Oxylipids are associated with higher disease risk in postpartum cows

A. K. Putman,* J. C. Gandy, G. A. Contreras, and L. M. Sordillo

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824

ABSTRACT

Postpartum diseases are a major animal welfare and economic concern for dairy producers. Dysregulated inflammation, which may begin as soon as the cessation of lactation, contributes to the development of postpartum diseases. The ability to regulate inflammation and mitigate postpartum health diseases relies, in part, on the production of inflammatory mediators known as oxylipids. The objective of this study was to examine associations between oxylipids and postpartum diseases. Plasma samples were collected from 16 cattle via coccygeal venipuncture at the following time points: 6 d before dry-off; dry-off (d 0); 1, 2, 6, and 12 d after dry-off; 14 ± 3 d before the expected calving date; and 7 ± 2 d after calving. After calving, cows were grouped according to if clinical disease was undetected throughout the sampling period ($n = 7$) or if they developed a disease postpartum ($n = 9$). Liquid chromatography-tandem mass spectrometry was used to analyze plasma concentrations of 63 oxylipid species. Of the 32 oxylipids detected, concentrations of 7 differed between cows with no detected disease and diseased cows throughout the sampling period. Thus, a variable oxylipid profile was demonstrated through 2 major physiological transitions of a lactation cycle. Further, the information gained from this pilot study using a small number of animals with diverse diseases from a single herd suggests that it may be possible to use oxylipids at early mammary involution to alert dairy producers of cows at risk for disease after calving. Future studies should be performed in larger populations of animals, including cows from diverse geographies and dairying styles, and focus on specific diseases to evaluate the utility of oxylipids as biomarkers. Furthermore, it is important to determine the clinical implications of variable oxylipid concentrations throughout the lactation cycle and if the oxylipid profile can be modulated to improve inflammatory outcomes.

Key words: dry cow, postpartum cow, oxylipid, inflammation

INTRODUCTION

Periparturient diseases, which include numerous metabolic and infectious diseases such as ketosis, displaced abomasum, mastitis, and metritis, cause severe animal welfare concerns and economic losses for dairy producers. The incidence and severity of periparturient diseases is associated with dysregulated inflammation (Sordillo and Raphael 2013). Specific physiological circumstances during the periparturient period, such as uterine involution and adipose tissue remodeling, invoke inflammation without the presence of a pathogen (Chapwanya et al., 2012; Contreras et al., 2017). To avoid periparturient diseases, dairy cows rely on a tightly regulated inflammatory response that is robust enough to promote tissue healing or pathogen elimination but not to an extent that tissue damage occurs (Sordillo, 2016). If inflammation is attenuated, such as when neutrophil function is diminished around the time of calving, it is more likely for diseases such as mastitis and metritis to occur (Cai et al., 1994; Sordillo, 2016). On the other hand, if inflammation is excessive or prolonged, substantial tissue damage may occur as exemplified during certain bovine mastitis cases where upregulation of proinflammatory substances and increased leukocyte infiltration can lead to apoptosis and mammary gland damage (Aitken et al., 2011). Navigating the periparturient period successfully necessitates tight control of every aspect of inflammation from its initiation to its termination, a process facilitated by numerous soluble mediators (Sordillo, 2018).

Oxylipids are potent soluble inflammatory mediators capable of regulating the onset, progression, and resolution of inflammation (Mavangira and Sordillo, 2018). Oxylipids are oxidized products of PUFA and the substrate they are generated from largely determines their biological action during inflammation (Figure 1). Oxylipids derived from omega-6 (n-6) PUFA, such as arachidonic acid, are most commonly associated with proinflammatory actions. In contrast, oxylipids derived from omega-3 (n-3) PUFA, including eicosapentaenoic acid and docosahexaenoic acid, tend to exert

Received July 27, 2021.

Accepted November 27, 2021.

*Corresponding author: putmanas@msu.edu

anti-inflammatory effects (Innes and Calder, 2018). For example, prostaglandin (**PG**) E_2 is a metabolite of arachidonic acid with well-documented proinflammatory effects such as mast cell degranulation and increased vascular permeability (Morimoto et al., 2014). The docosa-hexaenoic acid-derived 17-hydroxydocosa-hexaenoic acid (**17-HDoHE**) is a precursor to inflammation-resolving metabolites and has been associated with decreased nuclear factor kappa B and proinflammatory cytokine expression in models of adipose tissue inflammation (Neuhofer et al., 2013). The biological action of oxylipids also depends on the pathway with which they are produced. Enzymatic production occurs through cyclooxygenase (**COX**), lipoxygenase (**LOX**), and cytochrome P450 pathways, whereas nonenzymatic production occurs via mechanisms mediated by free radicals (e.g., hydroxyl radical, $OH^\bullet$; Mavangira and Sordillo, 2018). Reactions with free radicals form specialized oxylipids known as isoprostanes (**IsoP**), which are highly sensitive and specific biomarkers of lipid peroxidation (Milne et al., 2015). Given the diverse substrates and pathways available for biosynthesis, hundreds of oxylipids have been identified to date, each with its own unique but interrelated effect on inflammation (Wang et al., 2014). Thus, it is not merely the presence of any particular oxylipid but rather the relative abundance, potency, and timing of production of all oxylipids that influences inflammatory outcomes (Kuhn et al., 2017).

Although the dysregulated inflammation that occurs around the time of calving is recognized to contribute to risk of periparturient diseases, the early dry period is often overlooked. However, many of the factors that underlie clinical illnesses around parturition may begin weeks earlier when lactation is abruptly ceased, such as inflammation resulting from mammary involution (Putman et al., 2018; Wisnieski et al., 2019). For instance, previous work demonstrates that the oxylipid profile of clinically healthy dairy cattle is variable throughout the first 2 wk of the dry period (Putman et al., 2019). However, the potential of measuring oxylipids shortly after the day of dry-off to assess disease risk after calving has yet to be investigated. Therefore, this study aimed to characterize the oxylipid profile from the early dry period through early lactation in both cattle without detected disease and those that developed periparturient diseases.

MATERIALS AND METHODS

Animals

This study was approved by the Michigan State University Institutional Animal Care and Use Committee. Holstein cows from a commercial dairy herd

were free from clinical disease at enrollment, had SCC $<250 \times 10^3$ cells/mL at their last DHIA test date and were enrolled 56 d before expected calving date with owner consent. All cows were housed in a freestall barn, grouped according to stage of lactation, and lactating cows were milked 2 times/d. At the time of dry-off, cows were treated with intramammary cephalixin benzathine (Tomorrow, Boehringer Ingelheim) in all milking quarters. Average milk production at enrollment was ascertained from the last DHIA test day and was 32.5 kg/d (range: 17.8–40.4 kg/d), with the average DIM being 318 (range: 291–357). The average BCS at dry-off was 3.4 ± 0.3 out of 5 (range: 3–4; $\pm$ SD), and average parity was 2 (range 1–4). Cows had ad libitum access to a TMR and water. Animals were fed diets based on their lactation status (Table 1). Briefly, cows sampled at d –6 were fed a late-lactation diet, those sampled at d 0, 1, 2, 6, and 12 were fed a far-off dry cow diet, those in the close-up dry period were fed a close-up diet, and finally, animals sampled after calving were fed a fresh cow diet. After calving, herd records were inspected and cows were grouped into those without detectable clinical disease ($n = 7$) or those with detected disease ($n = 9$) as determined by trained farm staff. Mastitis was diagnosed in 3 cows due to abnormal milk with or without one or more of the following: redness and swelling of the udder, decreased appetite, depressed attitude, or rectal temperature greater than 39°C . The mastitis cows were diagnosed at 9, 18, and 16 DIM. Metritis was diagnosed in 1 cow (11 DIM) based on the presence of malodorous uterine discharge and a fluid-filled uterus on rectal palpation within 21 d of calving, with or without decreased appetite, a rectal temperature greater than 39°C , and depressed attitude. One cow (11 DIM) developed subclinical hypocalcemia, defined as a serum calcium concentration of <8.4 mg/dL, whereas the development of clinical hypocalcemia would have been defined as a serum calcium concentration of <8.4 mg/dL accompanied by muscle weakness, muscle shaking, or inability to rise. Lameness was detected in 1 cow (39 DIM), being diagnosed based on abnormal gait. Displaced abomasum was diagnosed based on auscultation of a characteristic ping in the region between the ninth rib and flank and was seen in 1 cow (8 DIM) of the present study. Finally, 2 cows displayed retained placenta (recorded at 7 and 10 DIM), which was diagnosed when the placenta had not been expelled within 24 h of calving. Other postpartum diseases, such as ketosis, were not diagnosed by farm staff in the present study. Each cow was only diagnosed with 1 disease during the study period according to farm records. For the remainder of the manuscript, cows where clinical disease was not detected after calving may be referred to as the apparently healthy (**AH**) group, whereas

Figure 1. Formation of the oxylipids detected in the present study. (A) Free radicals interact directly with PUFA in lipid membranes to form esterified isoprostanes, which are then released by phospholipases to generate free (unesterified) isoprostanes. (B) Phospholipases release PUFA from lipid membranes, which can then undergo enzymatic oxidation to produce a variety of oxylipid species. HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; DiHOME = dihydroxyoctadecadienoic acid; EpOME = epoxyoctadecadienoic acid; PG = prostaglandin; TX = thromboxane; HETE = hydroxyeicosatetraenoic acid; oxoETE = oxoeicosatetraenoic acid; HHTrE = hydroxyheptadecatrienoic acid; EET = epoxyeicosatrienoic acid; DHET = dihydroxyeicosatrienoic acid; 8-iso-PGA₂ = 8-isoprostane-PGA₂; 8,12-iso-iPF_{2α}-VI = 8,12-iso-isoprostane-F_{2α}-VI; 5-iPF_{2α}-VI = 5-iso prostaglandin F_{2α}-VI; DiHETE = dihydroxyeicosatetraenoic acid; HDoHE = hydroxydocosahexaenoic acid; DiHDPA = dihydroxydocosapentaenoic acid; EpDPE = epoxydocosapentaenoic acid.

those that developed clinical disease postpartum may be referred to as the clinical disease (CD) group.

Sample Collection and Processing

All samples were collected between the summer and fall of 2018. Sampling occurred at d -6, 0, 1, 2, 6, and 12 relative to the dry-off date, along with a sample 14 ± 3 d before the expected calving date (CU) and 7 ± 2 d after calving (C+7). Blood samples were collected aseptically into EDTA-containing Vacutainer tubes

via coccygeal venipuncture between 0800 and 1000 h. As lipid-containing biological samples are susceptible to ex vivo peroxidation, a mixture of 50% methanol, 25% ethanol, 25% water, 0.9 mM of butylated hydroxytoluene, 0.54 mM EDTA, 3.2 mM triphenylphosphine, and 5.6 mM indomethacin was added to each blood tube (10 µL/mL of blood) immediately following venipuncture (Morrow et al., 1990; O'Donnell et al., 2009). Samples were immediately stored on ice during transportation and processing. Upon returning to the laboratory, blood samples were centrifuged at 1,449 ×

g for 15 min at 4°C, and plasma was subsequently harvested, aliquoted, and flash-frozen in liquid nitrogen. Plasma samples were stored at −80°C pending analysis via liquid chromatography-tandem mass spectrometry (LC-MS/MS) within 1 mo of collection.

Targeted Lipidomics

Plasma was prepared for LC-MS/MS as described in Mavangira et al. (2015). Briefly, 1 mL of flash-frozen plasma was thawed on ice, diluted with 1 mL of 4% formic acid, and mixed with the antioxidant reducing agent listed above at 4 µL/mL to prevent degradation of preformed oxylipids and ex vivo lipid peroxidation (O'Donnell et al., 2009). Samples were combined with a 15-µL mixture of internal standards containing 0.25 µM 5(S)-HETE-*d*₈, 0.25 µM 15(S)-HETE-*d*₈, 0.5 µM 8(9)-EET-*d*₁₁, 0.5 µM PGE₂-*d*₉, and 0.25 µM 8,9-DHET-*d*₁₁. Solid phase extraction utilizing Waters Oasis Prime HLB 3-cc (150-mg) columns (Waters Corp.) was performed. Supernatants were loaded into the columns and pushed through with nitrogen. The columns were then washed with 3 mL of 5% methanol. Samples were eluted with 2.5 mL of 90:10 acetonitrile:methanol. Volatile solvents were removed using a Savant SpeedVac (Thermo Fisher Scientific) and residues were reconstituted in methanol, mixed at a 2:1 ratio with HPLC water and stored in chromatography vials at −80°C until analysis. A 6-point standard curve was created with a mix of standards and the internal standards mentioned above for quantification.

The LC-MS/MS protocol also has been reported previously by Mavangira et al. (2015). The quantification of oxylipid metabolites was accomplished on a Waters Xevo-TQ-S tandem quadrupole mass spectrometer using multiple reaction monitoring. Chromatography separation was performed with an Ascentis Express C18 HPLC column (Millipore Sigma) held at 50°C and autosampler held at 10°C. Mobile phase bottle A was water containing 0.1% acetic acid and mobile phase bottle B was acetonitrile with a flow rate of 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 8.01 min until 13.0 min; then returned to (99:1) at 13.01 min, and held at this condition until 15.0 min. Data analysis was performed by generating 6-point linear curves with commercial standards (Cayman Chemical) in 5-fold dilutions ranging from 0.01 to 100 nM. The generated linear curves produced R² values of 0.99 with percent deviations of less than 100%.

Quantification of IsoP was accomplished with a Waters Xevo TQ-S tandem quadrupole mass spectrometer using multiple reaction monitoring. Chromatography separation was performed with a Waters Acquity UPLC utilizing a BEH C18 1.7 µm (2.1 × 150 mm) column, held at 50°C and autosampler held at 10°C. Mobile phase bottle A was 0.1% acetic acid, mobile phase bottle B was acetonitrile, and mobile phase bottle C was methanol. The flow rate was 0.3 mL/min. The gradient initial phase (A:B, 80:20) to 1 min,

Table 1. Late lactation, early dry, close-up, and early lactation diet compositions on a DM basis of animals used in this study

Item	Late lactation	Far-off dry	Close-up	Fresh
Ingredients	Canola Corn gluten HMSC ¹ Alfalfa Corn silage Mineral supplement	Straw Grass silage Corn silage Salt Mineral supplement	Dehulled soymeal Soybean hulls Wheat middlings Molasses Yeast culture Wheat straw Corn silage Salt Mineral supplement	Canola HMSC ¹ Alfalfa Corn silage Mineral supplement
NE _L (Mcal/kg)	1.5	1.15	1.28	1.29
CP (%)	19	11	13.31	13.1
Fat (%)	5.3	2.3	2.29	24.8
NFC (%)	38	17	27.86	21.82
Calcium (%)	0.75	0.51	0.5	0.55
Phosphorus (%)	0.69	0.36	0.34	0.32
Magnesium (%)	0.78	0.31	0.26	0.13
Potassium (%)	1.4	2.5	1.34	0.95
Sodium (%)	0.29	0.34	0.11	0.01
Chloride (%)	0.53	0.99	0.2	0.16
Sulfur (%)	0.3	0.18	0.2	0.16
Selenium (mg/kg)	6.2	23	0.09	
Vitamin E (IU/animal)	290	1,064	1,200	540

¹HMSC = high-moisture shelled corn.

changing to (A:B:C, 50:30:20) to 7 min, changing to (A:B:C, 1:80:19) to 7.01 min, changing back to initial phase and holding until 10 min. All oxylipids and IsoP were detected using electrospray ionization in negative-ion mode. Cone voltages and collision voltages were optimized for each analyte using Waters QuanOptimize software and data analysis was carried out with Waters MassLynx software, version 4.1.

Statistical Analysis

Sample size was calculated a priori with the equation $n_i = 2 \left(\frac{Z\sigma}{ES} \right)^2$ and suggested that 7 animals were needed in each group to detect differences (based on preliminary data of one of the most abundant oxylipids detected in cattle, 13-HODE; n_i = sample size, Z = Z-score for $\alpha = 0.05$, $\sigma = 0.94$, effect size = 1; Ott and Longnecker, 2010; Putman et al., 2019). Repeated measures linear mixed effects models were constructed using the PROC MIXED procedure in SAS 9.4. (SAS Institute Inc.) for analysis of oxylipid and IsoP data over the study period. Each oxylipid and IsoP was tested in a separate model that included the fixed effect of sampling point and a random intercept for cow to account for the dependence between samples taken from the same cow. A spatial covariance residual matrix was used to account for unequal spacing between time points. Potential confounders, such as variation that may occur due to variable diets of cows at different stages of the lactation cycle, BCS, environmental factors, or stress related to handling, were accounted for by sampling from only one farm and having the same individuals handle the cattle. Each sampling occurred during the same timeframe to account for any potential variation due to time of day samples were taken. Similar numbers of primiparous and multiparous cows were used to minimize any effect age may have on the results. Normality of residuals was visually assessed with Q-Q plots and histograms. Data that violated the normality assumption were transformed either by the log or square root function. Estimated least squares means were then back-transformed and presented as geometric means. Levene's test and graphs of predicted residuals were used to assess heteroscedasticity. Degrees of freedom were estimated using the Kenward-Roger approximation if heteroscedasticity was present. Differences in concentrations of oxylipids and IsoP over the sampling period and between groups at each sampling point were tested using multiple pairwise comparisons with a Bonferroni adjustment. Statistical significance for differences between groups over the entire sampling period was set at $P < 0.0016$ to adjust for family-wise error

rate due to running multiple models ($P = 0.05/32$). Statistical significance for differences between groups at each sampling point were determined based on adjusted P -values calculated by SAS in the linear mixed effects model. As such, concentrations were deemed significantly different if the adjusted P -value on the SAS output was less than 0.05.

RESULTS

Sixty-three oxylipid species were targeted in this study, of which 32 were detectable (Table 2). Sampling 16 cows at 8 time points resulted in a total of 128 observations for each oxylipid, presented here as least squares means and standard error of the mean. The concentrations of 25 oxylipids were affected by time (from d -6 to C+7) in both AH and CD cows ($P < 0.05$). Of those 25 oxylipids, concentrations of 7 were different between the AH and CD groups ($P < 0.05$). Tables 3, 4, 5, and 6 indicate the concentrations of oxylipids that did not differ between AH and CD cows. Furthermore, the greatest concentrations of any given

Table 2. Names and corresponding abbreviation for the lipids analyzed in this study

Lipid	Abbreviation
Thromboxane B ₂	TXB ₂
Prostaglandin D ₂	PGD ₂
Prostaglandin F _{2α}	PGF _{2α}
6-Keto-prostaglandin F _{1α}	6-keto-PGF _{1α}
5-Hydroxyeicosatetraenoic acid	5-HETE
5-Oxo-eicosatetraenoic acid	5-oxoETE
5,6-Dihydroxyeicosatetraenoic acid	5,6-DiHETE
8,9-Dihydroxyeicosatrienoic acid	8,9-DHET
8,9-Epoxyeicosatrienoic acid	8,9-EET
9,10-Epoxyoctadecenoic acid	9,10-EpOME
9,10-Dihydroxyoctadecenoic acid	9,10-DiHOME
9-Hydroxyeicosatetraenoic acid	9-HETE
9-Hydroxyoctadecadienoic acid	9-HODE
9-Oxo-octadecadienoic acid	9-oxoODE
11,12-Dihydroxyeicosatrienoic acid	11,12-DHET
12,13-Epoxyoctadecenoic acid	12,13-EpOME
12,13-Dihydroxyoctadecenoic acid	12,13-DiHOME
12-Hydroxyheptadecatrienoic acid	12-HHTrE
13-Hydroxyoctadecadienoic acid	13-HODE
13-Oxo-octadecadienoic acid	13-oxoODE
14,15-Dihydroxyeicosatrienoic acid	14,15-DHET
14,15-Dihydroxyeicosatetraenoic acid	14,15-DiHETE
14,15-Epoxyeicosatrienoic acid	14,15-EET
15-Hydroxyeicosatetraenoic acid	15-HETE
15-Oxo-eicosatetraenoic acid	15-oxoETE
17,18-Dihydroxyeicosatetraenoic acid	17,18-DiHETE
17-Hydroxydocosahexaenoic acid	17-HDoHE
19,20-Dihydroxydocosapentaenoic acid	19,20-DiHDPA
19,20-Epoxydocosapentaenoic acid	19,20-EpDPE
20-Hydroxyeicosatetraenoic acid	20-HETE
8-iso-Prostaglandin A ₂	8-isoPGA ₂
8,12-iso-Isoprostane-F _{2α} -VI	8,12-iso-iPF _{2α} -VI
5-iso Prostaglandin F _{2α} -VI	5-iPF _{2α} -VI

Table 3. Mean plasma concentrations of cyclooxygenase-derived oxylipids in apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$) from 6 d before dry-off (d -6) to 7 d after calving (C+7); CU = sample taken 14 ± 3 d before expected calving date¹

Oxylipid ² (nM)	Sampling point							SE	<i>P</i> -value
	d -6	d 0	d 1	d 2	d 6	d 12	CU		
TXB ₂									0.08
Apparently healthy	0.75	0.91	1.62	1.92	0.77	0.78	3.81	1.08	0.05
Diseased	1.79	1.81	1.76	1.83	2.57	1.69	2.19	1.26	0.04
6-keto-PGF _{1α}									0.75
Apparently healthy	0.21	0.09	0.16	1.32	0.19	0.18	0.27	0.19	0.02
Diseased	0.19	0.44	0.38	0.27	0.36	0.23	0.33	0.17	0.01
PGD ₂									0.0001
Apparently healthy	0.12	0.13	0.27	0.22	0.14	0.08	0.28	0.06	0.006
Diseased	0.18	0.48	0.57	0.34	0.58	0.3	0.39	0.39	0.004
PGF _{2α}									0.001
Apparently healthy	0.16	0.2	0.19	0.24	0.16	0.27	0.54	0.17	0.01
Diseased	0.52	0.83	0.55	0.41	0.6	0.31	0.33	0.9	0.008
12-HHTrE									0.53
Apparently healthy	0.21	0.38	0.42	0.54	0.32	0.26	0.75	0.49	0.01
Diseased	0.5	0.55	0.41	0.38	0.64	0.29	0.53	0.36	0.01

¹Standard error and *P*-values listed for each row represents the SE and *P*-value for the treatment effect of undetected disease versus detected disease in the linear mixed model. After Bonferroni's correction to account for family-wise error, statistical significance was set at $P < 0.001$. Cyclooxygenase-derived oxylipids that differed between groups are also shown in Figure 3.

²TX = thromboxane; PG = prostaglandin; HHTrE = hydroxyheptadecatrienoic acid. Data log or square root transformed. Back-transformed values shown.

Table 4. Mean plasma concentrations of lipoxygenase-derived oxylipids in apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$) from 6 d before dry-off (d -6) to 7 d after calving (C+7); CU = sample taken 14 ± 3 d before expected calving date¹

Oxylipid ² (nM)	Sampling point							SE	<i>P</i> -value
	d -6	d 0	d +1	d +2	d +6	d +12	CU		
5-HETE ³									0.23
Apparently healthy	1.78	1.53	3.47	2.22	1.3	1.25	1.14	1.68	1.27
Diseased	2.09	2.04	2.92	2.37	2.13	2.07	1.17	2.11	1.24
5-oxoETE									0.49
Apparently healthy	0.09	0.1	0.14	0.11	0.12	0.11	0.16	0.16	0.03
Diseased	0.09	0.07	0.14	0.11	0.12	0.13	0.12	0.11	0.02
9-HODE ³									0.0003
Apparently healthy	10	3.5	4.9	4.2	5.8	3.2	2.4	6.1	1.2
Diseased	6.8	7.5	8.6	6.8	7.2	6.7	4.9	12	1.18
9-oxoODE ³									0.0001
Apparently healthy	8.5	6.8	7.3	11	8.0	5.9	5.3	15	0.05
Diseased	3.0	3.3	3.0	2.6	2.6	2.4	2.2	8.6	0.04
13-HODE ³									0.007
Apparently healthy	8.25	9.64	8.75	8.07	7.84	6.51	5.48	12.6	1.1
Diseased	11.6	12.6	10.6	8.78	9.86	8.69	7.66	16.3	1.1
13-oxoODE ³									0.0001
Apparently healthy	0.49	0.47	0.4	0.75	0.6	0.36	0.37	0.29	0.01
Diseased	4.8	4.0	5.4	6.4	6.8	5.2	4.0	4.4	0.009
15-HETE ³									0.4
Apparently healthy	1.5	1.81	1.97	2.3	1.69	1.61	1.83	2.38	1.21
Diseased	2	2.22	2.16	1.79	2.17	2.13	1.77	2.44	1.17
17-HDoHE									0.03
Apparently healthy	1.54	1.38	1.82	1.55	1.54	1.35	1.4	1.58	0.13
Diseased	1.65	1.77	1.81	1.62	1.8	1.72	1.58	1.84	0.11

¹Standard error and *P*-values listed for each row represents the SE and *P*-value for the treatment effect of undetected disease versus detected disease in the linear mixed model. After Bonferroni's correction to account for family-wise error, statistical significance was set at $P < 0.001$. Lipoxygenase-derived oxylipids that differed between groups are also shown in Figure 3.

²HETE = hydroxyeicosatetraenoic acid; oxoETE = oxoeicosatetraenoic acid; HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; HDoHE = hydroxydocosahexaenoic acid.

³Data log or square root transformed. Back-transformed values shown.

oxylipid were often not seen during the same sampling point between AH and CD animals (Figure 2).

The blood concentrations of COX-derived of TXB₂, 6-keto-PGF_{1α}, and 12-HHTrE are listed in Table 3.

Cyclooxygenase-derived PGD₂ and PGF_{2α}, differed between AH and CD cows (Figure 3; $P < 0.001$). Apparently healthy cows had the greatest concentrations at CU (PGD₂ = 0.28 nM; PGF_{2α} = 0.54 nM), whereas

Table 5. Mean plasma concentrations of cytochrome P450-derived oxylipids in apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$) from 6 d before dry-off (d -6) to 7 d after calving (C+7); CU = sample taken 14 ± 3 d before expected calving date¹

Oxylipid ² (nM)	Sampling point							SE	<i>P</i> -value
	d -6	d 0	d 1	d 2	d 6	d 12	CU		
5,6-DiHETE ³									0.94
Apparently healthy	3.82	6.21	39.1	8.15	5.86	5.44	6.01	5.18	1.4
Diseased	4.61	5.45	14.9	14.7	9.64	9.27	5.35	2.41	1.34
8,9-EET ³									0.59
Apparently healthy	0.05	0.06	0.29	0.1	0.1	0.09	0.09	0.19	0.003
Diseased	0.08	0.04	0.17	0.14	0.1	0.15	0.02	0.13	0.003
8,9-DHET ³									0.34
Apparently healthy	0.45	0.49	0.87	0.68	0.6	0.56	0.54	0.62	0.01
Diseased	0.52	0.63	0.75	0.72	0.79	0.84	0.6	0.71	0.004
9,10-EpOME ³									0.49
Apparently healthy	0.13	0.11	0.24	0.13	0.14	0.1	0.13	0.23	0.001
Diseased	0.12	0.13	0.15	0.11	0.11	0.11	0.12	0.26	0.001
9,10-DiHOME ³									0.004
Apparently healthy	31.4	29.6	13	9.29	8.79	6.38	12.9	59	1.14
Diseased	20.8	20	8.55	6.88	6.3	5.51	8.09	33	1.16
9-HETE ³									0.22
Apparently healthy	0.22	0.12	0.36	0.14	0.22	0.2	0.14	0.23	0.02
Diseased	0.22	0.39	0.37	0.32	0.19	0.27	0.27	0.33	0.01
11,12-DHET ³									0.14
Apparently healthy	1.84	2.19	2.7	2.02	2.26	2.37	2.31	2.68	1.15
Diseased	2.38	2.47	2.92	2.96	3.1	3.69	2.58	2.96	1.13
12,13-EpOME ³									0.14
Apparently healthy	5.72	6.3	5.08	5.33	3.49	3.06	3.81	13.3	0.03
Diseased	6.9	7.45	5.72	4.55	4.59	4.15	6.88	16.7	0.02
12,13-DiHOME ³									0.0001
Apparently healthy	1.2	1.6	1.3	0.85	0.76	0.61	1.8	4.4	1.4
Diseased	0.2	0.21	0.13	0.16	0.14	0.13	0.11	0.73	1.3
14,15-EET ³									0.39
Apparently healthy	0.11	0.08	0.36	0.19	0.15	0.17	0.14	0.2	0.002
Diseased	0.15	0.12	0.27	0.21	0.24	0.21	0.1	0.23	0.002
14,15-DHET ³									0.2
Apparently healthy	2.88	3.55	5.19	3.77	3.72	3.87	4.0	4.33	1.12
Diseased	3.6	3.83	4.93	4.62	4.48	5.23	4.1	4.57	1.1
14,15-DiHETE									0.4
Apparently healthy	6.41	7.46	9.41	9.45	9.53	8.31	7.81	7.86	1.24
Diseased	7.38	7.83	10.2	9.94	10.8	10.5	8.69	7.5	1.1
17,18-DiHETE									0.16
Apparently healthy	65.9	72.3	132	82.4	83.8	73	67	61.2	14.7
Diseased	83.9	85.6	114	108	126	125	84.6	59.9	13
19,20-DiHDPA ³									0.19
Apparently healthy	0.94	1.16	1.88	1.12	1.43	1.65	1.9	1.9	0.02
Diseased	1.9	1.85	2.39	1.65	2.06	1.36	2.65	1.87	0.01
19,20-EpDPE ³									0.33
Apparently healthy	1.53	1.67	1.97	1.9	2.15	1.69	1.69	1.79	0.01
Diseased	1.59	1.73	1.68	1.63	1.49	1.84	1.49	1.76	0.004
20-HETE ³									0.69
Apparently healthy	1.15	1.52	5.77	3.72	3.39	3.31	2.79	3.65	0.1
Diseased	1.69	1.64	2.92	2.8	2.92	3.56	1.61	4.58	0.08

¹Standard error and *P*-values listed for each row represents the SE and *P*-value for the treatment effect of undetected disease versus detected disease in the linear mixed model. After Bonferroni's correction to account for family-wise error, statistical significance was set at $P < 0.001$. Cytochrome P450-derived oxylipids that differed between groups are also shown in Figure 3.

²DiHETE = dihydroxyeicosatetraenoic acid; EET = epoxyeicosatrienoic acid; DHET = dihydroxyeicosatrienoic acid; EpOME = epoxyoctadecenoic acid; DiHOME = dihydroxyoctadecenoic acid; HETE = hydroxyeicosatetraenoic acid; DiHDPA = dihydroxydocosapentaenoic acid; EpDPE = epoxydocosapentaenoic acid; HETE = hydroxyeicosatetraenoic acid.

³Data log or square root transformed. Back-transformed values shown.

Table 6. Mean plasma concentrations of isoprostanes, nonenzymatically derived oxylipids, in apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$) from 6 d before dry-off (d -6) to 7 d after calving (C+7); CU = sample taken 14 ± 3 d before expected calving date¹

Oxylipid ² (nM)	Sampling point						CU	C +7	SE	P-value
	d -6	d 0	d +1	d +2	d +6	d +12				
8-iso-PGA ₂										0.0001
Apparently healthy	0.26	0.29	0.37	0.37	0.4	0.56	0.73	0.16	0.004	
Diseased	0.77	0.68	1.2	1.1	1.5	1.9	2.1	0.36	0.003	
8,12-iso-iPF _{2α} -VI										0.04
Apparently healthy	0.3	0.26	0.3	0.19	0.19	0.17	0.23	0.21	0.05	
Diseased	0.39	0.41	0.3	0.24	0.24	0.17	0.29	0.33	0.04	
5-iPF _{2α} -VI										0.04
Apparently healthy	0.09	0.07	0.09	0.07	0.07	0.06	0.06	0.06	0.02	
Diseased	0.14	0.13	0.13	0.13	0.04	0.09	0.06	0.16	0.02	

¹Standard error and *P*-values listed for each row represents the SE and *P*-value for the treatment effect of undetected disease versus detected disease in the linear mixed model. After Bonferroni's correction to account for family-wise error, statistical significance was set at $P < 0.001$. Isoprostanes that differed between groups are also shown in Figure 3.

²8-iso-PGA₂ = 8-iso-prostaglandin A₂; 8,12-iso-iPF_{2α}-VI = 8,12-iso-isoprostane-F_{2α}-VI; 5-iPF_{2α}-VI = 5-iso prostaglandin F_{2α}-VI.

CD cows had peak concentrations of PGD₂ at d +6 (0.58 nM) and PGF_{2α} at C +7 (0.9 nM).

Products of the LOX pathway are listed in Table 4. Out of 8 LOX-derived oxylipids, the concentrations of 3 differed between AH and CD animals throughout the sampling period (Figure 3; $P < 0.001$). Throughout the sampling period, peak concentrations of 9-HODE and its ketone derivative 9-oxoODE were greatest in the CD group at C +7 (12.5 nM and 8.59 nM, respectively). Concentrations of 9-HODE were greater in the CD group than the AH group at every sampling point except d -6. The opposite was true of 9-oxoODE in that concentrations were consistently greater in the AH group throughout the study. In contrast, the concentrations of 13-oxoODE were greater at every sampling point in CD animals compared with AH animals (Figure 3; $P < 0.001$).

Sixteen cytochrome P450-derived oxylipids were detected in this study, of which only 12,13-DiHOME concentrations differed between the AH and CD group over the study period (Table 5). Peak concentrations of 12,13-DiHOME were reached at C +7 for both groups, with greater concentrations seen in the AH cows (Figure 3; $P < 0.0001$).

Isoprostanes are a specialized oxylipid produced via a nonenzymatic mechanism. Although all 3 detected IsoP showed differences between AH and CD animals, only 8-iso-PGA₂ remained significant after accounting for family-wise error. Nonetheless, concentrations of IsoP were most commonly numerically or significantly greater in the CD group compared with AH cows. For 8-iso-PGA₂ in both groups, concentrations steadily rose from the early dry period to CU, where greatest concentrations were noted (AH = 0.73 nM; CD = 2.13 nM), before decreasing to the smallest concentrations of the study at C+7 (AH = 0.16 nM; CD = 0.36 nM).

DISCUSSION

Differences in oxylipid profiles between groups of the present study were detected weeks before the development of clinical disease and implies that oxylipids measured during early mammary involution may be useful in assessing risk of postpartum diseases. Currently, periparturient disease risk assessment uses biomarkers such as nonesterified fatty acids and BHB. These biomarkers are generally measured within 2 wk before or after calving (Ospina et al., 2010). Assessing disease risk near the time of calving often does not leave sufficient time for successful interventions because disease processes may be too advanced at that stage (Wisnieski et al., 2019). More recent work suggests that biomarkers of other contributors to disease risk, such as inflammation, strengthen the ability to predict disease around the time of calving. Measuring these biomarkers at cessation of lactation could effectively predict disease risk, potentially allowing more time to provide interventions to mitigate disease (Wisnieski et al., 2019). As oxylipids are intimately associated with dysregulated inflammation, it is reasonable to consider them for biomarker use to assess disease risk (Sordillo and Mavangira, 2014).

Recent studies identified specific oxylipids with potential as biomarkers for assessing disease risk. Ryman et al. (2015) reported that cows with *Streptococcus uberis* mastitis had increased concentrations of PGF_{2α} and 9-HODE in mammary tissue relative to healthy controls. In our study, the same oxylipids were increased in plasma of CD cows at most sampling points. Thus, PGF_{2α} and 9-HODE may be especially promising candidates for biomarker use as they are detected in several biological fluids and are associated with disease. Furthermore, PGF_{2α} and 9-HODE may be influencing

inflammatory outcomes via their biological actions. Well-described inflammatory roles of $\text{PGF}_{2\alpha}$ exist, such as stimulating COX-2 expression (Leimert et al., 2019). Expression of COX-2 is involved in inflammatory regulation, and it has been demonstrated that COX-2 is more active in milk leukocytes from bovine mammary quarters with mastitis compared with uninfected quarters (De and Mukherjee, 2014). However, the function of HODE is less clear (Leimert et al., 2019; Pecorelli et al., 2019). For instance, 9-HODE induced chemotaxis in bovine polymorphonuclear leukocytes in vitro but has also demonstrated decreased platelet adhesion in the vasculature (Henricks et al., 1991; Rolin and Maghazachi, 2014). Given the importance of $\text{PGF}_{2\alpha}$ and 9-HODE to leukocyte function during inflamma-

tion, future work should focus on the effect of these oxylipids in responses to environmental and contagious mastitis pathogens.

Among the oxylipids, IsoP may have the greatest potential as a biomarker. A specific isomer, 15- F_{2t} -IsoP, is currently considered the gold standard biomarker of in vivo oxidative stress in humans because it is highly specific for lipid peroxidation (Milne et al., 2015). Furthermore, 15- F_{2t} -IsoP has been established as a biomarker of several diseases in various species, including coliform mastitis in dairy cattle (Mavangira et al., 2016). Although we did not detect 15- F_{2t} -IsoP presently, we have provided evidence that other IsoP may be relevant as potential biomarkers of disease risk. One promising biomarker candidate may be 8-iso PGA_2 ,

	d -6	d 0	d 1	d 2	d 6	d 12	CU	C+7	Pathway
Cyclooxygenase									
Apparently healthy:				6-keto- $\text{PGF}_{1\alpha}$			TXB_2 12-HHTre PGD_2 $\text{PGF}_{2\alpha}$		
Diseased:		6-keto- $\text{PGF}_{1\alpha}$			TXB_2 12-HHTre PGD_2			$\text{PGF}_{2\alpha}$	
Lipoxygenase									
Apparently healthy:	9-HODE 13-HODE		5-HETE 17-HDoHE	13-oxoODE			5-oxoETE	5-oxoETE 15-HETE 9-oxoODE	
Diseased:			5-HETE 5-oxoETE		13-oxoODE			15-HETE 9-HODE 9-oxoODE 13-HODE 17-HDoHE	
Cytochrome P450									
Apparently healthy:			5, 6-DiHETE 8, 9-EET 8, 9-DHET 9, 10-EpOME 9-HETE 11, 12-DHET 14, 15-EET 14, 15-DHET 17, 18-DiHETE 20-HETE	14, 15-DiHETE 19, 20-EpDPE			19, 20-DiHDPA	12, 13-EpOME 19, 20-DiHDPA 9, 10-DiHOME 12, 13-DiHOME	
Diseased:		9-HETE	5, 6-DiHETE 8, 9-EET 14, 15-EET		14, 15-DiHETE 17, 18-DiHETE	8, 9-DHET 11, 12-DHET 14, 15-DHET 19, 20-EpDPE	19, 20-DiHDPA	9, 10-EpOME 12, 13-EpOME 20-HETE 9, 10-DiHOME 12, 13-DiHOME	
Non-enzymatic									
Apparently healthy:			8,12-iso- $\text{iPF}_{2\alpha}$ -VI 5- $\text{iPF}_{2\alpha}$ -VI				8-iso PGA_2		
Diseased:		8,12-iso- $\text{iPF}_{2\alpha}$ -VI					8-iso PGA_2	5- $\text{iPF}_{2\alpha}$ -VI	

Figure 2. Sampling point at which each oxylipid reached peak concentrations for apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$). Sampling points were as follows: d -6 = 6 d before dry-off; d 0 = dry-off; d 1, 2, 6, and 12 = 1, 2, 6, and 12 d after dry-off; CU = 14 ± 3 d before expected calving date; and C+7 = 7 ± 2 d postcalving. If an oxylipid is listed twice for the same group, peak concentrations were met at 2 sampling points.

Figure 3. Concentrations of oxylipids that differed between apparently healthy cows ($n = 7$) and those that developed a postpartum disease (diseased; $n = 9$) from 6 d before dry-off (d -6) to 7 d after calving (C+7). CU = 14 ± 3 d before expected calving date. (a) Cyclooxygenase (COX)-derived prostaglandin (PG) D₂ ($P < 0.0001$); (b) COX-derived PGF_{2α} ($P < 0.001$); (c) lipoxygenase (LOX)-derived 9-HODE ($P = 0.0003$); (d) LOX-derived 9-oxoODE ($P < 0.0001$); (e) LOX-derived 13-oxoODE ($P < 0.001$); (f) cytochrome P450-derived 12,13-DiHOME ($P < 0.0001$); (g) nonenzymatically derived 8-iso-PGA₂ ($P < 0.0001$). Data square root transformed; back-transformed values shown. After Bonferroni's correction to account for family-wise error, statistical significance between groups over the entire sampling period was set at $P < 0.001$. Error bars represent SE. *Pairwise differences between seemingly healthy and sick cows at a given sampling point after Bonferroni's correction for multiple comparisons (adjusted $P < 0.05$).

which is a stable metabolite of 8-iso-PGE₂. In environments replete of cellular reducing agents such as glutathione or α -tocopherol, 8-isoPGA₂ is made more readily than 15-F_{2t}-IsoP (Milne et al., 2011). Recent research demonstrated that α -tocopherol concentrations are decreased in dairy cattle around the time of calving relative to the day of dry-off, and therefore provides a plausible explanation for the increasing concentrations of 8-isoPGA₂ as calving approached but undetectable 15-F_{2t}-IsoP (Strickland et al., 2021). Another possible explanation is the use of updated analytical methods from previous studies in dairy cattle where antioxidant reducing agent was not added to the samples immediately after collection to prevent ex vivo peroxidation. As with other oxylipids, IsoP biosynthesis is complex and future studies are necessary to determine the implications of their presence or absence at varying times in the lactation cycle. Indeed, it would be beneficial to establish threshold concentrations of oxylipids that may indicate disease risk and determine when measurement should occur during the lactation cycle for best production outcomes.

Given that each oxylipid holds a unique function during inflammation, determining the relative abundance of several oxylipids rather than just measuring a single molecule may be best for assessing disease risk. As an example, downstream metabolites of oxylipids often have differing potencies and actions than the parent compound they are derived from. Although 9-HODE is a potent natural ligand for receptors such as peroxisome proliferator-activated receptor gamma, its metabolite 9-oxoODE is less potent and exerts an anti-inflammatory effect (Patwardhan et al., 2009; Vangaveti et al., 2010). In contrast, the metabolites of Ep-OME (DiHOME) are far more potent than their parent oxylipid (Powell et al., 1993; Moghaddam et al., 1997). The differences of timing and abundance of secondary oxylipid production between the groups of cows in this study may explain why AH cows did not develop disease but the CD group did. For example, DiHOME are produced during oxidative burst in inflammatory cells, which is a crucial component of the innate immune defense against pathogens (Powell et al., 1994; Thompson and Hammock, 2007; Mosca et al., 2020). Deficits in neutrophil oxidative burst are associated with retained placenta, metritis, and mastitis in dairy cattle, all of which were diseases noted in the present study (Cai et al., 1994). Hence, elevated concentrations of 12,13-DiHOME at CU and C+7 in the AH group may suggest these cows have stronger oxidative burst toward pathogens at these times (Powell and Rokach, 2015). In fact, the combination of increased concentrations of 9-oxoODE and 12,13-DiHOME after calving may sug-

gest that the oxylipid profile of AH cows in this study supported more favorable inflammatory outcomes. Thus, it may be more beneficial to measure a collective profile of oxylipids compared with measuring a single molecule for biomarker use. However, additional studies are required to identify the ideal composite of oxylipids to optimize inflammation.

The present study is an initial characterization of the oxylipid profile in dairy cattle during the dry and periparturient periods and therefore included a limited number of animals with diverse postpartum diseases. The variety of disease conditions coupled with diagnosis at a wide range of DIM represents a heterogeneous array of inflammatory processes. Furthermore, the observational nature of this study precludes the ability to establish causal relationships. As such, future studies should be conducted with a larger population from different geographic regions and dairying styles to validate oxylipids as biomarkers of disease risk. It would also be beneficial to perform studies focusing on specific postpartum diseases.

CONCLUSIONS

This study documents changes in the oxylipid profile of dairy cattle from the start of mammary gland involution through the first 7 d of the subsequent lactation. The data presented herein supports those differences in oxylipid concentrations between cows that developed disease after calving and those that remained apparently healthy can be detected during early mammary involution. This work has important implications for the dairy industry, as it suggests that factors contributing to disease after calving may begin as soon as the early dry period. Moreover, a collective measurement of the oxylipid pool during early mammary involution may represent a valuable diagnostic biomarker for postpartum diseases. Further elaboration of associations between oxylipid concentrations and specific postpartum diseases is warranted. Longer-term, it would be beneficial to determine if implementing interventions (e.g., dietary supplementation) at early mammary gland involution would mitigate the development of disease after calving via the alteration of oxylipid profiles.

ACKNOWLEDGMENTS

The authors acknowledge the entities that funded this project, including the Agriculture and Food Research Initiative Competitive Grants Program (2014-68004-21972 and 2017-67015-26676) from the USDA National Institute of Food and Agriculture (NIFA; Washington, DC), an endowment from the Matilda R. Wilson Fund

(Detroit, MI), and the Michigan Alliance for Animal Agriculture (Michigan State University, East Lansing). This material also is based upon work supported by the USDA NIFA under award number 2017-38420-26759. Jennifer Brown (Duke University, Durham, NC) provided technical support and assistance with data interpretation. Lauren Wisnieski (Lincoln Memorial University, Harrogate, TN) consulted with the authors regarding statistical analyses. We also thank the Michigan State University Mass Spectrometry and Metabolomics Core for their assistance in liquid chromatography. The authors have not stated any conflicts of interest.

REFERENCES

- Aitken, S. L., C. M. Corl, and L. M. Sordillo. 2011. Immunopathology of mastitis: Insights into disease recognition and resolution. *J. Mammary Gland Biol. Neoplasia* 16:291–304. <https://doi.org/10.1007/s10911-011-9230-4>.
- Cai, T. Q., P. G. Weston, L. A. Lund, B. Brodie, D. J. McKenna, and W. C. Wagner. 1994. Association between neutrophil functions and periparturient disorders in cows. *Am. J. Vet. Res.* 55:934–943.
- Chapwanya, A., K. G. Meade, C. Foley, F. Narciandi, A. C. O. Evans, M. L. Doherty, J. J. Callanan, and C. O'Farrelly. 2012. The postpartum endometrial inflammatory response: A normal physiological event with potential implications for bovine fertility. *Reprod. Fertil. Dev.* 24:1028–1039. <https://doi.org/10.1071/RD11153>.
- Contreras, G. A., C. Strieder-Barboza, and W. Raphael. 2017. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. *J. Anim. Sci. Biotechnol.* 8:41. <https://doi.org/10.1186/s40104-017-0174-4>.
- De, U. K., and R. Mukherjee. 2014. Activity of cyclooxygenase-2 and nitric oxide in milk leucocytes following intramammary inoculation of a bio-response modifier during bovine staphylococcus aureus subclinical mastitis. *Vet. Res. Commun.* 38:201–207. <https://doi.org/10.1007/s11259-014-9604-3>.
- Henricks, P. A., F. Engels, H. van der Vliet, and F. P. Nijkamp. 1991. 9- and 13-hydroxy-linoleic acid possess chemotactic activity for bovine and human polymorphonuclear leukocytes. *Prostaglandins* 41:21–27. [https://doi.org/10.1016/0090-6980\(91\)90101-K](https://doi.org/10.1016/0090-6980(91)90101-K).
- Innes, J. K., and P. C. Calder. 2018. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot. Essent. Fatty Acids* 132:41–48. <https://doi.org/10.1016/j.plefa.2018.03.004>.
- Kuhn, M. J., V. Mavangira, J. C. Gandy, C. Zhang, A. D. Jones, and L. M. Sordillo. 2017. Differences in the oxylipid profiles of bovine milk and plasma at different stages of lactation. *J. Agric. Food Chem.* 65:4980–4988. <https://doi.org/10.1021/acs.jafc.7b01602>.
- Leimert, K. B., B. S. E. Verstraeten, A. Messer, R. Nemati, K. Blackadar, X. Fang, S. A. Robertson, S. Chemtob, and D. M. Olson. 2019. Cooperative effects of sequential PGF $_{2\alpha}$ and IL-1 β on IL-6 and COX-2 expression in human myometrial cells. *Biol. Reprod.* 100:1370–1385. <https://doi.org/10.1093/biolre/iox029>.
- Mavangira, V., J. C. Gandy, C. Zhang, V. E. Ryman, A. Daniel Jones, and L. M. Sordillo. 2015. Polyunsaturated fatty acids influence differential biosynthesis of oxylipids and other lipid mediators during bovine coliform mastitis. *J. Dairy Sci.* 98:6202–6215. <https://doi.org/10.3168/jds.2015-9570>.
- Mavangira, V., M. J. Mangual, J. C. Gandy, and L. M. Sordillo. 2016. 15-F $_{2t}$ Isoprostane concentrations and oxidant status in lactating dairy cattle with acute coliform mastitis. *J. Vet. Intern. Med.* 30:339–347. <https://doi.org/10.1111/jvim.13793>.
- Mavangira, V., and L. M. Sordillo. 2018. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. *Res. Vet. Sci.* 116:4–14. <https://doi.org/10.1016/j.rvsc.2017.08.002>.
- Milne, G. L., Q. Dai, and L. J. Roberts 2nd.. 2015. The isoprostanes—25 years later. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851:433–445. <https://doi.org/10.1016/j.bbalip.2014.10.007>.
- Milne, G. L., H. Yin, K. D. Hardy, S. S. Davies, and L. J. Roberts 2nd.. 2011. Isoprostane generation and function. *Chem. Rev.* 111:5973–5996. <https://doi.org/10.1021/cr200160h>.
- Moghaddam, M. F., D. F. Grant, J. M. Cheek, J. F. Greene, K. C. Williamson, and B. D. Hammock. 1997. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat. Med.* 3:562–566. <https://doi.org/10.1038/nm0597-562>.
- Morimoto, K., N. Shirata, Y. Taketomi, S. Tsuchiya, E. Segi-Nishida, T. Inazumi, K. Kabashima, S. Tanaka, M. Murakami, S. Narumiya, and Y. Sugimoto. 2014. Prostaglandin e $_{2}$ -ep $_{3}$ signaling induces inflammatory swelling by mast cell activation. *J. Immunol.* 192:1130–1137. <https://doi.org/10.4049/jimmunol.1300290>.
- Morrow, J. D., T. M. Harris, and L. J. Roberts 2nd.. 1990. Noncyclooxygenase oxidative formation of a series of novel prostaglandins: Analytical ramifications for measurement of eicosanoids. *Anal. Biochem.* 184:1–10. [https://doi.org/10.1016/0003-2697\(90\)90002-Q](https://doi.org/10.1016/0003-2697(90)90002-Q).
- Mosca, F., A. R. Trachtman, J. Hattab, G. Marruchella, and P. G. Tiscar. 2020. Effect of hydrogen peroxide on the oxidative burst of neutrophils in pigs and ruminants. *Vet. World* 13:1934–1939. <https://doi.org/10.14202/vetworld.2020.1934-1939>.
- Neuhof, A., M. Zeyda, D. Mascher, B. K. Itariu, I. Murano, L. Leitner, E. E. Hochbrugger, P. Fraisl, S. Cinti, C. N. Serhan, and T. M. Stulnig. 2013. Impaired local production of proresolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation. *Diabetes* 62:1945–1956. <https://doi.org/10.2337/db12-0828>.
- O'Donnell, V. B., B. Maskrey, and G. W. Taylor. 2009. Eicosanoids: Generation and detection in mammalian cells. *Methods Mol. Biol.* 462:5–23. https://doi.org/10.1007/978-1-60327-115-8_1.
- Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern united states: Critical thresholds for prediction of clinical diseases. *J. Dairy Sci.* 93:546–554. <https://doi.org/10.3168/jds.2009-2277>.
- Ott, L., and M. Longnecker. 2010. Chapter 5: Inferences about population central values. Pages 232–300 in *An Introduction to Statistical Methods and Data Analysis*. Cengage Learning.
- Patwardhan, A. M., P. E. Scotland, A. N. Akopian, and K. M. Hargreaves. 2009. Activation of TRPV1 in the spinal cord by oxidized linoleic acid metabolites contributes to inflammatory hyperalgesia. *Proc. Natl. Acad. Sci. USA* 106:18820–18824. <https://doi.org/10.1073/pnas.0905415106>.
- Pecorelli, A., C. Cervellati, V. Cordone, F. Amicarelli, J. Hayek, and G. Valacchi. 2019. 13-hode, 9-hode and ALOX15 as potential players in Rett syndrome OxInflammation. *Free Radic. Biol. Med.* 134:598–603. <https://doi.org/10.1016/j.freeradbiomed.2019.02.007>.
- Powell, W. S., S. Gravel, R. J. MacLeod, E. Mills, and M. Hashefi. 1993. Stimulation of human neutrophils by 5-oxo-6,8,11,14-eicosatetraenoic acid by a mechanism independent of the leukotriene B $_4$ receptor. *J. Biol. Chem.* 268:9280–9286. [https://doi.org/10.1016/S0021-9258\(18\)98347-X](https://doi.org/10.1016/S0021-9258(18)98347-X).
- Powell, W. S., F. Gravelle, and S. Gravel. 1994. Phorbol myristate acetate stimulates the formation of 5-oxo-6,8,11,14-eicosatetraenoic acid by human neutrophils by activating nadph oxidase. *J. Biol. Chem.* 269:25373–25380. [https://doi.org/10.1016/S0021-9258\(18\)47259-6](https://doi.org/10.1016/S0021-9258(18)47259-6).
- Powell, W. S., and J. Rokach. 2015. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (hetes) and oxoeicosatetraenoic acids (oxo-etes) derived from arachidonic acid. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851:340–355. <https://doi.org/10.1016/j.bbalip.2014.10.008>.
- Putman, A. K., J. L. Brown, J. C. Gandy, A. Abuelo, and L. M. Sordillo. 2019. Oxylipid profiles of dairy cattle vary throughout the transition into early mammary gland involution. *J. Dairy Sci.* 102:2481–2491. <https://doi.org/10.3168/jds.2018-15158>.

- Putman, A. K., J. L. Brown, J. C. Gandy, L. Wisnieski, and L. M. Sordillo. 2018. Changes in biomarkers of nutrient metabolism, inflammation, and oxidative stress in dairy cows during the transition into the early dry period. *J. Dairy Sci.* 101:9350–9359. <https://doi.org/10.3168/jds.2018-14591>.
- Rolin, J., and A. A. Maghazachi. 2014. Implications of chemokines, chemokine receptors, and inflammatory lipids in atherosclerosis. *J. Leukoc. Biol.* 95:575–585. <https://doi.org/10.1189/jlb.1113571>.
- Ryman, V. E., G. M. Pighetti, J. D. Lippolis, J. C. Gandy, C. M. Applegate, and L. M. Sordillo. 2015. Quantification of bovine oxylipids during intramammary *Streptococcus uberis* infection. *Prostaglandins Other Lipid Mediat.* 121(Pt B):207–217.
- Sordillo, L. M. 2016. Nutritional strategies to optimize dairy cattle immunity. *J. Dairy Sci.* 99:4967–4982. <https://doi.org/10.3168/jds.2015-10354>.
- Sordillo, L. M. 2018. Symposium review: Oxylipids and the regulation of bovine mammary inflammatory responses. *J. Dairy Sci.* 101:5629–5641.
- Sordillo, L. M., and V. Mavangira. 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. *Anim. Prod. Sci.* 54:1204–1214. <https://doi.org/10.1071/AN14503>.
- Sordillo, L. M., and W. Raphael. 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. *Vet. Clin. North Am. Food Anim. Pract.* 29:267–278. <https://doi.org/10.1016/j.cvfa.2013.03.002>.
- Strickland, J. M., L. Wisnieski, T. H. Herdt, and L. M. Sordillo. 2021. Serum retinol, beta-carotene, and alpha-tocopherol as biomarkers for disease risk and milk production in periparturient dairy cows. *J. Dairy Sci.* 104:915–927. <https://doi.org/10.3168/jds.2020-18693>.
- Thompson, D. A., and B. D. Hammock. 2007. Dihydroxyoctadecamonoenoate esters inhibit the neutrophil respiratory burst. *J. Biosci.* 32:279–291. <https://doi.org/10.1007/s12038-007-0028-x>.
- Vangaveti, V., B. T. Baune, and R. L. Kennedy. 2010. Hydroxyoctadecadienoic acids: Novel regulators of macrophage differentiation and atherogenesis. *Ther. Adv. Endocrinol. Metab.* 1:51–60. <https://doi.org/10.1177/2042018810375656>.
- Wang, Y., A. M. Armando, O. Quehenberger, C. Yan, and E. A. Dennis. 2014. Comprehensive ultra-performance liquid chromatographic separation and mass spectrometric analysis of eicosanoid metabolites in human samples. *J. Chromatogr. A* 1359:60–69. <https://doi.org/10.1016/j.chroma.2014.07.006>.
- Wisnieski, L., B. Norby, S. J. Pierce, T. Becker, J. C. Gandy, and L. M. Sordillo. 2019. Predictive models for early lactation diseases in transition dairy cattle at dry-off. *Prev. Vet. Med.* 163:68–78. <https://doi.org/10.1016/j.prevetmed.2018.12.014>.

ORCIDS

- A. K. Putman <https://orcid.org/0000-0002-5532-3427>
 J. C. Gandy <https://orcid.org/0000-0003-0558-9611>
 G. A. Contreras <https://orcid.org/0000-0003-4969-2178>
 L. M. Sordillo <https://orcid.org/0000-0001-8873-3134>