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Adipose tissue remodeling in late-lactation dairy cows during feed-restriction-induced negative energy balance

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ABSTRACT

Excessive rates of demand lipolysis in the adipose tissue (AT) during periods of negative energy balance (NEB) are associated with increased susceptibility to disease and limited lactation performance. Lipolysis induces a remodeling process within AT that is characterized by an inflammatory response, cellular proliferation, and changes in the extracellular matrix (ECMT). The adipose tissue macrophage (ATM) is a key component of the inflammatory response. Infiltration of ATM-forming cellular aggregates was demonstrated in transition cows, suggesting that ATM trafficking and phenotype changes may be associated with disease. However, it is currently unknown if ATM infiltration occurs in dairy cows only during NEB states related to the transition period or also during NEB-induced lipolysis at other stages of lactation. The objective of this study was to evaluate changes in ATM trafficking and inflammatory phenotypes, and the expression of genetic markers of AT remodeling in healthy late-lactation cows during feed restriction-induced NEB. After a 14-d (d −14 to d −1) preliminary period, Holstein cows were randomly assigned to 1 of 2 feeding protocols, ad libitum (AL) or feed restriction (FR), for 4 d (d 1–4). Caloric intake was reduced in FR to achieve a targeted energy balance of −15 Mcal/d of net energy for lactation. Omental and subcutaneous AT samples were collected laparoscopically to harvest stromal vascular fraction (SVF) cells on d −3 and 4. The FR induced a NEB of −14.1 ± 0.62 Mcal/d of net energy for lactation, whereas AL cows remained in positive energy balance (3.2 ± 0.66 Mcal/d of NE_L). The FR triggered a lipolytic response reflected in increased plasma nonesterified fatty acids (0.65 ± 0.05 mEq/L on d 4), enhanced phosphorylation of hormone sensitive lipase, and reduced adipocyte diameter. Flow cytometry and immunohistochemistry analysis revealed that on d 4, FR cows had increased numbers of $CD172a^+$, an ATM (M1 and M2) surface marker, cells in SVF that were localized in aggregates. However, FR did not alter the number of SVF cells expressing M1 markers (CD14 and CD11c) or M2 markers (CD11b and CD163). This finding contrasts with the predominately M1 phenotype observed previously in ATM from clinically diseased cows. No changes were observed in the expression of ECMT-related or cell proliferation markers. In summary, an acute 4-d lipolytic stimulus in late-lactation dairy cows led to ATM infiltration with minimal changes in inflammatory phenotype and no changes in ECMT. These results underscore that physiological changes related to parturition, the onset of lactation, extended periods of lipolysis, or a combination of these can induce intense AT remodeling with enhanced ATM inflammatory phenotype expression that may impair the metabolic function of AT in transition dairy cattle.

Key words: adipose tissue remodeling, fatty acid, lipolysis, macrophages

INTRODUCTION

Adipose tissue (**AT**) lipolysis is a dynamic physiological response that involves not only the release of free fatty acids from adipocyte triglyceride reserves but also a remodeling process (Kosteli et al., 2010). The remodeling is accompanied by an inflammatory response with immune cell migration, proliferation of cellular components of the stromal vascular fraction (**SVF**), and changes in the extracellular matrix (**ECMT**) within AT (Rutkowski et al., 2015). An increased rate of total lipolysis, defined as the sum of basal and demand lipolysis, is the triggering stimulus for AT remodeling (Kosteli et al., 2010). The rate of basal lipolysis is determined by adipocyte TG content (Arner and Langin, 2014). In contrast, demand lipolysis is regulated hormonally in response to energy requirements. In dairy

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cattle, pronounced and protracted demand lipolysis in periods of negative energy balance (**NEB**), such as during the transition period, are associated with increased susceptibility to disease (Ospina et al., 2013). Despite this important epidemiological association, features of AT remodeling during lipolysis remain poorly understood. Although immune cell migration and activation are important components of the AT remodeling process in nonruminant species (Ferrante, 2013), little is known about these processes in ruminants. We recently demonstrated macrophage infiltration into AT of transition dairy cows with displaced abomasum (**DA**) and intense demand lipolysis (Contreras et al., 2015). In those transition cows, adipose tissue macrophages (**ATM**) infiltrated omental (**OM**) and subcutaneous (**SC**) fat, forming cellular aggregates similar to those seen in obese rodents and humans (Contreras et al., 2015). In healthy postpartum heifers, Akter et al. (2012) reported increased numbers of ATM in OM and mesenteric AT depots 1 d after parturition. Collectively, these findings suggest ATM trafficking changes occur in the bovine periparturient period and may be associated with the pathogenesis of metabolic diseases; however, it is currently unknown if ATM infiltration occurs in dairy cattle only during NEB states related to the transition period or also during NEB-induced demand lipolysis at other stages of lactation.

The macrophage is the most abundant immune cell type in the SVF of AT in domestic cattle, sheep, goats, cats, and dogs (Ampem et al., 2016). In nonpregnant, nonlactating cows, this mononuclear cell type represents around 10% of the SVF population (Contreras et al., 2015). During intense lipolytic events, ATM are the key component of the active inflammatory response and play a major role in the remodeling process of AT in rodents and humans (Kosteli et al., 2010; Suganami and Ogawa, 2010). The ATM remove fatty acids that are released in excess during lipolysis and reduce lipotoxicity in interstitial tissue. The ATM are also involved in the recruitment of new adipocyte progenitors by secreting chemotactic proteins such as osteopontin (Lee et al., 2013). The specific inflammatory phenotype of ATM is dependent on the types and concentrations of local cytokines, chemokines, and fatty acids that shape gene and protein expression profile during inflammation. Classical phenotype ATM (M1) stimulate pro-inflammatory pathways and secrete cytokines including tumor necrosis factor α (**TNFα**) and IL-6. Alternative phenotype ATM (M2) promote inflammation resolution by secreting cytokines such as IL-10 (Ferrante, 2013). Although it appears that M1 ATM are associated with intense periparturient lipolysis (Contreras et al., 2015), it is currently unknown if lipolysis during feed-restriction-induced NEB in mid and late lactation alters macrophage polarization and therefore could serve as a model for AT remodeling in transition cows.

Besides ATM infiltration, the AT remodeling process is also associated with changes in ECMT in monogastric animals. Increased deposition of collagen VI and thrombospondin during inflammatory responses in AT impairs adipocyte metabolic function by limiting lipid accumulation and insulin signaling (Varma et al., 2008; Pasarica et al., 2009). Less is known about the expression of ECMT proteins in dairy cows during lipolytic responses and their effect on AT metabolic function. In humans and rodents, AT remodeling with excessive infiltration of M1 ATM and increased deposition of ECMT impair adipocyte response to insulin, leading to enhanced total lipolysis that results in chronic elevation of circulating fatty acids. Characterizing AT remodeling in cows during NEB may identify mechanisms that lead to excessive rates of lipolysis that predispose transition cows to disease and poor lactation performance. Therefore, our objective was to evaluate changes in ATM trafficking and inflammatory phenotypes, and the expression of genetic markers of AT remodeling in healthy late-lactation cows during feed restriction-induced NEB.

MATERIALS AND METHODS

Cows

All animal procedures were approved by the Michigan State University Animal Care and Use Committee. Late-lactation (223 DIM ± 103 SD) bovine leukemia virus seronegative Holstein cows from the Michigan State University Dairy Field Laboratory (East Lansing, MI) were randomly assigned to 1 of 2 feeding protocols: ad libitum (**AL**) or feed-restricted (**FR**) in 2 separate blocks. In block 1, which was performed in August 2014, 8 cows in their second (n = 4), third (n = 2), and fourth (n = 2) lactations were selected. Cows were fed a common diet during a 14-d preliminary period (d −14 to −1) and then randomly assigned based on lactation number to AL (n = 4) or FR (n = 4) treatments. Average milk yields during the preliminary period were 35.6 ± 8.8 kg and 39.4 ± 6.2 kg for the AL and FR groups, respectively. The BCS scores before FR were AL = 3.1 ± 0.1 and FR = 3.3 ± 0.2. One cow from AL was removed from the study due to a health event before the beginning of the treatments. Caloric intake was reduced in FR cows for 4 d (d 1 to 4) to achieve a targeted NEB of −15 Mcal/d of NE_L similar to the transition period (3 wk before to 3 wk after calving;