Maternal Undernutrition During Gestation Programs Skeletal Muscle Development in Male Goat Offspring

Adel B. Ghnenis *, Jerome C. Peel II, Gemechu T. Dinegdie, John F. Odhiambo

Posted Date: 23 April 2026

doi: 10.20944/preprints202604.1658.v1

Keywords: boer goat; maternal undernutrition; nutrient restriction; skeletal muscle; muscle fiber number; insulin signaling

 doi:10.20944/preprints202604.1658.v1

Article

Maternal Undernutrition During Gestation Programs Skeletal Muscle Development in Male Goat Offspring

Adel B. Ghnenis [1,*], Jerome C. Peel II [2], Gemechu T. Dinegdie [2] and John F. Odhiambo [3]

[1] Department of Agriculture, Virginia State University, Petersburg, VA 23806, USA
[2] Department of Agriculture and Natural Resources, Langston University, Langston, OK 73050, USA
[3] Department of Food and Animal Sciences, Alabama A & M University, Normal, AL 35762, USA
* Correspondence: aghnenis@vsu.edu

Simple Summary

Nutrition during pregnancy plays an important role in the growth and development of the fetus. When pregnant animals do not receive enough nutrients, it can affect how tissues such as muscle develop in their offspring. This study examined how reduced maternal nutrition during early to mid-gestation affects skeletal muscle development in Boer goat offspring. The results showed that maternal undernutrition impairs muscle fiber development and alters key genes involved in muscle growth and development, which may influence animal growth efficiency and meat quality.

Abstract

Maternal undernutrition during gestation can impair fetal muscle development, inducing lasting consequences for offspring growth and carcass quality. This study evaluated the effects of early- to mid-gestation nutrient restriction on postnatal skeletal muscle development in Boer goat offspring. Pregnant does (n = 12 per treatment) were assigned to either a control diet (CON; 100% of NRC recommendations) or a nutrient-restricted diet (NR; 60% of CON) from days 45 to 100 of gestation, then all does were realimented to the CON diet until parturition; male offspring (n = 6 per treatment) were maintained on a CON diet until 5 months of age. Longissimus dorsi muscle samples were collected for histological evaluation of fiber number, diameter, and collagen content, and for gene expression analysis of insulin receptor (IR), insulin receptor substrate-1 (IRS1), glucose transporter-4 (GLUT4), myogenic regulatory factors (MYF5, MYF6, MYOD, MYOG), and collagen genes (COL1A1 and COL3A1) using RT-PCR. Plasma glucose and cortisol were also measured. Muscle fiber number tended to be reduced ($p = 0.06$) in NR offspring, accompanied by decreased MYOG expression ($p < 0.05$) and trends for reduced MYF5 and MYF6 expression ($p < 0.10$), as well as reduced IR expression ($p < 0.05$). Collagen content did not differ, although COL3A1 expression was increased in NR offspring. Plasma glucose was lower ($p < 0.05$) at 3 months, and cortisol tended to be higher ($p < 0.10$) at 5 months. These results indicate that maternal undernutrition during early- to mid-gestation alters postnatal skeletal muscle development in Boer goats by reducing muscle fiber number and affecting myogenic and metabolic signaling pathways. Such changes may negatively affect the efficiency of muscle growth and meat quality.

Keywords: Boer goat; maternal undernutrition; nutrient restriction; skeletal muscle; muscle fiber number; insulin signaling

1. Introduction

Demand for goat meat and products continues to increase in the US, whereas the domestic goat inventory has decreased over the past decade. This continued growth in demand has resulted in a significant importation of sheep and goat meat, costing about $1.53 billion in 2023 [1]. Rangelands

 doi:10.20944/preprints202604.1658.v1

where most small ruminants are produced often experience periods of scarcity in good quality and quantity of forage, leaving these animals predisposed to prolonged periods of undernutrition, especially during the winter months and times of drier conditions [2]. When animals become pregnant during these periods, the resulting maternal undernutrition during gestation (MUDG) represents a significant challenge in livestock production, with major problems for the development of vital organ systems in offspring, a phenomenon referred to as fetal programming [3,4]. Nutritional deprivation during critical periods of fetal development may lead to redirecting the nutritive supply to support development of vital organs such as the fetal brain and heart, at the expense of other organs, including skeletal muscle tissue, eventually leading to muscle fiber dysfunction and fewer fibers being formed in the offspring. This muscle fiber reduction may lead to low carcass weight and composition [5,6], significantly affecting the producer's economic returns [7]. Evidence from several ruminant breeds suggests that MUDG reduced offspring birth weight in sheep [8], goats [9], and beef cattle [10]. In addition, it may lead to intrauterine growth retardation (IUGR) [7,11], which is associated with increased neonatal morbidity and mortality, changes in postnatal growth rate, decreased carcass quality, adipose tissue dysfunction, and changes in feed efficiency [12] and, more importantly, programs the body to metabolic dysfunction postnatally [11,13,14]. These adverse outcomes could be due to a significant reduction in fetal blood supply [15]. However, in as much as the adverse effects of exposure to undernutrition during fetal life on skeletal muscle dysfunction phenotype are well established in ruminants [5,16,17], the early in utero mechanisms leading to fetal and offspring muscle dysfunction are poorly understood.

During early gestation, skeletal muscle development is governed by intricate processes of myoblast proliferation, differentiation, and fiber formation, all of which are highly susceptible to nutrient limitations [18–21]. Studies show that muscle fiber formation occurs early in life and only increases in size later [22]. Therefore, it is critical to manipulate muscle fiber early to increase carcass weight and quality at market age. Improving nutrition and optimizing environmental factors during gestation, especially during critical periods of cellular differentiation and myogenesis, can influence muscle fiber number and composition postnatally, impacting offspring growth and meat quality [12]. Considering the metabolic dysfunction outcomes induced by MUDG and its consequences on the offspring and the limited information available on the early mechanisms, especially on the skeletal muscle that determines carcass quantity and quality in meat goat, the objective of this study was to investigate the impact of gestational nutrient restriction from days 45–100 of gestation on skeletal muscle development in meat goat. Because NR fetuses are exposed to a low level of nutrition supply during early to mid-gestation[23], we hypothesized that MUDG would reduce offspring muscle fiber number and alter muscle myogenic regulatory factors (MRFs) gene expression.

2. Materials and Methods

2.1. Animals and Experimental Design

All experimental animal procedures were approved by Langston University Institutional Animal Care and Use Committee (Approval number 23 – 011). 24 Multiparous Boers goats of similar age and body weights were bred at the American Institute of Goat Research (AIGR-Langston University) as previously described [24]. Estrus was synchronized using a controlled internal drug release (CIDR) vaginal device (EAZI-breed™ CIDR®, Pfizer Animal Health, Auckland, New Zealand) and PGF2α (Lutalyse, 10 mg dinoprost tromethamine i.m., Zoetis Animal Health, Parsippany-Troy Hills, NJ, USA). Does were bred naturally using one of three genetically related Boer bucks (one buck per eight does). Pregnant does were assigned randomly into control (CON) and nutrient-restricted (NR) groups, adjusting for age (mean ± SEM: CON 4.22 ± 0.3 vs. NR 3.15 ± 0.5 years), body weight (CON 53.03 ± 2.5 vs. NR 57.61 ± 2.4 kg), and body condition score (CON 2.7 ± 0.05 vs. NR 2.6 ± 0.05). The diet consisted of a total mix of rations (TMR) (69.4% of total digestible nutrients (TDN) and 16.0% of crude protein (CP); dry matter (DM) basis) (**Table 1**).

 doi:10.20944/preprints202604.1658.v1

Table 1. The diet composition used in the experiment consists of a total mix of rations (TMR).

Diet Composition	
Ingredients (%)	
Mixed grass hay	50.00%
Ground corn	28.290%
Soybean meal	17.33%
Liquid molasses	2.500%
Limestone. Calcium carbonate	0.900%
Ammonium sulfate	0.252%
Mineralized salt	0.560%
Magnesium chloride	0.10
Vitamin A, D, E premix[1]	0.050%
Rumensin 90	0.011%
Estimated composition	
CP (% DM)	16.0
UIP (% DM)	5.6
UIP (% CP)	34.7
RDP (% DM)	10.15
TDN (% DM)	69.4
RDP (% TDN)	15.1
NDF (% DM)	37,0
eNDF (% DM)	33.4
Ca (% DM)	0.72
P (% DM)	0.37
Ca:P	1.97
S (% N)	10.0
[1] 8,800,000 IU/kg vitamin A, 1,760,000 IU/kg vitamin D3, and 1,100 IU/kg vitamin E; NB-8006, Nutra Blend, Nesosho, MO (air-dry basis).	

The diets were individually measured and delivered to both treatment groups twice daily (at 8:00 am and 5 pm) on a DM basis to meet the TDN required to maintain a pregnant doe (National Research Council (NRC) requirements) [25]. Body weights were recorded weekly, and rations were adjusted for each animal.

To generate offspring, pregnant does were housed in individual pens and fed either 60% of NRC (NR group, n=12) by restricting the total diet allowance or 100% NRC requirements (CON group, n=12) from day 45 to 100 of gestation. From day 101 till term ~150 days, both groups (CON and NR) were fed a diet as per NRC (2007) requirements. At parturition, only one randomly selected male offspring was used from each doe if there were multiple kids. Kids' body weights were measured once every 2 weeks till the end of the trial. Does and their kids were housed individually for the first 3 days after birth and then group-housed with other pairs of ids in a pen with a creep feeder till weaning (3 months of age). After weaning, male offspring were fed a control diet to meet their growth and maintenance requirements until five months of age. Animals (n = 6/group) were slaughtered for tissue collection (**Figure 1**).

 doi:10.20944/preprints202604.1658.v1

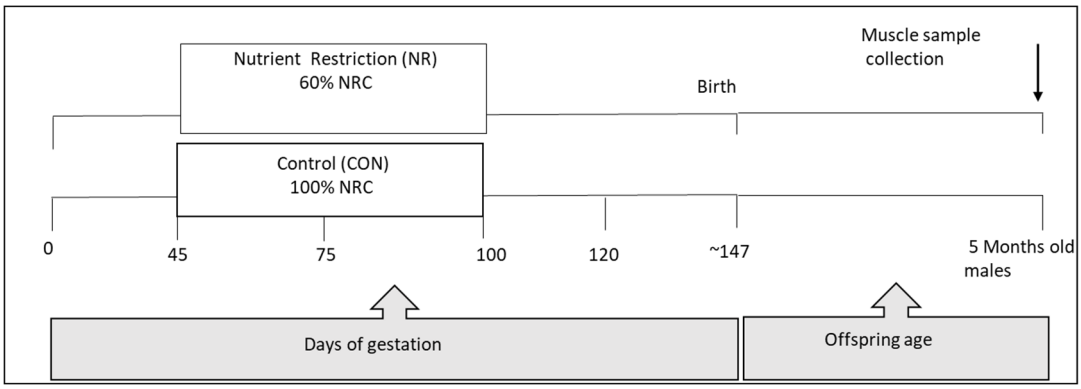


Figure 1. Experimental design and effects of MUDG on maternal and offspring body weight. Schematic diagram of the experimental design. Pregnant does (n = 12 per group) were assigned to either a nutrient restriction (NR) diet or a control (CON) diet to generate male offspring. At the experimental endpoint (5 months of age), animals were slaughtered, and longissimus dorsi muscle samples were collected. Tissues were either flash-frozen and stored at −80 °C for molecular analyses or fixed for histological analyses.

2.2. Blood and Muscle Sample Collection

Plasma samples were collected at 3 and 5 months of age after overnight fasting via jugular venipuncture in vacutainer tubes (blood was collected into tubes with sodium heparin as an anticoagulant, preventing blood clotting and driving plasma), then immediately centrifuged at 2,500 x g for 15 minutes at 4°C, and the plasma was stored at -80°C until analyzed. Male offspring were slaughtered for tissue harvest at Homestead Meats & Processing (Coyle, OK). Immediately after slaughter, the longissimus dorsi (LD) muscle samples (between the 12th and 14th ribs) were immediately collected from the left side of the carcass. Muscle samples (~3 grams each) were either snap-frozen in liquid nitrogen and stored at –80°C until utilized for molecular analysis or fixed in 10% formalin and paraffin-embedded for histological analyses.

2.3. Plasma Glucose and Cortisol Analysis

Plasma glucose was analyzed in triplicate using 96-well plates using validated colorimetric hexokinase assays (Pointe Scientific Glucose (Hexokinase) Liquid Reagents, Thermo Fisher Scientific, USA) according to the manufacturer's instructions as previously described [26]. The test was completed in a single assay with a sensitivity of 0.1 mg/dL and a mean intra-assay and CV of 4.83%. Plasma cortisol levels were measured in duplicate using 96-well plates using an ELISA kit. (Enzo Life Sciences; Farmingdale, NY, USA) According to the manufacturer's instructions. The test was completed in a single assay with a sensitivity of 56.72 pg/ml and a mean intra-assay and CV of 3.18%.

2.4. Skeletal Muscle Morphometry

Samples of the LD muscle were collected and fixed in 10% formalin for 48 hours, dehydrated with ethanol, cleared in xylene, and embedded in paraffin. Tissue sections were cut at 5 μm using a rotary microtome (Leica Biosystems). To determine muscle fiber number and diameter, two muscle sections per animal, separated by 100 μm, were stained with a hematoxylin and eosin staining kit (Abcam Inc., Waltham, MA). The number and diameter of muscle fibers were quantified by measuring fiber cross-sections from five randomly chosen images of each section, which were acquired by a THUNDER Imager 3D (Leica Microsystems) microscope at 40X magnification and quantified using Image J software (NIH, Bethesda, MD). To examine the development of fibrosis in muscle tissues, two sections 100 μm apart were stained with Masson's trichrome staining (Abcam, ab150686) according to the manufacturer's instructions. Ten randomly chosen images of each section were acquired by THUNDER Imager 3D microscope and analyzed using ImageJ software (NIH, Bethesda, MD). Fibrotic tissue quantification was performed as previously described [27].

 doi:10.20944/preprints202604.1658.v1

2.5. RNA Extraction, cDNA Synthesis, and mRNA Expression Analysis

Gene expression in LD muscle tissue was determined by Real-time PCR (RT-PCR, n=6/group). Total RNA in the muscle tissue was isolated by the TRIzol reagent (Invitrogen, Carlsbad, CA). The extracted RNA was purified by RNeasy mini kits (Qiagen, Germantown, MD) according to the manufacturer's instructions. The quantity and purity of the RNA were determined using a Nanodrop One spectrophotometer (Thermo Fisher Scientific, Hanover Park, Illinois), with an OD260/280 of 2.0, which indicates that good-quality RNA was achieved. One µg of RNA was reverse transcribed into cDNA using a cDNA synthesis kit (iScript™ Reverse Transcription Supermix Kit (Bio-Rad Systems, Hercules, CA). Considering MUDG may induce muscle fibrosis [20,28] and alter the insulin signaling pathway that regulates glucose uptake [29], the effects of MUDG on collagen types I and III (COLA1 and COLA3) and insulin receptor (IR), insulin receptor substrate 1 (IRS1), and glucose transporter protein type-4 (GLUT4) were determined. Since gestational undernutrition affects muscle development (myogenesis) [30,31], transcription factors known as MRFs, including MYF5, MYF6, MYOD, and myogenin (MYOG) [32], were examined. Gene expression levels were measured using SYBR Green-based QRT-PCR assay (SsoAdvanced Universal SYBR® Green Supermix, Bio-Rad Systems, Hercules, CA). RT-PCR was performed using CFX Duet Real-Time PCR System (Bio-Rad Systems, Hercules, CA). The primer sequences and their names are all previously published and listed in **Table 2**.

Table 2. Forward and reverse primer sequences (5′–>3′) of gene expression.

Gene ID	Forward Primer	Reverse Primer	Accession #
IR	CGGACGGATTCTGACTTTG	GCCTTTGAACCAGAGAGAAG	XM_018051135.1
IRS-1	GTCCCTCCACAGCTCTATAA	CACCTCCTCTCAGCAACTA	XM_018058864.1
Glut-4	GCTTGGCTTCTTCATCTTCACCTT	TGCTCAGACCACCCTTCCCTCCAG	NM_174604.1
COL1A1	GCTTCCTGTAAACTCCTTCC	GGCTTCAGTTTGGGTTGT	XM_018064893.1
COL3A1	AGGTGAACCCGGTAAGAA	CACCCTTAGGTCCTGGAATA	XM_005675869.3
MYF5	AGACGCCTGAAGAAGGTCAA	CTCCACCTGTTCCCTTAGCA	NM_001287037.1
MYF6	CAAGTCAGAGGCCAAGGAAG	TTCTAAGGGCTGCAGGGTAA	NM_001285602.1
MYOD	TGCAAACGCAAGACGACTAA	CTGGTTTGGGTTGCTAGACG	XM_018058990.1
MYOG	ACAATCTGCACTCCCTCACC	CATCCTGGCAGACAATCTCA	NM_001285733.1
GAPDH	AAGGTGGTGAAGCAGGTGTCA	TGGTCTTCAGTGTAGCCTAGAATGC	XM_005680968.3

To ensure accuracy and confidence in the results, each gene was tested in triplicate, and the average was calculated. The expression of each gene was determined after normalization with the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The relative amount of each transcript (in fold) was measured using the ΔΔCT method [33].

2.6. Statistical Analysis

Analyses were performed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC, USA) to account for the dependency of repeated measures. The model included the fixed effects of treatment, time, and their interaction. To verify the normality of the data, statistical outliers were excluded from the analysis using Grubbs' test [34]. Prism (GraphPad Software Inc., La Jolla, CA) was used to make graphs. Data are presented as least square means (LSM) ± standard error of the mean (SEM), and differences were considered significant at $P < 0.05$, tendencies at $P \leq 0.10$.

 doi:10.20944/preprints202604.1658.v1

3. Results

3.1. Effect of MUDG on Body Weights

Analyses of percentage (%) body weights (BW) revealed that CON dams gained more body weight than the NR group (15.04 ± 2.5 vs. 2.33 ± 1.5 % BW gain, respectively). Similar results were observed during the period from 100 to 135 days of gestation. CON dams also gained more ($P < 0.003$) % body weight than the NR group (29.3 ± 3.7 vs. 16.65 ± 1.6 % BW gain, respectively) (**Figure 2A**). Analyses of body weights for male offspring revealed a significant time effect ($P < .0001$), a trend in group effect ($P = 0.10$), and no significant group × time effect ($P = 0.15$). There was no significant difference in BW ($P = 0.72$) at birth between the NR vs. CON offspring. However, a significant difference ($P < 0.05$) in BWs was observed at time points of 10, 12, and 20 weeks of age (Male offspring from the NR group have lower body weight than controls) (**Figure 2B**).

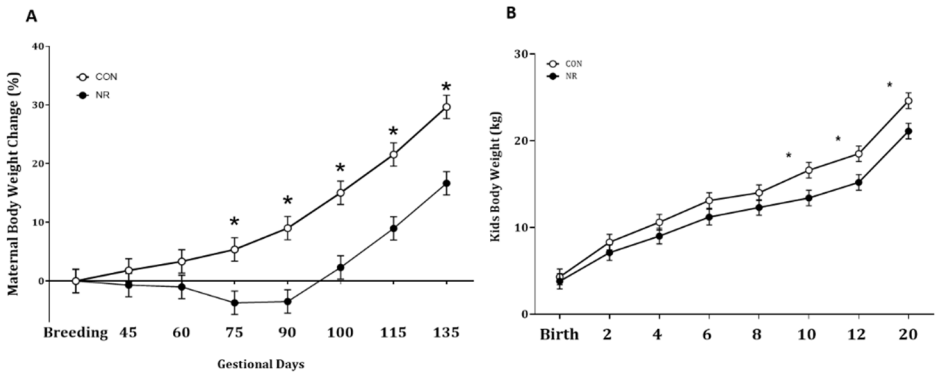


Figure 2. A: Maternal body weight change (%) during gestation in CON and NR groups. **B**: Body weights of offspring from birth to 20 weeks of age (n = 6 per group). Data were analyzed using mixed procedures in SAS, and values are presented as LS Means ± SEM. *P < 0.05 indicates a significant difference between the groups.

3.2. Effect of MUDG on Plasma Glucose and Cortisol Concentrations

There was a significant reduction ($P < 0.05$) in glucose concentration in the NR vs. CON offspring at three months of age, and glucose concentrations were similar between the groups at five months of age (**Figure 3A**). Cortisol concentration showed no significant differences at three months of age. However, there was a trend increase ($P = 0.068$) in cortisol concentration in the NR vs. CON offspring (**Figure 3B**).

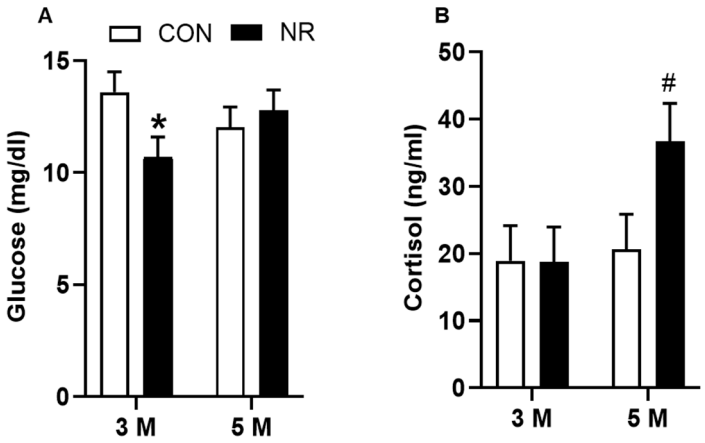


Figure 3. Effects of MUDG on **A**: offspring plasma glucose concentration and **B**: offspring plasma cortisol concentration (n=6 per group). Data were analyzed by mixed procedures of SAS, presented as LS Means ± SEM.*P ≤ 0.05; #P ≤ 0.10.

 doi:10.20944/preprints202604.1658.v1

3.3. Effect of MUDG on Muscle Fiber Number, Diameter, and Collagen Content

Histological analysis of the LD muscles showed a trend (P = 0.06) toward a decrease in the number of muscle fibers (**Figure 4B**). However, no significant differences were observed in muscle fiber diameter or collagen accumulation (**Figure 4C and 4E**, respectively).

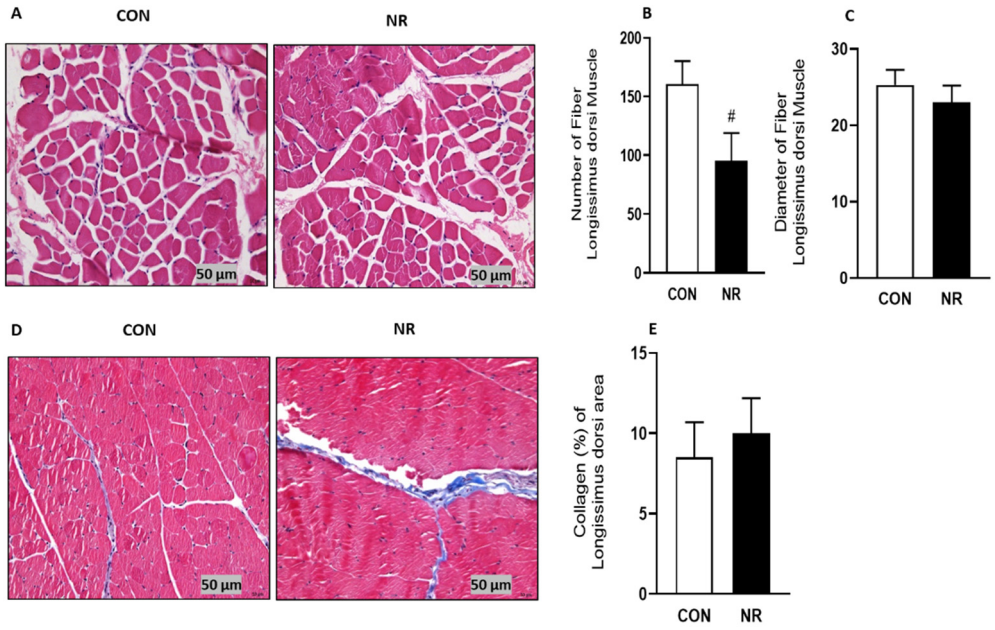
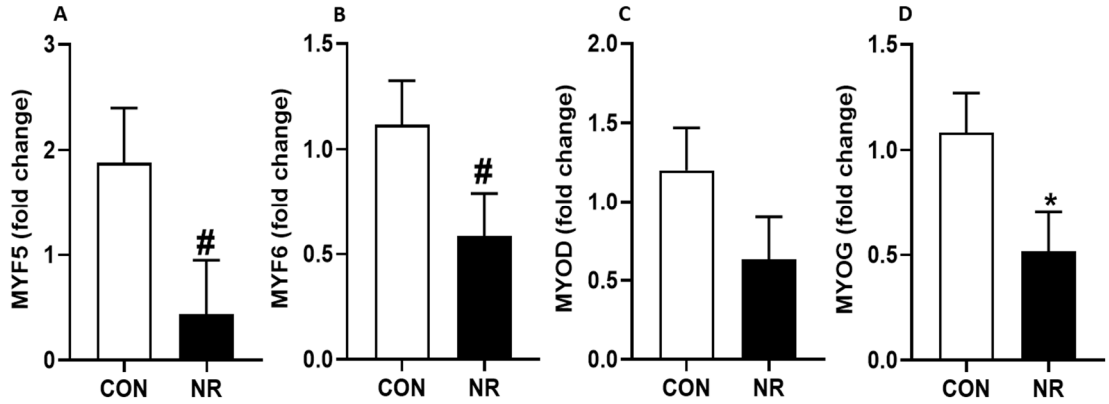


Figure 4. Effects of MUDG on muscle morphology and collagen deposition.

A: Representative hematoxylin and eosin–stained cross-sections of LD muscle from control (CON) and NR groups. **B**: Number of muscle fibers. **C**: Fiber diameter. **D**: Representative collagen staining of LD muscle sections. **E**: Quantification of collagen area (blue). Images were acquired at 40X magnification. Scale bar: 50 µm. Data was analyzed by mixed procedures of SAS, presented as LS Means ± SEM (2 sections/animal; 5 images per section; n = 6 per group) #P ≤ 0.10.

3.4. Effect of MUDG on Myogenic Determination Factors

MYF5 and MYF6 gene expression showed a trend toward reduced mRNA expression in NR compared with CON offspring (P = 0.07 and P = 0.09, respectively) (**Figure 5A and 5B**). However, MYOG gene expression was significantly decreased in NR compared with CON offspring (P < 0.05) (**Figure 5D**). No significant difference was observed in MYOD mRNA expression between the groups (P = 0.16) (**Figure 5C**).



 doi:10.20944/preprints202604.1658.v1

Figure 5. Effects of MUDG on Myogenic Regulatory Factors expression. mRNA expression levels of **A** MYF5, **B**: MYF6, **C**: MYOD, **D**: MYOG. Gene expression values were normalized to GAPDH, the housekeeping gene, to calculate fold changes. Data was analyzed by mixed procedures of SAS, presented as LS Means ± SEM (n = 6 per group) *P ≤ 0.05; #P ≤ 0.10.

3.5. Effect of MUDG on Insulin Signaling and Extracellular Matrix Gene Expression

Gene expression of IR was significantly reduced (P < 0.05) in NR offspring compared with CON (**Figure 6A**). However, no significant differences were observed in the mRNA expression of IRS1 and GLUT4 between the groups (**Figure 6B and 6C**, respectively). Gene expression analysis of fibrosis mediators showed no significant difference in COLA1 mRNA expression (P = 0.17), while COLA3 mRNA expression tended to increase (P = 0.06) in NR compared with CON offspring (**Figure 6D and 6E**, respectively).

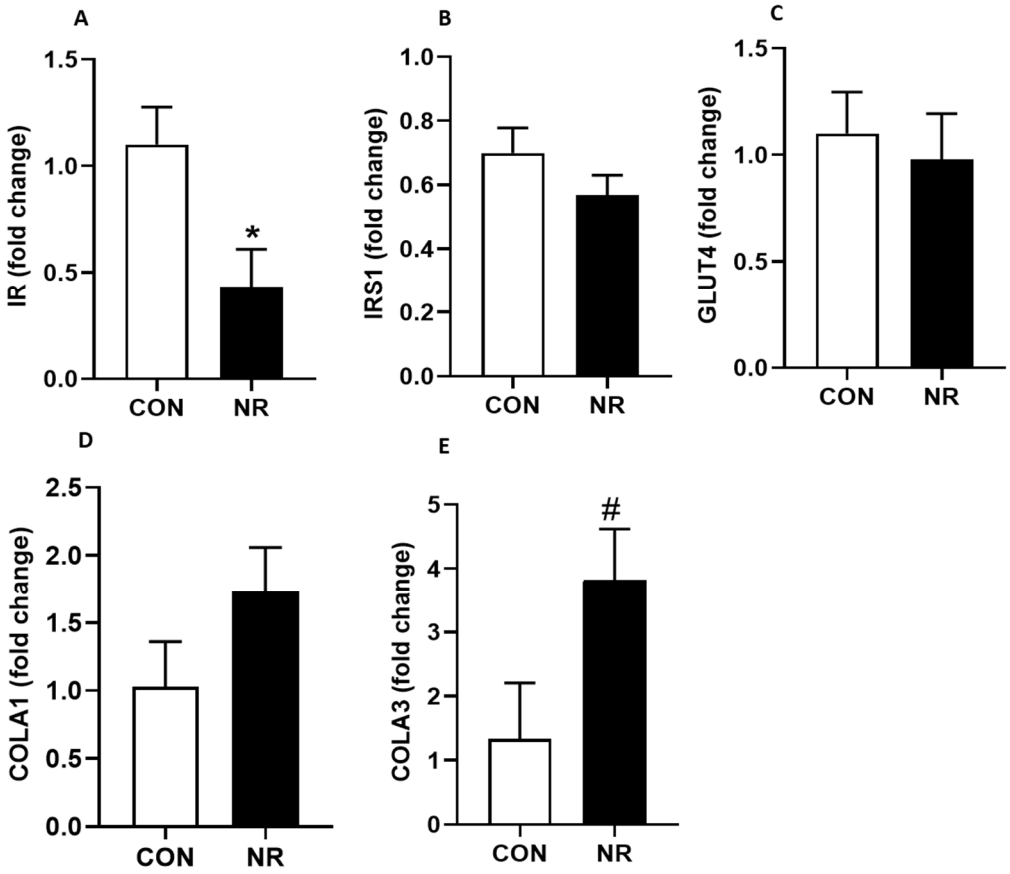


Figure 6. Effects of MUDG on insulin signaling and extracellular matrix gene expression. mRNA expression levels of **A**: IR, **B**: IRS1, **C**: GLUT4, **D**: COLA1, and **E**: COLA3. Gene expression values were normalized to GAPDH, the housekeeping gene, to calculate fold changes. Data was analyzed by mixed procedures of SAS, presented as LS Means ± SEM (n = 6 per group) *P ≤ 0.05; #P ≤ 0.10.

4. Discussion

The main findings of this study indicated that MUDG affects skeletal muscle development in the male offspring of Boer goats by decreasing the number of muscle fibers. This reduction was associated with decreased expression of the Myogenic regulatory factors (MRFs) MYF5, MYF6, and MYOG, as well as reduced IR gene expression in NR offspring compared to controls. Collectively, these results suggest impaired muscle development, altered glucose metabolism, and reduced insulin sensitivity. These changes likely induced metabolic dysfunction in the male kids, leading to decreased carcass yield at harvest.

 doi:10.20944/preprints202604.1658.v1

4.1. Impact of MUDG on Skeletal Muscle Morphology

The skeletal muscle development begins in the embryonic stage with mesenchymal cells committing to the myogenic lineage, which is regulated by several complex signaling pathways and myogenic regulatory factors (MRFs) [5,35,36] that eventually lead to the formation of muscle fibers [36]. During fetal development, myogenesis occurs in two stages, involving the primary and secondary myofibers, and no new muscle fibers are formed postnatally. After birth, muscle growth occurs through an increase in muscle fiber size only [5]. The effects of MUDG generally depend on several factors, including the level of nutrition restriction, its duration, and timing during gestation [37]. Consistent with other similar maternal nutrient restriction studies, our results showed no difference in neonatal body weights between the groups. However, shortly before and at slaughter (weeks 10, 12, and 20), body weights of restricted offspring were lower than those of the control [38]. Reduced fetal and offspring body weights resulting from gestational undernutrition have been widely reported [37]. Notably, gestational nutrient restriction at 50% of the control levels from conception to mid-gestation (day 75) in sheep resulted in fetuses with 30% less body weight compared to their controls. [39]. Interestingly, when these ewes were re-alimented to 100% of their nutritional requirements from day 75 of pregnancy until lambing, the resulting neonatal lambs from both NR and control ewes showed similar phenotypes, including no difference in bodyweight. Alterations in myofiber development and muscle growth induced by the poor maternal diet during gestation have also been reported in several studies [40,41]. In the present study, these effects were seen in a trend of reduction in muscle fiber number, while no differences were observed in muscle fiber diameter. The trend in reduced muscle fiber number may be related to the small sample size. In contrast, Zhou et al. [31], reported a significant increase in muscle fiber area and altered muscle fiber type in the Vastus Lateralis of restricted fetuses following a 40% dietary restriction in pregnant Liuyang black goats from days 45 to 100 of gestation. These findings were explained as possible evidence of compensatory hypertrophy. Compared with the findings of Zhou et al., our results suggest that compensatory mechanisms were absent or less pronounced in the present study. Differences in muscle groups may cause these differences to be realized; developmental stages (post-weaning vs. fetal and early postnatal), and genetic background (Boer vs. Liuyang black) may also lead to confounding results. Nevertheless, both studies support the concept that maternal undernutrition can cause adverse effects in muscle development in offspring [31,42].

4.2. Impact of MUDG on Muscle Myogenic Regulatory Factors

Myogenic regulatory factors (MRFs) are a family of transcription factors that regulate skeletal muscle development and play roles not only during fetal development but also in postnatal muscle fiber maturation and growth [43,44]. Our results showed a trend toward reduced MYF5, MYF6, and MYOD gene expression in NR offspring. These regulatory factors are known for their role in increasing and developing muscle fiber during the early life of embryonic and fetal development, as well as postnatal muscle maturation [45]. In addition, they are also involved in regulating myogenic precursor cells; therefore, their reduced expression may indicate impaired proliferation and expansion of myoblasts, which could partially explain the observed reduction in muscle fiber number in the NR offspring [46]. Myogenin (MYOG), a myogenic regulatory factor responsible for differentiation of myocytes, was significantly reduced in the NR offspring, suggesting impaired myogenic differentiation [47]. Previous studies have reported that maternal undernutrition can alter offspring MYF5 mRNA expression through increased DNA methylation of its promoter region [31], as well as downregulation of MYF5 and MYOG gene expression on days 85 and 135 of gestation after implementing 75% dietary restriction in the LD muscle [48]. These findings together indicate that maternal undernutrition disrupted the early and late phases of muscle fiber formation [21]. The rationale for applying feed restriction between days 45 and 100 of gestation was to target the period of myofibril formation. Previous ovine studies indicate that myofibril formation occurs approximately between days 50 and 90 of gestation, whereas after day 100, myofibrils mainly increase in size rather than number [49]. Severe undernutrition during pregnancy (greater than 50%) can

 doi:10.20944/preprints202604.1658.v1

reduce placental growth and often lead to pregnancy failure [50]. In contrast, the effects of moderate (30–40%) or mild (less than 20%) undernutrition are often less noticeable. By modifying the maternal diet from days 45-100 of gestation, this lines up with the second myofiber formation, which makes up for ~95% of total muscle fibers [51,52]. Nutrient deficiency during this period may therefore lead to a permanent reduction in fiber number, since myofiber hyperplasia only occurs during the prenatal period [53]. These findings support the hypothesis that mid-gestational undernutrition can have lasting effects on skeletal muscle development and may reduce muscle fiber number in the offspring.

4.3. Impact of MUDG on Insulin and Glucose Signaling

The skeletal muscle makes up to 40-50% of total body weight and plays a major role in metabolic function [54], particularly in glucose uptake and insulin signaling [55,56]. Several studies suggest a link between undernutrition during gestation and long-lasting adverse effects on the offspring's glucose and insulin homeostasis as well as metabolic programming [57]. In the present study, we observed a significant decrease in IR gene expression in the LD muscle of NR offspring, which may contribute to reduced insulin sensitivity or signaling efficacy in the skeletal muscle [58]. This contrasts with the expression of GLUT4 and IRS1, both downstream targets of insulin signaling, which did not show a significant difference. The reduction in the IR gene expression is evidence that early disruption at the receptor levels can result in permanent changes in skeletal muscles, including muscle structure, metabolism, and function [59]. This is consistent with other findings; studies from various animal models have reported a decreased IR expression in the offspring skeletal muscle induced by maternal undernutrition [60–62].

Furthermore, the plasma glucose concentration of the NR offspring was significantly lower than that of the control group at 3 months of age, indicating impaired glucose regulatory capacity potentially caused by disrupted Insulin signaling [63]. However, this contrasted with 5-month-old offspring, where there was no significance found in the glucose levels; this provides evidence of compensatory metabolic adaptations by restoring homeostasis. Cortisol concentrations were similar between the NR and the CON groups at 3 months. At 5 months of age, NR offspring exhibited a trend in an increase in cortisol concentration, which could be because NR animals experienced a greater stress response than the CON induced by MUDG. These findings support that mid-gestational maternal undernutrition can lead to an early metabolic phenotype in the offspring [31,42].

5. Conclusions

The significance of this research is that it helps understand how maternal undernutrition impacts offspring carcass value in the Boer goat, being the primary meat breed in the United States. This study is unique, as it represents the first investigation to pinpoint how nutritional stress during early-to-mid gestation permanently "reprograms" offspring muscle characteristics. It demonstrates that the reduction in offspring skeletal muscle fiber in NR offspring is driven by downregulation of the IR, which affects signaling required for myoblast proliferation, rather than by fibrosis, since the dominant collagen COLA1 was unchanged. Although COLA3 was upregulated, this did not affect muscle histology. By identifying this specific signaling defect, the study shows that future muscle growth is actually decided by biological switches during gestation on Boer goat. This gives farmers a new strategy by focusing on nutrition at the right time during pregnancy to ensure their kids are born with the highest possible potential for meat production.

Author Contributions: A B. G. conceived and designed the study: A. B. G. and J. C. P. conducted data collection and performed lab experiments. A B. G., J. C. P., G. T. D., and J. F. O. edited and revised the manuscript. J. C. P., G. T. D., J. F. O., and A. B. G. approved the final manuscript.

Funding: This work was supported by USDA NIFA Evans-Allen Projects (accession number 7008240)

 doi:10.20944/preprints202604.1658.v1

Institutional Review Board Statement: The study was conducted in accordance with the ethical standards of Langston University and approved by the Langston University Institutional Animal Care and Use Committee (IACUC; protocol code: 23-011). The author is currently affiliated with Virginia State University.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be made available from the corresponding author upon request.

Acknowledgments: The authors thank Mr. Dawit Woldemariam and farm staff for their assistance in breeding and animal care; Dr. Chanelle Hayes, a Lab Coordinator at Langston University, for assistance in supporting our research; and the students' support through Langston University Undergraduate Research for the help provided with animal feeding and tissue collection.

Conflicts of Interest statement: The authors declare no competing interests.

Abbreviations

Maternal Undernutrition During Gestation, MUDG; Nutrient Restriction, NR; Control, CON; National Research council, NRC; longissimus dorsi, LD; Intra Uterine Growth Retardation, IUGR; Collagen type A1, COLA1 and COLA3; insulin receptor, IR; insulin receptor substrate 1, IRS1; glucose transporter protein type-4, GLUT4; MRF, Myogenic Regulatory Factors; Myogenic Factors, MYF5, MYF6; Myogenic Myogenic Differentiation 1, MYOD; Myogenin, MYOG.

References

1. Meat of sheep, meat of goats | Imports and Exports | 2023 [Internet]. 2024 [cited 2025 Jul 21]. https://trendeconomy.com/data/commodity_h2/0204. Accessed 21 Jul 2025
2. Grings EE, Zampaligre N, Ayantunde A. Overcoming challenges to utilization of dormant forage in year-round grazing systems1. Journal of Animal Science. 2016;94:2–14. https://doi.org/10.2527/jas.2016-0517
3. Lakshmy R. Metabolic syndrome: role of maternal undernutrition and fetal programming. Rev Endocr Metab Disord. 2013;14:229–40. https://doi.org/10.1007/s11154-013-9266-4
4. Gauvin MC, Pillai SM, Reed SA, Stevens JR, Hoffman ML, Jones AK, et al. Poor maternal nutrition during gestation in sheep alters prenatal muscle growth and development in offspring. Journal of Animal Science. 2020;98:skz388. https://doi.org/10.1093/jas/skz388
5. Du M, Tong J, Zhao J, Underwood KR, Zhu M, Ford SP, et al. Fetal programming of skeletal muscle development in ruminant animals1. Journal of Animal Science. 2010;88:E51–60. https://doi.org/10.2527/jas.2009-2311
6. Larson DM, Martin JL, Adams DC, Funston RN. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. J Anim Sci. 2009;87:1147–55. https://doi.org/10.2527/jas.2008-1323
7. Wu G, Bazer FW, Wallace JM, Spencer TE. BOARD-INVITED REVIEW: Intrauterine growth retardation: Implications for the animal sciences1. Journal of Animal Science. 2006;84:2316–37. https://doi.org/10.2527/jas.2006-156
8. Nielsen MO, Kongsted AH, Thygesen MP, Strathe AB, Caddy S, Quistorff B, et al. Late gestation undernutrition can predispose for visceral adiposity by altering fat distribution patterns and increasing the preference for a high-fat diet in early postnatal life. Br J Nutr. 2013;109:2098–110. https://doi.org/10.1017/S0007114512004199
9. Bajhau HS, Kennedy JP. Influence of pre- and postpartum nutrition on growth of goat kids. Small Ruminant Research. 1990;3:227–36. https://doi.org/10.1016/0921-4488(90)90040-D
10. Greenwood PL, Cafe LM. Prenatal and pre-weaning growth and nutrition of cattle: long-term consequences for beef production. Animal. 2007;1:1283–96. https://doi.org/10.1017/S175173110700050X
11. Bell AW, Ehrhardt RA. Regulation of placental nutrient transport and implications for fetal growth. Nutr Res Rev. 2002;15:211–30. https://doi.org/10.1079/NRR200239

 doi:10.20944/preprints202604.1658.v1

12. Costa TC, Gionbelli MP, Duarte M de S. Fetal programming in ruminant animals: understanding the skeletal muscle development to improve meat quality. Anim Front. 2021;11:66–73. https://doi.org/10.1093/af/vfab061
13. Sharma D, Shastri S, Sharma P. Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. Clin Med Insights Pediatr. 2016;10:67–83. https://doi.org/10.4137/CMPed.S40070
14. Barker DJ, Clark PM. Fetal undernutrition and disease in later life. Rev Reprod. 1997;2:105–12. https://doi.org/10.1530/ror.0.0020105
15. Vasquez-Hidalgo MA, Grazul-Bilska AT, Swanson KC, Perry GA, Vonnahme KA. Timing and duration of nutrient restriction and its impacts on placental development and umbilical blood flow in adolescent sheep. Theriogenology. 2023;209:21–30. https://doi.org/10.1016/j.theriogenology.2023.06.016
16. Costa TC, Gionbelli MP, Duarte M de S. Fetal programming in ruminant animals: understanding the skeletal muscle development to improve meat quality. Animal Frontiers. 2021;11:66–73. https://doi.org/10.1093/af/vfab061
17. Du M. Prenatal Development of Muscle and Adipose and Connective Tissues and Its Impact on Meat Quality. Meat and Muscle Biology [Internet]. Iowa State University Digital Press; 2023 [cited 2025 Jul 21];7. https://doi.org/10.22175/mmb.16230
18. Ali AT, Hochfeld WE, Myburgh R, Pepper MS. Adipocyte and adipogenesis. Eur J Cell Biol. 2013;92:229–36. https://doi.org/10.1016/j.ejcb.2013.06.001
19. Kendall RT, Feghali-Bostwick CA. Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol. 2014;5:123. https://doi.org/10.3389/fphar.2014.00123
20. Yan X, Zhu M-J, Dodson MV, Du M. Developmental programming of fetal skeletal muscle and adipose tissue development. J Genomics. 2013;1:29–38. https://doi.org/10.7150/jgen.3930
21. Zhu M-J, Ford SP, Nathanielsz PW, Du M. Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. Biol Reprod. 2004;71:1968–73. https://doi.org/10.1095/biolreprod.104.034561
22. Fahey AJ, Brameld JM, Parr T, Buttery PJ. The effect of maternal undernutrition before muscle differentiation on the muscle fiber development of the newborn lamb. J Anim Sci. 2005;83:2564–71. https://doi.org/10.2527/2005.83112564x
23. Ruminants NRC (U S) C on NR of S, Council NR, Ruminants C on the NR of S, Resources B on A and N, Studies D on E and L. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. 中国法制出版社; 2007.
24. Patra AK, Puchala R, Animut G, Gipson TA, Sahlu T, Goetsch AL. Effects of acclimatization on energy expenditure by meat goats. Small Ruminant Research. 2009;81:42–54. https://doi.org/10.1016/j.smallrumres.2008.11.002
25. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids [Internet]. Washington, D.C.: National Academies Press; 2007 [cited 2025 Jul 21]. https://doi.org/10.17226/11654
26. Ford SP, Hess BW, Schwope MM, Nijland MJ, Gilbert JS, Vonnahme KA, et al. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring1. Journal of Animal Science. 2007;85:1285–94. https://doi.org/10.2527/jas.2005-624
27. Ghnenis A, Padmanabhan V, Vyas A. Sexual dimorphism in testosterone programming of cardiomyocyte development in sheep. American Journal of Physiology-Heart and Circulatory Physiology. American Physiological Society; 2022;322:H607–21. https://doi.org/10.1152/ajpheart.00691.2021
28. Costa TC, Du M, Nascimento KB, Galvão MC, Meneses JAM, Schultz EB, et al. Skeletal Muscle Development in Postnatal Beef Cattle Resulting from Maternal Protein Restriction during Mid-Gestation. Animals (Basel). 2021;11:860. https://doi.org/10.3390/ani11030860
29. Oliver MH, Bloomfield FH, Bansal A, Phua HH, Thorstensen EB, Harding JE, et al. Effects of maternal periconceptional undernutrition in sheep on offspring glucose-insulin axis function into adulthood. J Dev Orig Health Dis. 2021;12:714–20. https://doi.org/10.1017/S2040174420001063
30. Ithurralde J, Genovese P, Abud MJ, López-Pérez Á, Pérez-Clariget R, Bielli A. Maternal undernutrition affects secondary myogenesis in a muscle-dependent way across the major muscles of 70-day old ovine fetuses. Small Ruminant Research. 2020;191:106174. https://doi.org/10.1016/j.smallrumres.2020.106174

 doi:10.20944/preprints202604.1658.v1

31. Zhou X, Yan Q, Liu L, Chen G, Tang S, He Z, et al. Maternal undernutrition alters the skeletal muscle development and methylation of myogenic factors in goat offspring. Anim Biosci. 2022;35:847–57. https://doi.org/10.5713/ab.21.0285
32. Braun T, Gautel M. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev Mol Cell Biol. 2011;12:349–61. https://doi.org/10.1038/nrm3118
33. Livak KJ, 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. https://doi.org/10.1006/meth.2001.1262
34. Outlier calculator [Internet]. [cited 2025 Jul 21]. https://www.graphpad.com/quickcalcs/grubbs1/. Accessed 21 Jul 2025
35. Cossu G, Borello U. Wnt signaling and the activation of myogenesis in mammals. The EMBO Journal. John Wiley & Sons, Ltd; 1999;18:6867–72. https://doi.org/10.1093/emboj/18.24.6867
36. Kollias HD, McDermott JC. Transforming growth factor-beta and myostatin signaling in skeletal muscle. J Appl Physiol (1985). 2008;104:579–87. https://doi.org/10.1152/japplphysiol.01091.2007
37. Caton JS, Crouse MS, Reynolds LP, Neville TL, Dahlen CR, Ward AK, et al. Maternal nutrition and programming of offspring energy requirements. Transl Anim Sci. 2019;3:976–90. https://doi.org/10.1093/tas/txy127
38. Sen U, Sirin E, Ensoy U, Aksoy Y, Ulutas Z, Kuran M. The effect of maternal nutrition level during mid-gestation on postnatal muscle fibre composition and meat quality in lambs. Anim Prod Sci. CSIRO PUBLISHING; 2015;56:834–43. https://doi.org/10.1071/AN14663
39. Odhiambo JF, Pankey CL, Ghnenis AB, Ford SP. A Review of Maternal Nutrition during Pregnancy and Impact on the Offspring through Development: Evidence from Animal Models of Over- and Undernutrition. International Journal of Environmental Research and Public Health. Multidisciplinary Digital Publishing Institute; 2020;17:6926. https://doi.org/10.3390/ijerph17186926
40. 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. https://doi.org/10.1113/jphysiol.2006.112110
41. Reed SA, Raja JS, Hoffman ML, Zinn SA, Govoni KE. Poor maternal nutrition inhibits muscle development in ovine offspring. J Anim Sci Biotechnol. 2014;5:43. https://doi.org/10.1186/2049-1891-5-43
42. Sandoval C, Lambo CA, Beason K, Dunlap KA, Satterfield MC. Effect of maternal nutrient restriction on skeletal muscle mass and associated molecular pathways in SGA and Non-SGA sheep fetuses. Domestic Animal Endocrinology. 2020;72:106443. https://doi.org/10.1016/j.domaniend.2020.106443
43. Siqin Q, Nishiumi T, Yamada T, Wang S, Liu W, Wu R, et al. Relationships among muscle fiber type composition, fiber diameter and MRF gene expression in different skeletal muscles of naturally grazing Wuzhumuqin sheep during postnatal development. Anim Sci J. 2017;88:2033–43. https://doi.org/10.1111/asj.12848
44. Zammit PS. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Semin Cell Dev Biol. 2017;72:19–32. https://doi.org/10.1016/j.semcdb.2017.11.011
45. Bayrak B, Şen U, Gökçek D, Şirin E. Differential Myf5 and Myf6 expression and muscle fiber traits in Angora, Hair, Honamlı, and Kilis goats. Trop Anim Health Prod. 2025;57:36. https://doi.org/10.1007/s11250-025-04277-y
46. Ustanina S, Carvajal J, Rigby P, Braun T. The Myogenic Factor Myf5 Supports Efficient Skeletal Muscle Regeneration by Enabling Transient Myoblast Amplification. Stem Cells. 2007;25:2006–16. https://doi.org/10.1634/stemcells.2006-0736
47. Adhikari A, Kim W, Davie J. Myogenin is required for assembly of the transcription machinery on muscle genes during skeletal muscle differentiation. PLOS ONE. Public Library of Science; 2021;16:e0245618. https://doi.org/10.1371/journal.pone.0245618
48. Wang X, Shang M, Hu W, Zhang L. Multi-Omics Analysis Reveals the Potential Effects of Maternal Dietary Restriction on Fetal Muscle Growth and Development. Nutrients. 2023;15:1051. https://doi.org/10.3390/nu15041051

 doi:10.20944/preprints202604.1658.v1

49. Yan X, Zhu M-J, Dodson MV, Du M. Developmental Programming of Fetal Skeletal Muscle and Adipose Tissue Development. J Genomics. 2013;1:29–38. https://doi.org/10.7150/jgen.3930
50. Toschi P, Baratta M. Ruminant Placental Adaptation in Early Maternal Undernutrition: An Overview. Front Vet Sci. 2021;8:755034. https://doi.org/10.3389/fvets.2021.755034
51. Lee ASJ, Harris J, Bate M, Vijayraghavan K, Fisher L, Tajbakhsh S, et al. Initiation of primary myogenesis in amniote limb muscles. Developmental Dynamics. 2013;242:1043–55. https://doi.org/10.1002/dvdy.23998
52. Gundersen K, Anas M. Developmental Programming and Postnatal Modulations of Muscle Development in Ruminants. Biology. Multidisciplinary Digital Publishing Institute; 2025;14:929. https://doi.org/10.3390/biology14080929
53. Chang EI, Rozance PJ, Wesolowski SR, Nguyen LM, Shaw SC, Sclafani RA, et al. Rates of myogenesis and myofiber numbers are reduced in late gestation IUGR fetal sheep. J Endocrinol. 2019;244:339–52. https://doi.org/10.1530/JOE-19-0273
54. Trovato FM, Imbesi R, Conway N, Castrogiovanni P. Morphological and Functional Aspects of Human Skeletal Muscle. Journal of Functional Morphology and Kinesiology. Multidisciplinary Digital Publishing Institute; 2016;1:289–302. https://doi.org/10.3390/jfmk1030289
55. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes. 1981;30:1000–7. https://doi.org/10.2337/diab.30.12.1000
56. Govoni KE, Reed SA, Zinn SA. CELL BIOLOGY SYMPOSIUM: METABOLIC RESPONSES TO STRESS: FROM ANIMAL TO CELL: Poor maternal nutrition during gestation: effects on offspring whole-body and tissue-specific metabolism in livestock species,. J Anim Sci. 2019;97:3142–52. https://doi.org/10.1093/jas/skz157
57. Hoffman ML, Reed SA, Pillai SM, Jones AK, McFadden KK, Zinn SA, et al. PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: The effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism123. J Anim Sci. 2017;95:2222–32. https://doi.org/10.2527/jas.2016.1229
58. Dunlop K, Cedrone M, Staples JF, Regnault TRH. Altered fetal skeletal muscle nutrient metabolism following an adverse in utero environment and the modulation of later life insulin sensitivity. Nutrients. 2015;7:1202–16. https://doi.org/10.3390/nu7021202
59. Lopez-Pedrosa JM, Camprubi-Robles M, Guzman-Rolo G, Lopez-Gonzalez A, Garcia-Almeida JM, Sanz-Paris A, et al. The Vicious Cycle of Type 2 Diabetes Mellitus and Skeletal Muscle Atrophy: Clinical, Biochemical, and Nutritional Bases. Nutrients. 2024;16:172. https://doi.org/10.3390/nu16010172
60. De Blasio MJ, Gatford KL, Harland ML, Robinson JS, Owens JA. Placental Restriction Reduces Insulin Sensitivity and Expression of Insulin Signaling and Glucose Transporter Genes in Skeletal Muscle, But Not Liver, in Young Sheep. Endocrinology. 2012;153:2142–51. https://doi.org/10.1210/en.2011-1955
61. AWATA K, SHOJI H, ARAI Y, SANTOSA I, TOKITA K, MURANO Y, et al. Maternal Protein Restriction Inhibits Insulin Signaling and Insulin Resistance in the Skeletal Muscle of Young Adult Rats. Juntendo Iji Zasshi. 2024;70:142–51. https://doi.org/10.14789/jmj.JMJ23-0029-OA
62. Beauchamp B, Harper M-E. In utero Undernutrition Programs Skeletal and Cardiac Muscle Metabolism. Front Physiol. 2016;6:401. https://doi.org/10.3389/fphys.2015.00401
63. Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The crucial role and mechanism of insulin resistance in metabolic disease. Front Endocrinol (Lausanne). 2023;14:1149239. https://doi.org/10.3389/fendo.2023.1149239