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The Effects of Poor Maternal Nutrition During Gestation on Ewe and Offspring Plasma Concentrations of Leptin and Ghrelin

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Abstract

Poor maternal nutrition (restricted- and over-feeding) during gestation may alter leptin and ghrelin, key hormones in energy homeostasis and appetite control. They may also have a regulatory role in maternal metabolic adaptations critical during gestation to ensure optimal offspring growth and development. We hypothesized that restricted- and over-feeding during gestation would alter plasma concentrations of leptin and ghrelin in ewes and their offspring. Pregnant Western White-faced ewes were individually fed 60% (RES; n = 13), 100% (CON; n = 11), or 140% (OVER; n = 13) of NRC requirements for TDN starting on d 30 ± 0.02 of gestation. Blood samples were collected in the morning from fasted pregnant ewes weekly from d 20 of gestation until parturition and from offspring (n = 31) within 24 hours after birth. Leptin and ghrelin concentrations were determined by radioimmunoassay. Data were analyzed using the MIXED procedure in SAS with main effects of treatment, day of gestation and their interaction. Leptin ($P < 0.002$) and ghrelin ($P < 0.015$) concentrations were altered in pregnant ewes between treatments within a day of gestation. At d 100 ($P = 0.008$) and d 128 ($P = 0.04$), RES ewes (5.39 ± 2.58 ng/mL; 6.39 ± 2.50 ng/mL) had decreased leptin concentrations compared with OVER ewes (14.97 ± 2.48 ng/mL; 13.61 ± 2.47 ng/mL), with CON ewes intermediate. RES ewes (0.26 ± 0.04 ng/mL) had increased ghrelin concentrations compared with CON ewes at d 142 (0.15 ± 0.04 ng/mL; $P = 0.042$), with OVER ewes intermediate. Leptin ($P < 0.002$) and ghrelin ($P < 0.015$) concentrations were altered between days of gestation within a dietary treatment. Leptin concentration increased across gestation in OVER ewes. In RES ewes, leptin concentration decreased, and ghrelin concentration increased over gestation. Leptin ($P = 0.5$) and ghrelin ($P = 0.5$) concentrations in lambs were not different at birth. Alterations in leptin and ghrelin in ewes during gestation may disrupt critical metabolic adaptations that may contribute to suboptimal offspring growth and development.

Introduction

Leptin and Ghrelin

- Leptin is an anorexigenic peptide hormone synthesized and secreted predominantly in the adipocytes of white adipose tissue (Trayhurn and Bing, 2006).
- Ghrelin is an orexigenic peptide hormone synthesized and secreted primarily in the stomach (Horvath et al., 2001).
- Together, they help control energy regulation in mammals by coregulating hypothalamic peptidergic systems in opposing ways to prevent energy imbalances (Horvath et al., 2001).

Poor Maternal Nutrition During Gestation

- Maternal restricted nutrition and overnutrition during gestation can negatively impact fetal growth and development by altering the intrauterine environment (Wu et al., 2004), which can have permanent effects on offspring structure, physiology, and metabolism (Godfrey et al., 2001).

Hypothesis: restricted- and over-feeding during gestation would alter plasma concentrations of leptin and ghrelin in ewes and offspring.

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Materials & Methods

Experimental Design

- 37 pregnant ewes were randomly assigned to one of three diets: restricted-fed (RES; 60% NRC; n = 13), control-fed (CON; 100% NRC; n = 11), or over-fed (OVER; 140% NRC; n = 13) based on National Research Council (NRC) requirements for TDN.
- Blood samples (20 mL) were collected in heparin/EDTA coated tubes from fasted pregnant ewes in the morning from a jugular vein and immediately placed on ice until centrifugation for 30 minutes at 1800 x g at 4ºC starting on day 20 and weekly thereafter throughout the entirety of gestation and from the offspring within 24 hours after birth (n = 10 to 13 offspring per treatment).

Plasma Concentrations of Leptin and Ghrelin Analysis

- Plasma leptin concentrations were determined using a commercial multi-species leptin radioimmunoassay (RIA) kit (Millipore Corporation, Billerica, MA; Cat. # XL-85K).
- Plasma ghrelin concentrations were determined using a ghrelin total RIA kit (Millipore Corporation, Billerica, MA; Cat. # GHRT-89HK).
- The plasma samples were analyzed in duplicate and then averaged to give one value per animal per timepoint.

Statistics

- All data for the pregnant ewes were analyzed using the MIXED procedure in SAS with the main effects of dietary treatment, day of gestation, and the interaction between dietary treatment and day of gestation. Ewe was assigned as the subject of the repeated measures statement. All data for the offspring were analyzed using the MIXED procedure in SAS with dietary treatment as the main effect. Differences between means were determined using the PDIFF statement. Differences were considered significant at $P \leq 0.05$.

Results

Table 1. Maternal diet and day of gestation alter plasma leptin concentration.[1]

	Treatment[2]		
Day of Gestation	CON	OVER	RES
20	7.33 ± 1.92$_{a, b}$	7.12 ± 1.70$_{a}$	7.16 ± 1.67$_{a}$
30	19.28 ± 7.43$_{a}$	7.47 ± 6.43$_{a,b,c}$	4.62 ± 6.56$_{a,b}$
44	5.20 ± 3.10$_{b}$	11.09 ± 2.92$_{a,b,c}$	7.78 ± 2.88$_{a,b}$
72	7.66 ± 1.50$_{a,b}$	8.84 ± 1.46$_{a,c}$	5.74 ± 1.43$_{a,b}$
100	8.11 ± 2.61$_{a,b,x,y}$	14.97 ± 2.48$_{b,x}$	5.39 ± 2.58$_{a,b,y}$
128	8.73 ± 2.55$_{a,b,x,y}$	13.61 ± 2.47$_{b,c,x}$	6.39 ± 2.50$_{a,b,y}$
142	10.09 ± 0.90$_{a,b,x}$	6.10 ± 0.83$_{a,y}$	4.06 ± 0.84$_{b,z}$

[1] Blood was collected from pregnant ewes throughout gestation and the concentration of leptin was determined by RIA. Data are reported as least square means ± SEM in ng/mL.
[2] CON = 100% NRC for TDN, OVER = 140% NRC for TDN, RES = 60% NRC for TDN
[a,b] $P \leq 0.05$ between days of gestation within a dietary treatment
[x, y] $P \leq 0.05$ between treatments within a day of gestation

Table 2. Maternal diet and day of gestation alter plasma ghrelin concentration.[1]

	Treatment[2]		
Day of Gestation	CON	OVER	RES
20	0.18 ± 0.03$_{a,b}$	0.24 ± 0.03	0.18 ± 0.03$_{a}$
30	0.16 ± 0.03$_{a}$	0.23 ± 0.03	0.22 ± 0.03$_{b}$
44	0.17 ± 0.03$_{a,b}$	0.21 ± 0.03	0.22 ± 0.03$_{b}$
72	0.19 ± 0.03$_{a,b}$	0.23 ± 0.03	0.22 ± 0.03$_{b}$
100	0.17 ± 0.03$_{a}$	0.24 ± 0.03	0.22 ± 0.03$_{b}$
128	0.20 ± 0.03$_{b}$	0.20 ± 0.03	0.24 ± 0.03$_{b}$
142	0.15 ± 0.04$_{a,b,x}$	0.23 ± 0.04$_{x,y}$	0.26 ± 0.04$_{b,y}$

[1] Blood was collected from pregnant ewes throughout gestation and the concentration of ghrelin was determined by RIA. Data are reported as least square means ± SEM in ng/mL.
[2] CON = 100% NRC for TDN, OVER = 140% NRC for TDN, RES = 60% NRC for TDN
[a,b] $P \leq 0.05$ between days of gestation within a dietary treatment
[x, y] $P \leq 0.05$ between treatments within a day of gestation

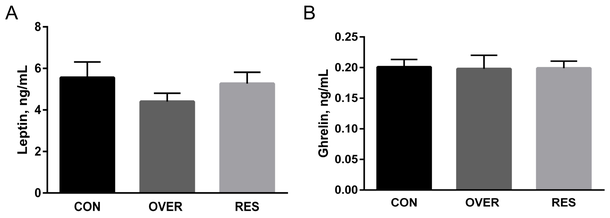

Figure 1. Maternal diet did not alter plasma leptin or ghrelin concentrations in the offspring at birth. Blood was collected from offspring within 24 hours after birth and the concentration of leptin and ghrelin were determined by RIA. No differences in the plasma concentration of (A) leptin or (B) ghrelin were observed between the dietary treatments ($P > 0.05$). All data are reported as means ± SEM.

Summary and Conclusions

- Plasma concentrations of leptin were decreased in the RES ewes (5.39 ± 2.58 ng/mL; 6.39 ± 2.50 ng/mL) compared with the OVER ewes (14.97 ± 2.48 ng/mL; 13.61 ± 2.47 ng/mL) at d 100 ($P = 0.008$) and d 128 ($P = 0.04$), respectively, with the CON ewes as intermediate.
- Plasma concentrations of leptin were decreased in the OVER ewes (6.10 ± 0.83 ng/mL; $P = 0.002$) and RES ewes (4.06 ± 0.84 ng/mL; $P < 0.0001$) compared with CON ewes (10.09 ± 0.90 ng/mL) at d 142.
- Plasma concentrations of ghrelin were increased in the RES ewes (0.26 ± 0.04 ng/mL) compared with the CON ewes (0.15 ± 0.04 ng/mL) at d 142 ($P = 0.042$), with the OVER ewes as intermediate (0.23 ± 0.04 ng/mL).
- Altered plasma concentrations of leptin ($P < 0.002$) and ghrelin ($P < 0.015$) were also observed between days of gestation within a dietary treatment.
- Maternal diet did not alter plasma leptin ($P = 0.5$) or ghrelin ($P = 0.5$) concentrations in the offspring.
- Leptin and ghrelin are important for energy regulation. Alterations in leptin and ghrelin concentrations in ewes during gestation due to poor maternal nutrition may disrupt critical metabolic adaptations that may contribute to suboptimal offspring growth and development, changing the physiology and metabolism of the offspring, potentially predisposing them to metabolic and endocrine diseases later in life (Wu et al., 2004).

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