Lipid Mobilization and Inflammation During the Transition Period

Andres Contreras[1]
College of Veterinary Medicine, Michigan State University

Introduction

Lipid mobilization is a metabolic process that includes lipogenesis and lipolysis. Within the adipose tissue (**AT**), lipogenesis is the assembly of triglycerides through a stepwise addition of fatty acids catalyzed by glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate acyltransferase, lipins, and diacylglycerol acyltransferase (Takeuchi and Reue, 2009). During lipolysis, adipocyte's adipose triglyceride lipase, hormone-sensitive lipase, and monoacylglycerol lipase hydrolysis the triglyceride molecule into glycerol and nonesterified fatty acids (**NEFA**) [reviewed by Arner and Langin (2014)]. Released NEFA are either re-esterified to triglycerides through lipogenesis or exported into circulation where they are transported by albumin and Fetuin-A for use in other tissues as fuel or secreted in milkfat (Strieder-Barboza et al., 2018). During the transition period, the net release of NEFA from AT into circulation is the result of reduced lipogenesis and enhanced lipolysis within adipocytes (De Koster et al., 2018). Normally, lipolysis decreases and lipogenesis replenishes adipocytes' triglyceride stores as lactation progresses. However, when AT exhibits an impaired response to the anti-lipolytic effects of insulin, lipolysis becomes intense and protracted, and lipogenesis is shut down. Excessive lipolysis increases the risk for inflammatory and metabolic diseases and reduces lactation and reproductive performance. Among the mechanisms driving these deleterious effects, there are alterations in the inflammatory responses that lead to dysregulation of metabolic and immune functions within the AT and systemically.

Periparturient Adipose Tissue Remodeling, An Inflammatory Process Induced by Lipolysis

The consequences of enhanced lipolysis and reduced lipogenesis in AT of transition cows go beyond the release of NEFA into circulation. Excessive lipolysis also induces a remodeling process in the adipose organ that is characterized by macrophage infiltration and changes in its extracellular matrix (Contreras et al., 2017b).

Macrophages are the most abundant immune cell type in the AT of ruminants and comprise 5-10% of its stromal vascular cell (i.e., non-adipocytes) fraction (Ampem et al., 2016). In dairy cows, lipolysis enhances macrophage trafficking into AT during the transition period and in feed restriction-induced negative energy balance (Contreras et al., 2016, Vailati-Riboni et al., 2017, Newman et al., 2018). In cases where lipolysis is severe, such

[1] Contact at: Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI; E-mail: contre28@cvm.msu.edu.

as in displaced abomasum and ketosis, macrophages account for 20% of cells in the stromal vascular fraction or 2% of the total number of cells in AT (Contreras et al., 2015, Häussler et al., 2017). The role of AT macrophages during lipolysis is to contain and eliminate the highly cytotoxic lipolytic products that include NEFA, diglycerides, and monoglycerides (Lee et al., 2013).

Adipose tissue macrophages are broadly classified as classical (**M1**), which have active pro-inflammatory responses, and alternative (**M2**), which promote inflammation resolution. At any given time, macrophages within the AT are a mixture of M1, M2, and intermediate phenotypes. In periparturient cows with excessive lipolysis, including those with displaced abomasum and ketosis, AT macrophages are predominantly of the M1 phenotype and accumulate in aggregates within omental and subcutaneous depots (Contreras et al., 2015, Newman et al., 2018). In contrast, during moderate lipolysis induced by a short four-day feed restriction protocol in late-lactation cows, macrophage infiltration into the same AT depots occurs, but without any change in phenotype (Contreras et al., 2016).

The total mass of the AT of dairy cows is drastically reduced during the transition period. As the size of the adipose organ is diminished, its extracellular matrix composition is altered. Transcriptomics studies indicate that ruminants with higher lipolysis rate immediately after parturition have a stronger expression of collagen type I and III in AT compared to those with low lipolysis intensity (Faulconnier et al., 2011, Akbar et al., 2014). Expression of thrombospondin 1, an important extracellular matrix protein, is also upregulated during the first two weeks after calving when lipolysis rate reaches its peak [reanalysis of microarray data from Sumner-Thomson et al. (2011)]. Higher content of collagen type III and thrombospondin 1 in AT has been associated with impaired sensitivity to insulin by adipocytes (Buechler et al., 2015, Matsuo et al., 2015).

In transition cows, the inflammatory responses driven by AT remodeling perpetuate the lipolytic stimuli. Adipose tissue macrophages and other immune cells that are active during AT remodeling express and secrete potent blockers of insulin signaling including interleukin (**IL**) 1-β, IL-6, resistin, and tumor necrosis factor (TNF) -α (Martinez-Santibanez and Lumeng, 2014). This AT-specific insulin resistance was demonstrated by Zachut and colleagues in a group of transition dairy cows (Zachut et al., 2013). In their study, cows that lost more body condition during early lactation exhibited a significant reduction in the phosphorylation of downstream insulin signaling pathways, such as IRS-1 and AKT, compared to cows with low lipolysis and reduced weight loss. Remarkably, the activation of these insulin signaling pathways remained intact in the liver.

Lipolysis Products as Modulators of Inflammation

Fatty acids released during lipolysis can modulate the inflammatory phenotype of macrophages and other immune cells. For example, saturated FA induce an M1 like inflammatory phenotype in macrophages of the AT and other organs through the activation of Toll-Like Receptors (**TLR**) 1, 2, 4, and 6 (Velloso et al., 2015). Saturated FA such as lauric, myristic, and palmitic strongly activate TLR4 and enhance the secretion of monocyte

chemotactic protein-1 (**MCP-1**) (Han et al., 2010). Importantly, these saturated FA are preferentially mobilized from AT during lipolysis (Douglas et al., 2007, Contreras et al., 2017a).

Polyunsaturated fatty acids released during lipolysis modulate immune function and inflammation through their oxidation products (oxylipids). For example, linoleic acid is oxidized by 15-lipoxygenase (**LOX**) and by other non-enzymatic reactions to produce hydroxyl-octadecadienoic acids (**HODEs**). The molecule 13-HODE, a product of lipoxygenases and cyclooxygenases, promotes M2 polarization during lipolysis and acts as a PPAR gamma ligand that promotes adipogenesis and lipogenesis (Lee et al., 2016). In contrast, 9-HODE, a product of non-enzymatic oxidation by reactive oxygen species, promotes M1 polarization in tissues with lipid infiltration like vessels with atherosclerotic vessels (Vangaveti et al., 2010).

In dairy cows, linoleic acid is the most abundant polyunsaturated fatty acid in plasma and in AT, and it is preferentially mobilized by lipolysis during the transition period (Contreras et al., 2010). The dynamics of the plasma and AT content of its derived oxylipids are linked with lipolysis intensity. In healthy transition cows, plasma 13-HODE increases at 1 week after parturition from its levels at 1 week before calving. In contrast, 9-HODE, an indicator of oxidative stress, remains unchanged. In AT, 9-HODE tends to increase after parturition and 13-HODE is higher than at either 1 or 4 weeks before calving. Adipose tissue content of 13-HODE is positively associated with plasma beta-hydroxybutyrate concentrations (Contreras et al., 2017a). In the future, HODEs and oxylipids derived from other polyunsaturated fatty acids could be used as disease risk or lactation performance predictors in transition dairy cows. However, the dynamics of the synthesis of lipolysis products in transition dairy cows are poorly understood and should be the focus of future research.

Lipolysis and Immune Function

Excessive lipolysis impairs the efficacy of the inflammatory responses of cells from both the innate and adaptive immune systems [reviewed by Contreras et al. (2018)]. For example, cows challenged with *Strep. uberis* (intramammary) and with high lipolysis rates induced by feed restriction, exhibit an increased number of immature polymorphonuclear cells in circulation that have lower phagocytic activity compared with cows in positive energy balance (Moyes et al., 2009). In transition cows, high lipolysis rates are associated with reduced chemotactic activity and impaired phagocytosis in neutrophils (Nonnecke et al., 2003, Hammon et al., 2006). The same population of cells has limited oxidative burst when circulating NEFA are above 500 µ*M* and its viability is drastically reduced when NEFA concentrations reach >1.0 m*M* (Scalia et al., 2006; Ster et al., 2012).

The inflammatory response of macrophages and lymphocytes are also affected by excessive lipolysis during the transition period. Exposure to high NEFA concentrations reduces the mitogenic capacity of these mononuclear cells and limits their secretion of IFN-γ and IgM (Lacetera et al., 2005, Ster et al., 2012). High NEFA affect the function of cells of the adaptive immune system. High lipolysis increases B lymphocytes

populations and reduces γδ T lymphocytes. Reduced γδ T lymphocytes are associated with deficient immune responses in epithelial tissues (Pollock and Welsh, 2002), Collectively, these studies indicate that excessive lipolysis augments disease susceptibility in transition dairy cows by impairing the inflammatory responses of innate and adaptive immune cells and reducing their capacity to clear pathogens.

Adipokines Modulate Immunity and Metabolism

Besides NEFA and other lipolysis products, AT also modulates inflammatory processes and the immune and metabolic function of transition dairy cattle through the secretion of adipocyte-derived peptides (i.e., adipokines). Although there are over 100 adipokines described in rodents and humans, only adiponectin, leptin, and resistin are well characterized in transition dairy cows.

Adiponectin enhances insulin sensitivity in adipocytes, hepatocytes, and muscle cells. At the same time, this adipokine promotes fatty acid β-oxidation in the liver and the skeletal muscle. In transition dairy cows, plasma adiponectin concentrations are reduced during the first week after parturition compared to levels observed during the dry period and peak lactation (Kabara et al., 2014). In addition to metabolic effects, adiponectin modulates the inflammatory responses of human and bovine macrophages by reducing their expression and secretion of TNF alpha and other pro-inflammatory cytokines (Kabara et al., 2014). Adiponectin is also an important modulator of adaptive immunity as it is required for dendritic cell activation and T-cell polarization (Jung et al., 2012). Excessive AT inflammation reduces the secretion of adiponectin by adipocytes thus impairing the use of NEFA as an energy substrate in the liver and skeletal muscle.

Leptin modulates the inflammatory responses locally and systemically. Hypoleptinemia impairs the efficacy of T cell immune responses by promoting a shift in the phenotype of these cells from type 1 (pro-inflammatory) to a T2 helper. This phenotype change reduces the capacity for pathogen clearance. Leptin is also necessary for adequate maturation and inflammatory responses in dendritic cells. In macrophages and polymorphonuclear cells, leptin signaling is required for phagocytosis in response to toll-like-receptor activation (Naylor and Petri Jr, 2016). Similar to adiponectin, leptin reaches its nadir during the first week after calving, while the highest plasma concentrations are observed early during the dry period (Chilliard et al., 2005).

Resistin is another adipokine with the capacity to modulate immune and inflammatory responses systemically. In dairy cows, plasma resistin peaks during the first week after calving and returns to prepartum levels by five weeks in milk (Reverchon et al., 2014). In humans and rodents, resistin expression in adipocytes is stimulated by IL-6, hyperglycemia and growth hormone. Resistin impairs insulin signaling in adipocytes and is characterized as a pro-inflammatory adipokine (AL-Suhaimi and Shehzad, 2013). In bovines, resistin promotes lipolysis in adipocytes, but its effects on immune cells are currently unknown.

Modulating Lipid Mobilization in the Transition Period

Reduced lipogenesis and increased lipolysis are homeorhetic adaptations to negative energy balance that maintain energy availability for milk production. Although the process of lipid mobilization is affected by physiological, nutritional, genetic, management factors, there are different on-farm management, nutritional, pharmacological tools that can be used to limit lipolysis and could potentially promote lipogenesis [reviewed in (Contreras et al., 2018)].

A basic management and nutritional strategy that reduces lipolysis and promotes lipogenesis in the transition period is maximizing dry matter intake (DMI). In addition, it is necessary to limit the sudden drop in feed intake commonly observed during the final weeks of the dry period (Grummer et al., 2004). It is also imperative to balance prepartum diets to meet but not exceed energy requirements. This is usually accomplished by feeding high levels of fiber (Allen and Piantoni, 2014). When balancing rations for dry cows, it is necessary to take into account that overfeeding energy in the last weeks of gestation enhances lipolysis postpartum and increases the risk of fatty liver (Douglas et al., 2006). Cows that gain excessive BCS during the dry period have larger adipocytes that are more sensitive to lipolytic stimuli postpartum (De Koster et al., 2016). An additional feeding strategy is to boost the production of ruminal propionate postpartum by feeding high amounts of moderately fermentable starch (van Knegsel et al., 2007). This nutritional intervention limits AT lipolysis by enhancing insulin secretion (McCarthy et al., 2015).

To complement ration balancing strategies, the inclusion of nutritional supplements that limit lipid mobilization in the diet of transition cows can be considered. Feeding niacin (as nicotinic acid) reduces AT lipolysis by limiting the phosphorylation of hormone-sensitive lipase (Kenez et al., 2014). However, niacin supplementation has shown inconsistent results (Schwab et al., 2005, Havlin et al., 2016). This may be related to the timing of niacin supplementation. When fed only post-partum, niacin does not have FFA-lowering effects (Havlin et al., 2016). However, supplementing niacin throughout the entire transition period was shown to reduce AT lipolysis effectively (Schwab et al., 2005).

Methyl donors are also nutritional supplements that when fed to transition cows limit lipid mobilization. Among these, choline and methionine are reported to reduce lipolysis in AT when fed alone (Cooke et al., 2007, Li et al., 2016) or combined (Sun et al., 2016). Chromium supplementation may promote lipogenesis in AT by enhancing the activity of the insulin receptor in adipocytes (Vincent, 2004). Nevertheless, reports on the pro-lipogenic activity of chromium are inconsistent with some studies demonstrating a NEFA lowering effect (Hayirli et al., 2001, Yasui et al., 2014) and others showing no changes in plasma lipids (McNamara and Valdez, 2005, Smith et al., 2008). The pool of available pharmacological and nutritional interventions that reduce lipolysis or enhance lipogenesis is still very limited. Exploring new drug targets that enhance insulin sensitivity and or block the lipolytic response in adipocytes will facilitate the management of transition dairy cows.

Assessing Adipose Tissue Function During the Transition Period

Transition cow management programs often include routine measures of clinical and production parameters that can directly or indirectly evaluate AT function. Body condition score (**BCS**) is a good measure of subcutaneous adiposity, and the dynamics of BCS changes around parturition subjectively describes lipolysis rates. Alternatively, the use of image biomarkers obtained during ultrasound examination of AT provides an objective evaluation of BCS avoiding the variability associated with subjective visual measurements. Subcutaneous AT depth is strongly correlated with BCS evaluation when measured by trained personnel and is highly sensitive and specific in predicting plasma NEFA concentrations immediately prepartum and at calving in dairy cattle (Strieder-Barboza et al., 2015). If using subjective BCS assessment, mature cows should approach calving with a BCS of 3.0 to 3.5 and heifers with 3.25 to 3.75 as excessively thin or over-conditioned cows are more susceptible to disease.

Currently, the most common direct measure of lipolysis is plasma NEFA. In preventive herd medicine, pre and post-calving plasma NEFA values are used as early lactation disease predictors (Table 1). Similar to plasma NEFA, post-partum plasma BHB indicate NEB and predict disease risk in early lactation (Ospina et al., 2013). Lipolysis can also be evaluated at the group or individual animal level using the milk fat to milk protein percentage ratio (Table 1). Milk fat increases as plasma NEFA rise. Cows with milk fat to milk protein ratio values higher than 2 during the first week after calving are at a higher risk for developing retained fetal membranes, DA, clinical endometritis, and being culled before the end of lactation (Toni et al., 2011).

Novel biomarkers of AT function are being explored. Low concentrations of the NEFA transporters albumin and fetuin-A are associated with low lipogenic activity in AT (Strieder-Barboza et al., 2018) and may indicate a higher risk for developing fatty liver. HODEs and other oxylipids that are markers of inflammation in AT may provide disease risk information regarding AT function but still require large epidemiological studies to be validated. It is necessary to note that single biomarkers do not provide enough information to support management decisions during the transition period. However, when multiple biomarkers are analyzed together and combined with health, production, nutritional, and environmental data, biomarkers become essential for identifying metabolic problems related to extended periods of intense lipolysis.

Conclusions

Excessive lipolysis impairs the inflammatory responses of transition dairy cows in their AT and systemically. A “lipolytic” environment around parturition exacerbates immune responses that are ineffective in clearing pathogens and affect the metabolic function. Within AT, macrophage infiltration, a key characteristic of AT remodeling is beneficial for the adaptation to the catabolic state characteristic of the transition period. However, when AT macrophage infiltration is excessive, it triggers a vicious cycle where

excessive lipolysis can exacerbate AT inflammation, which in turn further intensifies lipolytic responses.

The focus on adipose tissue biology research given by human obesity and diabetes epidemics in western countries has expanded our understanding of the role of lipid mobilization in metabolic and immune function. However, there are marked differences between ruminant and monogastric adipose organ physiology (Laliotis et al., 2010), demonstrating that focused research is required on specific inflammatory and metabolic pathways linking adipocyte and immune cells function in dairy cattle.

References

Akbar, H., F. C. Cardoso, S. Meier, C. Burke, S. McDougall, M. Mitchell, C. Walker, S. L. Rodriguez-Zas, R. E. Everts, H. A. Lewin, J. R. Roche, and J. J. Loor. 2014. Postpartal subclinical endometritis alters transcriptome profiles in liver and adipose tissue of dairy cows. Bioinform. Biol. Insights 8:45-63.

AL-Suhaimi, E. and A. Shehzad. 2013. Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. Eur. J. Med. Res. 18(1):12.

Allen, M. S. and P. Piantoni. 2014. Carbohydrate nutrition: managing energy intake and partitioning through lactation. Vet. Clin. North Am. Food Anim. Pract. 30(3):577-597.

Ampem, G., H. Azegrouz, A. Bacsadi, L. Balogh, S. Schmidt, J. Thuroczy, and T. Roszer. 2016. Adipose tissue macrophages in non-rodent mammals: a comparative study. Cell Tissue Res. 363(2):461-478.

Arner, P. and D. Langin. 2014. Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. Trends Endocrinol. Metab. 25(5):255-262.

Bertoni, G. and E. Trevisi. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Vet. Clin. North Am. Food Anim. Pract. 29(2):413-431.

Buechler, C., S. Krautbauer, and K. Eisinger. 2015. Adipose tissue fibrosis. World J. Diabetes 6(4):548-553.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest. Anim. Endocrinol. 29(1):3-22.

Contreras, G. A., E. Kabara, J. Brester, L. Neuder, and M. Kiupel. 2015. Macrophage infiltration in the omental and subcutaneous adipose tissues of dairy cows with displaced abomasum. J. Dairy Sci. 98(9):6176-6187.

Contreras, G. A., N. J. O'Boyle, T. H. Herdt, and L. M. Sordillo. 2010. Lipomobilization in periparturient dairy cows influences the composition of plasma nonesterified fatty acids and leukocyte phospholipid fatty acids. J Dairy Sci 93(6):2508-2516.

Contreras, G. A., C. Strieder-Barboza, and J. De Koster. 2018. Symposium review: Modulating adipose tissue lipolysis and remodeling to improve immune function

during the transition period and early lactation of dairy cows. J. Dairy Sci. 101(3):2737-2752.

Contreras, G. A., C. Strieder-Barboza, J. de Souza, J. Gandy, V. Mavangira, A. L. Lock, and L. M. Sordillo. 2017a. Periparturient lipolysis and oxylipid biosynthesis in bovine adipose tissues. PloS one 12(12):e0188621.

Contreras, G. A., C. Strieder-Barboza, and W. Raphael. 2017b. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J. Anim. Sci. Biotechnol. 8:41.

Contreras, G. A., K. Thelen, S. Schmidt, C. Strieder-Barboza, C. Preseault, R. Raphael, M. Kiupel, J. Caron, and A. Lock. 2016. Adipose tissue remodeling in late-lactation dairy cows during feed restriction-induced negative energy balance. J. Dairy Sci. 99(12):10009-10021

Cooke, R. F., N. S. Del Río, D. Z. Caraviello, S. J. Bertics, M. H. Ramos, and R. R. Grummer. 2007. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. J. Dairy Sci. 90(5):2413-2418.

De Koster, J., R. K. Nelli, C. Strieder-Barboza, J. De Souza, A. L. Lock, and G. A. Contreras. 2018. The contribution of hormone sensitive lipase to adipose tissue lipolysis and its regulation by insulin in periparturient dairy cows. Sci. Rep. *In Press* DOI: 10.1038/s41598-018-31582-4.

De Koster, J., W. Van Den Broeck, L. Hulpio, E. Claeys, M. Van Eetvelde, K. Hermans, M. Hostens, V. Fievez, and G. Opsomer. 2016. Influence of adipocyte size and adipose depot on the in vitro lipolytic activity and insulin sensitivity of adipose tissue in dairy cows at the end of the dry period. J. Dairy Sci. 99(3):2319-2328.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89(6):2141-2157.

Douglas, G. N., J. Rehage, A. D. Beaulieu, A. O. Bahaa, and J. K. Drackley. 2007. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. J. Dairy Sci. 90(6):2941-2959.

Faulconnier, Y., Y. Chilliard, M. B. Torbati, and C. Leroux. 2011. The transcriptomic profiles of adipose tissues are modified by feed deprivation in lactating goats. Comp. Biochem. Physiol. Part D Genomics Proteomics 6(2):139-149.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Vet. Clin. North Am. Food Anim. Pract. 20(3):447-470.

Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113(1–2):21-29.

Han, C. Y., A. Y. Kargi, M. Omer, C. K. Chan, M. Wabitsch, K. D. Brien, T. N. Wight, and A. Chait. 2010. Differential effect of saturated and unsaturated free fatty acids on

the generation of monocyte adhesion and chemotactic factors by adipocytes. Diabetes 59(2):386-396.

Häussler, S., D. Germeroth, L. Laubenthal, L. F. Ruda, J. Rehage, S. Dänicke, and H. Sauerwein. 2017. Short communication: immunohistochemical localization of the immune cell marker CD68 in bovine adipose tissue: impact of tissue alterations and excessive fat accumulation in dairy cows. Vet Immunol Immunopathol. 183:45-48.

Havlin, J., P. Robinson, and J. Garrett. 2016. Niacin feeding to fresh dairy cows: immediate effects on health and milk production. Anim. Prod. Sci. 57(6) 1069-1078.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. J. Dairy Sci. 84(5):1218-1230.

Jung, M. Y., H. S. Kim, H. J. Hong, B. S. Youn, and T. S. Kim. 2012. Adiponectin induces dendritic cell activation via PLCγ/JNK/NF- κB pathways, leading to Th1 and Th17 polarization. J. Immunol. 188(6):2592-2601.

Kabara, E., L. M. Sordillo, S. Holcombe, and G. A. Contreras. 2014. Adiponectin links adipose tissue function and monocyte inflammatory responses during bovine metabolic stress. Comp. Immunol. Microbiol. Infect. Dis. 37(1):49-58.

Kaneene, J. B., R. Miller, T. H. Herdt, and J. C. Gardiner. 1997. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. Prev. Vet. Med. 31(1–2):59-72.

Kenez, A., L. Locher, J. Rehage, S. Danicke, and K. Huber. 2014. Agonists of the G protein-coupled receptor 109A-mediated pathway promote antilipolysis by reducing serine residue 563 phosphorylation of hormone-sensitive lipase in bovine adipose tissue explants. J. Dairy Sci. 97(6):3626-3634.

Krogh, M. A., N. Toft, and C. Enevoldsen. 2011. Latent class evaluation of a milk test, a urine test, and the fat-to-protein percentage ratio in milk to diagnose ketosis in dairy cows. J. Dairy Sci. 94(5):2360-2367.

Lacetera, N., D. Scalia, U. Bernabucci, B. Ronchi, D. Pirazzi, and A. Nardone. 2005. Lymphocyte Functions in Overconditioned Cows Around Parturition. J. Dairy Sci 88(6):2010-2016.

Laliotis, G. P., I. Bizelis, and E. Rogdakis. 2010. Comparative approach of the de novo fatty acid synthesis (lipogenesis) between ruminant and non ruminant mammalian species: from biochemical level to the main regulatory lipogenic genes. Curr. Genomomics 11(3):168-183.

Lee, Y.-H., S.-N. Kim, H.-J. Kwon, K. R. Maddipati, and J. G. Granneman. 2016. Adipogenic role of alternatively activated macrophages in β-adrenergic remodeling of white adipose tissue. Am. J. Physiol. Regul. Integr. Comp. Physiol. 310(1):R55-R65.

Lee, Y.-H., A. Petkova, and J. Granneman. 2013. Identification of an adipogenic niche for adipose tissue remodeling and restoration. Cell Metab. 18(3):355-67

Li, C., F. Batistel, J. S. Osorio, J. K. Drackley, D. Luchini, and J. J. Loor. 2016. Peripartal rumen-protected methionine supplementation to higher energy diets elicits positive effects on blood neutrophil gene networks, performance and liver lipid content in dairy cows. J. Anim. Sci. Biotechnol. 7:18.

Martinez-Santibanez, G. and C. N. Lumeng. 2014. Macrophages and the regulation of adipose tissue remodeling. Ann. Rev. Nutr. 34:57-76.

Matsuo, Y., M. Tanaka, H. Yamakage, Y. Sasaki, K. Muranaka, H. Hata, I. Ikai, A. Shimatsu, M. Inoue, T. H. Chun, and N. Satoh-Asahara. 2015. Thrombospondin 1 as a novel biological marker of obesity and metabolic syndrome. Metabolism 64(11):1490-1499.

McCarthy, M. M., T. Yasui, C. M. Ryan, S. H. Pelton, G. D. Mechor, and T. R. Overton. 2015. Metabolism of early-lactation dairy cows as affected by dietary starch and monensin supplementation. J. Dairy Sci. 98(5):3351-3365.

McNamara, J. P. and F. Valdez. 2005. Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. J. Dairy Sci. 88(7):2498-2507.

Moyes, K. M., J. K. Drackley, J. L. Salak-Johnson, D. E. Morin, J. C. Hope, and J. J. Loor. 2009. Dietary-induced negative energy balance has minimal effects on innate immunity during a Streptococcus uberis mastitis challenge in dairy cows during midlactation. J. Dairy Sci. 92(9):4301-4316.

Naylor, C. and W. A. Petri Jr. 2016. Leptin regulation of immune responses. Trends Mol. Med 22(2):88-98.

Newman, A. W., A. Miller, F. A. Leal Yepes, E. Bitsko, D. Nydam, and S. Mann. 2018. The effect of the transition period and postpartum body weight loss on macrophage infiltrates in bovine subcutaneous adipose tissue. J Dairy Sci. 102(2):1693-1701.

Nonnecke, B. J., K. Kimura, J. P. Goff, and M. E. Kehrli, Jr. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. J Dairy Sci 86(7):2359-2368.

Ospina, P. A., J. A. McArt, T. R. Overton, T. Stokol, and D. V. Nydam. 2013. Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. Vet. Clin. North Am. Food Anim. Pract. 29(2):387-412.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. Journal of dairy science 93(2):546-554.

Pollock, J. M. and M. D. Welsh. 2002. The WC1+ γδ T-cell population in cattle: a possible role in resistance to intracellular infection. Vet. Immunol. Immunopathol. 89(3):105-114.

Reverchon, M., C. Ramé, J. Cognié, E. Briant, S. Elis, D. Guillaume, and J. Dupont. 2014. Resistin in dairy cows: plasma concentrations during early lactation, expression and potential role in adipose tissue. PLoS ONE 9(3):e93198.

Scalia, D., N. Lacetera, U. Bernabucci, K. Demeyere, L. Duchateau, and C. Burvenich. 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. J. Dairy Sci. 89(1):147-154.

Schwab, E., D. Caraviello, and R. Shaver. 2005. Review: A meta-analysis of lactation responses to supplemental dietary niacin in dairy cows. The Professional Animal Scientist 21(4):239-247.

Smith, K. L., M. R. Waldron, L. C. Ruzzi, J. K. Drackley, M. T. Socha, and T. R. Overton. 2008. Metabolism of dairy cows as affected by prepartum dietary carbohydrate source and supplementation with chromium throughout the periparturient period. J. Dairy Sci. 91(5):2011-2020.

Ster, C., M. C. Loiselle, and P. Lacasse. 2012. Effect of postcalving serum nonesterified fatty acids concentration on the functionality of bovine immune cells. J. Dairy Sci. 95(2):708-717.

Strieder-Barboza, C., J. de Souza, W. Raphael, A. L. Lock, and G. A. Contreras. 2018. Fetuin-A: A negative acute-phase protein linked to adipose tissue function in periparturient dairy cows. J. Dairy Sci. 101(3):2602-2616.

Strieder-Barboza, C., A. Zondlak, J. Kayitsinga, A. F. A. Pires, and G. A. Contreras. 2015. Lipid mobilization assessment in transition dairy cattle using ultrasound image biomarkers. Livest. Sci. 177:159-164.

Sumner-Thomson, J. M., J. L. Vierck, and J. P. McNamara. 2011. Differential expression of genes in adipose tissue of first-lactation dairy cattle. J. Dairy Sci. 94(1):361-369.

Sun, F., Y. Cao, C. Cai, S. Li, C. Yu, and J. Yao. 2016. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PLoS ONE 11(8):e0160659.

Takeuchi, K. and K. Reue. 2009. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. Am. J. Physiol. Endocrinol. Metab. 296(6):E1195-1209.

Toni, F., L. Vincenti, L. Grigoletto, A. Ricci, and Y. H. Schukken. 2011. Early lactation ratio of fat and protein percentage in milk is associated with health, milk production, and survival. J. Dairy Sci. 94(4):1772-1783.

Vailati-Riboni, M., G. Farina, F. Batistel, A. Heiser, M. D. Mitchell, M. A. Crookenden, C. G. Walker, J. K. Kay, S. Meier, J. R. Roche, and J. J. Loor. 2017. Far-off and close-up dry matter intake modulate indicators of immunometabolic adaptations to lactation in subcutaneous adipose tissue of pasture-based transition dairy cows. J. Dairy Sci. 100(3):2334-2350.

van Knegsel, A. T., H. van den Brand, J. Dijkstra, W. M. van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J. Dairy Sci. 90(7):3397-3409.

Vangaveti, V., B. T. Baune, and R. L. Kennedy. 2010. Hydroxyoctadecadienoic acids: novel regulators of macrophage differentiation and atherogenesis. Therap. Adv. Endocrinol. Metab. 1(2):51-60.

Velloso, L. A., F. Folli, and M. J. Saad. 2015. TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. Endocr. Rev. 36(3):245-271.

Vincent, J. B. 2004. Recent advances in the nutritional biochemistry of trivalent chromium. Proc. Nutr. Soc. 63(1):41-47.

Yasui, T., J. A. A. Mcart, C. M. Ryan, R. O. Gilbert, D. V. Nydam, F. Valdez, K. E. Griswold, and T. R. Overton. 2014. Effects of chromium propionate supplementation during the periparturient period and early lactation on metabolism, performance, and cytological endometritis in dairy cows. J. Dairy Sci. 97(10):6400-6410.

Zachut, M., H. Honig, S. Striem, Y. Zick, S. Boura-Halfon, and U. Moallem. 2013. Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J. Dairy Sci 96(9):5656-5669.

Table 1. Selected biomarkers of adipose tissue function in plasma and milk of transition dairy cows

Biomarker[1]	Suggested reference values
NEFA (Ospina et al., 2010)	< 0.50 mmol/L
BHB (Ospina et al., 2010)	1.20 mmol/L subclinical ketosis; 1.40 mmol/L clinical ketosis
Cholesterol (Kaneene et al., 1997)	1.7 to 4.3 mmol/L prepartum; 2.7 to 5.3 mmol/L postpartum
Triglycerides (Bertoni and Trevisi, 2013)	0.12 to 0.65 mmol/L
GOT/AST (Bertoni and Trevisi, 2013)	46.5 to 103 IU/L
GGT (Bertoni and Trevisi, 2013)	21 to 37 IU/L
Acetoacetate/acetone (Krogh et al., 2011)	> 10 mg/dL of acetoacetate and acetone
Albumin	3.2 to 3.7 g/dL
Fetuin A (Strieder-Barboza et al., 2018)	0.75 to 1.0 mg/mL
Milk fat: protein ratio (Toni et al., 2011)	1.00 to 1.25

[1] NEFA = nonesterified fatty acids; BHB = beta-hydroxybutyrate; GOT/AST = glutamic-oxaloacetic transaminase/aspartate aminotransferase; GGT = gamma-glutamyl transferase.

SESSION NOTES