Rapid Communication: Reduced maternal nutrition during early- to mid-gestation elevates newborn lamb plasma cortisol concentrations and eliminates the neonatal leptin surge

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ABSTRACT: Human epidemiological and animal studies show that maternal nutrient reduction (MNR) and maternal overnutrition/obesity (MO) alter fetal growth and development, predisposing offspring (F1) to endocrine and appetite dysregulation. Compared to F1 of control-fed ewes, F1 of MO ewes display hypercortisolemia at birth and fail to exhibit the neonatal leptin surge implicated in lifelong appetite regulation. Here, we determined if MNR also elevates newborn lamb plasma cortisol and eliminates the neonatal leptin surge. Starting 30 d prior to conception, nulliparous control (CON, $n = 6$) ewes ate 100% NRC recommendations through parturition. Nutrient-reduced (NR, $n = 6$) ewes ate a CON diet through day 27 of gestation. From gestational days 28 to 78, NR ewes ate 50% of the CON diet before

realimentation to 100% NRC recommendations. Jugular blood was collected daily from lambs from birth (day 0) through postnatal day 10, to determine plasma cortisol and leptin. Newborn NR plasma cortisol concentrations were increased ($P < 0.0001$) vs. CON and were similar to concentrations in MO lambs. Plasma leptin concentrations were similar between groups through postnatal day 7. The leptin surge, seen in CON lambs on postnatal days 8 to 10 was not present in NR lambs. These data show that, similar to MO lambs, early pregnancy MNR elevates newborn lamb plasma cortisol and eliminates the neonatal leptin surge. In the light of the similar elevation of neonatal cortisol in MNR and MO lambs, we conclude that cortisol plays a central role in regulating the neonatal lamb leptin surge.

Key words: early- to mid-gestation, fetal programming, hypercortisolemia, maternal nutrient reduction, maternal nutrition, neonatal leptin surge

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INTRODUCTION

Reduced fetal nutrition during early- to mid-gestation in rodents, sheep, and nonhuman primates leads to offspring hyperphagia, increased adiposity, hyperleptinemia, hypercortisolemia, pancreatic hypofunction, β -cell dysfunction, hyperinsulinemia, hyperglycemia, and

cardiovascular dysfunction at various points in later life (Vickers et al., 2000; Yura et al., 2005; Ford et al., 2007; Ford and Long, 2011; Li et al., 2013; Kuo et al., 2017a, 2017b). Rodent and sheep offspring born to overfed/obese mother's exhibit similar β -cell dysfunction, endocrine dysfunction (such as hypercortisolemia), and adverse health outcomes seen in undernourished fetuses and offspring (Gauguier et al., 1990; Ford et al., 2009; Kirk et al., 2009; Tamashiro et al., 2009; Long et al., 2010, 2011; Ford and Long, 2011; Zambrano and Nathanielsz, 2013).

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Hypercortisolemia exhibited by both offspring born to undernourished or overfed/obese mothers indicates elevated fetal stimulation of the hypothalamic–pituitary–adrenal axis, which may be associated with additional altered hypothalamic neuroendocrine activity. The rodent has been extensively studied to investigate the central mechanisms that control the neonatal surge of the adipocyte-derived hormone leptin, which programs lifelong activity of the appetitive control centers within the hypothalamus (Yura *et al.*, 2005; Bouret *et al.*, 2012). In sheep, as in rodents, a 2- to 3-fold surge release of leptin during the first 10 d of the postnatal period has been reported in lambs born to control-fed (CON) ewes (Long *et al.*, 2011). In contrast, lambs born to overfed/obese (maternal overnutrition/obesity; MO) ewes lack the neonatal leptin surge, display elevated plasma cortisol concentrations, are hyperphagic, and exhibit increased adiposity in postnatal life (Muhlhausler *et al.*, 2006; Long *et al.*, 2011). Given the similarities between altered appetitive phenotype in offspring born to both MO and maternal nutrient-reduced (MNR) ewes, we sought to determine if mid- to late-gestation MNR also produces similar changes in neonatal leptin physiology. We hypothesized that MNR from early- to mid-gestation alone will elevate newborn lamb plasma cortisol concentrations and eliminate the neonatal leptin surge, as seen in lambs born to MO ewes.

MATERIALS AND METHODS

Animals

All procedures were approved by the University of Wyoming Animal Care and Use Committee. Nulliparous Rambouillet-Columbia cross ewes (2–3 yr old) were studied. Ewes were fed a diet consisting of alfalfa hay and corn to meet NRC recommendations for replacement ewes until 30 d prior to the start of breeding. A single Rambouillet ram, carrying a marking harness, was housed with the ewes over 2 wk for natural breeding. Breeding dates were recorded and used to estimate stages of gestation. At the initiation of breeding, ewes were transitioned onto a highly palatable pelleted diet (NRC, 1985). The diet transition consisted of substituting 10% of their alfalfa/corn diet each day with a complete pelleted ration; thus, by the end of 10 d ewes were consuming only the pelleted diet. The pelleted diet consisted of: 88.54% DM; NDF, 24.09% of DM; ADF, 9.99% of DM; CP, 17.39%;

and in vivo DM digestibility, 93.92%. All ewes were fed a control diet at 100% NRC recommendations through day 27 of gestation. Ewes were then assigned randomly to receive either 50% of the control diet (nutrient-reduced; NR, $n = 6$) or were maintained at 100% NRC (CON, $n = 6$) from days 28 to 78 of gestation (NRC, 1985). From day 79 of gestation through parturition, all ewes were fed the control diet at 100% NRC recommendations. Ewes were weighed weekly and rations were adjusted on a metabolic BW basis ($BW^{0.75}$) for a gain or loss in weight. Ultrasonography (Chison 8300 VET Digital Ultrasound System, Chison Medical Imaging Co., Ltd., Wuxi, China) was performed at approximately days 50 and 80 of gestation for pregnancy confirmation. Ewes were allowed to lamb unassisted and were housed with their lamb in individual lambing pens until postnatal day 11. After parturition, ewes were fed a diet consisting of alfalfa hay and supplemented with corn to meet NRC recommendations for a lactating ewe (NRC, 1985). Lambs nursed freely on their mother after birth. Twelve singleton lambs were born. Of these offspring, one male and five female lambs were born to NR ewes, while three male and three female lambs were born to control-fed ewes.

Blood Collection

Blood samples were collected immediately after birth (day 0) and 24 h later on day 1 of postnatal life. Thereafter, daily blood samples were collected at 0600 h through postnatal day 10. Approximately 3 mL jugular venous blood samples were collected from lambs into 10 mL Vacutainer tubes with heparin (Becton, Dickinson and Co., Franklin Lakes, NJ) and immediately centrifuged at $2,500 \times g$ for 15 min at 4 °C; plasma was collected and stored at –80 °C until assayed via validated RIA.

Biochemical and Hormone Assays

Plasma cortisol and insulin were measured in duplicate using a commercially available ImmuChem RIA kit (MP Biomedicals, LLC., Solon, OH). Mean intra-assay and interassay CV for cortisol were 8.9% and 9.3%, respectively, with a sensitivity of 10.0 ng/mL. Mean intra-assay and interassay CV for insulin were 12.2% and 8.9%, respectively, with a sensitivity of 5.5 μ IU/mL. Plasma leptin was measured in duplicate using a commercially available RIA kit (Multi-Species Leptin, EMD Millipore Corporation, St. Charles, MO). Mean intra-assay and interassay CV for

leptin were 3.6% and 8.7%, respectively, with a sensitivity of 1.56 ng/mL. Glucose was measured colorimetrically in triplicate (Hexokinase Reagent Set, Pointe Scientific, Inc., Canton, MI) using a 96-well assay plate, as previously described (Ford et al., 2007). Mean intra-assay and interassay CV for glucose were 1.8% and 2.1%, respectively, with a sensitivity of 1 mg/dL.

Statistical Analysis

Ewe BW throughout gestation and percent BW change were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Lamb birth weight, and overall sex and treatment differences in plasma hormone concentrations were compared via a factorial analysis of variance using the MIXED procedure of SAS. Male and female interactions were removed from the final model as there was not enough male data to determine significance. Lamb plasma hormone concentrations from postnatal days 0–10 were analyzed as repeated measures using the MIXED procedure of SAS, where a treatment by day interaction was used in the model statement. Data are presented as mean $\pm$ SEM, and differences are considered significant when $P \leq 0.05$. GraphPad Prism (GraphPad Software Inc., La Jolla, CA) was used to represent changes in maternal BW and variations in lamb plasma hormone concentrations over time.

RESULTS

At the start of the experiment, 30 d prior to conception, maternal BW did not differ between CON and NR ewes (65.2 ± 2.7 vs. 66.5 ± 2.7 Kg, respectively). However, during the period of nutrient

reduction, from days 28 to 78 of gestation, the average BW of CON ewes was greater ($P < 0.01$) than NR ewes (70.9 ± 2.74 vs. 60.4 ± 2.74 Kg, respectively) as shown in Fig. 1A. From days 28 to 78 of gestation, CON ewes gained $8.5 \pm 2.07\%$ of their starting BW while NR ewes lost $10.9 \pm 2.07\%$ of their starting BW (Fig. 1B). After realimentation to the control diet, NR ewes showed a steady increase in BW whereby day 135 (0.9 of total gestation) NR and CON ewe BW were similar (72.8 ± 2.7 vs. 74.7 ± 2.7 Kg, respectively). There was no difference in birth weight between CON and NR lambs (5.81 ± 0.5 vs. 5.84 ± 0.5 Kg, respectively).

On the day of birth (day 0) NR lambs displayed elevated ($P < 0.0001$) plasma cortisol concentrations compared to CON lambs (115.1 ± 7.38 vs. 68.3 ± 7.38 ng/mL, respectively). However, by day 1, and continuing through day 10 of postnatal life, plasma cortisol concentrations were similar in NR and CON lambs (Fig. 2A). Plasma leptin concentrations were similar in NR and CON lambs through day 7 of postnatal life. Plasma leptin concentration of CON lambs was elevated on days 8–10, the final day of sampling ($P < 0.0001$) with no changes in NR over this period. (Fig. 2B). Plasma cortisol was significantly higher in NR lambs on the day of birth but thereafter values in CON and NR were similar. For the postnatal period of this experiment (days 0–10), overall plasma cortisol concentrations did not differ between male and female lambs (37.8 ± 4.4 vs. 32.3 ± 3.1 ng/mL, respectively).

Nutrient-reduced lambs displayed elevated ($P < 0.0001$) plasma glucose concentrations on day 0, compared to CON lambs (81.1 ± 5.07 vs. 44.1 ± 4.69 mg/dL, respectively), but concentrations were similar between groups by day 1 (101.7 ± 4.7 vs. 99.6 ± 4.7). Plasma insulin concentrations were

Figure 1. Maternal BW. Control-fed (CON) ewes (open circles, $n = 6$) and nutrient-reduced (NR) ewes (closed circles, $n = 6$) (A) Maternal BW throughout gestation. Values are mean $\pm$ SEM; $**P < 0.01$, $*P = 0.05$. (B) Percent weight variation from baseline maternal BW throughout gestation. Values are percent variation from baseline BW (mean $\pm$ SEM); $**P < 0.01$, $*P = 0.05$.

Figure 2. Lamb plasma hormone concentrations from days 0 to 10 of postnatal life. Lambs born to control-fed (CON) ewes (open circles, $n = 6$) and lambs born to nutrient-restricted (NR) ewes (closed circles, $n = 6$). (A) Lamb plasma cortisol concentrations. (B) Lamb plasma leptin concentrations. Means $\pm$ SEM differ; *** $P < 0.0001$.

similar between NR and CON lambs on day 0 (36.2 ± 15.9 vs. 52.6 ± 21.5 μ IU/mL, respectively) and day 1 (72.5 ± 15.9 vs. 43.1 ± 17.1).

DISCUSSION

Strong evidence exists to indicate that the adipocyte-derived hormone leptin signals the hypothalamus to regulate food intake by inhibiting appetite (Yura et al., 2005; Vickers et al., 2008; Bouret et al., 2015). A surge release of leptin in early life has been reported in rodents from postnatal days 8 to 21 (Proulx et al., 2001; Yura et al., 2005; Delahaye et al., 2008). The neonatal leptin surge has been shown to program normal development of appetitive centers in the hypothalamus and influence leptin sensitivity throughout life (Yura et al., 2005; Bouret et al., 2015). Modifications in timing and magnitude of the neonatal leptin surge are associated with altered leptin signaling, leptin sensitivity, development of hypothalamic appetitive control centers, and leads to leptin resistance in later life which associates with postnatal appetite dysregulation (Delahaye et al., 2008; Kirk et al., 2009; Vargas et al., 2017).

Both maternal undernutrition and overnutrition elevate maternal glucocorticoid levels in sheep (Ford and Long, 2011; Nathanielsz et al., 2013; Tuersunjiang et al., 2013). We have previously demonstrated that a stressful maternal metabolic state during gestation, characterized by either an obese diet or administration of the synthetic glucocorticoid dexamethasone, result in similar phenotypic changes in offspring which show intergenerational transmission (Long et al., 2011; Long et al., 2013; Shasa et al., 2015; Pankey et al., 2017). When offspring from MO ewes were subjected to a bout of ad libitum feeding during adulthood,

hyperphagia, increased adiposity, insulin resistance, hyperinsulinemia, and hyperleptinemia were observed in comparison to offspring from CON ewes (Long et al., 2010). In the current study, MNR was associated with neonatal hypercortisolemia and subsequent elimination of the neonatal leptin surge. At birth, the cortisol concentration in lambs born to MNR and MO ewes were similarly increased within a range of 100–150 ng/mL, while lambs born to CON ewes were within 50–60 ng/mL (data produced here and reported in Long et al., 2011). Further, we have previously reported that offspring from MNR ewes exhibit increased adiposity, hyperphagia, and glucose/insulin dysregulation, similar to offspring from MO ewes (Ford et al., 2007). Therefore, it is likely that elevated neonatal cortisol, in response to an altered intrauterine environment, is associated with the elimination of the neonatal leptin surge. Modification of the neonatal leptin surge is likely associated with appetite dysregulation in later life.

The elevated plasma cortisol concentration displayed by NR lambs on day 0 may also influence their increased day 0 plasma glucose concentration and lack of glucose-stimulated insulin response. It has been shown that fetal nutrient reduction in sheep and nonhuman primates, and fetal exposure to elevated glucocorticoids in rodents, induces a premature upregulation of gluconeogenic enzymes providing a potential mechanism to explain the elevated glucose on day 0 in NR lambs (Lemons et al., 1986; Seckl and Meaney, 2004; Nijland et al., 2010). Further, the lack of a glucose-stimulated insulin response seen by NR lambs on day 0 may stem from pancreatic hypofunction/ β -cell dysregulation, which we have previously shown in NR ovine offspring (Ford et al., 2007), or may be reflective of the associated hypercortisolemia on day 0.

Studies in multiple species have demonstrated that both MNR and MO lead to a collection of similar adverse health complications in offspring both before and after birth (Rajendram et al., 2017). Accumulating evidence suggests that elevated glucocorticoids are programming similar outcomes in the presence of both maternal nutritional challenges. Given the role of the late gestational rise in fetal cortisol on tissue maturation and programming, elevated fetal cortisol likely programs the adverse health complications in response to the challenges of poor maternal nutrition during pregnancy (Zambrano et al., 2014). Fetal programming from poor maternal nutrition may include control of the neonatal leptin surge and subsequent altered appetite control in our sheep model.

Evidence shows that fetal cortisol concentrations are elevated in response to the metabolic stressors and challenges of poor maternal nutrition during pregnancy (Ford and Long, 2011; Nathanielsz et al., 2013; Tuersunjiang et al., 2013; Zambrano et al., 2014). This rise in fetal cortisol has been proposed to orchestrate altered endocrine activity and appetite dysregulation. Further, the impact of cortisol on adipocyte differentiation and function and the hypercortisolemic state seen in newborn lambs of MNR and MO ewes indicates that cortisol plays a central role in regulating the neonatal lamb leptin surge.

Alternations in the neonatal lamb leptin surge have been associated with appetite dysregulation in postnatal life where predisposing offspring of MNR and MO ewes exhibit hyperphagia, increased adiposity and weight gain, and hyperleptinemia when subjected to ad libitum feeding challenge (Long et al., 2010; George et al., 2012; Long et al., 2015). Therefore, this study shows that early pregnancy is a specific period of vulnerability for programming of the leptin surge by decreased maternal and fetal nutrition.

Conflicts of interest statement. None declared.

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