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 $\times$ 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 centri-

fuged at $2,619 \times g$ 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; $\text{LSM} \pm \text{SEM}$ 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, $\text{SEM} = 1.4$ kg, respectively; $P = 0.03$) birth weight. However, no differences

($P = 0.52$) were observed for weaning weight (68.2 vs. 71.3 kg, SEM = 3.3 kg, respectively) or BW gain (28.0 vs. 26.3 kg, SEM = 2.2 kg, respectively) from birth to weaning between HT and CL calves. Basal plasma insulin (0.26 vs. 0.26 ng/mL, SEM = 0.02 ng/mL, respectively) and NEFA (434.6 vs. 442.8 μ Eq/dL, SEM = 55.3 μ Eq/dL, respectively) concentrations were similar ($P = 0.86$) between HT and CL calves after feed deprivation, but calves born to HT cows during the dry period tended ($P = 0.14$) to have higher basal circulating glucose (70.7 vs. 65.2 mg/dL, SEM = 2.5 mg/dL, respectively) compared with those born to CL cows.

No treatment effects were observed for insulin AUC to GTT (Table 1). However, relative to those born to CL cows, HT calves had lower ($P = 0.02$) glucose AUC at 30 min and tended ($P = 0.11$) to have lower glucose AUC 60 min after GTT (Table 1). In IC, no differences ($P > 0.15$) were observed for insulin AUC or NEFA AUC between treatments (Table 1). Relative to CL calves, HT calves had greater ($P < 0.03$) glucose AUC during IC (Table 1).

As in other reports (do Amaral et al., 2011; Tao et al., 2012b), although all dams were exposed to a similar thermal environment during the study, as indicated by temperature-humidity index, the decreases in rectal temperature and respiration rate suggest that the cooling system utilized in the present study efficiently minimized the heat strain on CL cows during the dry period compared with HT cows. Thus, the effectiveness of treatment; that is, dry period heat stress and in utero heat stress of the calves, was achieved in current experiment.

Smaller calf size at birth is a hallmark of late-gestation heat stress in cattle. Tao et al. (2012a) suggested that, in addition to the decrease in birth weight resulting from shorter gestation length, this phenomenon is also due to a combination of fetal hyperthermia and placental inefficiency-related intrauterine growth retardation (IUGR) under maternal heat stress. Given the fact that both groups of cows had similar gestation length, the reduced birth weight with maternal heat stress in the present study supports the concept of a negative effect of impaired placental development and hyperthermia in utero on fetal growth during late gestation. Consistent with previous reports (Monteiro et al., 2012; Tao et al., 2012a), BW gain from birth to weaning was similar in HT and CL calves.

Coupled with similar insulin responses, the more rapid glucose clearance during the GTT and IC of HT calves suggests a more robust whole-body insulin response compared with CL animals after weaning. Studies conducted in other ruminant species indicate that different uterine environments not only affect fetal growth but also carry over to postnatal life. Indeed, compared with lambs with normal birth weight, low-birth-weight lambs deposit more adipose tissue in postnatal life (Greenwood et al., 1998). Similarly, low-birth-weight lambs from ewes with experimentally induced placental restriction due to removal of endometrial caruncles before pregnancy exhibit compensatory growth and develop more body fat postnatally compared with normal lambs (De Blasio et al., 2007), and chemical embolization of placental vasculature during late gestation results in similar negative effects on fetal

Table 1. Insulin, glucose, and NEFA responses to glucose tolerance tests and insulin challenges of calves born to dams exposed to either heat stress ($n = 10$) or cooling ($n = 10$) during the dry period

Item	Heat stress	Cooling	SEM	P-value
Glucose tolerance test				
Insulin AUC ¹ (ng $\times$ min/mL)				
30 min	9.73	10.91	3.20	0.94
60 min	14.81	17.41	3.75	0.78
120 min	20.37	25.59	3.98	0.49
Glucose AUC (mg $\times$ min/dL)				
30 min	1,633.41	1,838.23	56.06	0.02
60 min	2,642.43	3,074.01	177.08	0.11
120 min	3,145.61	3,795.50	370.73	0.24
Insulin challenge				
Insulin AUC (ng $\times$ min/mL)				
30 min	42.02	46.46	2.53	0.23
60 min	48.24	54.12	3.11	0.20
Glucose AUC (mg $\times$ min/dL)				
30 min	-648.29	-505.13	41.35	0.03
60 min	-1,782.55	-1,391.78	97.58	0.01
NEFA AUC (μ Eq $\times$ min/dL)				
30 min	-5,681.45	-5,827.82	969.07	0.92
60 min	5,959.45	5,521.15	3,236.74	0.93

¹AUC = area under the curve.

growth and lamb body composition (Louey et al., 2005). Although the experimental design in the current study limits our ability to investigate the insulin responsive tissues individually, the enhanced whole-body insulin action of HT calves supports the possibility that calves born to heat-stressed dams are prone to develop obesity compared with CL calves in their postnatal life.

Compared with CL calves, HT calves had a similar insulin response to GTT and insulin clearance during IC, which indicates that both groups of animals had similar pancreatic insulin sensitivity and systemic insulin clearance after weaning. In contrast, neonatal lambs born to ewes exposed to heat stress during mid-gestation have enhanced insulin response to glucose stimulation (Yates et al., 2011). This discrepancy between studies may reflect the different responses of pancreatic development to heat stress at different stages of gestation. Development of the fetal pancreas in ruminants occurs during the first 2 trimesters of pregnancy and the weight of the fetal pancreas is constant during late gestation (see reviews by Hay, 2006; Guilloteau et al., 2009). Thus, the heat exposure during mid-gestation and the related placental restriction in the ovine model may exert more severe negative effects on pancreatic development relative to that in late gestation in the bovine model. Additionally, the metabolic tests were performed only after weaning in the current study; thus, the possibility that maternal heat stress affects the pancreatic function of the newborns before weaning cannot be excluded. Indeed, Tao and Dahl (2013) observed that calves born to HT cows during the dry period had a greater insulin response after colostrum ingestion during the first days of life compared with those from CL cows.

In addition to its important role in glucose metabolism, insulin is a potent antilipolytic factor and enhances lipogenesis (Hayirli, 2006), which is consistent with the decrease in NEFA concentration after insulin infusion observed in the current study. Compared with CL calves, HT calves had a similar NEFA response during IC, which indicates that both groups of calves had similar insulin response with regard to fatty acid metabolism after weaning. In early life, lambs with intrauterine growth retardation born to ewes heat-stressed during mid-gestation have lower NEFA responses to adrenaline challenge compared with control lambs, which suggests an impairment of lipid mobilization (Chen et al., 2010) and a propensity toward fat deposition (Yates et al., 2011). If a similar response exists in HT calves, it is unclear and deserves further examination.

The present study included a relatively small and unbalanced number of bulls or heifers within each treatment, which limited the power to detect sex $\times$ treatment effects on insulin actions. However, the pos-

sibility that bulls and heifers respond differently to maternal heat stress cannot be excluded. In future studies, a large number of animals may be included to further investigate the interaction between sex of offspring and maternal heat stress on the metabolic responses. Nevertheless, the data obtained in current study still suggest a possibility that calves born to heat-stressed dams during the dry period are more prone to develop obesity in the postnatal period compared with calves that develop under normothermic conditions in utero. Because body condition in dairy heifers is important for their future performance, greater rates of adipose accumulation during early life may lead to poorer performance. Indeed, the accelerated fat deposition in heifers during prepuberty is negatively correlated with mammary parenchymal content, and the excessive BCS at puberty is related to lower milk production in the first lactation (Silva et al., 2002). The effect of maternal heat stress during the dry period on the offspring's long-term performance was evaluated recently (Monteiro et al., 2013) and those data indicate that heifers heat-stressed in utero have lower milk production in their first lactation compared with those cooled in utero. However, it is unknown whether this compromised lactational performance by maternal heat stress is due to altered body growth or composition and deserves further investigation. Additionally, findings in the current study have implications for the performance of dairy steers because the possibility of altered body fat deposition also affects carcass composition and quality of feedlot cattle.

In conclusion, heat stress in utero during the dry period decreased calf birth weight but not weaning weight or BW gain from birth to weaning. However, data from GTT and IC indicated that, compared with CL calves, HT calves had an enhanced whole-body insulin response after weaning, but the pancreatic insulin response to glucose stimulation and systemic insulin clearance were not different between treatments.

REFERENCES

- Chen, X., A. L. Fahy, A. S. Green, M. J. Anderson, R. P. Rhoads, and S. W. Limesand. 2010. β 2-Adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. *J. Physiol.* 588:3539–3549.
- Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher, and C. J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. *J. Anim. Sci.* 54:309–319.
- De Blasio, M. J., K. L. Gatford, I. C. McMillen, J. S. Robinson, and J. A. Owen. 2007. Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. *Endocrinology* 148:1350–1358.
- Dikmen, S., E. Alava, E. Pontes, J. M. Fear, B. Y. Dikmen, T. A. Olson, and P. J. Hansen. 2008. Differences in thermoregulatory

- ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. *J. Dairy Sci.* 91:3395–3402.
- do Amaral, B. C., E. E. Connor, S. Tao, M. J. Hayen, J. W. Bubolz, and G. E. Dahl. 2011. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. *J. Dairy Sci.* 94:86–96.
- Greenwood, P. L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 1998. Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. *J. Anim. Sci.* 76:2354–2367.
- Guilloteau, P., R. Zabielski, and J. W. Blum. 2009. Gastrointestinal tract and digestion in the young ruminant: Ontogenesis, adaptations, consequences and manipulations. *J. Physiol. Pharmacol.* 60(Suppl. 3):37–46.
- Hay, W. W., Jr. 2006. Recent observations on the regulation of fetal metabolism by glucose. *J. Physiol.* 572:17–24.
- Hayirli, A. 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. *Vet. Res. Commun.* 30:749–774.
- Limesand, S. W., P. J. Rozance, G. O. Zerbe, J. C. Hutton, and W. W. Hay, Jr. 2006. Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. *Endocrinology* 147:1488–1497.
- Louey, S., M. L. Cock, and R. Harding. 2005. Long term consequences of low birthweight on postnatal growth, adiposity and brain weight at maturity in sheep. *J. Reprod. Dev.* 51:59–68.
- Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2012. Effect of heat stress during the dry period on immune function and growth performance of the offspring fed standardized pooled colostrum. *J. Anim. Sci.* 90(Suppl. 3):476. (Abstr.)
- Monteiro, A. P. A., S. Tao, I. M. Thompson, and G. E. Dahl. 2013. Effect of heat stress in utero on calf performance and health through the first lactation. *J. Anim. Sci.* 91(Suppl. 2):184. (Abstr.)
- Silva, L. F. P., M. J. Vandehaar, B. K. Whitlock, R. P. Radcliff, and H. A. Tucker. 2002. Short communication: Relationship between body growth and mammary development in dairy heifers. *J. Dairy Sci.* 85:2600–2602.
- Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bateman II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, and G. E. Goodier. 2002. Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. *J. Dairy Sci.* 85:2335–2343.
- Tao, S., and G. E. Dahl. 2013. Heat stress impacts during late gestation on dry cows and their calves. *J. Dairy Sci.* 96:4079–4093.
- Tao, S., A. P. Monteiro, I. M. Thompson, M. J. Hayen, and G. E. Dahl. 2012a. Effect of late gestation maternal heat stress on growth and immune function of dairy calves. *J. Dairy Sci.* 95:7128–7136.
- Tao, S., I. M. Thompson, A. P. A. Monteiro, M. J. Hayen, L. J. Young, and G. E. Dahl. 2012b. Effect of cooling heat-stressed dairy cows during the dry period on insulin response. *J. Dairy Sci.* 95:5035–5046.
- Wolfenson, D., I. Flamenbaum, and A. Berman. 1988. Dry period heat stress relief effects on prepartum progesterone, calf birth weight, and milk production. *J. Dairy Sci.* 71:809–818.
- Yari, M., A. Nikkhah, M. Alikhani, M. Khorvash, H. Rahmani, and G. R. Ghorbani. 2010. Physiological calf responses to increased chromium supply in summer. *J. Dairy Sci.* 93:4111–4120.
- Yates, D. T., A. S. Green, and S. W. Limesand. 2011. Catecholamines mediate multiple fetal adaptations during placental insufficiency that contribute to intrauterine growth restriction: Lessons from hyperthermic sheep. *J. Pregnancy* 2011:740408.