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Nutrient-sensing kinase signaling in bovine immune cells is altered during the postpartum nutrient deficit: A possible role in transition cow inflammatory response

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ABSTRACT

Transition dairy cows experience a nutrient deficit, particularly in the immediate postpartum period. At the same time, the inflammatory balance is altered and cows exhibit an immune response primed for inflammatory response rather than tolerance. The mechanistic link that might be underlying the immunological effects due to the lack in nutrients is not fully understood. Studies in other species demonstrate an orchestrating role of nutrient-sensing kinases in the determination of immune phenotypes and immune cell proliferation and differentiation. Our primary objective was to investigate changes in energy storage and signaling through the protein kinase B (AKT)/mechanistic target of rapamycin complex 1 (mTOR) pathway in bovine immune cells in the transition period, as well as the association with cytokine expression profiles. A secondary objective was to test if supplementation with branched-chain amino acids alone or in combination with oral propylene glycol had any effect on the measured parameters. To assess cellular energy storage, glycogen concentration was measured by an enzymatic-fluorometric method in peripheral blood mononuclear cells (PBMC) of multiparous Holstein cows (n = 72) at 3 time points in the transition period (21 d before, 7 and 28 d after calving). At the same time points, phosphorylation of proteins in the AKT/mTOR pathway was assessed by immunoblotting in PBMC from 60 animals. Whole-blood leukocyte cytokine gene expression of *IL12B*, *IL6*, *IL1B*, *TNF*, and *IL10* was measured in samples from 50 animals by reverse-transcription quantitative PCR with and without stimulation of samples with 10 ng/mL of lipopolysaccharide. Compared with glycogen concentration of prepartum PBMC, glycogen concentration decreased by 37% on d 7 postpartum. The activation of AKT/mTOR in bovine PBMC postpartum was reduced compared with prepartum values. Results of reverse-transcription quantitative PCR showed an increase in cytokine gene expression postpartum compared with prepartum values. Supplementation with branched-chain amino acids alone or in combination with oral propylene glycol did not alter glycogen storage, AKT/mTOR activity, or inflammatory balance as assessed by the measured parameters in this study. We conclude that the nutrient deficit of the immediate postpartum period is sensed by bovine immune cells, and that it affects their energy storage as well as cellular signaling pathways postpartum. Temporal associations with changes in cytokine gene expression are intriguing and warrant further investigation of the role of this pathway as a possible link between metabolism and immune phenotype postpartum.
Key words: transition, AKT kinase, mTOR kinase, inflammation

INTRODUCTION

The transition from late pregnancy to early lactation is a critical period for dairy cows that is associated with pronounced negative nutrient balance and an increase in the risk for metabolic and infectious diseases. At the same time, decreased efficiency in pathogen clearance and an increase in the magnitude and duration of inflammation is typically seen. The changes in nutrient availability early postpartum include a lack of energy substrates, as well as AA. This deficit is caused by a prioritization of nutrient use by the mammary gland, associated with a dramatic decrease in circulating glucose, insulin, and EAA (Bell et al., 2000; Kuhla et al., 2011; Mann et al., 2016a). At the same time, we observe an increase in plasma nonesterified fatty acids from lipolysis, as well as ketone bodies originating primarily from hepatic fatty acid metabolism (McCarthy et al., 2015).

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The degree and persistence of inflammation in the transition period show individual variation, which is associated with differences in disease risk and production (Bionaz et al., 2007; Bradford et al., 2015; Huzzey et al., 2015). Several factors are discussed as contributors to the inflammatory state. Adipose tissue mobilization is regarded as a contributor to immune dysfunction postpartum, as both fatty acids as well as ketone bodies can have direct effects on the functionality of immune cells in early lactation (Sordillo and Raphael, 2013). Reactive oxygen species are produced at an increased rate in early lactation and may have a direct effect on immune cell function when produced in excess (Sordillo and Raphael, 2013). Additionally, the availability of energy and AA changes rapidly during this time, although less is known about the effect of the lack of these macronutrients on bovine immune cells.

Immune cells require large amounts of glucose upon activation (Pearce and Pearce, 2013). This large demand for glucose upon activation of the innate immune system was recently demonstrated in lactating dairy cows by infusion of lipopolysaccharide and was estimated to require more than 1 kg of glucose in a 12-h period (Kvidera et al., 2017). Glucose is stored in the form of glycogen in white blood cells and increases with the maturity of the cell (Wachstein, 1949). This storage plays a crucial role in the rapid availability of glucose upon activation (Wei et al., 2017). It was previously demonstrated that neutrophils, which depend on glycolysis to perform phagocytosis and chemotaxis, have diminished glycogen stores early postpartum, and that this depletion is more pronounced in cows with metritis (Galvao et al., 2010). Glycogen storage in immune cell types other than neutrophils warrants investigation in peripartum dairy cows.

Decreased availability of glucose, insulin, and AA, particularly the branched-chain AA (**BCAA**) Leu, are known to decrease the activity of the protein kinase B (**AKT**)/mechanistic target of rapamycin complex 1 (**mTOR**) pathway (Beugnet et al., 2003; Atherton et al., 2010; Altomare and Khaled, 2012). The blood concentrations of free EAA experience a sudden drop around the time of calving and for the first weeks postpartum (Meijer et al., 1995) that occur parallel to the decreased concentration of glucose and insulin in the bloodstream. In addition, the cellular energy balance is sensed by the AMP-activated kinase as a ratio between AMP:ATP. Activation of AMPK leads to an increase in cellular catabolic pathways, and further inhibits the PI3K/AKT/mTOR-pathway upon activation (Blagih et al., 2012). The AKT/mTOR kinase signaling cascade is an important nutrient-sensing pathway in mammalian cells and this pathway is central in determining the type, magnitude, and duration of innate immune responses (Weichhart et al., 2015; Vergadi et al., 2017). We have shown previously that the activity of the AKT/mTOR pathway is decreased in tissues of postpartum dairy cows when the nutrient deficit is most severe (Mann et al., 2016b,c). The motivation of this work was to address the gap in knowledge by investigating temporal changes in nutrient-sensing pathways in the transition period that are known to play a critical role in regulating the magnitude and duration of the inflammatory response in other mammals. Our hypothesis was that changes in nutrient availability in the postpartum period are also associated with changes in energy storage and activity of the described nutrient-sensing cellular signaling pathways in bovine immune cells. In addition, and because of the well documented association of mTOR activity and BCAA availability, as well as the documented immunological benefits of BCAA in other species (Calder, 2006), we wanted to test if supplementation with these AA alone or in combination with a glucose precursor in the postpartum period has an effect on these signaling pathways.

Our primary objective was to investigate the glycogen storage in bovine peripheral blood mononuclear cells (**PBMC**) around the time of calving, as well as to describe the activity of nutrient-sensing cellular signaling pathways during the transition period and associate this with the inflammatory profile of bovine whole blood. Our secondary objective was to investigate if supplementation with BCAA alone, or BCAA in combination with the glucose precursor propylene glycol, in postpartum dairy cows is associated with changes in energy storage, cellular signaling, and inflammatory profile in the transition period.

MATERIALS AND METHODS

Animals and Treatments

Samples were collected from animals enrolled in a larger study investigating the role of BCAA supplementation alone or in combination with propylene glycol in postpartum dairy cows. All procedures for this experiment were approved by the Cornell University Institutional Animal Care and Use Committee (number 2014–0118). Cows were housed in the Cornell University Ruminant Center in Harford, New York, and samples were collected between February and June 2016. All cows were fed the same dry period and fresh period TMR ad libitum, and samples were analyzed throughout the study using near infrared analysis (Cumberland Valley, Maugansville, MD). Cows were fed once daily at 0700 h using individual feed buckets in a tiestall. Animals were enrolled in 1 of 3 treatment groups on the day of calving: cows that received 550 g