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Liver proteomic analysis of postpartum Holstein cows exposed to heat stress or cooling conditions during the dry period

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ABSTRACT

Heat stress negatively affects cow performance, compromises immune function, and increases susceptibility to metabolic disorders, particularly during the dry period and as cows transition from gestation to lactation. Metabolic adaptations of the liver are critical for successful transition, yet it is unclear how heat stress affects metabolic pathways within the liver at the proteomic level. The objective of this study was to investigate the liver proteome of postpartum cows that were cooled or heat stressed during the dry period to gain insight into how protein expression is altered by prior heat stress and may contribute to performance and disease outcomes. During the dry period, cows were either housed in shaded barns with fans and water soakers [cooled group (CL); n = 5] or in shaded barns lacking these cooling devices [heat-stressed group (HT); n = 5]. Liver biopsies were collected at 2 d postpartum, and protein content was analyzed by label-free quantitative shotgun proteomics (nanoscale liquid chromatography coupled to tandem mass spectrometry). In the most comprehensive bovine liver proteomics analysis completed to date, we identified 3,270 proteins, 75 of which were differentially expressed between HT and CL cows (fold change ±1.2). The top pathways differing between HT and CL cows were oxidative phosphorylation, mitochondrial dysfunction, farnesoid X receptor/retinoid X receptor (FXR/RXR) activation, and the methylmalonyl pathway. Cooling cows during the dry period likely improves ATP production, reduces oxidative stress, and prevents excessive accumulation of hepatic triglycerides and cholesterol, which may contribute to greater milk yield and lower susceptibility to transition-related diseases.

Key words: oxidative phosphorylation, mitochondria, transition, oxidative stress

INTRODUCTION

The transition between late gestation and the onset of lactation is a physiologically challenging process for dairy cows and the predominant time for occurrence of diseases and metabolic disorders (Goff and Horst, 1997; Drackley, 1999). During the periparturient period, increasing energy and nutritional demands for milk production and maintenance needs exceed DMI and a negative energy balance occurs (Bell, 1995; Drackley et al., 2005). To compensate for insufficient nutrient intake, a coordinated suite of physiological adaptations, including enhanced bone resorption, greater intestinal calcium transport, and increased hepatic gluconeogenesis, promotes delivery of substrates to the mammary gland to support milk synthesis (Reynolds et al., 2003; Horst et al., 2005). Furthermore, lower circulating insulin permits fat mobilization from adipose tissue induced by catabolic signaling and spares glucose for milk production (Bell, 1995; Rhoads et al., 2004).

The liver is a central organ coordinating transition-related adaptations in lipid, carbohydrate, and protein metabolism. First, circulating nonesterified fatty acids (**NEFA**) released during fat catabolism from adipose stores are taken up, oxidized, and used to produce ATP in the liver (Reynolds et al., 2003). Additionally, NEFA can be partially oxidized in the liver to produce ketone bodies and used as an alternate energy source in peripheral tissues (Drackley et al., 2005). Excessive NEFA, however, can lead to esterification of triglycerides and storage in the liver as well as greater conversion to ketone bodies, which can result in fatty liver disease and ketosis (Drackley et al., 2005; Schäff et al., 2012). Second, hepatic gluconeogenesis is enhanced, whereby glycerol from fat catabolism, propionate, and amino acids are converted to glucose (Reynolds et al., 2003). Finally, to a lesser extent, amino acids may be converted to pyruvate or tricarboxylic acid cycle intermediates within the liver and used for ATP synthesis (Schäff et al., 2012).

Various management practices, nutrition, and environmental factors, such as heat stress, may impede the

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metabolic shifts necessary for successful transition in otherwise healthy cows (Drackley et al., 2005). Heat stress induces several homeorhetic mechanisms that prioritize thermoregulation over other physiological processes (Baumgard and Rhoads, 2013). As a result, heat-stressed cows have markedly reduced milk production throughout lactation and are more susceptible to metabolic disorders during the transition period (Collier et al., 1982; Kadzere et al., 2002; Bernabucci et al., 2010; Tao and Dahl, 2013). Importantly, adverse effects of heat stress on lactation performance occur not only when cows are exposed to heat stress during lactation, but also when heat stress exposure is confined to the dry period, indicating carry-over effects of environmental stressors in the dry period to the subsequent lactation (Tao and Dahl, 2013). Limited evidence from targeted gene studies indicates that heat stress during the dry period alters mammary gland remodeling and hepatic lipid metabolism (do Amaral et al., 2009, 2011; Tao et al., 2011). For example, mRNA expression of carnitine palmitoyltransferase 1-A (*CPT1A*) and very long chain acyl-CoA dehydrogenase (*ACADVL*), which encode proteins involved in fatty acid β-oxidation, were downregulated in heat-stressed cows at 2 d postpartum. Nevertheless, relatively little is known about the effect of heat stress during the dry period on other metabolic pathways and hepatic function after calving. The objective of this study was to investigate the liver proteome of postpartum cows that were either cooled or heat stressed during the dry period to gain insight into how molecular pathways are altered by heat stress and may contribute to poor lactation performance and increased incidence of transition-related disorders observed in heat-stressed cows.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the University of Florida Dairy Unit (Hague, FL) in summer 2008, as described in detail elsewhere (do Amaral et al., 2011). Briefly, cows were dried off 46 d before expected calving and housed in shaded, sand-bedded, freestall barns. Cows were randomly assigned to 1 of 2 treatments for the duration of the dry period (dry-off to calving); the cooled group (**CL**; n = 9) was housed in a barn with fans and soakers, and the heat-stressed group (**HT**; n = 12) was housed in the same barn but lacked access to these cooling devices. Treatment groups were similar in mature-equivalent milk production in the previous lactation and parity (1.7 ± 1.1 lactations for CL and 1.7 ± 0.9 lactations for HT). After calving, all cows were housed in the same shaded, sand-bedded, freestall barn with soakers and fans. Air temperature and humidity in the barns were recorded with Hobo Pro series Temp probes (Onset Computer Corp., Pocasset, MA). The effectiveness of treatments was confirmed; temperature-humidity index between the 2 barns were similar, yet CL cows had lower rectal temperatures and respiration rates throughout the dry period relative to HT cows (do Amaral et al., 2011). Dry cows were fed a TMR once daily at 0900 h and after calving were fed a TMR for lactating cows twice daily at 0800 and 1200 h. Dry matter intake was recorded daily from dry off to 42 d postcalving. As reported in do Amaral et al. (2011), DMI of CL cows was higher than that of HT cows around the time of calving but not at any other time during the dry period or after calving and there was a tendency for CL cows to have greater milk yield relative to HT cows.

Liver Biopsy Collection

Liver tissue was collected at 2 d postpartum from the right side of the animal through the 10th or 11th intercostal space on an approximate line from the hooks to the elbow. Following sterilization of the area, liver tissue (0.5 to 1.5 g of wet weight) was collected using a stainless steel percutaneous liver biopsy tool (Aries Surgical, Davis, CA). Extracted liver tissue was rinsed with sterile saline, snap frozen in liquid nitrogen, and stored at −80°C until proteomics analysis. Tissue from 10 animals (5 per treatment) that were asymptomatic for ketosis, metritis, mastitis, or other health complications was used for proteomics analysis in the current study. Tissue from the remaining 4 CL cows and 7 HT cows were used to assess hepatic mRNA expression in a previous study (do Amaral et al., 2011). Sample size was based on previous studies with similar design in dairy cows (Zachut, 2015; Zachut et al., 2016).

Tissue Preparation for Proteomics Analysis

Approximately 30 mg of tissue was homogenized in 1 mL of lysis buffer that comprised 100 m*M* Tris-HCl, 4% SDS, 0.1 *M* dithiothreitol, 0.2 *M* phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Tissue was homogenized using 1-mm ceramic beads in a Precellys 24 Bead-Mill Tissue Homogenizer (Bertin Corp., MD) at 5,000 rpm for 15-s intervals, alternating with brief centrifugation (4°C, 10,000 × *g*) until completely homogenized. The homogenate was incubated for 1 h at 4°C followed by centrifugation (4°C, 10 min, 13,000 × *g*). The protein phase was transferred to a clean microcentrifuge tube