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Original article

Pharmacokinetics of omega-3 fatty acids in patients with severe sepsis compared with healthy volunteers: A prospective cohort study

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SUMMARY

Background: Pharmacokinetics (PK) of pharmaceuticals and pharmaconutrients are poorly understood in critically ill patients, and dosing is often based on healthy subject data. This might be particularly problematic with enteral medications due to metabolic abnormalities and impaired gastrointestinal tract absorption common in critically ill patients. Utilizing enteral fish oil, this study was undertaken to better understand and define PK of enteral omega-3 fatty acids (eicospentaenoic acid [EPA] and docosahexaenoic acid [DHA]) in critically ill patients with severe sepsis.

Materials and methods: Healthy volunteers (n = 15) and mechanically ventilated (MV) adults with severe sepsis (n = 10) were recruited and received 9.75 g EPA and 6.75 g DHA daily in two divided enteral doses of fish oil for 7 days. Volunteers continued their normal diet without other sources of fish oil, and sepsis patients received standard enteral feeding. Blood was collected at frequent intervals during the 14-day study period. Peripheral blood mononuclear cells (PMBCs) and neutrophils were isolated and analyzed for membrane fatty acid (FA) content. Mixed linear models and t-tests were used to analyze changes in FA levels over time and FA levels at individual time points, respectively. PK parameters were obtained based on single compartment models of EPA and DHA kinetics.

Results: Healthy volunteers were 41.1 ± 10.3 years; 67% were women. In patients with severe sepsis (55.6 ± 13.4 years, 50% women), acute physiologic and chronic health evaluation (APACHE) II score was 27.2 ± 8.8 at ICU admission and median MV duration was 10.5 days. Serum EPA and DHA were significantly lower in sepsis vs. healthy subjects over time. PBMC EPA concentrations were generally not different between groups over time, while PBMC DHA was higher in sepsis patients. Neutrophil EPA and DHA concentrations were similar between groups. The half-life of EPA in serum and neutrophils was significantly shorter in sepsis patients, whereas other half-life parameters did not vary significantly between healthy volunteers and sepsis patients.

Conclusions: While incorporation of n-3 FAs into PBMC and neutrophil membranes was relatively similar between healthy volunteers and sepsis patients receiving identical high doses of fish oil for one week, serum EPA and DHA were significantly lower in sepsis patients. These findings imply that serum concentrations and EPA and DHA may not be the dominant driver of leukocyte membrane incorporation of EPA and DHA. Furthermore, lower serum EPA and DHA concentrations suggest that either these n-3 FAs were being metabolized rapidly in sepsis patients or that absorption of enteral medications and pharmaconutrients, including fish oil, may be impaired in sepsis patients. If enteral absorption is impaired, doses of enteral medications administered to critically ill patients may be suboptimal.

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1. Introduction

Eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), two essential omega-3 (n-3) fatty acids found in fish oil, have several anti-inflammatory properties including: 1) replacement of arachidonic acid (AA) in white blood cell membranes thereby restricting metabolism of AA into more inflammatory eicosanoid mediators, 2) metabolism themselves into less inflammatory eicosanoids than those derived from n-6 fatty acids, and 3) metabolism into a series of mediators that participate in the resolution of inflammation (resolvins and protectins) [1]. Some prior studies investigating fish oil administration via continuous enteral feeding formula as a therapy in critical illness have found benefits (e.g. reduced mortality, risk of developing new organ failures, time on mechanical ventilation, and ICU length of stay), particularly in patients with ARDS [2–5], although other studies in which fish oil was administered by enteral boluses found no benefit [6,7]. A 2008 meta-analysis suggested reduction in mechanical ventilation duration in patients with sepsis who had been provided fish oil supplementation [8]. Among multiple other factors, potential reasons for the differences in outcomes of these studies could be dose, route of administration, and/or dosing frequency [8,9].

Although pharmaconutrients seldom undergo rigorous pharmacokinetics (PK) and phase I dose-finding studies similar to those required for pharmaceuticals, PK of EPA/DHA have been studied in healthy volunteers [9–16]. However, as is typical with both pharmaceuticals and pharmaconutrients, EPA/DHA PK have not been investigated in critically ill patients. In such patients, metabolism can be highly deranged due to multiple organ dysfunction, including reduced gastrointestinal (GI) motility and pancreatic and hepatic function. With prior literature suggesting that organ dysfunction alters drug metabolism and kinetics, it is biologically plausible that many agents, especially those delivered enterally, including EPA and DHA, exhibit different kinetics in critically ill patients than in healthy volunteers [17,18]. We undertook the present study to compare PK of enteral EPA and DHA in sepsis patients versus healthy volunteers.

2. Materials and methods

2.1. Study design

Patients ≥18 years old with severe sepsis at risk for acute respiratory distress syndrome (ARDS), but not meeting ARDS criteria, and 15 healthy volunteers were enrolled in this cohort study from January 2009 to July 2012 at the University of Vermont Medical Center (UVMMC) in Burlington, Vermont. As this study began in 2009, severe sepsis was defined as 1) meeting two or more of the following four criteria: (a) temperature >38.5 °C or <35.0 °C; (b) heart rate >90 beats/min; (c) respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg; and (d) WBC count >12,000 cells/mL or <4000 cells/mL, or >10% immature (band) forms; and 2) strongly suspected or documented infection (culture or Gram stain of blood, sputum, urine, or normally sterile fluid positive for pathogenic microorganism) [19]. All medical ICU patients at UVMMC were screened daily for eligibility. Once identified, eligible patients or surrogates were approached and underwent informed consent. Enrollment occurred within 36 h of severe sepsis diagnosis, and none had the diagnosis of ARDS at enrollment. Patients who met the aforementioned definition of severe sepsis and were ≥18 years of age were included. Exclusion criteria were the following: >36 h passed since meeting severe sepsis criteria, expected ICU length of stay ≤72 h, already met criteria for ARDS, unable to obtain enteral access, post cardiac arrest with significant anoxic brain injury, expected survival ≤28 days from underlying condition, platelet count <30,000, active bleeding, INR >3, known allergy to fish oil, taking fish oil supplement during past 3 months, or pregnant. Because shock may alter gut perfusion and fat absorption, patients with a mean arterial pressure (MAP) < 60 mmHg with or without vasopressors were excluded. If, however, a patient in shock subsequently stabilized with MAP >60 mmHg, s/he was considered eligible, provided that less than 36 h had passed since the diagnosis of sepsis.

Healthy volunteers were recruited locally via advertisement, underwent informed consent, and were compensated for participating. Healthy volunteers were gender- and age-matched ± 15 years to the sepsis patients. Healthy volunteers were excluded if they had a known allergy to fish oil, or were taking a fish oil supplement during past 3 months, pregnant, or ≤18 years of age. This study was approved by the University of Vermont Human Subjects Committee.

2.2. Study procedures

2.2.1. Intervention (Fig. 1)

All subjects received a total of 9.75 g EPA and 6.75 g DHA daily to mimic the total daily dose of EPA and DHA in prior studies that had demonstrated benefit [2–4]. Within 6 h of enrollment, patients with sepsis began receiving 15 ml of the concentrated fish oil product ProOmega (Nordic Naturals) via an oral or nasogastric feeding tube every 12 h for 7 days or until death. If the enteral feeding tube was removed when a patient was extubated, fish oil was given orally in capsular form if possible. Healthy volunteers were administered EPA/DHA (fish oil) twice a day for 7 days in capsular form in the same dose as patients with severe sepsis. Seven days was chosen for duration of fish oil administration due to 1) feasibility in both healthy volunteers and sepsis patients and 2) because we anticipated that patients with sepsis would be mechanically ventilated for approximately one week. Blood was collected for measurements and assays as shown in Fig. 1, which also shows the number of participants in each group whose samples were obtained at each time point. We began the study with the cohort of healthy volunteers, and after 7 volunteers, we analyzed all samples. This mid-study analysis demonstrated that concentrations followed a predictable pattern and allowed us to determine that we did not need to collect blood on several of the originally planned time points (e.g. we stopped collecting blood for PBMC and neutrophil isolation on study days 2, 5, and 11). Also of note, blood was not collected from critically ill patients if they died or if they were discharged from the ICU; therefore, the number of samples available decreases over the study period. Stool samples were also collected on study day #3 or later from both healthy volunteers and patients with severe sepsis.

2.2.2. Co-interventions

Participants with sepsis received standardized enteral feeding [20] started at 20 cc/hour and increased to goal rate as tolerated per the UVMMC enteral feeding protocol; patients did not receive other lipids beyond the study intervention. Volunteers were told to continue their regular diet but not to eat fish or other sources of EPA/DHA at the time of enrollment until the study concluded.

2.3. Measurements and assays

1) Blood collection: To study fatty acid pharmacokinetics in serum and leukocytes, blood was collected by venipuncture (or through central lines in ICU patients when applicable) as shown Fig. 1.

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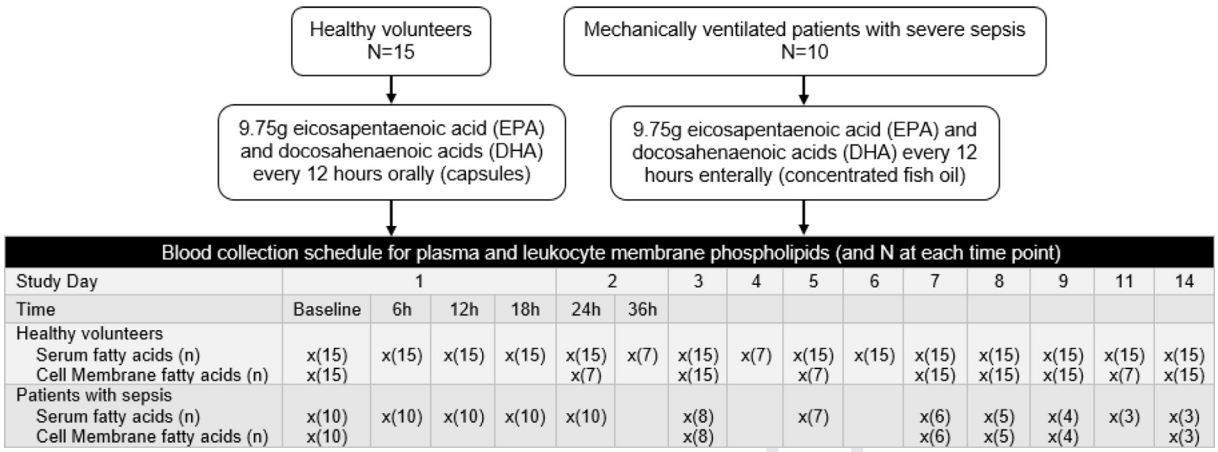

Blood collection schedule for plasma and leukocyte membrane phospholipids (and N at each time point)

Study Day	1				2		3	4	5	6	7	8	9	11	14
Time	Baseline	6h	12h	18h	24h	36h									
Healthy volunteers															
Serum fatty acids (n)	x(15)	x(15)	x(15)	x(15)	x(15)	x(7)	x(15)	x(7)	x(15)	x(15)	x(15)	x(15)	x(15)	x(15)	x(15)
Cell Membrane fatty acids (n)	x(15)				x(7)		x(15)		x(7)		x(15)	x(15)	x(15)	x(7)	x(15)
Patients with sepsis															
Serum fatty acids (n)	x(10)	x(10)	x(10)	x(10)	x(10)		x(8)		x(7)		x(6)	x(5)	x(4)	x(3)	x(3)
Cell Membrane fatty acids (n)	x(10)						x(8)				x(6)	x(5)	x(4)		x(3)

Fig. 1. EPA and DHA dose administration schedule, blood collection time points, and number of patients whose samples were obtained at each time point for both healthy volunteers and patients.

2) Leukocyte isolation: To measure EPA and DHA incorporation into leukocyte membranes, where most biologic activity of these fatty acids is thought to occur, peripheral blood mononuclear cells (PBMCs) and neutrophils were isolated from blood as previously described at the time points shown in Fig. 1 [21–24]. Briefly, venous blood was citrated (4.4 ml of 3.8% sodium citrate to 40 ml blood) and centrifuged at 1250 rpm for 20 min. The upper platelet rich plasma layer was aspirated and centrifuged at 3000 rpm for 15 min for production of platelet-poor plasma (PPP). To the remaining red and white cell layer, 5 ml of prewarmed 6% dextran and 0.9% saline to 50 ml were added, mixed gently, and allowed to sediment for 45 min. After sedimentation, the upper leukocyte-rich layer was harvested and centrifuged at 1000 rpm for 6 min, gently re-suspended in 2 ml PPP, and transferred to a 15-ml polystyrene tube. The cell suspension was then under-layered with 2 ml of 42% Percoll (Sigma) in PPP followed by 2 ml of 51% Percoll in PPP. The gradients were centrifuged at 200×*g* for 10 min. The PBMC-enriched fraction was harvested, washed three times with calcium and magnesium-free phosphate-buffered saline, and re-suspended in RPMI 1640 tissue culture media with 25 mM HEPES and L-glutamine, containing 10% fetal bovine serum (Gibco BRL, Grand Island, NY) as previously described [22]. The neutrophil-rich fraction was collected and washed three times in RPMI 1640 culture medium (BioWhittaker, Walkersville, MD) [23].
3) Phospholipid extraction, separation, and measurement: Total plasma, PBMC, and neutrophil lipids were extracted by methods previously described [25,26]. Briefly, 2:1 chloroform:methanol solution containing 0.05 mg/ml butylated hydroxytoluene was used for lipid extraction, after addition of internal standards. After centrifugation at 3500 rpm for 30 min at 4 °C, samples were dried under nitrogen, and lipids were resuspended in chloroform and applied to a thin layer chromatography (TLC) plate (Whatman, Maidstone, UK). The TLC plates were developed in hexane:diethyl ether:acetic acid (70:30:1). Lipid bands were visualized and scraped into glass vials. Samples were then transmethylated with 5% sulfuric acid in methanol, and methyl esters were extracted with petroleum ether after addition of distilled water. Samples were thoroughly mixed and centrifuged at 1500 g. The petroleum ether phase was transferred and dried under nitrogen. Methyl esters were then resuspended in dichloromethane and transferred to gas chromatography (GC) autosampler vials and analyzed on an Agilent 6890N Series Gas Chromatograph with an Agilent 7683 AutoSampler and Injector (Agilent Technologies, Santa Clara, CA) equipped with a 30 m × 0.25 mm fused silica capillary column (catalog number 24019; Supelco, Bellefonte, PA). To determine fatty acid concentration in the GC injection vial, we added fatty acid-specific methy ester standards. We chose to measure the actual serum concentrations of EPA and DHA in the total lipids, as most scientific data suggests that virtually all of the ethyl esters (prodrug forms) consumed are removed in the digestion/absorption process and only trace levels of omega-3 fatty acid ethyl esters are found in plasma [27]. Total serum, PBMC, and neutrophil arachidonic (AA) levels were also measured as above to discern if these might decrease concordant with increases in EPA/DHA concentrations.
4) Stool fat measurement: In order to ensure that EPA/DHA absorption is complete, we qualitatively measured stool neutral and split fats, with microscopy and stain, in patients and volunteers in stool samples sent to the clinical lab.

2.4. Data analysis

Based on prior studies [10–12] and presuming first order kinetics of omega-3 fatty acids, we hypothesized a putative model to describe the pharmacokinetics of fish oil (Fig. 2). EPA and DHA levels from blood samples were used to calculate PK parameters using this model. The development of the model equations and the

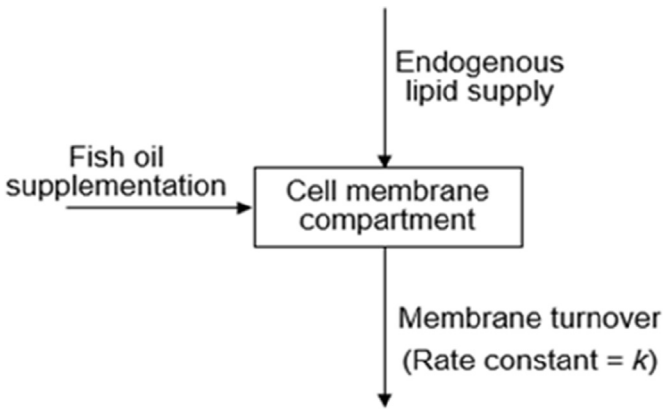

Fig. 2. Pharmacokinetic model of fish oil uptake and excretion (see Appendix).

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method used to fit the model to data are described in the Appendix (supplement).

We compared longitudinal changes in EPA, DHA, and AA in serum, PBMCs, and neutrophils between patients with sepsis and healthy volunteers by fitting mixed linear regression models with unstructured covariance matrices for the repeated measures. We also evaluated individual time points between the two groups using unpaired t-tests. $P < 0.05$ was considered statistically significant, and Stata version 14 (StataCorp LLC) was used for all analyses.

3. Results

Characteristics and outcomes of the 15 healthy volunteers and 10 patients with sepsis are shown in Table 1. All participants were Caucasian. Healthy volunteers were 41.1 ± 10.3 years and had body mass index (BMI) of 25.4 ± 4.2; 67% were women. Mechanically ventilated patients with severe sepsis were 55.6 ± 13.4 years, 50% women, and had acute physiologic and chronic health evaluation (APACHE) II score = 27.2 ± 8.8 and sequential organ failure assessment (SOFA) score = 10.5 ± 3.4 within 24 h of ICU admission. Patients with sepsis had BMI = 35.7 ± 8.3, and received MV for a median of 10.5 days (interquartile range [IQR] 3–15) days and were in the ICU for 11.5 (IQR 4–17) days. Of the 10 subjects with sepsis enrolled, 2 (20%) died. Blood was collected from all.

As shown in Fig. 3, panels A and D, PBMC EPA concentrations over time were not different between groups ($p = 0.56$), while PBMC DHA was higher in sepsis patients both over time ($p = 0.003$) and at several individual time points. Neutrophil EPA and DHA concentrations were also similar between the healthy volunteers and patients with sepsis (Fig. 3, panels B and E, $p = 0.12$ and 0.43 respectively).

Notably, serum EPA ($p < 0.001$) and DHA ($p < 0.001$) were significantly lower in patients with sepsis vs. volunteers over time (Fig. 3, panels C and F). After fitting the data to PK model (Fig. 2), we estimated the half-life of EPA and DHA in PBMCs, neutrophils, and serum (Table 2), and found that the half-life of EPA in serum (2.4 vs 3.2 days, $p = 0.0001$) and neutrophils (2 vs 2.7 days, $p = 0.007$) was significantly shorter in patients with sepsis than in volunteers. EPA half-life in PBMCs and all DHA half-lives were not different between the two groups, as shown in Table 2.

Arachidonic acid (AA) was also measured in PBMC and neutrophil membranes and in serum. As shown in Fig. 3, PBMC membrane AA concentrations (Panel G) were not different over time between the sepsis and healthy patient groups ($p = 0.19$), while neutrophil membrane AA concentrations (Panel H) were higher in healthy volunteers than in patients with sepsis over time ($p = 0.05$). In serum, similar to EPA and DHA, AA levels were greater in healthy volunteers than in sepsis patients (Panel I, $p < 0.001$). Of note, while EPA and DHA concentrations in leukocyte membranes generally rose over the study period in both healthy volunteers and patients with sepsis, we did not observe a corresponding decrease over time in AA in leukocyte membranes.

Qualitative (increased vs. normal) measurement of fecal neutral and split fats in our hospital clinical laboratory by microscopic examination of a fecal smear stained with Sudan III occurred in 5 patients with sepsis (of the remaining 5 patients, 3 did not produce any stool during the study period and 2 other stool samples were deemed inadequate for stool fat measurement by the clinical lab) and all 15 healthy volunteers. Though stool neutral fats were normal in all 15 healthy volunteers, they were increased in all 5 sepsis patients. Stool split fats were increased in 5 sepsis patients and 14 healthy volunteers.

There were no serious adverse events related to the study in any study participant. Of the 10 patients with sepsis, 7 had some degree of diarrhea measured qualitatively by asking critical care nurses. The number of distinct bowel movements was difficult to quality because the majority of our patients with sepsis had rectal tubes in place. One of these patients with diarrhea was found to have Clostridium difficile infection. An exact etiology for the other 6 cases was not determined, but as the diarrhea began in most patients approximately day 3–6 after beginning the study, it is reasonably likely to have been related to fish oil. None of the healthy volunteers reported any diarrhea. Fish oil delivery did not alter INR or platelet count in any study participant.

Table 1
Characteristics of control and sepsis patients.

	Control n = 15	Sepsis n = 10
Age (years ± SD)	41.1 ± 10.3	55.6 ± 13.4
Gender (% female)	67%	50%
Ethnicity (% White)	100%	100%
Body Mass Index (mean ± SD)	25.4 ± 4.2	35.7 ± 8.3
APACHE II Score	N/A	27.2 ± 8.8
SOFA Score (mean ± SD)	N/A	10.5±
Mechanical Ventilation Days (median [IQR])	N/A	10.5 [3–15]
ICU length of stay, days (median [IQR])	N/A	11.5 [4–17]
Hospital Mortality (% died)	N/A	20%
Baseline PBMC Fatty Acids (μg/10^6 cells)		
EPA	0.016 ± 0.017	0.017 ± 0.037
DHA	0.116 ± 0.075	0.224 ± 0.131
AA	1.042 ± 0.850	1.037 ± 0.887
Baseline Neutrophil Fatty Acids (μg/10^6 cells)		
EPA	0.003 ± 0.005	0.015 ± 0.003
DHA	0.023 ± 0.024	0.086 ± 0.036
AA	0.520 ± 0.227	0.428 ± 0.154
Baseline Serum Fatty Acids (μg/100 μL)		
EPA	6.84 ± 9.67	2.35 ± 4.04
DHA	11.11 ± 6.83	3.72 ± 3.45
AA	33.43 ± 24.74	6.92 ± 6.10

4. Discussion

The main finding of our study, seemingly the first to compare pharmacokinetics of EPA and DHA in critically ill patients to healthy volunteers, is that serum concentrations of EPA and DHA in patients with sepsis were significantly lower than in healthy volunteers receiving identical high doses of enteral fish oil for one week. Additional findings include that incorporation of EPA and DHA into PBMC and neutrophil membranes was relatively similar between healthy volunteers and sepsis patients, and that AA in PBMC membranes were not different between the groups over time, while neutrophil membrane AA concentrations were higher in healthy volunteers than in patients with sepsis.

Our results in healthy volunteers, to which our patients with sepsis are compared, are generally consistent with availably limited prior data. Prior studies on pharmacokinetics of EPA and DHA in healthy volunteers have demonstrated varied durations of reaching plasma steady state levels ranging from 4 days to 4 weeks (slower for DHA compared to EPA) [9,13–16,28–31]. DHA in erythrocyte membranes reached steady state in approximately 4 months, consistent with slow erythrocyte turnover [31]. DHA was also more slowly cleared from both plasma and red blood cells than EPA [31,32]. In another study, high dose EPA (6.0 g/day) and DHA (5.3 g/day) were administered twice daily for 7 days to five healthy volunteers [11]. Plasma levels of EPA peaked by day 4 and returned to baseline after 7 days of washout, while DHA increases were less pronounced. The estimated plasma half-life of EPA in normal subjects was 1.6–2.3 days. This estimated EPA half-life in plasma is slightly shorter than our finding of 3.2 days in serum of healthy volunteers, which may relate to differences in subject characteristics. For example, prior studies have demonstrated that both BMI and age, but not gender, affect EPA half-life [33–35]. The five

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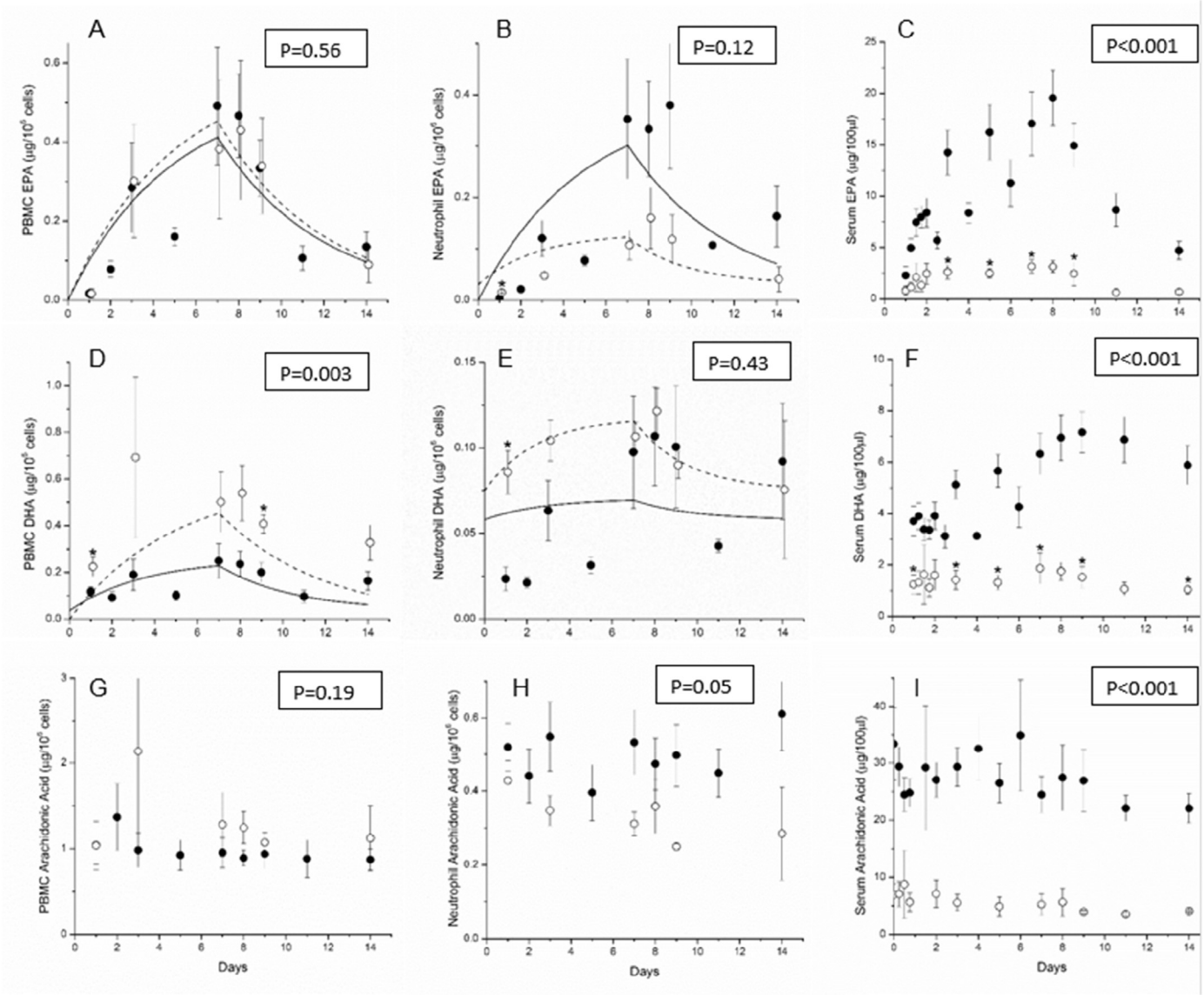

Fig. 3. EPA, DHA and arachidonic acid levels in PBMCs, neutrophils and serum. Closed and open circles and error bars indicate mean ± SD in healthy volunteers and patients with sepsis, respectively. Solid and dashed curves represent model fit to data from healthy volunteers and patients with sepsis, respectively. P-values in boxes indicates results from mixed linear regression modeling. Asterisks (*) indicate individual time points where concentrations are significantly different between groups.

Table 2
Half life ± estimated SD of EPA and DHA in PMBCs, neutrophils, and serum (days).

	PMBC EPA	PMBC DHA	Neutrophil EPA	Neutrophil DHA	Serum EPA	Serum DHA
Healthy Volunteers (n = 15)	2.7 ± 1	2.3 ± 0.5	2.7 ± 0.7	1.4 ± 0.3	3.2 ± 0.3	0.8 ± 1.1
Sepsis Patients (n = 10)	3.2 ± 1	1.9 ± 1.7	2 ± 0.3	1.6 ± 0.2	2.4 ± 0.4	0.3 ± 0.4
P value	0.20	0.40	0.007	0.08	0.0001	0.2

healthy volunteers in this particular prior investigation (11) were all of normal BMI (20–25 kg/m^2), while the mean BMI of our healthy volunteers was 25.4 kg/m^2. Additionally, our healthy volunteers were older (mean age 41.1 years compared to all participants in the prior study being ages 23–30 years).

While we found that serum concentrations of EPA and DHA were significantly lower in patients with sepsis than in healthy volunteers (and indeed did not increase much above baseline serum levels), we concomitantly found that, with the exception of DHA in PBMC membranes, the incorporation of EPA and DHA into leukocytes was generally not different in our sepsis patients compared with healthy volunteers, One potential explanation for the higher serum EPA and DHA levels in healthy volunteers compared with critically ill patients is poor intestinal absorption. For n-3 fatty acids to be absorbed (when provided as ethyl esters), the ethyl ester bond must undergo hydrolysis by pancreatic lipase to be converted to free fatty acids (EPA, DHA) for intestinal absorption [27,32]. To confirm absorption, we were able to measure qualitative levels of fecal neutral and split fats for 5 patients with sepsis (the remaining 5 patients did not produce any stool during

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the study period) as well as for all 15 healthy volunteers. Neutral fats include mono, di- and triglycerides, while split fats are the free fatty acids that are liberated from them. Impaired synthesis or secretion of pancreatic enzymes or bile may cause an increase in neutral fats while an increase in split fats suggests impaired absorption of nutrients [36–38]. Though neutral fats were normal in all 15 healthy volunteers, they were increased in all 5 patients with sepsis in whom we obtained stool samples. Biliary stasis is known to occur in sepsis and could explain the elevated neutral fat levels in sepsis patients [36–39]. The split fat levels were increased in 5 sepsis patients and 14 healthy volunteers, which implies some degree of impaired intestinal absorption in most participants, perhaps due to the large bolus of fat received. However, because this measurement is qualitative, we do not know if the degree of impairment in patients with sepsis exceeded that of healthy volunteers. Nonetheless, the higher serum EPA and DHA levels in healthy volunteers compared with critically ill patients suggest that reduced intestinal absorption may be important in patients with sepsis.

Another potential explanation for the lower serum EPA and DHA concentrations seen in patients with sepsis is that higher blood leukocyte counts could have led to a decrease in serum levels with the increased pool of circulating leukocytes incorporating these fatty acids. We therefore compared WBC counts in the two patient groups. In patients with sepsis, maximum WBC counts throughout the hospital stay varied between patients from 1.21 k/cmm to 30.01 k/cmm with a mean of 16.2 ± 9.8, whereas the WBC count in healthy volunteers ranged from 3.27 k/cmm to 8.97 k/cmm with a mean of 6.7 ± 1.4. The WBC count was significantly higher in the sepsis group compared to healthy volunteers ($p = 0.001$). Thus, it is possible that more rapid cell turnover and the greater number of circulating WBCs in sepsis patients could have contributed to lower serum EPA and DHA levels. Finally, a third potential explanation is that omega-3 fatty acids are rapidly oxidized in sepsis, which would be consistent with prior reports that whole body fat oxidation is increased in critical illness [40,41].

We also found that WBC membrane concentrations of omega-3 fatty acids were not different between the groups, suggesting that the mechanisms of cellular membrane incorporation are not necessarily impaired in critical illness. Explanations for this are perhaps less clear, but one possibility could certainly be that, as explained above, circulating WBCs are greater in number in patients with sepsis and work to scavenge available fatty acids from the blood – thus resulting in the concentration of omega-6 fats in the same volume of cell membrane appearing similar as in healthy patients but serum concentrations appearing lower.

This combination of findings – similar leukocyte membrane concentrations of EPA and DHA in healthy volunteers and patients with sepsis, yet much higher serum concentrations in healthy volunteers – implies that serum concentration is not the dominant driver of leukocyte membrane incorporation of EPA and DHA. Additionally we also found that the half-life of EPA in neutrophils and in serum was significantly shorter in patients with sepsis, while this was not true for PBMC half-life or for DHA in serum, neutrophils, or PBMCs. This reduced EPA half-life in neutrophils and serum suggests that clearance mechanisms are increased in patients with sepsis, although those mechanisms remain unclear. The clinical implications of these findings in terms of effects on innate or adaptive immunity are not clear, as available evidence from clinical research studies does not demonstrate a clear therapeutic benefit of n-3 FA administration in critically ill patients. However, perhaps reduced enteral absorption of n-3 FA is one explanation for some prior studies finding no benefit in patients who are critically ill.

We did not find that concentrations of AA in PBMC and neutrophil membranes decreased over the study period in either healthy volunteers or patients with sepsis. As one anti-inflammatory mechanism of action of EPA and DHA is replacement of arachidonic acid in leukocyte membranes, we might have expected to observe a corresponding drop in AA concentrations with our observed rise in EPA and DHA concentrations. Reasons for not finding a decrease in AA are unclear but are consistent with a prior study that found that AA in neutrophil lipids remained relatively constant across two different oral doses of omega-3 fatty acids (both of which were notably lower than our dose) [42]. Our results are similar to another study in critically ill patients where the intervention group received omega-3 fatty acid supplementation compared to a control group who received an isocaloric supplement with higher protein but less fat (and no omega-3 fatty acids), and there were no differences in serum AA between the treatment and control groups [5].

There are several limitations to our study. First, the sample size is relatively small, and we may have been able to detect differences in leukocyte membrane content with a larger study. Additionally, later data points on the sepsis patients are missing in some individuals because they dropped out of the study due to discharge from the ICU (with no enteral tube and inability to swallow large capsules) or death. We also excluded patients with MAP<60 mmHg, thus limiting generalizability of our results. Further, this cohort study was not intended to be a dose-finding study, so we do not have data from subjects receiving different doses. Finally, our stool fat measurement is qualitative rather than quantitative, so we do not specifically know the fat loss through stool and can therefore not gauge the degree of intestinal absorption impairment between the 2 patient groups.

In summary, while leukocyte membrane EPA and DHA concentrations appear not to differ between healthy volunteers and sepsis patients, serum concentrations are significantly lower in patients with sepsis. These results suggest that serum measurements of EPA and DHA alone are inadequate to assess PK. Additionally, our findings indicate that doses of enteral medications administered to critically ill patients may be suboptimal, and further pharmacokinetic studies of enteral medication in critically ill patients are warranted.

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Statement of authorship

Drs. Parikh, Bates, Poynter, Suratt, Parsons, Kien, Heyland, and Stapleton participation in conception and design of this work. Dr. Parikh, Dr. Stapleton, Ms. Garudathri, Ms. Crain, and Ms. Martin collected the data. Drs. Parikh, Bates, Poynter, Suratt, Parsons, Kien, Heyland, and Stapleton analyzed and interpreted the data. Drs. Parikh, Bates, and Stapleton drafted the manuscript. All 11 co-authors critically revised the manuscript and approved the final version.

Conflict of interest

The authors declare no conflict of interest.

Appendix

We modeled the fate of orally ingested fish oil in terms of a single compartment representing the lipid compartment in the cell

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membrane (Fig. A1). We assumed that the EPA and DHA components of the fish oil supplement each become incorporated into the cell membrane at a rate $a(t)$ over the period during which fish oil is being consumed. In addition, lipid is incorporated into the cell membrane compartment from endogenous sources at a fixed rate E. We further assume that each component is cleared from the cell membrane with time-constant k as a result of steady turnover.

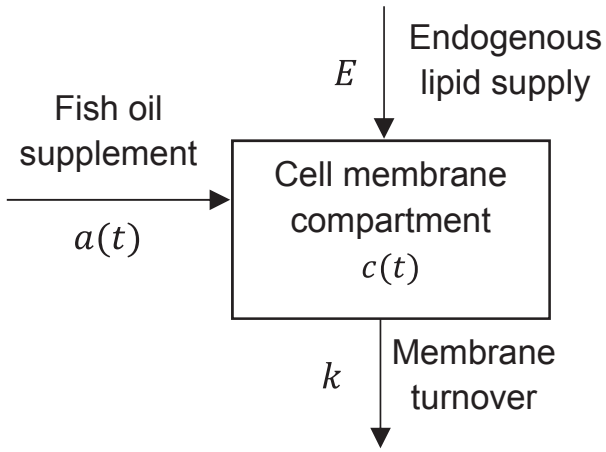

Figure A1. Model of fish oil incorporation into the plasma membrane following oral supplementation. Fish oil is provided over a finite time window $a(t)$. ainst a steady background, E.,f endogenous lipid supply to the cell membrane. Membrane turnover, and thus clearance of fish oil, occurs according to first-order kinetics with rate-constant k.

The equation describing the concentration $c(t)$ of either EPA or DHA in the cell membrane compartment is

$$\frac{dc(t)}{dt} = a(t) + E - kc(t) \quad \text{(A1)}$$

where

$$a(t) = A \text{ for } 0 < t < T \quad \text{(A2a)}$$

and

$$a(t) = 0 \text{ for } t < 0, t > T \quad \text{(A2b)}$$

The half-life, $t_{\frac{1}{2}}$, of clearance from the cell membrane compartment, is

$$t_{\frac{1}{2}} = k \cdot ln(2) \quad \text{(A3)}$$

The parameter A reflects the fish oil dose and T is the duration over which the fish oil supplementation was administered. Note that we assume here that the supplementation was a constant infusion even though it was actually administered as a pill twice a day. Importantly, because A is an adjustable parameter of the model that is evaluated during the model fitting process, we allow for differences between patient groups in the rates at which fish oil makes it way from the gut lumen to the cell membrane. Such differences might be due to, for example, differences in the rates of intestinal absorption between healthy subjects and septic patients.

Equations A1 and A2 were fit to the average EPA versus t and DHA versus t data sets for both control and patient groups. Fitting was achieved by finding the best-fit values of the model parameters E, k and A using a grid-search procedure that searched on a $10 \times 10 \times 10$ grid of values encompassing a wide range for each parameter. The ranges of the grid were then refined successively until they were within 1% of the parameter values that minimized the mean squared residual (RMSR) between the data and the fitted curve.

Standard deviations for the best-fit values of E, k and A were obtained using a Monte-Carlo procedure in which 512 synthetic data sets were constructed by adding to the model predictions at each time point a zero-mean Gaussian-distributed random number having variance equal to RMSR. The model was fit to each synthetic data set and the standard deviations of the resulting values of E, k and A were determined.

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