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Ingestion of (n-3) Fatty Acids Augments Basal and Platelet Activating Factor-Induced Permeability to Dextran in the Rat Mesenteric Vascular Bed[1–3]

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Abstract

Loss of intestinal barrier function and subsequent edema formation remains a serious clinical problem leading to hypoperfusion, anastomotic leakage, bacterial translocation, and inflammatory mediator liberation. The inflammatory mediator platelet activating factor (PAF) promotes eicosanoid-mediated edema formation and vasoconstriction. Fish oil-derived (n-3) fatty acids (FA) favor the production of less injurious eicosanoids but may also increase intestinal paracellular permeability. We hypothesized that dietary (n-3) FA would ameliorate PAF-induced vasoconstriction and enhance vascular leakage of dextran tracers. Rats were fed either an (n-3) FA-rich diet (EPA-rich diet; 4.0 g/kg EPA, 2.8 g/kg DHA) or a control diet (CON diet; 0.0 g/kg EPA and DHA) for 3 wk. Subsequently, isolated and perfused small intestines were stimulated with PAF and arterial pressure and the translocation of fluid and macromolecules from the vasculature to lumen and lymphatics were analyzed. In intestines of rats fed the EPA-rich diet, intestinal phospholipids contained up to 470% more EPA and DHA at the expense of arachidonic acid (AA). The PAF-induced increase in arterial pressure was not affected by the EPA-rich diet. However, PAF-induced fluid loss from the vascular perfusate was higher in intestines of rats fed the EPA-rich diet. This was accompanied by a greater basal loss of dextran from the vascular perfusate and a higher PAF-induced transfer of dextran from the vasculature to the lumen ($P = 0.058$) and lymphatics. Our data suggest that augmented intestinal barrier permeability to fluid and macromolecules is a possible side effect of (n-3) FA-rich diet supplementation. J. Nutr. 141: 1635–1642, 2011.

Introduction

Loss of intestinal endothelial or epithelial barrier functions with subsequent edema formation is a continuing problem in sepsis and multiple organ failure. In these patients, intestinal edema formation leads to disturbances in gut motility, increased intra-abdominal pressure, hypoperfusion, anastomotic leakage, translocation of bacteria, and liberation of proinflammatory mediators. One potent proinflammatory mediator that can promote several of these pathological responses is PAF[9]. It causes loss of fluid and protein from the vasculature, augments vascular resistance, and increases mucosal permeability (1–4). PAF stimulates the generation of a number of eicosanoids that have been implicated as second messengers in the events described above. For instance, PG of the E and F series stimulate intestinal secretion, LTC_4 and D_4 constrict the mesenteric vasculature, and TxA_2 results in vasoconstriction and LT production (5–7). These mediators, e.g. LTC_4/D_4, TxA_2, and PGE_2, are derived from the (n-6) FA AA, which is liberated by phospholipase A_2 from cell membrane phospholipids and transformed into the active mediators via cyclooxygenase or 5-lipoxygenase. The fish oil-derived (n-3) FA EPA is structurally similar to AA yet converts to far less potent mediators than AA (8,9). EPA

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[3] Supplemental Tables 1–5 and Figures 1 and 2 are available as Online Supporting Material with the online posting of this paper at http://jn.nutrition.org.
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[9] Abbreviations used: AA, arachidonic acid; CON, control; FA, fatty acid; FITC, fluorescein isothiocyanate; LT, leukotriene; PAF, platelet activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Tx, thromboxane.

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competes with the conversion of AA (10,11) and thereby dampens the inflammatory response. On the other hand, EPA and its elongation product DHA increase paracellular permeability of intestinal epithelial cell monolayers in vitro, probably via eicosanoid formation and lipid peroxidation (12–14). In an inflammatory setting, this might enhance fluid and protein extravasation from the vasculature to the lumen.

Reports of the clinical benefits of ingestion of (n-3) FA-rich fish oil on inflammatory diseases such as rheumatoid arthritis and ulcerative colitis (15–17) have led to the idea that EPA is antiinflammatory or immunomodulatory and has resulted in the introduction of fish oil-based lipid emulsions being given as parenteral immunonutrition to intensive care patients.

However, in studies on the clinical outcome of critically ill patients, results vary considerably. According to 2 meta-analyses, patients with acute respiratory distress syndrome, acute lung injury, or severe sepsis/septic shock benefited from a formulation with increased doses of EPA, γ-linolenic acid, and higher concentrations of antioxidants, whereas other fish oil-containing diets brought no improvement (18,19). One of the very few studies with no immunomodulating nutrients other than fish oil did not observe a beneficial effect in a mixed intensive care unit population (20). A rather small study in septic patients found a shortened length of hospital stay in the fish oil group only after excluding all patients who died (8 of 23) and after adjusting for age and glucose supply (21).

Considering the divergent effects of EPA on barrier function and its significance on critically ill patients, surprisingly little is known about the effect of fish oil supplementation on the extravasation of fluid and large molecules from the mesenteric vascular bed. This might be due to the fact that in patients, fluid shifts between compartments depend on a large number of variables and are difficult to observe. We have recently established an isolated perfused rat small intestine model allowing continuous access to all compartments involved in the complex events associated with the loss of intestinal barrier function (22). In the current study, we have used this model to determine the effects of dietary (fish oil-derived) (n-3) FA on the intestinal phospholipid composition and barrier function and have examined the PAF-induced arterial pressure rise, intestinal edema formation, and various other physiological, biophysical, biochemical, and histological aspects. We hypothesized that intestines from rats fed an EPA-rich diet would exhibit a smaller PAF-induced increase in arterial pressure and an augmented intestinal permeability to fluid and large molecules such as 150-kDa dextran.

TABLE 1 Composition of the diets

Ingredient, *g/kg*	CON[1] diet	EPA-rich diet
Casein	240	240
Modified starch	360	360
Maltodextrin	125	125
Saccharose	100	100
Cellulose product	50	50
L-Cystine	3	3
Vitamin mix[2]	12	12
Mineral nutrients/trace elements mix[3]	60	60
Oil mixture	50	50
12:0	0.1	0.1
14:0	0.2	2.1
16:0	5.0	6.4
16:1 (n-7)	0.1	2.4
18:0	1.5	1.7
18:1 (n-9)	10.7	6.8
18:2 (n-6)	29.4	15.6
18:3 (n-3)	1.9	0.4
20:0	0.2	0.1
20:1 (n-9)	0.1	0.6
20:4 (n-6) (AA)	0.0	0.2
20:5 (n-3) (EPA)	0.0	4.0
22:5 (n-3)	0.0	0.6
22:6 (n-3) (DHA)	0.0	2.8
Σ MUFA	10.9	9.8
Σ PUFA	31.3	23.6
Σ (n-6) FA	29.4	15.8
Σ (n-3) FA	1.9	7.8
(n-6):(n-3) PUFA ratio	15.5	2.0
Vitamins,[4] *mg/kg*		
Retinol	4.50	4.63
All-*rac*-α tocopheryl acetate	153	161

[1] AA, arachidonic acid; CON, control diet; FA, fatty acid.
[2] Containing the following (per kg diet): retinol, 4.5 mg; cholecalciferol, 37.5 μg; all-*rac*-α tocopheryl acetate, 150 mg; menadione nicotinamid bisulfite, 20 mg; thiamine mononitrate, 16.0 mg; riboflavin, 16.0 mg; pyridoxine HCl, 16.5 mg; cyanocobalamin, 30 μg; l-ascorbic acid, 30 mg; calcium d-pantothenate, 59.8 mg; nicotinic acid, 50 mg; folic acid, 20 mg; biotin, 300 μg; inositol, 80 mg; DL-methionine, 1.00 g.
[3] Containing the following (per kg diet): iron (as iron sulfate monohydrate), 163 mg; copper (as copper sulfate pentahydrate), 13 mg; iodine (as calcium iodate hexa hydrate), 1.12 mg; selenium (as sodium selenite), 0.11 mg; cobalt (as cobalt carbonate monohydrate), 0.12 mg; molybdenium (as sodium molybdate), 2.1 mg; calcium, 8.75 g; phosphorus, 5.90 g; sodium, 1.80 g; chlorine, 3.12 g; magnesium, 2.00 g; potassium, 9.40 g.
[4] Calculated based on oil content and vitamin mix.

Materials and Methods

Chemicals. All chemicals were obtained from Sigma-Aldrich if not otherwise stated.

Animals, diets, and isolated perfusion. Female Wistar rats weighing between 180 and 220 g were obtained from Charles River. All experiments were approved by the local authorities (Ministry of Agriculture, Environment and Rural Areas of Land Schleswig-Holstein). Housing of the animals at the Animal Care Facility of the Research Center Borstel conformed to the European Community regulations for animal use in research (CEE 86/609) (23). Rats had free access to 1 of 2 diets containing 50 g/kg soy bean oil/safflower oil (CON diet) or EPA-rich oil/safflower oil (EPA-rich diet) (ssniff Spezialdiäten) for 20–22 d (**Table 1**).

The jejunum and ileum of anesthetized rats were isolated and perfused both vascularly (7.5 mL/min) and luminally (0.15 mL/min) in a specialized perfusion chamber (Hugo-Sachs Elektronik-Harvard Apparatus) as described (22). Perfusates were mixed from stock solutions and solid components on a daily basis and were pH adjusted and sterile filtered. For the composition of perfusates, see **Supplemental Table 1**. The set-up was designed to monitor the shift of fluid from the mesenteric artery to the interstitial space, lymph, and lumen (**Fig. 1**). Venous and luminal effluents were collected via cannulation of the hepatic portal vein and the distal end of the intestine, respectively, in small containers. Lymph drained freely from the opened lymphatic vessels onto the dish containing the intestine. All 3 effluents were aspirated separately by roller pumps and collected on balances to allow the calculation of flow and transfer rates. In addition, the weight of the entire gut as well as arterial, venous, and luminal pressures were recorded. After an equilibration period of 60 min, the intestine was stimulated with a bolus of 0.5 nmol PAF or with solvent and data were recorded for another 80 min. At the end of the experiment, intestinal contents and mesentery were removed and histological stability (quantified as mucosal stability score), wet:dry weight ratio, and weight of the perfused organ were assessed as described (22).

In all experiments, the luminal perfusate contained lactose to assess metabolic competence as galactose uptake (Fig. 1). In one set of

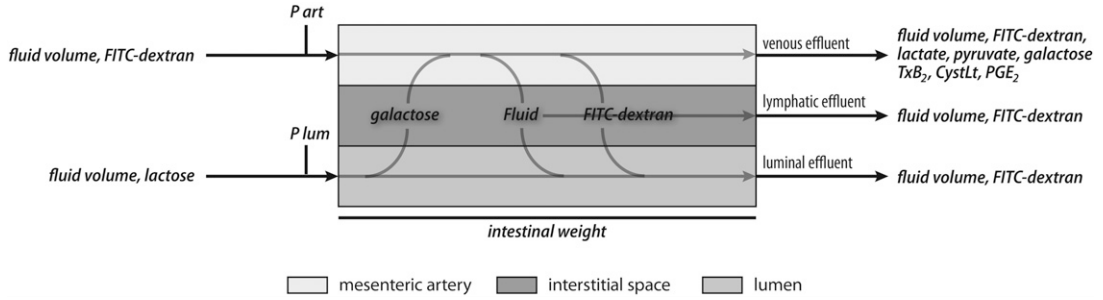

FIGURE 1 Diagram of fluid flows, data recorded, and metabolites collected for analysis during the isolated perfusion of rat intestines. For a detailed figure describing the set-up, see (22). FITC, fluorescein isothiocyanate; Tx, thromboxane.

experiments, FITC dextran (150 kDa, 40 mg/L) was included in the vascular buffer and fluorescence was analyzed in the venous, luminal, and lymphatic effluents.

Biochemical assays. Arterial pH and oxygen supply as partial pressure of O_2 were determined using an IL 1620 blood gas analyzer (Instrumentation Laboratory). FITC dextran was analyzed in an FL600FA (BIO-TEC Instruments, excitation: 485 nm; emission: 530 nm) against standard curves. The amount of FITC dextran in the lumen and lymph was calculated by multiplying the concentration by the volume flow in the respective time interval. Vascular galactose derived from luminal lactose was determined by a commercially available assay kit (Megazyme) with slight modifications. Pyruvate was determined using a standard photometric assay. Lactate was analyzed at the clinical laboratory of the Department of Clinical Medicine, Research Center Borstel.

Intestinal phospholipid analysis. Intestines were perfused vascularly and luminally with ice-cold saline (0.9%), frozen in liquid nitrogen after removal of fluid and mesenteric tissue, and ground up. Lipids were extracted according to Bligh and Dyer (24) and dissolved in chloroform/methanol (83/13). Phospholipids were analyzed by LC-MS (25). Solvents, gradient profiles, and flow rates are summarized in **Supplemental Table 2**. Phospholipids were identified on the basis of their accurate monoisotopic masses and HPLC retention times. Because only the total number of carbon atoms and double bonds in both FA chains can be determined from the exact mass, tandem MS was applied to more confidently assign the FA in PI. MS/MS resulted in identification of FA for PI16:0/20:4, PI18:0/20:4, PI18:0/20:5, and PI18:0/22:6. To account for any contamination by food lipids that might have caused differences between dietary groups, pure diets were extracted and analyzed in parallel.

Fourier transform infrared spectroscopy. Fourier transform infrared spectroscopy of intestinal lipid extracts was performed with an IFS-55 spectrometer (Bruker). Temperature scans were performed with a heating rate of 0.6°C/min. The symmetric stretching vibration of the methylene groups $\nu_s(CH_2)$ around 285 mm^{-1} was taken as the measure of the state of order (packing) of the acyl chains (26).

Eicosanoid ELISA. Eicosanoids were analyzed in the venous effluent samples with a monoclonal Thromboxane B_2 Express EIA kit, Cysteinyl Leukotriene Express EIA kit (both Cayman Chemical), and a Parameter PGE_2 assay (R&D Systems) according to the manufacturer's instruction.

Statistical analysis. Data are given as means ± SD. Prior to statistical analysis using JMP 5.0.1 (JMP Germany), Prism (Graph Pad Software), SAS 9.2 (SAS Institute), or R (27) data were transformed by the Box-Cox transformation to achieve homoscedasticity, if applicable. The data of arterial, venous, and luminal pressures, as well as for partial pressure of O_2, pH, venous galactose, and lactate/pyruvate, were compared at 60 and 135 min by mixed model analysis (PROC MIXED; SAS 9.2) with PAF and diet as the factors; the first order autoregressive covariance structure was selected by the AIC criterion. Data in Table 4 and **Supplemental Tables 3** (except galactose and lactate/pyruvate) and **4** (except perfusion pressures) were analyzed for 2 factors (diet and PAF) by 2-way ANOVA; only if at least one of the main effects or interaction terms was significant were *t* statistics based on the ANOVA output performed. Data in **Tables 2** and **3** and **Supplemental Table 5** were analyzed by individual *t* tests (for Table 2, phospholipid classes were tested separately). In all cases, the results of the *t* tests were corrected for multiple comparisons by the false discovery rate procedure ($f = 0.05$), "which may be the best practical solution to the problems of multiple comparisons that exist within physiology and other scientific disciplines" (28). $P < 0.05$ was considered significant.

Results

Body weight gain. Body weight gain did not differ between rats fed the CON (44 ± 18 g/3 wk; $n = 20$) or EPA-rich (45 ± 9 g/3 wk; $n = 22$) diet.

Viability of the isolated perfused intestinal model. Oxygen supply [691 ± 30 mm Hg (92.0 ± 6.5 kPa); $n = 268$)] and pH (7.36 ± 0.02; $n = 269$) were stable. At the end of the perfusion, the lactate:pyruvate ratio was within or slightly below the physiologic range (Supplemental Table 3) (22). In all groups, the perfused intestine preparations were capable of utilizing luminal lactose to increase galactose concentrations in the mesenteric vein (Supplemental Table 3). At the end of the experiment, after the transient effect of PAF, the wet:dry weight ratio and the mucosal stability score were comparable in intestines of all experimental groups (Supplemental Table 3) and no edema formation or histological alterations were observed (**Supplemental Fig. 1**).

Intestinal phospholipid composition and membrane fluidity. The composition of most PI, PS, PC, and PE species differed little between rats from both diet groups and differences were limited to PUFA ($P > 0.05$ for all other species) (Table 2). In the intestines of rats fed the EPA-rich diet, concentrations of all detectable EPA- and DHA-containing PI and PS species as well as PE16:0/22:6, PE18:0/22:6, and PC16:0/20:5 were higher compared to rats fed the CON diet. Apart from PI16:0/20:4 and PC18:0/20:4, all detectable AA-containing species were lower in the EPA-rich diet group. PI18:0/18:1, a major phospholipid in both diets (data not shown), was not detected in any intestinal lipid sample.

The peak positions of the symmetrical stretching vibrational band of the methylene groups $\nu_s(CH_2)$ had a typical temperature dependence of complex lipid mixtures with no clear phase transition in the temperature range investigated (**Fig. 2**). Both membranes were still fluid at 0°C (26). However, the wave numbers of the lipid extract of rats consuming the EPA-rich diet were consistently higher than those of rats fed the CON diet, indicating a lower state of order corresponding to higher membrane fluidity.

TABLE 2 Intestinal phospholipid composition of rats fed a control (CON) or EPA-rich diet for 3 wk[1–3]

	PI		PS		PE		PC[3]	
Molecular species	CON	EPA	CON	EPA	CON	EPA	CON	EPA
				mol %				
16:0/20:4	12 ± 1	12 ± 1	n.d.[4]	n.d.	6 ± 1	4 ± 0*	15 ± 1	12 ± 2*
18:0/20:4	68 ± 1	55 ± 3*	30 ± 2	16 ± 2*	34 ± 10	22 ± 3*	13 ± 4	9 ± 4
16:0/20:5	0 ± 0	2 ± 2*	n.d.	n.d.	1 ± 1	2 ± 1	4 ± 3	8 ± 1*
18:0/20:5	5 ± 1	13 ± 2*	1 ± 1	5 ± 0*	7 ± 6	13 ± 2	5 ± 2	8 ± 3
16:0/22:6	2 ± 0	4 ± 1*	n.d.	n.d.	3 ± 1	6 ± 1*	3 ± 1	4 ± 2
18:0/22:6	1 ± 1	5 ± 1*	8 ± 5	23 ± 1*	4 ± 3	17 ± 2*	1 ± 1	2 ± 1
16:1/22:6	n.d.	n.d.	n.d.	n.d.	1 ± 2	0 ± 0	2 ± 2	1 ± 1
18:1/22:6	n.d.	n.d.	n.d.	n.d.	5 ± 9	5 ± 3	1 ± 1	1 ± 1
18:0/18:2	2 ± 2	10 ± 1	33 ± 6	27 ± 3	24 ± 6	18 ± 2	26 ± 5	22 ± 2
18:0/18:1	n.d.	n.d.	29 ± 4	29 ± 1	5 ± 2	5. ± 2	n.d.	n.d.
16:0/18:2	n.d.	n.d.	n.d.	n.d.	4 ± 2	4 ± 1	27 ± 3	28 ± 6

[1] Values are means ± SD, $n = 4$. *Different from CON, $P < 0.05$.
[2] Omitted from the table: PE16:0/18:1 2 ± 2% (EPA) vs. 1 ± 1% (CON), PE18:1/18:2 3 ± 0% vs. 5 ± 1%, PC16:1/18:2 2 ± 0% vs. 2 ± 0%, PC16:0/16:1 3 ± 1% vs. 1 ± 0%, PC16:1/16:1 1 ± 0% vs. 0 ± 0%, PC16:0/14:0 1 ± 1% vs. 1 ± 0.%
[3] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.
[4] n.d., Not detected in any sample (absolute detection limit was 2.5 to 10 pmol).

Eicosanoid production. Venous effluent contained small amounts of TxB_2, cysteinyl LT, and PGE_2 throughout the experiment (**Fig. 3**). In response to PAF, all analyzed eicosanoids increased over baseline, peaked within 1–2 min, and subsided quickly. Differences in eicosanoid production of rats fed the EPA-rich or CON diet were not evaluated, because the EIA method was not entirely specific to (n-6) FA-derived eicosanoids.

Perfusion pressures. Under baseline conditions, e.g. before and 15 min after stimulation, perfusion pressures ranged in mean from 37 to 40 mm Hg (4.9–5.4 kPa) vascularly and from 1.5 to 2.6 mm Hg (0.2–0.4 kPa) luminally (Supplemental Table 4). After administration of a 0.5-nmol PAF bolus via the mesenteric artery, vascular and luminal pressures rose sharply [$\Delta_{max\ vasc}$: 30.3 ± 3.7 mm Hg (4.0 ± 0.5 kPa) vs. 31.2 ± 4.7 mm Hg (4.2 ± 0.6 kPa); $\Delta_{max\ lum}$: 8.8 ± 1.4 mm Hg (1.2 ± 0.2 kPa) vs. 8.6 ± 1.6 mm Hg (1.1 ± 0.2 kPa)] (Supplemental Table 5, EPA vs. CON) and subsequently declined toward baseline within 30 min (**Fig. 4**). Basal or stimulated arterial and luminal pressures did not differ between intestines of rats fed either the CON or EPA-rich diet (Supplemental Tables 4 and 5).

PAF-induced fluid loss from the vasculature. Before PAF stimulation, the venous effluent flow corresponded to the arterially applied perfusion rate. Luminal effluent flow was slightly lower than the administered perfusate flow, indicating (physiological) net liquid absorption. The lymph production was within the physiological range (29) (Supplemental Table 4). Intestines of rats fed either the CON or EPA-rich diet did not differ in these parameters.

After instillation of solvent, fluid fluxes remained at baseline levels in intestines of rats of both dietary groups (**Supplemental Fig. 2**). After PAF instillation, venous effluent flow dropped quickly toward 1 mL/min in all intestines (Supplemental Fig. 2*A*). In response, gut weight and luminal and lymphatic fluid fluxes increased (Supplemental Fig. 2*B–D*). This effect was temporary and fluid flows returned to baseline within 15 min. During this time, more fluid was lost from the intestinal vasculature from the EPA-rich diet-fed rats than from the CON diet-fed rats and the fluid balance analysis suggested that this extra fluid was drained mostly via the lumen ($P = 0.052$) (Table 3).

Intestinal permeability to dextran (150 kDa). At baseline, the FITC dextran concentration of the venous effluent reflected that of the arterially applied buffer. In nonstimulated intestines, luminal effluent contained hardly any FITC dextran (**Fig. 5***A*). However, the luminal FITC dextran concentration was greater in rats fed the EPA-rich diet compared to those fed the CON diet (Fig. 5*A*; **Table 4**). In the lymph, a steady-state concentration of 25 mg/L was reached 60–75 min after the onset of perfusion (Fig. 5*B*). The shift of FITC dextran to the lymphatics was slightly lower in intestines of rats fed the EPA-rich diet (Table 4); however, the effect is likely not physiologically relevant.

After PAF administration, luminal FITC dextran concentrations substantially increased, peaking at 6 min poststimulation and returning to baseline concentrations within the next 75 min (Fig. 5*A*). Likewise, lymphatic FITC dextran concentrations increased quickly and returned slowly back to the steady-state concentration (Fig. 5*B*). Within 15 min of PAF stimulation, the FITC dextran shift to the lumen and the lymph were greater in rats fed the EPA-rich diet than in rats fed the CON diet (Fig. 5*B*; Table 4).

Discussion

Parenteral nutrition containing (n-3) FA is receiving increasing interest owing to its proposed immunomodulatory effect. However, (n-3) FA have been shown to increase paracellular per-

TABLE 3 Platelet activating factor (PAF)-induced fluid shift between compartments in the isolated perfused intestine from rats fed a control (CON) or EPA-rich diet for 3 wk[1]

	CON		EPA	
	−PAF (3)	+ PAF (5)	−PAF (4)	+ PAF (5)
Vasculature, *mL/15 min*	−0.2 ± 1.6	−17.5 ± 1.6	−0.4 ± 0.6	−23.5 ± 6.1*
Lumen, *mL/15 min*	0.2 ± 0.2	11.7 ± 1.4	−0.2 ± 1.2	15.4 ± 3.9
Lymphatics, *mL/15 min*	−0.1 ± 0.0	4.3 ± 0.9	−0.2 ± 0.1	5.2 ± 1.9
Tissue, *g/15 min*	−0.0 ± 0.0	0.9 ± 0. 8	−0.1 ± 0.1	1.4 ± 0.6

[1] Data are mean ± SD, $n = 3–5$. *Different from CON + PAF, $P < 0.05$.

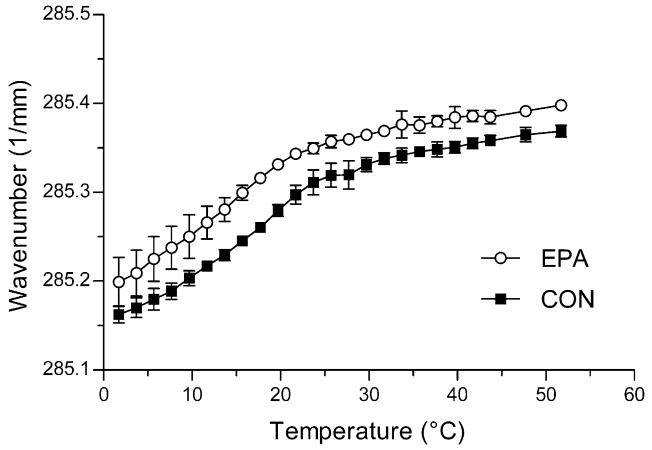

FIGURE 2 Fourier transform infrared spectroscopy of membranes from intestinal lipids of rats fed either the control (CON) or EPA-rich diet for 3 wk. Values are means ± SD, n = 3.

meability in cell monolayers in vitro, which, if applicable to humans, could be detrimental in severely ill patients. This study was designed to test the hypothesis that, in response to the archetypical inflammatory mediator PAF, intestines from rats fed an (n-3) EPA-rich diet would exhibit a less pronounced rise in arterial pressure but a greater vascular permeability to water and macromolecules in an intact organ model. In rats fed an EPA-rich diet, we found higher EPA and DHA levels in their intestines compared to controls. In response to PAF, intestines from both groups produced eicosanoids. As predicted from in vitro experiments (12–14), rats fed an EPA-rich diet had greater fluid loss and permeability of the intestines to dextra compared to the intestines of CON diet-fed rats. Unexpectedly, an increase in PAF-induced arterial pressure was not altered by the different diets.

Long chain (n-3) FA have multiple interactions with inflammatory pathways: EPA and DHA interfere with lipopolysaccharide signaling and subsequent NF-κB–mediated transcriptional events, DHA-derived protectin D1 and resolvin D1 are involved in the resolution of inflammation, and EPA competes with AA for cyclooxygenases and lipoxygenases but is converted to far less potent mediators (30). Owing to the time frame of the experiments and the absence of circulating neutrophils, the model system used here is primarily suited to examine the consequences of an EPA-rich diet on the immediate and direct effects of PAF such as increased vascular permeability, pressure responses, and eicosanoid generation. Therefore, this model allows separation of these rapid responses to PAF from those that require gene induction and activation of blood-borne leukocytes.

After 3 wk of dietary (n-3) FA incorporation (EPA-rich diet), EPA and DHA in rat intestinal phospholipids were up to 470% higher than after consuming the CON diet. These differences were not caused by contamination with food lipids, because the major dietary phospholipid PI18:1/18:1 was not found in any intestinal lipid sample. This shows that a relatively small change in the diet, namely a reduction in the (n-6):(n-3) PUFA ratio from 15.5 to 2.0, is enough to induce pronounced changes in the phospholipid composition of intestinal cell membranes. Incorporation of (n-3) FA in plasma and cell membranes is dose dependent (31) and even larger changes were found with diets containing more fat and a higher proportion of EPA or DHA, at least in the lung and liver (32,33), highlighting the flexibility of tissue FA composition.

Despite the substantial enrichment of intestinal membrane phospholipids in EPA-rich diet-fed rats, this did not dampen the

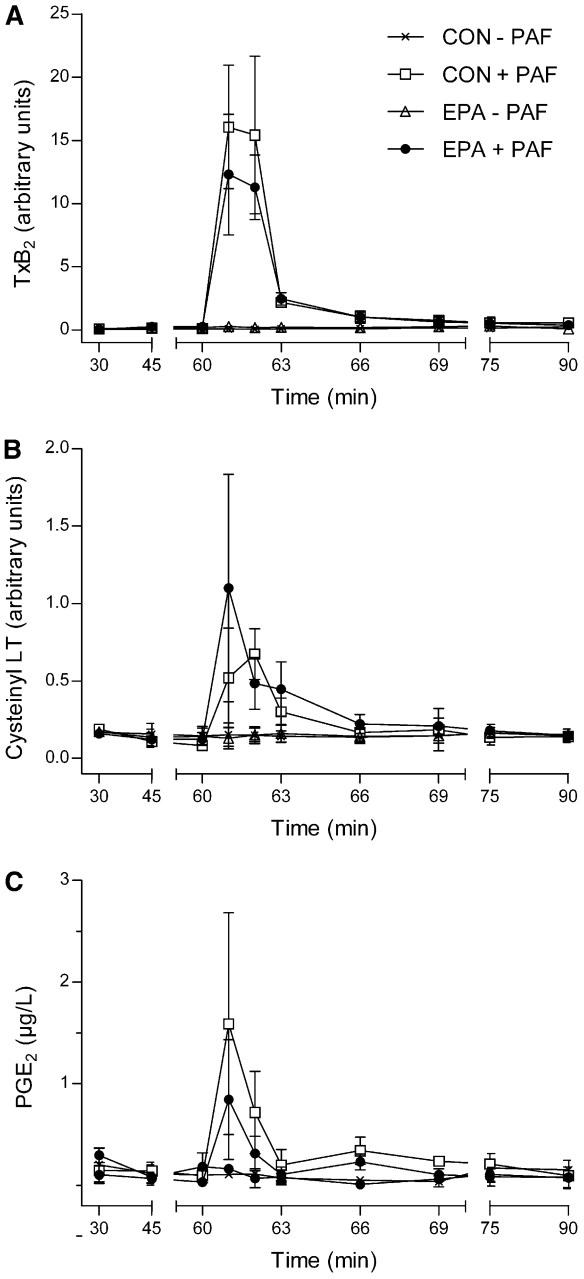

FIGURE 3 Concentrations of thromboxane B_2 (*A*), cysteinyl LT (*B*), and PGE_2 (*C*) in the venous effluent of isolated perfused intestines from rats fed a control (CON) or EPA-rich diet for 3 wk. Values are means ± SD, n = 3–4.

transient PAF-induced increase in arterial pressure compared to CON-fed rats. First, EPA accumulation, though fairly high, might have been insufficient, if only in the responsive cells or membrane compartments, to cause the necessary shift in eicosanoid production. For example, a previous study found that after 12 wk of high-dose fish oil supplementation, EPA was enriched 5- to 8-fold and LPS-stimulated PGE_2 production was reduced by ~50% in human peripheral blood mononuclear cells (31). This suggests that rather high concentrations of EPA are required to achieve measurable changes in eicosanoid production and, presumably, eicosanoid-dependent processes. Second, for conversion of phospholipid-bound AA or EPA to eicosanoids, the action of phospholipase A_2 is necessary. Phospholipase A_2 IIA has been closely linked to inflammation (34) and preferably acts on PE and PS, both highly enriched in EPA after

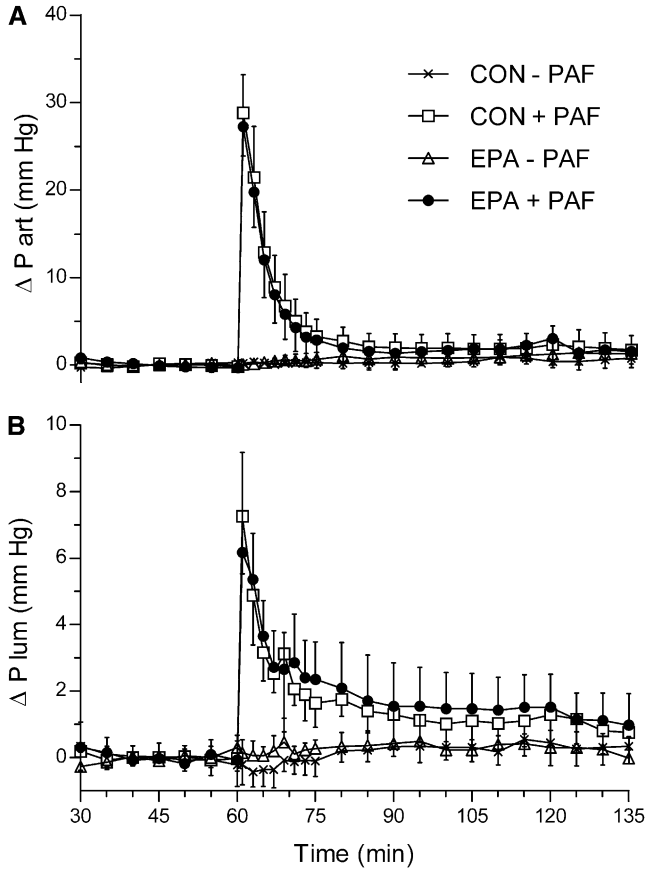

FIGURE 4 Arterial (art; *A*) and luminal (lum; *B*) pressure changes during isolated perfusion of intestines from rats fed a control (CON) or EPA-rich diet for 3 wk. Values are mean ± SD, n = 6–9. Data are displayed as pressure changes over baseline (pressure at respective time minus mean pressure from 30 to 60 min). Conversion factor for pressures: 1 mm Hg = 133.32 Pa. PAF, platelet activating factor.

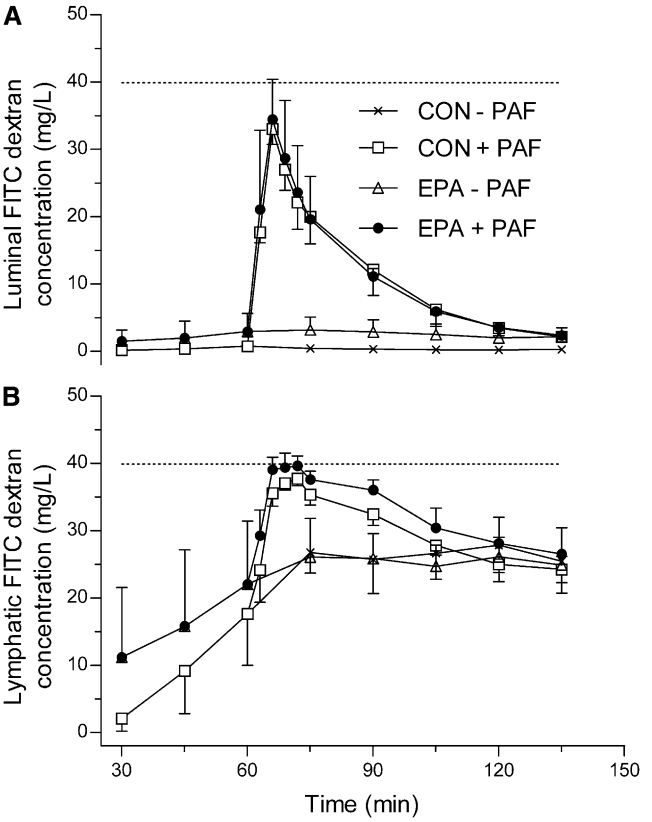

FIGURE 5 Fourier transform infrared spectroscopy dextran concentration in the luminal (*A*) and lymphatic (*B*) effluent of isolated perfused intestines from rats fed a control (CON) or EPA-rich diet for 3 wk. Values are means ± SD, n = 3–4. The dotted line represents the vascular Fourier transform infrared spectroscopy dextran concentration. FITC, fluorescein isothiocyanate; PAF, platelet activating factor.

an EPA-rich diet. As to its FA preference, only selectivity of AA over oleic acid has been described (35). Assuming nonpreferential conversion of EPA- and AA-containing phospholipids, our model system with phospholipids as the sole FA source should be suitable to show eicosanoid-mediated effects of the EPA-rich diet. And lastly, it is possible that TxA_2 and LTC_4/D_4 are not as important to PAF-mediated arterial pressure responses in the intestine as is generally thought. In our model, blocking the leukotriene and prostanoid receptor did not substantially alleviate the PAF-induced arterial pressure rise (36) despite the concomitant eicosanoid production. Experiments in isolated intestines and mesenteries with recirculating buffer suggest 2 consecutive events in response to PAF: first, a transient pressure peak abolished by Ca^{2+}-depletion, and second, a prolonged pressure increase transduced via eicosanoids fed back to the mesentery via the recirculating buffer (37,38). The vasoconstriction seen in our model could thus represent the early and possibly eicosanoid-independent response, and the second eicosanoid-mediated pressure increase could be prevented by the use of fresh buffer. It should be kept in mind that in vivo mediators released from the intestine do not immediately reenter the intestine.

PAF is well known to increase vascular permeability in a variety of vascular beds (39). Therefore, PAF is a critical mediator of hyperpermeability in inflammatory conditions such as sepsis, septic shock, and multiple organ failure (40,41). However, besides increasing vascular endothelial permeability, PAF can also increase edema formation via hydrostatic mechanisms and activation of neutrophils (39). Our experimental system allows for control of the following factors. Neutrophils were excluded by perfusion with a cell-free buffer. Overall edema formation in the intestine was assessed by continuously measuring the organ weight and PAF caused a strong but transient edema formation also reflected by the liquid shift from the vascular to the luminal and lymphatic compartments (Supplemental Fig. 2*D*; Table 3). Because the hydrostatic pressures were identical in both groups, we conclude that the more pronounced edema formation in the EPA-rich diet group was due to higher vascular permeability. To analyze permeability, the transfer of molecular tracers across the intestinal barrier is a frequently used method that works in cell

TABLE 4 Fluorescein isothiocyanate dextran mass shift from vasculature to lumen and lymphatics in the isolated perfused intestine from rats fed a control (CON) or EPA-rich diet for 3 wk[1]

	CON		EPA		*P* value		
	− PAF	+ PAF	− PAF	+ PAF	D	*P*	D x P
	μg/15 min						
Lumen	1 ± 0	297 ± 26[a]	6 ± 3[a]	441 ± 153[b]	<0.01	<0.0001	NS[2]
Lymphatics	12 ± 1	159 ± 8[a]	8 ± 2[a]	225 ± 11[b,c]	<0.01	<0.0001	<0.0001

[1] Values are mean ± SD of 3–4 experiments. [a]Different from CON − PAF (platelet activating factor), $P < 0.05$; [b]different from EPA − PAF, $P < 0.05$; [c]different from CON + PAF, $P < 0.05$.
[2] $P > 0.05$.

culture, isolated organs, and in vivo. Several groups have used 150-kDa FITC dextran for this purpose (42,43), because it is sufficiently large and it cannot cross the endothelium by transcytosis, as might happen with albumin, HRP (40 kDa), or smaller FITC dextran (4–10 kDa) in epithelial cell culture (44–46). Our present findings show that the loss of FITC dextran from the vasculature was greater in the EPA-rich diet group under both baseline conditions and when treated with PAF. Taken together, we conclude that the vascular permeability of the mesenteric vascular bed was increased in rats fed the EPA-rich diet, because intestinal weight gain, fluid shift, and transfer of FITC dextran were all greater, despite the fact that the hydrostatic pressure did not differ.

These findings might be explained by alterations in the lipid structure of the cells. The (n-3) FA increase the degree of unsaturation of the tight junctional lipid environment (47) and the paracellular permeability of Caco-2 monolayers, and eicosanoid formation and lipid peroxidation have been suggested to play a role in this process (12–14). However, whether the higher intestinal (n-3) FA concentration in this study is correlated with changes in the vicinity of tight junctions is unknown. The greater membrane fluidity in intestines from EPA-fed rats fits with the higher overall permeability but needs to be interpreted with caution, because the exact localization of the (n-3) FA within the organ or cells is unknown. As far as eicosanoids are concerned (especially PG of the E series), these seem to have important and diverse effects on intestinal permeability. The immediate response to PGE_2, and even more to PGE_3, is an increase in permeability (48). In the long run, PGE_2 seems to be necessary to preserve or repair intestinal barrier function and this effect has been linked to tight junctions (49,50). However, whether PGE_3 is more or less effective in this context is unknown.

Interestingly, enhanced vascular permeability (enhanced lung wet:dry weight ratio) was also found in mice infected with *Pseudomonas aeruginosa* after 3 wk of consuming an EPA-rich diet (51). This suggests that under certain circumstances, supplementation with (n-3) FA might lead to impaired barrier functions in susceptible organs such as intestine and lung. Translated to critically ill patients, this raises the question as to whether (n-3) FA should always be considered entirely safe. Numerous studies have identified antiinflammatory pathways of (n-3) FA and demonstrated favorable effects in different inflammatory diseases. However, our findings suggest that these beneficial effects need to be balanced against the possibility of enhanced vascular permeability as a side effect of fish oil supplementation.

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