Impact of Maternal Heat Stress in Late Gestation on Blood Hormones and Metabolites of Newborn Calves

J-R Guo, A. P. A. Monteiro, X-S. Weng, B. M. Ahmed, J. Laporta, M. J. Hayen, G. E. Dahl, J. K. Bernard, and S. Tao

ABSTRACT

Maternal heat stress alters immune function of the offspring, as well as metabolism and future lactational performance, but its impact on the hormonal and metabolic responses of the neonate immediately after birth is still not clear. The objective of this study was to investigate the blood profiles of hormones and metabolites of calves born to cows that were cooled (CL) or heat-stressed (HS) during the dry period. Within 2 h after birth, but before colostrum feeding, blood samples were collected from calves (18 bulls [HS: n=10; CL: n=8] and 20 heifers [HS: n=10; CL: n=10]) born to CL or HS dry cows, and hematocrit and plasma concentrations of total protein, prolactin, insulin-like growth factor-I, insulin, glucose, nonesterified fatty acid, and BHBA were measured. Compared with CL, HS calves had lower hematocrit, and tended to have lower plasma concentrations of insulin, prolactin, and insulin-like growth factor-I. However, maternal heat stress had no impact on plasma levels of total protein, glucose, nonesterified fatty acid, and BHBA immediately after birth. These results suggest that maternal heat stress desensitizes a calf's stress response and alters fetal development by reducing the secretion of insulin-like growth factor-I, prolactin, and insulin.

INTRODUCTION

Maternal heat stress during the dry period negatively affects offspring. Compared with those from cooled (**CL**) cows, calves born to heat-stressed (**HS**) dry cows have impaired passive and cell-mediated immune function (Tao et al., 2012; Monteiro et al., 2014), and altered metabolism during the preweaning period (Tao et al., 2014; Monteiro et al., 2016). Moreover, Monteiro et al. (2013) reported that maternal heat stress during the dry period reduced a heifer's milk production during the first lactation. However, no studies have attempted to examine the fetal response to maternal heat stress during late gestation in the bovine, perhaps due to the technical difficulty in assessing the fetal metabolic and hormonal responses in utero. Although confounded with the stress responses related to parturition, the hormonal responses and blood metabolite profiles of the calves immediately after birth prior to milk ingestion could provide important information related to the fetal responses to maternal heat stress before the complications of postnatal nutrition and management are encountered. Indeed, Tao et al. (2012) observed that calves born to HS dry cows had a lower plasma concentration of cortisol immediately after birth compared with calves born to CL cows, indicating that maternal heat stress alters the fetal development of the hypothalamus-pituitary-adrenal axis and related stress responses during the postnatal period. Thus, the hypothesis is that maternal heat stress during the dry period alters the offspring's metabolic and hormonal responses immediately after calving. The objective of the present study was to examine the blood hormone and metabolite profiles immediately after birth of calves born to CL and HS cows during the dry period.

MATERIALS AND METHODS

The animal trial was conducted at the Dairy Unit of the University of Florida during the summer of 2014, and the treatments and animal handling were approved by the University of Florida Institute of Food and Agricultural Sciences Animal Research Committee. Briefly, multiparous Holstein cows were dried off ~45 d before expected calving and randomly assigned to one of two treatments, HS or CL, based on parity and mature equivalent milk production of the lactation. All cows were housed in the same barn during the dry period, but CL cows were under shade and cooled by fans over the free stalls and feed bunks, as well as by soakers over the feed bunks, whereas HS cows were only provided with shade. The temperature-humidity index values during the dry period between stall areas for both groups of cows were similar and averaged 78 ± 4 (mean ± SD). Rectal temperature was measured daily at 1430 h, and the least squares means were 39.3 vs. 39.0°C, for HS and CL cows, respectively (SEM = 0.02°C, $P < 0.01$). The respiratory rate was counted at 1500 h once a day, and the least squares means were 66.7 vs. 49.1 breaths/min, for HS and CL cows, respectively (SEM = 3.3 breaths/min, $P < 0.01$) during the entire dry period. There were 38 calves delivered during the experiment including 18 bulls (HS: n = 10; CL: n = 8) and 20 heifers (HS: n = 10; CL: n = 10). There was no treatment effect for birth weight of the bulls (LSM = 42.6 vs. 39.8 kg, for CL and HS, respectively, SEM = 1.8 kg, $P = 0.28$, Ahmed and Dahl, unpublished, University of Florida) and heifers (LSM = 36.3 vs. 35.7 kg, for CL and HS, respectively, SEM = 1.5 kg, $P = 0.79$, Monteiro et al., 2016). Within 2 hours after birth and before colostrum feeding, a blood sample was collected via jugular venipuncture into sodium-heparinized vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) and immediately placed on ice. The hematocrit and total plasma protein were assessed using a micro capillary centrifuge and refractometer, respectively, and then plasma samples were harvested after centrifugation at 2,619 × g at 4°C for 30 min. The concentrations of glucose (Autokit Glucose; Wako Chemicals USA, Inc., Richmond, VA), nonesterified fatty acids (**NEFA,** HR Series NEFA-HR(2), Wako Chemicals USA, Inc.), and BHBA (Autokit 3-HB, Wako Chemicals USA, Inc., Richmond, VA) in plasma were measured by colorimetric methods (Monteiro et al., 2016). The insulin and prolactin (**PRL**) concentrations of plasma were determined by RIA (Malven et al., 1987; Miller et al., 2000). The IGF-I concentration of plasma was determined by chemiluminescent enzyme immunoassay according to the instruction provided by manufacturer (Immulite 1000, Siemens Medical Solutions Diagnostics, Los Angeles, CA), and the monoclonal murine anti-IGF-I antibody (Immulite 1000 IGF-I, Cat#: LKGF1, Siemens Medical Solutions Diagnostics) was used and the assay was validated for bovine serum previously (Brandão et al., 2016). The plasma concentrations of metabolites and hormones were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). The statistical model included fixed effects of treatment, gender, and treatment by gender, with calf (treatment×gender) as a random effect; least squares means ± standard error of the mean are reported. No treatment effects were observed for concentrations of total protein, glucose, NEFA, and BHBA in plasma, but HS calves had lower (P = 0.05) hematocrit and tended ($P \leq 0.09$) to have lower plasma concentrations of insulin, PRL, and IGF-I compared with CL calves (Table 1).

RESULTS AND DISCUSSION

During the dry period, the stall areas for both CL and HS cows had similar temperature-humidity index values, indicating that all cows were exposed to a similar degree of heat stress; however, the cooling system effectively reduced the heat strain of CL cows evidenced by the lower rectal temperature and respiration rate compared with HS cows. Thus, the effectiveness of the cooling treatment in this experiment was confirmed. The lower hematocrit of HS calves compared with CL is consistent with Tao et al. (2012), and may indicate a carryover effect from in utero hypoxia due to the impaired placental oxygen diffusion caused by maternal HS (Regnault et al., 2007). Compared with CL, the lower plasma concentration of PRL immediately after birth of HS calves is of interest and indicates that maternal stress alters the calf's PRL release in response to parturition. It is well known that PRL is a robust stress hormone in cattle

(Tucker, 1971), and the lower concentration of PRL in HS calves relative to CL after parturition may suggest that maternal heat stress attenuates the calf stress responses postnatally. Consistent with this theory, using a similar experimental design, Tao et al. (2012) reported that HS calves had lower serum cortisol concentrations than CL calves immediately after birth. The physiological mechanisms of the diminished PRL response of HS calves is unknown but may be related to the altered development of the fetal brain dopamine system of fetal brain by maternal stress. In rhesus monkeys, offspring that experience prenatal stress have higher dopamine D_2 receptor binding ability and synthesis of dopamine in the striatum of the brain compared with controls (Roberts et al., 2004), indicating a stronger dopaminergic response and weaker PRL release. It is unknown if the altered PRL and cortisol responses by maternal heat stress can persist into maturity. In rats, adult offspring born to dams that experienced a combination of heat and restraint stress during the late gestation have a reduced PRL response following restraint stress compared with controls (Kinsley et al., 1989), indicating a persistent effect on stress responses at maturity after prenatal stress. Whether a similar phenomenon occurs in the calf born to a cow that experiences heat stress during late gestation is unknown, but that may have important implications in offspring performance due to the important roles of cortisol and PRL in immune function, metabolism, and lactation. In the ovine fetus in late gestation, IGF-I is one of the determinants of fetal growth (Gluckman and Pinal, 2003) and its secretion during both the pre- and postnatal periods is reduced by prenatal insult (Thorn et al., 2009) and impaired fetal growth (Greenwood et al., 2002). In contrast to mature animals, regulation of fetal IGF-I is determined by fetal glucose and insulin levels rather than growth hormone (Oliver et al., 1996). In the current study, the lower plasma concentration of IGF-I of HS calves at birth may indicate lower concentrations of fetal glucose and insulin compared with CL. Further, a recent study (Fu et al., 2015) reports that prenatal stress in rats epigenetically alters the growth hormone response elements on the hepatic IGF-I gene of offspring, indicating a long term alteration of IGF-I expression postnatally. Although not examined in current study, the possible long-term impact of maternal HS on the development of the somatotrophic axis of the calf is of importance and interest due to its critical role in bovine growth and lactation.

Maternal heat stress during late gestation in cattle alters calf metabolism during the preweaning period (Tao et al., 2014; Monteiro et al., 2016), but the fetal metabolic shift with maternal HS is still unknown. In the current study, there were no differences in plasma glucose, NEFA, or BHBA between treatments immediately after birth, which is consistent with Monteiro et al. (2016) who reported that heifers from HS cows had similar plasma concentrations of metabolites in the first month of life compared with CL calves. However, the metabolite data in the current study must be interpreted with caution because the blood metabolites observed in current study are complicated by stress responses to parturition and are not solely a representation of fetal metabolism. Indeed, increased hormone concentrations, such as cortisol, PRL, and epinephrine, in response to stress have strong influences on metabolism. Lower insulin concentration of HS calves compared with CL is consistent with Limesand et al. (2006) who reported that intrauterine growth restriction in ovine fetuses caused by maternal heat stress during early to mid-gestation resulted in compromised insulin synthesis and secretion compared with those that develop under thermo-neutrality. In contrast, heifers born to dry period HS cows have similar basal plasma insulin concentration and pancreatic insulin sensitivity to glucose, but lower peripheral tissue insulin sensitivity during the preweaning period compared with those from CL cows (Monteiro et al., 2016). Further, similar to metabolites, the plasma insulin concentration after birth may also be confounded by the response to the stress of parturition. The metabolism of an animal is important to growth and body composition, which in turn alters her future productive and reproductive performance. Therefore, future study is needed to examine metabolic adaptation of HS calves during the transition from intra- to extra-uterine life and postnatal metabolic responses to stress and the impact on the calf's future performance.

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Table 1. Hematocrit and plasma concentrations of total protein, glucose, nonesterified fatty acids (NEFA), BHBA, insulin, prolactin, and IGF-I of calves born to dams exposed to either heat stress (n = 20) or cooling (n = 18) during the dry period immediately after birth

Variable	Heat stress	Cooling	SEM	*P*-value		
				TRT[1]	Gender	TRT×Gender
Hematocrit, %	32.00	34.94	1.57	0.05	0.85	0.62
Total Protein, g/dL	5.22	5.19	0.08	0.74	0.58	0.18
Glucose, mg/dL	68.04	61.51	6.99	0.97	0.67	0.15
NEFA, μEq/L	798.8	627.6	97.5	0.42	0.26	0.89
BHBA, mg/dL	0.75	1.06	0.28	0.14	0.64	0.76
Insulin, ng/mL	0.51	0.74	0.09	0.08	0.83	0.31
Prolactin, ng/mL	5.67	9.49	1.35	0.08	0.12	0.98
IGF-I, ng/mL	55.0	75.6	8.19	0.09	0.47	0.70

[1]TRT=treatment