Effects of early- to mid-gestational undernutrition with or without protein supplementation on offspring growth, carcass characteristics, and adipocyte size in beef cattle

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ABSTRACT: Angus × Gelbvieh cows with 2 to 3 previous pregnancies were used to evaluate effects of maternal nutrient restriction on offspring adipose tissue morphology at standard production endpoints. At 45 d after AI to a single sire, pregnancy was confirmed and cows randomly allotted into groups and fed a control (Con, 100% of NRC recommendations), nutrient-restricted (NR, 70% of Con diet), or nutrient-restricted + protein-supplemented (NRP, 70% of Con + essential AA supply to the small intestine equal to Con) diet. At d 185 of gestation, cows were commingled and received the Con diet thereafter. Bull calves were castrated at 2 mo of age. Calves were weaned at 210 d, backgrounded for 28 d, and then placed in the feedlot for 195 d. Steers and heifers were slaughtered at an average 12th-rib fat thickness of 7.6 mm. Adipose tissue from selected depots was collected for adipocyte size analysis. There was no significant difference in BW or BCS between Con, NRP, and NR cows at d 45 of gestation, which averaged 489.7 ± 17.7 kg and 5.35 ± 0.13, respectively. At d 185 of gestation, Con and NRP groups had similar BW (566.1 ± 14.8 and 550.2 ± 14.8 kg) and BCS (6.34 ± 0.27 and 5.59 ± 0.27), but NR cows exhibited reduced (P < 0.05) BW (517.9 ± 14.8 kg) and BCS (4.81 ± 0.27). Among offspring (steers and heifers) at slaughter, there were no significant differences in BW or organ weights among treatment groups. Yield grade was reduced (P < 0.05) and semitendinosus weight/HCW tended (P = 0.09) to be reduced in NR offspring compared with Con and NRP offspring. Average adipocyte diameter was increased (P < 0.05) in subcutaneous, mesenteric, and omental adipose tissue and tended (P = 0.09) to increase in perirenal adipose tissue in NR compared with Con offspring with NRP offspring adipocyte diameter being either intermediate or similar to Con calves. The adipocyte size alterations observed in NR offspring were confirmed by DNA concentration of the adipose tissue depots. There also was an increased mRNA expression (P < 0.05) of fatty acid transporter 1 in subcutaneous adipose tissue from NR offspring compared with Con and NRP offspring. Nutritional restriction during early and mid gestation increased or tended to increase (P < 0.09) adipocyte diameter in all adipose tissue depots in finished steer and heifer calves.

Key words: cattle, maternal nutrient restriction, offspring adipocyte size

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INTRODUCTION

Forage quality and quantity is decreased in summer and fall in the Western United States due to low precipitation (Vavra and Raleigh, 1976), leading many spring-calving cows to undergo periods of undernutrition at a time that often corresponds to the first one-half to two-thirds of gestation (DelCurto et al., 2000). Adequate maternal nutrition in early gestation is critical for establishing normal fetal development of all organs and tissues (Ford, 1995; Reynolds and Redmer, 1995; McGrady et al., 2006). Maternal undernutrition during early gestation results in fetal growth restriction, which reduces muscle mass and increases fatness in the offspring (Zhu et al., 2006; Long et al., 2009). Both fetal
skeletal muscle and adipose tissue are derived from the same pool of mesenchymal stem cells (Du et al., 2011); thus, attempts to enhance myogenesis are frequently associated with reduction in adipogenesis, increasing the lean-to-fat ratio of offspring. It has previously been reported that AA stimulate protein synthesis and muscle growth acting through mammalian target of rapamycin signaling (Zhu et al., 2004; Han et al., 2008). A protein supplement has been developed that can be fed to supply the intestine of a beef cow the same quantity of essential AA, even when she is experiencing restricted forage intake (Scholljegerdes et al., 2005). The objective of this study was to understand the impacts of maternal undernutrition and protein supplementation during early and mid gestation on offspring growth, adipocyte size, and expression of nutrient transporters and transcription factors in steer and heifer offspring slaughtered at normal production endpoints.

MATERIALS AND METHODS

The University of Wyoming Animal Care and Use Committee approved all animal procedures in this study.

Animals

Three- and 4-yr-old suckled Angus × Gelbvieh cows, previously exhibiting 2 and 3 successful pregnancies, respectively, were synchronized for estrus using a controlled internal drug-releasing device (CIDR, Pfizer, Exton, PA) for 7 d, and upon removal of the device, 25 mg of PGF$_{2\alpha}$ (Lutalyse, Pharmacia and Upjohn Co., Kalamazoo, MI) was administered intramuscularly in April. Cows were then observed for estrus every 12 h and AI approximately 12 h after the onset of estrus using semen from a single South Devon sire. Cows and their preweaned calves were sent to the University of Wyoming McGuire Ranch (56 km northeast of Laramie, 41°18′41″ N; 105°35′26″ W; 2,203 m elevation) and grazed summer pasture until d 33 of gestation, when cows were evaluated for pregnancy via rectal palpation. At d 34 of gestation, the most uniform pregnant cows in BW and BCS (n = 42, 18 triparous and 24 diparous) had their calves weaned and were transported to the University of Wyoming Livestock center in Laramie, placed in dry lots, and pen fed a diet of 10% CP composed of native grass hay (6.2% CP, DM basis; Scholljegerdes et al., 2005) to provide duodenal essential AA flow equivalent to the Con diet (Scholljegerdes et al., 2004). Diet composition and daily intakes are shown in Table 1.

At d 40 of gestation, cows were again evaluated for pregnancy by rectal palpation, and the 36 most uniform cows (12 triparous and 24 diparous) were selected on d 45 to be individually fed native grass hay and 1 of 3 supplements from d 45 through d 185 of gestation. The control (Con) diet consisted of a soybean meal-based supplement formulated for pregnant replacement heifers (590 kg of mature BW) to achieve 0.43 kg/d of BW gain (NRC, 2000), estimated to be comparable with a 0.51 kg/d BW gain for nonlactating diparous or triparous cows. The nutrient-restricted (NR) diet was 70% of NE$_m$ and CP provided to Con (NR), and 70% of NE$_m$ provided to Con plus a RUP supplement (NRP, 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal; DM basis). 227 g of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.

### Table 1. Ingredient and composition of diets fed to cows from d 45 to 180 of gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>Con</th>
<th>NRP</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, % as fed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native grass hay</td>
<td>86.6</td>
<td>77.7</td>
<td>86.6</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.3</td>
<td>7.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.4</td>
<td>—</td>
<td>2.4</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.4</td>
<td>—</td>
<td>1.4</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.2</td>
<td>—</td>
<td>0.2</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>—</td>
<td>9.5</td>
<td>—</td>
</tr>
<tr>
<td>Feathermeal</td>
<td>—</td>
<td>3.4</td>
<td>—</td>
</tr>
<tr>
<td>Blood meal</td>
<td>—</td>
<td>1.1</td>
<td>—</td>
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<tr>
<td>Diet composition</td>
<td></td>
<td></td>
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<tr>
<td>DM, %</td>
<td>92.3</td>
<td>92.6</td>
<td>92.3</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>10.0</td>
<td>17.1</td>
<td>10.0</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>62.7</td>
<td>58.8</td>
<td>62.7</td>
</tr>
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<td>IVDMC, %</td>
<td>47.2</td>
<td>50.9</td>
<td>47.2</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.0</td>
<td>8.0</td>
<td>7.0</td>
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<tr>
<td>NE$_m$ intake, Mcal</td>
<td>11.29</td>
<td>8.11</td>
<td>7.89</td>
</tr>
</tbody>
</table>

1Dietary treatments consisted of native grass hay plus a soybean meal-based supplement formulated to achieve 0.51 kg/d of BW gain (Con), 70% of NE$_m$ and CP provided to Con (NR), and 70% of NE$_m$ provided to Con plus a RUP supplement (NRP, 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal; DM basis).

2227 g of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.

3Premix: 68.3% KCl, 27.6% FeSO$_4$, 3.1% ZnO, 0.6% MnO, and 0.4% CuSO$_4$.

4Actual DMI from d 48 through d 163 of gestation.
ment had 8 male and 4 female calves, and NRP cows had 6 males and females, whereas NR cows had 8 male and 4 female calves. All cows and calves were sent to the University of Wyoming McGuire Ranch and grazed native grass during summer. Calves were weaned at 214 d of age and backgrounded for 28 d. After backgrounding, steers and heifers were placed in the University of Wyoming research center feedlot near Lingle, Wyoming, for 195 d. Heifers received 2 injections of PGF₂α (Lutalyse, Pharmacia & Upjohn Co.) administered intramuscularly 11 d apart, 14.5 ± 0.5 d before slaughter to synchronize their ovarian luteal cycles to allow comparisons of luteal size.

**Slaughter**

Calves were transported to Laramie, Wyoming, in 2 separate groups per sex, 24 h before slaughter, with heifers slaughtered 3 wk later than steers. Calves were alloted to slaughter groups by age and BW and slaughtered at an average 12th-rib backfat thickness of 7.6 ± 0.2 mm. Calves were allowed free access to water with a 24-h feed withdrawal before slaughter. Omental, perirenal, mesenteric, and subcutaneous adipose tissue were collected at slaughter. Omental adipose tissue was collected from each animal close to the dorsal rumen, perirenal adipose tissue from around the left kidney, and mesenteric adipose tissue from a point adjacent to the left cecum. Subcutaneous adipose tissue was collected from the brisket area. All samples were collected within 20 min of stunning. Adipose tissue samples for gene expression analyses were snap frozen in liquid nitrogen and maintained at −80°C until analysis. Ovaries were removed from heifers and placed on ice until weighed, and the corpora lutea (CL) removed and weighed. Ovarian tissue minus the CL was weighed, minced with a razor blade, blotted dry, and reweighed; follicular fluid weight was considered as the difference in these 2 weights as described previously by Ford et al. (1977). Carcass measurements, including semitendinosus (SEMT) weight, were collected after a 48-h chill.

**Histology**

Adipose samples for histology were processed by fixing tissue in 4% paraformaldehyde and then embedded in a paraffin block and sectioning at 10 μm using a MICROM HM310 microtome (MICROM Inc., Walldorf, Germany). Four 10-μm sections, 10 sections apart, were collected for evaluation. Sections were deparaffinized then stained using Harris Modified Hematoxylin (Fisher Scientific, Fair Lawn, NJ) and Eosin Y (EMD Chemicals, Gibbstown, NJ). Images were visualized using an Olympus BX50 microscope (Olympus, Center Valley, PA) and captured digitally using a Retiga EXiFast camera (Q Imaging, Burnaby, British Columbia, Canada). Pictures at 40× magnification were taken using QED Imaging software (Media Cybernetics, Silver Spring, MD). Five randomly chosen fields were taken per section for a total of 20 pictures per animal. Images were randomly selected for analysis, and 2 fields per section were analyzed for cell diameter by 2 trained investigators blinded to treatment. Each investigator completed one-half of each of the 4 adipose depot counts. Cell diameters were calculated for at least 50 cells per field area, and at least 800 adipocytes were measured per animal via image analysis using Image J Software (NIH, Bethesda, MD).

**DNA Quantification**

To confirm the histologic measure of cell size, concentrations of DNA were determined in duplicate samples in a fluorometer (VersaFluor, Bio-Rad, Hercules, CA) using a modified procedure that was outlined by Long et al. (2010a). Briefly, 500 μg of adipose tissue was homogenized (Polytron, Lauda-Brinkmann, Delran, NJ) in 5 mL of TEN buffer (100 mM Tris, 150 mM NaCl, 0.2 mM EDTA, 7.4 pH) on ice. Homogenates (10 μL) were then diluted with 2 mL of 0.1 μg/mL of Hoechst Dye 33258 (Bio-Rad) dissolved in TEN buffer and fluorescence measured. Calf thymic DNA (Bio-Rad) was utilized for a standard curve. All samples from an adipose tissue depot were analyzed in a single assay with an average CV of 4.3% between duplicate samples.

**Real-Time Quantitative PCR**

Total mRNA was extracted from subcutaneous adipose tissue using Trizol reagent (Invitrogen Corp., Carlsbad, CA) and then purified with an RNA binding mini column (Omega Bio-tek Inc., Norcross, GA) using manufacturers’ protocols. One microgram of RNA was reverse transcribed into cDNA using a kit (Qiagen, Valencia, CA). Reverse-transcribed cDNA were used for real-time quantitative PCR analyses using a SYBR Green RT-PCR kit from Bio-Rad. Primers used were fatty acid transporter 1 (FATP1) forward, 5’-ACTGTCTGGCCCTGTACCAC-3’ and reverse, 5’-GGCTGGCTGAAGACTTCTTG-3’; fatty acid transporter 4 (FATP4) forward, 5’-GGCACCAAC-GACAAGAAGAT-3’ and reverse, GCTCGTCCATT-CACTGAC; glucose transporter 4 (GLUT4) forward, 5’-AGATGCCCACAATGGAGA-3’ and reverse, 5’-AGATGCCCACAATGGAGA-3’. Each reaction yielded amplicons between 80 and 200 bp. Conditions of the PCR were as follows: 10 s at 95°C, 30 s at 60°C, and 20 s at 72°C for 40 cycles. After amplification, a melting curve (0.01°C/s) was used to confirm product purity. Results are expressed relative to 18S mRNA expression a housekeeping gene that is routinely utilized for adipose tissue gene expression (Ross et al., 2005; Long et al., 2010b), and in this study 18S cycle threshold (CT) values were similar (P > 0.29) within an adipose tissue depot.
**Statistical Analysis**

Four steers and 4 heifers per group were randomly chosen for use in this experiment after slaughter. Their data and that of their mothers were utilized for analysis. This was done to equalize the numbers of animals to that of the smallest treatment × sex group. All maternal BW and BCS were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC) with only treatment in the model. All offspring data including gestation lengths, calf birth weights and BW, carcass measurements, adipocyte diameter, and adipose tissue DNA content for all adipose depots were analyzed using the GLM procedure of SAS with treatment, sex, and their interaction in the model. Ovarian data were analyzed using the GLM procedure with treatment as the model main effects for carcass characteristics and selected organ weights are shown in Table 3. Yield grade was increased \( (P < 0.05) \) in NR offspring compared with NRP and Con offspring. There was also a tendency \( (P = 0.09) \) for the SEMT weight divided by the HCW to be reduced in the NR offspring compared with the Con and NRP offspring. However, heart weights were greater \( (P = 0.09) \) in steers compared with heifers, averaging 2.67 ± 0.08 vs. 2.37 ± 0.08 kg for the heart and 3.32 ± 0.15 vs. 2.94 ± 0.15 kg for the lungs. Dressing percentage was greater \( (P = 0.03) \) in steers compared with heifers \( (63.5 ± 0.3 \text{ vs. } 62.4 ± 0.3\%) \). Twelfth rib-fat thickness showed an opposite effect and tended to be greater \( (P = 0.07) \) in heifers compared with steers \( (1.21 ± 0.10 \text{ vs. } 0.94 ± 0.10 \text{ cm}) \).

**RESULTS**

Cow BW and BCS throughout gestation are shown in Table 2. At the initiation of the study on d 45 of gestation, BW and BCS were similar \( (P > 0.23) \) for cows assigned to all treatment groups. At d 129 of gestation, cow BW was similar among treatments \( (P = 0.49) \); however, BCS tended to be reduced \( (P = 0.06) \) in both the NR and NRP cows compared with Con cows. On d 185 of gestation, cow BW was reduced \( (P = 0.05) \) in the NR cows compared with the other 2 treatment groups. Cow BCS was reduced \( (P = 0.002) \) in NR cows compared with Con cows at d 185 of gestation, and tended to be reduced \( (P = 0.09) \) in NR cows compared with NRP cows. On d 251 of gestation, cow BW and BCS were similar between treatment groups \( (P = 0.16 \text{ and } 0.82, \text{ respectively}) \). There was a tendency \( (P = 0.06) \) for a treatment × sex of calf effect for gestation length with Con cows having a gestation length of 277.7 ± 2.2 and 278.0 ± 2.2 d for heifer and steer calves, respectively. Cows on the NRP had a gestation length of 278.3 ± 2.2 and 281 ± 2.2 d for heifer and steer calves, respectively, whereas the NR cows had a gestation length of 278.3 ± 2.2 d for heifer calves and 286.5 ± 2.2 d for steer calves.

Birth weight of calves was unaffected \( (P = 0.19) \) by maternal prenatal feeding level and was 36.4 ± 1.9, 39.2 ± 2.0, and 40.8 ± 1.6 kg for Con, NRP, and NR calves, respectively. At weaning, calf BW was unaffected \( (P = 0.83) \) by prenatal nutritional treatment, but there was a tendency \( (P = 0.06) \) for steers to be heavier than heifers \( (279.7 ± 6.6 \text{ vs. } 261.1 ± 6.6 \text{ kg}, \text{ respectively}) \). Body weight at slaughter was similar \( (P > 0.34) \) between treatment groups and sex of calf. Treatment main effects for carcass characteristics and selected organ weights were shown in Table 3. Yield grade was increased \( (P < 0.05) \) in NR offspring compared with NRP and Con offspring. There was also a tendency \( (P = 0.09) \) for the SEMT weight divided by the HCW to be reduced in the NR offspring compared with the Con and NRP offspring. However, heart weights were greater \( (P = 0.02) \) and lung weights tended to be greater \( (P = 0.09) \) in steers compared with heifers, averaging 2.67 ± 0.08 vs. 2.37 ± 0.08 kg for the heart and 3.32 ± 0.15 vs. 2.94 ± 0.15 kg for the lungs. Dressing percentage was greater \( (P = 0.03) \) in steers compared with heifers \( (63.5 ± 0.3 \text{ vs. } 62.4 ± 0.3\%) \). Twelfth rib-fat thickness showed an opposite effect and tended to be greater \( (P = 0.07) \) in heifers compared with steers \( (1.21 ± 0.10 \text{ vs. } 0.94 ± 0.10 \text{ cm}) \).

Average adipocyte diameter was increased \( (P = 0.02) \) in NR compared with Con offspring with NRP offspring being intermediate in both the subcutaneous and mesenteric adipose tissue depots (Figures 1a and 1c). For the omental adipose tissue depot, NR offspring had adipocytes with greater \( (P = 0.05; \text{ Figure 1d}) \) diameters compared with Con and NRP offspring, which were similar. The adipocyte diameters in perirenal adipose tissue tended to be increased \( (P = 0.09; \text{ Figure } 1c) \).
heifers. The weight of luteal tissue was greater in the Con heifers compared with the NR and NRP offspring. The weight of the ovary tended to be greater in the subcutaneous adipose tissue of NR offspring compared with Con and NRP offspring. However, was not different among treatment groups. Hence, FATP1 was increased in the subcutaneous adipose tissue of NR offspring compared with Con and NRP offspring.

The ovarian and luteal weights are shown in Table 5. The weight of the ovary tended to be greater in the Con heifers compared with the NR and NRP heifers. The weight of luteal tissue was greater in the Con heifers compared with NR and NRP heifers.

**DISCUSSION**

This is the first report in the cow utilizing both moderate maternal nutrient restriction from early to mid gestation and essential AA supplementation, thereby allowing comparison of the effects of global nutrient restriction with that of energy restriction only. Neither moderate global nutrient restriction nor energy restriction alone resulted in altered growth. However, moderate maternal nutrient restriction increased yield grades and tended to decrease SEMT muscle weights. Supplementation of nutrient-restricted cows with essential AA restored yield grade and SEMT muscle weight of their offspring compared with that of offspring from Con-fed cows. There were dietary effects on adipose tissue, with NR offspring exhibiting increased adipocyte size compared with those in the Con group, with NRP offspring either being intermediate or equal to Con offspring. The enlarged adipocytes in NR offspring also had greater expression of FATP1, an insulin-responsive fatty acid transporter (Wu et al., 2006), suggesting altered fatty acid transport and possible metabolism in these NR offspring. The adipocytes from NR offspring could be larger due to this altered fatty acid transport.

Similar birth weights of calves in the present study are consistent with Long et al. (2010b) and others (Doornbos et al., 1984; Goehring et al., 1989; Houghton et al., 1990) who showed that restriction of dietary energy before the last trimester of gestation did not influence birth weight or gestational length. Additionally, it has been reported that if postnatal nutrition is adequate, any effects of maternal diets during mid or late gestation on birth weights will not result in altered calf BW either before or at weaning (Hight, 1968; Freetry et al., 2000). In sheep, a severe (50%) maternal nutrient restriction during early and mid gestation results in increased postnatal growth of wethers compared with wethers of normal-fed ewes (Ford et al., 2007). Maternal protein restriction in heifers results in steer offspring that are heavier from 191 d of age until slaughter at 657 d of age (Micke et al., 2010). However, heifers exposed to the same maternal environment were lighter from d 552 of age until slaughter, indicating a possible sex effect. The lack of a sex effect in the present study could be due to the small number of animals in each treatment × sex group. Thus, differences in postnatal growth of offspring could depend both on the severity and timing of nutrient restriction along with the sex of the offspring.

Nutrient restriction did not affect organ weights and most carcass measurements in the present study. Tudor et al. (1980) reported that restriction of maternal nutrients from 180 d of gestation until parturition resulted in a 22% decrease in calf birth weight compared with cows.
fed to nutritional requirements. However, when steers and heifers were slaughtered at 370 to 400 kg of BW, no differences were observed in dressing percentage, weights of organs and muscles, or carcass composition (Tudor et al., 1980). Early-gestational undernutrition of heifers resulted in steer offspring with similar carcass measurements at normal production age and BW (Long et al., 2010b). These findings are consistent with studies that looked at postnatal performance of twin-born calves, a naturally occurring intrauterine growth restriction, compared with singletons (de Rose and Wilson, 1991; Gregory et al., 1996). These researchers reported that at equivalent slaughter weights or ages, carcasses were similar and any differences that were noted were small, with twins having a slightly leaner carcass than singletons. Micke et al. (2010) reported some effects of maternal protein restriction during either the first or second trimester of gestation on carcass measurements. However, there was a sex and timing of gestation interaction that makes these results less definitive. It should be pointed out that even if organ and muscle weights are similar in offspring subjected to prenatal nutrient restriction, it has been demonstrated that the cellular or functional composition of tissues may differ (Zhu et al., 2006; Long et al., 2009, 2010b).

Adipocyte size of offspring, across all 4 adipose tissue depots evaluated, was shown to be increased by global maternal nutrient restriction without altering the total amount of adipose tissue, regardless of sex. This increased in adipocyte size was determined both by histology and also by DNA concentrations. The DNA concentration is a reliable indicator of the number of mononuclear cells and, therefore, indirectly their size (Enesco and Lablond, 1962). As adipocytes get larger, the DNA concentration decreases because of a decreased number of cells per unit weight of adipose tissue (Enesco and Lablond, 1962; Long et al., 2010b). There are a limited number of studies evaluating the effects of maternal undernutrition on postnatal adipose tissue. Maternal undernutrition in the guinea pig has been shown to increase the amount of lipid in fetal adipocytes from underfed mothers compared with fetal adipocytes from mothers fed ad libitum (Nguyen et al., 2010). In a study by Jones and Friedman (1982), restricted feed intake during the first two-thirds of gestation in rats increased adipocyte size in both male and female offspring from these females compared with offspring from control mothers. In contrast, Shepherd et al. (1997) reported that maternal protein restriction in rats resulted in offspring exhibiting smaller adipocyte size than offspring from control-fed mothers. In this study, rats were euthanized at 6 wk of age, well before maturity and before adipocytes had a chance to fill, and the rats were only fed for normal growth. Jones and Friedman (1982) euthanized animals at an adult age and BW, and animals were fed for BW gain, which may account for the different results between these 2 studies. In cattle, maternal nutrient restriction from d 32 to 115 of gestation, resulted in increased adipocyte

![Figure 1](image-url)

**Figure 1.** Average adipocyte diameter from subcutaneous (A), perirenal (B), mesenteric (C), and omental (D) adipose tissue from steers and heifers whose dams were fed a diet formulated to achieve 0.51 kg/d of BW gain (control), fed 70% of NE<sub>m</sub> and CP provided to control (NR), and fed 70% of NE<sub>m</sub> provided to control plus a RUP supplement during early to mid gestation (NRP). a,bMeans without a common letter differ (P ≤ 0.05). c,dMeans without a common letter differ (P < 0.10).
size in the perirenal but not the subcutaneous adipose depot in steer offspring slaughtered at 22 mo of age (Long et al., 2010b). This could indicate that the timing of nutrient restriction influences the response on adipocyte size in a depot-specific manner.

Changes in adipocyte size have been associated with changes in fatty acid transporter activity. As shown in our experiment, mRNA expression of \textit{FATP1}, an insulin-responsive fatty acid transporter, was increased in adipose tissue from NR offspring. This is in contrast to a study conducted by Varlamov et al. (2010), who observed that as adipocytes became larger within an adipose depot, they progressively lost insulin-dependent fatty acid transporter activity (Varlamov et al., 2010). This suggests that maternal obesity may program larger adipocytes in offspring through an accumulation of excess fatty acids via elevated FATP1 activity. Further, when adipose tissue from NRP and control offspring were compared for \textit{LPL} expression, there was a tendency for NRP offspring to have reduced expression compared with controls. This could also support altered fatty acid delivery and transport into the adipocytes as a possible contributing factor for the enlarged adipocytes seen in this experiment. The lack of differences in \textit{GLUT4} mRNA among treatment groups is not unexpected. Perirenal adipose tissue GLUT4 protein content had been shown to be similar in adult offspring exposed to early maternal nutrient restriction and those from control-fed mothers (Gardner et al., 2005). Adipocyte size has also been shown to have no effect on GLUT4 protein content, but the ability of insulin to stimulate GLUT4 incorporation into the cell membrane had been shown to be reduced in large adipocytes compared with small adipocytes (Franck et al., 2007). However, there could have been changes in expression of growth factors that had already occurred that caused the differences in adipocyte size or numbers because we observed no increase in overall fat mass in this experiment. Adipose tissues from offspring from protein-restricted mothers have altered expression of components of the IGF system (Micke et al., 2011). This could have resulted in altered adipose tissue growth.

A potential reason for the increase in adipocyte size in NR offspring in this study may be that maternal nutrient restriction reduces skeletal muscle development and muscle mass in offspring (Ford et al., 2007). This altered skeletal muscle development is indicated in our experiment by the increased yield grade and tendency for a reduced SEMT muscle as a percentage of HCW in the NR offspring compared with the Con and NRP offspring. Yield grade is an estimate of the amount of closely trimmed boneless rib, loin, chuck, and round in the beef carcass (Murphey et al., 1960). A change in 1 yield grade results in a 3.4% change in retail prod-

### Table 4. Adipose tissue DNA concentration, milligrams per gram of tissue, at slaughter from 4 steers and 4 heifers per treatment group whose dams were exposed to nutrient restriction from early to mid gestation

<table>
<thead>
<tr>
<th>Depot</th>
<th>Con</th>
<th>NRP</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>1.29 ± 0.12(^a)</td>
<td>1.10 ± 0.12(^b)</td>
<td>0.89 ± 0.12(^b)</td>
</tr>
<tr>
<td>Perirenal</td>
<td>0.61 ± 0.05(^a)</td>
<td>0.57 ± 0.05(^a)</td>
<td>0.42 ± 0.05(^b)</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0.57 ± 0.04(^a)</td>
<td>0.51 ± 0.04(^b)</td>
<td>0.44 ± 0.04(^b)</td>
</tr>
<tr>
<td>Omental</td>
<td>0.56 ± 0.06(^a)</td>
<td>0.57 ± 0.06(^b)</td>
<td>0.38 ± 0.06(^b)</td>
</tr>
</tbody>
</table>

\(^a,b\)Means without a common superscript differ (\(P \leq 0.05\)).

### Table 5. Ovarian characteristics of heifers whose dams were exposed to nutrient restriction from early to mid gestation and slaughtered 14.5 ± 0.5 d after the second injection of PGF\(_{2\alpha}\) of a 2-injection sequence 11 d apart

<table>
<thead>
<tr>
<th>Item</th>
<th>Con</th>
<th>NRP</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Wet ovary wt, g</td>
<td>20.6 ± 1.3(^c)</td>
<td>16.6 ± 1.2(^d)</td>
<td>17.2 ± 1.6(^d)</td>
</tr>
<tr>
<td>Blotted ovary wt, g</td>
<td>8.9 ± 0.8</td>
<td>7.5 ± 0.7</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Follicular fluid wt, g</td>
<td>4.9 ± 0.5</td>
<td>4.2 ± 0.5</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Luteal tissue wt, g</td>
<td>6.8 ± 0.5(^b)</td>
<td>4.8 ± 0.5(^b)</td>
<td>5.0 ± 0.6(^b)</td>
</tr>
</tbody>
</table>

\(^a,b\)Means without a common superscript differ (\(P \leq 0.05\)).
\(^c,d\)Means without a common superscript differ (\(P < 0.10\)).

### Dietary treatments consisted of native grass hay plus soybean meal-based supplement formulated to achieve 0.51 kg/d of BW gain (Con), 70% of NE\(_{m}\) and CP provided to Con (NR), and 70% of NE\(_{m}\) provided to Con plus a RUP supplement (NRP; 6.8% porcine blood meal, 24.5% hydrolyzed feather meal, and 68.7% menhaden fish meal; DM basis).
uct from the whole carcass (Dikeman et al., 1998). Because skeletal muscle is the main tissue utilizing energy, the reduction in muscle mass is expected to enhance fat accumulation (Zhu et al., 2006; Du et al., 2011). Supplemental protein of dams in this study seemed to alleviate the altered adipocyte size and also restored yield grade and SEMT muscle weight as a percentage of HCW, resulting from maternal nutrient restriction in postnatal offspring, which may be due to the enhanced fetal muscle growth resulting from protein supplementation. Amino acids enhance protein synthesis and muscle growth through activating mammalian target of rapamycin signaling (Zhu et al., 2004), which is expected to enhance energy utilization by muscle and reduces fat accumulation.

The reduced CL weight on ovaries of both NR and NRP vs. Con heifers during the mid-luteal phase of their estrous cycles seems to contradict some of the other finding in this experiment. In the ovary, it appears that AA supplementation was unable to prevent alteration in CL weight. The number of animals in this study is small, so the finding of this study should be confirmed in a larger group of animals. However, others have shown alteration in offspring ovaries associated with maternal nutrient restriction. Mossa et al. (2009) reported that maternal nutrient restriction during the first trimester of pregnancy leads to female offspring with a reduced ovarian follicular reserve as indicated by antral follicular counts. In sheep, maternal nutrient restriction during early to mid gestation, a similar timing of gestation as our study, found DNA damage in oocytes from fetal ovaries (Murdock et al., 2003). As yearling and 2-yr-old ewes, these nutrient-restricted offspring had reduced plasma progesterone content during the estrous cycle, and when mated, had reduced fertility (Long et al., 2010a). As 6-yr-old ewes, offspring from NR ewes still tended to have reduced plasma progesterone during an estrous cycle along with reduced luteal progesterone content (Nurmamat et al., 2011). This was associated with reduced steroidalogenic acute regulatory enzyme and P450 side-chain cleavage enzyme mRNA and protein expression, thus indicating alterations in offspring luteal tissue function due to maternal nutrient restriction. Therefore, the results of this experiment tend to agree with other published results that maternal nutrient restriction does negatively affect offspring ovaries and possible fertility.

Our findings demonstrate a relationship between maternal undernutrition in the first two-thirds of gestation and increased adipocyte size in young adult offspring, which may contribute to altered adiposity and metabolism later in life. Although differences were not seen in BW or organ weights of offspring, significant differences were observed between Con and NR offspring in adipocyte size and muscle mass. Whereas fetal programming results in larger adipocytes and a reduction in muscle mass that could alter metabolism in later life, it seems to have limited negative effects on production of feedlot animals destined for slaughter at an early age, but may

Figure 2. Relative mRNA abundance for glucose transporter 4 (GLUT4; A), fatty acid transporter [FATP1 (B) and FATP4 (C)], and lipoprotein lipase (LPL; D) in subcutaneous adipose tissue from steers and heifers whose dams were fed a diet formulated to achieve 0.51 kg/d of BW gain (control), fed 70% of NE₉ and CP provided to control (NR), and fed 70% of NE₉ provided to control plus a RUP supplement during early to mid gestation (NRP). a,bMeans without a common letter differ (P ≤ 0.05).
negatively affect the long-term reproductive efficiency of replacement heifers. This concept is consistent with alterations in ovarian structures observed in this study. These alterations in female offspring reproductive characteristics could be an important management consideration for ranchers in the Western United States who graze winter pastures with poor-quality forage during the first two-thirds of gestation.

LITERATURE CITED


