IUGR alters muscle fiber development and proteome in fetal pigs

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1. ABSTRACT

Intrauterine growth restriction (IUGR) may have permanent stunting effects on muscle growth and development of the progeny. However, underlying mechanisms are largely unknown. Recent studies comparing muscle fiber development and proteomes in IUGR and normal-body-weight (NBW) fetal pigs indicated that muscle fiber diameter were smaller in IUGR fetal pigs than in NBW fetal pigs on all three stages (d 60, d 90 and d 110) of gestation. Although the number of primary fibers did not differ between these two fetal groups on d 60 of gestation, the total number of muscle fibers in IUGR fetal pigs was lower on d 90 and 110 of gestation, when compared with NBW fetal pigs. Further proteomic analysis has shown that 37 proteins involved in energy supply and protein metabolism, structure and type of muscle fibers, proliferation and differentiation of muscle fibers, nutrient transport, intracellular environment, and tissue integrity were differentially expressed between IUGR and NBW fetal pigs. These novel findings provide some implications on the mechanisms of reduced growth and impaired development of skeletal muscle in IUGR piglets.

2. INTRODUCTION

IUGR can be defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (1). Among domestic animals, pigs exhibit the most severe, naturally occurring IUGR (1). The growth of skeletal muscle in agricultural animals is of great interest because of its economic and social importance. IUGR may affect the total number of fibers in a given muscle (2). Available evidence shows that the number of muscle fiber can more accurately estimate the growth rate of pigs compared with birth weight (3). There is no increase in the number of muscle fibers after birth (4). Therefore, the fetal stage is crucial for skeletal muscle development and potential growth performance after birth. The postnatal growth of muscle fibers mainly refers to the muscle cell hypertrophy, such as the transverse section area and length increase. There is also evidence showing that IUGR is associated with altered distribution of muscle fiber type (5). Based on the biphasic nature theory of fiber formation (6), an initial population of fibers, called primary fibers, will provide surface for the attachment and fusion of
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Table 1. Body weights of IUGR and NBW fetal pigs during gestation

<table>
<thead>
<tr>
<th>Fetal pigs</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 60</td>
</tr>
<tr>
<td>IUGR</td>
<td>85.6 ± 3.5*</td>
</tr>
<tr>
<td>NBW</td>
<td>140.7 ± 2.9</td>
</tr>
</tbody>
</table>

Pregnant gilts were fed 2 kg/d of a corn and soybean meal-based diet and had free access to drinking water, as we previously described (48). On d 60, 90, and 110 of gestation, one IUGR fetus and one NBW fetus were obtained from each of 6 litters. Gilts were killed by jugular puncture after anesthesia, as previously described (49). The animal use protocol was reviewed and approved by the China Agricultural University Animal Care and Use Committee. Values, expressed as g, are means ± SEM, n = 6 per group. *P < 0.01 vs. the NBW group.

Myoblasts to form secondary fibers. The formation of primary fibers occurs on d 35-55 of gestation, followed by the proliferation of secondary fibers surrounding primary fibers till d 85-90 of gestation (5, 7). Both of these impacts will have a permanent effect on the postnatal growth of muscles in offspring. Additionally, our previous findings indicate that IUGR affects expression of muscle proteins (including enzymes) related to intracellular protein turnover, cellular redox balance, and signal transduction (8).

Due to altered expression of the fetal genome, fetal programming may influence fetal development and have life-long consequences (9). Prenatal muscle fiber number and size determine the growth rate of muscle after birth. This will have a profound impact on meat quality in animal production (10). Over the past two decades, nutritional scientists have directed much effort to enhance animal growth in the postnatal period. However, effective means to improve fetal growth remain limited.

3. THE HISTOLOGICAL AND COMPARATIVE PROTEOME ANALYSIS OF LONGISSIMUS DORSI MUSCLE IN IUGR AND NBW FETAL PIGS

3.1. Muscular fiber number and size

The body weight of selected IUGR fetal pigs was lower (P < 0.01) than that of NBW fetal pigs on d 60, 90, and 110 of gestation (Table 1). The histological analysis of muscle in IUGR and NBW fetal pigs are summarized in Figure 1. In addition, muscle fiber number and size in IUGR and NBW fetal pigs on d 60, 90 and 110 were compared (Figure 2). On d 60, primary fibers in the NBW group had a mean diameter of 22.0 m, compared with a value of 14.7 m in the IUGR group. Despite the difference in fiber size, the fiber number did not differ between IUGR and NBW fetal pigs on d 60. With the disappearance of the central myofibril-free region at d 90 and 110, it was difficult to distinguish primary fibers from secondary fibers. Therefore, the total number and mean diameter of muscle fibers on these two days were measured. On d 90 and 110, the mean diameter of muscle fibers was greater in the NBW group than in the IUGR group, with 8.08 m and 6.03 m for NBW and IUGR fetal pigs on d 90, respectively, and 11.7 m and 8.37 m for NBW and IUGR fetal pigs on d 110, respectively.

In our study, IUGR fetuses had a smaller size of primary fiber at d 60 as well as a lower number of total fibers and a smaller size at d 90 and 110, when compared with NBW fetuses. The difference in total fiber number between IUGR and NBW fetal pigs was greater than 25% on d 110 of gestation when the mass of longissimus dorsi muscle was 20% lower in the IUGR group. The smaller size of primary fibers in IUGR fetal pigs may lead to a decrease in the total number of fibers by birth because of the smaller surface area for secondary fibers to attach. Since muscle growth after birth is mainly based on cell hypertrophy and the transition of muscle fiber type, IUGR may have permanent adverse impact on growth potential, muscle growth, fat disposition, and meat quality.

The mechanism behind muscle growth and fiber formation during gestation was still unclear. The analysis was extended to muscle proteome changes in IUGR and NBW fetal pigs during three stages of gestation. Due to the muscle was in the time of rapid growth and development, the differential expression of protein was not consistent during three stages. A total of 37 protein spots were differentially expressed in muscle between IUGR and NBW fetal pigs at d 60, 90 and 110 of gestation. They were involved in energy supply and protein metabolism, structure and type of muscle fiber, proliferation and differentiation of muscle fibers, nutrient transport, the intracellular environment, and tissue integrity. Biochemical information about these protein spots is summarized in Table 2, whereas their abundances on the gel images are displayed in Figure 3.

3.2. Energy supply and protein metabolism

There were 10 differentially expressed proteins that participated in energy metabolism. These proteins included mitochondrial inner membrane protein-like protein (IMMT, Spot P502), isocitrate dehydrogenase 1 (IDH1, Spot L102), EH-domain containing protein 2 (EHD2, Spot P503) and dihydrolipoyl dehydrogenase, mitochondrial isoform 3 (DLD, Spot P163) were up-regulated in the muscle of IUGR fetal pigs, while citrate synthase (CS, Spot L271), pyruvate kinase isoforms M1/M2 (PKM, Spot M402), creatine kinase M chain (CKM, Spot M415), mitochondrial ATP synthase (ATPase, Spot M406), UDP-glucose pyrophosphorylase 2, isoform CRA -a (UGP2, Spot M239) and phosphoglycerate mutase 2 (PGAM2, Spot M425) were down-regulated in the IUGR group. IUGR affected expression of enzymes involved in protein metabolism. Compared with NBW fetuses, IUGR fetuses had a higher level of COP9 signalosome complex subunit 6 (COPS6, Spot M407) in the muscle.

During gestation, muscle fibers are in the stage of rapid growth, along with protein synthesis and degradation, and require a lot of energy supply. In this regard, it is noteworthy that IUGR significantly affects expression of
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Table 2. Biochemical properties of differentially expressed proteins in the muscle of fetal pigs

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein name</th>
<th>Abbr.</th>
<th>Accession. No.</th>
<th>IUGR/NBW</th>
<th>Protein score</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L196</td>
<td>Valosin containing protein, isform CRA-b</td>
<td>VCP</td>
<td>gi</td>
<td>148670554</td>
<td>-1.41</td>
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<tr>
<td>L401</td>
<td>Myosin-7</td>
<td>MYH7</td>
<td>gi</td>
<td>55741486</td>
<td>-1.38</td>
</tr>
<tr>
<td>L180</td>
<td>Transferrin</td>
<td>TF</td>
<td>gi</td>
<td>338000</td>
<td>-1.35</td>
</tr>
<tr>
<td>L102</td>
<td>Citrate synthase, mitochondrial precursor</td>
<td>CS</td>
<td>gi</td>
<td>753618</td>
<td>1.83</td>
</tr>
<tr>
<td>L271</td>
<td>Heat shock protein HSP 90-beta</td>
<td>HSP90</td>
<td>gi</td>
<td>197100267</td>
<td>-1.35</td>
</tr>
<tr>
<td>L408</td>
<td>Albumin</td>
<td>ALB</td>
<td>gi</td>
<td>833798</td>
<td>-5.57</td>
</tr>
<tr>
<td>L403</td>
<td>Albumin</td>
<td>ALB</td>
<td>gi</td>
<td>833798</td>
<td>-2.41</td>
</tr>
<tr>
<td>L404</td>
<td>Transferrin</td>
<td>TF</td>
<td>gi</td>
<td>189232884</td>
<td>1.31</td>
</tr>
<tr>
<td>L410</td>
<td>S-formylglutathione hydrolase</td>
<td>ESD</td>
<td>gi</td>
<td>752936</td>
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</tr>
<tr>
<td>L106</td>
<td>Similar to Septin-11</td>
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<td>gi</td>
<td>74001932</td>
<td>-1.37</td>
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<tr>
<td>L402</td>
<td>Alpha-2-HS-glycoprotein</td>
<td>AHS</td>
<td>gi</td>
<td>231467</td>
<td>+3.61</td>
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<tr>
<td>d 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M401</td>
<td>Bifunctional purine biosynthesis protein</td>
<td>PURH</td>
<td>gi</td>
<td>95539476</td>
<td>-1000000</td>
</tr>
<tr>
<td>M402</td>
<td>Pyruvate kinase isozymes M1/M2</td>
<td>PKM</td>
<td>gi</td>
<td>94377282</td>
<td>-1000000</td>
</tr>
<tr>
<td>M415</td>
<td>Creatine kinase M chain</td>
<td>CKM</td>
<td>gi</td>
<td>838363</td>
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</tr>
<tr>
<td>M406</td>
<td>mitochondrial ATP synthase</td>
<td>ATPase</td>
<td>gi</td>
<td>8887343</td>
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</tr>
<tr>
<td>M412</td>
<td>Albumin</td>
<td>ALB</td>
<td>gi</td>
<td>833798</td>
<td>-1.4</td>
</tr>
<tr>
<td>M407</td>
<td>COP9 signalosome complex subunit 6</td>
<td>COP9S6</td>
<td>gi</td>
<td>3690456</td>
<td>+5.88</td>
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<tr>
<td>M410</td>
<td>Nestin</td>
<td>Nestin</td>
<td>gi</td>
<td>94035987</td>
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</tr>
<tr>
<td>M170</td>
<td>Alpha-fetoprotein precursor</td>
<td>AFP</td>
<td>gi</td>
<td>7523700</td>
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</tr>
<tr>
<td>M413</td>
<td>Protein disulfide isomerase-associated 3 isofrom 1</td>
<td>PDI3A</td>
<td>gi</td>
<td>114656687</td>
<td>-1.29</td>
</tr>
<tr>
<td>M239</td>
<td>UGP-glucose pyrophosphorylase 2, isofrom CRA-a</td>
<td>UGP2</td>
<td>gi</td>
<td>848675899</td>
<td>-1.42</td>
</tr>
<tr>
<td>M424</td>
<td>Glutathione s-transferase omega 1</td>
<td>GSTO1</td>
<td>gi</td>
<td>752916</td>
<td>-1.79</td>
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<tr>
<td>M403</td>
<td>Similar to Septin-11</td>
<td>Septin</td>
<td>gi</td>
<td>2633693</td>
<td>3.75</td>
</tr>
<tr>
<td>M405</td>
<td>Porin 31HM</td>
<td>Porin</td>
<td>gi</td>
<td>238427</td>
<td>-1000000</td>
</tr>
<tr>
<td>M411</td>
<td>Prelamin/A-C</td>
<td>LMNA</td>
<td>gi</td>
<td>62139823</td>
<td>+1.42</td>
</tr>
<tr>
<td>M425</td>
<td>Phosphoglycerate mutase 2</td>
<td>PGAM2</td>
<td>gi</td>
<td>201086358</td>
<td>-1.43</td>
</tr>
<tr>
<td>d 110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P502</td>
<td>Low quality protein: mitochondrial inner membrane protein-like protein</td>
<td>IMM</td>
<td>gi</td>
<td>94386568</td>
<td>+3.13</td>
</tr>
<tr>
<td>P503</td>
<td>EH domain-containing protein 2</td>
<td>EHHD2</td>
<td>gi</td>
<td>114051716</td>
<td>+2.09</td>
</tr>
<tr>
<td>P380</td>
<td>Transferrin</td>
<td>TF</td>
<td>gi</td>
<td>189232884</td>
<td>-1.23</td>
</tr>
<tr>
<td>P178</td>
<td>Tripartite motif-containing 72</td>
<td>TRIM72</td>
<td>gi</td>
<td>19917044</td>
<td>+1.46</td>
</tr>
<tr>
<td>P505</td>
<td>MYH1 protein</td>
<td>MYH1</td>
<td>gi</td>
<td>15545466</td>
<td>+2.09</td>
</tr>
<tr>
<td>P510</td>
<td>Chain A, structures of actin-bound wh2 domains of spire and the impli filament nucleation</td>
<td>Wh2</td>
<td>gi</td>
<td>97343122</td>
<td>-1.46</td>
</tr>
<tr>
<td>P28</td>
<td>Heat shock protein beta-1</td>
<td>HSPB7</td>
<td>gi</td>
<td>55926209</td>
<td>+1.57</td>
</tr>
<tr>
<td>P290</td>
<td>Stress-70 protein, mitochondrial</td>
<td>GRP75</td>
<td>gi</td>
<td>154816188</td>
<td>+1.26</td>
</tr>
<tr>
<td>P163</td>
<td>Dihydropyrimidin dehydrogenase, mitochondrial isofrom 3</td>
<td>DLD</td>
<td>gi</td>
<td>296309969</td>
<td>+1.69</td>
</tr>
<tr>
<td>P512</td>
<td>Alpha-actinin-2-like isofrom 1</td>
<td>ACTN2</td>
<td>gi</td>
<td>94042529</td>
<td>+3.32</td>
</tr>
</tbody>
</table>

* Spot numbers refer to protein spot numbers that correspond to the labels in Figure 3. ' The signs (-) and (+) indicate a decrease and increase, respectively, compared with the value for NBW fetal pigs, n = 3 gels for both IUGR and NBW groups at each age. All the identified spots were differentially expressed (P < 0.05). ' Protein score generated by MS identification platform, with a score over 71 being considered as statistical significance.

Figure 1. A transverse section of the *longissimus dorsi* muscle of IUGR (D-F) and NBW (A-C) fetal pigs on d 60 (A and D), d 90 (B and E) and d 110 (C and F) of gestation. P and S represent primary and secondary fiber, respectively. Longissimus dorsi muscle was fixed in 4% formaldehyde (Sigma, St. Louis, MO) at 4°C. The formaldehyde fixed samples were embedded in paraffin, sectioned and mounted on glass slides for staining with hematoxylin and eosin. Muscle morphology was examined with a light microscope (Olympus BX50, Japan). The total number of fibers and fiber width were determined using the Medical Image Analysis System (MIAS) software. To examine each tissue slide in triplicate, 12 sections were randomly chosen, with each area being photographed at a 400 × magnification. The pictures were used to obtain the mean diameter of muscle fiber, the mean number of fiber per unit area. These values, along with the total section area, were used to estimate the total number of fibers.
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Figure 2. The number and mean diameter of *longissimus dorsi* muscle fiber. TNF means total number of fibers. Asterisk indicates statistical significance (*P* < 0.05, **P** < 0.01). Analysis was performed using SAS (version 8.2; SAS Institute, Cary, NC) and data was analyzed by *t*-test.

proteins related to energy and protein metabolism in skeletal muscle. Specifically, the levels of three rate-controlling enzymes in energy metabolism, citrate synthase (CS), isocitrrate dehydrogenase 1 (IDH1) and pyruvate kinase isoforms M1/M2 (PKM), were all affected in IUGR fetal pigs. CS catalyzes the first step of the citric acid cycle (11), while IDH1 participates in a subsequent reaction of the cycle. Both enzymes play a key role in intermediary metabolism and ATP production. PKM consists of M1-type (predominantly expressed in muscle, brain and heart) and M2-type (predominantly expressed in fetal tissues) (12). PKM is responsible for catalyzing the final step of glycolysis: the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP.

In addition to the enzymes mentioned above, IUGR also influenced the expression of some other key enzymes involved in glucose and energy metabolism, such as creatine kinase M chain (CKM), mitochondrial ATP synthase (ATPase), UDP-glucose pyrophosphorylase 2, isoform CRA-a (UGP2), EH-domain containing protein 2 (EHD2), and dihydrolipoyl dehydrogenase, mitochondrial isoform 3 (DLD). CKM, which is involved in cellular energy homeostasis, plays a central role in energy transduction in tissues with large and fluctuating energy demands, such as skeletal muscle (13). Mitochondrial ATP synthase catalyzes ATP synthesis (14). UGP2 (or glucose-1-phosphate uridyltransferase) is an enzyme associated with glycogenesis. EHD2 associates with insulin-induced glucose transporter-4 (Glut4). Insulin recruits Glut4 to the plasma membrane, thereby allowing Glut4 to bind glucose to increase the use of glucose by skeletal muscle (15). DLD, which functions as a component of the pyruvate dehydrogenase complex, localizes to the mitochondrial matrix to convert pyruvate to acetyl-CoA for oxidation by the Krebs cycle (16).

COP9 signalosome complex subunit 6 (COPS6) is a complex involved in various cellular and developmental processes. The structure and function of COPS6 signalosome is similar to that of the 19S regulatory particle of 26S proteasome. It is reported that concentrations of proteasome were markedly elevated in skeletal muscle of IUGR piglets, therefore enhancing ubiquitin-dependent protein degradation (17). Thus, it is possible that elevated protein degradation provides amino acids as a source of energy substrates in IUGR fetal pigs.

Another important finding of the study is that IUGR increased expression of some key enzymes involved
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Figure 3. Abundance of differentially expressed proteins in the muscle of IUGR and NBW fetal pigs on d 60, 90 and 110 of gestation. A portion of the tissue was rapidly placed in liquid nitrogen and stored at -80°C. Proteins were extracted from the muscle sample as we described (45). With one gel for each pair of IUGR and NBW samples in each of the 3 time-phases (d 60, d 90 and d 110), a total of 18 gels were run for the 2-dimensional electrophoresis (2-DE) using commercial IPG strips (pH 3-10 NL, 24 cm) (GE Healthcare, Piscataway, NJ) for isoelectric focusing (IEF), followed by standard vertical Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12.5%) for second dimension (46). High-resolution gel images (400 dpi) were obtained using an ImageScanner Model PowerLook 2100XL (UMAX Technologies, Atlanta, GA) and image analysis was performed using an Image-Master 2D Platinum Version 6.01 according to manufacturer’s protocol (GE Healthcare, Piscataway, NJ). Differentially expressed protein spots (P < 0.05) were cut from the gel and in-gel proteolytic digestion was performed, as we described (45). Peptides from in-gel digested proteins were identified by matrix assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF MS) as previous described (47).

in glucose metabolism (e.g., glycolysis), such as IMMT, IDH1, EHD2 and DLD. While, the expression of some enzymes related to gluconeogenesis and the generation of ATP was reduced in IUGR fetuses, such as UGP2, mitochondrial ATP synthase, PKM. Therefore, the conditions of fetal growth restriction may lead to increased glucose utilization and reduced glucose production, leading to the deprivation of energy in IUGR fetal pigs. In support of this view, expression of phosphoglycerate mutase 2 (PGAM2) was down-regulated in the muscle of IUGR fetal pigs. PGAM2 is an enzyme of the glycolysis pathway. In humans, a deficiency of PGAM causes metabolic myopathy, which is one of the many forms of syndromes formerly referred to as muscular dystrophy (18).

3.3. Structure and type of muscle fiber

Five spots of proteins were structural proteins or related to muscle fiber type. The abundance of myosin-7 (MYH-7, Spot L401), alpha 3-actin (ACTN3, Spot M403) and actin-bound wh2 domains of spire and the impli filament nucleation (Wh2, Spot P510) was lower in the muscle of IUGR fetal pigs, when compared with NBW fetal pigs. Additionally, the levels of MYH1 protein (MYH1, Spot P505) and alpha-actinin-2-like isoform 1
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(ACTN2, Spot P512) were higher in IUGR than in NBW fetuses.

Due to the programmed development of the fetus, prenatal structure and the proportion of different type of muscle fiber may affect the growth potential of skeletal muscle and even meat quality when slaughtered. Skeletal muscle is composed of two major types of muscle fibers: slow- and fast-twitch fibers, which can be distinguished by myosin heavy chain isoforms (19). Myosin-7 (MYH7) and myosin-1 (MYH1) are two myosin heavy chain isoforms.

Actin-bound wh2 domains of spire and the impli filament nucleation (Wh2) is one of the three classes of proteins that are known to nucleate new filaments. The principal feature of these structures is their loose, open conformations, in which the sides of actins that normally constitute the inner interface core of a filament are flipped inside out (20). Each muscle fiber is composed of long tubes called myofibrils which in turn are composed of filaments. There are two types of filaments: actin (thin filaments) and myosin (thick filaments) which are arranged in parallel. Alpha-actinins belong to a family of actin-binding and crosslinking proteins, including spectrin and dystrophin. Alpha-actinin-2-like isoform 1 (ACTN2) and alpha 3-actin (ACTN3) are the two main types which function as anti-parallel homodimers (21). Our findings indicated that IUGR affects the development of muscle fiber type and its structure.

3.4. Proliferation and differentiation of muscle fiber

IUGR affected the expression of proteins involved in proliferation and differentiation of muscle fibers. Specifically, valosin containing protein, isoform CRA-b (VCP, Spot L196), nestin (Nestin, Spot M410) and septin-11 (Sep11, Spot L106) were down-regulated in IUGR fetal pigs, while prelamin-A/C (LMNA. Spot M411) and heat shock protein beta-1 (HSP27, Spot P28) were up-regulated in IUGR fetuses.

During myogenesis, mitosis is increased markedly. Valosin-containing protein, isoform CRA-b (VCP), also designated TERA (transitional endoplasmic reticulum ATPase), is involved in a variety of cellular activities, such as cell cycle and biogenesis (22). Nestin is expressed by dermalomal cells and myoblasts during the early stage of myogenesis (23). Septins are a highly conserved family of membrane-associated GTPases with functions in cell division (24). Sep11 displays GTP-binding and GTPase activity and may regulate cytokinesis (25). Prelamin-A/C (LMNA) is a protein that plays an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics (26). LMNA can accelerate senescence of smooth muscle cells by disrupting mitosis and inducing DNA damage, leading to mitotic failure, genomic instability, and premature senescence (27).

Heat shock protein beta-1 (Hsp27) appears in many cell types, especially all types of muscle cells. High expression levels may be in inverse relation with cell proliferation (28), which can be observed in muscle diseases (29). Therefore, the elevated level of Hsp27 in IUGR fetal pigs provides another line of evidence for the adverse effect of IUGR on muscle growth and development. Additionally, some proteins related to mitosis in muscle were reduced in IUGR fetuses, ultimately leading to the decreased number of muscle fibers. Our findings on the differential expression of VCP, nestin, septins, LMNA and HSP27 between IUGR and NBW fetal pigs may provide new molecular mechanisms for the regulation of muscle growth and development in mammals.

3.5. Nutrient transport

Nine spots of proteins play important roles in nutrient transport, including transferrin (TF, Spot L180, L404, P380), albumin (ALB, Spot L403, L408, M412), alpha-fetoprotein precursor (AFP, Spot M170), bifunctional purine biosynthesis protein (PURH, Spot M401) and porin 31HM (Porin, Spot M405). All of these proteins were reduced in the muscle of IUGR fetuses, in comparison with NBW fetuses.

Muscle growth and developmental needs a large number of building blocks, including iron, calcium and other minerals as well as amino acids, glucose and other nutrients. Transferrin (TF), an iron-binding transport protein, is responsible for iron transport from sites of absorption and heme degradation to those of storage and utilization (30). Interestingly, TF has been shown to interact with insulin-like growth factor 2 and IGFBP3; therefore, transferrin may play an important role in regulating IGF/IGFBP-3 functions (31). Porins are beta barrel proteins that cross a cellular membrane and act as a pore through which molecules can diffuse. Porins typically control the diffusion of small metabolites like sugars, ions, and amino acids (32). TF and porins were more abundant in skeletal muscle of NBW than IUGR fetuses. We suggest that the deficiency of TF and porins in IUGR fetuses contributes to iron deficiency and impair fetal myogenesis and glucose metabolism.

A number of serum transport proteins, including serum albumin (ALB, alpha-fetoprotein precursor (AFP), are evolutionarily related (33). They are the major proteins in plasma and bind various cations, fatty acids and bilirubin. The reduced level of these two proteins may result from malnutrition and, therefore, impaired the synthesis of proteins, including hemoglobins and myoglobins, in IUGR fetuses.

PURH, a bifunctional protein, catalyzes the second to the last step in purine biosynthesis. This enzyme may play a role in stimulating glucose transport in the absence of insulin in isolated rat muscle (34). Therefore, the reduced level of the PURH may impair cell proliferation during myogenesis and glucose transport, leading to the deprivation of glucose in IUGR fetal pigs. The decline in transport function will inevitably result in the obstruction of growth and differentiation of muscle fibers.

3.6. Intracellular environment

Compared with NBW fetuses, glutathione s-transferase omega-1 (GSTO1, Spot M424) and s-formylglutathione hydrolase (ESD, Spot L410) were down-
regulated in the muscle of IUGR fetuses. These proteins were related to the detoxification of xenobiotics. Harmful substances in the cellular metabolic processes require their timely clearance in order to ensure a good environment for the growth of muscle fibers. S-formylglutathione hydrolase, also known as ESD, is an enzyme that catalyzes the chemical reaction using s-formylglutathione and H₂O to produce glutathione and formate. The main function of ESD is to detoxify formaldehyde (35). Glutathione s-transferase omega-1 (GSTO1) catalyzes the reaction of glutathione with a wide variety of organic compounds to form thioureas, a process that is essential for the metabolism and detoxification of a variety of xenobiotics (36). Interestingly, levels for these two proteins were both decreased in the muscle of IUGR fetal pigs, indicating an impaired capacity for xenobiotic detoxification in the runts.

3.7. Muscle tissue integrity

Expression of five muscle proteins related to tissue health, such as cell repairing, stress response as well as the processing and degradation of unwanted or harmful proteins were affected by IUGR. Heat shock protein HSP 90-beta (HSP90, Spot L407) and protein disulfide isomerase-associated 3 isoform 1 (PDIA3, Spot M413) were reduced in the muscle of IUGR fetuses, compared with the NBW fetuses. In contrast, Alpha-2-HS-glycoprotein (AHSG, Spot L402), tripartite motif-containing 72 (TRIM72, Spot P176), and stress-70 protein (GRP75, Spot P290) were increased in the muscle of the IUGR group.

Cell repair, as well as the processing and degradation of unwanted or harmful proteins, is important to maintain normal tissue development. Alpha-2-HS-glycoprotein (AHSG) has been shown to function as an acute phase antiinflammatory mediator that is critical to regulating the innate immune response following tissue injury (37). Tripartite motif-containing 72 (TRIM72) is exclusively expressed in cardiac and skeletal muscle (38). Acting as a sensor of oxidation on membrane damage, TRIM72 plays a central role in cell membrane repair by nucleating the assembly of the repair machinery at injury sites (39). The increased expression of fetuin and TRIM72 in the muscle of IUGR fetal pigs suggests the presence of metabolic or oxidative injury in this tissue.

Heat shock protein HSP 90-beta (Hsp90) plays a number of important roles, including assisting folding (40). This chaperone functions to stabilize the 26S proteasome, which enables the cell to degrade unwanted and/or harmful proteins (41). Protein disulfide isomerase-associated 3 isoform 1 (PDIA3) localizes to the lumen of the endoplasmic reticulum (ER), where in conjunction with folding-helper proteins, mediating tertiary and quaternary protein-processing (42). The major function of PDIA3 is that it aids wrongly-folded proteins to reach a correctly-folded state (43). The reduced level of hsp90 and PDIA3 in IUGR fetal pigs may impair the processing and degradation of abnormally-folded proteins, thereby influencing the protein structure and function. Another protein affecting tissue integrity is stress-70 protein (GRP75), whose expression is restricted to the mitochondrial matrix. This protein aids in the translocation and folding of nascent polypeptide chains of both nuclear and mitochondrial origin (44). Results of recent studies have shown that intestinal expression of Hsp70 can be enhanced by functional amino acids [including glutamine and arginine (50, 51)] and related metabolites (e.g., alpha-ketoglutarate) in young pigs (52-57). This may provide a biochemical basis for the use of these nutrients to improve growth, health, and well-being of animals and humans (58-63).

4. CONCLUSION AND PERSPECTIVES

Currently, few studies are available linking maternal nutrition to epigenetic modifications in fetal muscle. The proteomic study provides the first line of evidence for possible defects in molecular mechanisms that regulate growth and development of fetal skeletal muscle. The differentially expressed proteins are related to energy supply and protein metabolism, muscle structure and type, proliferation and differentiation of muscle fibers, nutrient transport, intracellular environment, as well as tissue integrity. These results indicate impaired metabolism of nutrients and reduced growth and development of muscle in the IUGR fetus. The findings have important implications for improving muscle growth and development in both humans and animals. This new knowledge can be translated into animal production to improve feed efficiency and meat quality.

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**Abbreviations:** IUGR, intrauterine growth restriction; NBW, normal-body-weight; IEF, isoelectric focusing; 2-DE, 2-dimensional electrophoresis; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MALDI-TOF/TOF MS, matrix assisted laser desorption ionization-time of flight/time of flight mass spectrometry

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