

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

Table 1. Body weights of IUGR and NBW fetal pigs during gestation^a

Fetal pigs	Body weight		
	d 60	d 90	d 110
IUGR	85.6 ± 3.5 ^{b**}	515.0 ± 41.5 ^{**}	858.3 ± 100.6 ^{**}
NBW	140.7 ± 2.9	758.3 ± 47.8	1528.0 ± 77.6

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. ^a Values, expressed as g, are means ± SEM, n = 6 per group. ^{b**}, $P < 0.01$ 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 μm, compared with a value of 14.7 μm 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 μm and 6.03 μm for NBW and IUGR fetal pigs on d 90, respectively, and 11.7 μm and 8.37 μm 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 isozymes 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

Table 2. Biochemical properties of differentially expressed proteins in the muscle of fetal pigs

	Spot No. ^a	Protein name	Abbr.	Accession. No.	IUGR/NBW ^b	Protein score ^c
d 60						
	L196	Valosin containing protein, isform CRA-b	VCP	gi 148670554	-1.41	123
	L401	Myosin-7	MYH7	gi 55741486	-1.38	107
	L180	Transferrin	TF	gi 833800	-1.35	103
	L102	Isocitrate dehydrogenase 1	IDH1	gi 89573979	+1.33	172
	L271	Citrate synthase, mitochondrial precursor	CS	gi 47523618	-1.83	136
	L407	Heat shock protein HSP 90-beta	HSP90	gi 197100267	-1.35	416
	L408	Albumin	ALB	gi 833798	-5.57	525
	L403	Albumin	ALB	gi 833798	-2.41	475
	L404	Transferrin	TF	gi 189232884	-1.31	198
	L410	S-formylglutathione hydrolase	ESD	gi 47522936	-1000000	166
	L106	Similar to Septin-11	Sep11	gi 74001932	-1.37	206
	L402	Alpha-2-HS-glycoprotein	AHSG	gi 231467	+3.61	174
d 90						
	M401	Bifunctional purine biosynthesis protein	PURH	gi 195539476	-1000000	259
	M402	Pyruvate kinase isozymes M1/M2	PKM	gi 194377282	-1000000	82
	M415	Creatine kinase M chain	CKM	gi 4838363	-1.37	225
	M406	mitochondrial ATP synthase	ATPase	gi 148887343	-1000000	220
	M412	Albumin	ALB	gi 833798	-1.4	317
	M407	COP9 signalosome complex subunit 6	COPS6	gi 2360945	+1.58	171
	M410	Nestin	Nestin	gi 194035987	-1.22	112
	M170	Alpha-fetoprotein precursor	AFP	gi 47523700	-1.28	126
	M413	Protein disulfide isomerase-associated 3 isoform 1	PDIA3	gi 114656687	-1.29	77
	M239	UDP-glucose pyrophosphorylase 2, isoform CRA-a	UGP2	gi 148675899	-1.42	210
	M424	Glutathione s-transferase omega-1	GSTO1	gi 47522916	-1.29	140
	M403	Similar to alpha 3-actin	ACTN3	gi 126333693	-3.75	295
	M405	Porin 31HM	Porin	gi 238427	-1000000	74
	M411	Prelamin-A/C	LMNA	gi 162139823	+1.42	591
	M425	Phosphoglycerate mutase 2	PGAM2	gi 201066358	-1.43	267
d 110						
	P502	Low quality protein: mitochondrial inner membrane protein-like protein	IMMT	gi 194386568	+1.31	93
	P503	EH domain-containing protein 2	EHD2	gi 114051716	+2.09	239
	P380	Transferrin	TF	gi 189232884	-1.23	179
	P176	Tripartite motif-containing 72	TRIM72	gi 119917044	+1.46	170
	P505	MYH1 protein	MYH1	gi 115545466	+2.09	119
	P510	Chain A, structures of actin-bound wh2 domains of spire and the impli filament nucleation	Wh2	gi 297343122	-1.46	112
	P28	Heat shock protein beta-1	HSP27	gi 55926209	+1.57	147
	P290	Stress-70 protein, mitochondrial	GRP75	gi 154816168	+1.26	134
	P163	Dihydrolipoyl dehydrogenase, mitochondrial isoform 3	DLD	gi 296209969	+1.69	239
	P512	Alpha-actinin-2-like isoform 1	ACTN2	gi 194042529	+1.32	503

^a Spot numbers refer to protein spot numbers that correspond to the labels in Figure 3. ^b 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 ($P < 0.05$). ^c Protein score generated by MS identification platform, with a score over 71 being considered as statistical significance.

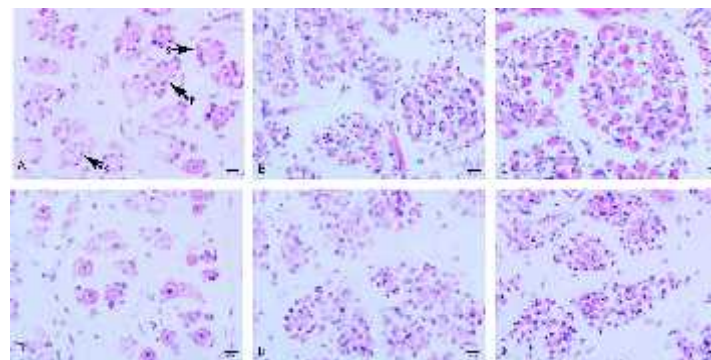


Figure 1. A transverse section of the *longissimus dorsi* muscle of IUGR (D-F) and NBW (A-C) fetal pigs on d 60 (A and D), d 90 (B and E) and d 110 (C and F) of gestation. P and S represent primary and secondary fiber, respectively. *Longissimus dorsi* muscle was fixed in 4% formaldehyde (Sigma, St. 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.

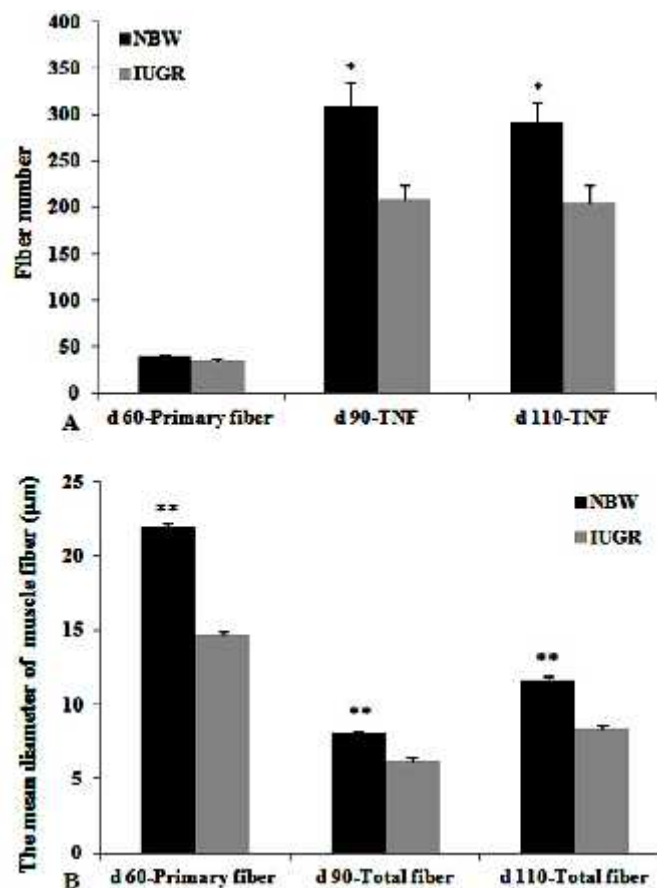


Figure 2. The number and mean diameter of *longissimus dorsi* muscle fiber. TNF means total number of fibers. Asterisk indicates statistical significance (* $P < 0.05$, ** $P < 0.01$). Analysis was performed using SAS (version 8.2; SAS Institute, Cary, NC) and data was analyzed by *t*-test.

proteins related to energy and protein metabolism in skeletal muscle. Specifically, the levels of three rate-controlling enzymes in energy metabolism, citrate synthase (CS), isocitrate dehydrogenase 1 (IDH1) and pyruvate kinase isozymes 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 uridylyltransferase) 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

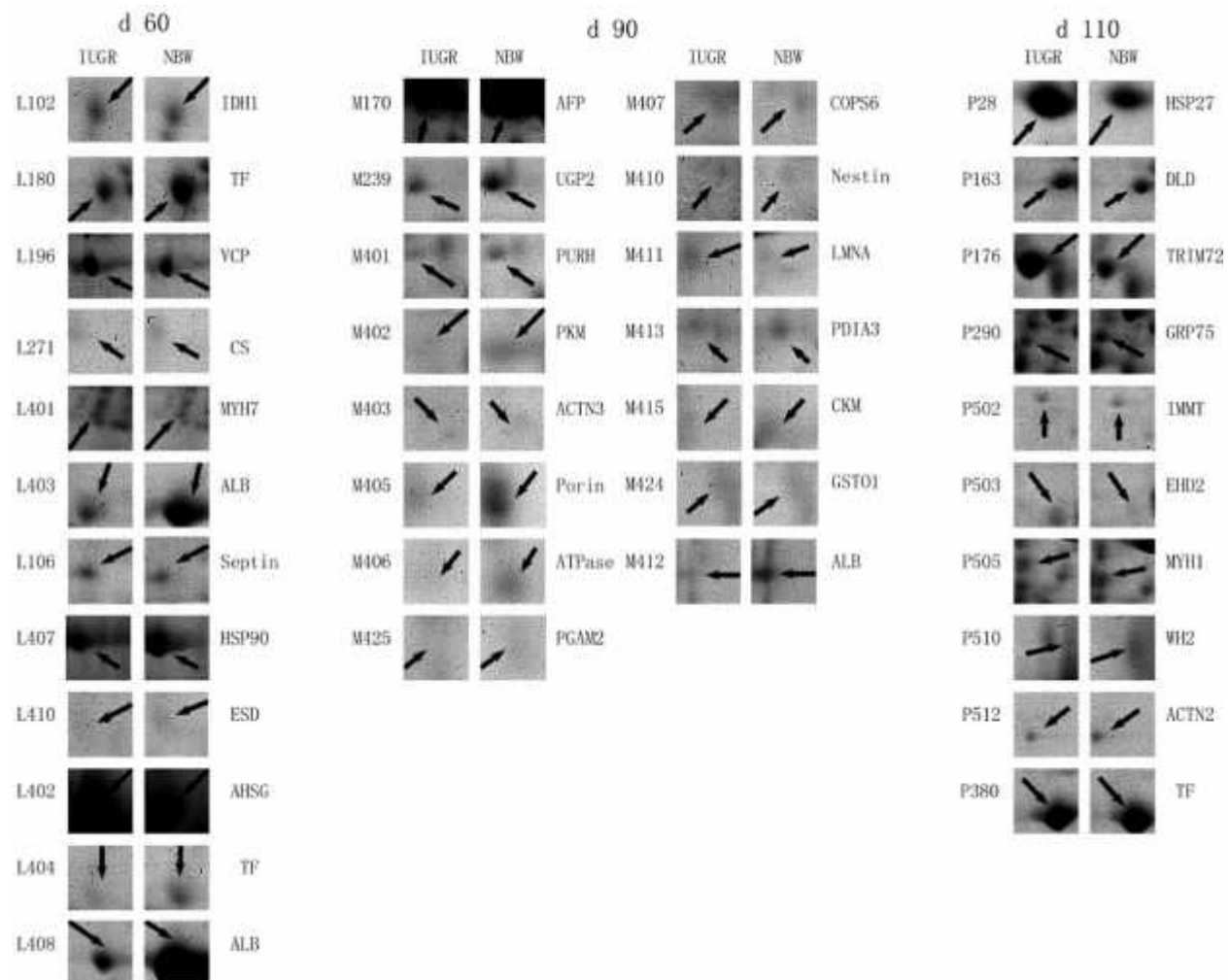


Figure 3. Abundance of differentially expressed proteins in the muscle of IUGR and NBW fetal pigs on d 60, 90 and 110 of gestation. A portion of the tissue was rapidly placed in liquid nitrogen and stored at -80°C . Proteins were extracted from the muscle sample as we described (45). With one gel for 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 previously 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

(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 dermatomal 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 thioethers, 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.

5. ACKNOWLEDGEMENTS

This work was financially supported by National Basic Research Program of China (no. 2012CB124703), and the National Natural Science Foundation of China (no. 30972156 and 31129006).

6. REFERENCES

1. G. Wu, F. W. Bazer, J. M. Wallace and T. E. Spencer: Board-invited review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci*, 84, 2316-2337 (2006)
2. S. E. Powell and E. D. Aberle: Skeletal muscle and adipose tissue cellularity in runt and normal birth weight swine. *J Anim Sci*, 52, 748-756 (1981)
3. C. M. Dwyer, J. M. Fletcher and N. C. Stickland: Muscle cellularity and postnatal growth in the pig. *J Anim Sci*, 71, 3339-3343 (1993)
4. N. C. Stickland, and G. Goldspink: A possible Indicator Muscle for Fiber Content and Growth Characteristics of Porcine Muscle. *Anim Prod*, 16, 135-146 (1973)
5. P. M. Wigmore and N. C. Stickland: Muscle development in large and small pig fetuses. *J Anat*, 137, 235-245 (1983)
6. H. J. Swatland: Muscle growth in the fetal and neonatal pig. *J Anim Sci*, 37, 536-545 (1973)

7. N. C. Stickland and S. E. Handel: The numbers and types of muscle fibres in large and small breeds of pigs. *J Anat*, 147, 181-189 (1986)
8. J. Wang, L. Chen, D. Li, Y. Yin, X. Wang, P. Li, L. J. Dangott, W. Hu and G. Wu: Intrauterine growth restriction affects the proteomes of the small intestine, liver, and skeletal muscle in newborn pigs. *J Nutr*, 138, 60-66 (2008)
9. D. J. Barker and P. M. Clark: Fetal undernutrition and disease in later life. *Rev Reprod*, 2, 105-112 (1997)
10. G. Bee: Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. *J Anim Sci*, 82, 826-836 (2004)
11. G. Wiegand and S. J. Remington: Citrate synthase: structure, control, and mechanism. *Annu Rev Biophys Chem*, 15, 97-117 (1986)
12. V. Gupta and R. N. Bamezai: Human pyruvate kinase M2: a multifunctional protein. *Protein Sci*, 19, 2031-2044 (2010)
13. Y. Q. Shen, L. Tang, H. M. Zhou and Z. J. Lin: Structure of human muscle creatine kinase. *Acta Crystallogr, D Biol Crystallogr*, 57, 1196-1200 (2001)
14. M. Prescott, N. C. Bush, P. Nagley and R. J. Devenish: Properties of yeast cells depleted of the OSCP subunit of mitochondrial ATP synthase by regulated expression of the ATP5 gene. *Biochem Mol Biol Int*, 34, 789-799 (1994)
15. S. Y. Park, B. G. Ha, G. H. Choi, J. Ryu, B. Kim, C. Y. Jung and W. Lee: EHD2 interacts with the insulin-responsive glucose transporter (GLUT4) in rat adipocytes and may participate in insulin-induced GLUT4 recruitment. *Biochemistry*, 43, 7552-7562 (2004)
16. E. Nishimoto, Y. Aso, T. Koga and S. Yamashita: Thermal unfolding process of dihydrolipoamide dehydrogenase studied by fluorescence spectroscopy. *J Biochem*, 140, 349-357 (2006)
17. S. H. Lecker, R. T. Jagoe, A. Gilbert, M. Gomes, V. Baracos, J. Bailey, S. R. Price, W. E. Mitch and A. L. Goldberg: Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J*, 18, 39-51 (2004)
18. S. DiMauro, A. F. Miranda, S. Khan, K. Gitlin and R. Friedman: Human muscle phosphoglycerate mutase deficiency: newly discovered metabolic myopathy. *Science*, 212, 1277-1279 (1981)
19. N. Okumura, A. Hashida-Okumura, K. Kita, M. Matsubae, T. Matsubara, T. Takao and K. Nagai: Proteomic analysis of slow- and fast-twitch skeletal muscles. *Proteomics*, 5, 2896-2906 (2005)
20. A. M. Ducka, P. Joel, G. M. Popowicz, K. M. Trybus, M. Schleicher, A. A. Noegel, R. Huber, T. A. Holak and T. Sitar: Structures of actin-bound Wiskott-Aldrich syndrome protein homology 2 (WH2) domains of Spire and the implication for filament nucleation. *Proc Natl Acad Sci USA*, 107, 11757-11762 (2010)
21. Y. Chan, H. Q. Tong, A. H. Beggs and L. M. Kunkel: Human skeletal muscle-specific alpha-actinin-2 and -3 isoforms form homodimers and heterodimers in vitro and in vivo. *Biochem Biophys Res Commun*, 248, 134-139 (1998)
22. R. M. Dai, E. Chen, D. L. Longo, C. M. Gorbea and C. C. Li: Involvement of valosin-containing protein, an ATPase Co-purified with IkappaBalpha and 26 S proteasome, in ubiquitin-proteasome-mediated degradation of IkappaBalpha. *J Biol Chem*, 273, 3562-3573 (1998)
23. A. M. Kachinsky, J. A. Dominov and J. B. Miller: Myogenesis and the intermediate filament protein, nestin. *Dev Biol*, 165, 216-228 (1994)
24. W. S. Trimble: Septins: a highly conserved family of membrane-associated GTPases with functions in cell division and beyond. *J Membr Biol*, 169, 75-81 (1999)
25. N. Hanai, K. Nagata, A. Kawajiri, T. Shiromizu, N. Saitoh, Y. Hasegawa, S. Murakami and M. Inagaki: Biochemical and cell biological characterization of a mammalian septin, Sept11. *FEBS Lett*, 568, 83-88 (2004)
26. W. H. De Vos, F. Houben, R. A. Hoebe, R. Hennekam, B. van Engelen, E. M. Manders, F. C. Ramaekers, J. L. Broers and P. Van Oostveldt: Increased plasticity of the nuclear envelope and hypermobility of telomeres due to the loss of A-type lamins. *Biochim Biophys Acta*, 1800, 448-458 (2010)
27. C. D. Ragnauth, D. T. Warren, Y. Liu, R. McNair, T. Tajsic, N. Figg, R. Shroff, J. Skepper and C. M. Shanahan: Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. *Circulation*, 121, 2200-2210 (2010)
28. L. M. Vargas-Roig, M. A. Fanelli, L. A. Lopez, F. E. Gago, O. Tello, J. C. Aznar and D. R. Ciocca: Heat shock proteins and cell proliferation in human breast cancer biopsy samples. *Cancer Detect Prev*, 21, 441-451 (1997)
29. C. Sarto, P. A. Binz and P. Mocarelli: Heat shock proteins in human cancer. *Electrophoresis*, 21, 1218-1226 (2000)
30. E. C. Theil: Iron, ferritin, and nutrition. *Annu Rev Nutr*, 24, 327-343 (2004)
31. S. Storch, B. Kubler, S. Honing, M. Ackmann, J. Zapf, W. Blum and T. Bräulke: Transferrin binds insulin-like growth factors and affects binding properties of insulin-like growth factor binding protein-3. *FEBS Lett*, 509, 395-398 (2001)
32. T. Schirmer: General and specific porins from bacterial outer membranes. *J Struct Biol*, 121, 101-109 (1998)

33. F. Schoentgen, M. H. Metz-Boutigue, J. Jolles, J. Constans and P. Jolles: Complete amino acid sequence of human vitamin D-binding protein (group-specific component): evidence of a three-fold internal homology as in serum albumin and alpha-fetoprotein. *Biochim Biophys Acta*, 871, 189-198 (1986)
34. N. Fujii, M. F. Hirshman, E. M. Kane, R. C. Ho, L. E. Peter, M. M. Seifert and L. J. Goodyear: AMP-activated protein kinase alpha2 activity is not essential for contraction- and hyperosmolarity-induced glucose transport in skeletal muscle. *J Biol Chem*, 280, 39033-39041 (2005)
35. C. F. Gonzalez, M. Proudfoot, G. Brown, Y. Korniyenko, H. Mori, A. V. Savchenko and A. F. Yakunin: Molecular basis of formaldehyde detoxification. Characterization of two S-formylglutathione hydrolases from *Escherichia coli*, FrmB and YeiG. *J Biol Chem*, 281, 14514-14522 (2006)
36. Y. J. Li, S. A. Oliveira, P. Xu, E. R. Martin, J. E. Stenger, C. R. Scherzer, M. A. Hauser, W. K. Scott, G. W. Small, M. A. Nance, R. L. Watts, J. P. Hubble, W. C. Koller, R. Pahwa, M. B. Stern, B. C. Hiner, J. Jankovic, C. G. Goetz, F. Mastaglia, L. T. Middleton, A. D. Roses, A. M. Saunders, D. E. Schmechel, S. R. Gullans, J. L. Haines, J. R. Gilbert, J. M. Vance, M. A. Pericak-Vance, C. Hulette and K. A. Welsh-Bohmer: Glutathione S-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease. *Hum Mol Genet*, 12, 3259-3267 (2003)
37. J. P. Lebreton, F. Joisel, J. P. Raoult, B. Lannuzel, J. P. Rogez and G. Humbert: Serum concentration of human alpha 2 HS glycoprotein during the inflammatory process: evidence that alpha 2 HS glycoprotein is a negative acute-phase reactant. *J Clin Invest*, 64, 1118-1129 (1979)
38. C. Cai, H. Masumiya, N. Weisleder, N. Matsuda, M. Nishi, M. Hwang, J. K. Ko, P. Lin, A. Thornton, X. Zhao, Z. Pan, S. Komazaki, M. Brotto, H. Takeshima and J. Ma: MG53 nucleates assembly of cell membrane repair machinery. *Nat Cell Biol*, 11, 56-64 (2009)
39. C. Cai, H. Masumiya, N. Weisleder, Z. Pan, M. Nishi, S. Komazaki, H. Takeshima and J. Ma: MG53 regulates membrane budding and exocytosis in muscle cells. *J Biol Chem*, 284, 3314-3322 (2009)
40. Y. Miyata and I. Yahara: The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. *J Biol Chem*, 267, 7042-7047 (1992)
41. J. Imai, M. Maruya, H. Yashiroda, I. Yahara and K. Tanaka: The molecular chaperone Hsp90 plays a role in the assembly and maintenance of the 26S proteasome. *EMBO J*, 22, 3557-3567 (2003)
42. M. Mayer, U. Kies, R. Kammermeier and J. Buchner: BiP and PDI cooperate in the oxidative folding of antibodies in vitro. *J Biol Chem*, 275, 29421-29425 (2000)
43. J. K. Burgess, K. A. Hotchkiss, C. Suter, N. P. Dudman, J. Szollosi, C. N. Chesterman, B. H. Chong and P. J. Hogg: Physical proximity and functional association of glycoprotein 1balpha and protein-disulfide isomerase on the platelet plasma membrane. *J Biol Chem*, 275, 9758-9766 (2000)
44. I. G. Haas: Protein-mediated protein maturation in eukaryotes. *FEBS Lett*, 369, 72-75 (1995)
45. J. Wang, T. Ying, H. Wang, Z. Shi, M. Li, K. He, E. Feng, J. Yuan, T. Li, K. Wei, G. Su, H. Zhu, X. Zhang, P. Huang and L. Huang: 2-D reference map of *Bacillus anthracis* vaccine strain A16R proteins. *Proteomics*, 5, 4488-4495 (2005)
46. X. Wang, D. Ou, J. Yin, G. Wu and J. Wang: Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of zinc oxide-supplemented piglets. *Amino Acids*, 37, 209-218 (2009)
47. X. Wang, W. Wu, G. Lin, D. Li, G. Wu and J. Wang: Temporal proteomic analysis reveals continuous impairment of intestinal development in neonatal piglets with intrauterine growth restriction. *J Proteome Res*, 9, 924-935 (2010)
48. G. Wu, F. W. Bazer and W. Tou: Developmental changes of free amino acid concentrations in fetal fluids of pigs. *J Nutr*, 125, 2859-2868 (1995)
49. G. Wu, D. A. Knabe, W. Yan and N. E. Flynn: Glutamine and glucose metabolism in enterocytes of the neonatal pig. *Am J Physiol Regulatory Integrative Comp Physiol*, 268, R334-342 (1995)
50. G. Wu: Functional amino acids in growth, reproduction and health. *Adv Nutr*, 1, 31-37 (2010)
51. X. Li, R. Rezaei, P. Li and G. Wu: Composition of amino acids in feed ingredients for animal diets. *Amino Acids*, 40, 1159-1168 (2011)
52. Y. Hou, L. Wang, B. Ding, Y. Liu, H. Zhu, J. Liu, Y. Li, P. Kang, Y. Yin and G. Wu: Alpha-ketoglutarate and intestinal function. *Front Biosci*, 16, 1186-1196 (2011)
53. P. Xi, Z. Jiang, C. Zheng, Y. Lin and G. Wu: Regulation of protein metabolism by glutamine: implications for nutrition and health. *Front Biosci*, 16, 578-597 (2011)
54. B. Tan, Y. Yin, X. Kong, P. Li, X. Li, H. Gao, X. Li, R. Huang and G. Wu: L-Arginine stimulates proliferation and prevents endotoxin-induced death of intestinal cells. *Amino Acids*, 38, 1227-1235 (2010)
55. Y. Hou, L. Wang, B. Ding, Y. Liu, H. Zhu, J. Liu, Y. Li, X. Wu, Y. Yin and G. Wu: Dietary -ketoglutarate supplementation ameliorates intestinal injury in

lipopolysaccharide-challenged piglets. *Amino Acids*, 39, 555-564 (2010)

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56. J. R. McKnight, M. C. Satterfield, W. S. Jobgen, S. B. Smith, T. E. Spencer, C. J. Meininger, C. J. McNeal and G. Wu: Beneficial effects of L-arginine on reducing obesity: Potential mechanisms and important implications for human health. *Amino Acids*, 39, 349-357 (2010)

57. G. Wu, F. W. Bazer, R. C. Burghardt, G. A. Johnson, S. W. Kim, D. A. Knabe, P. Li, X. Li, J. R. McKnight, M. C. Satterfield and T. E. Spencer: Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids*, 40, 1053-1063 (2011)

58. X. Wu, Z. Ruan, Y. Gao, Y. Yin, X. Zhou, L. Wang, M. Geng, Y. Hou and G. Wu: Dietary supplementation with L-arginine or N-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed a corn- and soybean meal-based diet. *Amino Acids*, 39, 831-839 (2010)

59. G. Wu, F. W. Bazer, G. A. Johnson, D. A. Knabe, R. C. Burghardt, T. E. Spencer, X. Li and J. Wang: Important roles for L-glutamine in swine nutrition and production. *J Anim Sci*, 89, 2017-2030 (2011)

60. F. Li, Y. Yin, B. Tan, X. Kong and G. Wu: Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino Acids*, 41, 1185-1193 (2011)

61. M. Geng, T. Li, X. Kong, X. Song, W. Chu, R. Huang, Y. Yin and G. Wu: Reduced expression of intestinal N-acetylglutamate synthase in suckling piglets: a novel molecular mechanism for arginine as a nutritionally essential amino acid for neonates. *Amino Acids*, 40, 1513-1522 (2011)

62. G. Wu, S. A. Meier, and D. A. Knabe: Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. *J Nutr*, 126, 2578-2584 (1996)

63. J. Wang, L. Chen, P. Li, X. Li, H. Zhou, F. Wang, D. Li, Y. Yin and G. Wu: Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. *J Nutr*, 138, 1025-1032 (2008)

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

Key Words: IUGR, Muscle, Fetal Pigs, Proteome, Review

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