

Review: Lipid biology in the periparturient dairy cow: contemporary perspectives

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Coordinated changes in energy metabolism develop to support gestation and lactation in the periparturient dairy cow. Maternal physiology involves the partitioning of nutrients (i.e. glucose, amino acids and fatty acids (FA)) for fetal growth and milk synthesis. However, the inability of the dairy cow to successfully adapt to a productive lactation may trigger metabolic stress characterized by uncontrolled adipose tissue lipolysis and reduced insulin sensitivity. A consequence is lipotoxicity and hepatic triglyceride deposition that favors the development of fatty liver disease (FLD) and ketosis. This review describes contemporary perspectives pertaining to FA surfeit and complex lipid metabolism in the transition dairy cow. The role of saturated and unsaturated FA as bioactive signaling molecules capable of modulating insulin secretion and sensitivity is explored. Moreover, the metabolic fate of FA as influenced by mitochondrial function is considered. This includes the influence of inadequate mitochondrial oxidation on acylcarnitine status and the use of FA for lipid mediator synthesis. Lipid mediators, including the sphingolipid ceramide and diacylglycerol, are evaluated considering their established ability to inhibit insulin signaling and glucose transport in non-ruminant diabetics. The mechanisms of FLD in the transition cow are revisited with attention centered on glycerophospholipid phosphatidylcholine and triglyceride secretion. The relationship between oxidative stress and oxylipids within the context of insulin antagonism, hepatic steatosis and inflammation is also reviewed. Lastly, periparturient hormonal involvement or lack thereof of adipokines (i.e. leptin, adiponectin) and hepatokines (i.e. fibroblast growth factor-21) is described. Similarities and differences in ruminant and non-ruminant physiology are routinely showcased. Unraveling the lipidome of the dairy cow has generated breakthroughs in our understanding of periparturient lipid biology. Therapeutic approaches that target FA and complex lipid metabolism holds promise to enhance cow health, well-being and productive lifespan.

Keywords: acylcarnitine, ceramide, fatty acid, metabolism, oxylipid

Implications

Metabolic stress may compromise the ability of the dairy cow to successfully adapt to a productive lactation and remain fertile and healthy. This review discusses changes in lipid metabolism that define maternal adaptation and metabolic stress in the cow. The narrative describes the roles of fatty acids, mitochondria and acylcarnitines, lipid mediators of insulin antagonism, including ceramide, glycerophospholipids and triglyceride secretion, and oxylipids during oxidative imbalance. The influence of leptin, adiponectin and fibroblast growth factors is considered. Defining the periparturient lipidome is important to refine existing or identify new therapeutic strategies to improve cow health and production efficiency.

Introduction

The dairy cow transitioning from gestation to lactation experiences dynamic changes in energy metabolism that develop as a means to support fetal and neonatal development (Bauman and Currie, 1980; Roche *et al.*, 2013). At the onset of the periparturient period (i.e. late gestation), the utilization of glucose and amino acids to support the growth of the fetus increases. Moreover, the demand for these nutrients as well as fatty acids (FA) and minerals only accelerates as milk production initiates. Although the survival of offspring demands nutrients for reproduction and lactation, the periparturient cow experiences a decline in energy intake immediately prior to parturition. Energy intake also remains inadequate throughout early lactation. The consequence is the cow's intrinsic reliance on coordinated metabolic adaptations to ensure that nutrient requirements for maintenance, conceptus growth, mammaryogenesis and lactogenesis are met.

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The classic understanding of physiological adaptations that support nutrient partitioning in dairy cattle have been summarized (Baumgard *et al.*, 2017). In brief, *de novo* fat synthesis and FA uptake and esterification are blunted in adipose tissue. Whereas, lipolysis is accelerated. Circulating FA are then oxidized in liver and skeletal muscle tissues and incorporated into milk triglycerides in the mammary gland. In muscle, protein synthesis is reduced and amino acid mobilization is enhanced. In turn, amino acids are utilized to support increased rates of gluconeogenesis (i.e. alanine) or milk protein synthesis. Hepatic glycogenolysis and ketogenesis are enhanced. Glucose utilization by skeletal muscle and adipose tissue is reduced to spare glucose for milk lactose synthesis. These metabolic events are controlled in part by a reduction in peripheral insulin sensitivity and responsiveness, as well as reduced pancreatic insulin secretion. Although the mechanisms of reduced insulin-stimulated glucose disposal are not entirely defined in the cow, uncoupling of the somatotrophic axis is involved (Lucy *et al.*, 2001, 2009). Specifically, plasma somatotropin concentrations increase during early lactation, whereas the concentration of the insulin-sensitizer insulin-like growth factor-1 decline. The cause is likely attributed to a decrease in hepatic growth hormone receptor second messenger signaling in early-lactation dairy cows (Lucy *et al.*, 2001). Nevertheless, low circulating insulin-like growth factor-1 concentrations help reduce glucose transport in skeletal muscle and adipose tissues, and enhance hepatic glucose output (Clemmons, 2004; Wang *et al.*, 2012). Enhanced adipose tissue responsiveness to catecholamines, and elevations in circulating glucocorticoids and glucagon are also evident in postpartum cows. It is important to emphasize that these adaptations are innate and should not be used to describe metabolic stress if milk production, health or well-being are not compromised. Indeed, cows with high productive efficiency rely heavily on these adaptations. Moreover, one could argue that delayed conception in a cow that maintains high milk production is not due to metabolic stress but simply reflects the metabolic decision of the cow to support one nutrient sink over another.

Metabolic stress is a consequence of inadequate metabolic adaptation (Sordillo and Raphael, 2013). By definition, unresolved metabolic stress triggers metabolic disorders such as subclinical or clinical ketosis, fatty liver disease (FLD) characterized by triglyceride deposition in the absence or presence of inflammation, and hypocalcemia. These disorders are linked to lower milk production, lower conception rates and increased calving intervals, lameness and compromised well-being and productive lifespan in the herd (Mulligan and Doherty, 2008). Implications for metabolic stress include increased replacement rates, inefficient nutrient utilization and economic loss.

This review explores contemporary perspectives in bovine lipid biology defined as the study of lipid metabolism, structure and bioactive functionality and the interactions lipids have with changes in physiology in the dairy cow. The intent is to expand our understanding of nutrient partitioning and

metabolic stress in the transition cow. The unique bioactive properties of FA, the physiological relevance of functional mitochondria, emerging insights in sphingolipid and glycerophospholipid biology, the consequences of oxidative imbalance and the involvement of adipokines are examined.

Fatty acids, and insulin secretion and sensitivity

In the transition cow, negative energy balance develops with increased concentrations of palmitic, stearic, oleic and linoleic acids in serum (Rukkwamsuk *et al.*, 2000; Douglas *et al.*, 2007). These changes reflect the FA profile of adipose tissue. As the proportion of saturated FA increase in plasma, the proportion of long-chain polyunsaturated FA decreases (e.g. arachidonic acid; Douglas *et al.*, 2007). In parallel with increased lipolysis, the postpartum liver of the cow accumulates greater amounts of palmitic, oleic and linoleic acids, but not stearic acid (Rukkwamsuk *et al.*, 2000). Enrichment of palmitic acid, oleic acid and linoleic acid is observed in hepatic triglycerides and phospholipids, whereas polyunsaturated FA (i.e. arachidonic and eicosapentaenoic acids) depletion is evident (Douglas *et al.*, 2007). Although not completely understood, the modulation of plasma FA status has implications for insulin secretion and sensitivity as well as liver health in the dairy cow.

During early lactation, insulin secretion in response to insulinotropic agents (i.e. glucose and propionate) is suppressed (Lomax *et al.*, 1979). This action advantageously removes the inhibitory signal on lipolysis, and thus circulating FA concentrations increase for oxidation and milk fat production. Therefore, it would be counterintuitive for FA to promote insulin secretion and lipogenesis during this stage of lactation. However, this concept deserves consideration especially in the mid- or late-lactation cow that is more responsive to insulinotropic compounds. The reason is that FA promote insulin secretion in non-ruminants (Poitout *et al.*, 2006). Evidence also suggests that these responses are uniquely influenced by the type of FA studied. In rats, glucose-stimulated insulin secretion from perfused pancreas is higher in response to co-stimulation with saturated palmitic or stearic acids, relative to oleic or linoleic acids (Stein *et al.*, 1997). The ability of saturated FA supplementation to increase circulating insulin concentrations has been observed in peak-lactation dairy cows (Harvatine and Allen, 2006). One notable exception is that chronic excess of palmitic acid reduces insulin synthesis and secretion in human pancreatic islets via mechanisms that may depend on ceramide-mediated inhibition of insulin gene expression as well as reduced β -cell turnover and apoptotic activation (Maedler *et al.*, 2003). Considering that circulating palmitic acid and ceramide is elevated when plasma insulin is low during early lactation, might palmitic acid-induced lipotoxicity and ceramide-dependent mechanisms explain suppressed insulin secretion during early lactation? Although this question remains unanswered, it should be mentioned

that monounsaturated FA (i.e. palmitoleic or oleic acids) counteract the harmful effects of palmitic acid on human pancreatic β -cell turnover and function (Maedler *et al.*, 2003). Of potential relevance in the cow, feeding a blend of palmitic and oleic acids increased circulating insulin concentrations in mid-lactation Holstein cows, relative to those fed high palmitic acid or a blend of palmitic and stearic acids (de Souza *et al.*, 2018).

In the cow, the alteration of peripheral insulin sensitivity by FA may influence lipolysis and the mammary utilization of glucose for milk synthesis. Specifically, diet- or lipolytic-derived FA may control insulin signaling, although regulation likely depends on the degree of FA saturation, chain length and concentration, and whether the cow is under homeostatic control. In non-ruminants, it is hypothesized that saturated FA inhibit insulin sensitivity by promoting lipid mediator synthesis (e.g. ceramide or diacylglycerol (DAG)), inflammation via toll-like receptor-4 signaling, oxidative stress or endoplasmic reticulum stress (Boden, 2011). In contrast, n-3 FA such as docosahexaenoic acid (DHA) appear to counteract insulin resistance development by stimulating mitochondrial function, reducing reactive oxygen species (ROS) production and preventing inflammation in non-ruminants, as reviewed by Lepretti *et al.* (2018). Similar benefits are observed when studying monounsaturated FA (i.e. oleic acid; Palomer *et al.*, 2018). In dairy cattle, an intravenous infusion of saturated tallow caused insulin resistance (Pires *et al.*, 2007). Evidence suggests that palmitic acid partitions nutrients to the mammary gland, whereas oleic acid shifts energy away from milk production and toward body fat accretion (de Souza *et al.*, 2018). In addition, abomasal infusion of linseed oil rich in polyunsaturated linolenic acid appeared to enhance the anti-lipolytic effects of insulin in feed-restricted cows (Pires *et al.*, 2008). In growing steers, long-chain eicosapentaenoic and DHA increases insulin sensitivity, relative to animals unsupplemented with n-3 FA (Cartiff *et al.*, 2013). Although a better mechanistic understanding is required, the current literature suggests that saturated FA reduce insulin sensitivity in dairy cattle, whereas unsaturated FA (particularly n-3 FA) enhance insulin action. Future research should determine whether and how saturated and unsaturated FA influence insulin signaling and inflammation in early-lactation cow, and whether these changes are associated with the advancement of FLD, the incidence of metabolic disorders and long-term lactation and reproductive success of the cow.

Acylcarnitines and mitochondrial β -oxidation

A key step in the β -oxidation of FA is the formation of acylcarnitines as a means to shuttle fatty acyl-CoA from the cytosol into the mitochondrion. This process is facilitated by carnitine palmitoyltransferase-1, which is inhibited by malonyl-CoA generated by the lipogenic enzyme acetyl-CoA carboxylase. In the study of obesity and type 2 diabetes, acylcarnitines have received attention as biomarkers of

lipid-induced mitochondrial dysfunction (Schooneman *et al.*, 2013). The accumulation of acylcarnitines reflects a reduction in FA oxidation capacity and the partitioning of FA toward the synthesis of triglyceride and lipid mediators of insulin antagonism, including ceramide and diacylglycerol. Types of acylcarnitines include ketone-derived (e.g. the carnitine ester of β -hydroxybutyrate), branched-chain amino acid-derived (e.g. C4-dicarboxylic-carnitine), and acylcarnitines varying in FA chain length (e.g. acetyl-, myristoyl- or palmitoyl-carnitine, respectively). Focus has centered on long-chain acylcarnitines because they are linked to insulin resistance (Schooneman *et al.*, 2013). In the study of Koves *et al.* (2008), chronic high-fat feeding increased post-prandial serum total FA as well as C8:1-, C10:3-, C16:0-, C18:0- and C18:1-carnitine concentrations in rats. In the same study, acylcarnitine levels were elevated in gastrocnemius muscles in *ad libitum* high-fat fed obese Zucker diabetic fatty rats experiencing elevations in FA oxidation to acid-soluble metabolites (a measure of incomplete FA catabolism), relative to lean animals. More recent data suggest that acylcarnitines may directly modulate insulin signaling. In differentiated myotubes of non-ruminant origin, C16:0-carnitine treatment inhibited insulin-stimulated protein kinase B (AKT) activation and glucose uptake (Aguer *et al.*, 2015). In addition, lowering of acetyl-carnitine concentrations by mildronate (an inhibitor of acylcarnitine transferase) prevents palmitate-induced reductions in insulin-stimulated glucose utilization by human primary myotubes derived from lean subjects (Aguer *et al.*, 2015). Interestingly, mildronate also prevented palmitic acid-induced accumulation of ROS, which suggests that acylcarnitines may promote oxidative stress concomitantly with impaired insulin sensitivity.

Acylcarnitine metabolism has also been implicated in the development of FLD. Specifically, hepatic acylcarnitine levels are elevated in humans with non-alcoholic FLD (e.g. C16:0- and C12:0-carnitines in steatosis and steatohepatitis, respectively; Lake *et al.*, 2015). Albeit *de novo* lipogenesis plays a role in non-ruminant FLD, mitochondrial dysfunction and FA excess also provides FA substrate for excess triglyceride storage, which favors steatosis. The advancement of hepatic injury in the form of inflammation (steatosis to steatohepatitis) may be provoked by acylcarnitines. In support, C14:0-carnitine supplementation stimulated the expression and secretion of pro-inflammatory tumor necrosis factor- α (TNF- α) from RAW 264.7 macrophage cells (Rutkowski *et al.*, 2014). This is of potential significance considering that TNF- α is associated with FLD in humans and dairy cows (Ohtsuka *et al.*, 2001; Marcellini *et al.*, 2007). Interestingly, hepatic branched-chain amino acid concentrations (i.e. leucine, isoleucine and valine) are elevated with amplified acylcarnitine status during advanced FLD (Lake *et al.*, 2015). Moreover, elevations in branched-chain amino acid concentrations in the presence of high saturated fat intake may overload mitochondrial fuel oxidation via anaplerosis to form incompletely oxidized lipid-derived metabolites such as acylcarnitines and exacerbate insulin resistance (Newgard *et al.*, 2009). Lastly, although the accrual of hepatic acylcarnitines

promotes their accumulation in circulation via mechanisms that may involve organic cation/carnitine transporter-2, it deserves mentioning that the plasma acylcarnitine profile may inadequately reflect hepatic acylcarnitine metabolism in mammals (i.e. plasma levels do not reflect tissue levels; Schooneman *et al.*, 2014).

In dairy cattle transitioning from gestation to lactation, the capacity to completely oxidize FA to CO₂ is lowered, whereas incomplete oxidation to acid-soluble metabolites is elevated (Litherland *et al.*, 2011). In the muscle of transition cow, long-chain acylcarnitines accumulate with short- and medium-chain acylcarnitines during the peripartum (Yang *et al.*, 2019), suggesting that β -oxidation to acetyl-CoA increased but the tricarboxylic acid cycle was downregulated. In sick transition cows (i.e. those with mastitis, metritis, retained placenta and/or laminitis; $n = 6$), plasma short-chain propionyl-carnitine concentrations were elevated, relative to six healthy controls (Hailemariam *et al.*, 2014). In over-conditioned transition cows that mobilize more FA postpartum, plasma C14:0-, C16:0-, C18:0- and C20:0-carnitine levels are elevated, relative to lean cows (Rico *et al.*, 2018b). In the same study, plasma total acylcarnitine levels were positively correlated with circulating total FA concentrations, as well as total and C24:0-linked ceramide. Although this study supports that long-chain acylcarnitines were formed and exported out of the mitochondrion, the work did not assess FA oxidation or functionality of the TCA cycle. Regardless, hepatic FA that are not oxidized can serve as substrate for triglyceride esterification and are potentially partitioned toward ceramide synthesis in over-conditioned cows prone to accelerated lipolysis and FLD.

Our understanding of acylcarnitine status within the context of productivity and health requires further study. The reason is because elevations in serum carnitine or long-chain acylcarnitine (e.g. C16:0- or 18:2-carnitine) levels during the transition period or peak lactation have been observed in cows that remain clinically healthy beyond 100 days in milk, relative to those culled beyond peak milk production (Huber *et al.*, 2016). It remains to be determined whether elevations in acylcarnitines during transition are predictive of metabolic disorders (e.g. ketosis) during early lactation. It is conceivable that fully functional mitochondria that manage FA surfeit during early lactation would protect the cow from lipotoxicity and FLD to enhance performance. In support, dietary carnitine supplementation has been shown to stimulate palmitate β -oxidation and decrease liver triglyceride concentrations in postpartum cows (Carlson *et al.*, 2007). Cows with a single nucleotide polymorphism in mitochondrial transcription factor A, an autosomal gene essential for transcription and replication of mitochondrial DNA, are less fertile and more likely to be culled, albeit they produce more milk (Clempton *et al.*, 2011). Moreover, evidence in growing steers suggests that increased mitochondrial function may contribute to enhanced feed efficiency during compensatory growth that follows feed restriction (Connor *et al.*, 2010).

Lipid mediators, insulin sensitivity and nutrient partitioning

Sphingolipids including ceramides

Sphingolipids are composed of a sphingoid base backbone (i.e. D-erythro-sphingosine) and an FA linked via an amide bond. Types of sphingolipids include ceramide and those with a polar head group, including monohexosylceramide (i.e. glucosyl- or galactosyl-ceramide; **GlcCer**), lactosylceramide and phosphocholine-containing sphingomyelin. Even more complex in structure are gangliosides, which are glycosphingolipids containing one or more sialic acid residues. Although sphingolipids are diverse in structure, their *de novo* synthesis begins with ceramide controlled in part by serine palmitoyltransferase and ceramide synthase (**CerS**). Serine palmitoyltransferase controls the condensation of palmitoyl-CoA and serine to produce 3-ketodihydro-sphingosine. Whereas, one of six CerS isoforms attaches a second FA (most often saturated) to sphinganine to synthesize the ceramide precursor dihydroceramide. For instance, CerS6 and CerS2 are involved in the production of the highly abundant C16:0- and C24:0-ceramide in mammals, respectively (Levy and Futerman, 2010). An alternative pathway that produces ceramide is controlled by sphingomyelinase (**SMase**; acid or neutral) which hydrolyzes sphingomyelin. Once ceramide is formed, it may be used by glucosylceramide synthase to form GlcCer and eventually gangliosides, converted to sphingomyelin by sphingomyelin synthase, phosphorylated by ceramide kinase, or degraded by ceramidase to form sphingosine and FA (McFadden and Rico, 2019).

The diversity of sphingolipid structure dictates their equally diverse function. Sphingolipids are structurally important as aggregates within cellular membrane rafts and caveolae that often include caveolin (Liu and Anderson, 1995). Ceramide, glycosylated ceramide and sphingomyelin are also components of lipoproteins. Specifically, ceramides are primarily enriched in low-density lipoproteins (**LDL**) as observed in humans (Wiesner *et al.*, 2009) and dairy cows (Davis *et al.*, 2019); however, their incorporation within very-low-density lipoproteins (**VLDL**) increases with fasting (Wiesner *et al.*, 2009). Bioactive ceramides and glycosylated ceramides behave as second messengers and interact with proteins to modulate cell signaling. From an historical perspective, ceramide is recognized for its role in the downregulation of cell proliferation, induction of cell differentiation and initiation of caspase-mediated programmed cell death (Pushkareva *et al.*, 1995). Today, sphingolipids have received attention for their respective role within the progression of type 2 diabetes, non-alcoholic FLD and cardiovascular disease in humans. Emerging evidence also suggests that sphingolipids decrease insulin signaling in dairy cattle, which may accelerate glucose and FA partitioning toward liver and the mammary gland (Rico *et al.*, 2015, 2017a and 2018c).

The ability of ceramide to inhibit insulin-stimulated glucose utilization is a feature that defines lipotoxicity and insulin resistance in skeletal muscle and adipose tissue of non-ruminants (Chavez and Summers, 2012). The mechanisms

by which ceramide influences insulin sensitivity are multifaceted. It was first discovered that excess long-chain FA promote lipoapoptosis (i.e. apoptosis caused by FA overaccumulation otherwise known as steatosis) of pancreatic β -cells to contribute to inadequate insulin secretion in Zucker diabetic fatty rats (Shimabukuro *et al.*, 1998). The apoptotic effect was due, in part, to enhanced *de novo* ceramide synthesis. Subsequent research demonstrated that ceramide inactivates AKT to downregulate the translocation of glucose transporter-4 to the plasma membrane in myotubes and adipocytes challenged by insulin (Chavez and Summers, 2012). Although AKT inactivation appears fundamentally involved, ceramide-mediated insulin resistance also appears to include the activation of phosphatase and tensin homolog and protein phosphatase 2A, as well as the caveolin-enriched microdomain-recruitment of protein kinase C- ζ (Chavez and Summers, 2012). Recent evidence also suggests that extracellular-derived ceramide within LDL may also downregulate insulin signaling in the muscle (Boon *et al.*, 2013). Interestingly, pharmacological inhibition of serine palmitoyltransferase or glucosylceramide synthase (*de novo* ceramide and GlcCer synthesis, respectively) is an effective means to improve insulin sensitivity in the muscle of obese rodents (Aerts *et al.*, 2007; Holland *et al.*, 2007). A similar outcome has been observed in cultured C2C12 myotubes that overexpress acid ceramidase (Chavez *et al.*, 2005) or in CerS6-deficient mice that exhibit reduced C16:0-ceramide concentrations in white adipose tissue and liver (Turpin *et al.*, 2014).

Our understanding of sphingolipid biology in the dairy cow has expanded in recent years. It was first observed that plasma ceramide (e.g. total ceramide, and C18:0-, C20:0-, C22:0- and C24:0-ceramide) and GlcCer (e.g. C16:0- and C18:0-GlcCer) concentrations increase with the transition from gestation to lactation, more so for cows with elevated prepartum body condition and postpartum circulating total FA concentrations (Rico *et al.*, 2015 and 2017a). During the transition period, postpartum liver total and C24:0-ceramide concentrations increase progressively in over-conditioned dairy cows with hepatic lipid accumulation, and C16:0-ceramide concentrations increase in skeletal muscle tissue and plasma LDL fractions in all cows independent of prepartum body condition status (Rico *et al.*, 2017a; Davis *et al.*, 2019). The enhanced supply of circulating and hepatic ceramide was also observed in non-pregnant and non-lactating Holstein cows intravenously infused a triglyceride emulsion, or feed-restricted to enhance circulating total FA concentrations (Davis *et al.*, 2017; Rico *et al.*, 2018a). In these studies, ceramide supply is positively related to circulating total FA supply, and inversely related to indirect and direct measures of systemic insulin sensitivity in cows (Rico *et al.*, 2015 and 2017a; Davis *et al.*, 2017). These findings suggest that ceramide may antagonize insulin-stimulated glucose uptake. In support, the treatment of primary bovine-differentiated adipocytes with hydrophilic C2:0-ceramide decreased AKT Ser-473 phosphorylation (i.e. activation) and insulin-stimulated 2-deoxy-d-[3 H]-glucose uptake (Rico *et al.*, 2018c). While in contrast, the treatment of bovine adipocytes with the serine palmitoyltransferase

inhibitor myriocin lowered intracellular ceramide concentrations, and enhanced AKT phosphorylation and 2-deoxy-d-[3 H]-glucose uptake in the presence of insulin (Rico *et al.*, 2018c). Collectively, our findings suggest that adipose ceramide accrual may promote lipolysis via the inhibition of insulin signaling. In support, glucose-stimulated reductions in circulating total FA levels are inversely related to plasma ceramide concentrations in lactating cows (Rico *et al.*, 2016).

It is likely that increases in saturated FA uptake by tissues drives *de novo* ceramide synthesis in cows. In support, dietary palmitic acid feeding increases plasma and hepatic ceramide levels in mid-lactation cows, relative to no added fat or stearic acid (Rico *et al.*, 2016 and 2017b). Moreover, treatment of primary bovine neonatal hepatocytes with a *de novo* synthesis inhibitor prevents intracellular ceramide accumulation in response to palmitic acid incubation (McFadden *et al.*, 2018). Potentially relevant in the transition cow experiencing FLD, inflammation and ceramide accrual, the induction of sphingomyelin hydrolysis by SMase may also contribute to ceramide synthesis. Several lines of evidence support this hypothesis. First, plasma sphingomyelin levels are lowest at calving (Rico *et al.*, 2017a and 2018b). Second, pro-inflammatory TNF- α induces acid SMase activation to generate ceramide and inhibit insulin signaling (i.e. insulin receptor substrate-1; Peraldi *et al.*, 1996). Third, serum TNF- α activity is negatively correlated with insulin-stimulated glucose disposal in dairy cows with FLD (Ohtsuka *et al.*, 2001). The net contributions of *de novo* ceramide synthesis and sphingomyelin hydrolysis to ceramide pools within the context of FA supply and inflammation should be further evaluated in the periparturient cow.

Diacylglycerols

It deserves to be stated that the underlying molecular mechanisms of insulin resistance associated with obesity and type 2 diabetes are complex. Case in point, DAG is another lipid mediator and activator of protein kinase C in skeletal muscle and liver (Erion and Shulman, 2010). In both tissues, DAG accumulation decreases insulin-stimulated insulin receptor substrate tyrosine phosphorylation, and phosphoinositide 3-kinase activation. The result is the downregulation of glucose transporter-4 translocation and glucose uptake in response to insulin in muscle, and the inhibition of glycogen synthesis and induction of gluconeogenesis in liver. The question of whether ceramide or DAG is the leading cause of insulin resistance in diabetics is debated (Petersen and Jurczak, 2016; Summers and Goodpaster, 2016). In the dairy cow, the ability of DAG to modulate insulin signaling has not been tested. However, concurrent with hepatic triglyceride accumulation, liver DAG concentrations increase postpartum, relative to prepartum (Qin *et al.*, 2017). In the study of Qin *et al.* (2017), prepartum overfeeding did not modulate hepatic DAG concentrations. Because evidence does not support the development of insulin resistance in the liver of postpartum cows (Zachut *et al.*, 2013), the observed elevation in hepatic DAG concentrations may only represent an

intermediate in triglyceride synthesis and not a causal agent of insulin antagonism. Future studies will need to confirm the role of DAG in the liver and muscle of transition cows.

Phosphatidylcholines and hepatic triglyceride disposal

In humans, non-alcoholic FLD is attributed to enhanced *de novo* lipogenesis involving the induction of sterol regulatory element-binding protein (Kohjima *et al.*, 2008). In contrast, hepatic triglyceride deposition in peripartal cows with FLD is due to enhanced esterification of lipolytic-derived FA. This process is likely exacerbated by the aforementioned inadequate mitochondrial β -oxidation of FA in the liver as well as adipose insulin antagonism, which would enhance FA supply for hepatic uptake. Additionally, the pathophysiology of FLD in cows likely involves a limited capacity to secrete triglyceride within VLDL. Indeed, triglyceride secretion is limited in ruminants, relative to non-ruminants (Pullen *et al.*, 1990), and the rate of hepatic VLDL secretion plateaus as intrahepatic fat content becomes severe in humans with non-alcoholic FLD (Fabbrini *et al.*, 2008). In transition cows, a downregulation of hepatic apolipoprotein B₁₀₀ mRNA expression and protein abundance may limit VLDL assembly and export to promote FLD (Bernabucci *et al.*, 2009). An alternative hypothesis focuses on phosphatidylcholine (PC), a glycerophospholipid and principal component of the VLDL monolayer. Studies employing rodent models and choline-deficient diets have demonstrated the essential requirement of PC synthesis for VLDL secretion as a means to prevent steatosis (Fast and Vance, 1995). Although the rapid ruminal degradation of unprotected choline and incorporation of choline into milk phospholipids may partially explain why plasma total choline and PC concentrations are low in postpartum cows (Artegoitia *et al.*, 2014), reduced choline availability has a potential to limit hepatic PC synthesis and VLDL secretion to favor the advancement of steatosis in the ruminant.

Hepatic synthesis of PC involves the cytidine diphosphate (CDP)-choline pathway (i.e. the Kennedy pathway) and the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway. Initiating the CDP-choline pathway, choline kinase utilizes free choline and ATP to form the PC-precursor phosphocholine. As observed in rat hepatocytes, choline kinase serves a critical function considering that ~70% of total PC are produced by the CDP-choline pathway (DeLong *et al.*, 1999). In liver, the PEMT pathway is recognized as a compensatory pathway that relies on coupled folate and transmethylation cycles, which fundamentally define one-carbon metabolism. Methyl donors, including methionine, choline and betaine, and one-carbon donors glycine and serine are utilized to generate *S*-adenosylmethionine for the transformation of phosphatidylethanolamine to PC by PEMT. Although undefined in cows, the CDP-choline pathway prefers DAG enriched in saturated and monounsaturated FA (i.e. palmitic and oleic acids, respectively). Instead, PEMT prefers phosphatidylethanolamine enriched in long- and very-

long-chain polyunsaturated FA, including eicosatetraenoic acid and DHA (DeLong *et al.*, 1999). Indeed, circulating PC containing DHA has been recognized as a biomarker for PEMT activation in humans (da Costa *et al.*, 2011). In the transition cow, lipolytic-derived FA (i.e. palmitic and oleic acids) may support CDP-choline pathway activation; however, it is hypothesized that hepatic polyunsaturated FA depletion may prevent PEMT activation (Myers *et al.*, 2019).

Associative relationships between circulating PC and the severity of hepatic steatosis have been repeatedly observed in dairy cows. For example, the levels of serum PC with short-chain FA moieties are low in cows with severe hepatic lipodosis, relative to clinically healthy cows (Imhasly *et al.*, 2014). Moreover, suppressed hepatic levels of highly unsaturated PC have been observed in Holstein cows with moderate FLD (Saed Samii *et al.*, 2018). With the intent to increase hepatic PC synthesis, feeding strategies that emphasize rumen-protected choline or methionine supplementation are routinely studied and applied as a potential means to prevent FLD in transition cows. For example, peripartal dietary rumen-protected choline supplementation has been shown to reduce hepatic triglyceride concentrations in postpartum cows (Zom *et al.*, 2011). Recent *in vitro* work has aimed to delineate how choline and methionine are utilized by the CDP-choline and PEMT pathways (Zhou *et al.*, 2018). The potential of methyl donor feeding to enhance hepatic PC synthesis and lipid disposal may also have implications for improving inflammatory and immune status in cows (Zhou *et al.*, 2016). Moving forward, the interplay between dietary FA and methyl donor efficacy to enhance hepatic PC synthesis and VLDL secretion should be examined. In support, Myers *et al.* (2019) hypothesize that DHA may selectively induce PEMT activation in cows.

Oxylipids and oxidative stress

Mitochondrial overload of saturated FA triggers the loss of redox homeostasis and accelerates the formation of oxygen radicals (i.e. ROS). This lipotoxic condition called oxidative stress has been implicated in the development of insulin resistance as well as non-alcoholic FLD in non-ruminants (Videla *et al.*, 2006). As reviewed by Videla *et al.* (2006), persistent ROS production activates serine/threonine kinase signaling cascades that inhibit insulin-stimulated insulin receptor substrate induction. In turn, insulin resistance may enhance intrahepatic lipid content to promote simple steatosis (i.e. non-inflammatory phenotype). However, oxidative stress may also promote Kupffer cell activation to activate redox-sensitive transcription factors, including nuclear factor- κ B, and upregulate pro-inflammatory TNF- α . Unfortunately, inflammatory steatohepatitis is also characterized by the upregulation of NADPH oxidase and cytochrome P450 (family 2, subfamily E, polypeptide 1; otherwise known as CYP2E1), which further reduce antioxidant capacity and promote hepatocellular damage. Another consequence of rampant ROS generation includes n-3 long-chain

polyunsaturated FA depletion caused by defective FA desaturation and enhanced peroxidation in the liver (Videla *et al.*, 2004). In addition, polyunsaturated FA subjected to enzymatic (via cyclooxygenase, lipoxygenase and CYP2E1) or non-enzymatic (via ROS) oxidation form oxylipids with diverse inflammatory functions. For instance, oxylipids derived from n-6 arachidonic acid and linoleic acid include pro-inflammatory hydroxyl-octadecadienoic acid and hydroxyl-eicosatetraenoic acid, respectively.

In the transition cow experiencing reduced antioxidant potential (Sordillo and Aitken, 2009), ROS accumulation likely develops in part because of rampant mitochondrial FA oxidation, albeit incomplete breakdown. In turn, oxidative stress may enhance adipose tissue lipolysis to exacerbate oxidant status (Krawczyk *et al.*, 2012). One fate of lipolytic-derived unsaturated FA is ROS oxidation, which results in lipid hydroperoxide and isoprostane formation. Fatty acids released by adipose tissue are also used for hepatic β -hydroxybutyrate generation, which may promote hepatocyte apoptosis via ROS-mediated p38 mitogen-activated protein kinase activation (Song *et al.*, 2016). In addition, lipolytic FA may induce nuclear factor- κ B in hepatocytes via ROS-dependent mechanisms that trigger inflammation (Li *et al.*, 2015). These findings suggest that oxidative stress is involved in FLD pathology.

As previously reviewed by Sordillo and Aitken (2009), the accumulation of ROS or oxylipids likely influences bovine immune responses. For instance, plasma oxylipid levels are correlated with the expression of interleukin-12 β and inducible nitric oxide synthase-2 in peripheral blood mononuclear cells derived from healthy transition cows (Raphael *et al.*, 2014). In endothelial cells, elevations in the arachidonic acid metabolite 15-hydroxy-peroxyeicosatetraenoic acid develops with apoptosis and caspase-3 activation (Sordillo *et al.*, 2005), and leukocyte recruitment and inflammatory cytokine release (Whatling *et al.*, 2007). Cytochrome P450- and lipoxygenase-derived oxylipids accumulate in plasma (e.g. 11,12-epoxyoctadecanoic acid) and adipose tissue (e.g. 5-hydroxy-eicosatetraenoic acid) of postpartum cows, respectively, and may act as positive or negative modulators of inflammation and immune cell trafficking during intense lipolysis (Contreras *et al.*, 2017). Inflammatory dysfunction is a key attribute of inflammatory disorders including mastitis and metritis; therefore, the role of oxidized lipids has been considered. For instance, *Streptococcus uberis* mastitis is characterized by mammary inflammation and the accrual of oxylipids, including hydroxyl-octadecadienoic acid (Ryman *et al.*, 2015). The amount of dietary antioxidants (i.e. vitamin E and β -carotene) and trace minerals (i.e. copper or zinc) that play a role in antioxidant defense mechanisms, and the type and amount of FA fed to transition cows, may influence oxylipid formation and affiliated health outcomes.

Leptin, adiponectin and fibroblast growth factor-21

Although the role of insulin within the framework of glucose utilization and lipolysis is described, adipokines and

hepatokines deserve consideration to understand lipid metabolism in the transition cow. First, the adipokine leptin is produced by adipocytes and mediates its action through the Janus kinase signal transducer and activator of transcription pathway (Yadav *et al.*, 2013). The hormone regulates energy metabolism, insulin sensitivity, thyroid hormone secretion, immunity and appetite. In a demonstration of function, insulin resistance and inflammatory non-alcoholic FLD develops in obese leptin-deficient *ob/ob* mice (Perfield *et al.*, 2013). Moreover, *ob/ob* mice experience lipotoxicity including muscle ceramide and DAG accrual (Turpin *et al.*, 2009); however, chronic treatment of *ob/ob* mice with myriocin decreases circulating ceramides, steatosis and improves glucose homeostasis (Yang *et al.*, 2009). In hyperlipidemic animals, leptin acts by preventing the accumulation of ceramide and DAG to protect against insulin resistance (Dube *et al.*, 2007). Such results may be explained by a stimulatory effect of leptin on FA breakdown (Shimabukuro *et al.*, 1997). However, the lipid mediator-lowering effect of leptin is not always observed. In hyperleptinemic lean animals, for example, soleus and superficial vastus muscle concentrations of ceramide are increased, DAG unchanged, and triglyceride decreased (Dube *et al.*, 2007). These findings suggest that mechanisms by which leptin modulate insulin action likely vary by adiposity phenotype.

In the late-gestating cow, fatter cows exhibit higher plasma leptin concentrations, relative to lean cows (Kokkonen *et al.*, 2005), whereas all cows experience a decline in circulating leptin and white adipose tissue leptin mRNA expression at the onset of lactation (Block *et al.*, 2001). With this physiology in mind, it can be argued that the observed elevations in muscle FA oxidation occur independent of leptin signaling (Schäff *et al.*, 2013). In ruminants, leptin may only be responsive during positive energy balance. In support, an intravenous infusion of a triglyceride emulsion increases circulating FA and leptin concentrations during late gestation but not early lactation (Chelikani *et al.*, 2003). The authors suggest that this effect may be due to elevations in insulin-like growth factor-1, which is known to regulate leptin secretion. Although current evidence suggests a limited role of leptin in postpartum dairy cattle, the administration of human leptin to early-lactation cows decreases liver triglyceride content (Ehrhardt *et al.*, 2016); therefore, strategies that elevate leptin status during the periparturient period may be therapeutic.

Adiponectin is predominantly expressed in adipose tissue, and the expression of adiponectin decreases with increasing adiposity (Yadav *et al.*, 2013). This adipokine increases insulin sensitivity, reduces circulating glucose, enhances FA catabolism and prevents inflammation (Yadav *et al.*, 2013). The mechanisms of adiponectin action involve the activation of peroxisome proliferator activator receptor- α and AMP-activated protein kinase. In the muscle, downstream outcomes include enhanced FA transport and oxidation, reduced intracellular triglyceride content and enhanced insulin-stimulated translocation of glucose transporters to the plasma membrane.

In multiparous dairy cow transitioning to lactation, circulating adiponectin levels are lowest at parturition

(Giesy *et al.*, 2012). Suppressed adiponectin secretion in the multiparous transition cow could limit FA oxidation and glucose utilization in response to insulin. In support, enhanced plasma adiponectin levels are associated with reduced plasma concentrations of long-chain acylcarnitines (Rodríguez-Gutiérrez *et al.*, 2012), and adiponectin decreases ceramide accumulation in obese mice (Holland *et al.*, 2013). It deserves to be emphasized that serum adiponectin concentrations appear to increase after parturition in primiparous cows (Urh *et al.*, 2019). It has been hypothesized that greater adiponectin concentrations in primiparous cows may enhance peripheral insulin sensitivity and nutrient partitioning toward growth (Koster *et al.*, 2017; Urh *et al.*, 2019). If this were observed, it can be further hypothesized that circulating acylcarnitines and ceramides would be lower in primiparous cows, relative to multiparous cows.

Studies have also focused on the expression of adiponectin receptor-1 and -2 in bovine tissues during the transition period. Giesy *et al.* (2012) observed elevated mRNA expression of adiponectin receptor-1 and -2 during early lactation in the muscle and liver, respectively, relative to late pregnancy. Similar changes in hepatic adiponectin receptor-2 expression have been confirmed (Saremi *et al.*, 2014). In adipose tissue, Giesy *et al.* (2012) did not observe a change in adiponectin receptor expression, which contrasts with other work demonstrating reduced adiponectin receptor-1 and -2 expression in adipose tissue in early lactation, relative to late gestation (Lemor *et al.*, 2009; Saremi *et al.*, 2014). Future work should define how changes in adiponectin receptor mRNA expression influence adiponectin signaling.

Fibroblast growth factor-21 (FGF21) lowers blood glucose, insulin and triglyceride concentrations, and reduces body weight in non-ruminants (Holland *et al.*, 2013). Interestingly, FGF21 stimulates adiponectin secretion, and adiponectin mediates FGF21-induced increases in energy expenditure (Holland *et al.*, 2013). In periparturient cow, plasma FGF21 concentrations peak at parturition. Moreover, plasma FGF21 levels and hepatic FGF21 mRNA are increased by feed restriction in non-pregnant, late-lactating dairy cows (Schoenberg *et al.*, 2011); however, elevations in circulating FGF21 does not appear to prevent FA-induced increases in liver triglyceride in non-pregnant, non-lactating dairy cows (Caixeta *et al.*, 2017). Because plasma FGF21 and ceramide concentrations are elevated and adiponectin secretion is suppressed in transition cows, it would appear that the FGF21–adiponectin–ceramide axis observed in non-ruminants (Holland *et al.*, 2013) is not intact in periparturient ruminants but may explain metabolic dysfunction (i.e. FLD and impaired insulin antagonism).


Conclusion

The periparturient cow relies on well-described biochemical and hormonal changes in nutrient metabolism to meet metabolic demands; however, the advent of lipidomics technologies has contemporized our understanding of lipid biology

and lipotoxicity during the periparturient period. Today, FA are recognized as bioactive signaling molecules that uniquely influence nutrient utilization and production. The same certainly applies to many of tens of thousands of complex lipids that constitute the bovine lipidome. Therefore, we should be careful not to generalize acylcarnitines, ceramides, diacylglycerols, phosphatidylcholines and oxylipids. Rather, the mechanistic role of these lipids at the species level should be elucidated in the transition cow. Then, if deemed appropriate, they should be targeted to advantageously control nutrient partitioning for therapeutic gain. Such insight has potential to result in the development of optimized dairy cattle nutrition strategies designed to modulate cow metabolism and productive lifespan.

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Declaration of interest

The authors declare that there is no conflict of interest for this manuscript.

Ethics statement

None.

Software and data repository resources

None of the data were deposited in an official repository.

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