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Effect of fish oil on monoepoxides derived from fatty acids during cardiac surgery[1] [S]

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Abstract Our objective was to assess the dynamics of monoepoxides derived from polyunsaturated fatty acids (MEFAs), and their response to n-3 PUFA supplementation, in the setting of acute tissue injury and inflammation (cardiac surgery) in humans. Patients (479) undergoing cardiac surgery in three countries were randomized to perioperative fish oil (EPA + DHA; 8–10 g over 2–5 days preoperatively, then 2 g/day postoperatively) or placebo (olive oil). Plasma MEFAs derived from n-3 and n-6 PUFAs were measured 2 days postoperatively. Based on serial measures in a subset of the placebo group, levels of all MEFAs declined substantially following surgery (at postoperative day 2), with declines ranging from 37% to 63% ($P < 0.05$ each). Compared with placebo at postoperative day 2, levels of EPA- and DHA-derived MEFAs were 40% and 18% higher, respectively ($P \leq 0.004$). The n-3 PUFA supplementation did not significantly alter the decline in n-6 PUFA-derived MEFAs. Both enrollment level and changes in plasma phospholipid EPA and DHA were associated with their respective MEFAs at postoperative day 2 ($P < 0.001$). [JLR] Under the acute stress of cardiac surgery, n-3 PUFA supplementation significantly ameliorated the reduction in postoperative n-3 MEFAs, but not n-6 MEFAs, and the degree of increase in n-3 MEFAs related positively to the circulating level of their n-3 PUFA precursors.—Akintoye, E., J. H. Y. Wu, T. Hou, X. Song, J. Yang, B. Hammock, and D. Mozaffarian. **Effect of fish oil on monoepoxides derived from fatty acids during cardiac surgery. *J. Lipid Res.* 2016.** 57: **492–498.**

Supplementary key words diet and dietary lipids • inflammation • omega-3 fatty acids • phospholipids • vascular biology • randomized controlled trial

Growing evidence highlights the importance of endogenous anti-inflammatory mediators in appropriate modulation of inflammatory responses and suggests that breakdowns in the metabolism of these highly bioactive molecules may underlie disease development (1–5). One key class of these metabolic regulators is the monoepoxides derived from polyunsaturated fatty acids (MEFAs), synthesized from long-chain omega-3 (n-3) and omega-6 (n-6) PUFAs by cytochrome P450 (CYP450) enzymes (**Fig. 1**) (6). Once synthesized, MEFAs can be incorporated into membrane phospholipids or further metabolized by soluble epoxide hydrolase to their less active corresponding diols. In experimental and animal models, MEFAs have potent anti-inflammatory and vascular protective properties, suggesting potential for development of MEFA-based therapies to target inflammatory disorders and cardiovascular diseases (3, 5, 7). However, MEFAs have rarely been measured in humans, and important questions remain regarding their usual physiological concentrations, the interrelationship between different MEFAs derived from different classes of PUFAs, and if and by how much endogenous levels of

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Abbreviations: AA, arachidonic acid; CV, coefficient of variation; CYP450, cytochrome P450; EET, epoxyeicosatrienoic acid; LA, linoleic acid; MEFA, monoepoxide derived from polyunsaturated fatty acid; n-3 PUFA, omega-3 PUFA; n-6 PUFA, omega-6 PUFA; OPERA, Omega-3 Fatty Acids for Prevention of Postoperative Atrial Fibrillation; PoAF, postoperative atrial fibrillation.

[1] This trial is registered at www.clinicaltrials.gov as #NCT00970489.

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[S] The online version of this article (available at http://www.jlr.org) contains a supplement.

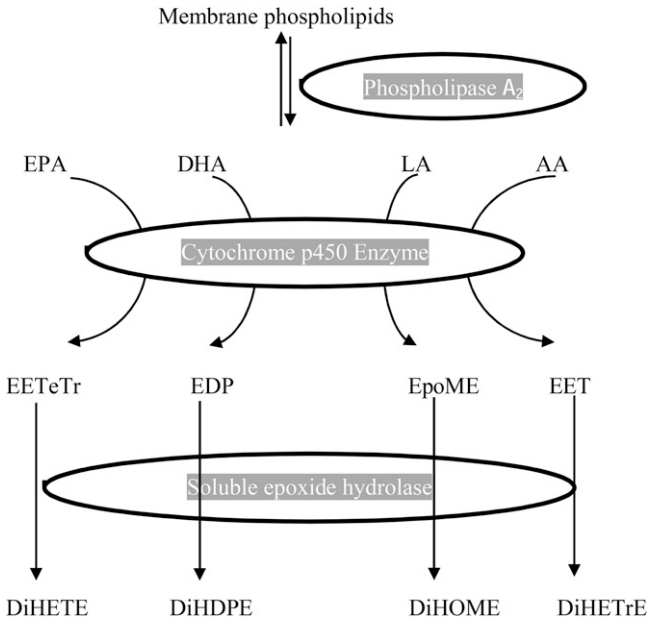

Fig. 1. Metabolism of MEFAs. MEFAs are CYP450-derived oxylipins that are formed when members of CYP450 epoxygenases act at any of the double bonds of PUFAs. Principal MEFAs include regioisomers of epoxyeicosatetraenoic acids (EETeTrs) from EPA, epoxydocosapentaenoic acids (EDPs) from DHA, epoxyoctadecenoic acid (EpoME) from linoleic acid (LA), and epoxyeicosatrienoic acids (EETs) from arachidonic acid (AA). Once synthesized, MEFAs can be incorporated into membrane phospholipids or further metabolized by soluble epoxide hydrolase to their respective diols including dihydroxyeicosatetraenoic acid (DiHETE); dihydroxydocosapentaenoic acid (DiHDPE); dihydroxyoctadecenoic acid (DiHOME); and dihydroxyeicosatrienoic acid (DiHETrE).

MEFAs could be altered by changing dietary intake of their PUFA precursors. These questions are particularly relevant in the setting of acute tissue injury and stress, when their inflammation-resolving activities may be most relevant.

To address these gaps in knowledge, we aimed to investigate the interrelationships between dietary n-3 PUFAs and circulating MEFAs in a human model of acute tissue injury and stress, namely cardiac surgery. Cardiac surgery is known to elicit both systemic and cardiac tissue proinflammatory responses (8, 9). However, ensuing levels of novel anti-inflammatory mediators such as MEFAs have not been established in this clinically important population. In a prospectively designed ancillary trial nested within the Omega-3 Fatty Acids for Prevention of Postoperative Atrial Fibrillation (OPERA) trial, we quantified the postoperative circulating levels of MEFAs derived from n-3 and n-6 PUFAs, and determined their response to n-3 PUFA supplementation. We also tested the hypothesis that increased availability of PUFA precursors, measured as plasma phospholipid PUFA levels, would positively associate with postoperative plasma concentrations of their downstream MEFA metabolites.

METHODS

Study design and patients

This trial was a prospectively designed ancillary investigation nested within the OPERA trial (10), a randomized, double-blind, placebo-controlled trial that tested the effect of perioperative fish oil supplementation on postoperative atrial fibrillation (PoAF) in patients undergoing cardiac surgery. The design and primary results of OPERA have been described (10, 11). Briefly, 1,516 patients undergoing cardiac surgery across 28 centers in the United States, Italy, and Argentina were recruited between August 2010 and June 2012. Broad inclusion criteria captured a generalizable patient population: age ⩾18 years; scheduled for cardiac surgery (any combination of coronary artery bypass, valve surgery, or other cardiac surgery opening the pericardium); and sinus rhythm at enrollment. Exclusion criteria were regular use (⩾3 days/week) of fish oil within the prior 4 weeks; known allergy to fish oil or olive oil (placebo); current pregnancy; or inability to provide informed written consent. The OPERA trial showed that perioperative fish oil supplementation did not significantly reduce the incidence of PoAF. The present investigation utilized 479 subjects from 17 centers in the OPERA trial who provided blood samples and informed written consent to participate in this ancillary biological study. Characteristics of these patients were generally similar to the remainder of the patients in the OPERA trial, except that these patients were more likely to be from the United States and less likely to have congestive heart failure or undergo bypass graft surgery (supplementary Table 1). The study was approved by the human subjects committees of all participating institutions.

Intervention

Patients were randomized to oral n-3 PUFA (1 g capsules of fish oil, each containing 465 mg of EPA + ∼375 mg of DHA, for a total of ∼840 mg of n-3 PUFA) or matched placebo (olive oil, 1 g capsules), provided to have identical appearance. Preoperatively, a total loading dose of 10 g was provided over 3 to 5 days (or 8 g total divided over 2 days), depending on the time period between enrollment and planned surgery. Postoperatively, all patients received 2 g/day until discharge or postoperative day 10, whichever was sooner.

Plasma MEFA and fatty acid measurements

Fasting blood was collected at enrollment, the morning of cardiac surgery, and on postoperative day 2 by trained staff using standardized kits and techniques. EDTA anticoagulated blood samples were centrifuged within 10 min at 4°C to collect plasma. Immediately after centrifugation, plasma was frozen and stored at −70°C to −80°C at each study site, until being express-couriered on dry ice to the centralized OPERA biobank (Fisher Bioservices, Rockville, MD) for storage at −80°C. Plasma samples were subsequently shipped on dry ice to the UC Davis Metabolomics Center for analysis of MEFAs. Prior studies indicate that MEFA levels are stable under these collection and storage conditions (12).

Total MEFA concentrations were measured in all patients at postoperative day 2. In a subset of the placebo group, we also assessed total MEFA levels at enrollment to determine trajectories from preoperative to postoperative day 2. We assessed circulating MEFAs derived from two n-3 PUFAs (EPA and DHA) and two n-6 PUFAs (LA and AA) (6). Due to multiple double bonds, each PUFA gives rise to multiple MEFA regioisomers and respective diol metabolites (Fig. 1). MEFAs and their diol metabolites were analyzed using validated LC/MS/MS as previously described (13, 14). Briefly, nine deuterated internal standards including d4 prostaglandin F1a, d4 thromboxane B2, d4 prostaglandin E2, d4 leukotriene B4, d8 20 HETE, d8 5 HETE, d11 14,15 dihydroxyeicosatrienoic acid, d11 11,12 EET, d4 9 HODE were added to plasma samples, which were then saponified using 1 M Na_2CO_3 in methanol, and MEFAs were extracted by solid-phase extraction method after being neutralized by formic acid followed by the protocol described previously (13). MEFAs were subsequently analyzed using LC/MS/MS. Individual epoxide and diols were identified based on retention

time as well as highly specific multiple reaction monitoring. Quantification was achieved by calculating the ratio of the peak area of each MEFA to deuterated internal standards and relating the ratio to a calibration curve run on the same day as sample analyses. Recovery of MEFAs were excellent (85–94%), with interassay coefficients of variation (CVs) for EPA-, DHA-, LA-, and AA-derived MEFAs ≤15%.

Individual plasma phospholipid fatty acids including PUFAs were measured at enrollment and on the morning of cardiac surgery as weight percentage of total phospholipid fatty acids by the Fred Hutchinson Cancer Research Institute (Seattle, WA), using a validated gas chromatography method as previously described (15). Interassay CVs were ≤2% for EPA, DHA, LA, and AA.

Statistical analysis

Our primary end points were the sum of MEFAs (epoxides plus diol metabolites) derived from each of EPA, DHA, LA, and AA. These sums provide an index of the overall CYP450-mediated epoxygenation toward each of the fatty acids (6, 16). We also secondarily assessed each MEFA regioisomer derived from the same PUFA, as well as the epoxide:diol ratio for each MEFA, an index that is inversely correlated with soluble epoxide hydrolase activity and has been suggested to be a proxy for the enzyme activity in vivo (17, 18). We evaluated this ratio in our study because n-3 PUFA supplementation has been shown in animal studies to change soluble epoxide hydrolase expression (19).

We assessed the influence of n-3 PUFA supplementation on MEFA levels in two ways. First, we assessed differences in postoperative MEFA levels in intention-to-treat analysis between treatment groups using the Wilcoxon rank sum test, providing a randomized, placebo-controlled evaluation of the influence of dietary n-3 PUFA supplementation. Because patients have differing background levels of circulating PUFAs, and also variation in the response in circulating PUFAs following supplementation (15), we additionally evaluated the relationship of enrollment levels and change in levels (from enrollment to the morning of cardiac surgery) of plasma phospholipid PUFAs with their respective MEFAs on postoperative day 2. These relationships were evaluated using multivariable-adjusted linear regression with log-transformed MEFA concentrations as the dependent variable and plasma phospholipid EPA, DHA, LA, or AA as the independent variable. Because lifestyle and demographic characteristics related to circulating MEFAs are not well established, covariates were included via backward stepwise selection (P-exclusion = 0.20, P-inclusion = 0.10), with forcing of major demographics including age, sex, and body mass index, and model-based selection of hypertension, diabetes, smoking, history of heart failure, renal failure, and use of anti-inflammatory medications.

Analyses were performed using STATA 13 (StataCorp, College Station, TX), two-tailed $\alpha = 0.05$.

RESULTS

Mean (SD) participant age was 63 (12) years, and 73% were men. Coronary artery bypass grafting and valvular surgery were the most common types of cardiac surgery performed (**Table 1**). Demographics, cardiovascular risk factors, and surgery characteristics were comparable between treatment groups. MEFA concentrations at enrollment were also similar between treatment groups (supplementary Fig. 1). At enrollment prior to surgery, mean n-6 MEFA levels were ∼2-fold higher than that of n-3 MEFAs, whereas among the n-3 MEFAs, mean DHA-derived MEFA levels were ∼8-fold higher than that of EPA-derived MEFAs.

Based on serial measurements in a subset of the placebo group, levels of EPA-, DHA-, LA-, and AA-derived MEFAs

TABLE 1. Baseline characteristics of 479 patients undergoing cardiac surgery in the OPERA trial, according to treatment assignment

	Placebo (n = 230)	n-3 PUFA (n = 249)	P^a
Age, mean (SD)	63 (12)	62 (13)	0.46
Men, no. (%)	167 (73)	184 (74)	0.75
White race, no. (%)	219 (95)	235 (94)	0.84
Country, no. (%)			
Italy	78 (34)	92 (37)	0.75
United States	123 (53)	125 (50)	
Argentina	29 (13)	32 (13)	
BMI, mean (SD), kg/m^2	29 (6.0)	29 (5.6)	0.45
Waist circumference, mean (SD), cm	101 (15)	100 (17)	0.59
Diabetes mellitus, no. (%)	72 (31)	61 (25)	0.10
Hypertension, no. (%)	178 (77)	190 (77)	0.84
Current smoking, no. (%)	32 (14)	33 (14)	0.89
Dyslipidemia, no. (%)	152 (67)	158 (63)	0.46
Total cholesterol, mean (SD)	177 (40)	173 (40)	0.62
Congestive heart failure, no. (%)	43 (19)	41 (16)	0.52
Chronic renal failure, no. (%)	17(7.4)	12(4.8)	0.24
Type of cardiac surgery, no. (%)[b]			
Valvular	127 (55)	138 (55)	0.96
CABG	115 (50)	114 (46)	0.36
Others	41 (2.2)	46 (18)	0.71

CABG, coronary artery bypass graft surgery. Patients were randomized to oral n-3 PUFA (1 g capsules of fish oil, each containing 850 mg of EPA + DHA) or matched placebo (olive oil, 1 g capsules). Preoperatively, a total loading dose of 10 g was provided over 3 to 5 days (or 8 g total divided over 2 days), depending on the time period between enrollment and planned surgery. Postoperatively, all patients received 2 g/day until discharge or postoperative day 10, whichever was sooner.

[a]Differences between the two groups were evaluated using an unpaired *t*-test or Wilcoxon rank sum test, as appropriate, for continuous variables, and Chi-square test or Fisher's exact (for expected count <5) for categorical variables.

[b]Numbers for each type of cardiac surgery are not mutually exclusive, as some patients underwent multiple procedures.

all declined from presurgery to postoperative day 2, with declines ranging from 37% to 63% (supplementary Table 2). Compared with placebo at postoperative day 2, levels of EPA- and DHA-derived MEFAs were 40% and 18% higher, respectively ($P \leqslant 0.004$ for each; **Table 2**). In contrast, n-3 PUFA treatment did not significantly affect levels of LA-derived (% difference = −2.5, $P = 0.71$) or AA-derived (% difference = −7.3, $P = 0.89$) MEFAs. These effects were generally similar for each MEFA regioisomer derived from the same PUFA precursor. For example, MEFAs derived from DHA were all significantly higher in the n-3 PUFA relative to the placebo group ($P \leqslant 0.02$ for each), with the magnitude of increase varying from 21% to 31%. The n-3 PUFA supplementation also increased the epoxide:diol ratio for EPA-derived MEFAs ($P < 0.001$), with a nonsignificant higher epoxide:diol ratio for DHA-derived ($P = 0.19$), LA-derived ($P = 0.69$), and AA-derived ($P = 0.51$) MEFAs (**Fig. 2**).

When we evaluated the association of plasma phospholipid PUFAs at enrollment with their respective MEFAs at postoperative day 2, levels of plasma phospholipids EPA, DHA, and AA, but not LA, were each positively associated with their respective MEFAs (**Table 3**). For example, each 1 SD higher level of EPA, DHA, and AA concentration at enrollment was associated with 12, 34, and 22% higher levels of EPA-, DHA-, and AA-derived MEFAs, respectively, at postoperative day 2 ($P < 0.001$ each).

From enrollment to postoperative day 2, n-3 PUFA supplementation caused a significant increase in mean plasma phospholipid levels of EPA and DHA, a decrease in LA, and no significant effect on AA (not shown). However, interindividual variability was apparent in these changes following supplementation (supplementary Figs. 2–5). When we evaluated how changes in plasma phospholipid levels of PUFAs from enrollment to the morning of cardiac surgery related to their respective MEFA at postoperative day 2, the change in EPA, DHA, and LA, but not AA, was each positively associated with their respective MEFAs (supplementary Table 3). Results were generally similar when evaluated among only the intervention group, although with lower statistical power as expected (data not shown). Across all analyses, similar findings were observed for each respective MEFA isomers.

DISCUSSION

In this randomized trial of general cardiac surgery patients from the United States, Italy, and Argentina, perioperative n-3 PUFA supplementation significantly increased postoperative levels of n-3 MEFAs, including those derived from EPA and DHA, but had no appreciable effect on levels of n-6 MEFAs. In addition, enrollment level and change (from enrollment to the morning of cardiac surgery) in plasma phospholipid concentrations of EPA and DHA positively associated with their respective MEFAs at postoperative day 2. These findings provide novel evidence that short-term supplementation with n-3 PUFA significantly augments

TABLE 2. Effect of perioperative n-3 PUFA (fish oil) supplementation versus placebo on circulating MEFA concentrations in 479 subjects undergoing cardiac surgery, at postoperative day 2

Plasma MEFA	Placebo (n = 230) Median (IQR) (nM)	n-3 PUFA[a] (n = 249) Median (IQR) (nM)	Percentage Difference between the Two Groups	P^b
EPA derived:				
Sum of MEFAs	7.0 (6)	9.8 (13.7)	40	<0.001
17,18 EETeTr	2.0 (2.2)	3.3 (5.8)	65	<0.001
14,15 EETeTr	1.4 (1.7)	2.7 (4)	93	<0.001
11,12 EETeTr	0.7 (2.1)	1.1 (3.0)	57	0.10
8,9 EETeTr	1.8 (1.4)	2.0 (1.7)	11	0.03
DHA derived:				
Sum of MEFAs	106 (109)	125 (117)	18	0.004
19,20 EDP	15 (16.8)	19 (25)	27	0.003
16,17 EDP	5.8 (6.8)	7.6 (9.8)	31	0.003
13,14 EDP	4.7 (5.2)	5.7 (7.2)	21	0.02
10,11 EDP	5.6 (5.6)	7.0 (7.2)	25	0.004
7,8 EDP	63 (74)	78 (89)	25	0.006
LA derived:				
Sum of MEFAs	159 (149)	155 (165)	−2.5	0.71
12,13 EpoME	89 (84)	86 (84)	−3.4	0.81
9,10 EpoME	73 (66)	68 (72)	−6.8	0.53
AA derived:				
Sum of MEFAs	151 (140)	140 (150)	−7.3	0.89
14,15 EET	44 (64)	47 (65)	6.8	0.71
11,12 EET	36 (39)	35 (37)	−2.8	0.99
8,9 EET	12 (22)	10 (20)	−17	0.52
5,6 EET	48 (42)	47 (43)	−2.1	0.53

IQR, interquartile range.

[a]Patients were randomized to oral n-3 PUFA (1 g capsules of fish oil, each containing 850 mg of EPA + DHA) or matched placebo (olive oil, 1 g capsules). Preoperatively, a total loading dose of 10 g was provided over 3 to 5 days (or 8 g total divided over 2 days), depending on the time period between enrollment and planned surgery. Postoperatively, all patients received 2 g/day until discharge or postoperative day 10, whichever was sooner.

[b]For each class of MEFA, parent MEFAs and diols were summed (the latter representing 1–12% of the total). The statistical significance of differences between the treatment groups was analyzed using Wilcoxon rank sum test.

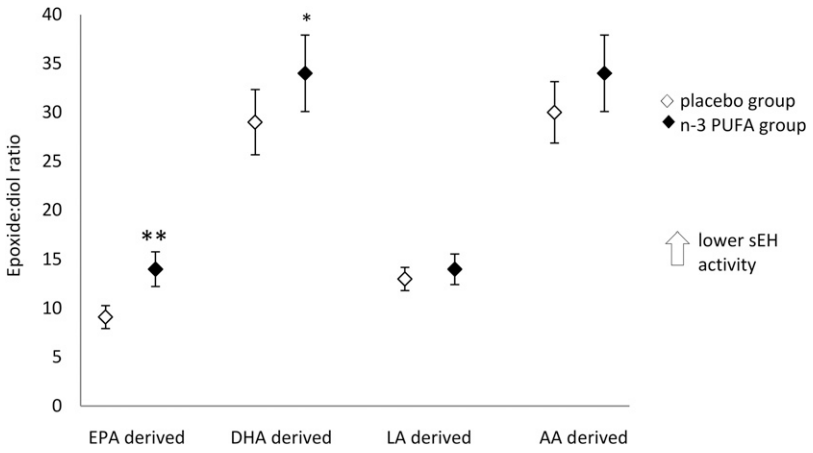

Fig. 2. Epoxide:diol ratio on postoperative day 2 among 479 patients undergoing cardiac surgery, randomized to perioperative n-3 PUFA (fish oil) supplementation or matched placebo. The diamonds correspond to the group mean, and the lines to the 95% confidence limits. A higher epoxide:diol ratio indicates lower soluble epoxide hydrolase (sEH) activity. * $P = 0.18$, ** $P < 0.001$, and $P \leqslant 0.69$ (for the n-6 metabolites) comparing placebo (n = 230) to n-3 PUFA (n = 249) using Wilcoxon rank sum test.

circulating levels of MEFAs after the acute stress of cardiac surgery. We demonstrate, for the first time to our knowledge, that the availability of dietary-derived n-3 PUFA substrate in phospholipids contributes to the in vivo regulation of MEFA concentrations in the setting of major acute tissue injury/inflammation in humans.

Dietary n-3 PUFAs likely increase the availability of fatty acid substrate to CYP450 enzymes, which in experimental studies can efficiently epoxidize the n-3 double bonds in EPA and DHA (20). This mechanism is further supported by our findings demonstrating a positive relationship between both enrollment level and change in plasma phospholipid concentration of n-3 PUFAs and their corresponding MEFA levels. Additional mechanisms could also be contributing. For example, n-3 PUFA supplementation upregulates CYP450 gene expression (21), which could contribute to enhanced synthesis of MEFAs. The n-3 PUFA may also inhibit or downregulate the expression of soluble epoxide hydrolase (19), which would reduce the conversion of epoxides to their diol metabolites. This is further supported by the increase in epoxide:diol ratio in our trial, especially in light of the apparent additional increase in n-6 epoxide:diol ratio. The epoxide:diol ratio provides a measure of the abundance of the MEFAs relative to their metabolites, the diols.

TABLE 3. Association of plasma phospholipid levels of parent PUFAs at enrollment with their respective MEFA metabolites at postoperative day 2 (n = 452)

	Univariate Model		Multivariate Model	
MEFA[a]	% Change per 1 SD Higher Parent PUFA[b]	*P*	% Change per 1 SD Higher Parent PUFA[b]	*P*[c]
EPA derived:				
Sum of MEFAs	16	<0.001	12	<0.001
17,18 EETeTr	25	<0.001	20	<0.001
14,15 EETeTr	23	<0.001	20	0.001
11,12 EETeTr	−1.2	0.91	−2.0	0.85
8,9 EETeTr	15	0.02	16	0.02
DHA derived:				
Sum of MEFAs	33	<0.001	34	<0.001
19,20 EDP	28	<0.001	30	<0.001
16,17 EDP	33	<0.001	35	<0.001
13,14 EDP	31	<0.001	33	<0.001
10,11 EDP	31	<0.001	33	<0.001
7,8 EDP	35	<0.001	37	<0.001
LA derived:				
Sum of MEFAs	5.0	0.11	2.5	0.41
12,13 EpoME	5.7	0.08	2.8	0.37
9,10 EpoME	4.3	0.17	2.0	0.51
AA derived:				
Sum of MEFAs	18	<0.001	22	<0.001
14,15 EET	24	<0.001	29	<0.001
11,12 EET	19	<0.001	24	<0.001
8,9 EET	2.3	0.77	7.2	0.41
5,6 EET	13	<0.001	16	<0.001

[a]For each class of MEFA, parent MEFAs and diols were summed (the latter representing 1–12% of the total).
[b]Represents percentage difference in postoperative MEFA level for each 1 SD higher level of parent PUFA at enrollment.
[c]Each parent PUFA was measured as percent of total plasma phospholipid fatty acids, normalized, and association with their respective downstream log-transformed MEFA assessed by multivariate linear regression. Covariates were included via backward stepwise selection (P-exclusion = 0.20, P-inclusion = 0.10), with forcing of treatment assignment (fish oil vs. placebo) and major demographics age, sex, and BMI, and model-based selection of hypertension, diabetes, smoking, history of heart failure, renal failure, and use of anti-inflammatory medications.

The biological activities of MEFAs are inactivated by their conversion to their diol metabolites, and the main enzyme responsible for this conversion is the soluble epoxide hydrolase (6). This enzyme is therefore a potential therapeutic target, and the manipulation of its expression or activity (and therefore the epoxide:diol ratio) in animal studies influences inflammation and other physiological parameters (16–18).

We demonstrated no increase (or decrease) in postoperative n-6 PUFA-derived MEFAs with n-3 PUFA supplementation. This suggests that the observed additional physiological capacity for synthesis of n-3 PUFA-derived MEFAs, which becomes engaged with increasing availability of n-3 PUFA substrate, does not apparently compete with production of n-6 PUFA-derived MEFAs.

Our findings of variable increases in n-3 plasma phospholipid fatty acid concentratations following supplementation are consistent with accumulating evidence for substantial variability in responses in circulating levels of n-3 PUFA following dietary supplementation (15, 22, 23). There is increasing interest in how such variation might be a determinant of the impact of n-3 PUFA on cardiovascular risk factors and clinical outcomes (24) because incorporation of n-3 PUFA into phospholipids could influence physical properties of cellular membranes and associated protein (e.g., ion channel) functions. Our novel results indicate that the variability in plasma phospholipid response to n-3 PUFA supplementation may also be an important determinant of the availability of circulating MEFA following tissue injury. Given the potent anti-inflammatory and cardiovascular-protective properties of MEFAs observed in experimental studies (25–27), our findings suggest a potential explanation for variable cardiovascular effects of n-3 PUFA supplementation in different individuals and highlight the need for additional studies to determine biological and dietary determinants that may enhance or weaken MEFA production in response to n-3 PUFA consumption.

Among the n-6 PUFAs, plasma phospholipid level of AA at enrollment was associated with its MEFA at postoperative day 2, but the change in AA was not. The reverse was true for LA and its MEFA. These new findings suggest that regulation of AA-derived MEFAs may be more tightly regulated and less influenced by substrate availability, whereas change in LA substrate influence production of its MEFA metabolites. Our results support the need for further investigation of the molecular pathways underlying these findings.

In prior experimental studies involving generally healthy subjects in the absence of acute tissue stress or injury (28–30), n-3 PUFA supplementation for 4 to 8 weeks increased the levels of n-3 PUFA-derived, but not n-6 PUFA-derived, MEFAs. Similar results have also been demonstrated in patients with chronic stable disease, including asthma and IgA nephropathy (14, 31). Each of these prior studies was quite small (n = 10 to 30) and did not include evaluation of MEFA responses following acute tissue stress/inflammation, when their function may be most relevant. Our study builds on and considerably extends these prior findings by providing the largest and most comprehensive assessment of the effects of n-3 PUFA supplementation on circulating MEFA levels in a human model of acute injury and inflammation (i.e., cardiac surgery). Notably, in contrast to these prior studies that reported increased MEFA levels from baseline following n-3 supplementation in healthy or stable subjects, we found that a major event such as surgery significantly lowers physiological MEFA levels in the absence of supplementation, and that n-3 supplementation helps to restore this depletion. Other strengths of our study include the randomized design to define causal effects of supplementation; highly accurate and reproducible assays to assess plasma phospholipid PUFAs and MEFAs, which reduced misclassification; and recruitment of subjects from 17 centers across 3 countries with varied population characteristics, which augments generalizability.

Potential limitations should be considered. Long-term effects of habitual fish or fish oil consumption on MEFA responses may differ from short-term effects. In this light, our results for the relationship of plasma phospholipid levels at enrollment with post-operative MEFA levels may best reflect habitual dietary exposure and provide relevant findings for this question. As it may take weeks to months to reach steady-state n-3 PUFA in various lipid pools (32), a longer supplementation period might achieve even larger changes in MEFAs. Conversely, short-term effects in the context of acute injury and proinflammatory responses, such as seen in cardiac surgery, may be most physiologically relevant given the key role of MEFAs in resolution of acute inflammation. Although there is no known reason to believe that MEFA responses may differ in other acute proinflammatory states, our findings might not be fully generalizable to other setting of acute tissue injury and inflammation or in healthy subjects not undergoing cardiac surgery. Finally, although the increased MEFAs in the n-3 supplemented group were not associated with any differences in PoAF in the larger study (OPERA), we did not evaluate the association between MEFA levels and inflammation-related clinical outcomes. This is an important but separate question that must be evaluated in future studies.

In summary, we have demonstrated, for the first time in the setting of acute tissue stress/injury in humans, that short-term n-3 PUFA supplementation significantly increases plasma n-3 MEFA concentrations, without effects on plasma n-6 MEFAs, and that the magnitude of these increases is influenced by both starting levels and changes in phospholipid levels of the n-3 PUFA precursors. These findings provide the first evidence in humans of MEFA responses to dietary interventions in the setting of acute injury, emphasizing the importance of further studies to investigate the biological interplay between n-3 PUFAs and MEFAs.

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