Domestic Animal Endocrinology 49 (2014) 39–48

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

# Poor maternal nutrition during gestation in sheep reduces circulating concentrations of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in offspring

M.L. Hoffman, M.A. Rokosa, S.A. Zinn, T.A. Hoagland, K.E. Govoni*

*Department of Animal Science, University of Connecticut, Storrs, CT, USA*

ARTICLE INFO

*Article history:*
Received 4 February 2014
Received in revised form 15 May 2014
Accepted 18 May 2014

*Keywords:*
Adipose
Maternal nutrition
Muscle
Sheep
Somatotropic axis

ABSTRACT

To determine if poor maternal nutrition alters growth, body composition, circulating growth factors, and expression of genes involved in the development of muscle and adipose of offspring, 24 Dorset and Shropshire ewes were fed either 100% (control fed), 60% (restricted fed), or 126% (over fed) of National Research Council requirements. Diets began at day 116 ± 6 of gestation until parturition. At parturition, 1 lamb from each control fed (CON), restricted fed (RES), and over fed (OVER) ewe was necropsied within 24 h of birth (1 d; n = 3/treatment) or reared on a control diet for 3 mo (CON = 5, RES = 5, and OVER = 3/treatment) and then euthanized. Body weights and blood samples were collected from lambs from 1 d to 3 mo. Organ weights, back fat thickness, loin eye area, and tissue samples (quadriceps, adipose, and liver) were collected at 1 d and 3 mo of age. The RES lambs weighed 16% less than CON ($P = 0.01$) between 1 d and 3 mo of age. In RES, there was a tendency for reduced heart girth at 1 d and 3 mo ($P < 0.07$) and back fat was reduced 36% at 3 mo ($P = 0.03$). Heart weight was 30% greater in OVER at 1 d when compared with RES lambs ($P = 0.02$). Serum IGF-I and IGFBP-3 were reduced in RES and OVER lambs ($P < 0.05$). Leptin tended to be greater in OVER lambs compared with CON at 1 d and 3 mo ($P \leq 0.08$). Triiodothyronine was reduced in RES at 1 d ($P = 0.05$) and triglycerides tended to be greater in OVER at 3 mo ($P = 0.07$). In liver, there was a tendency for increased expression of *IGF-I* in OVER ($P = 0.06$) and decreased *IGFBP-3* in RES ($P = 0.09$) compared with CON lambs at 1 d. In adipose tissue, adiponectin expression was decreased in RES ($P = 0.05$) at 3 mo. At 1 d of age, muscle expression of *IGF-I* tended to increase in RES ($P = 0.06$). In conclusion, poor maternal nutrition during gestation reduced growth rate in offspring which may be because of reduced circulating IGF-I and IGFBP-3 and decreased expression of IGFBP-3 in the liver.



## 1. Introduction

Fetal development is a period of rapid growth that is tightly regulated by several systemic and local factors. Therefore, alterations to the maternal environment during gestation can negatively impact the normal growth and development patterns of the offspring during postnatal growth. Maternal nutrition can greatly impact fetal development and has persistent effects throughout postnatal growth and into adulthood. In sheep, poor maternal nutrition during gestation due to either undernutrition or overnutrition can alter growth rate, reduce muscle development, and increase fat deposition in offspring [1–5]. Specifically, poor maternal nutrition reduces myofiber diameter and myofiber number and increases muscle connective tissue content in the offspring [1,2,6–8]. Similarly, increased

* Corresponding author. Tel.: +1 860 486 2480; fax: +1 860 486 2413.
*E-mail address:* kristen.govoni@uconn.edu (K.E. Govoni).

0739-7240/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.domaniend.2014.05.002

adipose accumulation within muscle tissue and other adipose tissue depots has been observed in the offspring [9,10]. Consequently, these modifications can affect the carcass quality and predispose the offspring to metabolic disorders. Although some of the phenotypic changes in offspring that result from poor maternal nutrition have been identified, the mechanisms through which these modifications occur are not fully understood.

The somatotropic axis, including GH, IGF-I, and IGFBP, is an important regulator of growth and development of several tissues, including muscle and adipose. Knockout-mouse models in which body weight (BW) is reduced 25% and 67% in GH- and IGF-I-deficient mice, respectively have demonstrated the critical roles of GH and IGF-I in development [11]. In addition, we and others have shown that components of the GH/IGF pathway are altered by nutritional status in cattle and humans [12–14]. Specifically, in a state of reduced growth and/or restricted nutrition, serum concentrations of IGF-I and IGFBP-3 are reduced and IGFBP-2 is increased [12]. Intrauterine growth retardation is associated with reduced circulating IGF-I and IGFBP-3 and increased GH and IGFBP-2 [15]. Similarly, both circulating GH and IGF-I concentrations are reduced in the offspring of mothers who have been undernourished [16]. Poor maternal nutrition can impact circulating concentrations of several other hormones in offspring including leptin, insulin, and thyroid hormones [17–20]. These data provide potential mechanisms by which poor maternal nutrition alters offspring growth and metabolism.

In addition to changes in circulating hormones and growth factors, poor maternal nutrition may affect local production of key genes involved in tissue development. Specifically, over-feeding during gestation alters expression of factors involved in the canonical Wnt signaling pathway and myogenesis in the fetus [2]. Therefore, changes in gene expression may be a potential mechanism through which poor maternal nutrition alters tissue development in offspring. However, little is known about how these changes may be reflected during postnatal muscle growth and development. In addition, many studies target fetal time points or only one variation in diet (overfed or underfed). Based on the negative effects of poor maternal nutrition on offspring development and the potential role of the somatotropic axis, the Wnt signaling pathway and metabolic factors, we hypothesized that poor maternal nutrition would alter offspring growth, body composition, circulating growth factors, and expression of genes involved in the development of muscle and adipose tissue of lambs at 2 early postnatal time points. To test our hypothesis, we underfed or overfed ewes during gestation and determined effects on early postnatal growth of offspring at 1 d and 3 mo of age.

## 2. Materials and methods

All procedures were approved by the University of Connecticut's Institutional Animal Care and Use Committee (protocol number; A10–040).

### 2.1. Animals

Twenty-four multiparous (n = 18) and primiparous (n = 6) Dorset (n = 21) and Shropshire ewes (n = 3), confirmed pregnant with twins by ultrasound (85 ± 10 d of gestation), were placed in individual pens at day 95 ± 10 of gestation (average BW: 102.0 ± 2 kg) and fed a control diet (100% of National Research Council [NRC] requirement) for an initial acclimation period of 12 d. Ewes were transitioned to treatment diets over a 9-d period. Beginning at day 116 ± 6 of gestation ewes began treatment diets and were fed either 100% (control fed), 60% (restricted fed), or 126% (overfed) of the NRC requirements for total digestible nutrients for a ewe in late gestation and pregnant with twin lambs [21]. Ewes were balanced across treatments for parity and breed. We chose to start diets at 116 d of gestation to determine the effects of maternal diet on offspring during the last one-third of gestation. Our target diet for overfed was 140% of NRC, but based on actual feed intake, the overfed group consumed 126% of control fed. Diets for ewes were calculated on a BW basis and adjusted accordingly with changes in BW and stage of gestation. Second-cutting hay was fed to control-fed and overfed ewes (daily average of 2.2 kg per ewe, respectively), and first-cutting hay was fed to restricted-fed ewes (daily average of 1.3 kg per ewe). The overfed ewes received 0.45 kg of cracked corn per ewe daily in addition to hay. During the last 6 wk of gestation, the amount of corn fed to overfed ewes was increased to 0.66 kg/d, whereas, control-fed and restricted-fed ewes received cracked corn for the last 6 wk of gestation (daily average of 0.17 kg and 0.10 kg per ewe, respectively). All feed was weighed daily and any residual feed was weighed and removed the following morning. Nutrient analysis of feeds was performed by Dairy One (Ithaca, NY; Table 1).

A total of 52 lambs were born to all ewes (Dorset = 47; Shropshire = 5). Contrary to earlier ultrasound information, the actual occurrence of singletons, twins, and triplets, respectively was 2, 5, and 1 for control fed, 1, 5, and 2 for restricted fed and 0, 4, and 4 for overfed ewes. On parturition, lambs from control-fed ewes (CON), overfed ewes (OVER), and restricted-fed ewes (RES) remained with the mother for up to 24 h to allow adequate colostrum intake. A commercially available colostrum supplement was provided to the lamb as needed (Lamb's Choice Total Colostrum; Saskatoon Colostrum Co; Saskatoon, Canada). One lamb born to a restricted-fed ewe had to be supplemented with colostrum. After 24 h, 1 lamb was selected from each ewe and was euthanized for sample collection at birth (1 d; n = 7 males; n = 2 females) or 3 mo (n = 10 males; n = 3 females). The remaining lambs stayed with the ewe and were removed from the study and returned to the flock.

**Table 1**
Ewe diet feed composition[a].

| Feed | % CP | % ADF | % NDF | % NFC | % TDN |
|---|---|---|---|---|---|
| Mixed grass hay; first cutting | 8.2 | 43.7 | 66.4 | 18.9 | 55.0 |
| Mixed grass hay; second cutting | 14.2 | 37.5 | 56.8 | 23.0 | 58.0 |
| Corn, cracked | 9.0 | 4.0 | 9.0 | 75.0 | 90.0 |

Abbreviations: ADF, acid detergent fiber; CP, crude protein; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; TDN, total digestible nutrients.

[a] Diets for ewes were based on NRC requirements for TDN for a ewe pregnant with twins during the late gestation [21]. Diets for each ewe were adjusted weekly for changes in body weight.

The larger of the 2 lambs was chosen when lambs were the same gender. If lambs were different genders, the male lamb was chosen for use on the study because males are often slaughtered for market and females kept for breeding. One-half of the lambs from each diet group were slaughtered within 1 d of birth. The remaining lambs were maintained on a control diet until 3 mo of age. These lambs were fed milk replacer (1.7% of BW; Land O'Lakes Animal Milk Product Company; Shoreview, MN) from a bottle until weaning at 60 d of age and allowed ad libitum access to water, creep feed (Lamb BT, Blue Seal Feeds; Litchfield, CT), and second-cutting hay for the entire 3 mo period. The eventual gender distribution of lambs within treatment groups for CON, RES, and OVER, respectively, at 1 d of age was 2 males/1 female, 2 males/1 female and 3 males, and 3 males/2 females, 4 males/1 female, and 1 male/2 females for 3 mo of age. Each 3 mo group had 1 Shropshire lamb.

### 2.2. Sample collection

Lambs were weighed twice per wk and blood samples (10 mL) were collected from a jugular vein every other wk until 3 wk of age when blood samples were collected weekly. Samples were evenly distributed among 3 tubes (no anticoagulant, heparin, and ethylenediaminetetraacetic acid; Becton Dicjson, Franklin Lakes, NJ) for later analyses. Serum tubes were kept at room temperature for 4 to 6 h to allow blood to clot, then stored overnight at 4°C and centrifuged the next morning. Plasma tubes were inverted several times after blood collection and kept on ice until centrifugation, which was completed within 1 h of collection. Blood was centrifuged for 30 min at 1,800*g* (Sorvall RT7; Kendro Laboratory Products, Newtown, CT) at 4°C, and serum and plasma were harvested and stored at −20°C until hormone and metabolite analyses were performed. Animals were euthanized with an intravenous injection of Beuthanasia-D Special followed by exsanguination (0.22 mL/kg; Merck Animal Health, Summit, NJ) within 24 h after birth (1 d) or at 3 mo of age. Organs (heart and liver) were removed and weighed. Loin eye area was determined by evaluating the cross-sectional area ($cm^2$) of the longissimus dorsi muscle between the 12th and 13th rib. Back fat was also measured at this region for lambs at 3 mo. Muscle tissue (quadriceps and longissimus dorsi), adipose tissue (perirenal fat), and liver were collected, immediately snap frozen in liquid nitrogen and stored at −80°C for RNA isolation. The muscle samples were collected from the midpoint of the quadriceps to avoid the fascia and connective tissue. A cross section of the longissimus dorsi was obtained and samples collected from the midpoint. These 2 sites were chosen based on their use as meat products in the sheep industry.

### 2.3. Serum analysis

Circulating concentrations of GH [22] and IGF-I [23–25] were quantified using radioimmuno assay as previously described. Briefly, GH was determined using anti-ovine GH with ovine GH standard (National Hormone & Peptide Program, Harbor-UCLA Medical Center, Torrance, CA). Intra and inter assay CV were 9.5% and 13.4%, respectively with a minimum detectable level of 1.8 ng/mL. Serum IGF-I was determined using anti-human IGF-I with human standard (National Hormone & Peptide Program). Intra and inter assay CV were 6.4% and 10.8%, respectively with a minimum detectable level of 40 ng/mL. Concentrations of IGFBP-2 and 3 were determined by ligand blot following polyacrylamide gel electrophoresis [23–25]. Membranes were incubated overnight with approximately 1.6 MBq of $^{125}$Ilabeled IGF-I (Amersham Pharmacia Biotech, Piscataway NJ). After incubation, membranes were washed to remove unbound 125I-labeled IGF-I and then exposed to a multipurpose phosphor screen (Packard Instrument Company, Meriden CT). The remaining radioactivity bound to blots was imaged with a Cyclone Storage Phosphor System (Packard Instrument Company), and quantified with OptiQuant software (Packard Instrument Company). Both IGFBP-2 and 3 were quantified as digital light units per $mm^2$ and expressed in arbitrary units (AU) as a percentage of the pooled ovine standard sera IGFBP-3 included on each gel. Commercially available ELISA kits were used to determine circulating concentrations of triiodothyronine ($T_3$; Calbiotech, Spring Valley, CA), thyroxine ($T_4$; Calbiotech), and glucose (Cayman Chemical Company, Ann Arbor, MI). Minimum detectable level of $T_3$ and $T_4$ are 0.04 ng/mL and 0.45 ng/mL, respectively. Standard intra and inter assay CV for glucose was 4.6% and 1.7%, respectively. Circulating concentrations of insulin were determined by ELISA (ovine insulin kit; ALPCO Diagnostics, Salem, NH), and the intra assay CV was 4.9%. Circulating concentrations of leptin were determined using a commercially available multi-species radioimmuno assay (EMD Millipore Corporation, Billerica, MA). This kit has been successfully used to determine circulating leptin in sheep [18]. Leptin assay intra assay CV was 4.4%. Circulating total cholesterol and triglycerides (TG) were determined via standard enzymatic analysis [26].

### 2.4. RNA extraction

Tissue was ground in a mortar cooled with liquid nitrogen and cells lysed with 1 mL TriReagent (Sigma, Aldrich, Valencia, MO). Adipose, muscle, and liver RNA was extracted using a Qiagen Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, CA). Genomic DNA was removed from samples using a Turbo DNA Free kit (Ambion, Foster City, CA). The quality of RNA was determined using an Agilent analysis system (Agilent Technologies, Santa Clara, CA). Quantity of RNA was determined using a Nanodrop spectrophotometer (Thermoscientific, Lafayette, CO).

### 2.5. Real-time reverse transcription polymerase chain reaction

Reverse transcription polymerase chain reaction (RT-PCR) was performed using 300 ng total RNA with OligodT primer (Ambion) and master mix containing 5.5 μL of 5× buffer (Invitrogen; Carlsbad, CA), 1.0 μL dNTP (Promega; Madison, WI), 2.0 μL DL-dithiothreitol (DTT), and 0.5 μL Superscript II (Invitrogen) for a total reaction volume of 20 μL. Reverse transcription was performed with a standard protocol starting at 70°C for 10 min, 4°C for 20 min, 37°C for 3 min, 42°C for 1 h, 4°C for 3 min, and 90°C for 2.5 min. Primers were designed using Primer3 and NCBI BLAST,

**Table 2**
Primer sequences for real-time RT-PCR.

| Gene | Primer sequences (5′ to 3′) | Reference |
|---|---|---|
| *ADIPOQ* | | |
| Forward | ATCAAACTCTGGAACCTCCTATCTAC | [52] |
| Reverse | TTGCATTGCAGGCTCAAG | |
| *β-Catenin* | | |
| Forward | GGATGTGGATACCACCCAAG | [2] |
| Reverse | CCCTCATCTAGCGTCTCAGG | |
| *CEBPα* | | |
| Forward | AGTCCGTGGACAAGAACAGC | XM_004015623.1 |
| Reverse | TTGTCACTGGTCAGCTCCAG | |
| *CEBPβ* | | |
| Forward | GACAAGCACAGCGACGAGT | [53] |
| Reverse | GTGCTGCGTCTCCAGGTG | |
| *CHREBP* | | |
| Forward | CTCCGCTCCACATACTGGAT | NM_001205408.1 |
| Reverse | GTTGTTGAGGCGGATCTTGT | |
| *GAPDH* | | |
| Forward | GGCGTGAACCACGAGAAGTATAA | [54] |
| Reverse | CCTCCACGATGCCAAAGTG | |
| *GHR* | | |
| Forward | AAATTCACCAAGTGCCGTTC | NM_00100323.2 |
| Reverse | TGTTTTCACCAGCAGAGACG | |
| *GLUT-4* | | |
| Forward | AGGACGTTTGACCAGATCTCA | AB_005283.1 |
| Reverse | CAGTTCTGTGCTGGGTTTCA | |
| *GSK-3β* | | |
| Forward | TCCGACCCCGAACTCCACCC | NM_001129740.1 |
| Reverse | GTGCAGGTGTGTCTCGCCCA | |
| *IGFBP1* | | |
| Forward | TGATGACCGAGTCCAGTGAG | NM_001145177.1 |
| Reverse | GCTCCTTCCACTTCTTGACG | |
| *IGFBP2* | | |
| Forward | CCCTACACATCCCCAACTGT | NM_0010009436.1 |
| Reverse | CAGTGTTGGGGTTCACACAC | |
| *IGFBP3* | | |
| Forward | CAGAGCACAGACACCCAGAA | NM_001159276.1 |
| Reverse | CACAGTTGGGAATGTGGATG | |
| *IGF-I* | | |
| Forward | CCAGACAGGAATCGTGGATG | NM_001009774.3 |
| Reverse | ACTTGGCGGGCTTGAGAG | |
| *IGF-IR* | | |
| Forward | ACCTACACAGCCCGGATCCA | XM_004018023.1 |
| Reverse | ACACAGGCTCCGTCCATGAC | |
| *Leptin* | | |
| Forward | TGACACCAAAACCCTCATCA | U84247.1 |
| Reverse | CCAAACCAGTGACCCTCTGT | |
| *Myf-5* | | |
| Forward | AGACGCCTGAAGAAGGTGAA | XM_004006219.1 |
| Reverse | AGCAGCTCCTGCAGACTCTC | |
| *MyoD* | | |
| Forward | CCCTGGTGACTTCAGCTGTT | [2] |
| Reverse | CCTGCCTGCCGTATAAACAT | |
| *Myogenin* | | |
| Forward | TGGGCGTGTAAGGTGTGTAA | [2] |
| Reverse | TGCACAGGATCTCCACTTTG | |
| *PAX-7* | | |
| Forward | GAGACCGACTGCTGAAGGAC | XM_002685738 |
| Reverse | ATGCTGTGCTTGGCTTTCTT | |
| *PPAR-γ* | | |
| Forward | CTTGCTGTGGGGATGTCT | NM_001100921.1 |
| Reverse | GGTCAGGAGACTCTGGGTC | |
| *Tbx2* | | |
| Forward | CTT GCA GTG CTC CTC CTA | NM_001191443.1 |
| Reverse | CAC GCA GCT TAA GAT CGA CA | |
| *Tbx3* | | |
| Forward | ATC GCT GTG ACT GCA TAC CA | XM_002694588.3 |
| Reverse | TCT CTC CTG CCA TTT CCA GT | |
| *TSC-2* | | |
| Forward | TGCAAGCTGTCTTCCACATC | XM_003587808.2 |
| Reverse | AACTTGAAGTCCTCGCCAGA | |

validated as previously described (Govoni et al., 2006) and synthesized by Integrated DNA Technologies (Coralsville, IA; Table 2). Four endogenous control genes (pentidylprolyl isomerase [*PPIA*], tubulin, 18s, and glyceraldehyde 3-phosphatase [*GAPDH*]) were tested, and variation in cycle threshold (Ct) values between treatment and time points were determined. Expression of *GAPDH* did not vary between treatments or time points and was used as the endogenous control gene for gene expression analysis. Genes of interest for adipose tissue and myogenic factors are listed in Table 2. Primers were designed for genes involved in the somatotropic axis (*GH*, *GH* receptor [*GHR*], *IGF-I*, *IGF-I* receptor [*IGF-IR*], *IGFBP-1*, *IGFBP-2*, and *IGFBP-3*), the Wnt signaling pathway (*TSC-2*, *β-catenin*, and glycogen synthase kinase [*GSK*]-*3 β*), myogenesis (paired box protein [*Pax*]-*7*, myogenic factor [*myf*]-*5*, myogenin, and *myoD*), adipogenesis (peroxisome proliferator-activated receptor [*PPAR*]-*γ*, *CCAAT*/enhancer binding protein *(C/EBP)-α*, and *C/EBP-β*), metabolism (leptin, glucose transporter type 4 [*GLUT4*], and carbohydrate-responsive element-binding protein [*CHREBP*]), and cell function (T-box [*Tbx*]-2 and -3). Real-time RT-PCR was performed using Power SybrGreen Master Mix (Invitrogen) and the ABI 7900 HT Fast Real-time PCR machine (Applied Biosystems, Foster City, CA). The total volume of the reaction mixture was 25 μL containing 5 μL of complementary DNA, 3 μL of nuclease free water, 1 μL each of 10 nM forward and reverse primer, and 10 μL of Sybrgreen. For the Tbx3 primer, 0.5 μL of each forward and reverse primer was used. Real-time RT-PCR was performed using standard cycling conditions (stage 1: 50°C for 2 min and 95°C for 10 min, stage 2: 95°C for 15 s and 60°C for 40 cycles). Ct values were used to calculate the $^{\Delta\Delta}$Ct values to determine changes in gene expression [27]. Changes in gene expression are expressed relative to the control.

### 2.6. Statistical analysis

All statistical analysis was performed using Statistical Analysis Software version 9.2 (SAS Inst. Inc, Cary, NC). Circulating GH, IGF-I, IGFBP-2, IGFBP-3, and BW were analyzed using the mixed models procedure with repeated measures. The covariance structure used was determined by the lowest Akaike information criterion values. Covariance structure used for GH and IGF-I were compound symmetry and for IGFBP-2 and 3, variance component and autoregression were used, respectively. Back fat data were analyzed as a percentage of BW of the animal. All other data were analyzed using the mixed models procedure. Where appropriate, mean comparisons were made using least square means. Statistical significance was considered at $P \leq 0.05$ and a tendency at $P > 0.05$ and $\leq 0.10$. Because of the limited number of females and Shropshire lambs in each treatment and time point, we were not able to test for

Abbreviations: *ADIPOQ*, adiponectin; *ChREBP*, carbohydrate-responsive element-binding protein; *GAPDH*, glyceraldehyde 3-phosphatase; *GH*, growth hormone; *GHR*, GH receptor; *GLUT4*, glucose transporter type 4; *GSK-3β*, glycogen synthase kinase; *IGF-IR*, IGF-I receptor; *TSC2*, tuberous sclerosis-2; *Myf5*, myogenic factor-5; *MyoD*, myogenic differentiation-1; *Pax-7*, paired box protein; RT-PCR, reverse transcription polymerase chain reaction; *Tbx-2 and -3*, T-box.

breed or gender differences. Therefore, all animals were included and the main effect of treatment was tested.

## 3. Results

### 3.1. Body composition and body parameters

Over the duration of the study control-fed, restricted-fed or overfed ewes consumed 1.43, 0.79, or 1.83 kg of total digestible nutrients (standard error of the mean ± 0.09) and 0.30, 0.10, and 0.31 kg of CP (standard error of the mean ± 0.02) per day, respectively. Ewes remained on study until parturition (time on treatment = 32 ± 10 d). At parturition, restricted-fed ewes weighed 14% less than control-fed ewes (95 ± 4 kg vs 111 ± 3 kg; $P = 0.002$). Body weights for overfed ewes were not different compared with control-fed ewes (118 ± 3 vs 111 ± 3 kg; $P = 0.18$).

Overall, for all time points between 1 d and 3 mo of age, RES lambs weighed 16% less compared with CON lambs ($P = 0.01$; Fig. 1). A difference in BW was not observed between OVER and CON between 1 d and 3 mo of age ($P = 0.24$). However, between 7 and 10 wk of age, OVER lambs tended to weigh 11% less than CON lambs ($P \leq 0.08$; Fig. 1). Heart girth size was reduced in RES lambs at 1 d ($P \leq 0.01$) and tended to be reduced 3 mo of age ($P = 0.07$; Table 3). At 1 d, there tended to be an effect of maternal diet on heart weight ($P = 0.08$; Table 3). Specifically, heart weight was 30% greater in OVER compared with RES lambs ($P = 0.02$; Table 3) but at 3 mo were not different ($P = 0.61$; Table 3). Back fat was reduced 36% in RES compared with CON lambs at 3 mo of age ($P = 0.03$; Table 3). No differences in liver weight ($P \geq 0.76$), crown rump length ($P \geq 0.29$), and loin eye area ($P = 0.61$) were observed at 1 d or 3 mo of age (Table 3).

### 3.2. Circulating hormones and growth factors

Average serum concentrations of IGF-I between 1 d and 3 mo of age were 48% less in RES ($P = 0.01$), but not different in OVER ($P = 0.19$) compared with CON lambs (Fig. 2; 308.1 ± 37.5 ng/mL, 158.8 ± 36.5 ng/mL, and 227.5 ± 40.6 ng/mL for CON, RES, and OVER, respectively). Similarly, average serum concentrations of IGFBP-3 between 1 d and

**Table 3**
Growth traits in lambs born to ewes fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins.

| Item | Treatment[a] | | | | $P$[b] |
|---|---|---|---|---|---|
| | CON | RES | OVER | SEM | |
| BW, kg | | | | | |
| 1 d | 5.33[c] | 4.15[d] | 5.12[c] | 0.25 | **0.04** |
| 3 mo | 35.21 | 31.29 | 33.59 | 0.72 | 0.17 |
| HG, cm | | | | | |
| 1 d | 40.80[c] | 36.90[d] | 40.40[c] | 1.18 | **< 0.01** |
| 3 mo | 79.44 | 70.04 | 72.81 | 2.22 | 0.17 |
| CR, cm | | | | | |
| 1 d | 49.37 | 47.18 | 50.88 | 1.03 | 0.27 |
| 3 mo | 97.66 | 93.82 | 97.68 | 1.17 | 0.29 |
| LEA, $cm^2$ | | | | | |
| 1 d | ND | ND | ND | ND | ND |
| 3 mo | 27.90 | 26.65 | 25.25 | 1.13 | 0.61 |
| BF, mm | | | | | |
| 1 d | ND | ND | ND | ND | ND |
| 3 mo | 2.19[c] | 1.40[d] | 2.80[c] | 0.30 | **0.03** |
| Heart, g | | | | | |
| 1 d | 36.32[x] | 30.27[x] | 43.89[y] | 2.55 | **0.08** |
| 3 mo | 170.00 | 153.64 | 159.09 | 6.80 | 0.61 |
| Liver, g | | | | | |
| 1 d | 116.53 | 99.88 | 119.53 | 10.43 | 0.76 |
| 3 mo | 623.64 | 594.55 | 616.67 | 21.94 | 0.88 |

Abbreviations: BF, back fat; CR, crown rump; LEA, loin eye area; HG, heart girth; ND, not determined; SEM, standard error of the mean.
One lamb from each ewe was used for sample collection at 1 d (n = 3/treatment) and 3 mo of age (n = 3–5/treatment). Bold values demonstrate a significant difference ($P \leq 0.05$ or trend $P \leq 0.10$ and $\geq 0.05$).
[c,d] Means within a row with different superscripts differ ($P \leq 0.05$).
[x,y] Means within a row with different superscripts differ ($P \leq 0.10$).
[a] Values given as mean ± SEM.
[b] Values provided for treatment effect.

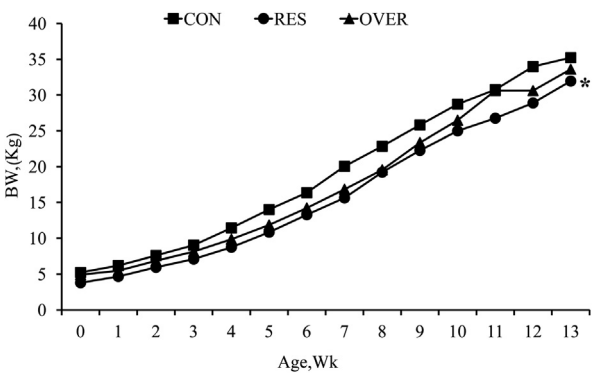


**Fig. 1.** Effects of maternal diet during the last third of gestation on offspring BW. Ewes were fed 100% (control-fed, CON), 60% (restricted-fed, RES), or 126% (overfed, OVER) of NRC requirements for ewes pregnant with twins and 1 lamb from each ewe was necropsied at 1 d (n = 3/treatment) or 3 mo (n = 3 to 5/treatment) of age. Data presented are means ± SEM of lambs born to CON, RES, and OVER ewes. * $P = 0.01$ vs CON lambs. Average daily gains for animals were 0.33 ± 0.10 kg, 0.29 ± 0.01 kg, and 0.32 ± 0.10 for CON, RES, and OVER, respectively. Abbreviation: SEM, standard error of the mean.

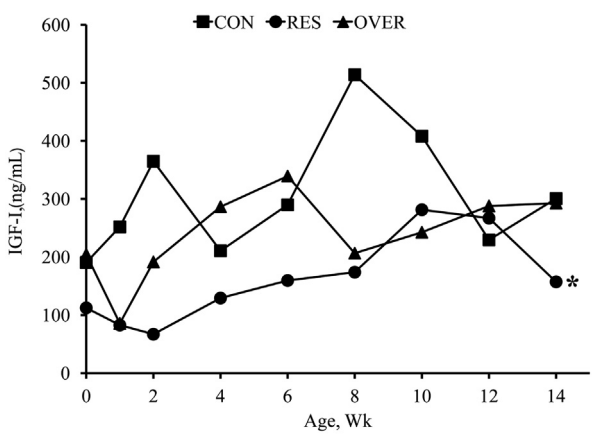


**Fig. 2.** Effects of maternal diet during the last third of gestation on offspring circulating IGF-I. Ewes were fed 100% (control-fed, CON), 60% (restricted-fed, RES), or 126% (overfed, OVER) of NRC requirements for ewes pregnant with twins and 1 lamb from each ewe was necropsied at 1 d (n = 3/treatment) or 3 mo (n = 3 to 5/treatment) of age. Data presented are means ± SEM of lambs born to control-fed (CON), restricted-fed (RES), and overfed (OVER) ewes. * $P = 0.01$ vs CON lambs. Average serum concentrations of IGF-I over time were 308.1 ± 37.5 ng/mL, 158.8 ± 36.5 ng/mL, and 227.5 ± 40.6 ng/mL for CON, RES, and OVER lambs, respectively. Abbreviation: SEM, standard error of the mean.

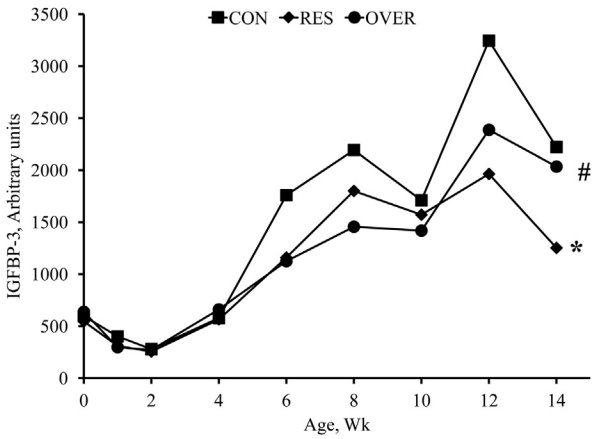


**Fig. 3.** Effects of maternal diet during the last third of gestation on offspring circulating IGFBP-3. Ewes were fed 100% (control-fed, CON), 60% (restricted-fed, RES), or 126% (overfed, OVER) of NRC requirements for ewes pregnant with twins and 1 lamb from each ewe was necropsied at 1 d (n = 3/treatment) or 3 mo (n = 3–5/treatment) of age. Data presented are means ± SEM of lambs born to control-fed (CON), restricted-fed (RES), and overfed (OVER) ewes. * $P = 0.02$ vs CON lambs. # $P = 0.07$ vs CON lambs. Average serum concentrations of IGFBP-3 over time were 1,735 ± 123 AU, 1,366 ± 145 AU, 1, 301 ± 125 AU for CON, RES, and OVER lambs, respectively. Abbreviation: SEM, standard error of the mean.

3 mo of age were 24% less in RES ($P = 0.02$) and tended ($P = 0.07$) to be 21% less in OVER compared with CON lambs (Fig. 3; 1,735 ± 123 AU, 1,366 ± 145 AU, and 1, 301 ± 125 AU for CON, RES, and OVER, respectively). Across all time points, average serum concentrations of GH (1.55 ± 0.29 ng/mL, 2.00 ± 0.23 ng/mL, 1.92 ± 0.29 ng/mL for CON, RES and OVER, respectively; $P = 0.49$) or IGFBP-2 (193 ± 18 AU, 211 ± 18 AU, 196 ± 18 AU for CON, RES, and OVER, respectively; $P = 0.87$) were not affected by maternal diet. At 1 d, concentrations of leptin were 62% greater in OVER ($P = 0.02$; Table 4) compared with CON, but no difference was detected ($P = 0.12$) in RES lambs. At 3 mo of age, serum concentrations of leptin were 39% greater in OVER ($P = 0.03$; Table 4) compared with CON, but no difference was detected ($P = 0.13$) in RES lambs. Serum concentrations of $T_3$ were reduced in RES ($P = 0.05$) compared with CON lambs at 1 d, but no differences were observed at 3 mo of age ($P = 0.35$; Table 4). Serum concentrations of $T_4$ were not different in RES, OVER, and CON groups ($P \geq 0.29$; Table 4). We did not detect an effect of maternal diet on circulating glucose ($P \geq 0.56$; Table 4), insulin ($P \geq 0.23$; Table 4), or serum concentrations of TG or total cholesterol at 1 d and 3 mo ($P \geq 0.17$; Table 4).

### 3.3. Gene expression in liver from offspring

Based on the changes in circulating IGF-I and IGFBP-3, we determined expression of genes involved in the somatotropic axis in the liver at 1 d and at 3 mo of age. At 1 d, expression of *IGF-I* tended to increase in OVER ($P = 0.06$), but did not change in RES ($P = 0.69$; Table 5) compared with CON lambs. No effects of dietary treatment were observed in expression of *GHR*, *IGFBP-1*, *IGFBP-2*, and *IGFBP-3* at 1 d of age ($P \geq 0.15$; Table 5). At 3 mo of age, no change

**Table 4**
Concentrations of metabolic hormones and metabolites in lambs born to ewes fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins.

| Item | Treatment[a] | | | | $P$[b] |
|---|---|---|---|---|---|
| | CON | RES | OVER | SEM | |
| Leptin, mg/dL | | | | | |
| 1 d | $1.28^{x}$ | $1.73^{x}$ | $2.08^{y}$ | 0.24 | **0.07** |
| 3 mo | $0.92^{x}$ | $1.14^{x}$ | $1.28^{y}$ | 0.15 | **0.08** |
| $T_3$, ng/mL | | | | | |
| 1 d | $5.95^{x}$ | $5.58^{y}$ | $5.94^{x}$ | 0.07 | **0.08** |
| 3 mo | 2.98 | 3.38 | 2.87 | 0.18 | 0.53 |
| $T_4$, ng/mL | | | | | |
| 1 d | 16.28 | 14.88 | 16.44 | 0.44 | 0.29 |
| 3 mo | 7.19 | 6.50 | 5.95 | 0.55 | 0.75 |
| Insulin, mg/dL | | | | | |
| 1 d | 0.52 | 0.40 | 0.55 | 0.03 | 0.23 |
| 3 mo | 0.62 | 0.68 | 0.97 | 0.09 | 0.52 |
| Glucose, mg/dL | | | | | |
| 1 d | 59.00 | 56.00 | 65.00 | 3.69 | 0.62 |
| 3 mo | 93.00 | 73.00 | 83.00 | 8.65 | 0.56 |
| TG, mg/dL | | | | | |
| 1 d | 30.01 | 27.48 | 19.35 | 3.07 | 0.35 |
| 3 mo | 12.68 | 13.85 | 17.86 | 2.80 | 0.17 |
| TC, mg/dL | | | | | |
| 1 d | 39.52 | 45.76 | 45.70 | 1.98 | 0.37 |
| 3 mo | 45.42 | 37.65 | 49.80 | 1.10 | 0.25 |

Abbreviations: $T_3$, triiodothyronine; $T_4$, thyroxine; TC, total cholesterol; TG, triglyceride; SEM, standard error of the mean.
One lamb from each ewe was used for sample collection at 1 d (n = 3/treatment) and 3 mo of age (n = 3–5/treatment).
Shaded values demonstrate a significant difference ($P \leq 0.05$ or trend $P \leq 0.10$ and $\geq 0.05$).
[x,y] Means within a row with different superscripts differ ($P \leq 0.10$).
[a] Values given as mean ± SEM.
[b] Values provided for treatment effect.

was observed in messenger RNA (mRNA) expression of factors involved in the somatotropic axis (*GHR*, *IGF-I*, *IGFBP-1*, *IGFBP-2*, and *IGFBP-3*; $P \geq 0.11$; Table 6). Maternal diet did not alter expression of *GLUT4* or *CHREBP* in offspring at 1 d or 3 mo of age ($P \geq 0.13$; Table 5).

### 3.4. Gene expression in adipose tissue from offspring

Maternal diet did not alter expression of markers of adipogenesis (*PPARγ*, *C/EBPα*, and *C/EBPβ*) at 1 d or at 3 mo of age ($P \geq 0.34$; Tables 5 and 6). Similarly, maternal diet did not alter expression of leptin at 1 d or 3 mo of age compared with CON ($P \geq 0.20$; Tables 5 and 6). Maternal diet did not alter expression of adiponectin, *CHREBP*, or *TSC2* at 1 d or at 3 mo of age ($P \geq 0.12$; Tables 5 and 6).

### 3.5. Gene expression in muscle from offspring

In quadriceps muscle, expression of *β-catenin* tended to be 1.9-fold greater in RES and OVER at 1 d compared with CON ($P = 0.07$; Table 5), but no differences were observed at 3 mo of age ($P = 0.89$; Table 6). Maternal diet did not affect expression of markers of myogenesis (*Pax7*, *Myf5*, and myogenin), *IGF-I*, *IGF-IR*, *GSKβ*, *GLUT4*, *TSC2*, *Tbx2*, and *Tbx3* at 1 d or 3 mo of age ($P \geq 0.31$; Tables 5 and 6). We did not detect a change in expression of any genes in longissimus dorsi at 1 d or 3 mo of age.

**Table 5**
mRNA expression at 1 d in liver, adipose, and muscle of lambs born to ewes fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins.

| Gene | Treatment[a] | | | | $P^{b}$ |
|---|---|---|---|---|---|
| | CON | RES | OVER | SEM | TRT |
| Liver | | | | | |
| *GHR* | 1.03 | 0.78 | 0.84 | 0.06 | 0.27 |
| *IGF-I* | 1.01$^{x}$ | 0.95$^{x}$ | 1.22$^{y}$ | 0.06 | **0.07** |
| *IGFBP-1* | 1.05 | 1.97 | 0.60 | 0.23 | 0.15 |
| *IGFBP-2* | 1.11 | 0.73 | 1.16 | 0.25 | 0.81 |
| *IGFBP-3* | 1.03$^{x}$ | 0.68$^{y}$ | 0.93$^{x}$ | 0.08 | 0.19 |
| *CHREBP* | 1.10 | 0.89 | 1.05 | 0.21 | 0.95 |
| *GLUT4* | 1.87 | 1.54 | 1.21 | 0.39 | 0.93 |
| Adipose | | | | | |
| *PPAR-γ* | 1.05 | 1.21 | 0.93 | 0.07 | 0.58 |
| *CEBPα* | 1.43 | 1.91 | 1.82 | 0.33 | 0.78 |
| *CEBPβ* | 1.02 | 0.86 | 0.80 | 0.22 | 0.47 |
| *Leptin* | 2.34 | 4.82 | 7.25 | 0.30 | 0.30 |
| *CHREBP* | 1.31 | 1.41 | 1.41 | 0.30 | 0.86 |
| *GLUT4* | 1.35 | 1.23 | 2.06 | 0.19 | 0.77 |
| *ADIPOQ* | 1.09 | 1.49 | 0.97 | 0.16 | 0.55 |
| Quadriceps | | | | | |
| *β-Catenin* | 1.12 | 1.90 | 1.91 | 0.18 | 0.11 |
| *GSKβ* | 1.32 | 1.43 | 1.67 | 0.21 | 0.63 |
| *PAX7* | 1.66 | 1.18 | 0.91 | 0.41 | 0.73 |
| *Myf5* | 1.29 | 2.08 | 1.77 | 0.23 | 0.38 |
| *Myogenin* | 1.92 | 1.34 | 1.73 | 0.55 | 0.98 |
| *IGF-I* | 1.27 | 2.92 | 1.94 | 0.24 | 0.16 |
| *IGF-IR* | 1.23 | 1.56 | 1.42 | 0.30 | 0.64 |
| *GLUT4* | 1.40 | 1.25 | 1.33 | 0.24 | 0.80 |
| *TSC2* | 1.20 | 0.97 | 1.19 | 0.25 | 0.61 |
| *TBX2* | 1.23 | 1.56 | 1.54 | 0.21 | 0.63 |
| *TBX3* | 1.08 | 1.12 | 1.01 | 0.11 | 0.94 |

Abbreviations: *ADIPOQ*, adiponectin; *GHR*, growth hormone receptor; *IGFBP*, insulin like growth factor binding protein; mRNA, messenger RNA; *Myf-5*, myogenic factor-5; *PAX-7*, paired box protein-7; TRT, treatment; SEM, standard error of the mean.
Ewes were fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins. Tissue samples were collected from lambs at 3 mo (3–5/trt) necropsies.
Bold value demonstrates a significant difference ($P \leq 0.05$ or trend $P \leq 0.10$ and $\geq 0.05$).
$^{x,y}$ Means within a row with different superscripts differ ($P \leq 0.10$).
[a] Values given as mean ± SEM.
[b] Values provided for treatment effect.

**Table 6**
mRNA expression at 3 mo in liver, adipose, and muscle of lambs born to ewes fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins.

| Gene | Treatment[a] | | | | $P^{b}$ |
|---|---|---|---|---|---|
| | CON | RES | OVER | SEM | TRT |
| Liver | | | | | |
| *GHR* | 1.37 | 0.80 | 1.10 | 0.24 | 0.80 |
| *IGF-I* | 1.17 | 0.85 | 0.77 | 0.14 | 0.68 |
| *IGFBP-1* | 2.45 | 1.68 | 0.92 | 0.62 | 0.92 |
| *IGFBP-2* | 1.76 | 2.03 | 1.55 | 0.37 | 0.83 |
| *IGFBP-3* | 1.04 | 0.80 | 1.29 | 0.10 | 0.25 |
| *CHREBP* | 1.06 | 1.51 | 0.74 | 0.25 | 0.24 |
| *GLUT4* | 1.07 | 1.80 | 1.90 | 3.97 | 0.13 |
| Adipose | | | | | |
| *PPAR-γ* | 1.02 | 0.91 | 1.20 | 0.07 | 0.37 |
| *CEBPα* | 1.13 | 0.67 | 1.74 | 0.28 | 0.34 |
| *CEBPβ* | 1.16 | 1.28 | 0.65 | 0.22 | 0.42 |
| *Leptin* | 1.21 | 0.92 | 2.59 | 0.30 | 0.19 |
| *CHREBP* | 1.60 | 0.88 | 1.82 | 0.30 | 0.54 |
| *GLUT4* | 1.33 | 1.18 | 1.54 | 0.19 | 0.66 |
| *ADIOPQ* | 1.00 | 0.69 | 0.92 | 0.07 | 0.12 |
| Quadriceps | | | | | |
| *β-Catenin* | 1.06 | 1.19 | 1.19 | 0.09 | 0.89 |
| *GSKβ* | 1.02 | 0.99 | 1.10 | 0.08 | 0.74 |
| *PAX7* | 1.36 | 1.29 | 2.00 | 0.50 | 0.86 |
| *Myf5* | 1.13 | 0.98 | 1.43 | 0.23 | 0.59 |
| *Myogenin* | 1.04 | 1.46 | 0.90 | 0.26 | 0.49 |
| *IGF-I* | 1.06 | 1.22 | 1.23 | 0.16 | 0.95 |
| *IGF-IR* | 1.46 | 1.80 | 1.12 | 0.47 | 0.90 |
| *GLUT4* | 1.28 | 1.55 | 1.05 | 0.21 | 0.65 |
| *TSC2* | 1.02 | 0.74 | 1.18 | 0.16 | 0.52 |
| *TBX2* | 1.29 | 1.09 | 0.83 | 0.30 | 0.57 |
| *TBX3* | 1.09 | 1.39 | 1.33 | 0.45 | 0.85 |

Abbreviations: *ADIPOQ*, adiponectin; *GHR*, growth hormone receptor; *IGFBP*, insulin like growth factor binding protein; mRNA, messenger RNA; *Myf-5*, myogenic factor-5; *PAX-7*, paired box protein-7; TRT, treatment; SEM, standard error of the mean.
Ewes were fed 100% (CON), 60% (RES), or 126% (OVER) of NRC requirements for ewes in late gestation and pregnant with twins. Tissue samples were collected from lambs at 3 mo (3–5/trt) necropsies.
[a] Values given as mean ± SEM.
[b] Values provided for treatment effect.

## 4. Discussion

Proper maternal nutrition is critical for fetal development, and there is increasing evidence that negative effects of poor maternal diet can persist into adulthood contributing to poor growth and metabolic disorders in offspring. Poor maternal nutrition can result from many factors including, inadequate intake of calories, protein, or specific vitamins and minerals. In addition to impaired growth because of inadequate nutrients, overfeeding of mothers during gestation also negatively impacts growth and health of the offspring leading to increased fat deposition [6,28–30]. We used both underfeeding and overfeeding models in the same study to allow for these direct comparisons.

Underfeeding ewes during gestation reduced BW of offspring at birth. These findings are similar to George et al [31] in which nutrient restriction occurred during the first half of gestation (d 28–78), whereas in the present study ewes were restricted for the last 32 d of gestation. Therefore, regardless of timing of maternal nutrient restriction, it leads to reduced BW and early postnatal growth. Typically, offspring born small for gestational age undergo compensatory growth, which is because of increased adipose tissue accretion [32–34]. However, in the present study, the reduced BW persisted until 3 mo of age. In fact, similar to reduced BW, there was a reduction in back fat in the RES offspring at 3 mo suggesting that, although they were fed ad libitum postnatally, we did not observe a compensatory weight gain in these lambs during early postnatal growth. Alternatively, previous studies [32,35] quantified BW and body composition of the offspring for longer periods of time (eg, 6–16 mo of age). Therefore, compensatory growth may occur during later stages of postnatal growth.

Similar to previous studies [30,36], a difference in BW between CON and OVER was not observed. This is not surprising based on the 26% increase in feed intake in mothers during the last third of gestation. Although a difference in BW was not observed, there was a tendency for increased heart size in OVER at birth. These findings are similar to a previous study in sheep in ewes fed an obesogenic diet had offspring with increased heart size during gestation [36]. These findings in the present study, along

with previous studies, demonstrate that maternal diet can impact development of critical organs.

The somatotropic axis, including GH, IGF-I, and IGFBP, is required for normal growth. We and others [12,37–39] previously demonstrated that circulating components of the somatotropic axis are altered by the nutritional status of the animal. Specifically, with reduced nutrition and reduced growth rate, GH and IGFBP-2 are increased and IGF-I and IGFBP-3 are reduced [12]. However, it is not well understood how reduced or excess nutrient intake of the mother affects the somatotropic axis during early postnatal growth of the offspring. Consistent with reduced BW in RES offspring, circulating concentrations of IGF-I and IGFBP-3 were reduced. Insulin-like growth factor-I is required for optimal growth and is primarily found in serum bound to IGFBP-3 to prevent rapid degradation [40]. Therefore, the reduced IGF-I and IGFBP-3 may contribute to the lack of increased growth in the RES offspring by 3 mo of age. Similar to RES lambs, IGFBP-3 tended to be reduced in OVER offspring. These findings are similar to recent findings by Smith et al [41] in rats, in which 2 models of maternal nutrition (underfeeding and overfeeding) have similar effects on the somatotropic axis in offspring [41]. Based on the knowledge that reduced nutrition can alter circulating IGFBP [12], we anticipated that IGFBP-2 would be increased at birth. However, no changes in IGFBP-2 were observed. It is possible that other mechanisms besides nutrient availability of the mother, such as hormone changes in the mother, program the circulating factors in the offspring during gestation and may persist into postnatal growth. Although it is well established that nutrition affects circulating GH [12] additional studies with more frequent sample collection, because of the pulsatile nature of GH, are needed to fully understand the effects of poor maternal nutrition on GH concentrations in offspring [42].

To determine if changes in circulating IGF-I and IGFBP were because of changes in gene expression in the liver, we determined mRNA expression at 1 d and at 3 mo of age. Consistent with reduced circulating IGF-I and IGFBP-3, mRNA expression of IGFBP-3 tended to be reduced at birth in RES suggesting that reduced production of IGFBP-3 from liver may have contributed to reduced BW at birth. It is not clear why liver IGF-I mRNA did not parallel circulating concentrations because this is the primary source of IGF-I in circulation. However, the parallel in changes in circulating IGF-I and IGFBP-3 are consistent with previous reports demonstrating that both factors are affected by exogenous GH administration and critical for optimal growth [43]. Therefore, the reduction in IGF-I and IGFBP-3 in the circulation are consistent with the critical role they play in growth. Additionally, this may be a potential mechanism contributing to reduced growth in the RES offspring during the first 3 mo of age. The lack of change in expression of genes involved in the somatotropic axis could be because of the limited number of animals. Additionally, it could also be possible that changes in circulating factors, IGF-I and IGFBP-3, were not affected similarly at the local level in response to maternal nutrition. Future studies with increased animal numbers and global gene expression analysis are needed to fully elucidate the mechanisms by which poor maternal nutrition impair offspring growth.

In addition to changes in circulating growth factors, changes in local expression in key tissues, such as muscle and fat, may contribute to altered growth. Although a difference in loin eye area in RES and OVER lambs was not detected in the present study, previous studies have demonstrated that poor maternal nutrition, because of both underfeeding and overfeeding, reduced muscle development in offspring during gestation and early postnatal development [1–3,8,20]. The canonical Wnt signaling pathway has been shown to enhance bone and muscle formation and inhibit adipogenesis [10,44]. Expression of *β-catenin* tended to increase in muscle of RES and OVER lambs at 1 d of age. This is in contrast to a previous study in which expression was reduced [2]. The previous study evaluated effects of maternal obesity and also observed reduced muscle fiber diameter and expression of markers of myogenesis [2]. It is not clear why the increased expression was observed at 1 d; however, it was not present at 3 mo suggesting that this is not a primary mechanism by which poor maternal nutrition alters postnatal muscle growth. Since these are early time points, it is possible that these changes in expression may contribute to changes that would occur later in life as the animal continues to grow.

As expected, there is very little subcutaneous fat at birth. Therefore, for consistency, we evaluated expression of genes in renal fat at both time points. Although changes in gene expression are not observed at 1 d of age, consistent with reduced back fat in RES lambs at 3 mo, expression of adiponectin tended to be reduced in renal fat of RES lambs at 3 mo of age. Previous studies also demonstrate that reduced expression and circulating concentrations of adiponectin are associated with obesity and metabolic disorders [45,46]. Although the RES offspring were not growing as well as CON and have reduced fat mass during early postnatal growth, the reduced expression of adiponectin could be indicative of programed altered metabolism, which could contribute to increased fat later in life. Future studies determining circulating adiponectin and long-term body condition of offspring are needed to determine if adiponectin is a key factor in increased obesity of offspring. Leptin is an important factor in regulating feed intake, metabolism and development of key tissues [47,48]. Previous studies have demonstrated that poor maternal nutrition can alter circulating leptin in offspring as early as the first week of life [18].These effects can persist into adulthood and may contribute to altered metabolism later in life because of hyperleptinemia [18,49]. Similar to previous studies, circulating leptin increased in both OVER offspring. Consistent with these findings, expression of leptin in adipose tissue was numerically greater in RES and OVER at 3 mo. At these early time points, we were not able to determine if the increased leptin would lead to altered metabolism later in life, but based on previous reports [30,50] this is a likely scenario. Consistent with potential changes in metabolism of offspring, circulating TG tended to be increased in OVER lambs at 3 mo of age, which may indicate a change in lipid accumulation in older animals [51].Triglycerides are known indicators of future fat accumulation and/or metabolic disease [51]. It is likely that the overfeeding during gestation has programed these

offspring to be prone to obesity later in life [49], and these changes in circulating factors associated with metabolism are detectable as early as 3 mo of age when the animal is rapidly growing.

## 5. Conclusions

Poor maternal nutrition during the last third of pregnancy in sheep negatively affects the growth of the fetus and these effects persist during early postnatal growth. The reduction in BW and growth associated with restricted maternal nutrition is associated with reduced circulating IGF-I and IGFBP-3. In addition, metabolic factors are also altered both systemically and locally at this early postnatal time point. Changes in specific circulating growth factors and local gene expression may contribute to the altered growth of the offspring, and explain how these phenotypic changes persist during the rapid growth period when the offspring are provided ad libitum feed. There are limited studies available following offspring from poorly nourished mothers into adulthood. Long-term studies as well as evaluating global changes in gene expression and epigenetic modifications are need to fully understand the negative impact poor maternal nutrition has on the offspring.

## Acknowledgments

The authors thank the UCONN Sheep Barn staff, David Schreiber, Victor Delaire, Elizabeth Glynn, and Katelyn McFadden. We thank the many undergraduates that assisted with feeding the ewes, lambs and sample collection. We thank Dr Ji-Young Lee from the Nutritional Sciences Department at the University of Connecticut for triglyceride and total cholesterol analysis. Funding was provided by a UCONN Research Foundation Grant (K.E. Govoni) and the Storrs Agriculture Experiment Station (CONS00844; W2112).

## References

[1] Yan X, Huang Y, Zhao JX, Long NM, Uthlaut AB, Zhu MJ, Ford SP, Nathanielsz PW, Du M. Maternal obesity-impaired insulin signaling in sheep and induced lipid accumulation and fibrosis in skeletal muscle of offspring. Biol Reprod 2011;85:172–8.
[2] Tong JF, Yan X, Zhu MJ, Ford PW, Nathanielsz PW, Du M. Maternal obesity down regulates myogenesis and β-catenin signaling in fetal skeletal muscle. Am J Physiol Endocrinol Metab 2009;296:917–24.
[3] Daniel ZCTR, Brameld JM, Craigon J, Scollan ND, Buttery PJ. Effect of maternal dietary restriction during pregnancy on lamb carcass characteristics and muscle fiber composition. J Anim Sci 2007;85:1565–76.
[4] Ford SP, Hess BW, Schwope MM, Nijland MJ, Gilbert JS, Vonnahme KA, Means WJ, Han H, Nathanielsz PW. Maternal under nutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J Anim Sci 2007;85:1285–94.
[5] Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. J Nutr 2004;139:2169–72.
[6] Yan X, Huang X, Zhao JX, Rogers CJ, Zhu MJ, Ford SP, Nathanielsz PW, Du M. Maternal obesity down regulates microRNA let-7g expression, a possible mechanism for enhanced adipogenesis during ovine fetal skeletal muscle development. Int J Obes 2013;37:568–75.
[7] Huang Y, Yan X, Zhu MJ, McCormick RJ, Ford SP, Nathanielsz PW, Du M. Enhances transforming growth factor-β signaling and fibrogenesis in ovine fetal skeletal muscle of obese dams at late gestation. Am J Physiol Endocrinol Metab 2010;298:1254–60.
[8] Zhu MJ, Ford SP, Means WJ, Hess BW, Nathanielsz PW, Du M. Maternal nutrient restriction affects properties of skeletal muscle in offspring. J Physiol 2006;575:241–50.
[9] McNamara LB, Gibin L, Markham T, Sickland NC, Berry DP, O'reilly JJ, Lynch PB, Kerry JP, Lawlor PG. Nutritional intervention during gestation alters growth, body composition and gene expression patterns in skeletal muscle of pig offspring. Animal 2011;5:1195–206.
[10] Du M, Tong J, Zhao J, Underwood KR, Zhu M, Ford SP, Nathanielsz PW. Fetal programming of skeletal muscle development in ruminant animals. J Anim Sci 2010;88:51–60.
[11] Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J, Baylink DJ. Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and –independent mechanisms. Endocrinology 2003;144:929–36.
[12] Rausch MI, Tripp MA, Govoni KE, Zang W, Webert WJ, Crooker BA, Hoagland TA, Zinn SA. The influence of level of feeding on growth and serum insulin-like growth factor I and insulin-like growth factor-binding proteins in growing beef cattle supplemented with somatotropin. J Anim Sci 2002;80:94–100.
[13] Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Kilbanski A. Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. J Clin Endocrinol Metab 1996;81:3864–70.
[14] Elsasser TH, Rumsey TS, Norton SA. Relationships between the thyroid and somatotropic axes in steers. I: effects of propylthiouracil-induced hypothyroidism on growth hormone, thyroid stimulating hormone and insulin-like growth factor I. Domest Anim Endocrinol 1992;9:261–71.
[15] de Zegher F, Butenandt O, Chatelain P, Albertsson-Wickland K, Jonsson B, Lofstrom A, Chaussain JL. Growth hormone treatment of short children born small for gestational age: reappraisal of the rate of bone maturation over 2 years and metanalysis of height gain over 4 years. Acta Pediatr Suppl 1997;423:207–12.
[16] Gluckman PD, Pinal CS. Maternal-placental-fetal interactions in the endocrine regulation of fetal growth: role of somatotropic axes. Endocrine 2002;19:81–9.
[17] Limesand SW, Rozance PJ, Macko AR, Anderson MJ, Kelly AC, Hay WW. Reductions in insulin concentrations and β-cell mass precede growth restriction in sheep fetuses with placental insufficiency. Am J Physiol Endocrinol Metab 2012;304:516–23.
[18] Long NM, Ford SP, Nathanielsz PW. Maternal obesity eliminates the neonatal lamb plasma leptin peak. J Physiol 2011;589:1455–62.
[19] Ford SP, Zhang L, Zhu M, Miller MM, Smith DT, Hess W, Moss GW, Nathanielsz PW, Nijland MJ. Maternal obesity accelerates fetal pancreatic β cell but not α cell development in sheep: prenatal consequences. Am J Physiol Regul Integr Comp Physiol 2009;297:835–43.
[20] Wu G, Bazer FW, Wallace JM, Spencer TE. Intrauterine growth retardation: implications for the animal sciences. J Anim Sci 2006;84:2316–37.
[21] National Research Council (NRC). Nutrient requirements of sheep. Washington, DC: National Academic Press; 1992.
[22] Kazmer GW, Canfield RW, Bean B. Characteristics of somatotropin and prolactin profiles in young dairy sires before and after a 72-hour fast during different seasons. J Anim Sci 1992;70:503–7.
[23] Govoni KE, Tian XC, Kazmer GW, Taneja M, Enright BP, Rivard AL, Yang X, Zinn SA. Age-related changes of the somatotropic axis in cloned Holstein calves. Biol Reprod 2002;66:1293–8.
[24] Freake HC, Govoni KE, uada K, Huang C, Zinn SA. Actions and interactions of thyroid hormone and zinc status in growing rats. J Nutr 2001;131:1135–41.
[25] Funston RN, Roberts AJ, Hixon DL, Hallford DM, Sanson DW, Moss GE. Effect of acute glucose antagonism on hypophyseal hormones and concentrations of insulin like growth factor (IGF)-I and IGF-I binding protein in serum, anterior pituitary, and hypothalamus of ewes. Biol Reprod 1995;52:1179–86.
[26] Rasmussen HE, Blobaum KR, Jesch ED, Ku CS, Park YK, Lu F, Carr TP, Lee JY. Hypocholesterolemic effect of Nostoc commune var. sphaeroides Kützing, an edible blue-green algae. Eur J Nutr 2009;48:387–94.
[27] Livak JK, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–8.
[28] Parlee SD, Macdougald OA. Maternal nutrition and risk of obesity in offspring: the Trojan horse of developmental plasticity. Biochim Biophys Acta 2014;1842:495–506.
[29] Nielsen MO, Kongsted AH, Thygesen MP, Strathe AB, Caddy S, Quistorff B, Jorgensen W, Christensen VG, Husted S, Chwalibog A, Sejrsen K, Purup S, Svalastoga E, McEnvoy FJ, Johnsen L. Late gestation under nutrition can predispose for visceral adiposity by

altering fat distribution patters and increasing the preference for a high fat diet in early postnatal life. Br J Nutr 2013;109:2098–110.
[30] Long NM, George LA, Uthlaut AB, Smith DT, Nigland MJ, Nathanielsz PW, Ford SP. Maternal obesity and increased nutrient intake before and during gestation in the ewe results in altered growth, adiposity, and glucose tolerance in adult offspring. J Anim Sci 2010;88:3546–53.
[31] George LA, Zhang L, Tuersunjiang N, Ma Y, Long NM, Uthlaut AB, Smith DT, Nathanielsz PW, Ford SP. Early maternal under nutrition programs increased feed intake, altered glucose metabolism and insulin secretion, and liver function in aged female offspring. Am J Physiol Regul Integr Comp Physiol 2012;302:795–804.
[32] Neville TL, Caton JS, Hammer CJ, Reed JJ, Luther JS, Taylor JB, Redmer DA, Reynolds LP, Vonnahme KA. Ovine offspring growth and diet digestibility are influenced by maternal selenium supplementation and nutritional intake during pregnancy despite a common post-natal diet. J Anim Sci 2010;88:3645–56.
[33] De Blasio MJ, Gatford KJ, McMillen IC, Robinson JS, Owens JA. Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. Endocrinology 2007;148:1350–8.
[34] Mericq V, Ong KK, Bazaes R, Pena V, Avila A, Salazar T, Soto N, Iniquez G, Dunger DB. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. Diabetologia 2005;48:2609–14.
[35] Sebert SP, Dellshaft NS, Chan LL, Street H, Henry M, Francois C, Sharma V, Fainberg HP, Patel N, Roda N, Keisler D, Budge H, Symonds ME. Maternal nutrient restriction during late gestation and early postnatal growth in sheep differentially reset the control of energy metabolism in the gastric mucosa. Endocrinology 2001;152:2816–26.
[36] George LA, Uthlaut AB, Long NM, Zhang L, Ma Y, Smith DT, Nathanielsz PW, Ford SP. Different levels of over nutrition and weight gain during pregnancy have differential effects on fetal growth and organ development. Reprod Biol Endrocrinol 2010;8:75.
[37] Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocr Rev 1994;15:80–101.
[38] Dawson JM, Craigon J, Buttery PJ, Beever DE. Influence of diet and beta-agonist administration on plasma concentrations of growth hormone and insulin-like growth factor-1 in young steers. Br J Nutr 1993;70:93–102.
[39] McGuire MA, Vicini JL, Bauman DE, Veenhuizenn JJ. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J Anim Sci 1992;70:2901–10.
[40] Jones JL, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
[41] Smith T, Sloboda DM, Saffery R, Joo E, Vickers MH. Maternal nutritional history modulates the hepatic IGF-IGFBP axis in adult male rat offspring. Endocrine 2013;46:70–82.
[42] Tuggle CK, Trenkle A. Control of growth hormone synthesis. Domest Anim Endocrinol 1996;13:1–33.
[43] Govoni KE, Hoagland TA, Zinn SA. The ontogeny of the somatotropic axis in male and female Hereford calves from birth to one year of age. J Anim Sci 2003;16:3–34.
[44] Shang YC, Wang SH, Xiong F, Zhao CP, Peng FN, Feng SW, Li MS, Li Y, Zhang C. Wnt3a signaling promotes proliferation, myogenic differentiation, and migration of rat bone marrow mesenchymal stem cells. Acta Pharmacol Sin 2007;28:1761–76.
[45] Shankar K, Kang P, Harell A, Zhong Y, Marecki JC, Ronis MJ, Badger TM. Maternal overweight programs insulin and adiponectin signaling in the offspring. Endocrinology 2010;151:2577–89.
[46] Kern PA, Di Gregorio GB, Tong L, Rassouli N, Ranganathen G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 2003;52:1779–85.
[47] Forhead AJ, Lamn CA, Franko KK, O'Connor DM, Wooding FBP, Cripps RL, Blache D, Shen QW, Du M, Fowden AL. Role of leptin in the regulation of growth and carbohydrate metabolism in the ovine fetus during late gestation. J Physiol 2008;586:2393–403.
[48] Chilliard Y, Delavaud C, Bonnet M. Leptin expression in ruminants: nutritional and physiological regulation in relation to energy metabolism. Domest Anim Endocrinol 2005;29:3–22.
[49] Stocker CJ, Arch JR, Cawthorne MA. Fetal origins of insulin resistance and obesity. Proc Nutr Soc 2005;64:143–51.
[50] Coupe B, Grit I, Hulin P, Randuineau G, Parnet P. Postnatal growth after intrauterine growth restriction alters central leptin signal and energy homeostasis. PLos One 2012;7.
[51] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415–28.
[52] Muhlhausler BS, Duffield JA, McMillen IC. Increased maternal nutrition stimulates peroxisome proliferator activated receptor-γ, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. Endocrinology 2007;148:878–86.
[53] Dervishi E, Serrano C, Joy M, Serrano MRC, Calvo JH. The effect of feeding system in the expression of genes related with fat metabolism in semitendinous muscle in sheep. Meat Sci 2011;89:91–7.
[54] Buza JJ, Mori Y, Bari AM, Hikono A, H H, Hirayama S, Shu Y, Momotani E. Mycobacterium avium subsp. paratuberculosis infection causes suppression of RANTES, monocyte chemoattractant protein 1, and tumor necrosis factor alpha expression in peripheral blood of experimentally infected cattle. Infect Immun 2003;71:7223–7.