Adipose tissue as an integrator of metabolic and inflammatory signals in periparturient cows

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The adipose tissue (AT) is a multisite organ that participates in the endocrine and metabolic adaptations to the onset of lactation in periparturient dairy cows. Three primary AT functions secure a continuous supply of energy to maintain milk secretion and bodily function during periparturient negative energy balance. First, AT releases free fatty acids from triglycerides molecules stored in its adipocytes through lipolysis. Second, AT cellular components secrete peptides, also termed adipokines, that directly and indirectly adapt other organs to use free fatty acids (FFA) as energy substrates (Contreras et al., 2017). Third, the AT undergoes a remodeling process during the periparturient period due to the rapid loss of lipid reserves. This process includes infiltration of anti-inflammatory macrophages that promote the differentiation of new adipocytes capable of buffering the FFA excess accumulated during the first 2-3 weeks after parturition (Contreras et al., 2018). This summary paper highlights metabolic and endocrine functions of AT that are necessary for an effective transition from the dry period to early lactation in dairy cows.

Lipid mobilization

Within the adipocytes, FFA are constantly esterified (i.e., lipogenesis) and hydrolyzed (i.e., lipolysis) to and from triglyceride molecules. This process is known as lipid or fat mobilization. Around parturition due to the negative energy balance, the rate of lipolysis surpasses that of lipogenesis. Consequently, the AT releases FFA into circulation. Lipolysis can be broadly divided into basal and demand lipolysis (Lafontan and Langin, 2009). The rate of basal lipolysis increases with adipocyte volume. For this reason, over-conditioned cows that have large adipocytes release more FFA at basal conditions than lean cows (De Koster et al., 2016). Around parturition, demand lipolysis is regulated hormonally. Its primary activators are catecholamines, growth hormone, angiopoietin-like 4, and prolactin (Figure 1). However, lipolytic signals can be exacerbated during infectious and metabolic diseases by the presence of endotoxins in blood that directly activate adipocyte lipases and impair the response of adipocytes to insulin (Chirivi et al., 2021).

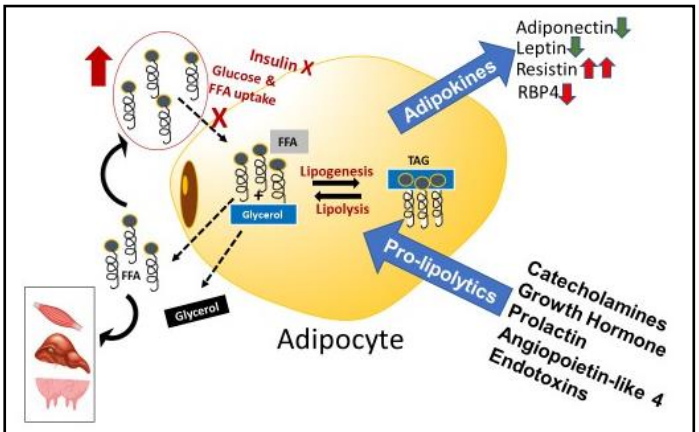

Figure 1. **Lipid mobilization** includes lipogenesis and lipolysis. Lipolysis during the periparturient period is induced by pro-lipolytic agents and releases free fatty acids (FFA) and glycerol from triglycerides (TAG). Adipocytes secrete adipokines that modulate metabolism systemically prioritizing the use of FFA for lactogenesis and thus supporting lactational homeorhesis.

The rapid increase in demand lipolysis during the periparturient period coincides with a drastic reduction in lipogenesis. This change is related to low plasma insulin, adipocyte insulin resistance, and the AT's inflammatory responses. All these factors inhibit the transcription of genes that promote de novo lipogenesis and triglyceride assembly. In healthy cows, as lactation progresses, energy balance becomes positive, plasma insulin returns to pre-calving levels, and AT lipolysis and inflammation are reduced. In contrast, cows that do not transition well into lactation exhibit an impaired response to the anti-lipolytic effects of insulin driven by chronic AT inflammation leading to lipolysis dysregulation (Contreras et al., 2015).

Adipokines as regulators of metabolic function

The AT controls systemic energy homeostasis by modulating the availability of energy-dense FFA and by secreting adipokines that have endo-, para-, and autocrine functions. These peptides are produced by the adipocytes and immune and vascular cells that reside in AT. Although there are over 200 adipokines described, only adiponectin, leptin, resistin, and retinol-binding protein 4 (RBP4) have been studied in dairy cattle in detail. It is important to note that the periparturient secretion patterns of these adipokines support lactation energy needs by redirecting glucose to the mammary gland, increasing FFA flow to the liver and epithelial cells in the mammary gland, and modulating energy intake [(Giesy et al., 2012), Figure 1].

Adiponectin enhances systemic insulin sensitivity and reduces lipolysis. Around parturition, its plasma content drops from 35 µg/mL during the dry period to <20 µg/mL immediately postpartum (Singh et al., 2014). Also, the expression of its receptors is reduced during the first three weeks postpartum (Saremi et al., 2014). Reflecting its anti-lipolytic effects, plasma adiponectin is negatively associated with circulating FFA (Kabara et al., 2014). Similar to adiponectin, plasma *leptin* peaks during the dry period (>6 ng/mL), and then its plasma concentration is reduced to <4 ng/mL by the first week after calving (Holtenius et al., 2003). Importantly, over-conditioned cows exhibit higher plasma leptin pre-calving than lean animals (Leon et al., 2004). This difference explains, in part, the more dramatic drop in dry matter intake and higher rates of lipolysis observed in cows with high body condition scores. Since leptin reduces appetite, its low postpartum levels promote the return of DMI to pre-calving levels.

In contrast to adiponectin and leptin, the synthesis of *resistin* increases during the periparturient period. Adipocytes and AT macrophages are the primary sources of this adipokine. Resistin promotes lipolysis by inhibiting insulin signaling and promoting inflammatory responses within the AT (Park et al., 2017). In dairy cows, plasma resistin concentrations rise from 45 ng/mL during the dry period to values above 75 ng/mL postpartum (Reverchon et al., 2014). Body condition score is positively associated with resistin production by AT macrophages (Reverchon et al., 2014). Therefore, over-conditioned cows will have higher circulating resistin compared to lean cows, making them more susceptible to excessive lipolysis and AT inflammation. Finally, *RBP4* is a potent inhibitor of adipocyte glucose uptake that also impairs the differentiation of preadipocytes into adipocytes (Romacho et al., 2014). Plasma

levels of this adipokine fall from >50 mg/mL one week before parturition to <30 mg/mL immediately after calving (Abd Eldaim et al., 2010). By inhibiting AT glucose utilization, RBP4 ensures energy prioritization to the mammary gland; however, impairing adipogenesis reduces the capacity of AT to buffer FFA excess predisposing to lipolysis dysregulation.

Adipose tissue remodeling

Lipolysis in AT induces a remodeling process within the organ characterized by a moderate inflammatory response with infiltration of immune cells, the proliferation of cells that are precursors of adipocytes, and production of lipid mediators of inflammation (Contreras et al., 2015, Contreras et al., 2017). Macrophages are the primary immune cell infiltrating AT during lipolysis. The specific inflammatory phenotype of these mononuclear cells has been broadly classified in classical (M1), which have active pro-inflammatory responses, and alternative phenotype (M2), which promote inflammation resolution. The central role of M2 macrophages in AT is to remove products of lipolysis that can be toxic to the cell, such as FFA and triglycerides (Kosteli et al., 2010). For this reason, moderate infiltration of M2 macrophages into AT is beneficial for periparturient cows. During negative energy balance states, healthy dairy cows have a balanced mixture of M1 and M2 phenotype macrophages in AT (Contreras et al., 2016). When periparturient lipolysis is excessive and protracted, macrophages aggregate, forming crown-like structures and polarizing towards the M1 phenotype [Figure 2, (Contreras et al., 2015, Newman et al., 2019)]. These macrophages secrete potent cytokines such as TNFa and interleukin 6 that impair insulin signaling leading to a vicious circle where AT inflammation exacerbates lipolysis, aggravating AT inflammation. It is important to note that TNFa and interleukin-6 can activate lipolysis directly in adipocytes (Chirivi et al., 2021).

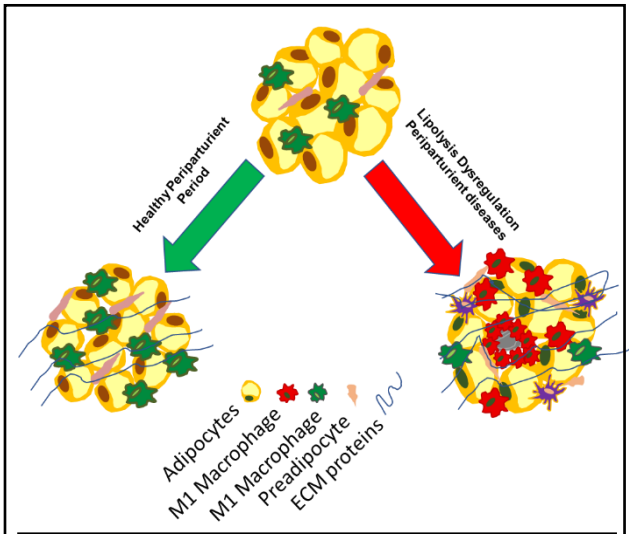

Figure 2. Adipose tissue remodeling during the periparturient period of dairy cows. In healthy cows, lipolysis induces infiltration of M2 macrophages and minimal changes in the structure and composition of the extracellular matrix (ECM). In cows that succumb to periparturient diseases, M1 macropahges infiltrate AT forming aggregates (crown-like structures) leading to

Regarding the proliferation of adipocyte precursors, this change is directly associated with the drastic changes in the volume of fat depots. During the first 40 days after calving, AT mass is reduced by 25-35% (Akter et al., 2011). Although not demonstrated in dairy cattle, rapid body weight loss induced by extended caloric restriction causes adipocyte death and the release of lipid remnants (Kosteli et al., 2010). As a response, preadipocytes proliferate to generate new adipocytes that replenish fat cell populations in a process termed adipogenesis. An adequate

adaptation to periparturient negative energy balance requires adipogenesis to support the buffering of FFA and other products of lipolysis that are toxic to cells.

Lipolysis induces the production of lipid mediators of inflammation in AT that are released into circulation. The activity of the lipases that break down triglycerides, such as hormone-sensitive lipase, releases linoleic, arachidonic, and other polyunsaturated fatty acids that are the substrate for prostaglandins and oxylipids (Contreras et al., 2020). The synthesis of these mediators of inflammation in the AT is probably one of the significant mechanisms sustaining the low-grade inflammation described by several authors in periparturient cows (Bradford and Swartz, 2020).

Adipose tissue dysregulation during periparturient diseases

The periparturient period is the lactation stage with the highest risk for metabolic and infectious diseases in dairy cows. Periparturient health events pose severe welfare issues and result in significant economic losses associated with decreased milk production, cost of treatment, and culling (USDA, 2015). To make things more complicated, periparturient illnesses often are presented as complexes of metabolic and inflammatory/infectious diseases (Probo et al., 2018). Two significant risk factors for increased disease susceptibility around parturition are lipolysis dysregulation (described above) and the dramatic increase in circulating endotoxins (e.g., Lipopolysaccharide (LPS) and lipoteichoic acids). Remarkably, common periparturient diseases such as mastitis, metritis, pneumonia, and metabolic events such as ruminal acidosis, heat stress, and parturition, often result in high circulating levels of LPS (Dickson et al., 2019). In humans and rodent models of disease, the inflammatory response to endotoxins, especially LPS, impairs the metabolic function of AT (Hersoug et al., 2018). In periparturient cows, experimental LPS exposure was associated with a higher incidence of displaced abomasum and placental retention and changes in metabolic parameters, including low plasma cholesterol and high β-hydroxybutyrate and FFA (Zebeli et al., 2011). The profile of these parameters indicates that endotoxemia possibly induces the development of lipolysis dysregulation in bovine AT.

The possible mechanisms by which the endotoxemia associated with multiple periparturient diseases triggers AT dysfunction are twofold. First, endotoxins activate lipolysis in dairy cows by three mechanisms (Chirivi et al., 2021): 1) binding to TLR4 increases the levels of intracellular cAMP through a calcium-dependent pathway (Song et al., 2007, Moon et al., 2011), leading to the activation of hormone-sensitive lipase. 2) TLR4 binding to LPS stimulates the activation of NF-κB that triggers the synthesis of pro-inflammatory cytokines, including TNFα (Lu et al., 2008). The latter promotes lipolysis by impairing the expression/function of perilipin, causing the thinning of the protein envelop of the lipid droplet and making it more susceptible to the action of hormone-sensitive lipase (Laurencikiene et al., 2007). 3) The activation of the mitogen-activated protein kinase /extracellular signal-regulated kinase (MAPK and ERK1/2). This pathway activates beta-adrenergic receptors that ultimately trigger the lipolytic activity of hormone-sensitive lipase (Zu et al., 2009, Hong et al., 2018). In contrast to bovines, in rodent adipocytes, LPS activates lipolysis preferentially by ERK1/2, as these species are resistant to NFκB triggered lipolysis (Zu et al., 2009, Bergan et al., 2013, Chi et al., 2014). In periparturient

dairy cows, lipolytic responses in AT are increased upon LPS exposure indicating that endotoxemia can potentiate AT responses to common stimulants of postpartum lipolysis such as catecholamines.

The second mechanism by which endotoxins may induce AT dysfunction is by altering the inflammatory phenotype of AT macrophages. Endotoxins promote macrophage M1 polarization; therefore, exposure to these bacterial byproducts early in the periparturient period may predispose cows to excessive lipolytic response during NEB postpartum. However, endotoxin-driven M1 polarization in AT may be affected by the degree of adiposity and by the development of endotoxin tolerance (Komegae et al., 2019). Therefore, future studies need to evaluate the effects of endotoxins on the phenotype of AT immune cells and its impact on metabolic function during the periparturient period.

Conclusion

Our knowledge of AT biology in periparturient dairy cows has advanced dramatically since the 1990s. However, there are still gaps in our understanding of the changes that occur during the periparturient period in AT. The role of AT remodeling on the homeorhetic adaptation to lactation, including the responses of AT to infectious and inflammatory diseases is unclear. Also, the impact of the anatomical differences on the immunobiology of AT depots and the endocrine function of fat tissues is unknown. Filling these gaps will support the development of preventive and corrective nutritional or pharmacological interventions to maintain an effective periparturient AT function.

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