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Short communication: Effects of body fat mobilization on macrophage infiltration in adipose tissue of early lactation dairy cows

Jenne De Koster,* Clarissa Strieder-Barboza,* Jonas de Souza,† Adam L. Lock,† and G. Andres Contreras*[1]
*Department of Large Animal Clinical Sciences, and
†Department of Animal Science, Michigan State University, East Lansing MI 48824

ABSTRACT

Intense lipolysis triggers an inflammatory response within adipose tissue characterized by adipose tissue macrophage (ATM) infiltration; however, the mechanisms triggering this process are poorly characterized in transition dairy cows. The aim of this study was to determine the association between ATM infiltration and body fat mobilization in the transition period, markers of excessive lipolysis, and adipose tissue expression of genes related to chemotactic and inflammatory responses. Subcutaneous adipose tissue samples were taken from the tailhead of 9 multiparous Holstein cows, 27 ± 2.2 d (far-off) and 10 ± 1.5 d (close-up) before and 9 ± 0.3 d after calving (fresh). Blood samples were collected by coccygeal venipuncture 2 h before adipose sample collections. Body condition score (BCS) was assessed independently by 3 experienced technicians at every time point. Based on BCS loss intensity between the close-up and fresh period, cows were divided into 2 groups: low BCS loss (LBCSL, change in BCS <0.25 units, n = 5) and high BCS loss (HBCSL, change in BCS >0.25 units, n = 4). Although none of the LBCSL cows had a health event, all cows in the HBCSL group suffered from one or more clinical disorder (retained placenta, milk fever, or ketosis) in the transition period. The number of ATM was determined by immunohistochemistry, and expression of selected chemotactic and inflammatory genes was determined by reverse-transcription quantitative real-time PCR in subcutaneous adipose tissue samples. The proportion of ATM in subcutaneous adipose tissue increased in HBCSL during the postpartum period. The proportion of ATM was not associated with serum β-hydroxybutyrate or free fatty acid concentrations on the day of adipose tissue collection. The ATM infiltration in the fresh period was associated with local expression of the chemotactic genes, C-C motif chemokine ligand 22 (*CCL22*), osteopontin (*SPP1*), and the receptor for SPP1, cluster of differentiation 44 (*CD44*). This supports a potential chemotactic role of *CCL22* and *SPP1* for ATM in bovine adipose tissue. None of the genes encoding pro- or anti-inflammatory mediators, tumor necrosis factor (*TNF*), *IL6*, and *IL10* were associated with the proportion of ATM. Our results indicate that ATM infiltration of subcutaneous adipose tissue is associated with body fat mobilization in early-lactation dairy cows and supports a role for ATM in the adaptation of adipose tissues to the metabolic challenges of the transition period.
Key words: adipose tissue remodelling, adipose tissue macrophage, immune response, transition cow

Short Communication

Adipose tissue (**AT**) is the main energy reserve in the body of cows. It is not uncommon that in the first weeks after calving, 30% to 50% of the triglycerides stored in adipocytes are broken down and their fatty acids (**FA**) mobilized (McNamara, 1991; von Soosten et al., 2011). Because FA are an important energy source for different tissues, their mobilization is a physiological mechanism to support the energy needs of lactation, thereby preserving glucose to be used for milk production (De Koster and Opsomer, 2013). Periparturient FA mobilization intensity is determined by the balance between lipogenesis and lipolysis within adipocytes and is mainly regulated by catecholamines, growth hormone, and insulin (Roche et al., 2009).

In humans and rodents, intense lipolysis triggers an inflammatory response within AT. Part of this inflammatory response is mediated by AT macrophages (**ATM**) that reside or infiltrate the adipose organ (Hotamisligil, 2006). These inflammatory changes within AT influence whole-body metabolic function, and when chronic, may lead to type 2 diabetes mellitus and cardiovascular disease (Cornier et al., 2008). In dairy cows, where adipocyte volume decreases in the first weeks after calving due to increased lipolysis and decreased lipogenesis, inflammatory changes develop in AT (Contreras et al., 2017b). Different research groups

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[1] Corresponding author: contre28@msu.edu

have documented ATM infiltration in adipose depots of dairy cows around parturition and during early lactation using immunohistochemistry (Akter et al., 2012), flow cytometry (Clark, 2014; Contreras et al., 2015), and microRNA markers (Vailati-Riboni et al., 2017). The ATM were abundantly present in AT from cows suffering from abomasal displacement and ketosis, 2 conditions that are associated with excessive lipolysis (Contreras et al., 2015). In the same study, expression of several pro-inflammatory genes, tumor necrosis factor (*TNF*), *IL6*, and C-C motif chemokine ligand 2 (*CCL2*), provided further evidence for an important interrelationship between lipolysis and inflammatory pathways within AT of transition cows (Contreras et al., 2015). We hypothesized that lipolysis and inflammation in AT are involved in a vicious cycle where ATM infiltration is increased by FA mobilization and production of inflammatory mediators by ATM further exacerbates lipolysis (Contreras et al., 2017b). To evaluate the crosstalk between lipolysis and inflammatory events within AT, the aim of our current study was to quantify ATM infiltration and adipose expression of selected chemotactic and inflammatory genes in subcutaneous AT from dairy cows during the transition period.

All animal procedures were approved by the Michigan State University Animal Care and Use Committee. A convenience sample of 9 multiparous (2.6 ± 0.73 lactations, mean ± SD) Holstein dairy cows from the Michigan State University Dairy Teaching and Research Center (East Lansing) were enrolled in the study. Cows were nonlactating and pregnant (>235 d of gestation) and housed in tiestall barns bedded with sawdust. Cows were fed a dry period TMR diet from 60 to 21 d prepartum, a close-up diet from 21 d until calving and an early-lactation diet following parturition (Supplemental Table S1; https://doi.org/10.3168/jds.2017-14318).

Blood and subcutaneous AT samples were taken at 27 ± 2.2 d (far-off) and 10 ± 1.5 d (close-up) before calving and 9 ± 0.3 d after parturition (fresh). The AT samples were collected from the tailhead using minimally invasive surgical techniques as previously described (Contreras et al., 2017a). Three to five grams of subcutaneous AT were obtained from the surgical site. Subsequent biopsies were obtained from opposite sites. Immediately after sampling, a part of the AT sample was fixed in 4% formaldehyde for 24 h, a part was snap frozen in liquid nitrogen and stored at −80°C until further analysis, and a part was collected in RNA Later (Life Technologies, Carlsbad, CA), snap frozen in liquid nitrogen, and stored at −80°C for gene expression analysis.

Blood samples were collected 2 h before adipose sample collections via coccygeal venipuncture using uncoated serum collection tubes, centrifuged for 20 min at 3,000 × *g* (15°C) for serum fraction collection, and stored at −80°C until further analysis. Serum concentrations of free fatty acids (**FFA**) and BHB were determined as described in Contreras et al. (2017a).

Body condition score of the cows was assessed at every sampling by 3 experienced technicians using a 5-point scale with 0.25-point increments according to Wildman et al. (1982). Cows were divided into 2 groups based on the change in BCS between the close-up and fresh period: low BCS loss (**LBCSL**, change in BCS <0.25 units, n = 5) and high BCS loss (**HBCSL**, change in BCS >0.25 units, n = 4). During the experiment, cows were monitored and treated for health disorders by farm personnel. Ketotic cows were diagnosed by depressed attitude, decreased feed intake, and decreased milk production, and the presence of moderate (40 mg/dL) to large (80 to 160 mg/dL) urinary acetoacetate concentrations detected by Ketostix (Bayer Corp., Elkhart, IN). Retained placenta was diagnosed by its attachment to the uterus for longer than 12 h after calving. Milk fever was diagnosed by clinical signs such as recumbency, muscle tremors, unsteadiness, cool extremities, and depression.

Immunohistochemistry was carried out as described by Contreras et al. (2015). Sections (5 μm) of formalin-fixed AT samples were stained with a mouse monoclonal antibody to detect bovine CD172a (1:100; DH59B, Washington State University Monoclonal Antibody Center, Pullman). Positive (bovine spleen) and negative controls were included. Sections were counterstained with hematoxylin, and CD172a-positive cells were detected using an Olympus BX-40 microscope (Olympus, Center Valley, PA). This anti-bovine CD172a antibody has been used previously to detect ATM using immunohistochemistry and flow cytometry in different studies, and has proven to be consistent in the detection of ATM (Contreras et al., 2015, 2016). For this study, 500 adipocytes were counted and the number of CD172a-positive cells detected while counting these adipocytes was used as a measure of macrophage infiltration into AT. Although CD172a is expressed by myeloid cells including monocytes, macrophages, and dendritic cells, and by neuronal cells (Ring et al., 2017), the proportion of dendritic cells and neuronal cells in AT is lower compared with the number of macrophages and monocytes (Ampem et al., 2016); therefore, for our statistical analyses, we defined ATM as CD172a-positive cells. The proportion of ATM was calculated as the number of CD172a-positive cells divided by 500 and given as a percentage.