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Short communication: Maternal heat stress during the dry period alters postnatal whole-body insulin response of calves

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ABSTRACT

Heat stress during the dry period not only negatively affects a cow's performance but also affects her offspring. Previous studies indicate that calves born to cows heat-stressed during late gestation have lower birth weight but similar overall weight gain during the prepubertal period compared with those cooled in utero. However, it is unclear if whole-body insulin response, and thus metabolism, of calves is altered in their postnatal life after in utero heat stress. The aim of the present study was to examine the effects of maternal heat stress during the dry period on whole-body insulin response of calves after weaning. Calves (10/treatment) were born to cows exposed to heat stress (HT) or cooling (CL) when dry. Calves were immediately separated from their dams and fed 3.8 L of high-quality colostrum within 1 h after birth and then 1.9 L 12 h later. All calves were fed 1.9 to 3.8 L of pasteurized milk in the morning and afternoon from 2 to 42 d of age and then only in the morning until weaning at 49 d. Calf starter and water were offered ad libitum starting at 2 d of age. All calves were managed in the same manner throughout the study. All calves were subjected to a glucose tolerance test (GTT) and an insulin challenge (IC) at 55 d of age. Calves heat-stressed in utero were born lighter (40 ± 1.4 vs. 45 ± 1.4 kg) compared with CL calves. Both groups of calves had similar weaning weights (HT: 68 ± 3.2 kg; CL: 71 ± 3.3 kg) and body weight gain from birth to weaning (HT: 28 ± 2.2 kg; CL: 26 ± 2.3 kg). Compared with those cooled in utero, HT calves had a similar insulin response to GTT and insulin clearance during IC but faster glucose clearance during GTT and IC. In conclusion, in addition to impaired fetal growth, maternal heat stress during the dry period enhances the whole-body insulin response of calves after weaning, which suggests the possibility of accelerated lipogenesis and fat deposition in early life.
Key words: heat stress, insulin response, dairy calf

Short Communication

Heat stress during late gestation affects not only a cow's performance but also her offspring. For example, heat stress during the dry period reduces calf birth weight (Collier et al., 1982; Wolfenson et al., 1988) which suggests compromised fetal growth. Additionally, maternal heat stress during late gestation has no effect on overall whole-body growth rate of offspring during the prepubertal period (Monteiro et al., 2012; Tao et al., 2012a); however, whether animal metabolism and body composition are affected postnatally is unknown (Tao and Dahl, 2013). Insulin plays critical roles in glucose and fatty acid metabolism (Hayirli, 2006), and its secretion and responses are altered in sheep fetuses and lambs by heat stress during mid-gestation (Limesand et al., 2006; Chen et al., 2010). Therefore, examination of insulin responses of calves born to heat-stressed dry cows is warranted to further understand the carryover effect of maternal heat stress on neonatal metabolism and to provide some clarity related to tissue developmental adaptations during early life of the calf. Our hypothesis was that heat stress in utero during the final stages of development alters the insulin response of calves after weaning. The objective of the present study was to examine the effects of maternal heat stress during the dry period on insulin response of calves after weaning.

This study was conducted at the Calf Unit of the University of Florida (Gainesville) from August to December 2012. The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved the treatments and animal handling. Multiparous Holstein cows were dried off at ~45 d before expected calving date and randomly assigned to 1 of 2 treatments: heat stress (**HT**) or cooling (**CL**), based on mature-equivalent milk production of the previous lactation. All cows were housed in a freestall barn during the dry period. The stall areas for CL cows were equipped with soakers over feed bunks and fans over feed bunks and freestalls, whereas those for HT cows were not. Air temperature and relative humidity were measured using Hobo Pro Series Temp probes (Onset Computer Corp., Pocasset, MA) every 15 min during

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the dry period at stall areas for both HT and CL cows, and temperature-humidity index was calculated based on Dikmen et al. (2008). Rectal temperature was measured once a day (1430 h) using a GLA M700 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) on a daily basis and respiratory rate was counted thrice weekly (1500 h, Monday, Wednesday, and Friday) during the dry period (I. M. Thompson and G. E. Dahl, University of Florida, Gainesville, unpublished data). The treatments of calves reflected the treatments of their dams during the dry period. Five bulls and 5 heifers were delivered from HT cows and 6 bulls and 4 heifers were born to CL cows. Calves were removed from their dams immediately after birth and housed and managed in the same manner throughout the study. Within 1 h of birth, calves were fed 3.8 L of colostrum (colostrum score: heifer >70; bull = 50–65), and the second colostrum feeding (1.9 L) was performed around 12 h after birth. From 2 d of age, calves were fed 1.9 to 3.8 L of pasteurized milk in the morning and afternoon until 42 d of age and then only in the morning until weaning at 49 d of age. Calf starter (Cornerstone, Land O'Lakes Purina Feed, Shoreview, MN) and water were offered ad libitum starting at 2 d of age.

Metabolic tests were performed at 55 d (SD = 1.6 d) of age, shortly after weaning. All calves showed no sign of illness at the day of metabolic tests. Animals were fasted overnight before the metabolic tests. A catheter (14-gauge × 5.1 cm Abbocath-T, Hospira, Finisklin Business Park, Sligo, Ireland) was inserted into the jugular vein of each calf at least 1 h before metabolic tests. An intravenous glucose tolerance test (**GTT**) and an insulin challenge (**IC**) were performed following catheterization, with a 1-h washout period between the 2 tests. The sequence of the GTT and IC was switched for alternate calves within treatment and balanced by sex to balance the possible carryover effect from one metabolic test to the other. The means of plasma insulin and metabolite concentrations of samples collected before glucose or insulin administration during the first metabolic test were considered as basal fasting concentrations. For the GTT, 0.3 g/kg of BW (Stanley et al., 2002; Yari et al., 2010) of glucose (dextrose 50%, wt/vol; Phoenix Scientific Inc., St. Joseph, MO) was infused into the jugular vein through the catheter, followed by 10 mL of sterile saline solution to flush the catheter. Glucose infusion took less than 1 min. Blood samples were drawn through the catheter at −15, −5, and 0 min relative to the starting point of glucose infusion and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120 min relative to the ending point of glucose infusion into Vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed in the ice and centrifuged at 2,619 × g at 4°C for 15 min after all samples were collected. The catheter was flushed with sterile saline containing sodium heparin between samplings to avoid clotting and the first 2 mL of blood collected was discarded before each subsequent sample. During IC, 0.1 IU of insulin/kg of BW (100 IU/mL, human insulin, rDNA origin, Eli Lilly and Co., Indianapolis, IN) was administered through the jugular catheter followed by 10 mL of sterile saline solution. Blood samples from the IC were collected through the catheter at −30 and 0 min relative to the starting point of insulin infusion and at 5, 10, 15, 20, 25, 30, 45, and 60 min relative to the ending point of insulin administration.

Glucose (Autokit Glucose; Wako Chemicals USA Inc., Richmond, VA) and NEFA (HR Series NEFA-HR(2), Wako Chemicals USA Inc., Richmond, VA) concentrations of plasma were measured by enzymatic methods. The inter- and intraassay coefficients were 2.5 and 3.5%, respectively, for glucose assays, and 2.2 and 1.7%, respectively, for NEFA assays. Plasma insulin concentration was determined by RIA, and the inter- and intraassay coefficients were 18.2 and 3.1%, respectively.

The mean value of the insulin or metabolite concentrations of the samples collected at −15, −5, and 0 min relative to glucose infusion or at −30 and 0 min relative to insulin administration were considered the baseline values of GTT or IC, respectively. The area under curve (**AUC**) was calculated by the trapezoidal method, in which the hormone or metabolite concentration was calculated by subtracting the actual value from the baseline value. The GLM procedure (SAS 9.3, SAS Institute Inc., Cary, NC) was used to analyze birth weight; weaning weight; BW change from birth to weaning; basal fasting plasma concentrations of glucose, insulin, and NEFA; and AUC of plasma insulin and metabolites during GTT and IC; LSM ± SEM were reported. For the statistical analyses of AUC, the sequence of metabolic tests was included in the SAS model to further account for possible carryover effects.

Cows involved in current experiment were from a larger study reported elsewhere (I. M. Thompson and G. E. Dahl, University of Florida, Gainesville, unpublished data). Briefly, the average temperature-humidity index in a day at the stall areas for HT and CL cows during the dry period was similar (75.2 vs. 74.4, respectively), but HT cows had higher rectal temperature (39.0 vs. 38.7°C, respectively) and respiration rate (69.7 vs. 49.1 breaths/min, respectively) compared with CL cows. Additionally, both groups of cows had similar gestation lengths (HT: 277 d; CL: 279 d).

Compared with CL calves, those born to HT cows had lower (45.0 vs. 40.2 kg, SEM = 1.4 kg, respectively; $P = 0.03$) birth weight. However, no differences