Polyunsaturated fatty acids influence differential biosynthesis of oxylipids and other lipid mediators during bovine coliform mastitis

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ABSTRACT

Coliform mastitis is a severe and sometimes fatal disease characterized by an unregulated inflammatory response. The initiation, progression, and resolution of inflammatory responses are regulated, in part, by potent oxylipid metabolites derived from polyunsaturated fatty acids. The purpose of this study was to characterize the biosynthesis and diversity of oxylipid metabolites during acute bovine coliform mastitis. Eleven cows diagnosed with naturally occurring acute systemic coliform mastitis and 13 healthy control cows, matched for lactation number and days in milk, were selected for comparison of oxylipid and free fatty acid concentrations in both milk and plasma. Oxylipids and free fatty acids were quantified using liquid chromatography-tandem mass spectrometry. All polyunsaturated fatty acids quantified in milk were elevated during coliform mastitis with linoleic acid being the most abundant. Oxylipids synthesized through the lipoxygenase and cytochrome P450 pathways accounted for the majority of the oxylipid biosynthesis. This study demonstrated a complex and diverse oxylipid network, most pronounced at the level of the mammary gland. Substrate availability, biosynthetic pathways, and degree of metabolism influence the biosynthesis of oxylipids during bovine coliform mastitis. Further studies are required to identify targets for novel interventions that modulate oxylipid biosynthesis during coliform mastitis to optimize inflammation.

Key words: inflammation, mastitis, oxylipid, lipids

INTRODUCTION

Gram-negative bacteria are a predominant cause of clinical mastitis infections in dairy cows (Erskine et al., 1988; Oliveira et al., 2013). Whereas some infec-

tions are subclinical and self-limiting, a proportion of affected cows develop an acute and severe systemic disease resulting in significant decrease in milk production and sometimes death (Erskine et al., 1988; Hogan and Smith, 2003). Subclinical and self-limiting infections are associated with a controlled inflammatory response with a prompt return to normal tissue homeostasis and milk production (Vangroenweghe et al., 2005). Acute gram-negative mammary infections, however, are characterized by dysregulated inflammatory responses elicited by LPS (Sordillo et al., 2009). Binding of LPS to toll-like receptor 4 (TLR4) results in activation of nuclear factor κ B (NF- κ B) with subsequent transcription and translation of proteins important for inflammatory mediator biosynthesis. Potent phospholipid mediators crucial for onset and progression of inflammation following LPS exposure also are released including platelet activating factor and oxidized lipids known as oxylipids (Corl et al., 2008).

The biosynthesis of oxylipids is preceded by release of their PUFA precursors, including linoleic acid (LA), arachidonic acid (ArA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) from the *sn*-2 esterification site in the phospholipid glycerol backbone (Rosenthal et al., 1995). The abundance of PUFA in phospholipids is dependent on and can be modified by dietary FA composition in several species (Calder, 2008; Childs et al., 2008). Metabolism of PUFA may occur in situ on esterified forms in phospholipids or, more commonly, following their release and availability as FFA in the cytosol (Kuhn et al., 2015). The release of PUFA as free or pre-oxygenated fatty acids from the *sn*-2 ester linkage is mediated by phospholipase enzymes (Murakami et al., 2011). Several classes of phospholipase enzymes are known, but the cytosolic PLA₂ (cPLA₂) is the predominant isoform class responsible for *sn*-2 ester linkage hydrolysis (Buczynski et al., 2009). The activation of cPLA₂ is mediated by an increase in intracellular calcium in response to several stimuli, including LPS from gram-negative bacteria (Rosenthal et al., 1995). Activation of other classes of

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lipases releases other bioactive lipid mediators from cell membrane phospholipids, including endocannabinoids (Zubrzycki et al., 2014a).

Regardless of PUFA type, oxygenation can occur through enzymatic pathways such as cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP) and nonenzymatic auto-oxidation mediated by some reactive metabolites (Buczynski et al., 2009). The COX enzymes are bifunctional, initially abstracting a hydrogen atom mediated by a tyrosine radical at the oxygenase site and followed by addition of dioxygen to generate prostaglandin (PG) G_2 (Wu et al., 2011). Then, PGG₂ is reduced at the peroxidase site of COX into the more stable PGH₂, which undergoes further metabolism by various prostaglandin synthases to a variety of bioactive prostanoids. Lipoxygenases utilize their non-heme Fe²⁺ to form ferrous hydroxide, which subsequently abstracts a hydrogen atom followed by insertion of molecular oxygen, forming a peroxy radical that is further converted to peroxy fatty acids (Kuhn et al., 2015). Similarly, cytochrome enzymes use their non-heme Fe²⁺ in mediating the oxidation, peroxidation, and hydroxylation of PUFA and some of the COX-derived prostaglandins including PGE₂ and PGD₂ (Spector et al., 2004).

The nonenzymatic oxidation of PUFA is primarily orchestrated by pro-oxidant metabolites such as reactive oxygen species (ROS) that include superoxide and hydrogen peroxide. Although physiologically important at low levels, ROS concentrations exceeding the cellular antioxidant capacity can induce oxidative stress where there is peroxidation of cell membrane PUFA (Sordillo et al., 2009). In dairy cows, the transition period is associated with oxidative stress related to increased ROS production by enhanced mitochondrial metabolism (Bernabucci et al., 2005). The induction of the oxidative burst mechanism used by leukocytes in killing bacteria is another major source of ROS during infections such as mastitis (Sordillo et al., 2009). The highly reactive unpaired electrons of ROS free radicals target the double bonds in PUFA and generate oxylipids, including hydroperoxy fatty acids and isoprostanes. Such auto-oxidation of PUFA differs from the enzymatic-derived pathways, by producing an assortment of both positional and enantiomeric isomeric products because of lack of specificity as exhibited by enzymes (Milne et al., 2011).

The initial oxygenation FA products from either enzymatic or nonenzymatic oxidation are subjected to further downstream metabolism to form a diverse network of oxylipids. Prostaglandin H₂ metabolites are converted to downstream prostaglandins by specific prostaglandin synthases. For example, specific prostaglandin synthases metabolize PGH₂ to prostaglandins

D₂, E₂, F_{2 α} , and I₂. The LOX-derived peroxy FA are further converted into leukotrienes, lipoxins, resolvins, and protectins (Kuhn et al., 2015), whereas CYP-derived fatty acid epoxides are further converted to fatty acid diols by soluble epoxide hydrolases. At least 140 oxylipids have been characterized to date, most with potent regulatory effects on inflammation (Tam, 2013).

Oxylipid biosynthesis is complex and is regulated by available PUFA substrates, activation of oxygenation pathways, and the extent to which initial oxygenation products are metabolized. A broader understanding of oxylipid diversity, changes in patterns of oxylipid accumulation, and their direct effects on inflammatory responses during bovine mastitis is required to enable evaluation of efficacious intervention modalities that promote resolution of inflammation. The purpose of this study, therefore, was to investigate the biosynthesis of oxylipid metabolites during acute naturally occurring coliform mastitis infection in dairy cows. Based on previous studies documenting increased FFA during mastitis (Atroschi et al., 1989a,b), the hypothesis for this study was that oxylipid biosynthesis would be correlated with their FFA substrates in both milk and plasma during naturally occurring bovine coliform mastitis.

MATERIALS AND METHODS

Chemicals

Acetonitrile, methanol, and formic acid of liquid chromatography-mass spectrometry grade were purchased from Sigma-Aldrich (St. Louis, MO). Deuterated and nondeuterated oxylipid standards were purchased from Cayman Chemical (Ann Arbor, MI). Butylated hydroxy toluene was purchased from ACROS (Thermo Fisher, Fair Lawn, NJ), EDTA and triphenylphosphine were purchased from Sigma-Aldrich, and indomethacin was purchased from Cayman Chemical.

Animals

This study was approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC) and cows were enrolled with client consent. The study was conducted at a commercial dairy operation in Michigan with about 3,300 lactating cows with an approximate rolling herd average milk production of 12,250 kg. Cows were housed in freestall barns and grouped according to lactation number, DIM, and milk yield. Cows were milked 2 times daily. Diets were formulated to meet the energy requirements based on production (Table 1) and feed was delivered 2 times/d as TMR.

Table 1. Nutrient composition (% of DM unless otherwise noted) of the diet for the experimental animals¹

Item	Nutrient analysis
DM	47.64
Forage DM	40.87
CP	17.23
MP	11.97
ME, Mcal/kg	2.98
NE _L , Mcal/kg	1.79
ADF	16.23
NDF	29.56
Forage ADF	10.92
Forage NDF	17.46
NFC	42.36
Starch (nonsugar)	29.85
Fat	4.34

¹Supplemented with: vitamin A, 8,369 IU/kg, vitamin D, 1,455 IU/kg, and vitamin E, 33 IU/kg; minerals (% of DM): 1.04 Ca, 0.48 P, 1.1 K, 0.34 Mg, 0.26 S, 0.56 Na, 0.32 Cl, 0.35 Salt; Ca:P ratio = 2.17; trace minerals (mg/kg): 51.8 Zn, 7.6 Fe, 11.5 Cu, 37.0 Mn, 0.6 Co, 0.6 I, 0.37 Se; DCAD = 27.6 mEq/100 g; Rumensin 9.3 g/t (Elanco, Indianapolis, IN).

Study Design

Twenty-four multiparous dairy cows of at least third lactation were enrolled and divided into coliform mastitis ($n = 11$) and healthy control ($n = 13$) groups. The coliform mastitis group cows had cultured positive for *Escherichia coli* in milk samples and exhibited signs of systemic clinical disease. Negative bacterial cultures on milk, absence of overt clinical signs, and an SCC of <250,000 cells/mL on last test day were required for the healthy control group. Bacterial milk cultures were performed on blood agar and selective media for coliform bacteria according to the National Mastitis Council guidelines (Hogan et al., 1999). Briefly, individual mammary gland quarter milk (10 μ L) was streaked onto a sheep blood agar and selective media using a sterile loop inoculator. Cultures were incubated at 37°C, read at 24 and 48 h, and considered positive for coliform growth based on presence of 3 or more colonies on both blood and selective media. Clinical signs for diagnosis of acute systemic coliform mastitis included elevated rectal temperature (>39.2°C), tachycardia (heart rate >80 beats/min), tachypnea (respiratory rate >30 breaths/min), episcleral injection, local signs of mammary gland inflammation including discoloration, swelling, heat, pain on palpation, and typical serum-like watery milk. Cows in the mastitis group had heavy coliform bacterial growth (>100 cfu) on milk culture and at least 2 of the clinical signs of systemic disease. Milk and blood samples were collected at the same time from each cow within 12 h following a clinical diagnosis of systemic coliform mastitis and from the lactation and DIM matched healthy control cows.

Lipidomics

Targeted PUFA, MUFA, SFA, and oxylipids were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS). Plasma from whole blood collected in EDTA anticoagulant and milk samples were mixed with an antioxidant reducing agent mixture to prevent degradation of preformed oxylipids and prevent ex vivo lipid peroxidation (O'Donnell et al., 2009). The antioxidant reducing agent mixture consisted of 50% methanol, 25% ethanol, and 25% water with 0.9 mM butylated hydroxy toluene, 0.54 mM EDTA, 3.2 mM triphenylphosphine, and 5.6 mM indomethacin. Samples were flash frozen in liquid nitrogen and stored at -80°C until analyses.

Internal Standards

A mixture of internal standards containing 5(S)-hydroxyeicosatetraenoic-d₈ acid [5(S)-HETE-d₈], 15(S)-hydroxyeicosatetraenoic-d₈ acid [15(S)-HETE-d₈], 8(9)-epoxyeicosatrienoic-d₁₁ acid [8(9)-EET-d₁₁], prostaglandin E2-d₉ (PGE2-d₉), 8,9-dihydroxyeicosatrienoic-d₁₁ acid (8,9-DHET-d₁₁), arachidonic acid-d₈ (AA-d₈), 2-arachidonoyl glycerol-d₈ (2-AG-d₈), and arachidonoyl ethanolamide-d₈ (AEA-d₈) was prepared to final concentrations of 0.25, 0.25, 0.5, 0.5, 0.25, 50, 2, and 0.25 μ M, respectively. The internal pure standards mixture was added into each sample and used to generate a 6-point standard curve ranging from 500 to 0.001 μ M in concentration of unlabeled fatty acid and oxylipid standards.

Sample Processing

Frozen plasma and whole milk samples were thawed on ice and processed as follows: 4 mL of milk was combined with 9.15 mL of 4°C acetonitrile, and 2 mL of plasma was combined with 5 mL of 4°C methanol and 2 μ L of 88% formic acid. To each sample mixture, 15 μ L of internal standard cocktail was added, and the sample was vortexed for 2 min, incubated at room temperature for 15 min, and centrifuged at $4,816 \times g$ for 20 min at 4°C. For plasma, the supernatant was diluted with 95 mL of HPLC-grade water and 95 μ L of formic acid, and supernatant in milk samples was diluted with 170 mL of HPLC-grade water and 170 μ L of formic acid.

Solid-Phase Extraction

Solid-phase extraction was performed for each sample with Oasis HLB 12cc (500 mg) LP Extraction Columns (Waters, Milford, MA). Columns were conditioned with

6 mL of methanol followed by 6 mL of HPLC water. Extracts were loaded onto the columns, which were then washed with 6 mL of 20% methanol and dried under full vacuum for 4 min. Analytes were eluted with 6 mL of methanol:acetonitrile 50:50 (vol:vol). The volatile solvents were removed under vacuum using a Savant SpeedVac (ThermoQuest, Holbrook, NY). The residues were reconstituted in 100 μ L of methanol and transferred to a microcentrifuge tube containing 50 μ L of water. The mixture was centrifuged at $14,000 \times g$ for 10 min at 4°C and then the supernatant was transferred to an auto-sampler vial with a low volume insert and stored at -20°C until analyzed.

LC-MS/MS Analyses

The quantification of metabolites was accomplished on a Waters Acquity UPLC coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters) using multiple reaction monitoring. Chromatography separation was performed with an Ascentis Express C18 HPLC column, 10 cm $\times$ 2.1 mm, 2.7 μ m (Supelco, Bellefonte, PA) held at 50°C. The autosampler was held at 10°C. Mobile phase A was water with 0.1% formic acid, and mobile phase B was acetonitrile. Flow rate was fixed at 0.3 mL/min. Liquid chromatography separation took 15 min with linear gradient steps programmed as follows (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. All oxylipids and fatty acids were detected using electrospray ionization in negative-ion mode, whereas the endocannabinoids were detected using positive-ion mode. Cone voltages and collision voltages were optimized for each analyte using Waters QuanOptimize software (see Appendix for multiple reaction monitoring parameters).

Statistical Analyses

Statistical analyses were performed using SAS software (version SAS 9.4; SAS institute Inc., Cary, NC). Data were expressed as means $\pm$ standard errors of the mean and tested for normality. Where Gaussian distribution was satisfied, *t*-tests for independent means were performed. Data deviating from the Gaussian distribution were either square root- or log-transformed before analyses. An ANOVA and Tukey test for comparison between means were performed to determine differences among FA concentrations in the same sample type (milk or plasma). Pearson correlations were calculated for multiple relationships among FA substrates;

FA substrates and their oxylipid metabolites; related upstream and downstream oxylipid metabolites and concentrations of the same substrate/metabolite in milk versus plasma. The levels of significance for correlation analyses were adjusted for multiple comparisons. Statistical significance was set at $\alpha = 5\%$, and data are presented as means $\pm$ SEM.

RESULTS

Fatty acids quantified in milk and plasma included PUFA (ArA, LA, EPA, and DHA), MUFA (oleic acid), and SFA (palmitic and stearic acids). All PUFA concentrations were greater in milk during coliform mastitis; however, only the elevations in LA concentrations were reflected systemically in plasma (Figure 1). Fold differences of PUFA in milk between mastitis and healthy cows were highest for LA (5.1) followed, in decreasing order, by ArA (3.8), DHA (3.0), and EPA (2.4). Linoleic acid was the most abundant PUFA in both milk and plasma in both mastitis and healthy cows (Figure 1). Higher concentrations of oleic acid in milk during coliform mastitis were reflected systemically in plasma, whereas no differences in the SFA were detected (Figure 2).

A lipidome of 63 oxylipid species was targeted by the LC-MS/MS protocol used in this study. Thirty oxylipids were detected in milk and 27 were detected in plasma. The oxylipids leukotriene B₄ (**LTB₄**), 8,9-epoxyeicosatrienoic acid (**8,9-EET**), and 17-hydroxydocosahexaenoic acid (**17-HDoHE**) were detected in milk only and accounted for the differences in the number of detected oxylipids between milk and plasma. A significantly ($P < 0.05$) higher proportion of the fraction of total oxylipid changes was observed in milk oxylipids (60%) compared with plasma oxylipids (32%) during coliform mastitis. Oxylipids that were not detectable in 50% or more cows in the study groups were excluded from all statistical analyses and are reported together with their corresponding limits of detection (Table 2). The limit of detection was defined as the concentration at which the peak response for an analyte was 3 times that of the noise (signal:noise = 3).

Oxylipids detected were representative of the known enzymatic pathways (COX, LOX, and CYP) and non-enzymatic pathways. The COX pathway was represented by prostaglandin metabolites from ArA metabolism, accounting for only 8 and 9% of the fraction of total oxylipids in milk and plasma, respectively. Concentrations of PGE₂ and 6-ketoPGF_{1 α} were elevated significantly in milk (Table 3), but only the concentrations of 6-ketoPGF_{1 α} (a stable metabolite of PGI₂) were reflected systemically in plasma.

Figure 1. Concentrations (mean $\pm$ SEM, μ M) of PUFA differed for all PUFA in milk (A) but only for linoleic acid (LA) in plasma (B) between coliform mastitis (solid bars) and healthy control (open bars) cows. Linoleic acid was most abundant among PUFA but similar to arachidonic acid (ArA) in control milk and plasma and mastitis plasma) within an experimental group; EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; bars with different letters (a–d) differ ($P < 0.05$) among PUFA within an experimental group (mastitis or control).

Lipoxygenase-derived oxylipids (Table 4) accounted for the highest proportion of the fraction of total oxylipid metabolites detected in both milk (48%) and plasma (45%). The hydroxy metabolite concentrations, 9- and 13-hydroxyoctadecadienoic acids (**HODE**) from LA and 5- and 15-hydroxyeicosatetraenoic acids (**HETE**) from ArA were elevated in milk during coliform mastitis; however, their respective downstream ketone derivatives 9- and 13-oxooctadecadienoic acid (**oxoODE**) from LA and 5- and 15-oxoeicosatetraenoic acid (**oxoETE**) from ArA were not different. Lipoxygenase-derived LTB₄ and lipoxin A₄ (**LXA₄**) similarly were elevated in milk, but LXA₄ was significantly lower in plasma during mastitis. The DHA metabolite resol-

vin D₂ (**RvD₂**) was significantly decreased in plasma during mastitis.

The CYP enzymatic metabolism of LA, ArA, and EPA generated oxylipids that accounted for 36 and 36% of the fraction of total oxylipids detected in milk and plasma, respectively (Table 5). The epoxidation metabolites of LA, epoxyoctadecenoic acids (**9,10-** and **12,13-EpOME**), were significantly elevated in milk of coliform mastitis cows but the epoxide hydration products (dihydroxyoctadecenoic acids; **9,10-** and **12,13-DiHOME**) were significantly lower in plasma of mastitis cows. In contrast, the ArA epoxide 11,12-epoxyeicosatrienoic (**EET**) was elevated in plasma of control cows. The hydroxylation product of ArA, 20-HETE,

Figure 2. Milk and plasma concentrations (mean $\pm$ SEM; μ M) of oleic acid were greater in coliform mastitis ($n = 11$) than control ($n = 13$) cows but no differences were detected for stearic and palmitic acids. Solid and open black bars represent milk samples; diagonally striped and gray bars represent plasma samples. * $P < 0.05$.

Table 2. Concentrations and limits of detection (mean $\pm$ SEM; nM) for oxylipids inconsistently detected in milk and plasma from coliform mastitis (total n = 11) and control (total n = 13) cows

Sample	Oxylipid ¹	Mastitis group			Control cows			LOD ²
		No./total (%) of cows	Mean	SEM	No./total (%) of cows	Mean	SEM	
Milk	14,15-EET	0/11 (0)	—	—	0/13 (0)	—	—	0.94
	8,9-EET	5/11 (45)	0.15	0.10	0/13 (0)	—	—	0.02
	19,20-EpDPE	2/11 (18)	0.87	0.16	0/13 (0)	—	—	0.60
	15-dPGJ ₂	2/11 (18)	0.10	0.02	0/13 (0)	—	—	0.05
	14,15-EpETE	2/11 (18)	0.06	0.06	0/13 (0)	—	—	0.01
Plasma	LTB ₄	0/11 (0)	—	—	0/13 (0)	—	—	0.003
	14,15-EET	0/11 (0)	—	—	0/13 (0)	—	—	0.94
	8,9-EET	0/11 (0)	—	—	0/13 (0)	—	—	0.02
	19,20-EpDPE	0/11 (0)	—	—	1/13 (8)	0.76	0.00	0.60
	17-HDoHE	5/11 (45)	0.30	0.11	0/13 (0)	—	—	0.11
	6-ketoPGF _{1α}	5/11 (45)	1.1	0.23	0/13 (0)	—	—	0.44
	15-dPGJ ₂	2/11 (18)	0.07	0.01	0/13 (0)	—	—	0.05
	14,15-EpETE	1/11 (9)	0.01	0.00	0/13 (0)	—	—	0.01

¹EET = epoxyeicosatrienoic acid; EpDPE = epoxydocosapentaenoic acid; 15-dPGJ₂ = 15-deoxyprostaglandin J₂; EpETE = epoxyeicosatetraenoic acid; LTB₄ = leukotriene B₄; HDoHE = hydroxy-docosahexaenoic acid; 6-ketoPGF_{1α} = 6-keto prostaglandin F_{1α}.

²Limit of detection.

was elevated in both milk and plasma during coliform mastitis. Although the EPA vicinal diols 14,15- and 17,18-dihydroxyeicosatetraenoic acid (**DiHETE**) were increased in milk during coliform mastitis, significantly lower concentrations of the 14,15-DiHETE isomer were detected in plasma.

The nonenzymatic metabolites from AA (hydroxyeicosatetraenoic acids; **9-** and **11-HETE**) were abundant in milk, but not in plasma during mastitis. Consequently, nonenzymatic metabolites accounted for only 8 and 9% of the fraction of total oxylipids in milk and plasma, respectively (Table 6). The relative contribution of nonenzymatic auto-oxidation of LA into 9- and 13-HODE was not determined.

Relative contributions of metabolic pathways to oxylipid biosynthesis were assessed from ratios of metabolites of different pathways from the same PUFA substrate (Table 7). In both milk and plasma, the lack of differences between mastitis and control cows in the ratios 13-HODE to 9-HODE and 9- and 11-HETE to

15-HETE suggested lack of pathway preference for LA and ArA, respectively. However, there was a shift from epoxidation of ArA toward hydroxylation into 20-HETE in milk during coliform mastitis.

Ratios representing the progressive metabolism of oxylipids such as the conversion of hydroxy oxylipids to ketone derivatives (HODE to oxoODE; HETE to oxoETE) and epoxides to vicinal diols (EpOME to DiHOME) were compared between groups (Table 8). Ratios suggesting greater (when >1) abundance of hydroxy metabolites were detected for both milk and plasma in all cows; however, only the ArA-derived metabolites in coliform mastitis milk samples and 13-HODE:13-oxoODE in plasma of control cows were significant. The within-group epoxide-to-vicinal diol ratios suggested abundant 12,13-EpOME (>1) and lesser 9,10-EpOME (<1) isomers in coliform mastitis cows and greater vicinal diols (<1) in control cows. Numerical values of the epoxide-to-vicinal diol ratios were greater significantly in coliform than in control cows.

Table 3. Cyclooxygenase-derived oxylipids in milk and plasma from coliform mastitis (n = 11) and mid-lactation control (n = 13) cows (mean $\pm$ SEM; nM)

Sample	Oxylipid ¹	Substrate ²	Mastitis		Control		Significance
			Mean	SEM	Mean	SEM	
Milk	6-KetoPGF _{1α}	ArA	1.19	0.36	0.44	0.00	*
	PGE ₂	ArA	2.28	0.56	0.05	0.06	***
Plasma	6-KetoPGF _{1α}	ArA	0.74	0.21	0.44	0.00	*
	PGE ₂	ArA	0.24	0.06	0.20	0.07	

¹Data log-transformed; results expressed as geometric mean $\pm$ SEM; PGE₂ = prostaglandin E₂; 6-ketoPGF_{1α} = 6-keto prostaglandin F_{1α}.

²ArA = arachidonic acid.

P* < 0.05; **P* < 0.001.

Table 4. Lipoygenase-derived oxylipids in milk and plasma from coliform mastitis (n = 11) and mid-lactation control (n = 13) cows (mean $\pm$ SEM; nM)

Sample	Oxylipid ¹	Substrate ²	Mastitis		Control		P-value
			Mean	SEM	Mean	SEM	
Milk	13-HODE	LA	32.6	9.0	6.8	1.7	*
	9-HODE ³	LA	5.9	0.29	2.0	0.29	*
	13-oxoODE ³	LA	1.6	0.47	0.67	0.22	
	9-oxoODE ³	LA	1.3	0.40	0.67	0.37	
	15-oxoETE ³	ArA	0.03	0.63	0.019	0.58	
	5-oxoETE ³	ArA	0.04	0.80	0.010	0.61	
	5-HETE ³	ArA	0.23	0.28	0.06	0.17	***
	LTB ₄ ³	ArA	0.07	1.03	0.003	0.00	**
	LXA ₄ ³	ArA	0.09	0.45	0.01	0.3	**
	15-HETE ³	ArA	3.0	0.26	0.07	0.27	***
	17-HDoHE ³	DHA	0.26	0.22	0.11	0.00	
	RvD ₂ ³	DHA	0.02	0.80	0.02	0.3	
Plasma	13-HODE ³	LA	4.27	0.17	4.61	0.16	
	9-HODE ³	LA	1.34	0.21	1.41	0.20	
	13-oxoODE	LA	0.38	0.05	0.51	0.16	
	9-oxoODE	LA	0.86	0.17	1.3	0.34	
	15-oxoETE	ArA	0.02	0.005	0.03	0.01	
	5-oxoETE	ArA	0.01	0.002	0.02	0.005	
	5-HETE ³	ArA	0.06	0.32	0.08	0.29	
	LXA ₄ ³	ArA	0.01	0.12	0.04	0.41	**
	15-HETE ³	ArA	0.36	0.30	0.20	0.27	
	RvD ₂ ³	DHA	0.003	0.02	0.10	0.08	*

¹HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; oxoETE = oxoeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LTB₄ = leukotriene B₄; LXA₄ = lipoxin A₄; HDoHE = hydroxydocosahexaenoic acid; RvD₂ = resolvin D₂.

²ArA = arachidonic acid; LA = linoleic acid; DHA = docosahexaenoic acid.

³Data log-transformed, results expressed as geometric mean $\pm$ SEM.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5. Cytochrome P450-derived oxylipids in milk and plasma from coliform mastitis (n = 11) and mid-lactation control (n = 13) cows (mean $\pm$ SEM; nM)

Sample	Oxylipid ¹	Substrate ²	Mastitis		Control		P-value
			Mean	SEM	Mean	SEM	
Milk	9,10-DiHOME ³	LA	2.0	0.54	6.6	0.17	
	12,13-DiHOME ³	LA	2.7	0.45	5.0	0.37	
	9,10-EpOME	LA	0.95	0.20	0.46	0.05	*
	12,13-EpOME	LA	2.3	0.57	0.40	0.06	**
	11,12-EET ³	ArA	0.02	0.28	0.01	0.00	
	8,9-EET ³	ArA	0.05	0.43	0.02	0.00	
	20-HETE ³	ArA	0.72	0.66	0.01	0.24	***
	14,15-DiHETE	EPA	1.1	0.20	0.10	0.01	***
	17,18-DiHETE	EPA	4.2	0.87	0.16	0.02	**
	9,10-DiHOME	LA	2.1	0.50	19.3	2.0	***
Plasma	12,13-DiHOME ³	LA	0.8	0.20	10.9	0.44	***
	9,10-EpOME	LA	1.0	0.25	1.9	0.46	
	12,13-EpOME ³	LA	1.0	0.26	1.8	0.24	
	11,12-EET	ArA	0.02	0.003	0.05	0.01	*
	20-HETE ³	ArA	0.53	0.31	0.24	0.15	*
	14,15-DiHETE	EPA	1.1	0.19	1.5	0.16	
	17,18-DiHETE	EPA	8.0	1.3	19.6	3.3	**

¹DiHOME = dihydroxyoctadecenoic acid; EpOME = epoxyoctadecenoic acid; EET = epoxyeicosatrienoic acid; HETE = hydroxyeicosatetraenoic acid; DiHETE = dihydroxyeicosatrienoic acid.

²LA = linoleic acid; ArA = arachidonic acid; EPA = eicosapentaenoic acid.

³Data log-transformed, results expressed as geometric mean $\pm$ SEM.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$.

Table 6. Nonenzymatic-derived oxylipids in milk and plasma from coliform mastitis (n = 11) and mid-lactation control (n = 13) cows (mean $\pm$ SEM; nM)

Sample	Oxylipid ¹	Substrate ²	Mastitis		Control		P-value
			Mean	SEM	Mean	SEM	
Milk	11-HETE ³	ArA	0.57	0.62	0.04	0.22	**
	9-HETE ³	ArA	0.10	0.61	0.02	0.36	**
Plasma	11-HETE	ArA	0.20	0.05	0.14	0.02	
	9-HETE	ArA	0.06	0.02	0.03	0.004	

¹HETE = hydroxyeicosatetraenoic acid.²ArA = arachidonic acid.³Data log-transformed, results expressed as geometric mean $\pm$ SEM.***P* < 0.01.**Table 7.** Milk and plasma oxylipids in coliform mastitis (n = 11) and mid-lactation control (n = 13) cows expressed as ratios of select pathways based on substrate

Sample	Ratio ¹	Substrate ²	Mastitis cows	Control cows	P-value
Milk	13-HODE ³ /9-HODE ⁴	LA	4.12 $\pm$ 0.13	2.60 $\pm$ 0.26	**
	Sum EET/20-HETE	ArA	0.10 $\pm$ 0.51	5.32 $\pm$ 0.71	
	(9-HETE + 11-HETE) ⁵ /15-HETE ³	ArA	0.25 $\pm$ 0.65	0.84 $\pm$ 0.12	
Plasma	13-HODE/9-HODE	LA	3.28 $\pm$ 0.26	3.50 $\pm$ 0.36	
	(9-HETE + 11-HETE)/15-HETE	ArA	0.59 $\pm$ 0.08	0.60 $\pm$ 0.07	

¹HODE = hydroxyoctadecadienoic acid; EET = epoxyeicosatrienoic acid; HETE = hydroxyeicosatetraenoic acid.²ArA = arachidonic acid; LA = linoleic acid.³Predominantly enzyme derived.⁴Both enzymatic and nonenzymatic derived.⁵Predominantly nonenzymatic derived.***P* < 0.01.**Table 8.** Milk and plasma oxylipids in coliform mastitis (n = 11) and mid-lactation control (n = 13) cows expressed as ratios of select upstream:downstream metabolites based on substrate

Sample	Ratio ¹	Substrate ²	Mastitis		Control		P-value
			Mean	SEM	Mean	SEM	
Milk	5-HETE/5-oxoETE ³	ArA	37.2	1.08	8.9	0.43	*
	15-HETE/15-oxoETE	ArA	277.68	81.73	6.12	2.44	**
	9-HODE/9-oxoODE ³	LA	5.7	0.45	4.4	0.41	
	13-HODE/13-oxoODE ³	LA	18.22	0.56	8.74	0.32	*
	9,10-EpOME/9,10-DiHOME ³	LA	0.42	0.10	0.07	0.02	**
	12,13-EpOME/12,13-DiHOME ⁴	LA	0.94	0.34	0.07	0.33	***
Plasma	5-HETE/5-oxoETE	ArA	10.18	1.65	7.44	1.33	
	15-HETE/15-oxoETE	ArA	88.50	27.82	56.86	19.70	
	9-HODE/9-oxoODE	LA	1.97	0.21	1.61	0.25	
	13-HODE/13-oxoODE	LA	14.26	2.62	40.19	13.40	*
	9,10-EpOME/9,10-DiHOME	LA	0.57	0.10	0.09	0.01	***
	12,13-EpOME/12,13-DiHOME	LA	1.46	0.25	0.23	0.05	***

¹HETE = hydroxyeicosatetraenoic acid; oxoETE = oxoeicosatetraenoic acid; HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; EpOME = epoxyoctadecenoic acid; DiHOME = dihydroxyoctadecenoic acid.²ArA = arachidonic acid; LA = linoleic acid.³Square-root transformed data reported as geometric mean $\pm$ SEM.⁴Log transformed data reported as geometric mean $\pm$ SEM.**P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Table 9. Correlations between FA substrates and their oxylipid metabolites in milk and plasma from coliform mastitis cows

Substrate and metabolite ¹	Milk		Plasma	
	r	P-value	r	P-value
ArA vs. 15-oxoETE	0.921	**		
EPA vs. 14,15-DiHETE			0.863	**
EPA vs. 17,18-DiHETE			0.756	*

¹ArA = arachidonic acid; oxoETE = oxoeicosatetraenoic; EPA = eicosapentaenoic acid; DiHETE = dihydroxyeicosatrienoic acid.

* $P < 0.05$; ** $P < 0.01$.

Several significant correlations were detected among quantified metabolites (FA, FA and oxylipids, oxylipid to oxylipid) in milk and plasma from coliform mastitis cows (Tables 9, 10, and 11). Interestingly, no correlations could be detected for several metabolites between the milk and plasma samples, especially in the coliform mastitis group. Endocannabinoid metabolites derived from ArA and DHA only were detected with significantly higher concentrations of arachidonyl ethanolamide (mastitis, 1.5 ± 0.2 nM vs. control 0.07 ± 0.01 nM; $P < 0.0001$) and docosahexanoyl ethanolamide (mastitis, 1.5 ± 0.05 nM vs. control 1.1 ± 0.004 nM; $P < 0.001$) in milk, but not in plasma, during coliform mastitis.

DISCUSSION

Polyunsaturated fatty acids are essential substrates that play a critical role in the synthesis of oxylipids during health and inflammatory processes. The elevations of all PUFA assessed in milk in this study agree with previous findings of increased LA, ArA, and EPA in milk from bovine mastitis (Atroshi et al., 1989b). Mastitis in humans is also associated with increased long-chain FA in milk (Hunt et al., 2013). The increased

PUFA suggest abundance of oxylipid precursor substrates whose sources may include increased lipolysis, dietary lipids, and de novo biosynthesis from essential FA. Although sources of FA were not evaluated in this study, increased lipolysis related to elevated tumor necrosis factor α (TNF α), may have contributed to increased PUFA substrate (Hoebe et al., 2000). For example, previous studies showed that plasma TNF α concentrations increase significantly with bovine coliform mastitis (Sordillo and Peel, 1992). Experimental injection of TNF α and the associated decrease in DMI increased adipose tissue lipolysis in cows (Kushibiki et al., 2003). Although plasma NEFA, an indicator of lipolysis, were not measured in this study, increased lipolysis could be supported by significant elevations in oleic acid in this study, a known abundant component of plasma NEFA in dairy cows during times of increased lipolysis (Contreras and Sordillo, 2011).

The simultaneous evaluation of metabolites in milk and plasma during coliform mastitis showed substantial differences in oxylipid profiles even with the presence of systemic disease. Similarly, mice with LPS-induced sepsis demonstrated different oxylipid profiles among various tissue locations (Balvers et al., 2012). Consequently, oxylipid concentrations in one biological sample cannot be used to infer oxylipid profiles in a different anatomic area. The lack of correlations in concentrations of several oxylipids between milk and plasma during mastitis (Table 11) suggests that the microenvironment of the affected tissues can greatly influence oxylipid biosynthesis. Concentrations of milk and plasma PUFA substrates differed during coliform mastitis and can be explained by several factors. First, milk production decreases precipitously during coliform mastitis, resulting in elevated concentrations of milk components, including FFA (Shuster et al., 1991). Second, the disruption of the blood mammary endothelial

Table 10. Correlations between degree of metabolism for select oxylipids in milk and plasma from coliform mastitis cows

Substrate ¹	Degree of metabolism ²	Milk		Plasma	
		r	P-value	r	P-value
LA	13-HODE vs. 9-HODE	0.864	*	0.834	*
LA	13-HODE vs. 13-oxoODE	0.736	*	0.26	NS
LA	9-HODE vs. 9-oxoODE	0.791	*	0.882	**
LA	9,10-DiHOME vs. 9,10-EpOME	0.724	*	0.387	NS
LA	12,13-EpOME vs. 12,13-DiHOME	0.382	NS	0.75	*
ArA	5-HETE vs. 5-oxoETE	0.639	*	0.678	*
ArA	15-HETE vs. 15-oxoETE	0.639	NS	0.531	NS

¹ArA = arachidonic acid; LA = linoleic acid.

²HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; DiHOME = dihydroxyoctadecadienoic acid; EpOME = epoxyoctadecadienoic acid; HETE = hydroxyeicosatetraenoic acid; oxoETE = oxoeicosatetraenoic acid.

* $P < 0.05$; ** $P < 0.01$.

Table 11. Correlations between fatty acid:fatty acid substrate and oxylipid:oxylipid between samples (e.g., milk LA correlated with plasma LA) within each experimental groups

Substrate ¹	Oxylipid metabolite	Mastitis group		Control group	
		r	P-value	r	P-value
LA				0.843	***
ArA				0.903	***
EPA		0.789	**	0.755	**
LA	13-HODE			0.844	***
LA	9-oxoODE			0.976	***
LA	13-oxoODE			0.793	**
LA	12,13-EpOME			0.753	**
LA	9,10-DiHOME	0.730	*		
LA	12,13-DiHOME	0.906	***	0.841	***
ArA	15-HETE	0.751	**		
ArA	15-oxoETE			0.845	**
ArA	2-AG			0.758	**

¹LA = linoleic acid; ArA = arachidonic acid; EPA = eicosapentaenoic acid.

²HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; EpOME = epoxyoctadecenoic acid; DiHOME = dihydroxyoctadecenoic acid; HETE = hydroxyeicosatetraenoic acid; oxoETE = oxoeicosatetraenoic acid; AG = arachidonoyl glycerol.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

barrier and increased blood flow to the mammary gland during coliform mastitis could result in increased transfer of long-chain FA into the mammary gland (Wenz et al., 2001). For example, previous studies in mice with LPS-induced peritonitis showed that increased n-3 fatty acids intravenously pre-infused were detected in peritoneal exudates (Kasuga et al., 2008). Increasing plasma concentrations of select PUFA types such as the n-3 FA may be a direct source of PUFA for localized inflammatory sites. Thus, plasma PUFA may be increased by their dietary supplementation or precursor essential FA such as LA and α -linoleic acid to support de novo biosynthesis of PUFA.

The variety of oxylipids expressed in both milk and plasma in this study represented some of the well-known enzymatic and nonenzymatic oxylipid biosynthetic pathways. Earlier studies on bovine coliform mastitis reported a few select prostanoids including PGE₂, PGF_{2 α} , and thromboxane B₂ in milk using immunoassay based detection techniques (Atroschi et al., 1989a). In the current study, the use of LC-MS/MS provided a more robust methodology to identify an expanded oxylipid network during acute bovine coliform mastitis.

Oxylipids generated from the COX pathway in the current study were represented only by PGE₂ and the inactive downstream metabolite of PGI₂, 6-ketoPGF_{1 α} . Both PGI₂ and PGE₂ are produced by constitutive COX-1 and inducible COX-2 isoforms and are important for the vasodynamic responses during initial stages of inflammation (Bulger and Maier, 2000). In addition to maintaining basal prostanoid production, COX-1 isoforms are responsible for the initial prostanoid synthesis during inflammation before induction of the

transcriptionally and translationally dependent COX-2 isoform (Poher and Sessa, 2007). Previous experimental and natural bovine coliform mastitis studies reported increased concentrations of PGE₂, PGI₂, PGF_{2 α} , and thromboxane B₂. Inhibition of COX enzymes using non-steroidal anti-inflammatory drugs (NSAID) reduces prostanoid and thromboxane concentrations, with subsequent improved clinical signs (Anderson et al., 1986). Use of NSAID in veterinary medicine, however, is associated with complications such as abomasal ulceration and renal toxicity (Anderson and Muir, 2005). Defining the variety and temporal generation of COX-derived oxylipids during coliform mastitis may identify optimal times for pharmacologic inhibition of COX enzymes. In acute bovine coliform mastitis, for example, early NSAID use may not be optimal as oxylipid products of COX enzymes mediate crucial processes during early stages of acute inflammation.

The LOX-derived metabolites accounted for the majority of oxylipids detected in the present study. Several lines of evidence showed that LOX generates both pro- and anti-inflammatory oxylipids. For example, development of atherosclerosis in humans is associated with increased 15LOX mRNA expression and hydroxy metabolites including 15-HETE and 9- and 13-HODE (Gertow et al., 2011). Several oxylipids, including lipoxin A₄, resolvins, protectins, and maresins in several murine models of sepsis, are associated with shorter time to inflammation resolution and improved survival (Serhan et al., 2008). In dairy cows, 15LOX1 gene expression was increased in mammary gland tissues during the periparturient period, suggesting a potential contribution to oxidative stress in transition cows (Aitken et

al., 2009). Oxidative stress was suggested as contributing to predisposing periparturient cows to dysregulated inflammatory conditions including mastitis (Sordillo and Mavangira, 2014). Nonspecific inhibition of LOX in combination with flunixin meglumine, a COX inhibitor, was associated with improved bovine mammary gland inflammation (Rose et al., 1991). Taken together, these data demonstrate the need to clearly define the role of LOX-derived oxylipids in the pathophysiology of coliform mastitis to explore potential targets and optimal times for therapy or interventions.

The metabolism of PUFA through CYP enzymes showed significant elevations in various epoxides of ArA, LA, and EPA. Several epoxide metabolites were identified as unexplored targets for therapy with potent anti-inflammatory effects (Morisseau and Hammock, 2013). For example, with LPS-induced peritonitis, inhibiting hydration of epoxides decreased pro-inflammatory cytokines, abrogated hypotension, and diminished mortality compared with control mice (Schmelzer et al., 2005). Furthermore, pro-inflammatory metabolites of COX and LOX pathways such as PGE₂ and 5-HETE, respectively, were decreased, suggesting an indirect influence of CYP epoxides on acute inflammatory pathways (Schmelzer et al., 2005). Conversely, CYP hydroxylation of ArA produces 20-HETE, a potent hypertensive oxylipid during inflammation (Konkel and Schunck, 2011). Epoxides of LA origin are associated with acute respiratory distress syndrome following sepsis in humans (Konkel and Schunck, 2011), suggesting that FA substrate and relative abundance of their derived oxylipids may affect the inflammatory phenotype. Defining the relative contributions of CYP derived epoxides from different PUFA is critical to balance between the beneficial anti-inflammatory species and the pro-inflammatory 20-HETE and LA epoxides. The apparent shift from epoxidation into hydroxylation of ArA (Table 7) in the current study suggests a contribution of pro-inflammatory 20-HETE to the acute inflammatory phase during bovine coliform mastitis. The ability of epoxides to downregulate pro-inflammatory metabolites from other biosynthetic pathways may make them particularly attractive because of a multimodal mechanism that may be very effective at resolving inflammation.

Several PUFA metabolites exhibit different levels of metabolism producing distinct oxylipid species with specific effects on the inflammatory process. For example, LA is initially oxygenated to hydroperoxy acids (9- and 13-HPODE) that are subsequently reduced by peroxidases to hydroxy acids (9- and 13-HODE). Dehydrogenase enzymes eventually convert the hydroxyacid metabolites into ketone derivatives (9- and 13-oxoODE). The hydroperoxy oxylipid 13-HPODE

activates the pro-inflammatory transcription factor NFκB in vascular smooth muscle cells (Natarajan et al., 2001). In addition, another hydroperoxy metabolite, 15HPETE, mediates inflammation and apoptosis in bovine aortic endothelial cells (Sordillo et al., 2005). Hydroxy metabolites (HODE and ArA-derived HETE) are associated with either pro- or anti-inflammatory states in cardiovascular and other diseases (Kuhn et al., 2015). The ketone derivatives have predominantly anti-inflammatory effects. For example, 13-oxoODE reduces pro-inflammatory IL-8 through peroxisome proliferator-activated receptor gamma activation in human colonic epithelial cells (Altmann et al., 2007). Together, these data suggest that the degree of PUFA catabolism during inflammation may affect the duration, phenotype, and outcome of inflammatory process. Although upstream oxylipids were positively correlated with their downstream derivatives in the current study (Tables 9 and 10), the abundance of ArA- and LA-derived hydroxy oxylipids suggests an overproduction or decreased dehydrogenation to the ketone derivatives, indicating a pro-inflammatory oxylipid profile. Studying the temporal patterns of the degree of metabolism of PUFA and the specific effects of their oxylipid metabolites during inflammation may uncover targets for enhancement or blockade of metabolic pathways to modulate inflammation.

The roles for endocannabinoid metabolites from ArA and DHA that accumulated during coliform mastitis are not immediately clear. In a rat model of LPS-induced uveitis, activation of cannabinoid CB2 receptors was associated with reduction in the pro-inflammatory transcription factors, pro-inflammatory cytokines, and leukocyte endothelial adhesion (Toguri et al., 2014). The endocannabinoid system was reviewed recently as a potential inflammation modulatory target in various human diseases (Zubrzycki et al., 2014a,b). In cows, endocannabinoids were detected in milk from healthy animals (Gouveia-Figueira and Nording, 2014). Further investigations may be useful to determine the presence and influence of cannabinoid receptors on mammary gland inflammation.

CONCLUSIONS

In conclusion, oxylipid generation during acute bovine coliform mastitis is influenced by substrate availability, multiple biosynthetic pathways, and different degrees of metabolism. The use of LC-MS/MS offers a sensitive and specific technique that can be utilized to evaluate novel targets in oxylipid biosynthesis for diagnostic and therapeutic interventions in inflammatory conditions, including coliform mastitis. Additionally, biomarkers that reflect oxylipid profiles and inflammatory pheno-

type during disease can be developed. The detection of several localized changes in oxylipid biosynthesis in the presence of systemic disease suggests focusing specifically on the mammary gland environment in developing and evaluating interventions to modulate inflammation during mastitis.

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APPENDIX

Table A1. Optimized multiple reaction monitoring parameters for metabolites

Metabolite ¹	Parent ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Cone voltage	Collision energy	Precursor ²	Class
12 HHTrE	279.2	179	45	10	ArA	Alcohol
20-HETE	319.2	245	55	16	ArA	Alcohol
15-HETE	319.2	219	45	10	ArA	Alcohol
11-HETE	319.2	167	51	16	ArA	Alcohol
9-HETE	319.2	151	15	10	ArA	Alcohol
5-HETE	319.2	115	33	10	ArA	Alcohol
14,15-DHET	337.2	207	33	16	ArA	Diol
11,12-DHET	337.2	167	51	22	ArA	Diol
8,9-DHET	337.2	127	15	22	ArA	Diol
AEA	348.2	62	21	16	ArA	Endocannabinoid
2-AG	379.3	287	45	16	ArA	Endocannabinoid
14,15-EET	319.2	219	33	10	ArA	Epoxide
11,12-EET	319.2	179	27	10	ArA	Epoxide
8,9-EET	319.2	155	27	10	ArA	Epoxide
8 iso PGF _{2α}	353.2	193	21	22	ArA	Isoprostane
8 iso PGA ₂	333.2	175	55	22	ArA	Isoprostane
8 iso PGA ₁	335.2	235	39	16	ArA	Isoprostane
LTB ₄	335.2	195	21	16	ArA	Leukotriene
5(S),6(R)-Lipoxin A ₄	351.2	115	55	16	ArA	Lipoxin
15-OxoETE	317.2	113	45	16	ArA	Ketone
5-OxoETE	317.2	203	45	22	ArA	Ketone
6-keto-PGF _{1α}	369.3	163	21	22	ArA	Prostanoid
PGE ₂	351.2	271	55	16	ArA	Prostanoid
PGD ₂	351.2	189	55	22	ArA	Prostanoid
15d-PGJ ₂	315.2	271	39	10	ArA	Prostanoid
TXB ₂	369.2	169	51	16	ArA	Thromboxane
17-HDoHE	343.2	281	45	10	DHA	Alcohol
19,20-DiHDPA	361.2	273	27	16	DHA	Diol
10,17-DiHDoHE	359.2	153	15	16	DHA	Protectin
7,17-dh-DPA	361.2	143	27	16	DHA	Protectin
Resolvin D ₂	375.2	175	21	22	DHA	Resolvin
17,18-DiHETE	335.2	247	39	16	EPA	Diol
14,15-DiHETE	335.2	207	27	16	EPA	Diol
17,18-EpETE	317.2	255	15	10	EPA	Epoxide
14,15-EpETE	317.2	207	27	10	EPA	Epoxide
13-HODE	295.2	195	30	16	LA	Alcohol
9-HODE	295.2	171	45	16	LA	Alcohol
12,13-DiHOME	313.2	183	45	22	LA	Diol
9,10-DiHOME	313.2	201	27	22	LA	Diol
12,13-EpOME	295.2	195	30	16	LA	Epoxide
9,10-EpOME	295.2	171	45	16	LA	Epoxide
13-OxoODE	293.2	113	15	22	LA	Ketone
9-OxoODE	293.2	185	27	16	LA	Ketone
LTD ₄	495.3	177	51	22	ArA	Leukotriene
Resolvin D ₁	375.2	141	21	10	DHA	Resolvin
LA	279.2	59	45	16		PUFA
ArA	303.2	259	21	10		PUFA
Eicosapentaenoic acid	301.2	257	39	10		PUFA
Docosahexaenoic acid	327.2	283	51	10		PUFA
Stearic acid	283	283	33	22		SFA
Oleic acid	281	281	45	22		MUFA
Palmitic acid	255	255	33	22		SFA
PGE _{2-d9}	360.2	280	21	16		
8,9-DHET- <i>d</i> ₁₁	348.3	127	55	22		
15-HETE- <i>d</i> ₈	327.2	226	15	10		
8,9-EET- <i>d</i> ₁₁	330.3	268	15	10		
AEA- <i>d</i> ₈	356.3	63	33	10		
2-AG- <i>d</i> ₈	387.3	294	21	16		
γ-Linoleic acid	277.2	233	27	10		
AA- <i>d</i> ₈	311.2	267	21	10		
5-HETE- <i>d</i> ₈	327.3	116	55	16		
7-Maresin1	359.2	177	51	16	DHA	

¹HHTrE = hydroxyheptadecatrienoic acid; HETE = hydroxyeicosatetraenoic acid; DHET = dihydroxyeicosatrienoic acid; AEA = arachidonoyl ethanolamide; AG = arachidonoyl glycerol; EET = epoxyeicosatrienoic acid; PG = prostaglandin; LTB₄ = leukotriene B₄; oxoETE = oxoeicosatetraenoic acid; 15d-PGJ₂ = 15-deoxyprostaglandin J₂; TXB₂ = thromboxane B₂; HDoHE = hydroxydocosahexaenoic acid; DiHDPA = dihydroxydocosapentaenoic acid; DiHDoHE = dihydroxydocosahexaenoic acid; 7,17-dh-DPA = 7,17-dihydroxydocosapentaenoic acid; DiHETE = dihydroxyeicosatrienoic acid; EpETE = epoxyeicosatetraenoic acid; HODE = hydroxyoctadecadienoic acid; DiHOME = dihydroxyoctadecenoic acid; EpOME = epoxyoctadecenoic acid; oxoODE = oxooctadecadienoic acid; LTD₄ = leukotriene D₄; PGE_{2-d9} = prostaglandin E₂-d₉; 8,9-DHET-*d*₁₁ = dihydroxyeicosatrienoic-d₁₁ acid; 15-HETE-*d*₈ = hydroxyeicosatetraenoic-d₈ acid; 8,9-EET-*d*₁₁ = epoxyeicosatrienoic-d₁₁ acid; AEA-*d*₈ = arachidonoyl ethanolamide-d₈; 2-AG-*d*₈ = 2-arachidonoyl glycerol-d₈; AA-*d*₈ = arachidonic acid-d₈; 5-HETE-*d*₈ = hydroxyeicosatetraenoic-d₈ acid.

²ArA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid.