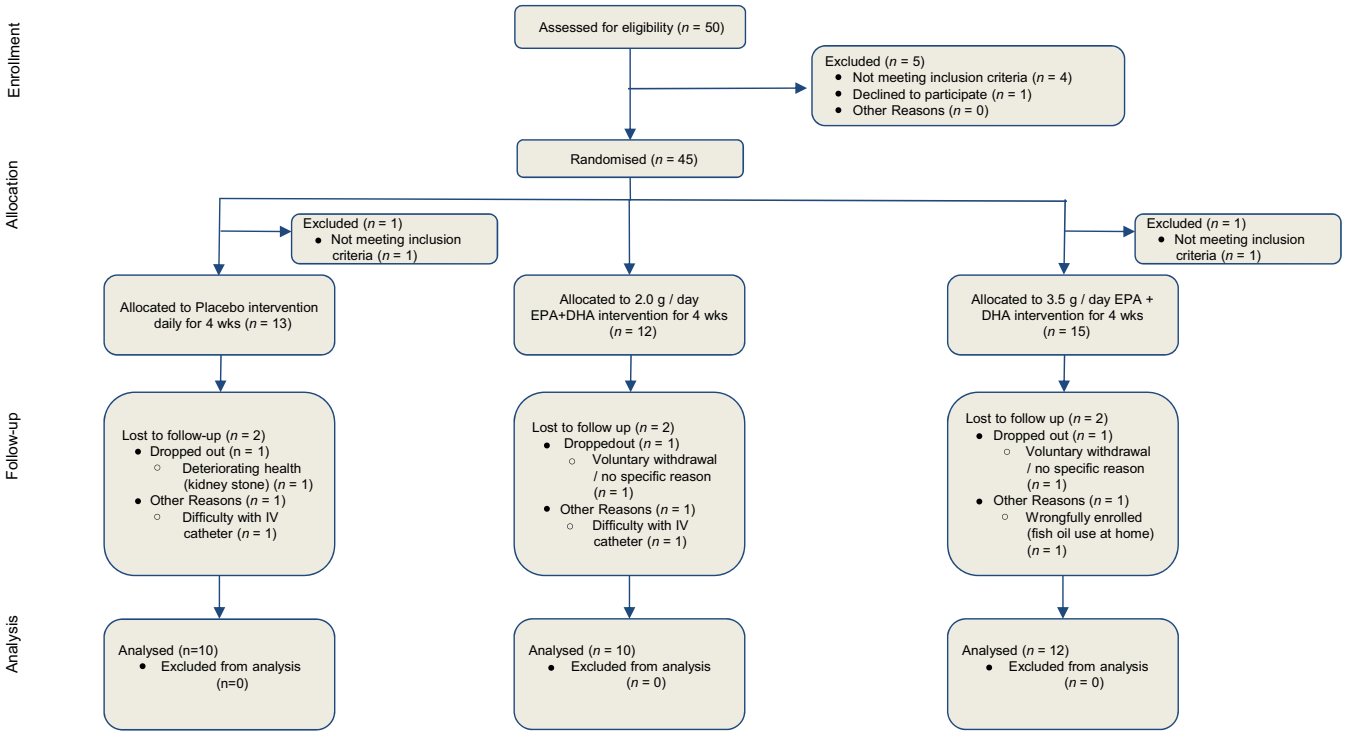

FIGURE 1 Participant flow through the study.

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 n–3 PUFAs EPA (20:5n–3) and 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 patients with COPD, daily intake of 1–2 g n–3 PUFAs [as part of oral nutritional support (ONS)] for 5–12 wk resulted in reduced symptoms after a 6-min walk test (16) and enhanced exercise capacity (17). but inflammatory markers, muscle mass, or function were not improved. In malnourished patients with COPD, 1.2 g/d n–3 PUFAs for 12 wk (alongside low-intensity exercise) resulted in increased muscle mass, strength, and functional performance (18), but no changes were found after 4 mo 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 ∼2 g/d in chronic conditions (19), although the optimal daily dose and period of intervention remain unclear. Four weeks of 3.2 g/d 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 wk 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 protein breakdown. 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 EPA + DHA/d as part of ONS resulted in some normalization of the metabolic response in both the fasted and fed states (26–28).

In the present study, we examined whether 4 wk 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 wk 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/d 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.

Methods

Study population

In the present study, 45 clinically stable patients with a diagnosis of COPD (grades 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 preexistent untreated metabolic or renal disease, malignancy, recent surgery, daily use of supplements containing >1000 mg EPA + DHA in the 3 mo prior to the first test day, use of protein- or amino acid–containing nutritional supplements within 5 d 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 wk preceding the first test day. Written informed

TABLE 1 Disease characteristics of the COPD groups[1]

Characteristic	Total COPD group ($n = 32$)	COPD placebo ($n = 10$)	COPD low EPA + DHA ($n = 10$)	COPD high EPA + DHA ($n = 12$)
Age, y	66.79 ± 4.35	62.10 ± 11.09	70.70 ± 7.85	67.58 ± 7.48
Sex, female/male, No.	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 O_2 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, y	11.30 ± 1.08	10.40 ± 5.86	12.50 ± 7.63	11.00 ± 6.81
COPD exacerbations past year, No.	0.84 ± 0.25	0.60 ± 0.52	1.10 ± 1.29	0.83 ± 0.94
COPD hospitalization for exacerbation past year, No.	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

[1] Values are presented as mean ± SD unless otherwise indicated. COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

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 to June 2016.

Study design

All participants 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 participants 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 h per test day) and a 4-wk intervention period in between.

Nutritional intervention.

The 4-wk nutritional intervention was performed according to a double-blind, randomized, placebo-controlled 3-group design and consisted of *1*) 3.5 g EPA + DHA per day [7 capsules of 1000 mg Swanson EFAs superior essential fatty acids (Super EPA), consisting of 300 mg EPA, 200 mg DHA, 50 mg other n–3 PUFAs per capsule] or *2*) 2 g EPA + DHA (4 capsules) + 3 g 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 *3*) 7 g placebo per day (7 capsules of 1000 mg olive oil). The total energy content provided was equal among all groups (10 cal/capsule). A total of 2 g EPA + DHA daily was used as this is the commonly used dose in previous studies (30). The higher dose of 3.5 g EPA + DHA daily has been approved by the FDA for lowering plasma triglyceride concentrations in hypertriglyceridemic patients and has therefore previously been shown to be physiologically relevant in human participants. As we previously observed in a pilot study that ~3–4 h 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 number. 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 postintervention 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 DXA (Hologic QDR 4500/Version 12.7.3.1). Forced expiratory volume in 1 s and forced vital capacity 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 handheld mouth pressure device (Micro Respiratory Pressure Meter; MD spiro). Peak handgrip force (Vernier dynamometry; Vernier Software and Technology) was used as a marker of muscle strength (32). Habitual physical activity level was assessed using the Physical Activity Scale for the Elderly questionnaire (33). The modified

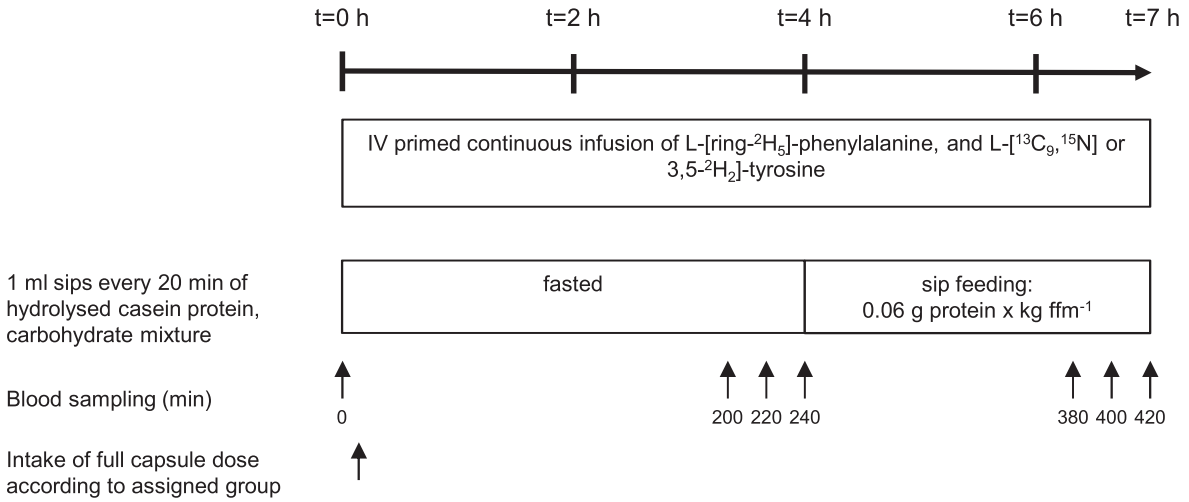

FIGURE 2 Overview of study design to measure protein metabolism in the postabsorptive and prandial states.

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

Protein metabolism in the postabsorptive and prandial state. After an overnight fast (**Figure 2**), 1 catheter was inserted in a peripheral vein of the lower arm for stable isotope tracer (Cambridge Isotope Laboratories) primed constant infusion of phenylalanine (PHE-[ring-$^2H_5$]: 0.61 mg/kg/h, prime 0.37 mg/kg) and tyrosine (TYR-[3,5-$^2H_2$] or TYR-[U-$^{13}C_9$, ^{15}N]: 0.02 mg/kg/h, prime 0.02 mg/kg), and enteral TYR-[ring-$^2H_4$] prime was simultaneously provided (0.063 mg/kg). In addition, 1 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 high-sensitivity C-reactive protein (hs-CRP)], followed by intake of a full dose of capsules according to the assigned group, arterialized venous blood was drawn at 200, 220, and 240 min (fasted state) and 380, 400, and 420 min (fed state) for analysis of tracer enrichments and concentrations of amino acids. Three hours after the start of the infusion, all participants received every 20 min for 3 h a mixture of 0.06 g/kg ffm (Fat-free mass) 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) and 20.0 g 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 × *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 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, and 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 semiautomatic 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 50°C for 2 h. 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 described elsewhere (42). Fatty acid methyl esters were detected by a flame ionization detector. Each fatty acid was expressed as a percentage of total fatty acids measured in the sample. Plasma concentrations of HDL, VLDL, and LDL cholesterol and triglycerides were analyzed by Labcorp.

Statistical analysis

Calculations of protein kinetics were done for each participant using a horizontal regression line robust fitting procedure using measurements taken at 200, 220, and 240 min for the

postabsorptive phase and 380, 400, and 420 min for the prandial phase.

ANCOVA, using multiple linear regression with, if needed, weighing to obtain homoscedasticity and log transformation to obtain a normal distribution of the residuals (43), was applied to compare the 3 groups on each dependent variable, including the primary outcome (postintervention anabolic response to the meal) and the secondary outcomes [postintervention whole-body production rates (in the postabsorptive and prandial state), body composition, laboratory clinical characteristics, muscle function, physical activity, quality of life, and plasma amino acid concentrations]. Independent variables included intake of 2.0 g EPA + DHA (0 = no, 1 = yes) and 3.5 g EPA + DHA (0 = no, 1 = yes) as dummy variables, baseline value of the dependent variable, and BMI (in kg/m^2; not in case of body composition measurements). The level of significance was set at $P < 0.05$ compared with placebo or a low (2.0 g) compared with a high (3.5 g) dose of EPA + DHA, and GraphPad Prism (version 9.3; GraphPad Software) was used for data analysis.

As we used ANCOVA to estimate the effect of EPA + DHA supplementation on the primary outcome (anabolic response to a protein meal), we adjusted the power calculation by using a previously calculated correlation coefficient of 0.8 between pre- and postmeasurements (44). Our previous study on COPD showed an anabolic response to a protein meal (net protein synthesis) of 226 ± 42 μmol/kg ffm/min (45). An anticipated 10% increase in net protein synthesis was used to calculate the estimated sample size per group [independent samples, power: 0.80; α: 0.05 (46)]. We calculated a sample size of 11.1 participants per group.

Results

Population characteristics

Forty-five patients with COPD (24 male/21 female) 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 participants were no longer meeting the inclusion criteria, 3 were early discontinuation [2 had difficulty with an intravenous catheter; 1 had 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) and characterized by having slightly elevated fasting glucose concentrations (6.0 ± 0.5 mmol/L) and low-grade systemic inflammation (CRP: 4.5 ± 3.5 mg/L).

Effects of 4 wk 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,

TABLE 2 Plasma phosphatidylcholine fatty acids of the COPD groups before and at the end of 4 wk of low compared with high EPA + DHA supplementation as compared with placebo[1]

	COPD placebo ($n = 10$)			COPD low EPA + DHA ($n = 10$)			COPD high EPA + DHA ($n = 12$)		
Fatty acid	Baseline	4 wk	*P* value	Baseline	4 wk	*P* value	Baseline	4 wk	*P* value
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)	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)	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 (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)	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 (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

[1]Data are mean with 95% CI as percentage of sum of all measured fatty acids. Mixed-effect analysis was applied to compare the 4-wk intervention and baseline values for the 3 groups with daily intake of 2.0 g EPA + DHA and 3.5 g EPA + DHA. Underline is $P < 0.05$ as compared to placebo or low compared with high-dose EPA + DHA. COPD, chronic obstructive pulmonary disease.

TABLE 3 Clinical characteristics and body composition of the COPD groups at the end of the 4-wk intervention in response to the low compared with high EPA + DHA supplementation as compared with placebo[1]

	COPD placebo ($n = 10$)	COPD low EPA + DHA ($n = 10$)			COPD high EPA + DHA ($n = 12$)			Δ estimate: high vs. low EPA + DHA	
Characteristic	Predictive mean	Predictive mean	Δ estimate vs. placebo	*P* value	Predictive mean	Δ estimate vs. placebo	*P* value		*P* value
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 pressure, mm Hg	80.57 [75.96, 85.17]	74.52 [70.33, 78.71]	–6.043 [–12.37, 0.2824]	0.0603	77.41 [73.18, 81.63]	–3.158 [–9.420, 3.104]	0.3089	2.885 [–3.063, 8.833]	0.3273
CRP, mg/L	4.410 [1.483, 7.337]	7.094 [4.091, 10.096]	1.405 [–2.974, 5.784]	0.1572	5815 [2.829, 8.801]	1.405 [–2.974, 5.784]	0.6141	–1.279 [–5.634, 3.076]	0.3665
Glucose, mmol/L	5.689 [5.396, 5.982]	5.876 [5.559, 6.193]	0.1879 [–0.2003, 0.5739]	0.3298	5.902 [5.582, 6.221]	0.2124 [–0.2251, 0.6500	0.3269	0.0256 [–0.4275, 0.4788]	0.9082
Cholesterol total, mg/dL	163.0 [147.7, 178.2]	178.8 [157.0,200.5]	15.78 [–9.935, 41.49]	0.2170	166.9 [151.2,182.6]	3.876 [–17.21, 24.97]	0.7073	–11.0 [–38.40, 14.59]	0.3624
Triglycerides, mg/dL	104.1 [87.61, 120.6]	84.18 [68.86, 99.50]	–19.96 [–32.12, –7.794]	0.0025	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]	0.4392	63.67 [58.37, 68.98]	1.560 [–4.858, 7.978]	0.6192	4.006 [–2.337, 10.35]	0.2038
VLDL cholesterol, mg/dL	20.82 [17.46, 24.19]	16.82 [13.71, 19.93]	–4.001 [–6.486, –1.515]	0.0029	20.19 [16.82, 23.56]	–0.6318 [–4.954, 3.690]	0.7651	3.369 [–0.7477, 7.486]	0.1040
LDL cholesterol, mg/dL	77.41 [66.74, 88.08]	95.22 [77.75,112.7]	17.81 [–1.238, 36.85]	0.0655	88.63 [75.24,102.0]	11.22 [–5.298, 27.74]	0.1733	–6.583 [–28.14, 14.98]	0.5338
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	47,616 [46,981,48,250]	48,096 [47,463, 48,730]	480.4 [–249.7, 1210]	0.1875	48,495 [47,843, 49,146]	878.9 [91.31, 1666]	0.0302	398.5 [–416.3, 1213]	0.3235
Lean mass extremities, g	19,113 [18,741,19,485]	19,745 [19,369, 20,121]	631.6 [241.0, 1022]	0.0027	19,653 [19,267, 20,040]	540.0 [86.57, 993.4]	0.0215	–91.61 [–561.3, 378.0]	0.6913
Fat mass, g	27,192 [26,238,28,146]	27,914 [26,903, 28,925]	722.0 [–484.7, 1929]	0.2293	27,526 [26,550, 28,502]	333.8 [–326.4, 994.1]	0.3077	–388.2 [–1630, 853.3]	0.5255
Fat mass extremities, g	11,957 [11,534,12,379]	11,766 [11,365, 12,167]	–190.3 [–626.0, 245.3]	*0.4470*	11,917 [11,474, 12,360]	–39.98 [–345.6, 265.7]	*0.2121*	150.4 [–309.6, 610.4]	*0.5933*

[1]Data are expressed as postintervention predicted mean and difference between groups with 95% CI. ANCOVA, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g 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. Underline is $P < 0.05$ as compared with placebo or low as compared with high-dose EPA + DHA, and *P* value in italics was obtained after log transformation. COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; FeNO, exhaled nitric oxide.

diarrhea). Eighteen 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-γ-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 h of intake for any of the 3 groups (**Supplemental 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 compared with placebo for exhaled nitric oxide (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 ($P < 0.003$), whereas other plasma lipid measurements were not significantly affected. Lean mass in the extremities was significantly increased after 4 wk of EPA + DHA, independent of the dose (Table 3). Total lean mass was also increased after the high dose of EPA + DHA intake compared with the placebo ($P = 0.030$). Extremity lean mass was increased after 4 wk 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 ($P = 0.033$) compared with the placebo group (**Table 4**).

Plasma amino acid concentrations in the postabsorptive state

The plasma amino acid profile was relatively unaffected after 4 wk of EPA + DHA supplementation as only a few changes were observed (**Table 5**). For instance, plasma glutamate was higher and hydroxyproline lower after the high EPA + DHA intervention ($P = 0.02$) compared with the low EPA + DHA. Tau methylhistidine concentration was higher after the high EPA + DHA dose compared with the low dose of EPA + DHA and the placebo ($P = 0.012$). No significant differences were found in sum of branched-chain amino acid (BCAA), essential amino acid (EAA), nonessential amino acid (NEAA), or amino acid plasma concentrations.

TABLE 4 Muscle function, physical activity, and quality of life of the COPD groups at the end of the 4-wk intervention in response to the low compared with high EPA + DHA supplementation as compared with placebo[1]

	COPD placebo ($n = 10$)	COPD low EPA + DHA ($n = 10$)			COPD high EPA + DHA ($n = 12$)			Δ estimate: high vs. low EPA + DHA	
Characteristic	Predictive mean	Predictive mean	Δ estimate vs. placebo	*P* value	Predictive mean	Δ estimate vs. placebo	*P* value		*P* value
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 (score)	62.64 [54.89, 70.41]	64.21 [54.75, 73.64]	1.568 [–10.52, 13.66]	0.7909	59.03 [51.96, 66.10]	–3.616 [–12.85, 5.615]	0.4261	–5.184 [–16.46, 6.096]	0.3517
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*

[1]Data are expressed as postintervention predicted mean and difference between groups with 95% CI. ANCOVA, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g 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. Underline is $P < 0.05$ as compared with placebo or low as compared with high-dose EPA + DHA, and *P* value in italics was obtained after log transformation. COPD, chronic obstructive pulmonary disease.

TABLE 5 Postabsorptive plasma amino acid concentration of the COPD groups at the end of the 4-wk intervention in response to the low compared with high EPA + DHA supplementation as compared with placebo[1]

	COPD placebo ($n = 10$)	COPD low EPA + DHA ($n = 10$)			COPD high EPA + DHA ($n = 12$)			Δ estimate: high vs. low EPA + DHA	
Characteristic	Predictive mean	Predictive mean	Δ estimate vs. placebo	*P* value	Predictive mean	Δ estimate vs, placebo	*P* value		*P* value
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, 58.43]	51.08 [45.52, 56.64]	–2.114 [–9.884, 5.626]	*0.8738*	52.97 [47.70, 58.23]	–0.2280 [–7.362, 6.906]	*0.6706*	1.886 [–5.935, 9.707]	*0.5761*
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, 34.10]	29.94 [26.38, 33.50]	–0.7200 [–5.545, 4.105]	0.7611	31.33 [27.73, 34.93]	0.6718 [–4.103, 5.446]	0.7744	1.392 [–3.554, 6.338]	0.5674
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*

[1]Data (μM) are expressed as postintervention predicted mean and difference between groups with 95% CI. ANCOVA, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g 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. Underline is $P < 0.05$ as compared to placebo or low as compared with high-dose EPA + DHA, and *P* value in italics was obtained after log transformation. BCAA, sum of branched-chain amino acids; COPD, chronic obstructive pulmonary disease; EAA, sum of essential amino acids; NEAA, sum of nonessential amino acids; SumAA, sum of all measured amino acids.

Whole-body protein metabolism in the postabsorptive and prandial states

Postabsorptive phenylalanine production (marker of protein turnover) was lower after the high EPA + DHA compared with placebo supplementation ($P = 0.026$) (**Table 6**). Moreover, the phenylalanine to tyrosine conversion (marker of postabsorptive net protein breakdown) (**Figures 3** and **4**, Supplemental Figure 1) was 10% ($P = 0.037$) and 13% ($P = 0.026$) lower after 4 wk of low and high EPA + DHA, respectively, with no difference between the high compared with the low dose of the EPA + DHA groups. Prandial netPS was 15% ($P = 0.031$) higher after 4 wk of high-dose EPA + DHA but not different after the low-dose EPA + DHA.

Discussion

In the present randomized controlled trial, the daily low (2.0 g) and high (3.5 g) doses of n–3 fatty acids for 4 wk 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 a low or a high EPA + DHA intervention compared with placebo.

We measured fatty acids in plasma phosphatidylcholine, the main circulating phospholipid. Mean baseline values in the 3 groups were 0.75%, 0.75%, and 0.79% for EPA and 2.25%, 2.66%, and 2.80% for DHA. Hodson et al. (47) combined data for EPA and DHA from multiple studies, mainly in healthy participants. For total plasma (combination of triglycerides, phospholipids, cholesteryl esters, and nonesterified 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 that contained 66% oleic acid. At an oil dose of 7 g/d, this would provide 4.62 g 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 indicate an average daily intake of oleic acid among US adults of 27 g (48). Thus, the amount of oleic acid provided in the placebo is ∼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

TABLE 6 Whole-body production in postabsorptive compared with prandial state of the COPD groups at the end of the 4-wk intervention in response to the low compared with high EPA + DHA supplementation as compared to placebo[1]

	COPD placebo ($n = 10$)	COPD low EPA + DHA ($n = 10$)			COPD high EPA + DHA ($n = 12$)			Δ estimate: high vs. low EPA + DHA	
Characteristic	Predictive mean	Predictive mean	Δ estimate vs. placebo	*P* value	Predictive mean	Δ estimate vs. placebo	*P* value		*P* value
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

[1]Data are expressed in μmol/h as postintervention predicted mean and difference between groups with 95% CI. ANCOVA, using multiple linear regression, was applied to compare the 3 groups with intake of 2.0 g 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 covariates to correct for difference in body size. Underline is $P < 0.05$ as compared to placebo or low as compared with high-dose EPA + DHA. COPD, chronic obstructive pulmonary disease; PB, protein breakdown.

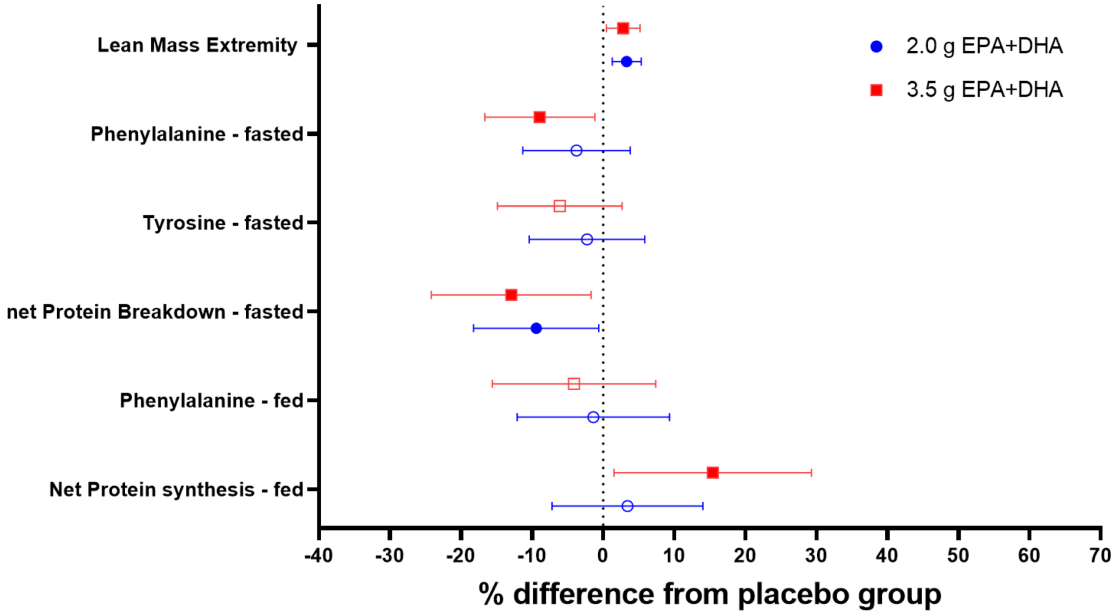

FIGURE 3 Predicted percent difference from placebo (with 95% CI) ($n = 10$) for lean mass extremities, postabsorptive rates of appearances (Ra) of phenylalanine and tyrosine, net protein breakdown, prandial rate of appearance of phenylalanine, and net protein synthesis at 4 wk of supplementation of 2.0 g/d EPA + DHA (circles, $n = 10$) compared with 3.5 g/d EPA + DHA (squares, $n = 12$). Closed circles or squares mean statistical significance ($P < 0.05$) was obtained.

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 compared with the low EPA + DHA dose. No differences were observed in plasma BCAAs, EAAs, or NEAAs. 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 8 wk 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

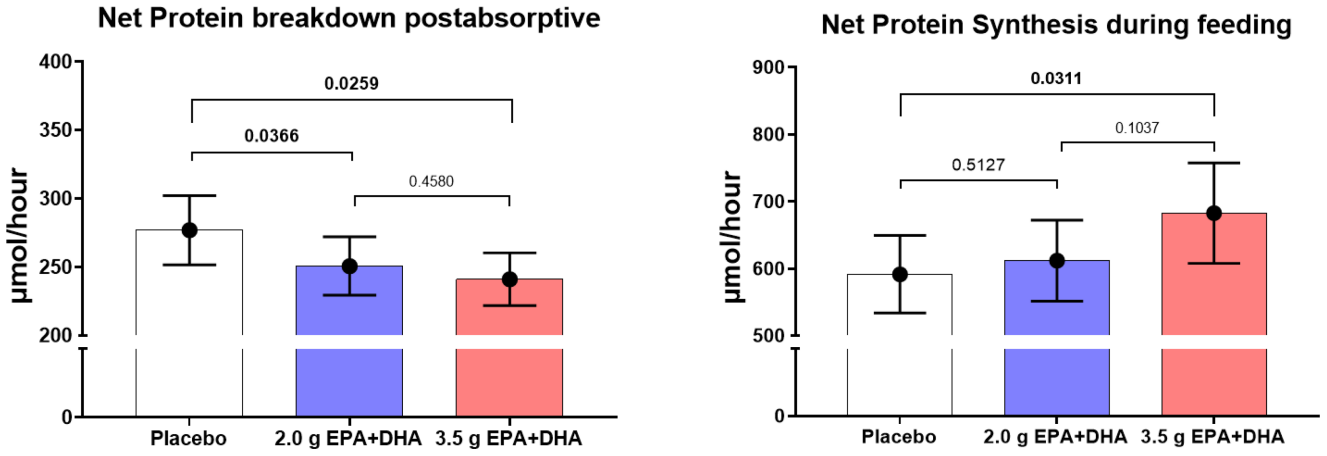

FIGURE 4 Predicted means with 95% CI of postabsorptive net protein breakdown (left panel) and net protein synthesis during feeding (protein anabolism; right panel) after 4 wk of intake of placebo (dark gray bar, $n = 10$), 2.0 g/d EPA + DHA (light gray bar, $n = 10$), and 3.5 g/d EPA + DHA (white bar, $n = 12$). ANCOVA, 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.

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 nonsteroidal anti-inflammatory drugs was able to improve postprandial protein synthesis (18, 51). No significant changes were observed in hs-CRP after 4 wk of EPA + DHA intervention in the current 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 wk 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-κB (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 able to study the potential positive effects on muscle endurance or exercise capacity. No differences were observed in handgrip strength, but our study was not adequately powered to detect a statistical difference in muscle strength, and the intervention period of 4 wk might be too short to see such an effect (55). To improve functional performance, incorporation of exercise training is likely needed during n–3 PUFA supplementation as an enhanced anabolic response to exercise was observed in older adult women (56). In line with this, following 16 wk of 3.9 g/d of n–3 PUFA supplementation in healthy participants (24), postabsorptive mixed muscle protein synthesis rates were increased in older adults, and mitochondrial and myofibrillar protein synthesis were elevated (15–18 h) following a bout of resistance exercise. The enhanced anabolic response to exercise following n–3 PUFAs was attributed to transcriptional changes, and changes to the activation of anabolic signaling proteins in skeletal muscle as lower levels of postexercise myostatin, a negative regulator of muscle growth and differentiation, and altered phosphorylation of signaling transduction proteins in muscle such as mTOR and p70s6k (25) have been found. Further research is required to what extent n–3 PUFAs are also able to improve the anabolic responsiveness to exercise in COPD and to characterize the patients with COPD who are not able to increase their anabolic response to feeding after n–3 PUFA supplementation (24, 57). Future studies are also needed in COPD to determine if the observed adaptations translate into meaningful improvements in physical function when n–3 PUFAs are given over longer periods of time or in combination with an exercise training program.

Limitations

The number of participants per group was small, and the results might not be applicable to the general COPD population as the participants were normal weight to overweight with preserved muscle mass, and the group who might benefit the most from n–3 PUFA support, patients with COPD with impaired muscle health and exercise performance, were not specifically included. Data on the anabolic response to a high-quality protein were provided so it remains unclear whether the enhanced anabolic response would also be found with lower-quality proteins. Furthermore, 71% of the randomly allocated participants 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.

In conclusion, daily oral intake of n–3 PUFAs (EPA + DHA) for 4 wk 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 concentrations are well tolerated and lead to protein gain in patients with COPD even after a short period (4 wk) 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.

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The authors' responsibilities were as follows—HS: had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. HS: was involved in the recruitment of subjects and conduct of the study. MPKJE, RJ, PCC, and NEPD: had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; MPKJE, RJ, and NEPD: designed the research and involved in the recruitment of participants and conduct of the study, involved in the data analysis, sample analysis, and writing of the manuscript; HLF and PCC: involved in sample analysis, writing, and reviewing of the manuscript; and all authors: read and approved the final version of the manuscript. The authors report no conflicts of interest.

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