J. Dairy Sci. 98:1–13
http://dx.doi.org/10.3168/jds.2014-9034

Hepatic metabolic response of Holstein cows in early and mid lactation is altered by nutrient supply and lipopolysaccharide in vitro

M. Garcia, B. J. Bequette,[1] and K. M. Moyes[2]
Department of Animal and Avian Sciences, University of Maryland, College Park 20742

ABSTRACT

The metabolic response of the liver during periods of inflammation is poorly understood. The objective of this study was to characterize the effects of nutrient supply and lipopolysaccharide (LPS) challenge on hepatic intermediate metabolism of early- and mid-lactation cows by employing gas chromatography-mass spectrometry with stable isotope tracer. Twelve multiparous Holstein-Friesian cows in early (n = 6; 12 ± 4.2 d in milk) and mid (n = 6; 115 ± 13.5 d in milk) lactation were used for this study. Liver biopsies were performed on all cows. Liver slices (40–60 mg) were incubated in a 37°C water bath for 2 h with either control (phosphate buffered saline), pyruvate (PYR; 1 m*M* unlabeled pyruvate and 1 m*M* [$^{13}C_3$]pyruvate), pyruvate + propionate (PYR+PRO; 1 m*M* unlabeled pyruvate, 1 m*M* [$^{13}C_3$] pyruvate, and 2 m*M* sodium propionate), or pyruvate + AA (PYR+AA; 1 m*M* unlabeled pyruvate, 1 m*M* [$^{13}C_3$] pyruvate, and 2 m*M* AA solution), and LPS (0.0 or 0.2 μg/mL) was added to flasks per treatment. Enrichment of isotopomers in metabolic equilibrium with Krebs cycle intermediates was assessed. Pyruvate fluxes and the enzymatic activity of pyruvate carboxylase (PC) versus pyruvate dehydrogenase (PDH) and phosphoenol pyruvate carboxykinase (PEPCK) were calculated. Media were analyzed for concentrations of tumor necrosis factor-α (TNF-α), glucose, and haptoglobin. Data were analyzed as randomized block (stage of lactation) design in a factorial arrangement of nutrient treatments by LPS dose. Challenge with LPS increased the mRNA abundance of TNF-α, haptoglobin, and serum amyloid A 2, and the concentration of TNF-α in media. Challenge with LPS increased mRNA abundance of PC but reduced the enrichment of $^{13}C_1$[M1] and $^{13}C_2$[M2] alanine and tended to reduce the enzymatic activity of PEPCK. Incubation with PYR+PRO and PYR+AA increased the flux of pyruvate to acetyl CoA. However, only PYR+PRO increased the enzymatic activity of PEPCK and PDH versus PC and decreased the mRNA abundance of PC. Cows in early lactation tended to receive a greater contribution of pyruvate to the oxaloacetate flux via the lower PDH versus PC activity and a higher mRNA abundance of PC than cows in mid lactation. Our results suggest that regardless of stage of lactation and nutrient supplement, hepatic gluconeogenesis was impaired during inflammation. Further research examining how various nutrients support liver function and improve the immunometabolic response of liver during inflammation is warranted.

Key words: Holstein cow, isotope tracer, liver metabolism, lipopolysaccharide challenge

INTRODUCTION

In periparturient cows, metabolic stress occurs as consequence of marked endocrine, neurologic, and immune system changes to divert the partitioning of nutrients from the fetus to the mammary gland for milk synthesis (Ingvartsen and Moyes, 2013). Moreover, the periparturient period is characterized by a period of immunosuppression, with reduced function of immune cells and development of oxidative stress that increases the risk of several diseases at this time (Sordillo et al., 2009). Although, the mechanisms are still not well understood, this transition period has been associated with an increased susceptibility to inflammatory diseases resulting in reduced animal health and economic outcome to the farmer (Mallard et al., 1998; Hammon et al., 2006). To meet the nutrient demands for lactation, most cows experience a period of negative energy balance (Ingvartsen, 2006), i.e., −1 wk before ∼3 wk relative to parturition (Grummer, 1995). Under conditions of negative energy balance, elevated concentrations of circulating NEFA, BHBA, and lower glucose have been associated with an increased risk of digestive disorders, uterine problems (Ospina et al., 2010; Chapinal et al., 2011), and mastitis (Moyes et al., 2009b). From a global gene expression perspective, cows with severe negative energy balance have marked alterations in hepatic metabolism and pro-inflammatory response (Loor et al., 2005; McCarthy et al., 2010).

Received October 28, 2014.
Accepted May 10, 2015.
[1]Deceased.
[2]Corresponding author: kmoyes@umd.edu

In addition to its central role in metabolism, the liver secretes inflammatory mediators, i.e., acute phase proteins (serum amyloid A, **SAA**; haptoglobin, **Hp**) and cytokines [i.e., tumor necrosis factor-α (**TNF-α**)], during inflammation (Jørgensen et al., 2012) and in response to intramammary *Escherichia coli* endotoxins such as LPS (Vels et al., 2009). Recent work suggests that the ability of the liver to metabolize FA is reduced and key genes associated with AA, glucose, lipid, and FA metabolic processes are downregulated after LPS challenge (Jiang et al., 2008). Reduced hepatic function may increase risk of subsequent disease, especially during early lactation (Drackley et al., 2005).

The relationship between hepatic metabolism, inflammation, and risk of subsequent disease is poorly understood. We have previously shown that glucose regulation is altered during the early response to mastitis (Moyes et al., 2009a, 2014). Furthermore, during early lactation when glucose availability is low, cows use AA derived from skeletal muscle degradation for energy and hepatic gluconeogenesis (Ingvartsen and Moyes, 2013). To our knowledge, no studies have examined whether increased AA or glucose supply improve hepatic function during inflammation.

The use of stable isotopes and GC-MS technologies in metabolic flux analysis provides unique measures of the dynamics of cellular metabolism and its regulation, which otherwise would not be accessible from static measurements of mRNA and protein expression or metabolite concentrations (Bequette et al., 2006; Griffin and Des Rosiers, 2009). However, a limited number of studies report using fluxomic technologies to evaluate the intermediary metabolism of nutrients and their utilization by tissues and cells (Bequette et al., 2006; El-Kadi et al., 2009). To our knowledge, no studies have examined how nutrients alter the inflammatory and metabolic responses of liver after LPS challenge in vitro and how stage of lactation may alter this response. Consequently, the objective of the current study was to characterize the effects of nutrient supply and LPS challenge on hepatic inflammatory and intermediate metabolism for cows in early and mid lactation by employing GC-MS with stable isotope tracers coupled with both protein- and transcription-level responses in vitro.

MATERIALS AND METHODS

Cow Management and Biopsy Procedure

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland. Twelve multiparous Holstein-Friesian cows in early (n = 6, 12 ± 4.2 DIM) and mid (n = 6, 115 ± 13.5 DIM) lactation were used for this study. To be eligible, cows were free of clinical signs of disease, including normal heart and respiratory rate, with milk SCC <200,000 cells/mL before biopsy. Cows were fed twice daily a standard TMR formulated to meet NRC (2001) requirements and were milked twice daily at 0700 and 1900 h. Liver biopsies were performed on all cows after the morning milking and before the morning feeding (~0800 h). Cows were housed and fed in free stalls and had access to water ad libitum. At the time of biopsy, 20 mL of blood was collected into vacutainer tubes without coagulant (Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA) via coccygeal venipuncture. After the biopsy procedure, cows were placed in tie-stalls for 10 d for daily monitoring. Blood was allowed to clot at room temperature for ~2 h. Tubes were centrifuged at 2,095 × *g* for 15 min at 4°C (model 5810R, Eppendorf, Hauppauge, NY) and serum was frozen at −20°C for metabolite analysis.

Liver tissue (~1 to 2 g) was obtained under local anesthesia via puncture biopsy as described previously (Carlson et al., 2006). Liver tissue was placed in a 50-mL tube containing ice-cold sterile PBS and transferred to the laboratory on ice (~2 h).

Liver Explant Treatments and Incubation

Details of chemicals and reagents used are available in the Supplemental Material (http://dx.doi.org/10.3168/jds.2014-9034). Treatments were prepared using Krebs ringer bicarbonate buffer (**KRBB**) as basal medium, as previously reported (Drackley et al., 1991; Carlson et al., 2006), for a final volume of 2.8 mL in 25-mL flasks. Treatments were control (**CTL**; KRBB only), pyruvate (**PYR**; 1 m*M* unlabeled pyruvate and 1 m*M* [$^{13}C_3$]pyruvate), pyruvate + propionate (**PYR+PRO**; 1 m*M* unlabeled pyruvate, 1 m*M* [$^{13}C_3$]pyruvate, and 2 m*M* sodium propionate), and pyruvate + AA (**PYR+AA**; 1 m*M* unlabeled pyruvate, 1 m*M* [$^{13}C_3$]pyruvate, and 2 m*M* AA solution). Liver tissue (~20 mg) was sliced using a Krumdieck automated tissue slicer (Alabama Research and Development, Munford, AL). Each flask (25 mL) contained 40 to 60 mg of tissue. Treatments were run in duplicate. Flasks were transferred to a sterile hood, and 200 μL of PBS or LPS (3 μg/mL working solution, to provide 0.2 μg of LPS/mL of total culture medium, L055, Sigma Aldrich) was added. Flasks were gassed with 95 CO_2:5 O_2 for 10 s and immediately sealed with a rubber septum. Flasks were incubated in a shaking water bath (VWR International, Philadelphia, PA) at 37°C for 2 h. A pilot study revealed that 2-h incubation time was sufficient to establish metabolic equilibrium of Krebs cycle intermediates for GC-MS analyses described below. After incubation,