Bovine Metabolic Stress and the Generation of Inflammatory Lipid Mediators

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Michigan State University
Metabolic & Infectious Diseases in Periparturient Cows

- Displaced Abomasum
- Ketosis
- Mastitis
- Milk Fever
- Retained Fetal Membranes
- Metritis
- Lameness

Inflammatory Dysfunction
Inflammation
(Essential Innate Immune Response)

- Purposes of inflammation:
  - eliminate or neutralize source of injury
  - repairs damaged tissues
  - restores normal tissue function
  - complex network of factors
  - tightly regulated response
  - robust onset and timely resolution

“Balance is Essential”
Periparturient Inflammatory Dysfunction

**Immunopathogenesis**

► Inflammatory responses help disease progression

- Inability of local defenses to adequately detect and eliminate pathogen (immune evasion)
- Uncontrolled recruitment and activation of inflammatory response
- Delicate balance of robust response and inflammatory resolution mechanisms is lost
- Increased metabolic and infectious disease severity

*Metabolic Stress Triad is the Common Link*
The nexus between nutrient metabolism, oxidative stress and inflammation in transition cow

L. M. Sordillo\textsuperscript{A,B} and V. Mavangira\textsuperscript{A}

Destructive feedback loops that enhance disease susceptibility.
Altered Nutrient Metabolism

- Transition from late gestation to early lactation
- Fetal demands
- Onset of copious milk production
- Linked to changes in nutrient requirements

Increased Need:
- Energy
- Proteins
- Glucose
- Minerals
- Vitamins
Altered Nutrient Metabolism

Negative Energy Balance

Energy (Megajoules Equivalent/day)

Days from Calving

Energy Required

Energy Ingested

Babcock Institute
Altered Nutrient Metabolism

Metabolic Adaptations to NEB

- Lipid mobilization
- Alterations in blood lipids
- Increased nonesterified fatty acids (NEFA)
- Increased beta-hydroxybutyrate (BHB)

Suriyasathaporn et al., 2000
Metabolic Adaptations During the Periparturient Period

*Balance is Essential*

Lipid Mobilization and Inflammation

Impact of NEFA Concentration

Plasma NEFA

Low Concentrations (<0.3 mM/L)
- Normal immune cell functions
- Regulated inflammation
- Decreased risk of metabolic and infectious diseases

High Concentrations (>0.7 mM/L)
- Abnormal immune cell functions
- Increased inflammation
- Increased risk of metabolic and infectious diseases

### Consequences of Altered Nutrient Metabolism

**Impact of lipid mobilization on inflammation**

<table>
<thead>
<tr>
<th>Transition Period</th>
<th>Observed Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative Energy Balance</strong></td>
<td>● Reduces antibody formation <em>(van Knegsel, 2007)</em> &amp; neutrophil functions <em>(Hammon et al., 2006)</em></td>
</tr>
<tr>
<td><strong>Elevated NEFA</strong></td>
<td>● Compromised lymphocyte functions <em>(Lacetera et al 2004)</em>, impaired cytokine production <em>(Scalia et al 2006)</em>, reduced neutrophil function <em>(Ster et al 2012)</em> and altered endothelial cell adhesion <em>(Contreras et al 2012)</em></td>
</tr>
<tr>
<td><strong>Ketosis (BHB levels)</strong></td>
<td>● Impairs neutrophil function <em>(Grinberg et al., 2008)</em></td>
</tr>
<tr>
<td><strong>Body Condition Score</strong></td>
<td>● Alters cytokine production <em>(O’Boyle et al., 2006)</em> and reduces lymphocyte functions <em>(Lacetera et al., 2005)</em></td>
</tr>
</tbody>
</table>
Fatty Acids and Inflammation

• Composition of NEFA can influence inflammation
  • Omega 6 (n6) fatty acids (linoleic acid and derivatives)
  • Omega 3 (n3) fatty acids (linolenic acid and derivatives)

Corn, sunflower seed, cottonseed, legumes

Fish oil and Flaxseed

Pro-inflammatory

Anti-inflammatory

Fatty Acids and Inflammation

• Cows fed omega-3 fatty acids (linseed and fish oil)
  • Improved lymphocyte function during negative energy balance (*Lacetera et al.*, 2007, *J. Dairy Sci.* 74:323)
  • Enhanced immunity during high ambient temperatures (*Caropese et al.*, 2009, *J. Dairy Sci.* 92:2796)
  • Improved milk production and reproductive performance in transition cows (*Silvestre et al.*, 2011)
Fatty Acids and Inflammation

Direct effects on immune cells

• Modifies membrane phospholipids and normal cell functions
• Regulates intracellular signaling and gene expression (cytokines and adhesion molecules)
• Influences oxylipid biosynthesis
Fatty Acids and Inflammation

*Oxylipid Biosynthesis*

- Regulates onset and resolution of inflammation
  - Products of polyunsaturated fatty acid oxidation
  - Derived from fatty acid substrates (omega-6; omega-3)
  - Catabolism by enzymatic (Cyclooxygenase; Lipoxygenase; Cytochrome P450)
  - Non-enzymatic pathways (ROS during oxidative stress)
  - Prostaglandins, thromboxanes & leukotrienes
  - Complex network of >150 lipid metabolites
Complexity of Oxylipid Biosynthesis

Substrate → Oxidation Pathway → Degree Metabolism

α – Linolenic (18:3 n-3) → *Δ6 desaturase
*Elongase
*Δ5 desaturase

Linoleic (18:2 n-6) → ROS or LOX
CYP2J2
13-HODE

Docosahexaenoic (DHA, 22:6 n-3) → 5/15LOX → 17s-HpDHA
17sHDHA → 5/15LOX → COX2 → 18R-HEPE

Eicosapentaenoic (EPA, 20:5 n-3) → 5/15LOX → COX2 → 18R-HEPE

CYP4A → 5/15LOX → 5LOX → 20-HETE
5-HEPE
15-HEPE

Arachidonic Acid (20:4 n-6) → 5LOX → COX1/2 → 5-HPETE
5-HETE

DiHOME → Pro-inflammatory

15LOX → 15-HEPE

Pro-inflammatory

Resolving

Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids


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

**Fatty Acid Composition (g/100g) of Leukocytes**

<table>
<thead>
<tr>
<th>Days relative to calving</th>
<th>Palmitic</th>
<th>Stearic</th>
<th>Linoleic</th>
</tr>
</thead>
<tbody>
<tr>
<td>-14</td>
<td>30.86±1.85&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.61±1.53</td>
<td>0.32±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>-7</td>
<td>30.96±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.82±1.82</td>
<td><strong>0.32±0.03&lt;sup&gt;a&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>Calving</td>
<td>31.87±1.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>32.63±1.87</td>
<td><strong>0.88±0.28&lt;sup&gt;ab&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>7</td>
<td>34.75±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.39±1.11</td>
<td>0.77±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>35.79±1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.49±1.60</td>
<td>0.89±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>35.04±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.67±1.31</td>
<td><strong>0.97±0.28&lt;sup&gt;b&lt;/sup&gt;</strong></td>
</tr>
</tbody>
</table>
Oxylipid Biosynthesis from Linoleic Acid

Linoleic Acid (substrate)

PRO-INFLAMMATORY
- Hydroperoxyl 9-HPODE; 13-HPODE
- Reduction
  - Hydroxyl 9-HODE; 13-HODE
  - Dehydrogenation
    - Ketone 9-oxoODE; 13-oxoODE

ANTI-INFLAMMATORY

Cyclooxygenase Lipoxygenase
(oxgenation pathway)

ROS

Degree Metabolism

hydroperoxyoctadecadienoic acid (HPODE)
Quantification of Linoleic-derived Metabolites

*Liquid Chromatography-Mass Spectrophotometry*

**Oxylipid biosynthesis**
- Linoleic acid
  - 9,13-HPODE
  - 9,13-HODE
  - 9,13-oxoODE

**Sample preparation for LC-MS/MS analysis**
- Addition of antioxidant/reducing agent
  - HPODE $\rightarrow$ HODE

**LC-MS/MS product quantification**
- 9,13-HODE
- 9,13-oxoODE
Oxylipid Biosynthesis
(Streptococcus uberis infected mammary glands)

Omega-6 Metabolites (Linoleic Acid)

- Descriptive findings from cows with mastitis
  - Targeted analyses of 25 oxylipids using LC/MS/MS
  - Omega-6 metabolites were most abundant (pro-inflammatory)
  - Correlations between the HODEs and inflammatory markers (cytokines & adhesion molecules)

hydroxyoctadecadienoic acid (HODE)

Ryman et al., 2015 Prostaglandins & Lipid Mediat. 121:207-217
Oxylipid Biosynthesis
(Streptococcus uberis infected mammary glands)

Greater HODE, but no change in oxoODE

Ryman et al., 2015 Prostaglandins & Lipid Mediat. 121:207-217
What is the contribution of linoleic acid derived metabolites to inflammatory dysfunction?
Endothelial Cells Regulate Inflammation

*In vitro model to mimic changes in fatty acid composition and concentration during early lactation*

- **Experimental Details**
  - Culture endothelial cells with fatty acid mixtures similar to plasma NEFA content
  - Vary concentration of fatty acid mixture to reflect degree of lipid mobilization in cows
  - Assess changes in oxylipids and inflammatory markers

### NEFA Concentration

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Lactation Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM</td>
<td>Control</td>
</tr>
<tr>
<td>0.25mM</td>
<td>Mid Lactation</td>
</tr>
<tr>
<td>0.5mM</td>
<td>Early Lactation</td>
</tr>
<tr>
<td>0.75mM</td>
<td>Transition Period</td>
</tr>
</tbody>
</table>

Contreras et al., 2012. J. Dairy Sci.
Fatty Acids and Inflammation

NEFA Alters Endothelial Inflammatory Responses

Pro-inflammatory Cytokines

Contreras et al., 2012. J. Dairy Sci.
Fatty Acids and Inflammation

NEFA Alters Inflammatory Responses

Omega-6 Pro-inflammatory Oxylipids

Contreras et al., 2012. J. Dairy Sci.
Can changes in the composition of fatty acids during lipid mobilization affect inflammatory responses in dairy cattle?
### Fatty Acids and Inflammation

#### NEFA Complex Compositions

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Symbol</th>
<th>Transition Cow</th>
<th>0.75Ω3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic</td>
<td>C14:0</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Palmitic</td>
<td>C16:0</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Stearic</td>
<td>C18:0</td>
<td>45%</td>
<td>45%</td>
</tr>
<tr>
<td>Oleic</td>
<td>C18:1n9c</td>
<td>16%</td>
<td>9%</td>
</tr>
<tr>
<td>Linoleic</td>
<td>C18:2n6c</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>EPA</td>
<td>C20:5n3c</td>
<td>0%</td>
<td>4%</td>
</tr>
<tr>
<td>DHA</td>
<td>C22:6n3c</td>
<td>1%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Contreras et al., 2012. J. Dairy Sci.95:7137.
Fatty Acids and Inflammation

Omega-3 Alters Inflammatory Responses

Reduces Pro-inflammatory Cytokines

Contreras et al., 2012. J. Dairy Sci. 95:7137.
Fatty Acids and Inflammation

Omega-3 Alters Inflammatory Responses
Reduces Pro-inflammatory Oxylipids

9-HPODE/9-HODE

Contreras et al., 2012. J. Dairy Sci. 95:7137.
Fatty Acids and Inflammation

Omega-3 Alters Inflammatory Responses
Increases Anti-inflammatory Oxylipids

Contreras et al., 2012. J. Dairy Sci. 95:7137.
Oxylipids and Dysfunctional Inflammation

Loss of Endothelial Cell Barrier Integrity

Normal inflammatory response:
- Activated endothelium
- Leukocyte migration
- Vascular permeability

Endothelial dysfunction:
- Tight junction disruption
- Endothelial contraction
- Cell death

Does 13-HPODE and 13-HODE reduce barrier integrity?

13-HPODE and 13-HODE

Primary BMEC

Endothelial barrier integrity:
- Electric Cell-substrate Impedance Sensing (ECIS)
- Apoptosis

Up to 24 hours

Measures Resistance

Appliedbiophysics.com
Linoleic-derived Oxylipids and Barrier Integrity

Electric Cell-substrate Impedance Sensing (ECIS)

Normalized Resistance

13-HODE

13-HPODE

Ryman et al., 2016. Mediators Inflamm. In press
Linoleic-derived Oxylipids and Barrier Integrity

*Endothelial Cell Apoptosis*

**Flow Cytometry**

**A**

YOPRO®-1

Early apoptosis

<table>
<thead>
<tr>
<th></th>
<th>0 µM</th>
<th>0.5 µM</th>
<th>30 µM</th>
<th>150 µM</th>
<th>2mM H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>13-HPODE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td></td>
<td></td>
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**B**

Propidium iodide

Late apoptosis/primary necrosis

<table>
<thead>
<tr>
<th></th>
<th>0 µM</th>
<th>0.5 µM</th>
<th>30 µM</th>
<th>150 µM</th>
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<tr>
<td>13-HPODE</td>
<td></td>
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<tr>
<td>6 hr</td>
<td></td>
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Ryman et al., 2016. Mediators Inflamm. In press
Antioxidant Treatment Reduces 13-HPODE Impact

*N*-acetylcysteine (.1mM to 10 mM NAC)

**Endothelial Cell Apoptosis**

150 µM 13-HPODE
0.1 mM NAC
1 mM NAC
10 mM NAC

**Endothelial Cell Integrity**

Hours relative to stimulation

0 2 4 6 8 10 12

0.8 0.9 1.0 1.1 1.2 1.3

* % FBS media control
13HPODE 150µM
13HPODE + 1mM NAC

Ryman et al., 2016. Mediators Inflamm. In press
Conclusions

Lipid Mobilization Alters Inflammatory Responses

• Plasma and cellular fatty acid concentration and composition changes during transition period
• The type of fatty acids (n6 vs n3) can alter oxylipid profiles (pro-inflammatory or resolving)
• Oxylipids impact endothelial cell inflammatory phenotype and barrier function
• Targeting the oxylipid network to control metabolic stress in transition cows
Practical Considerations

- **Reduce intense lipid mobilization during the transition period**
  - Minimize reductions in dry matter intake (DMI)
  - Design diets to increase energy without affecting DMI
  - Prevent over-conditioning in the dry period

- **Optimize inflammatory responses**
  - Nutritional strategies to increase n3 fatty acids (ratios??)
  - Reduces sources of stress (heat stress, exposure to pathogens)
  - Micronutrient supplementation (Se, Vitamin E, etc.)

- **Comprehensive Approach to Reduce Metabolic Stress**
  - Nutrient metabolism
  - Immune function
  - Oxidative balance
Questions?