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Dietary Fish Oil Substitution Alters the Eicosanoid Profile in Ankle Joints of Mice during Lyme Infection^{1–3}

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Abstract

Dietary ingestion of (n-3) PUFA alters the production of eicosanoids and can suppress chronic inflammatory and autoimmune diseases. The extent of changes in eicosanoid production during an infection of mice fed a diet high in (n-3) PUFA, however, has not, to our knowledge, been reported. We fed mice a diet containing either 18% by weight soybean oil (SO) or a mixture with fish oil (FO), FO:SO (4:1 ratio), for 2 wk and then infected them with *Borrelia burgdorferi*. We used an MS-based lipidomics approach and quantified changes in eicosanoid production during Lyme arthritis development over 21 d. *B. burgdorferi* infection induced a robust production of prostanoids, mono-hydroxylated metabolites, and epoxide-containing metabolites, with 103 eicosanoids detected of the 139 monitored. In addition to temporal and compositional changes in the eicosanoid profile, dietary FO substitution increased the accumulation of 15-deoxy PGJ₂, an antiinflammatory metabolite derived from arachidonic acid. Chiral analysis of the mono-hydroxylated metabolites revealed they were generated from primarily nonenzymatic mechanisms. Although dietary FO substitution reduced the production of inflammatory (n-6) fatty acid-derived eicosanoids, no change in the host inflammatory response or development of disease was detected. J. Nutr. 142: 1582–1589, 2012.

Introduction

Eicosanoids constitute a diverse class of bioactive signaling molecules and are involved in many biological processes (1). Although eicosanoids are derived from PUFA containing 20 carbons, we use the term loosely to encompass eicosanoid-like metabolites derived from PUFA with varying carbon lengths. They are an integral part of the innate and adaptive immune systems and mediate signals for inflammation, pain, fever, vasodilation, vasoconstriction, and chemotaxis. Following their liberation from membrane phospholipids by phospholipase A₂, PUFA are substrates for a variety of biosynthetic pathways, especially the production of PG and leukotrienes (LT)⁹ via

cyclooxygenase (COX) and 5-lipoxygenase (LOX) pathways, respectively (2).

Supplementation of fish oil (FO) into the human diet appears to be beneficial for certain chronic inflammatory conditions such as cardiovascular disease, diabetes, rheumatoid arthritis, cystic fibrosis, and cancer, although the molecular mechanisms responsible for these benefits are unclear (3–8). FO contains high concentrations of the (n-3) PUFA EPA [20:5(n-3)] and DHA [22:6(n-3)] and these are considered the primary contributors to the antiinflammatory properties of dietary FO. EPA and DHA compete with arachidonic acid (AA) [20:4(n-6)] for incorporation into membrane phospholipids and for use as substrates for COX and LOX enzymes (9–12). Upon release from membrane stores, metabolism of EPA and DHA results in the generation of (n-3) eicosanoids that are generally less potent than analogous (n-6) eicosanoids (13). EPA-derived PGE₃ and LTB₅ are less bioactive than analogous AA-derived eicosanoids PGE₂ and LTB₄ (10,14). Although the production of 3-series prostanoids and 5-series LT was reported in several studies, the overall extent

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³ Supplemental Figures 1–3 and Table 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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⁹ Abbreviations used: AA, arachidonic acid; COX, cyclooxygenase; FO, fish oil; HDcHE, hydroxy-DHA; HEPE, hydroxy-EPA; HETE, hydroxy-eicosatetraenoic acid; LA, linoleic acid; LC, liquid chromatography; LOX, lipoxygenase; LT, leukotriene; MRM, multiple reaction monitoring; p.i., postinfection; ROS, reactive oxygen species; SO, soy oil; TXB₂, thromboxane B₂.

of changes in eicosanoid production due to dietary FO intake has not been reported.

In association with the LIPID MAPS consortium, we developed a high-throughput mass spectrometric methodology capable of monitoring 139 unique eicosanoid species (15,16). This systems biology approach allows us to globally and temporally monitor changes in the eicosanoid profile during disease processes and identify compounds associated with disease development or resolution. We used the well-characterized murine model of experimental Lyme borreliosis as our experimental model system (18). The effect of dietary FO on Lyme disease has not, to our knowledge, been reported. Lyme disease is the most prevalent vector-borne disease in the United States, with >35,000 new cases reported each year (17). The spirochete, *Borrelia burgdorferi*, is the etiologic agent of Lyme disease and is transmitted to the mammalian host through the bite of an infected *Ixodes* tick. When not treated with antibiotics early, the infection can manifest in joint, heart, and central nervous system disorders (18). Patients with Lyme arthritis are routinely treated with nonsteroidal antiinflammatory drugs or COX-2-specific inhibitors, suggesting that products of the AA pathway can modulate arthritis severity (18). Indeed, using a murine model of experimental Lyme arthritis, we previously demonstrated that blocking PG production via COX-2 inhibition or genetic deficiency resulted in the normal development of arthritis, accompanied by a failure of disease resolution (19). Other models of arthritis, such as collagen-induced arthritis or the K/BxN serum-transfer model, are also dependent upon products of the AA metabolic pathway for development of disease (20–23). Thus, regulation of inflammatory responses by AA-derived bioactive lipids may be a common pathogenic mechanism in arthritis.

In the present study, we investigated the impact of substituting (n-6) PUFA-containing soy oil (SO) with (n-3) PUFA-containing FO on the eicosanoid profile in the murine model of experimental Lyme arthritis.

Methods

Chemicals and reagents. Liquid chromatography (LC)-grade solvents were from EMD Biosciences. Synergy C18 reverse-phase HPLC column and Strata-X solid phase extraction columns were from Phenomenex. Eicosanoids were from Cayman Chemicals and Biomol.

Mice and infections. Female C3H/HeJ mice 4–6 wk old were from The Jackson Laboratory. The mice were housed in a specific pathogen-free facility. The room was maintained on a 12-h-light/dark cycle at 23°C and 40–50% relative humidity. Mice had free access to autoclaved water and commercial rodent diet (Purina Mills). After a 5-d acclimation period, the mice were randomly assigned to 1 of 2 experimental dietary treatment groups. The experimental diets were nutritionally complete and based on the semipurified AIN-93G diet (24) modified to contain 18% fat (wt:wt) while maintaining the nutrient:energy ratio of the original, lower fat diet. Ingredients and fatty acid composition of the experimental diets are shown in Table 1. We chose this level of fat to provide a greater level of (n-3) PUFA enrichment from a widely used FO source. The 2 experimental diets were identical, except the major dietary fat sources were either SO or refined menhaden FO (a generous gift from Omega Protein). A small amount of SO was added to the FO to ensure essential fatty acid [i.e., linoleic acid (LA)] requirements of the mice were met. These diets represent 2 extremes relative to the proportion and absolute amounts of (n-6) and (n-3) PUFA, respectively. The FO diet provided ~10% energy of EPA and DHA combined. This represents a level of (n-3) PUFA intake that greatly exceeds what is possible in free-living human beings, where 3 g/d (~0.5% energy) is considered a safe upper limit.

After 2 wk of consuming the experimental diets, mice were infected with 5×10^4 virulent low-passage N40 strain *B. burgdorferi* spirochetes

TABLE 1 Experimental diet fatty acid profiles¹

Fatty acid	SO	FO
	% of total fatty acids	
14:0	<0.2	4.5
16:0	10.9	17.6
18:0	5.0	3.5
18:1	23.8	16.8
18:2(n-6)	52.8	12.7
18:3(n-3)	6.4	1.6
20:5(n-3) (EPA)	—	15.9
22:5(n-3)	—	2.9
22:6(n-3) (DHA)	—	12.0

¹ Experimental diet formulations were based on the AIN-93G diet but with modifications to allow the incorporation of a higher amount of fat without changing the nutrient:energy ratio. Diets contained (g/kg): corn starch (375), casein (230), sucrose (110), fiber (α -cellulose) (57), AIN-93 mineral mix (40.2), AIN-93G vitamin mix (11.5), L-cysteine (3.4), choline bitartrate (2.9), and 1 of 2 fat sources (180). The dietary fat sources were either SO or a mixture of menhaden FO and SO (in a 4:1 ratio). Both fat sources were stabilized against auto-oxidation with the inclusion of 0.02% (wt:wt) tertiary butylhydroquinone. Fatty acids present in the FO diet at levels >1% of the total, but not listed above, include: 16:1(n-7) (7.1%), 18:1(n-7) (1.6%), 18:4(n-3) (2.7%), and 20:4(n-3) (1.0%). FO, fish oil; SO, soy oil.

(19). All studies were conducted in accordance with the Animal Care and Use Committee of the University of Missouri.

Fatty acid analysis. Serum and liver fatty acid analysis was performed as described (25). Briefly, total fatty acids were extracted and phospholipids isolated by TLC. Base-catalyzed methylation prepared FAME for analysis using GC. Identification of the FAME was completed by comparing relative retention times to commercial standards.

Assessment of disease progression. Development of arthritis was monitored by weekly measurements of ankle diameters for 25 d using a spring-loaded caliper and comparison to preinfection diameters. Mice were killed via cervical dislocation and one ankle joint was randomly chosen for histology and the other used for eicosanoid extraction. For carditis assessment, the hearts were sagittally bisected through both ventricles and atria and prepared for histology. Arthritis and carditis severity scores were determined by 2 independent, trained veterinary pathologists who were unaware of the study design. The severity scores were graded on a scale of 0–4, with 0 representing no inflammation and 4 representing severe inflammation (26). Inflammation type scores were graded on a scale of 0–4 and represented the ratio of neutrophils:macrophages within the inflammatory lesion as described (27).

Assessment of bacterial loads in tissues. Knee joints are colonized by *B. burgdorferi* and develop severe arthritis similar to ankle joints (28). We used one knee joint and quantitative multiplex real-time PCR to assess the numbers of spirochetes present in tissues as described (29). Murine DNA was quantified using the single copy nidogen gene, and *B. burgdorferi* DNA in the samples was quantified using the flagellin gene (29). The PCR was performed in duplicate and levels of flagellin were normalized to nidogen levels in the same tube.

LC and MS. The MS-based lipidomics approach used was described in Dumlao et al. (16). Briefly, 50 μ L of 50 pg in 1 μ L solution of deuterated eicosanoid internal standards was added to each homogenized ankle joint and eicosanoids were extracted into 1 mL methanol using solid-phase extraction columns (Phenomenex) (30). A 40- μ L aliquot of the sample was separated by LC using a reverse-phase column (Phenomenex) and eicosanoids were subsequently detected using a tandem quadrupole MS (ABI 4000 Q-TrapR, Analyst 1.5 software, Applied Biosystems) via scheduled multiple reaction monitoring (MRM) in negative-ion mode. Eicosanoids were identified in samples by matching their individually optimized MRM signal and LC retention time with those of a pure standard.

Chiral chromatography and MS. Lipid chiral analysis was completed as described in Harkewicz et al. (43). Briefly, 10- μ L aliquots of lipids extracted from mouse ankle joints were separated on an amylose chiral column (Chiralpak AD-H) under normal phase conditions and detected using a triple quadrupole MS with a chemical ion source. Eicosanoids were identified in samples by matching their individually optimized MRM signal and LC retention time with those of a pure standard.

Quantification and statistics. Eicosanoids were quantified using MultiQuant 1.1 software (ABI Sciex). Quantified values were determined from a stable isotope dilution method using pure eicosanoid standards. All data were filtered using Grubb's test to remove outliers. Values represent means \pm SEM unless indicated. Statistical analysis was calculated using Prism 5 (GraphPad Software). Significance was determined by 2-way ANOVA ($P < 0.05$) for effects of diet (or metabolite), time, and diet \times time interaction. Significance for specific comparisons was determined by ANOVA followed by Dunnett's post hoc test for metabolite changes over time on a given diet or by Bonferroni's post hoc multiple comparison test for metabolite changes between diets. Between-group means were analyzed for significance using the Mann-Whitney Rank Sum test when comparing disease severity. Significance levels were set at $\alpha = 0.05$.

Results

Serum and liver fatty acids. After feeding mice either a SO or a FO diet for 2 wk, the fatty acid profile was measured to determine the effectiveness of the dietary treatment. EPA and DHA concentrations in the serum and liver were higher in the FO- than in the SO-fed mice ($P < 0.05$) (Table 2), indicating that 2 wk of consuming the FO diet was sufficient to alter the total tissue fatty acid profile. Conversely, LA [18:2(n-6)] and AA concentrations were lower ($P < 0.05$) in tissues from the FO-fed mice.

Eicosanoid production in response to infection. Following 2 wk of priming with the experimental diets, the mice were infected with *B. burgdorferi* and the concentrations of eicosanoids in ankle tissue were determined at 0, 10, and 21 d postinfection (p.i.). Using a lipidomics approach, we detected 103 lipid metabolites of 139 total metabolites monitored throughout the experimental time-course. Quantified amounts were obtained for 89 of the 103 metabolites detected and these are

TABLE 2 Serum and liver fatty acid profiles of mice infected with *B. burgdorferi* and fed a SO or FO diet for 2 and 3 wk p.i.¹

Fatty acid	Serum		Liver	
	SO	FO	SO	FO
	<i>mol% of total fatty acids</i>			
16:0	23.8 \pm 0.4	30.1 \pm 0.6*	32.7 \pm 0.7	41.8 \pm 0.7*
18:0	12.8 \pm 0.3	11.2 \pm 0.3*	23.0 \pm 0.7	19.9 \pm 0.7*
18:1(n-9)	8.4 \pm 0.6	8.8 \pm 0.4	7.7 \pm 0.5	6.3 \pm 0.5*
18:2(n-6)	32.8 \pm 0.4	20.0 \pm 1.1*	16.2 \pm 0.2	10.0 \pm 0.5*
18:3(n-3)	0.5 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.0	0.0 \pm 0.0
20:3(n-6)	0.6 \pm 0.1	0.3 \pm 0.1	0.7 \pm 0.1	0.6 \pm 0.0
20:4(n-6)	17.0 \pm 0.5	9.6 \pm 0.5*	14.0 \pm 0.5	6.1 \pm 0.3*
20:5(n-3) (EPA)	0.0 \pm 0.0	7.7 \pm 0.6*	0.2 \pm 0.0	3.6 \pm 0.4*
22:5(n-3)	0.0 \pm 0.0	0.3 \pm 0.1	0.1 \pm 0.0	0.4 \pm 0.0
22:6(n-3) (DHA)	3.3 \pm 0.1	8.7 \pm 0.5*	5.0 \pm 0.2	8.4 \pm 0.4*

¹ Values are means \pm SEM, $n = 10$. Values for fatty acids $<0.5\%$ of the total fatty acids were excluded from this table. *Within a given tissue FO mean differs from SO mean, $P < 0.05$. FO, fish oil; p.i., postinfection; SO, soy oil.

summarized in Supplemental Table 1. At the time of infection (d 0), the joint tissue eicosanoid concentrations did not differ between the groups (Table 3). However, by d 10 postinfection (p.i.), there were 30 metabolites that differed ($P < 0.05$) and this increased to 76 metabolites at d 21 p.i.

A heat map containing the major metabolites derived from AA, LA, EPA, and DHA displays the relative global fold changes to the eicosanoid profile in the ankle joint tissue of mice consuming either diet in response to a Lyme infection (Fig. 1A). In SO-fed mice, *B. burgdorferi* infection caused a relative-fold increase (>4 -fold in most cases) in AA- and LA-derived eicosanoids from all major subclasses (PG, mono-hydroxylated metabolites, and epoxide-containing metabolites) from baseline to d 21 (Fig. 1A). The EPA- and DHA-derived metabolites did not display the same trend as the AA- and LA-derived metabolites in the SO-fed mice. In contrast, most AA- and LA-derived metabolites increased 1.5–2 fold in the FO-fed mice compared with those fed SO (Fig. 1A). Additionally, the majority of EPA- and DHA-derived metabolites were 4-fold greater than baseline at d 10 and 21 in the FO-fed mice. To demonstrate the impact of SO and FO on the global ankle joint eicosanoid profile, we compared the relative eicosanoid concentrations between the FO- and SO-fed mice (Fig. 1B). At d 21, a majority of the (n-6) fatty acid-derived eicosanoids were relatively reduced in the FO-compared with the SO-fed mice. Most of the (n-3) fatty acid-derived eicosanoids were 4-fold greater in the FO-fed mice than in the SO-fed mice at d 10 and 21 p.i. Collectively, the (n-3) fatty acid-derived eicosanoids accounted for 47 and 59% of the total eicosanoids in the FO-fed mice at d 10 and 21 p.i., respectively, whereas concentrations were 11 and 9% in the SO-fed mice. Dietary FO substitution thus resulted in a global shift of the eicosanoid profile toward EPA- and DHA-derived metabolites during a bacterial infection.

Production of mono-hydroxylated metabolites. The heat map shows the relative fold changes to the global eicosanoid profile but lacks a magnitude component to distinguish between the major and minor metabolites. In the SO-fed mice, the EPA- and DHA-derived metabolites accounted for 8 and 12% of the total mono-hydroxylated metabolites monitored at d 10 and 21 p.i. In contrast, EPA and DHA metabolites in FO-fed mice accounted for more than one-half (56 and 58%) of the total mono-hydroxylated metabolites at the respective days. The 5-hydroxy-eicosatetraenoic acid (HETE, AA-derived) and the analogous 5-hydroxy-EPA (5-HEPE, EPA-derived) and 4-hydroxy-DHA (HDoHE, DHA-derived) are used to illustrate the effect of FO substitution on the mono-hydroxylated metabolites (Fig. 2). Concentrations of 5-HETE increased ($P < 0.05$) over the course of infection in SO-fed mice (Fig. 2A). In FO-fed mice, 5-HETE levels were reduced ($P < 0.05$) at d 21 p.i. compared with SO-fed mice (Fig. 2A). In contrast, concentrations of 5-HEPE and 4-HDoHE were unchanged during infection of SO-fed mice but were increased ($P < 0.05$) in FO-fed mice at d 10 and 21 p.i. (Fig. 2B,C). Epoxide-containing metabolites were affected in a similar manner as the mono-hydroxylated metabolites in response to FO substitution (Supplemental Fig. 1).

Chiral analysis of mono-hydroxylated metabolites. Mono-hydroxylated metabolites can be generated enzymatically via LOX or nonenzymatically via reactive oxygen species (ROS). PUFA molecules are particularly prone to hydroxylation through oxidative stress mechanisms. The dramatic increase of EPA- and DHA-derived mono-hydroxylated metabolites in FO-fed mice was surprising, because 5-HETE was relatively low

TABLE 3 Total eicosanoid concentrations in ankle joints of mice infected with *B. burgdorferi* and fed a SO or FO diet for 2 and 3 wk p.i.¹

	SO			FO		
	0 d	10 d	21 d	0 d	10 d	21 d
<i>n</i>	4	4	9	4	4	9
Eicosanoid subclass	<i>ng/mg of tissue</i>					
PG	0.03 ± 0.01	0.04 ± 0.01	#0.14 ± 0.02	0.04 ± 0.01	*0.11 ± 0.03	#0.13 ± 0.02
Mono-hydroxylated	0.32 ± 0.15	0.92 ± 0.15	#2.03 ± 0.22	0.47 ± 0.22	#1.42 ± 0.44	*#1.44 ± 0.22
Di-hydroxylated	0.00 ± 0.00	0.01 ± 0.00	#0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*0.01 ± 0.00
Hepoxilins	0.00 ± 0.00	#0.05 ± 0.01	#0.11 ± 0.01	0.00 ± 0.00	#0.04 ± 0.01	*#0.05 ± 0.01
Lipoxins, resolvins, and protectins	0.00 ± 0.00	#0.00 ± 0.00	#0.01 ± 0.00	0.00 ± 0.00	*#0.01 ± 0.00	*#0.01 ± 0.00
Epoxide	0.06 ± 0.03	0.05 ± 0.01	#0.15 ± 0.02	0.09 ± 0.00	*0.21 ± 0.06	0.18 ± 0.02
Nonenzymatic	0.05 ± 0.02	0.03 ± 0.01	0.07 ± 0.01	0.05 ± 0.02	*#0.27 ± 0.06	*#0.29 ± 0.04
Totals	0.05 ± 0.02	1.10 ± 0.18	#2.35 ± 0.28	0.66 ± 0.29	#2.08 ± 0.60	#2.09 ± 0.32

¹ Values are total eicosanoid concentrations from ankle joints (ng/mg tissue) ± SEM. #Different from d 0, $P < 0.05$; *different between diets at that time, $P < 0.05$. FO, fish oil; p.i., postinfection; SO, soy oil.

in SO-fed mice. To investigate whether enzymatic or nonenzymatic mechanisms were driving the production of these mono-hydroxylated metabolites, we subjected our samples to chiral LC-MS analysis. Enzymatic production of these metabolites will yield only the S-isomer, whereas a racemic mixture (R- and S-isomers) is indicative of nonenzymatic production. The ratio between the R:S isomers of 5-HEPE and 4-HDoHE was ~1:1, indicating that both metabolites were predominantly nonenzymatically generated (Supplemental Fig. 2). Furthermore, chiral analysis revealed that the most abundant mono-hydroxylated metabolites (9- and 13-hydroxy-octadecadienoic acid, both LA derived) were also nonenzymatically generated. These findings suggest that the majority of mono-hydroxylated metabolites were the result of nonenzymatic mechanisms, possibly from ROS generated by immune cells to combat the bacterial infection.

Additionally, our methodology monitored a subset of eicosanoids (9-HETE, 9-HEPE, 8-HDoHE, 16-HDoHE, 20-HDoHE, and isoprostanes) that are generated by only nonenzymatic mechanisms and can be used to gauge the contribution of oxidative stress to the overall eicosanoid pool. The total amount of oxidative stress metabolites increased in the FO-fed mice at d 10 and 21 p.i. compared to d 0 ($P < 0.05$) (Table 3). Furthermore, 9-HEPE, 8-HDoHE, 16-HDoHE, and 20-HDoHE accounted for the majority of these metabolites and were all elevated ($P < 0.05$) at d 10 and 21 p.i. in FO-fed mice compared with d 0, suggesting that EPA and DHA are more readily oxidized than AA (Supplemental Table 1). However, isoprostanes, which are nonenzymatically derived PG, were detected at relatively low levels during infection regardless of the dietary regimen, suggesting that the detected PGs were enzymatically generated via COX.

Production of COX-derived metabolites. Eicosanoids generated from the COX pathway have been extensively studied and associated with a proinflammatory response (31). 6-Keto PGF_{1α}, a breakdown product of the highly unstable vasodilator, PGI₂, increased ($P < 0.05$) over the course of infection in SO-fed mice (Fig. 3A). However, in FO-fed mice 6k-PGF_{1α}, concentrations were reduced ($P < 0.05$) by 71% at d 21 p.i. compared with SO-fed mice. A similar trend was observed ($P < 0.01$) for the COX AA-derived vasoconstrictive metabolite, thromboxane B₂ (TXB₂) (Fig. 3B). TXB₂ was monitored as a stable surrogate for the unstable bioactive metabolite, thromboxane A₂. The

corresponding EPA eicosanoids (Δ17,6k-PGF_{1α}, and TXB₃) were not detected during the course of the infection.

Other COX-catalyzed, AA-derived, proinflammatory PG (PGD₂ and PGE₂) increased throughout the 21 d following infection with *B. burgdorferi* (Fig. 4A,B). At d 10 p.i., the concentrations of these PG were similar between mice fed either diet. However, by d 21 p.i., concentrations of PGD₂ and PGE₂ were 73 and 60% lower in FO-fed mice compared with SO-fed mice, respectively ($P < 0.05$) (Fig. 4A,B). The corresponding EPA-derived PG (3-series) were detected at a low abundance in SO-fed mice at any time following infection, but PGD₃ and PGE₃ was higher ($P < 0.05$) in FO-fed mice compared with SO-fed mice at d 10 and 21 p.i. (Fig. 4C,D).

Surprisingly, FO substitution led to the accumulation ($P < 0.05$) of the PGD₂ dehydration product 15-deoxy PGJ₂ at d 10 and 21 p.i. compared with SO-fed mice (Fig. 4E). The most abundant PGE₂ degradation product detected (15-keto PGE₂) did not accumulate to the same extent as 15-deoxy PGJ₂. The levels of 15-deoxy PGJ₂ in FO-fed mice were comparable with the levels of PGE₂ in SO-fed mice (compare Fig. 4B to E). These data suggest that dietary FO substitution caused AA to be redirected from PGE₂ synthesis to PGD₂ synthesis. To further investigate this phenomenon, we examined the total amount of metabolites detected from the PGE₂ or PGD₂ pathway in either diet group (Fig. 5). PGE₂ and related degradation products (PGE_{2total}) were more abundant ($P < 0.05$) than PGD_{2total} at d 10 and 21 p.i. in SO-fed mice (Fig. 5A). This trend was reversed in FO-fed mice, where PGD_{2total} was greater than PGE_{2total} (Fig. 5B). Moreover, the ratio (PGE_{2total}:PGD_{2total}) differed ($P < 0.05$) between each diet group at d 10 and 21 p.i. (Fig. 5C).

Development of Lyme arthritis and carditis. All mice were susceptible to infection with *B. burgdorferi* and developed characteristic inflammation of the ankle joints and heart typical of experimental Lyme disease. Ankle joint swelling (measured as increased ankle diameter) was monitored throughout a 25-d time course (Supplemental Fig. 3A). Ankle diameters reached a maximum at ~10 d p.i. and returned to near preinfection levels by d 25, which is a typical time course of joint swelling in this experimental model. No differences in ankle diameters were observed between mice fed the 2 diets, indicating that FO substitution did not affect the inflammatory response to *B. burgdorferi* infection. Arthritis and carditis scores between the

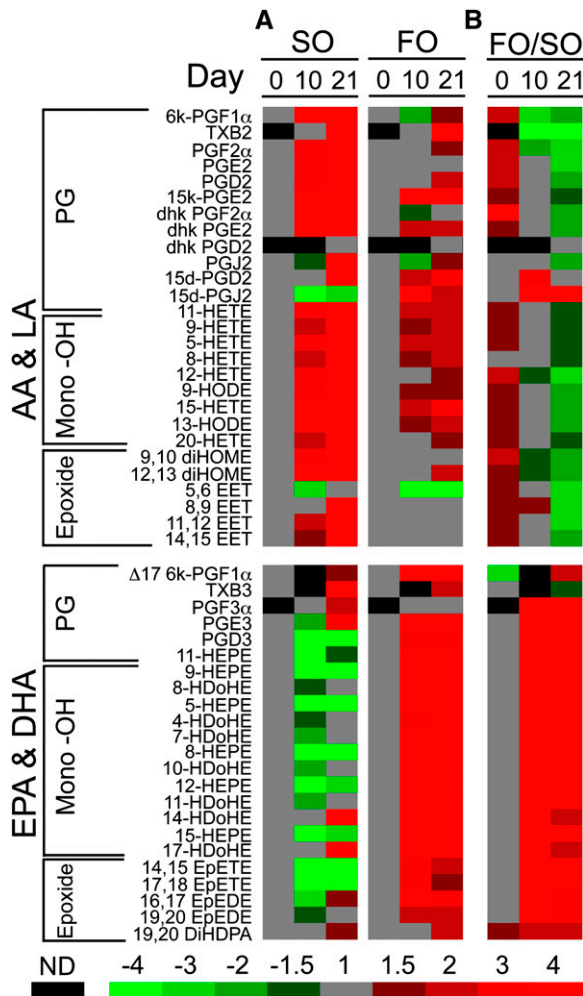


FIGURE 1 Heat map displaying the relative fold changes of selected AA-, LA-, EPA-, and DHA-derived eicosanoids in ankle joints of mice infected with *B. burgdorferi* and fed a SO or FO diet. Eicosanoids are grouped based on their subclassification. (A) Fold changes in the eicosanoid profile relative to d 0 in response to Lyme infection are shown for mice fed a SO or FO diet. For eicosanoids not detected on d 0, relative fold changes were determined from the day they first appeared. (B) Direct comparison between relative eicosanoid concentrations detected in SO- and FO-fed mice. ND (black) indicates the eicosanoid was either not detected or below our level of detection. $n = 4$ (d 0 and 10) and 9 (d 21). AA, arachidonic acid; FO, fish oil; LA, linoleic acid; SO, soy oil.

SO- and FO-fed mice were similar in both their overall severity and in the cellular makeup of the inflammatory infiltrate (Supplemental Fig. 3B,C). Similarly, we found no differences in the numbers of spirochetes present in the joints or hearts of mice fed the 2 diets (Supplemental Fig. 3D,E, respectively). Thus, we saw no effect of the FO diet on the development of Lyme arthritis or carditis. Extended dietary supplementation for up to 5 wk prior to infection with *B. burgdorferi* similarly did not affect the subsequent development of disease (data not shown).

Discussion

We used a high-throughput lipidomics methodology to assess the impact of substituting dietary (n-6) PUFA-containing SO with (n-3) PUFA-containing FO on the eicosanoid profile in mice in response to an infectious agent. Our results demonstrate that

dietary FO substitution had extensive compositional and temporal effects on enzymatically and nonenzymatically generated eicosanoids during the host response to infection. We fed mice the FO diet for 2 wk prior to infecting them with *B. burgdorferi*. Fatty acid analysis of liver and serum samples indicated that 2 wk on the FO diet was sufficient for EPA and DHA incorporation into membrane phospholipids ($P < 0.05$). Dietary fatty acids are incorporated into membrane phospholipids in proportion to their dietary concentration and can significantly affect membrane composition in as little as 1 wk (9,12). High concentrations of dietary fatty acids continue to increase their presence in membrane phospholipids until an equilibrium is reached. In the mouse model of Lyme arthritis, the inflammatory response develops during wk 2 of infection and peaks during wk 3 and 4. Thus, there was adequate time for incorporation of dietary EPA and DHA into the membranes of immune cells in our study. However, caution should be exercised in interpreting our findings, because the high level of EPA and DHA provided in our experimental diet exceeded by 10- to 20-fold what is reasonable in humans.

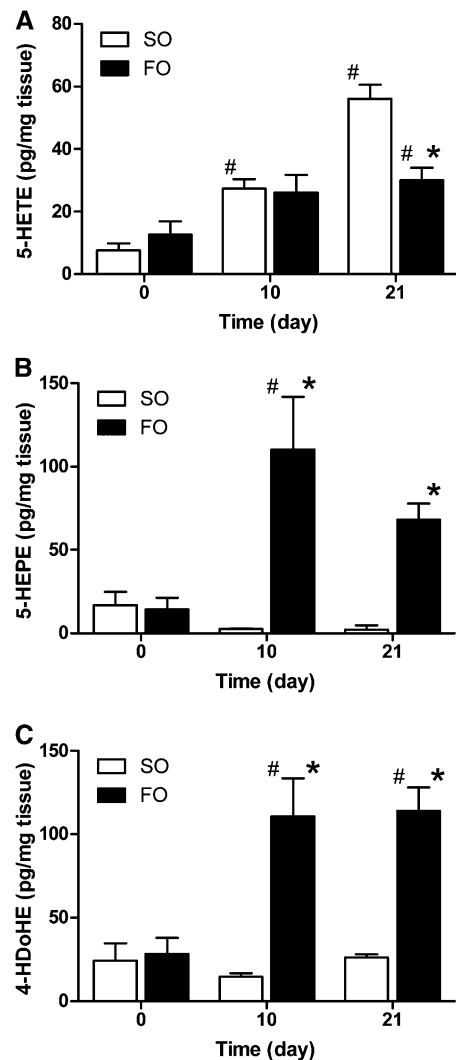


FIGURE 2 Quantitative amounts of representative mono-hydroxylated eicosanoids in ankle joints of mice infected with *B. burgdorferi* and fed a SO or FO diet. Concentrations of 5-HETE (A), 5-HEPE (B), and 4-HDoHE (C). Values are means \pm SEM, $n = 4$ (d 0 and 10) or 9 (d 21). #Different from d 0; *different from SO at that time, $P < 0.05$. FO, fish oil; HDoHE, hydroxy-DHA; HEPE, hydroxy-EPA; HETE, hydroxy-eicosatetraenoic acid; SO, soy oil.

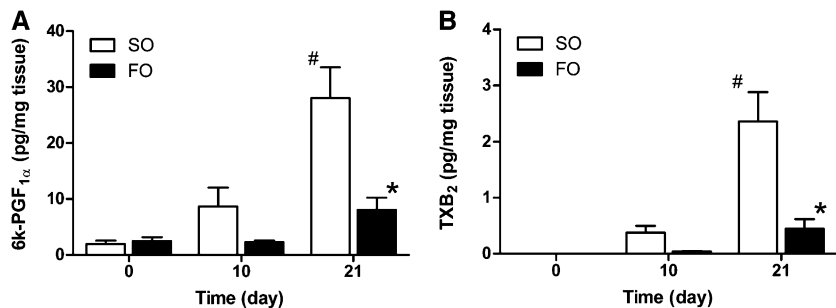


FIGURE 3 Quantitative amounts of COX-derived 6-keto $\text{PGF}_{1\alpha}$ (A) and TXB_2 (B) in ankle joints of mice infected with *B. burgdorferi* and fed a SO or FO diet. Values are means \pm SEM, $n = 4$ (d 0 and 10) or 9 (d 21). #Different from d 0; *different from SO at that time, $P < 0.05$. COX, cyclooxygenase; FO, fish oil; SO, soy oil; TXB_2 , thromboxane B_2 .

Whereas dietary FO had a significant impact on the composition of membrane fatty acids, it had little effect on the production of eicosanoids in the joints of uninfected mice. Following infection, however, dietary FO had a profound global effect on the production of eicosanoids in infected joints. Infection of SO-fed mice had the expected result of increasing the production of AA-derived eicosanoids, with PGE_2 being the most abundant COX product (30). Compositional changes to the eicosanoid profile in response to dietary FO were seen in every eicosanoid subclass. Whereas FO substitution caused a decrease in many AA-derived eicosanoids, unexpectedly, it led to the accumulation of the PGD_2 -derived, antiinflammatory mediator 15-deoxy PGJ_2 . Numerous in vitro studies have implicated 15-deoxy PGJ_2 as an antiinflammatory mediator that activates $\text{PPAR}\gamma$ and blocks $\text{NF-}\kappa\text{B}$ activation via I κB kinase (IKK) inhibition (32–36). 15-Deoxy PGJ_2 is generated from PGD_2 by undergoing 2 subsequent dehydration steps. We speculate that the elevated 15-deoxy PGJ_2 levels were directly related to increased oxidative stress in the FO-fed mice. FO substitution led to a significant increase in nonenzymatically generated eicosanoids, presumably from ROS. Under these oxidative stress conditions, we propose that PGD_2 underwent subsequent dehydration steps, yielding 15-deoxy PGJ_2 .

Although the accumulation of 15-deoxy PGJ_2 can possibly be explained by increased oxidative conditions, we cannot conclusively describe the increased amounts of the precursor PGD_2 in

FO-fed mice. Altering the prostanoids derived from COX-2 via inhibitor treatment or gene knockout affected the normal immune response in Lyme-infected mice (19). Changing the prostanoid profile caused infiltrating neutrophils to remain in the ankle joint; thus, the mice did not resolve the Lyme infection. Although we did not observe differences in neutrophil infiltration into the infected joint between mice on either diet, we cannot rule out that dramatically altering the eicosanoid profile did not affect other cell types present at the site of infection. Additionally, changes in the eicosanoid profile could affect the expression and activity of eicosanoid synthases.

Mono-hydroxylated metabolites made up the majority of eicosanoids detected in response to Lyme infection. Chiral analysis of major and minor mono-hydroxylated metabolites strongly suggested they were generated through nonenzymatic pathways. ROS can attack PUFA molecules at their double bonds, resulting in the formation of peroxyated and hydroxylated species. Immune cells (like neutrophils) infiltrate the site of infection and release ROS as a first line of defense against invading bacteria. 5-HEPE is a major metabolite produced by stimulated neutrophils when supplied with EPA (37,38). It is thus not surprising to find a large percentage of these molecules generated by nonenzymatic means given the amount of ROS at the site of infection. Regardless, bioactive isomers are being generated along with nonbioactive isomers, which could have 2 possible effects: 1) the nonbioactive R isomers could compete with bioactive

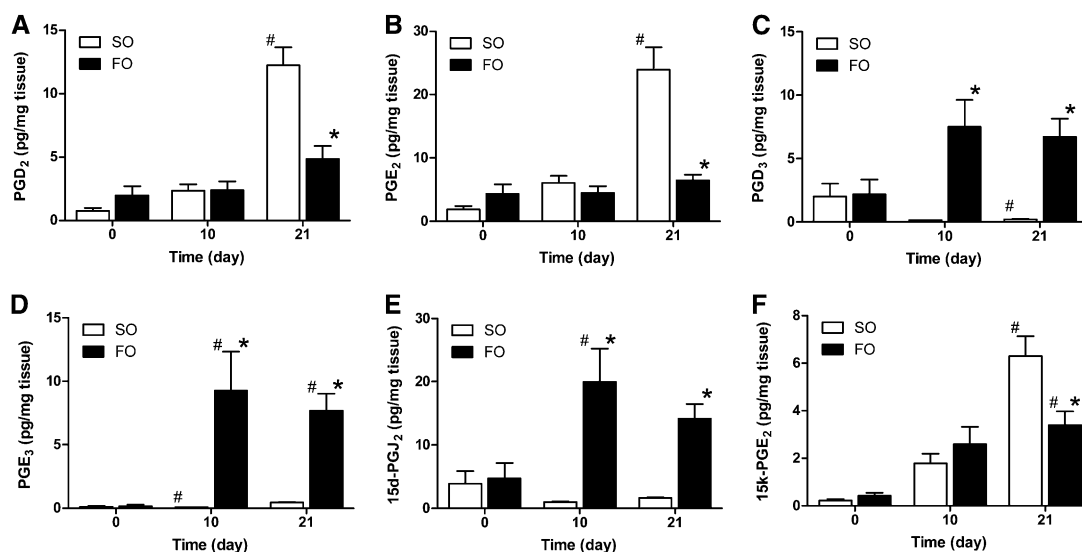


FIGURE 4 Quantitative amounts of COX-derived PGD_2 , PGE_2 , and related metabolites in ankle joints of mice infected with *B. burgdorferi* and fed a SO or FO diet. Concentrations of AA-derived PGD_2 (A) and PGE_2 (B), EPA-derived PGD_3 (C) and PGE_3 (D), and AA-derived 15d- PGJ_2 (E) and 15k- PGE_2 (F). Values are means \pm SEM, $n = 4$ (d 0 and 10) or 9 (d 21). #Different from d 0; *different from the SO at that time, $P < 0.05$. AA, arachidonic acid; COX, cyclooxygenase; FO, fish oil; SO, soy oil.

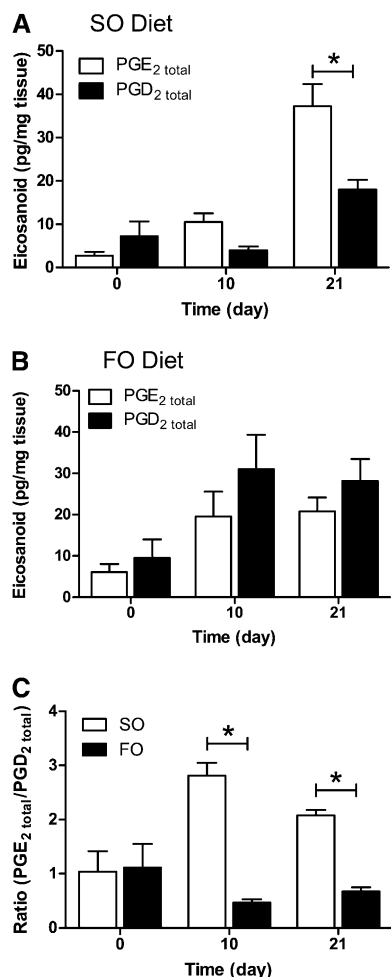


FIGURE 5 Quantitative amounts of PGD₂ and derived metabolites, and PGE₂ and derived metabolites in ankle joints of mice infected with *B. burgdorferi* and fed an SO (A) or FO (B) diet. (C) The ratio of (PGE₂total:PGD₂total) is shown for mice fed the SO or FO diet. Values are means ± SEM, n = 4 (d 0 and 10) or 9 (d 21). *Different between PGD₂total and PGE₂total (A,B) and between diets (C), P < 0.05. FO, fish oil; SO, soy oil.

S isomers for their cognate receptors, resulting in a reduction of receptor activation; or 2) the R isomers could not be inhibitory and the S isomers could propagate their proinflammatory signal.

Cytochrome P450-generated, epoxide-containing EPA- and DHA-derived eicosanoids were also dramatically more abundant in FO-fed mice than in SO-fed mice. Recently, EPA and DHA were reported to be antihyperalgesic in an inflammatory rat pain model (39). The cytochrome P450 metabolite profile from FO-fed mice is very similar to the mono-hydroxylated metabolite profile, suggesting that nonenzymatic mechanisms may also be involved in their production. Unfortunately, the lack of commercial + and - isomer standards prevented us from determining the +/- ratio of these molecules. A similar speculation on the impact of the mono-hydroxylated metabolites can be applied to these metabolites as well.

Despite a definite global shift in the eicosanoid profile to a more antiinflammatory/proresolution tone in mice fed the FO diet, we found no effects on the amount of joint inflammation or severity of Lyme arthritis or carditis. Dietary (n-3) PUFA have been reported to both improve and impair host responses to a number of pathogens (40). Although the increased production of antiinflammatory metabolites derived from EPA and DHA and

decreased production of proinflammatory AA-derived metabolites are thought to be the major mechanisms involved, there are clearly other nuances to the system that we have yet to understand. One possible explanation is that reductions in concentrations of the more bioactive AA-derived eicosanoids may be negated by the overall increase in eicosanoid production. 3-Series PG are bioactive but are reportedly only 30% as effective as analogous 2-series compounds in inducing inflammatory responses (13). Any antiinflammatory effects from the (n-3) fatty acid-derived metabolites could potentially be masked if the increase in total eicosanoids was large enough to reach an inflammatory threshold. Also, the increased amount of oxidative stress in the FO-fed mice could be a result of the extreme nature of the diet. Feeding the mice a diet containing a lower composition of FO may reduce the amount of oxidative stress and allow the beneficial effects of FO to be observed. Some of the beneficial effects of FO may be attributed to its use in the production of less bioactive molecules (41), which could lessen the intensity of the immune response. Immune suppression can also protect against chronic inflammatory conditions but comes at the price of increased susceptibility to infection. A previous study showed that dietary FO-supplemented mice had higher mortality rates when challenged with influenza (42). In our study, we show that mice fed a FO-diet were capable of mounting a full immune response against *B. burgdorferi*. Thus, shifting the eicosanoid profile to a more antiinflammatory/proresolution tone does not necessarily impede the immune system from functioning properly.

Finally, although the translatability of these findings may be limited by the extreme nature of the dietary intervention employed, we were successful in demonstrating for the first time, to our knowledge, the power of using a systems biology approach to examine the effects of FO substitution on the eicosanoid profile in response to a bacterial infection. Monitoring a few select eicosanoids would not have revealed the full extent to which dietary FO affected the eicosanoid profile. Eicosanoid changes were observed in every eicosanoid subclass monitored, revealing a change in the inflammatory tone. Eicosanoids are a complex and diverse class of bioactive mediators with overlapping signaling properties. These molecules can alter their signaling properties depending on the cell types present and the specific receptors being expressed. To further complicate the picture, both proinflammatory and antiinflammatory mediators can be produced concomitantly (1,30). A systems approach allows the behavior of these bioactive metabolites to be viewed as a class. This methodology can be applied to investigating the role of eicosanoids in other models of acute and chronic inflammation.

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E.A.D. and C.R.B. designed the research project; D.S.D., A.M.C., L.E.W., R.H., K.M.L., and V.A.B. conducted the experiments; K.L.F. formulated the diets; D.S.D., P.C.N., J.H.H., W.J.M., and C.R.B. analyzed the data; D.S.D., A.M.C., and C.R.B. wrote the manuscript; and D.S.D., K.L.F., E.A.D., and C.R.B. edited the manuscript. All authors read and approved the final manuscript.

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