Supplementation of linoleic acid (C18:2n-6) or α -linolenic acid (C18:3n-3) changes microbial agonist-induced oxylipid biosynthesis

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ABSTRACT

Oxylipids are derived from polyunsaturated fatty acids (PUFA) in cellular membranes and the relative abundance or balance may contribute to disease pathogenesis. Previous studies documented unique oxylipid profiles from cows with either coliform or *Streptococcus uberis* mastitis, suggesting that lipid mediator biosynthesis may be dependent on the type of microbial-derived agonist. Changing the fatty acid content of peripheral blood leukocytes also may be critical to the relative expression of oxylipid profiles and the outcome of bacterial infection. No information is available in dairy cows describing how changing cellular PUFA content will modify oxylipids in the context of a microbial agonist challenge. Therefore, the hypothesis for the current study was that PUFA supplementation would change bovine leukocyte fatty acid content and respective oxylipid profiles from ex vivo microbial agonist-challenged leukocytes. Fatty acid content of leukocytes and plasma was quantified in (1) samples from cows not supplemented with PUFA, (2) cows supplemented with linoleic acid (LnA), and (3) cows supplemented with α -linolenic acid (ALA). Plasma oxylipids were assessed after *S. uberis* or lipopolysaccharide exposure and was compared with unstimulated oxylipid profiles. Fatty acid supplementation with ALA significantly increased ALA content of blood leukocytes and plasma relative to LnA. Fatty acid supplementation affected several *S. uberis*-induced oxylipids, but only *S. uberis*-induced 15-oxoETE was greater with ALA supplementation compared with LnA. Notably, only LPS-induced 5,6 LXA₄ was altered with fatty acid supplementation, but no significant effect of LnA vs. ALA treatment was identified. Future studies are needed to understand how leukocyte activation and membrane PUFA availability collectively contribute to differential oxylipid profiles.

Key words: oxylipin, lipid mediator, polyunsaturated fatty acid, *Streptococcus uberis*, lipopolysaccharide

INTRODUCTION

The outcome of bovine mastitis may be dependent, in part, on potent lipid mediators derived from PUFA. Specifically, the oxygenation of PUFA following cleavage from the phospholipid membrane generates a profile of pro- and anti-inflammatory oxylipids. The profile of pro- and anti-inflammatory oxylipids may depend on the specific causative pathogen and its capability to induce different immune recognition and signaling pathways (Bannerman et al., 2004a; Mavangira et al., 2015; Ryman et al., 2015). For example, a diverse oxylipid profile was robustly upregulated in milk during severe gram-negative coliform mastitis, whereas oxylipid profiles were not changed in milk during clinical gram-positive *Streptococcus uberis* mastitis (Mavangira et al., 2015; Ryman et al., 2015). Coliform mastitis is characterized by an acute, robust inflammatory response with clinical symptoms appearing in as little as 8 h after challenge predominantly as a result of LPS exposure (Eberhart, 1977; Bannerman et al., 2004b; Moyes et al., 2014). In contrast, the uncontrolled inflammatory response to *S. uberis* does not result in clinical symptoms until several days after infection and may develop into a chronic inflammatory disease (Thomas et al., 1994; Pedersen et al., 2003). Thus, understanding oxylipid biosynthesis in the context of different microbial agonist challenges may be critical for developing optimal nutritional and pharmacological strategies to manage disease prevalence and severity.

Several PUFA, including linoleic acid (LnA, C18:2n-6), arachidonic acid (ArA, C20:4n-6), eicosapentaenoic acid (EPA, C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), are the predominant substrates for oxylipid biosynthesis (Raphael and Sordillo, 2013). The PUFA are esterified to the phospholipid membrane and are cleaved by calcium-dependent cytosolic phospholipase A₂ in response to a variety of agonists, such as recognition of pathogen-associated molecular

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patterns by host pathogen receptors (Buczynski et al., 2009; Chiang et al., 2012). Enzymatic and nonenzymatic pathways oxidize cleaved PUFA to create a complex network of oxylipids (Buczynski et al., 2009). Enzymatic oxidation pathways include the cyclooxygenase, lipoxygenase (**LOX**), and cytochrome P450 (**CYP**) pathways. Nonenzymatic oxidation is mediated by reactive oxygen species and free radicals. Initial peroxidation products including hydroperoxyoctadecadienoic acids are immediately reduced to stable hydroxyl analogs, such as hydroxyeicosatetraenoic acids (**HETE**; Sordillo et al., 2005; Serhan and Petasis, 2011; Smith et al., 2011). Reduced oxylipids, including hydroxyls and epoxides, can serve as substrates for metabolism to dehydrogenase (e.g., oxoeicosatetraenoic acids, **oxoETE**) and hydrolase products (e.g., dihydroxyeicosapentaenoic acid, **DiHETE**; Ramsden et al., 2012). The effects of oxylipids on inflammation may depend on method of oxidation and the degree of metabolism. For example, 15-LOX-derived lipoxin A₄ (**LXA₄**) prevented disruption of the rat small bowel mesenteric endothelial barrier during LPS challenge (Ereso et al., 2009). However, the precursor to LXA₄, 15-hydroperoxyeicosatetraenoic acid, induced apoptosis of bovine aortic endothelial cells in an oxidative stress model (Sordillo et al., 2005).

In addition to the method of oxidation and degree of metabolism, the availability of PUFA substrate can directly alter oxylipid profiles. Linoleic acid and α -linolenic acid (**ALA**, C18:3n-3) are essential fatty acids that must be provided in the diet because de novo synthesis requires desaturase enzymes that are absent in mammals (Nakamura and Nara, 2003). Dietary LnA contributes to lipid membrane LnA content but also can be used for de novo ArA synthesis. Similarly, ALA can be used for EPA and DHA de novo synthesis. The de novo synthesis requires a series of desaturation, elongation, and potentially β -oxidation events (Marcel et al., 1968; Sinclair et al., 2002). In monogastrics, feeding essential fatty acids or other PUFA changed the fatty acid profile of plasma and various tissues (Ramsden et al., 2012). Similarly, previous research in monogastrics and ruminants showed that abundance and type of PUFA within the phospholipid membrane can be altered by the diet and influence disease status (Calder, 2008; Raphael and Sordillo, 2013; Min et al., 2014). For example, obese pregnant women supplemented with 1,200 mg of EPA and 800 mg of DHA resulted in decreased systemic markers of inflammation, such as C-reactive protein and pro-inflammatory cytokines (Haghiac et al., 2015). One of the ways in which PUFA supplementation is thought to mediate disease severity is through the biosynthesis of oxylipids. Supplementation of humans with 1,008 mg of EPA and 672 mg of DHA contributed to greater EPA-derived, but not

DHA-derived, oxylipids (Schuchardt et al., 2014). Conversely, reducing nutritional LnA in humans decreased LnA-derived plasma oxylipids (Ramsden et al., 2012). In dairy cows, dietary PUFA supplementation is more challenging because isomerization and biohydrogenation of PUFA by rumen microbes results in conversion of PUFA to SFA (Glasser et al., 2008; Jenkins et al., 2008). Recent studies used abomasal supplementation of PUFA to modify milk fat, but PUFA content of cells and tissues was not investigated, so it is unclear if abomasal supplementation changes cellular PUFA content as well as if there is any effect on oxylipids during inflammation (Lock et al., 2007). Thus, the hypothesis for the current study was that PUFA supplementation would change bovine leukocyte fatty acid content and respective oxylipid profiles from ex vivo microbial agonist-challenged leukocytes.

MATERIALS AND METHODS

Supplementation of Fatty Acids by Abomasal Infusion

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Six ruminally fistulated multiparous (2+) mid-lactation Holstein cows (91.7 $\pm$ 8.36 d in milk) from the Michigan State University Dairy Teaching and Research Center (East Lansing, MI) were blocked by milk yield. The cows were then randomly assigned to a treatment sequence in a replicated 3 $\times$ 3 Latin square design experiment with a 7-d preliminary period and three 7-d treatment periods separated by 7-d washout periods (Lock et al., 2007). Prior to the first treatment period, all cows were fitted with an abomasal infusion line. All lines were flushed daily with water and ethanol during the treatment periods to ensure proper delivery of the fatty acid infusion treatments into the abomasum. Treatments consisted of an ethanol carrier infusion (**CON**), LnA infusion, and ALA infusion. The LnA infusion blend consisted of 82% safflower oil, 17% high omega flax oil, and 1% palm oil. The ALA infusion blend consisted of 93% high omega flax oil, 6% palm oil, and 2% safflower oil. Fat blends were dissolved in ethanol before infusion and administered at a dose of approximately 62 g/d with LnA or ALA comprising approximately 40 g/d. The fatty acid profile of infusion treatments displayed in Table 1 were determined by GLC according to methods previously described (Lock et al., 2013). Daily infusions were divided and administered at 6-h intervals throughout each 7-d treatment period. The infusions were administered at 0500, 1100, 1700, and 2300 h. Blood was collected in 4 mM EDTA anticoagulant prior

Table 1. Fatty acid composition of linoleic acid (LnA) and α -linolenic acid (ALA) infusions

Fatty acid	LnA (g/100 g)	ALA (g/100 g)
Myristic acid (C14:0)	0.09	0.09
Palmitic acid (C16:0)	6.40	6.36
Palmitoleic acid (C16:1 <i>cis</i> -9)	0.17	0.05
Stearic acid (C18:0)	2.44	2.52
Oleic acid (C18:1 <i>cis</i> -9)	16.05	15.22
LnA (C18:2n-6)	60.92	13.41
ALA (C18:3n-3)	10.68	60.49
Σ SFA	1.62	1.42
Σ MUFA	9.63	9.43
Σ PUFA	17.05	16.06
Σ n-3 PUFA	71.65	74.07
Σ n-6 PUFA	10.70	60.62

to treatment, after a 7-d washout period (prior to next treatment), and 7 d after treatment with PUFA. Blood samples were transported back to the laboratory on ice for immediate collection to quantify fatty acid content of blood leukocytes or for ex vivo challenge with microbial agonists. Ex vivo challenge with microbial agonists is described in the next section.

Cows were housed individually in tie stalls throughout the experiment. The cows were milked twice daily at 0330 and 1630 h. The SCC was monitored before the beginning of the trial and was an average of 177,667 SCC/mL. Enrolled cows were monitored for symptoms of mastitis and other diseases. Abnormalities in milk and mammary gland appearance were assessed for detection of clinical mastitis. Mammary gland abnormalities included pain, swelling, or hardening of the mammary gland, heat, or redness, and milk abnormalities included a watery consistency, clots, or pus. Access to feed was blocked from 0800 to 1000 h daily to allow for collection of orts and distribution of feed. Feed intake was measured daily and cows were fed at 115% of expected DMI at 1000 h each day. Water was available ad libitum at all times. Stalls were bedded with sawdust and cleaned twice daily. The composition of the diet was a standard TMR with mineral and vitamin supplementation (Table 2). The DM content of the forages was determined twice weekly and the diets were adjusted as needed.

Microbial Agonist Ex Vivo Challenge of Blood Leukocytes from Nonsupplemented and Supplemented Animals

Heat-killed *S. uberis* strain 0140J was prepared by collecting 3 pure *S. uberis* colonies from a blood agar plate and adding them to 100 mL of RPMI 1640 medium (Cellgro, Manassas, VA) containing 5% fetal bovine serum, 300 mg/mL of L-glutamine, and 0.1 μ M

selenium. The bacterial suspension was shaken at 37°C for 13 h. After that time, the suspension was heat-killed in a 65°C water bath for 45 min. Suspensions diluted in 1 $\times$ PBS were aliquoted at 10⁹ cfu of *S. uberis*/mL and frozen at -20°C until use. Ultrapure LPS derived from *Escherichia coli* (InvivoGen, San Diego, CA) was purchased at a stock of 5 mg/mL and kept at -20°C until use, at which time LPS was diluted in 1 $\times$ PBS to 1 μ g/mL before experimental agonist challenge. Blood samples from cows abomasally infused with ethanol carrier, LnA, or ALA were aliquoted into pre-labeled tubes and either 10⁷ cfu of *S. uberis*/mL, 1 μ g/mL of LPS, or 1 $\times$ PBS were added. The dose of LPS was chosen based on previous literature (Sellers et al., 2013). The dose of *S. uberis* was chosen based on previous research demonstrating that pathogen load following *S. uberis* intramammary challenge may reach up to 10⁷/mL (Leigh et al., 2010). All samples, including unstimulated 1 $\times$ PBS control, were gently rotated for mixing then incubated in a 37°C water bath for 4 h. The 4-h time point was selected based on previous studies with whole blood LPS challenge (Røntved et al., 2005) and replicated with heat-killed *S. uberis* so as to compare differences in fatty acid and oxylipid profiles at a similar time point.

Whole Blood Processing for Quantification of Fatty Acids and Oxylipids

Whole blood collected from animals in the 3 treatment groups (CON, LnA, and ALA), but not cultured

Table 2. Ingredient and nutrient composition of the treatment diet¹

Item	Value
Ingredient (% of DM)	
Corn silage	28.4
Alfalfa silage	16.7
Wheat straw	3.2
Dry ground corn	17.5
High moisture corn	8.5
Soybean meal	14.8
Cottonseed with lint	7.4
Vitamin-mineral mix ¹	2.1
Limestone	0.76
Sodium bicarbonate	0.75
Nutrient composition (% of DM)	
DM (%)	58.7
NDF	30.6
CP	17.5
Starch	26.3
Fatty acid	3.3

¹Vitamin and mineral mix contained 34.1% dry ground shell corn, 25.6% white salt, 21.8% calcium carbonate, 9.1% Biofos (Mosaic Crop Nutrition, Riverview, FL), 3.9% magnesium oxide, 2% soybean oil, and <1% of each of the following: manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, iodine, cobalt carbonate, vitamin E, vitamin A, vitamin D, and selenium.

with microbial agonists, was collected and immediately spun at $4,816 \times g$ for 20 min at room temperature to enable separation of buffy coat. The buffy coat fraction, containing primarily monocytes, neutrophils, and lymphocytes, was collected and deposited into a separate 50-mL tube. A volume of 20 mL of Ammonium-Chloride-Potassium lysing buffer (Qiagen, Hamburg, Germany) was added and incubated for 4 min at room temperature to remove contaminating erythrocytes. To neutralize excess lysis buffer, 20 mL of $1 \times$ PBS was added after incubation. The samples were spun at $4,816 \times g$ for 5 min at 4°C and the supernatant containing lysed erythrocyte fractions was discarded. The pellet was resuspended in 20 mL of $1 \times$ PBS and washed twice. Last, the supernatant was discarded and the leukocyte pellet was stored at -80°C until further analysis.

Whole blood collected from animals in the 3 treatment groups (CON, LnA, and ALA), then cultured with microbial agonists (LPS and heat-killed *S. uberis*) for 4 h, was centrifuged at $4,816 \times g$ for 20 min at room temperature. A control sample, not stimulated with microbial agonists, was simultaneously incubated at 4 h, then centrifuged at $4,816 \times g$ for 20 min at room temperature. A 2-mL aliquot of plasma then was collected and immediately flash-frozen in liquid nitrogen until solid phase extraction.

Chemicals and Reagents

Liquid chromatography/mass spectrometry (LC-MS) grade acetonitrile, LC-MS methanol, formic acid, EDTA, and triphenylphosphine (TPP) were purchased from Sigma-Aldrich (St. Louis, MO). Standards, deuterated and nondeuterated, and indomethacin were purchased from Cayman Chemical (Ann Arbor, MI). Butylated hydroxy toluene (BHT) was purchased from ACROS (Morris Plains, NJ).

Solid Phase Extraction of Fatty Acids and Oxylipids in Leukocyte and Cultured Plasma

The leukocyte pellets were thawed on ice and resuspended in 600 μL of PBS. A 40- μL aliquot was collected for quantification of total DNA in each sample to normalize fatty acid levels (Silva et al., 2013; Mattmiller et al., 2014). Total DNA was quantified using Broad Range Quant-iT DNA Assay kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Samples were sonicated after addition of 15 μL of internal standards and 4 μL of antioxidant reducing agent. The antioxidant reducing agent mixture was 0.9 mM of BHT, 0.54 mM EDTA, 3.2 mM TPP, and 5.6 mM indomethacin in 50% methanol, 25% ethanol, and

25% water. Samples were immediately frozen in liquid nitrogen and stored at -80°C . The internal standard mixture contained 0.25 μM 5(S)-HETE- d_8 , 0.25 μM 15(S)-HETE- d_8 , 0.5 μM 8(9)-epoxyeicosatrienoic acid (EET)- d_{11} , 0.5 μM prostaglandin E2- d_9 , 0.25 μM 8,9-dihydroxyeicosatrienoic acid (DHET)- d_{11} , 50 μM AA- d_8 , 2 μM 2-arachidonoyl glycerol- d_8 , and 0.2 μM arachidonoyl ethanolamide- d_8 . A volume of 900 μL of methanol was added to each sample. To release fatty acids from cell membranes, samples were hydrolyzed with 3 mM potassium hydroxide and incubated at 45°C for 45 min. Samples were cooled, acidified to a pH <3 using 1 N hydrochloric acid, and centrifuged at $4,816 \times g$ for 45 min at 4°C . Supernatants were collected for solid phase extraction.

For flash-frozen plasma collected from blood following 4 h of microbial agonist challenge, all plasma aliquots were thawed on ice. A volume of 4 μL of antioxidant reducing agent, 15 μL of internal standards, and 2 μL of formic acid was added. The antioxidant reducing agent contained 0.9 mM of BHT, 0.54 mM EDTA, 3.2 mM TPP, and 5.6 mM indomethacin in 50% methanol, 25% ethanol, and 25% water. Internal standards in ethanol were 0.25 μM 5(S)-HETE- d_8 , 0.25 μM 15(S)-HETE- d_8 , 0.5 μM EET- d_{11} , 0.5 μM prostaglandin E2- d_9 , 0.25 μM DHET- d_{11} , 50 μM AA- d_8 , 2 μM 2-arachidonoyl glycerol- d_8 , and 0.2 μM arachidonoyl ethanolamide- d_8 . A volume of 5 mL of methanol was added and the sample was vigorously vortexed for 2 min. Samples were incubated for 15 min at room temperature and then centrifuged at $14,000 \times g$ for 10 min at 4°C . The supernatant was aspirated and added to 95 mL of HPLC-grade water and 95 μL of formic acid. Solid phase extraction was conducted with Oasis HLB 12cc (500 mg) LP Extraction Columns (Waters, Milford, MA) pre-conditioned with 6 mL of methanol followed by 6 mL of HPLC water. Sample extracts from plasma and blood leukocytes were loaded onto the columns and then washed with 6 mL of 20% methanol. Columns were dried under full vacuum for 4 min and extracts were eluted with 6 mL of methanol:acetonitrile 50:50 (vol:vol). Solvents were removed under vacuum using a Savant SpeedVac. Oxylipid extracts were reconstituted in 150 μL of methanol:water (2:1) and centrifuged at $14,000 \times g$ for 15 min at 4°C . The supernatant was transferred to a chromatography vial, topped with argon, and stored at -20°C until analysis.

LC-MS Quantification of Fatty Acids

All fatty acids, both leukocyte-derived and plasma-derived, were quantified based on previously described techniques (Ryman et al., 2015). Briefly, reverse-phase

LC on a Waters Acquity ultra performance LC BEH C18 1.7 μ M column (2.1 $\times$ 100 mm) was used at a flow rate of 0.6 mL/min at 35°C. The quadrupole MS was in electrospray negative ionization mode and voltage was -3 kV with the turbo ion spray source temperature at 450°C. The isocratic mobile phase for method 1 was acetonitrile:water:formic acid (45:55:0.01 vol/vol/vol) and 15 min of analysis time. Method 2 had an isocratic mobile phase consisting of acetonitrile:methanol:water:formic acid (47.4:15.8:26.8:0.01 vol/vol/vol/vol) and 10 min of analysis time. A total of 5 PUFA, 2 SFA, and 1 MUFA were quantified by matching mass-1 and retention time with corresponding deuterated internal standard abundance and calibrated to a linear 5-point standard curve ($R^2 > 0.99$). Fatty acids quantified by Waters Empower Z software (Waters) for plasma samples and hydrolyzed leukocytes were as follows: LnA, ALA, ArA, EPA, DHA, palmitic acid, oleic acid, and stearic acid. The limit of detection was established as the lowest point of each standard curve.

LC-MS/MS Quantification of Oxylipids

Plasma oxylipids were quantified based on techniques previously described (Mavangira et al., 2015). Briefly, a Waters Acquity UPLC coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters) was employed. Mobile phase A was HPLC water with 0.1% formic acid, and mobile phase B was acetonitrile. Flow rate across the C18 HPLC column was 0.3 mL/min. Linear gradient steps were programmed in the following manner (A/B ratio): time 0 to 0.5 min (99/1), to (60/40) at 2.0 min, to (20/80) at 8.0 min, to (1/99) at 9.0 min, 0.5 min, held at (1/99) until min 13.0, then return to (99/1) at 13.01 min, and held at this condition until 15.0 min. Oxylipids were detected using electrospray ionization in negative-ion mode. Cone voltages and collision voltages were optimized using the Waters QuanOptimize software (Mavangira et al., 2015).

Statistical Analysis

Statistical analyses were performed using commercially available software (SAS 9.3, SAS Institute Inc., Cary, NC). The efficacy of abomasal supplementation treatment groups on absolute fatty acid content of leukocyte and plasma without ex vivo microbial agonist challenge or in the context of microbial agonist challenge was analyzed as a balanced, 2-square, 3×3 Latin square design, including treatment and period as fixed effects. Delta values for changes in microbial agonist-induced fatty acid and oxylipid concentrations relative to abomasal supplementation were tested with the same model. Delta values for microbial agonist-induced

fatty acid and oxylipid concentrations were calculated by subtracting the concentration of fatty acids or oxylipids detected in unstimulated samples from concentrations in challenged samples. Parameterization of the model was $y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$, where y_{ijk} was the observation at treatment i and period j on the k th cow, μ is the overall mean, α_i is the effect of the i th treatment ($i = 1, 2, 3$), β_j was the effect of the j th period ($j = 1, 2, 3$), e_{ijk} is the random residual effect for all i, j, k , and e_{ijk} is supposed normally, independently identically distributed $NIID(0, \sigma_e^2)$, with a mean of 0 and a variance σ_e . The compound symmetry covariance structure was used to model the repeated measurement of cows in different periods, and Kenward-Roger adjustment of degrees-of-freedom was applied (West et al., 2015). Model diagnostics included visual assessment of the normality assumption of residuals and homogeneous variance among treatment groups. To determine if there was an effect of PUFA infusion, we compared the control group versus the average of the LnA and ALA group using the orthogonal contrast (1 -0.5 -0.5). Further, to determine the effect of PUFA type, we compared the LnA group with the ALA group using the following orthogonal contrast and (0 1 -1). Differences in fatty acid and oxylipid concentrations due to *S. uberis* or LPS exposure compared with unstimulated CON samples were determined by Student's t -tests. Significance was set at $P \leq 0.05$ for all tests.

RESULTS

Blood Leukocyte and Plasma Fatty Acid Concentrations Following Supplementation of LnA and ALA

To determine the efficacy of LnA and ALA supplementation (in the absence of ex vivo microbial agonist challenge), leukocyte and plasma fatty acid concentrations were analyzed. Though LnA and ALA supplementation numerically altered fatty acid content in the leukocyte, fatty acid supplementation did not significantly change leukocyte fatty acid content relative to nonsupplemented cows (Figure 1, left panel of graph, and Table 3). Plasma changes were in contrast to leukocyte where ALA, palmitic acid, oleic acid, stearic acid, n-3 PUFA, the ratio of plasma n-6 PUFA/n-3 PUFA, and the ratio of plasma LnA/ALA were all significantly changed by fatty acid supplementation (Table 3). Some of these plasma changes were source dependent for ALA with greater ALA ($P = 0.005$), increased sum of n-3 PUFA ($P = 0.003$), and reduced ratios of n-6 PUFA/n-3 PUFA ($P < 0.0001$) and LnA/ALA ($P = 0.0002$) relative to LnA supplementation (Figure 1, 2 right panel of graph, and Table 3).

Figure 1. Normalized amount (ng of fatty acids/ng of DNA) of PUFA, SFA, and MUFA in bovine white blood cells is displayed in the left panel of each graph and plasma concentration (mM) of PUFA, SFA, and MUFA is displayed in the right panel of each graph following supplementation of the ethanol carrier (CON), linoleic acid (LnA) at 45 g/d, or α-linolenic acid (ALA) at 45 g/d. Panels A–E depict quantified PUFA [LnA, ALA, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)]. Panels F–H depict quantified MUFA and SFA (oleic acid, palmitic acid, and stearic acid). Asterisks denote $P \leq 0.05$ for CON versus the average of LnA and ALA treatments or LnA versus ALA. Error bars indicate $\pm$ SEM.

Table 3. *P*-values for comparisons of fatty acid (FA) concentrations, sum of FA, and ratio of FA between treatment groups (CON, LnA, ALA) following abomasal FA supplementation in white blood cells and plasma¹

Fatty acid, Σ PUFA, or ratio of PUFA	<i>P</i> -value ²			
	White blood cells		Plasma	
	CON vs. Avg ³	LnA vs. ALA	CON vs. Avg ³	LnA vs. ALA
LnA	0.44	0.75	0.007	0.61
ArA	0.56	0.32	0.10	0.43
ALA	0.11	0.03	0.01	0.005
EPA	0.49	0.16	0.08	0.11
DHA	0.62	0.30	0.34	0.93
Palmitic acid	0.87	0.52	0.02	0.99
Oleic acid	0.59	0.55	0.002	0.89
Stearic acid ⁴	0.54	0.89	0.01	0.51
Σ n-6 PUFA	0.46	0.40	0.009	0.43
Σ n-3 PUFA ⁴	0.33	0.12	0.0001	0.003
Ratio of n-6 PUFA/n-3 PUFA	0.24	0.01	0.0004	<0.0001
Ratio of LnA/ALA	0.06	0.001	0.0009	0.0002

¹LnA = linoleic acid, ArA = arachidonic acid, ALA = α -linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, CON = ethanol carrier, Σ n-6 PUFA = sum of n-6 PUFA (ArA and LnA), Σ n-3 PUFA = sum of n-3 PUFA (ALA, EPA, and DHA).

²*P*-values represent level of significance when analyzing orthogonal contrasts CON vs. Avg and LnA vs. ALA.

³CON vs. the average of LnA and ALA treatments.

⁴Nonnormal residuals.

Fatty Acids and Oxylipids Following Microbial Agonist Challenge Without PUFA Supplementation

Whole blood exposed to heat-killed *S. uberis* for 4 h resulted in numerical decreases in all plasma PUFA, but only plasma ArA was significantly decreased (Table 4). In contrast, whole blood exposed to LPS for 4 h did not reveal any differences in plasma fatty acids (Table 4). Heat-killed *S. uberis* exposure of whole blood induced an increase in LnA-derived, ArA-derived, and EPA-derived oxylipids from the LOX and CYP oxidation pathways (Table 5). Heat-killed *S. uberis* increased biosynthesis of 13-oxooctadecadienoic acid (**oxoODE**, $P = 0.03$), 15-oxoETE ($P = 0.01$), 12-HETE ($P = 0.01$), 14,15-DiHETE ($P = 0.05$), and 17,18-DiHETE ($P = 0.05$, Table 5). Oxylipids not significantly changed in response to *S. uberis* are displayed in Appendix Table A1.

All oxylipids changed by LPS exposure were derived from ArA and represented all 3 enzymatic pathways: cyclooxygenase, LOX, and CYP (Table 5). Lipopolysaccharide challenge increased 12-hydroxyheptadecatrienoic acid ($P = 0.03$), but decreased 5,6-LXA₄ ($P = 0.05$) and 11,12-epoxyeicosatrienoic acid ($P = 0.03$). The ratio of 11,12-EET/11,12-DHET was also decreased ($P = 0.05$), suggesting a greater abundance of the downstream DHET compared with EET during LPS exposure relative to unstimulated plasma oxylipid profiles from bovine leukocytes. However, because the ratio of 11,12-EET/11,12-DHET is below 1, DHET is abundant regardless of inflammatory challenge. Oxy-

lipids not significantly changed in response to LPS are displayed in Appendix Table A1.

Microbial Agonist Challenge-Induced Fatty Acids and Oxylipid Changes Following LnA and ALA Supplementation

Plasma fatty acids were not altered following *S. uberis* stimulation (Table 6). In contrast, fatty acid supplementation significantly affected the *S. uberis*-induced synthesis of 9-HETE, 8-iso-PGF_{2 α} , and 15-oxoETE, but only the *S. uberis*-induced 15-oxoETE ($P = 0.02$) was source dependent between LnA and ALA supplemented groups (Table 7). *Streptococcus uberis*-induced oxylipids that were not changed by treatments are in Appendix Table A2.

The LPS-induced plasma fatty acids were not altered with fatty acid supplementation (Table 8). Fatty acid supplementation, regardless of source, affected the LPS-induced synthesis of 5,6-LXA₄ ($P = 0.03$), though no difference ($P = 0.20$) was found between LnA and ALA groups (Table 9). The LPS-induced oxylipids that were not changed by treatments are in Appendix Table A3.

DISCUSSION

The availability or relative abundance of cellular PUFA content contributes to distinct oxylipid profiles during homeostasis and disease. Indeed, altering PUFA content in host cells is being pursued as a strategy to

Table 4. Concentration (mM) of plasma fatty acids (FA) following ex vivo microbial exposure¹

Fatty acid ²	Unstimulated		<i>Streptococcus uberis</i>			LPS		
	Mean ³	SEM	Mean ³	SEM	<i>P</i> -value ⁴	Mean ³	SEM	<i>P</i> -value ⁴
LnA	225.56	44.13	188.15	41.84	0.52	273.01	82.56	0.52
ArA	28.68	7.41	13.26	2.25	0.06	30.48	12.22	0.90
ALA	54.09	10.34	45.76	9.46	0.51	57.04	16.00	0.82
EPA	5.69	1.81	2.52	0.52	0.08	5.72	2.33	0.99
DHA	1.61	0.33	1.26	0.26	0.36	1.84	0.47	0.68
Palmitic acid	933.34	326.46	744.19	201.83	0.33	1,861.58	1,010.93	0.27
Oleic acid	105.49	18.89	95.94	32.72	0.71	144.16	56.42	0.46
Stearic acid	39.23	14.67	59.27	38.8	0.48	90.90	46.85	0.36

¹Blood samples from the nonsupplemented group (CON) were collected and exposed to heat-killed *S. uberis* or LPS for 4 h. Plasma then was collected and plasma FA were quantified by liquid chromatography-MS.

²LnA = linoleic acid, ArA = arachidonic acid, ALA = α -linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

³Arithmetic means are shown for unstimulated, heat-killed *S. uberis*-challenged, and LPS-challenged plasma FA concentrations.

⁴*P*-values correspond to microbial exposure (*S. uberis* or LPS) relative to unstimulated as determined by paired Student's *t*-tests ($P \leq 0.05$).

Figure 2. Normalized average sum of n-6 fatty acids (FA; ng of FA/ng of DNA), n-3 FA (ng of FA/ng of DNA), ratio of n-6/n-3, and ratio of linoleic acid (LnA)/ α -linolenic acid (ALA) in bovine white blood cells is displayed in the left panel of each graph and average sum concentration of n-6 PUFA, n-3 PUFA, ratio of n-6/n-3, and ratio of LnA/ALA in plasma is displayed in the right panel of each graph following supplementation of the ethanol carrier (CON), LnA at 45 g/d, or ALA at 45 g/d. Panel A represents the normalized sum of n-6 PUFA (LnA and arachidonic acid). Panel B represents the normalized sum of n-3 PUFA [ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)]. Panel C represents the ratio of total n-6 to n-3 (n-6/n-3). Panel D represents the ratio of LnA to ALA (LnA/ALA). Asterisks denote $P \leq 0.05$ for CON versus the average of LnA and ALA treatments or LnA versus ALA. Error bars indicate $\pm$ SEM.

Table 5. Concentration (μM) of significantly changed plasma oxylipids following ex vivo microbial agonist exposure^{1,2}

Oxylipid ³	PUFA substrate ³	Oxidation pathway ³	Unstimulated		<i>Streptococcus uberis</i>			LPS		
			Mean ⁴	SEM	Mean ⁴	SEM	<i>P</i> -value ⁵	Mean ⁴	SEM	<i>P</i> -value ⁵
13-oxoODE	LnA	LOX	13.62	4.97	19.78	5.63	0.03	15.08	1.76	0.89
15-oxoETE	ArA	LOX	0.25	0.06	0.92	0.19	0.01	0.37	0.08	0.24
12-HETE	ArA	LOX	48.12	10.56	87.28	15.91	0.01	81.48	27.07	0.14
14,15-DiHETE	EPA	CYP	25.32	3.55	38.65	6.29	0.05	37.60	7.71	0.19
17,18-DiHETE	EPA	CYP	244.40	33.69	328.55	44.80	0.05	291.70	28.07	0.37
5,6-LXA ₄	ArA	LOX	1.60	0.35	1.18	0.31	0.45	0.68	0.18	0.05
11,12-EET	ArA	CYP	0.10	0.00	0.09	0.04	0.63	0.04	0.02	0.03
12-HHTre	ArA	COX	2.58	0.79	2.67	0.86	0.91	3.83	0.76	0.03
11,12-EET/ 11,12-DHET	EPA		0.042	0.011	0.018	0.006	0.18	0.009	0.003	0.05

¹Blood samples from the nonsupplemented group (CON) were collected and exposed to heat-killed *S. uberis* or LPS for 4 h. Plasma then was collected and oxylipids were quantified by liquid chromatography-MS/MS.

²Only oxylipids significantly changed following *S. uberis* or LPS exposure relative to control are displayed.

³oxoODE = oxooctadecadienoic acid, oxoETE = oxoeicosatetraenoic acid, HETE = hydroxyeicosatetraenoic acids, LX = lipoxin, EET = epoxyeicosatrienoic acid, DHET = dihydroxyeicosatrienoic acid, HHTre = hydroxyheptadecatrienoic acid, DiHETE = dihydroxyeicosatetraenoic acid, LnA = linoleic acid, ArA = arachidonic acid, EPA = eicosapentaenoic acid, LOX = lipoxygenase, CYP = cytochrome P450, COX = cyclooxygenase.

⁴Arithmetic means are shown for unstimulated, heat-killed *S. uberis*-challenged, and LPS-challenged plasma oxylipid concentrations.

⁵*P*-values correspond to microbial exposure (*S. uberis* or LPS) relative to unstimulated as determined by paired Student's *t*-tests ($P \leq 0.05$).

modify oxylipid biosynthesis in hopes of mitigating inflammation and reducing disease severity (Duda et al., 2009; Raphael and Sordillo, 2013; Min et al., 2014). Increasing n-3 fatty acids, such as ALA, is a common approach to mitigate disease primarily given its availability for conversion to EPA and DHA, which may be oxidized to a variety of anti-inflammatory oxylipids. Supplementation of ALA relative to LnA treatment significantly increased ALA content of leukocyte in the present trial. However, supplementation of ALA relative to LnA treatment did not result in increased EPA and DHA content of leukocyte, perhaps as a consequence of

insufficient dose or length of time with supplementation. A recent review concluded, based on a summary of ALA-supplementation trials, up to 40 g/d (a high dose for humans) and as many as 42 wk was required to consistently modify EPA content, with little to no appreciable change in DHA content (Brenna et al., 2009). Additionally, elongase and desaturase enzyme activities were not evaluated in the study but may affect ALA conversion because the same elongase and desaturase enzymes may metabolize both LnA and ALA (Guilou et al., 2010). Thus, the abundance of LnA in the diet and in the leukocyte may outcompete ALA for

Table 6. Changes in plasma fatty acids (FA) from PUFA-supplemented cows following ex vivo *Streptococcus uberis* challenge¹

Fatty acid	Treatment						<i>P</i> -value ²	
	CON		LnA		ALA		CON vs. Avg ³	LnA vs. ALA
	Δ Mean ⁴	SEM	Δ Mean	SEM	Δ Mean	SEM		
LnA	-37.41	54.73	-93.37	68.90	+86.07	113.24	0.78	0.28
ArA	-15.42	6.27	-8.19	5.18	+9.30	6.62	0.16	0.09
ALA	-8.34	11.82	-51.76	13.86	-3.95	93.56	0.86	0.70
EPA	-3.18	1.43	-1.25	0.98	+0.74	0.90	0.15	0.22
DHA	-0.36	0.35	-0.18	0.40	+0.58	0.55	0.36	0.36
Palmitic acid	-189.16	175.84	+59.86	543.60	+399.64	238.69	0.43	0.52
Oleic acid	-9.55	24.11	-34.59	27.34	+72.61	76.61	0.68	0.28
Stearic acid	+20.04	26.05	+1.22	9.63	+58.04	24.75	0.82	0.03

¹LnA = linoleic acid, ArA = arachidonic acid, ALA = α -linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, CON = ethanol carrier.

²*P*-values represent level of significance when analyzing orthogonal contrasts CON vs. Avg and LnA vs. ALA.

³CON vs. the average of LnA and ALA treatments.

⁴ Δ Mean for each replicate was quantified by subtracting *S. uberis*-induced plasma FA concentration (mM) from unstimulated plasma FA concentration (mM). Means in table represent arithmetic means. A + indicates greater plasma FA following *S. uberis* exposure compared with unstimulated samples, whereas - indicates less plasma FA following *S. uberis* exposure compared with unstimulated samples.

Table 7. Changes in plasma oxylipids from PUFA-supplemented cows following ex vivo *Streptococcus uberis* challenge¹

Oxylipid ²	PUFA substrate ²	Oxidation pathway ²	Treatment						P-value ³	
			CON ²		LnA		ALA		CON vs. Avg ⁴	LnA vs. ALA
			Δ Mean ⁵	SEM	Δ Mean	SEM	Δ Mean	SEM		
9-HODE	LnA	LOX/Non	+13.50	9.31	−7.62	10.34	−4.58	9.31	0.07	0.80
9,10-EpOME	LnA	CYP	−1.33	3.09	−1.20	3.20	−6.33	3.09	0.21	0.05
9-HETE	ArA	Non	−0.36	0.26	+0.44	0.28	+0.68	0.26	0.03	0.54
8-iso-PGA ₂	ArA	Non	−0.05	0.12	+0.14	0.12	−0.12	0.12	0.44	0.03
8-iso-PGF _{2α}	ArA	Non	−0.25	0.43	+0.63	0.48	+0.34	0.42	0.03	0.44
PGD ₂	ArA	COX	−0.13	0.14	+0.41	0.15	−0.04	0.14	0.13	0.09
6-keto PGF _{1α}	ArA	COX	+0.47	0.24	−0.30	0.27	+0.47	0.24	0.18	0.04
15-oxoETE	ArA	LOX	+0.67	0.17	−0.17	0.18	+0.23	0.17	0.009	0.02
LTB ₄	ArA	LOX	−0.10	0.26	+0.54	0.29	−0.30	0.26	0.42	0.03
20-HETE	ArA	CYP	−0.07	0.22	+0.37	0.24	−0.30	0.22	0.87	0.02
9-HODE/ 9-oxoODE ^{6,7}	LnA		+0.98	1.02	−0.40	1.19	+2.58	1.02	0.93	0.09

¹Only oxylipids demonstrating a significant or trending difference are displayed.²HODE = hydroxyoctadecadienoic acid, EpOME = epoxyoctadecenoic acid, oxoODE = oxooctadecadienoic acid, HETE = hydroxyeicosatetraenoic acid, PG = prostaglandin, LT = leukotriene, oxoETE = oxoeicosatetraenoic acid, LnA = linoleic acid, ArA = arachidonic acid, CYP = cytochrome P450, LOX = lipoxygenase, Non = nonenzymatic, COX = cyclooxygenase, CON = ethanol carrier, ALA = α-linolenic acid.³P-values represent level of significance when analyzing orthogonal contrasts CON versus Avg and LnA versus ALA.⁴CON vs. the average of LnA and ALA treatments.⁵Δ Mean for each replicate was quantified by subtracting *S. uberis*-induced plasma oxylipid concentrations (μM) from unstimulated plasma oxylipid concentrations (μM). Means in table represent arithmetic means. A + indicates greater plasma oxylipids following *S. uberis* exposure compared with unstimulated samples, whereas − indicates less plasma oxylipids following *S. uberis* exposure compared with unstimulated samples.⁶Nonnormal residuals.⁷Heterogenous variance.

elongation and desaturation. Although our data do not fully support this claim because no change occurred in LnA or ArA content, we also did not measure all intermediary PUFA that may compete for elongases and desaturases (Patterson et al., 2012). In addition to the possibility of differential elongase and desatu-

rase activity affecting PUFA content, the ability for fatty acid incorporation into the cell can influence fatty acid content changes with supplementation. Previous research also suggested there was no discrimination between incorporation of n-6 or n-3 PUFA and that the availability of PUFA in the phospholipid membrane is

Table 8. Changes in plasma fatty acids (FA) from PUFA-supplemented cows following ex vivo LPS challenge¹

Fatty acid	Treatment						P-value ²	
	CON		LnA		ALA		CON vs. Avg ³	LnA vs. ALA
	Δ Mean ⁴	SEM	Δ Mean	SEM	Δ Mean	SEM		
LnA	+47.45	68.94	−29.59	45.98	+120.79	27.12	0.94	0.06
ArA	+1.80	14.25	+8.51	10.88	+4.27	2.66	0.69	0.75
ALA	+2.94	12.31	−21.05	9.25	+60.83	26.00	0.54	0.01
EPA	+0.02	2.88	+1.80	1.60	+0.67	0.60	0.64	0.73
DHA	+0.23	0.52	−0.05	0.21	+1.07	0.29	0.58	0.06
Palmitic acid	+928.23	745.06	+37.33	413.70	+678.18	292.73	0.36	0.37
Oleic acid ⁵	+8.67	48.62	−22.28	30.73	+74.13	25.23	0.82	0.15
Stearic acid	+51.67	51.58	+2.38	17.26	+72.20	55.02	0.73	0.25

¹LnA = linoleic acid, ArA = arachidonic acid, ALA = α-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, CON = ethanol carrier.²P-values represent level of significance when analyzing orthogonal contrasts CON vs. Avg and LnA vs. ALA.³CON vs. the average of LnA and ALA treatments.⁴Δ Mean for each replicate was quantified by subtracting LPS-induced plasma FA concentration (mM) from unstimulated plasma fatty acid concentration (mM). Means in table represent arithmetic means. A + indicates greater plasma FA following LPS exposure compared with unstimulated samples, whereas − indicates less plasma fatty acids following LPS exposure compared with unstimulated samples.⁵Nonnormal residuals.

reflective of dietary intake (Lands et al., 1982; Dougherty et al., 1987). Though the potential for a maximal level of PUFA incorporation has yet to be thoroughly considered, an in vitro epithelial cell line study demonstrated that 50% of maximal incorporation of ALA was achieved by 10 to 20 $\mu\text{g/mL}$, whereas 50% of maximal incorporation of LnA was at only 5 to 10 $\mu\text{g/mL}$ (Bryan et al., 2001). Furthermore, previous research also indicated a potential negative effect of excess PUFA on the ability to incorporate PUFA by acyltransferases. In humans pre-fed a high LnA diet before receiving LnA or ALA, acyltransferase activity appeared reduced compared with humans pre-fed a high SFA diet (Emken et al., 1994). Authors hypothesized that the high LnA in the prefed diet might decrease acyltransferase mRNA synthesis, support increase transport to the mitochondria for β -oxidation, or divert excess PUFA to adipose tissue for storage. Although conjecture, current and previous data suggest supplementation of PUFA already in abundance may prohibit further incorporation or changes in leukocyte PUFA content and thus affect oxylipid biosynthesis.

A complex network of oxylipids is synthesized in response to a variety of stimuli, including bacteria and bacterial-derived agonists. Previous studies supported the differential biosynthesis of oxylipids to gram-negative and gram-positive agonists, perhaps as a result of pathogen-associated molecular patterns recognition during bovine mastitis (Tam, 2013; Mavangira et al., 2015; Ryman et al., 2015). Thus, it was not surprising that ex vivo exposure to LPS and *S. uberis* resulted in different oxylipid profiles because LPS mediates the

inflammatory response through toll-like receptor-4, whereas the response to *S. uberis* is generally considered to be dependent on lipoteichoic acid binding to toll-like receptor-2 (Bannerman et al., 2004a). It is possible that other *S. uberis*-derived secreted toxins could contribute to differential oxylipid biosynthesis (Oliver et al., 1998). The most striking difference in oxylipid profiles within the current study was that LPS contributed to a reduction in oxylipids with anti-inflammatory properties, whereas the inverse was true for *S. uberis*. Exposure to LPS in the current study reduced anti-inflammatory LXA₄ and 11,12-EET biosynthesis, which previous studies demonstrated are necessary for maintenance of the endothelial barrier and control of pro-inflammatory cytokine expression (Schmelzer et al., 2005; Ereso et al., 2009). In contrast, *S. uberis* exposure induced biosynthesis of oxylipids that are ligands for activation of a nuclear receptor called peroxisome proliferator-activated receptor- γ that can inhibit pro-inflammatory nuclear factor- κB signaling (Zhao et al., 2004; Shiraki et al., 2005; Snyder et al., 2015). The significance of these changes in the context of mastitis was not elucidated in the current study, but highlighted a need to understand if different pathogen recognition receptor signaling pathways can directly mediate oxylipid biosynthesis (Strandberg et al., 2005).

The inflammatory response to LPS during coliform mastitis contributes to the production of lipid mediators from many different cell types. Thus, it was not surprising that fewer changes in oxylipids were induced by ex vivo LPS exposure of leukocytes compared with milk and plasma profiles during coliform mastitis

Table 9. Changes in plasma oxylipids from PUFA-supplemented cows following ex vivo LPS challenge¹

Oxylipid ²	PUFA substrate ²	Oxidation pathway ²	Treatment						P-value ³	
			CON ²		LnA		ALA		CON vs. Avg ⁴	LnA vs. ALA
			Δ Mean ⁵	SEM	Δ Mean	SEM	Δ Mean	SEM		
5,6 LXA ₄	ArA	LOX	+0.92	0.35	−0.23	0.39	+0.37	0.35	0.03	0.20
PGE ₂	ArA	COX	−0.95	0.55	+0.46	0.63	+0.33	0.55	0.09	0.89
9-oxoODE	LnA	LOX/Non	−12.48	8.30	−30.25	9.59	+30.42	8.3	0.32	0.06
9-HODE/9-oxoODE ^{6,7}	LnA		+0.01	0.93	+0.29	1.03	−3.01	0.93	0.29	0.08
13-HODE/13-oxoODE ⁷	LnA		−6.39	5.31	−6.31	5.90	−22.16	5.31	0.29	0.08

¹Only oxylipids demonstrating a significant or trending difference are displayed.

²LX = lipoxin, PG = prostaglandin, HODE = hydroxyoctadecadienoic acid, oxoODE = oxooctadecadienoic acid, LnA = linoleic acid, ArA = arachidonic acid, LOX = lipoxygenase, Non = nonenzymatic, COX = cyclooxygenase, CON = ethanol carrier.

³P-values represent level of significance when analyzing orthogonal contrasts CON vs. Avg and LnA vs. ALA.

⁴CON vs. the average of LnA and ALA treatments.

⁵ Δ Mean for each replicate was quantified by subtracting LPS-induced plasma oxylipid concentrations (μM) from unstimulated plasma oxylipid concentrations (μM). Means in table represent arithmetic means. A + indicates greater plasma oxylipids following LPS exposure compared with unstimulated samples, whereas − indicates less plasma oxylipids following LPS exposure compared with unstimulated samples.

⁶Nonnormal residuals.

⁷Heterogenous variance.

(Mavangira et al., 2015). However, the contribution of replicating *E. coli* bacteria, sustained exposure to LPS release, and the participation of a variety of cells and tissues probably led to a more diverse oxylipid profile. Furthermore, the ex vivo model could not capture the contribution of adipose tissue lipolysis, which may be important for oxylipid biosynthesis as it is proposed to be the primary source of nonesterified fatty acids during LPS-mediated inflammation (Zu et al., 2009; Moyes et al., 2014). This limitation may be supported by the absence of any changes in nonesterified LnA in the current study compared with an almost 3-fold increase measured in milk and plasma during bovine coliform mastitis (Mavangira et al., 2015). Additionally, a different LPS dose or duration of challenge could contribute to a more diverse ex vivo oxylipid profile, recapitulating in vivo data more closely. Though the current model had limitations, ex vivo exposure of leukocyte may be critical in understanding the specific contributions to oxylipid biosynthesis of blood leukocytes during LPS exposure. In fact, some similarities in oxylipid biosynthesis were detected between ex vivo and in vivo studies. Decreased anti-inflammatory 5,6-LXA₄ and 11,12-EET in the current study were consistent with plasma oxylipids during coliform mastitis, but inverse to the change in milk oxylipids (Mavangira et al., 2015). The authors hypothesized that the inverse relationship between milk and plasma oxylipids may be the result, in part, of a breakdown in the blood-milk barrier. Our findings may suggest the oxylipid production from blood cells also decreased, which could contribute to reduced plasma oxylipid expression during coliform mastitis.

Oxylipid profiles induced by *S. uberis* in the current study also were in contrast to previous work. For example, intramammary *S. uberis* challenge did not induce changes in milk oxylipids, whereas the current study demonstrated increases in ArA-, LnA-, and EPA-derived oxylipids (Ryman et al., 2015). The lack of changes in milk oxylipids following intramammary *S. uberis* challenge compared with current findings may be attributed, in part, to the difference in the size of the quantified oxylipid panel. For example, the current study included a much wider oxylipid profile, especially including more CYP- and EPA-derived oxylipids than the previous work (Ryman et al., 2015). A wider profile in the current study may be the reason we were able to report increased 14,15-DiHETE and 17,18-DiHETE, though their functions in the inflammatory response are unknown. Similarly, 12-HETE was not previously quantified in milk or tissue from *S. uberis* challenge, yet our ex vivo results demonstrated an *S. uberis*-induced increase. However, the inflammatory properties of 12-HETE are contradictory depending on the disease and cell model and warrant further investigation (Bolick et

al., 2005; Kronke et al., 2009). Whereas 13-oxoODE and 15-oxoETE, both ligands for PPAR- γ , were quantified in milk and tissue from *S. uberis* challenge animals similar to the current study, only ex vivo exposure of leukocytes to *S. uberis* resulted in increased biosynthesis. The inconsistency of 13-oxoODE and 15-oxoETE biosynthesis between the current study and intramammary *S. uberis* challenge may be a function of different stages of inflammation, in the case of *S. uberis* tissue, or dilution of oxylipids in milk limiting the detection of appreciable changes. A final important finding was that, in general, significantly changed *S. uberis*-induced oxylipids (oxoODE, oxoETE, and DiHETE) represented a greater degree of metabolism from their precursors. It is unknown how *S. uberis* recognition and the subsequent immune response may influence the activity of downstream enzymatic metabolism in leukocytes, but may be important when developing pharmacologic intervention strategies during mastitis.

Aside from the likely contribution of pathogen recognition pathways, oxylipid profiles depend on abundance and proportion of cellular PUFA content. In the current study, the primary goal of supplementation was to modify cellular PUFA content, and thus, alter microbial agonist-induced oxylipid profiles. As discussed earlier, we demonstrated in the present study that fatty acid supplementation for 7 d did not modify PUFA leukocyte content. However, fatty acid supplementation could have affected other levels of the oxylipid biosynthetic pathway besides substrate availability during microbial exposure, including method of oxidation and degree of metabolism (Ramsden et al., 2012; Mavangira et al., 2015). Our data support a potential effect of fatty acid supplementation on oxidation pathway during *S. uberis* exposure because oxylipids that were significantly affected by fatty acid supplementation represented both enzymatic (15-oxoETE from 15-LOX) and nonenzymatic oxidation pathways (9-HETE and 8-iso-PGF_{2 α}). The effect of fatty acid supplementation on degree of metabolism was not conclusive because even though 15-oxoETE was decreased with fatty acid treatment, no change occurred in the ratio of upstream hydroxyl 15-HETE to its downstream ketone 15-oxoETE. The reason for a decrease 15-oxoETE is unclear, but previous data demonstrated an inhibitory effect of PUFA on some CYP isoforms and the ability of 15-LOX-1 to undergo suicide inactivation due to its own metabolites (Wiesner et al., 2003; Yao et al., 2006). Additional studies are required to understand how supplemental PUFA, without a change in leukocyte PUFA content, may affect method of fatty acid oxidation during a microbial agonist challenge.

Interestingly, our findings from evaluating *S. uberis*-induced oxylipids suggested an increase in lipid per-

oxidation with fatty acid supplementation because *S. uberis*-induced nonenzymatically derived 9-HETE and 8-iso-PGF_{2α} was greater with fatty acid supplementation relative to no PUFA supplementation. Lipid peroxidation is implicated in dysfunctional inflammatory responses and inflammatory-based diseases (Catala, 2009). Though no difference was found between LnA and ALA supplemented groups for 9-HETE, the *S. uberis*-induced biosynthesis was numerically higher with ALA supplementation relative to LnA supplementation, which is counterintuitive to the perceived positive attributes of ALA. Feeding whole linseed as a source of ALA is a common approach in dairy cows to increase anti-inflammatory n-3 fats, such as EPA and DHA, in milk because milk composition is negatively affected by fish oil supplementation (Petit et al., 2002). However, our findings suggest that supplementation with either PUFA could contribute to increased peroxidation of lipid membranes during *S. uberis* infection. Increased fatty acid availability, with no appreciable decreases in other PUFA, would increase the availability of PUFA for lipid peroxidation (Guido et al., 1993). Demonstrating a PUFA-induced increase in markers of lipid peroxidation indicates that further studies are required to understand how both excess LnA and ALA may affect pathogen-induced immune responses. Although outside the scope of the current study, our data suggest that supplementing any PUFA could have an unintended negative consequence on disease outcome.

In contrast to *S. uberis* exposure, markers of lipid peroxidation were not increased by fatty acid supplementation during LPS challenge; however, the biosynthesis of LPS-induced ArA-derived 5,6-LXA₄ was significantly affected by fatty acid supplementation. Biosynthesis of LXA₄ is instrumental in decreasing leukocyte recruitment and migration, as well as promoting resolution of inflammation (Serhan, 1994). Though no significant difference was found between LnA- and ALA-supplemented groups in LPS-induced 5,6-LXA₄ biosynthesis, a substantial numerical decrease occurred with LnA supplementation. Similarly, LPS-induced 9-oxoODE was trending lower with LnA supplementation compared with an increased in LPS-induced biosynthesis during ALA supplementation ($P = 0.06$, Table 9). Perhaps manipulating the ratio of LnA/ALA with fatty acid supplementation contributed to the increased presentation of an anti-inflammatory oxylipid during LPS exposure (Poulsen et al., 2008). Similarly, 9-oxoODE are ligands for anti-inflammatory PPAR- γ and adding exogenous 9-oxoODE to a macrophage model of oxidative stress deficient in 9-oxoODE decreased TNF- α release (Nagy et al., 1998; Itoh et al., 2008; Mattmiller et al., 2014). Perhaps decreasing the

ratio of LnA/ALA with ALA supplementation relative to LnA, as the present study demonstrated, contributed to the presentation of a more anti-inflammatory profile during LPS exposure regardless of the oxylipid substrate (Poulsen et al., 2008). Given the contrasting findings with LPS- and *S. uberis*-induced oxylipid profiles with fatty acid supplementation, positive or negative effects of fatty acid supplementation may be different in the context of mastitis caused by either gram-negative or gram-positive pathogens. Additionally, given the use of linseed for ALA rather than fish oil for EPA and DHA in dairy cattle, future studies are needed to determine its efficacy in improving disease outcome in variety of inflammatory conditions, such as mastitis and metritis. Perhaps increased EPA- and DHA-derived oxylipids are not required if increasing the abundance of anti-inflammatory n-6 derived oxylipids, such as lipoxins and oxoODEs, can be consistently achieved.

CONCLUSIONS

Oxylipid biosynthesis during microbial exposure is dependent on several factors, including type of agonist and PUFA availability. Supplementation of ALA increased ALA content of leukocyte and decreased the ratio of LnA/ALA compared with LnA supplementation, but no change in EPA and DHA content suggested that dose or duration of ALA supplementation may be important for enhanced EPA and DHA content. Consistent with no change in EPA and DHA content after ALA supplementation, microbial-induced EPA- and DHA-derived oxylipids were not increased with ALA treatment relative to LnA. However, LPS-induced anti-inflammatory oxylipids from n-6 fatty acids were numerically increased following ALA supplementation. Perhaps the ratio of LnA/ALA content is equally, if not more, important in mediating oxylipid biosynthesis during microbial agonist challenge than absolute abundance. Furthermore, data indicated that fatty acid supplementation could affect *S. uberis*-induced oxylipid biosynthesis without changing PUFA content in bovine leukocytes, suggesting that supplemental PUFA can affect other levels of oxylipid biosynthesis, like method of oxidation or degree of oxylipid metabolism. Though oxylipid profiles are inherently dependent on phospholipid PUFA content, the interaction with pathogen recognition pathways could contribute to modification of other regulatory levels of oxylipid biosynthesis, such as method of oxidation. The data presented in this manuscript are essential to enhancing our understanding of PUFA and oxylipid biosynthesis during different microbial exposures in the dairy cow.

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APPENDIX

Table A1. Concentration (μM) of plasma oxylipids not significantly changed following ex vivo microbial exposure^{1,2}

Oxylipid ³	PUFA substrate ³	Oxidation pathway ³	Unstimulated		<i>Streptococcus uberis</i>			LPS	
			Mean ⁴	SEM	Mean	SEM	P-value ⁵	Mean	P-value ⁵
9-HODE	LnA	LOX/Non	69.00	6.07	82.50	13.94	0.26	60.00	0.18
9-oxoODE	LnA	LOX/Non	38.88	9.56	64.20	17.28	0.20	26.40	0.17
13-HODE	LnA	LOX	221.25	21.28	243.25	33.99	0.59	203.08	0.22
9,10-EpOME	LnA	CYP	24.67	2.29	23.33	2.46	0.59	21.00	0.36
9,10-DiHOME	LnA	CYP	142.00	29.68	163.50	48.84	0.57	156.83	0.56
12,13-EpOME	LnA	CYP	73.00	6.38	75.00	10.34	0.74	69.67	0.74
12,13-DiHOME	LnA	CYP	2,305.5	374.1	2,812.8	767.3	0.35	3,020.5	0.22
PGE ₂	ArA	COX	2.17	1.19	0.67	0.28	0.26	1.22	0.32
PGD ₂	ArA	COX	0.42	0.09	0.29	0.06	0.29	0.83	0.19
6-keto PGF _{1α}	ArA	COX	0.88	0.33	1.35	0.42	0.21	1.13	0.45
TXB ₂	ArA	COX	2.22	1.23	3.13	1.24	0.45	3.37	0.14
8-iso-PGA ₁	ArA	Non	1.27	0.57	1.50	0.24	0.71	1.83	0.65
8-iso-PGA ₂	ArA	Non	0.23	0.08	0.18	0.09	0.71	0.37	0.30
8-iso-PGE ₂	ArA	Non	1.58	0.71	1.13	0.53	0.24	1.97	0.39
8-iso-PGF _{2α}	ArA	Non	0.85	0.16	0.60	0.23	0.30	0.88	0.90
5-HETE	ArA	LOX	4.85	0.62	5.15	0.84	0.69	4.67	0.86
5-oxoETE	ArA	LOX	1.43	0.44	1.08	0.46	0.66	0.74	0.21
15-HETE	ArA	LOX	5.55	0.56	2.35	0.49	0.09	5.78	0.80
11-HETE	ArA	Non	2.48	0.27	2.68	0.29	0.47	2.78	0.63
20-HETE	ArA	CYP	0.93	0.23	1.00	0.12	0.78	0.38	0.12
9-HETE	ArA	Non	1.75	0.85	0.26	0.15	0.19	0.50	0.24
LTB ₄	ArA	LOX	0.63	0.20	0.53	0.16	0.78	0.33	0.17
14,15-EET	ArA	CYP	0.53	0.16	0.58	0.15	0.79	0.28	0.16
14,15-DHET	ArA	CYP	25.32	3.55	38.65	6.29	0.07	37.60	0.45
8,9-DHET	ArA	CYP	0.60	0.12	0.54	0.14	0.75	0.48	0.49
11,12-DHET	ArA	CYP	3.50	0.94	4.53	1.43	0.25	3.90	0.55
5,6-DiHETE	EPA	CYP	3.45	1.03	10.77	4.79	0.24	6.60	0.09
19,20-EpDPE	DHA	CYP	2.08	0.51	3.21	1.22	0.25	2.32	0.66
19,20-DiHDPa	DHA	CYP	1.45	0.20	1.25	0.37	0.50	1.20	0.36
RD1	DHA	LOX	0.25	0.06	0.38	0.11	0.31	0.18	0.49
RD2	DHA	LOX	0.63	0.15	0.93	0.27	0.46	1.22	0.28
5-HETE/5-oxoETE	ArA		13.29	6.81	14.74	8.57	0.86	26.25	0.10
15-HETE/15-oxoETE	ArA		28.38	7.10	9.59	1.79	0.06	18.36	0.31
14,15-EET/14,15-DHET	ArA		0.020	0.004	0.055	0.02	0.81	0.010	0.41
9-HODE/9-oxoODE	LnA		2.44	0.68	1.46	0.16	0.23	2.45	0.98
13-HODE/13-oxoODE	LnA		20.50	8.00	12.09	5.00	0.22	14.16	0.43
9,10-EpOME/9,10-DiHOME	LnA		0.218	0.056	0.174	0.02	0.40	0.154	0.14
12,13-EpOME/12,13-DiHOME	LnA		0.036	0.006	0.031	0.01	0.32	0.026	0.11

¹Blood samples from the nonsupplemented group (CON) were collected and exposed to heat-killed *S. uberis* or LPS for 4 h. Plasma then was collected and oxylipids were quantified by liquid chromatography-MS/MS.

²Oxylipids not significantly changed following *S. uberis* or LPS exposure relative to unstimulated are displayed.

³HODE = hydroxyoctadecadienoic acid, oxoODE = oxooctadecadienoic acid, oxoETE = oxoeicosatetraenoic acid, EpOME = epoxyoctadecenoic acid, DiHOME = dihydroxyoctadecenoic acid, PG = prostaglandin, HETE = hydroxyeicosatetraenoic acid, LT = leukotriene, EET = epoxyeicosatrienoic acid, DHET = dihydroxyeicosatrienoic acid, DiHETE = dihydroxyeicosatetraenoic acid, EpDPE = epoxydocosapentaenoic acid, DiHDPa = dihydroxydocosapentaenoic acid, RD = Resolvin, HOTrE = hydroxyoctadecatrienoic acid, HETrE = hydroxyeicosatrienoic acid, LnA = linoleic acid, ArA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ALA = α -linolenic acid, DGLA = dihomogamma-linolenic acid, LOX = lipoxygenase, Non = nonenzymatic, CYP = cytochrome P450, COX = cyclooxygenase, TXB2 = thromboxane B2.

⁴Arithmetic means are shown for unstimulated, heat-killed *S. uberis*-challenged, and LPS-challenged plasma oxylipid concentrations.

⁵P-values correspond to microbial exposure (*S. uberis* or LPS) relative to unstimulated as determined by paired Student's *t*-tests ($P \leq 0.05$).

Table A2. Changes in plasma oxylipids from PUFA-supplemented cows following ex vivo *Streptococcus uberis* challenge¹

Oxylipid ²	PUFA substrate ²	Oxidation pathway ²	Treatment						P-value ³	
			CON ²		LnA		ALA			
			Δ Mean ⁵	SEM	Δ Mean	SEM	Δ Mean	SEM		
9-oxoODE	LnA	LOX	+25.3	11.5	-47.6	13.96	+11.12	11.5	0.12	0.12
13-HODE	LnA	LOX	+22.00	31.46	-28.13	35.85	-36.42	31.46	0.18	0.86
13-oxoODE ^{6,7}	LnA	LOX	+6.17	9.58	-3.47	10.63	+9.08	9.58	0.51	0.49
9,10-DiHOME ⁶	LnA	CYP	+21.50	25.67	-18.26	28.93	-1.67	25.67	0.30	0.64
12,13-EpOME	LnA	CYP	+2.00	4.49	-12.26	4.53	-8.33	4.49	0.17	0.96
12,13-DiHOME ^{6,7}	LnA	CYP	+507.3	431.7	-477.2	478.2	+258.8	431.7	0.19	0.20
PGE ₂	ArA	COX	-1.50	0.65	+0.52	0.72	+0.18	0.65	0.06	0.76
TXB ₂	ArA	COX	0.92	0.77	-0.66	0.87	+0.12	0.77	0.18	0.45
12-HHTrE	ArA	COX	+0.08	0.55	+0.08	0.62	+0.25	0.55	0.90	0.82
8-iso-PGA ₁	ArA	Non	+0.23	0.41	+0.14	0.46	+0.58	0.41	0.31	0.20
8-iso-PGE ₂ ^{6,7}	ArA	Non	-0.45	0.73	+1.28	0.83	-0.53	0.73	0.35	0.11
5-HETE	ArA	LOX	+0.30	1.24	+0.76	1.37	+2.18	1.24	0.56	0.49
5-oxoETE	ArA	LOX	-0.35	0.70	-1.32	0.80	-0.18	0.70	0.65	0.29
15-HETE	ArA	LOX	+1.87	0.79	+0.06	0.88	+0.12	0.79	0.14	0.95
11-HETE	ArA	Non	+0.20	0.30	-0.02	0.33	+0.53	0.30	0.88	0.30
12-HETE ^{6,7}	ArA	LOX	+39.17	17.88	-8.08	19.83	+13.52	17.88	0.16	0.47
5,6 LXA ₄	ArA	LOX	-0.42	0.43	+0.13	0.48	+0.23	0.43	0.32	0.87
14,15-EET	ArA	CYP	+0.05	0.16	-0.13	0.03	+0.16	0.11	0.53	0.69
14,15-DHET	ArA	CYP	+3.02	1.40	-0.93	1.56	+1.72	1.40	0.25	0.35
11,12-EET ^{6,7}	ArA	CYP	-0.02	0.06	-0.05	0.07	+0.02	0.06	0.53	1.00
11,12-DHET	ArA	CYP	+1.03	0.84	-0.75	0.97	+0.83	0.84	0.34	0.22
8,9-DHET	ArA	CYP	-0.06	0.18	-0.20	0.20	-0.35	0.18	0.38	0.88
5,6-DiHETE ⁶	EPA	CYP	+7.32	3.37	+3.05	3.58	+1.65	3.37	0.08	0.65
14,15-DiHETE	EPA	CYP	+13.33	5.57	-1.34	6.68	+6.03	5.57	0.18	0.42
17,18-DiHETE	EPA	CYP	+84.15	46.82	-26.20	51.94	+61.7	46.82	0.50	0.51
19,20-EpDPE ^{6,7}	DHA	CYP	+1.13	0.57	+0.28	0.64	+0.42	0.57	0.32	0.89
19,20-DiHDP	DHA	CYP	-0.20	0.54	-0.32	0.62	-0.35	0.54	0.84	0.97
RD1 ⁷	DHA	LOX	+0.13	0.29	+0.51	0.32	+0.13	0.29	0.63	0.43
RD2	DHA	LOX	+0.30	0.32	+0.64	0.35	+0.33	0.32	0.74	0.66
5-HETE/5-oxoETE	Ara		-1.45	30.72	-10.88	35.08	-52.14	30.72	0.45	0.39
15-HETE/15-oxoETE	Ara		-18.79	5.89	-4.62	6.53	-9.96	5.89	0.15	0.65
11,12-EET/11,12-DHET	Ara		-0.02	0.02	-0.007	0.02	+0.003	0.02	0.42	0.78
14,15-EET/14,15-DHET	Ara		-0.005	0.02	-0.00	0.02	-0.009	0.02	1.00	0.75
13-HODE/13-oxoODE ^{6,7}	LnA		+14.71	9.11	+8.82	10.11	-0.24	9.11	0.51	0.49
9,10-EpOME/9,10-DiHOME ⁷	LnA		-0.04	0.03	+0.004	0.03	-0.05	0.03	0.46	0.22
12,13-EpOME/12,13-DiHOME	LnA		+0.003	0.004	-0.006	0.005	-0.004	0.004	0.18	0.85

¹Changes in table represent oxylipids not significantly changed due to PUFA supplementation.

²HODE = hydroxyoctadecadienoic acid, oxoODE = octadecadienoic acid, EpOME = epoxyoctadecadienoic acid, DiHOME = dihydroxyoctadecadienoic acid, PG = prostaglandin, TXB₂ = thromboxane B₂, LX = lipoxin, HETE = hydroxyeicosatetraenoic acid, oxoETE = oxoeicosatetraenoic acid, LT = leukotriene, EET = epoxyeicosatrienoic acid, DHET = dihydroxyeicosatrienoic acid, DiHETE = dihydroxyeicosatetraenoic acid, EpDPE = epoxydocosapentaenoic acid, DiHDP = dihydroxydocosapentaenoic acid, RD = resolvin, HOTE = hydroxyoctadecatrienoic acid, HHTe = hydroxyheptadecatrienoic acid, LnA = linoleic acid, ArA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ALA = α-linolenic acid, DGLA = dihomogamma-linoleic acid, LOX = lipoxygenase, Non = nonenzymatic, CYP = cytochrome P450, COX = cyclooxygenase, CON = ethanol carrier, LnA = linoleic acid supplementation, ALA = α-linolenic acid supplementation.

³P-values represent level of significance when analyzing orthogonal contrasts CON vs. Avg and LnA vs. ALA.⁴CON vs. the average of LnA and ALA treatments.

⁵Δ Mean for each replicate was quantified by subtracting *S. uberis*-induced plasma oxylipid concentrations (μM) from unstimulated plasma oxylipid concentrations (μM). Means in table represent arithmetic means. A + indicates greater plasma oxylipids following *S. uberis* exposure compared with unstimulated samples, whereas - indicates less plasma oxylipids following *S. uberis* exposure compared with unstimulated samples.

⁶Nonnormal residuals.⁷Heterogenous variance.

Table A3. Changes in plasma oxylipids from PUFA-supplemented cows following ex vivo LPS challenge¹

Oxylipid ²	PUFA substrate ²	Oxidation pathway ²	Treatment				P-value ³	
			CON ²		LnA			
			Δ Mean ⁵	SEM	Δ Mean	SEM	CON vs. Avg ⁴	LnA vs. ALA
9-HODE	LnA	LOX/Non	-9.00	6.46	+1.94	7.17	0.36	0.93
13-HODE	LnA	LOX	-18.17	20.73	+26.51	23.00	0.66	0.18
13-oxoODE	LnA	LOX	-1.77	3.93	-0.75	4.54	0.15	0.10
9,10-EpOME ^{6,7}	LnA	CYP	-3.67	2.72	+3.15	3.02	0.50	0.09
9,10-DiHOME ^{6,7}	LnA	CYP	+14.83	25.00	+53.67	27.73	0.71	0.25
12,13-EpOME	LnA	CYP	-3.33	7.10	-0.38	7.87	0.89	0.42
12,13-DiHOME ⁶	LnA	CYP	+715.0	412.2	+1571.3	476.0	0.96	0.40
PGD ₂	ArA	COX	+0.42	0.20	+0.12	0.22	0.34	0.98
6-keto PGF _{1α}	ArA	COX	+0.25	0.22	+0.31	0.25	0.48	0.14
TXB ₂ ^{6,7}	ArA	COX	+1.15	1.72	+2.01	1.96	0.45	0.57
12-HHTe ^{6,7}	ArA	COX	+1.25	1.47	+2.08	1.63	0.44	0.57
8-iso-PGA ₁	ArA	Non	+0.57	0.80	-0.68	0.89	0.39	0.61
8-iso-PGA ₂	ArA	Non	+0.13	0.15	-0.09	0.17	0.15	0.65
8-iso-PGE ₂	ArA	Non	+0.38	0.26	+0.19	0.30	0.66	0.72
8-iso-PGF ₂ ^{6,7}	ArA	Non	+0.03	0.65	+0.65	0.72	0.37	0.73
5-HETE ⁷	ArA	LOX	-0.18	1.39	-0.06	1.54	0.77	0.71
5-oxoETE	ArA	LOX	-0.69	0.54	-1.02	0.60	0.51	0.08
15-HETE	ArA	LOX	+0.23	0.91	+2.09	1.00	0.20	0.21
15-oxoETE	ArA	LOX	+0.12	0.15	-0.10	0.17	0.66	0.20
11-HETE	ArA	Non	+0.30	0.53	+1.04	0.59	0.28	0.95
12-HETE ⁷	ArA	LOX	+33.37	14.78	-5.80	16.40	0.18	0.35
20-HETE	ArA	CYP	-0.55	0.31	+0.19	0.36	0.13	0.63
9-HETE	ArA	Non	-1.25	0.62	-0.13	0.71	0.82	0.19
LTB ₄	ArA	LOX	-0.30	0.17	+0.06	0.19	0.57	0.26
14,15-EET	ArA	CYP	-0.25	0.12	-0.11	0.14	0.31	0.82
14,15-DHET	ArA	CYP	+1.35	1.63	+1.79	1.86	0.42	0.33
11,12-EET ⁷	ArA	CYP	-0.06	0.05	-0.01	0.05	0.46	0.81
11,12-DHET	ArA	CYP	+0.40	0.73	+1.13	0.83	0.15	0.25
8,9-DHET	ArA	CYP	-0.12	0.22	+0.03	0.26	0.76	0.21
5,6-DiHETE ^{6,7}	EPA	CYP	+3.15	2.52	+5.63	2.86	0.99	0.21
14,15-DiHETE	EPA	CYP	+12.28	5.42	+8.87	6.12	0.98	0.37
17,18-DiHETE	EPA	CYP	+47.30	42.59	+58.68	47.25	0.42	0.35
19,20-EpDPE	DHA	CYP	+0.23	0.40	+0.02	0.46	0.63	0.16
19,20-DiHDP	DHA	CYP	-0.25	0.26	-0.02	0.30	0.12	0.14
RD1	DHA	LOX	-0.07	0.14	+0.06	0.16	0.82	0.15
RD2 ^{6,7}	DHA	LOX	+0.58	0.42	+0.49	0.44	0.58	0.65
5-HETE/5-oxoETE ⁷	ArA		+12.96	5.12	9.35	5.76	0.24	0.33
15-HETE/15-oxoETE	ArA		-10.02	6.82	+1.48	7.78	0.23	0.91
11,12-EET/11,12-DHET	ArA		-0.03	0.02	-0.00	0.02	0.37	0.74
14,15-EET/14,15-DHET	ArA		-0.01	0.00	-0.01	-0.01	0.62	0.78
9,10-EpOME/9,10-DiHOME	LnA		-0.06	0.03	-0.00	0.03	0.19	0.65
12,13-EpOME/12,13-DiHOME	LnA		-0.01	0.00	-0.00	-0.01	0.48	0.97

¹Changes in table represent oxylipids not significantly changed due to PUFA supplementation.²HODE = hydroxyoctadecadienoic acid, oxoODE = oxooctadecadienoic acid, EpOME = epoxyoctadecadienoic acid, DiHOME = dihydroxyoctadecadienoic acid, PG = prostaglandin, TXB₂ = thromboxane B₂, HHTe = hydroxyheptadecatrienoic acid, HETE = hydroxyheptadecatrienoic acid, oxoETE = oxooctadecatrienoic acid, LT = leukotriene, EET = epoxyeicosatrienoic acid, DHET = dihydroxyeicosatrienoic acid, DiHETE = dihydroxyeicosatrienoic acid, EpDPE = epoxydocosapentaenoic acid, DiHDP = dihydroxydocosapentaenoic acid, RD = resolvin, LnA = linoleic acid, ArA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, ALA = α-linolenic acid, DGLA = dihomo-γ-linolenic acid, LOX = lipoxygenase, Non = nonenzymatic, CYP = cyclooxygenase, CON = ethanol carrier.³P-value represent level of significance when analyzing orthogonal contrasts CON vs. LnA, CON vs. ALA, LnA vs. ALA, and CON vs. Avg.⁴CON vs. the average of LnA and ALA treatments.⁵Δ Mean for each replicate was quantified by subtracting LPS-induced plasma oxylipid concentrations (μM) from unstimulated plasma oxylipid concentrations (μM). Means in table represent arithmetic means. A + indicates greater plasma oxylipids following LPS exposure compared with unstimulated samples, whereas - indicates less plasma oxylipids following LPS exposure compared with unstimulated samples.⁶Nonnormal residuals.⁷Heterogenous variance.