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Altered tissue mineralization, increased hepatic lipid and inhibited autophagy in intrauterine growth retardation piglets

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Abstract

Mineral homeostasis is often disrupted in intrauterine growth retardation (IUGR) infants. Most studies focus on calcium or phosphorus metabolism of IUGR infants via determining serum mineral concentrations instead of tissues. This study was conducted to investigate the effects of IUGR on the mineralization and physiological functions of tissue in a piglet model. Six normal birth weight (NBW) and six IUGR neonatal piglets were slaughtered at 35 days. Mineral concentrations in blood and selected tissues (liver, kidney, lungs, heart, and *longissimus dorsi* muscle (LDM)), hepatic lipid, mRNA expressions of magnesium (Mg) metabolism, and autophagy were analysed. Results showed that IUGR pigs showed significantly lower phosphorus (P) in LDM, and lower Mg in the liver and LDM, and higher Mg in lungs than NBW pigs. There were no significant differences in concentrations of selenium (Se), calcium (Ca), copper (Cu), aluminium (Al), and lithium (Li) in selected tissues. IUGR pigs had similar mRNA expression of TRPM7 and MagT1 to NBW pigs, but significantly lower expressions of HNF1B and Mrs2 in the liver than NBW pigs. Hepatic triglyceride was significantly increased, and MAP1LC3B expression was significantly decreased in IUGR pigs compared with those of NBW pigs. These result suggested that IUGR pigs had tissue mineralization disturbance, especially for Mg, and liver dysfunction (increased hepatic lipid and inhibited autophagy). Hepatic Mg deficiency might result from increased Mg efflux via reducing HNF1B expression.

Keywords: Hepatic triglyceride, magnesium metabolism, mineral homeostasis, physiological function
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Introduction

Intrauterine growth restriction (IUGR) refers to restricted growth and inhibited development of the embryo/foetus or impaired organs during pregnancy. Commonly, foetal weight less than 10th percentile of a given population at the same gestational age is regarded as IUGR (Wu *et al*., 2006; Roman *et al*., 2013). IUGR is a severe problem in livestock. For example, in the swine industry IUGR affects more than 15% of new-born piglets (Wu *et al*., 2006). IUGR is associated with neonatal death and metabolic dysfunctions, such as mineral and lipid metabolic dysfunctions (Blaga *et al*., 2008; Li *et al*., 2016). Epidemiological studies indicate that IUGR exhibits side effects on postnatal growth, health and lipid metabolism for a long time, and is closely linked to hypertension, insulin resistance, and obesity in adult (Vickers, 2014).

Minerals are commonly divided into major minerals, for example Ca, Mg and P, and trace or micro-minerals for example iron (Fe) and Cu. They are involved in various enzyme activities and nutrient metabolism, which have been summarized by Gharibzahedi & Jafari (2017). Therefore, small amounts of minerals play an important role in livestock and the human body. Complicated processes and hormones, such as the parathyroid hormone, calcitriol, and sex steroids, to keep mineral homeostasis (Kovacs, 2014), regulate the mineral metabolism. However, IUGR is closely associated with disturbance in mineral homeostasis. IUGR infants fed human milk or commercial formulas show severely disturbed mineral homeostasis (Schanler *et al*., 1985; Schanler & Garza, 1988; Mataloun & Leone, 2000). Most of these studies focus on bone development and calcium or phosphorus metabolism via determining mineral concentrations in serum or bone (Prestridge *et al*., 1993; Li *et al*., 2016). However, essential minerals are abundant in tissues, in which they are critical for electrolyte balance and nutrient metabolism through being

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involved in the activities of enzymes (Moltu *et al*., 2013; Gupta *et al*., 2014; Gharibzahedi & Jafari, 2017). For example, Mg-deficient foetal livers of IUGR mice are accompanied by low monounsaturated fatty acids (MUFAs), high polyunsaturated fatty acids (PUFAs), and low desaturase and elongase mRNA expression (Gupta *et al*., 2014). Therefore, it seems necessary to determine the mineral contents and their metabolism in tissues. However, few studies have investigated the effects of IUGR on tissue mineralization.

It was hypothesized that IUGR might impair tissue mineralization and affect the physiological functions of tissues with mineral disturbance. To test this hypothesis, a pig model of IUGR was used, because pigs are the optimal model for human (Darragh & Moughan, 1995; Kues & Niemann, 2004; Pearce *et al*., 2007). After the liver mineral disturbance (especially for Mg) was found, Mg metabolism-related gene expressions, hepatic lipid, and autophagy were analysed. This study could provide additional information for neonatal nutrition research, especially for the IUGR infants.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University, China. At the time of parturition (114 days gestation), six normal birth weight (NBW) (1.54 ± 0.01kg) and six IUGR (0.93 ± 0.02kg) Duroc × Landrace × Large White piglets were chosen from six similar birth order (third and fourth) sows with the same litter size (each litter 10 piglets). Each IUGR piglet and one same sex sibling of NBW piglets were chosen, as discussed in previous reports (Xu *et al*., 1994; Wu *et al*., 2006). Piglets stayed with their own mothers until 14 days. After weaning, piglets were fed individually with water and feed *ad libitum*. The feed was formulated to meet or exceed the nutritional requirements of NRC (1998), as shown in Table 1.

Table 1 Composition of diet (as-fed basis) for weaning piglets

Ingredients (g / 100 g)		**Nutrient composition (%)**	
Corn	40.00	Crude protein	20.20
Rice, broken	15.00	Digestible energy (Mcal/kg)	3.40
Soybean meal, fermented	10.00	Total calcium	0.85
Soybean meal, de-hulled	6.00	Total phosphorus	0.70
Spray dried animal plasma	5.00	Digestible lysine	1.45
Whey powder	7.00	Digestible methionine +cystine	0.79
Fish meal	4.00	Digestible threonine	0.81
Sugar	4.50	Digestible tryptophan	0.23
Glucose	3.00	Digestible isoleucine	0.74
Soybean oil	1.50	Digestible leucine	1.45
L-Lysine-HCl	0.30	Digestible valine	0.89
L-Methionine	0.15		
L-Threonine	0.20		
L-Tryptophan	0.05		
L-Isoleucine	0.05		
L-Valine	0.05		
Salt	0.30		
Limestone	1.10		
Dicalcium phosphate	0.80		
Vitamin mixture[a]	0.20		
Mineral mixture[b]	0.80		
Total	100.00		

[a]Vitamin mixture supplied per kg complete diet: vitamin A, 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 150 mg; vitamin K_3, 3.00 mg; vitamin B_1, 3.00 mg; vitamin B_2, 6.00 mg; vitamin B_6, 5.00 mg; vitamin B_{12}, 0.03 mg; niacin, 45.00 mg; vitamin C, 250 mg; calcium pantothenate, 9.00 mg; folic acid, 1.00 mg; biotin, 0.30 mg; choline chloride, 500 mg. [b]Mineral mixture supplied per kg complete diet: Fe, 170 mg; Cu, 150 mg; I, 0.90 mg; Se,0.20 mg; Zn, 150 mg; Mg, 68 mg; Mn, 80 mg; Co, 0.30 mg

At 0, 14, and 35 days, bodyweight and average daily feed intake (ADFI) (14–35 days) were recorded. ADG and feed/gain ratio (F : G) were calculated. At the end of this experiment (35 days), whole blood was collected with heparinized tubes two hours after their last meal. All pigs were slaughtered via electrical stunning. Samples to determine mineral concentrations and mRNA expression were collected as described by Wang *et al*. (2016). Briefly, lungs, heart, kidney, liver, and *longissimus dorsi* muscle (LDM) were collected and stored at -20 °C to determine mineral concentration. Liver samples were frozen quickly and stored at -80 °C to determine mRNA expression and lipid metabolism.

The mineral concentrations (Se, P, Fe, chromium (Cr), Mg, Ca, Cu, Al, and Li) were determined as described by Demirbaş (1999). Briefly, tissues (2–3 g) and blood (3–3.5 ml) were digested using a mixture of HNO3 : HClO4 (v/v = 4 : 1). After cooling, the digest was dissolved with demineralized water to 25 ml. Subsequently, it was diluted to the optimal concentrations. Mineral concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent, USA).

Total RNA of the liver was extracted with Trizol reagents, and reverse transcription was conducted as described by Dong *et al*. (2014). Briefly, after the RNA quality had been verified by a Thermo Fisher Scientific Nanodrop 2000 spectrophotometer (ratios of absorption including 260/280 nm and 260/230 nm between 1.90 and 2.05) and by agarose gel electrophoresis, 2 μg RNA were incubated at 72 °C with random primer (Promega, Belgium) for 5 min, then incubated for 1 hour with reverse transcription mixture (Takara, Dalian, China). Finally, the reverse transcription was inactivated at 90 °C for 10 min.

In this study, Mg metabolism-related gene expressions of TRPM7, MagT1, Mrs2, HNF1B, and autophagy-related gene expressions of MAP1LC3A, MAP1LC3B and Atg5 in the liver were determined. The related gene primers are listed in Table 2 and were synthesized in Invitrogen (Shanghai) Biotech Co. Ltd. (Shanghai, China). GAPDH was used as a housekeeping gene. Reverse transcription polymerase chain reaction (RT-PCR) assays were conducted with the ABI 7300 RT-PCR system with a SYBR Premix Ex TaqTM Kit (TakaRa, Dalian, China) according to the manufacturer's instructions. The mRNA expressions were examined with ABI software and calculated with the 2-△△Ct, as previous reports (Livak & Schmittgen, 2001).

Table 2 Primer sequences used in quantitative real-time polymerase chain reaction assays

Genes	Accession No.	Primers	Sequences(5'--3')	bp
GAPDH	NM_001206359.1	Forward	CATTGCCCTCAACGACCACT	84
		Reverse	ATGAGGTCCACCACCCTGTT	
TRPM7	XM_003121515.2	Forward	CCCGATAGATGGCTACAGGC	85
		Reverse	CTGGGACATTCTCCTCACGG	
MagT1	XM_003135205.4	Forward	GCCTGTTTTTGTTACGCCCC	77
		Reverse	TGGCCTGAGGCAAGTACAAG	
HNF1B	NM_213956.1	Forward	CGACAAACCACGGAAGAGGA	157
		Reverse	GGTGGCTGATGTTTACAGTGTG	
Mrs2	XM_001928036	Forward	GGCGTTTGCTGTCATTCCTC	126
		Reverse	CATCCGGTCTGAAGCTGTGT	
MAP1LC3A	NM_001170827.1	Forward	GTCTACGCCTCCCAGGAAAC	127
		Reverse	CAGGGGCAGAGACAGCTTAG	
MAP1LC3B	NM_001190290.1	Forward	CCACGTCCATCCCAGTGTAT	200
		Reverse	GGTTCCTGTTGAGCAGTGGT	
Atg5	NM_001037152	Forward	GACCTTCTGCACTGTCCATCA	181
		Reverse	TCCGGTTGATGGTCCAAAACT	

Hepatic lipid content was analysed as described by Ahn *et al*. (2008). Briefly, portions of liver samples from each pig were weighed and homogenized in Tris-HCl solution with a glass Dounce homogenizer on ice. The TG and CHO contents in the liver were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

All the data were processed with the student t-test of the SPSS statistical package (Version 20.0, SPSS Inc., Chicago, IL) as described by Dong *et al*. (2014).Results were shown as mean ± SE. P value below 0.05 was regarded as significant.

Results

In this study, the bodyweights of IUGR pigs were lower than NBW pigs at 0, 14 and 35 days (P < 0.01). Compared with the NBW pigs, IUGR pigs exhibited significantly lower ADGs (0–14 days and 14–35 days), less ADFI (14–35 days), and higher F: G (14–35 days), as shown in Table 3.

Table 3 Effects of intrauterine growth retardation on growth performance in pigs

Items	NBW	IUGR
Bodyweight (kg)		
0 days	1.54 ± 0.01^{A}	0.93 ± 0.02^{B}
14 days	5.38 ± 0.06^{A}	3.45 ± 0.05^{B}
35 days	9.95 ± 0.40^{A}	6.03 ± 0.23^{B}
ADG (kg/d)		
0–14 days	0.27 ± 0.01^{A}	0.18 ± 0.01^{B}
14–35 days	0.20 ± 0.02^{A}	0.12 ± 0.01^{B}
ADFI (kg/d, 14–35d)	0.28 ± 0.02^{a}	0.22 ± 0.01^{b}
F:G (14–35d)	1.40 ± 0.06^{A}	1.82 ± 0.11^{B}

Data were expressed as means ± SE (n = 6); a–b (*P* <0.05) or A–B (*P* <0.01) with different superscripts between values for NBW and IUGR pigs mean significant differences
NBW: normal birth weight; IUGR: intrauterine growth retardation; ADG: average daily gain; ADFI: average daily feed intake; F : G: feed/gain ratio

The effects of IUGR on the mineral concentrations of the liver, LDM, heart, lungs and kidney are shown in Table 4. There were no significant differences in the Se, Ca, Cu, Al, and Li levels of these tissues between IUGR and NBW pigs (*P* >0.05). However, IUGR pigs had lower Cr (*P* <0.05) and Mg (*P* <0.01) in the liver and LDM, and higher Mg in the lungs (*P* <0.01) than NBW pigs. Lower P concentration in the LDM (*P* <0.05) and Fe concentration in the lungs (*P* <0.05) were found in IUGR pigs than in those of NBW pigs.

Table 4 Effect of intrauterine growth retardation on mineral concentrations of selected organs in pigs

Items	Liver		LDM		Heart		Lung		Kidney	
	NBW	IUGR	NBW	IUGR	NBW	IUGR	NBW	IUGR	NBW	IUGR
Se[1]	0.023 ± 0.002	0.025 ± 0.002	0.018 ± 0.007	0.009 ± 0.002	0.017 ± 0.002	0.018 ± 0.002	0.019 ± 0.003	0.023 ± 0.003	0.063 ± 0.003	0.069 ± 0.007
P[2]	357.02 ± 5.68	367.35 ± 6.79	350.77 ± 5.38a	324.48 ± 7.20b	245.19 ± 4.56	244.72 ± 4.84	294.31 ± 17.06	321.81 ± 8.76	326.47 ± 7.88	327.55 ± 7.71
Fe[1]	25.12 ± 3.12	50.07 ± 7.99	48.88 ± 2.93	43.57 ± 3.32	11.41 ± 0.51	9.79 ± 0.70	91.81 ± 5.51	21.93 ± 8.76**	90.74 ± 7.78	102.84 ± 18.78
Cr[1]	24.50 ± 1.05	21.52 ± 0.79*	15.83 ± 1.45	11.96 ± 1.12	51.05 ± 4.60	43.26 ± 0.71	26.65 ± 1.30	31.49 ± 2.65	25.18 ± 2.72	20.71 ± 0.84
Mg[2]	15.63 ± 0.30A	14.47 ± 0.25B	18.55 ± 0.50A	16.45 ± 0.34B	21.49 ± 0.41	22.12 ± 0.43	14.16 ± 0.63A	16.70 ± 0.56B	16.10 ± 0.34	16.35 ± 0.41
Ca[2]	3.50 ± 0.29	3.65 ± 0.14	2.62 ± 0.14	2.61 ± 0.16	4.29 ± 0.12	4.88 ± 0.29	7.12 ± 0.42	7.58 ± 0.29	5.61 ± 0.29	5.54 ± 0.19
Cu[2]	1.25 ± 0.11	1.40 ± 0.24	0.063 ± 0.001	0.065 ± 0.003	0.319 ± 0.012	0.315 ± 0.017	0.078 ± 0.001	0.088 ± 0.001	5.76 ± 0.28	5.99 ± 0.93
Al[1]	208.20 ± 30.29	172.00 ± 9.03	304.22 ± 47.73	349.75 ± 47.17	173.37 ± 79.95	144.55 ± 14.24	219.29 ± 27.16	213.46 ± 31.04	204.22 ± 10.60	230.16 ± 33.15
Li[1]	4.22 ± 0.06	4.39 ± 0.17	0.63 ± 0.06	0.65 ± 0.07	3.45 ± 0.15	3.74 ± 0.09	1.68 ± 0.14	1.84 ± 0.13	1.74 ± 0.10	2.04 ± 0.01

Data were expressed as means ± SE (n = 6); a–b (P <0.05) or A–B (P <0.01) with different superscripts between values for NBW and IUGR pigs means significant differences; 1μg/100g wet tissue weight; 2mg/100g wet tissue weight
NBW: normal birth weight; IUGR: intrauterine growth retardation; LDM: *longissimus dorsi* muscle

There were no significant differences in concentrations of Se, P, Fe, Mg, Ca, Cu, Al and Li in blood between IUGR and NBW pigs (P >0.05) as stated in Table 5. However, IUGR pigs had lower blood Cr level compared with NBW pigs (P <0.05).

Table 5 Effects of intrauterine growth retardation on the mineral concentration of blood in pigs

Items	NBW	IUGR
Se (μg/L)	0.06 ± 0.02	0.06 ± 0.02
P (mg/L)	606.89 ± 14.53	600.18 ± 44.56
Fe (μg/L)	549.32 ± 60.29	696.29 ± 47.70
Cr (μg/L)	458.22 ± 35.62[a]	328.11 ± 41.41[b]
Mg (mg/L)	61.84 ± 1.23	61.37 ± 3.09
Ca (mg/L)	67.97 ± 1.25	69.26 ± 1.98
Cu (mg/L)	1.54 ± 0.19	1.25 ± 0.10
Al (μg/L)	499.23 ± 21.43	483.71 ± 31.00
Li (μg/L)	36.15 ± 0.67	34.95 ± 1.67

Data are expressed as means ± SE (n =6)
[a-b] (P <0.05) different superscripts between values for NBW and IUGR pigs mean significant differences
NBW: normal birth weight; IUGR: intrauterine growth retardation

There were no differences in relative mRNA levels of TRPM7 and MagT1 between the two groups (P >0.05) (Figure 1). However, the relative mRNA levels of Mrs2 and HNF1B were significantly reduced in IUGR pigs compared with NBW pigs (P <0.05).

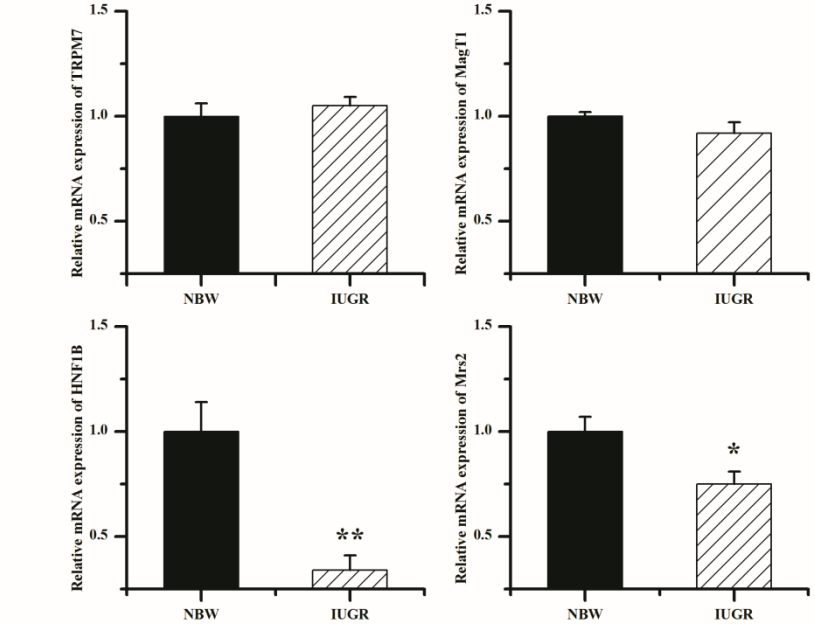

Figure 1 mRNA abundance of magnesium metabolism-related genes in liver of intrauterine growth retardation and normal birth weight pigs
Data expressed relative to the housekeeping gene GAPDH, normalized to the NBW group, and represent means ± SE (n = 6). *P <0.05, **P <0.01
NBW: normal birth weight; IUGR: intrauterine growth retardation

IUGR pigs had a significantly higher hepatic TG level relative to the NBW pigs (P =0.01), while hepatic CHO levels in IUGR and NBW pigs were similar (P >0.05), as shown in Figure 2. The relative mRNA expressions of MAP1LC3B and MAP1LC3B : MAP1LC3A ratio were increased in IUGR pigs compared with NBW pigs (P <0.05), as shown in Figure 3. There were no significant differences in relative mRNA expressions of MAP1LC3A and ATG5 in the liver between IUGR and NBW pigs (P >0.05).

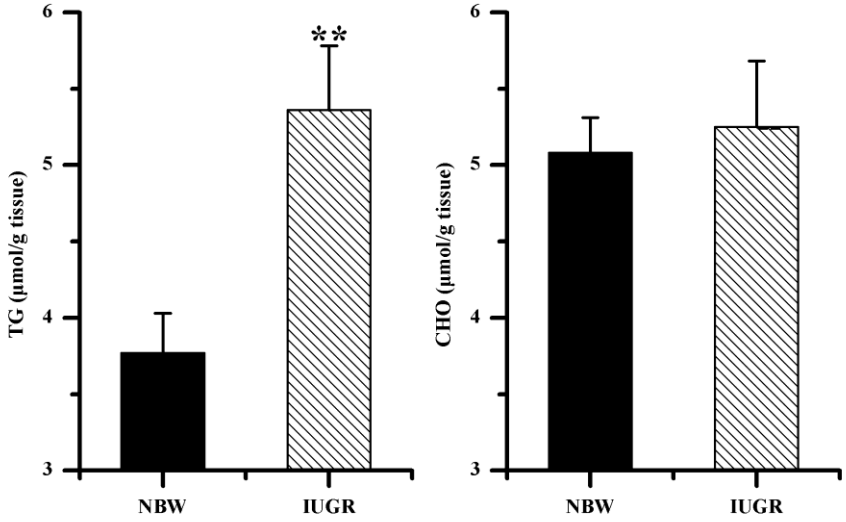

Figure 2 Hepatic lipid in intrauterine growth retardation and normal birth weight pigs
Data represent means ± SE (n = 6); **P <0.01
NBW: normal birth weight; IUGR: intrauterine growth retardation; TG: triglyceride; CHO: cholesterol

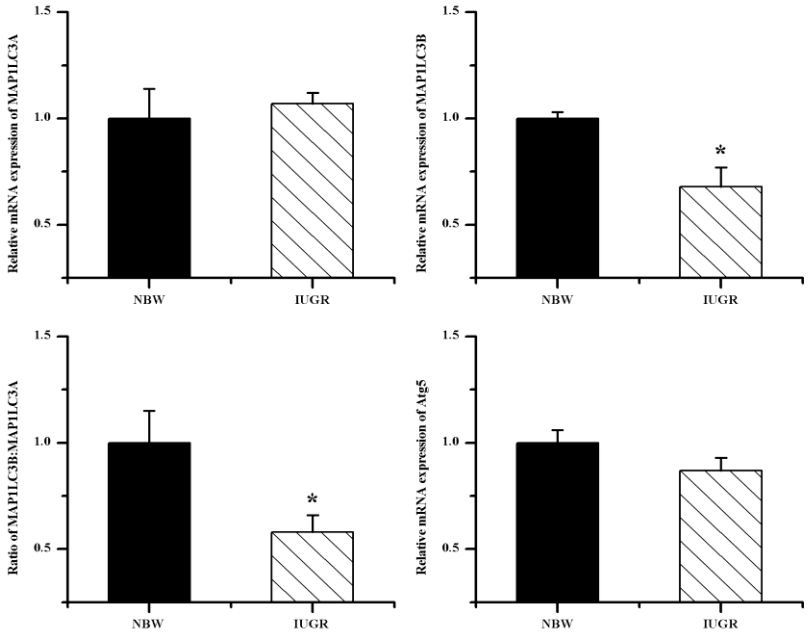

Figure 3 mRNA abundance of autophagy related genes in liver of intrauterine growth retardation and normal birth weight pigs
Data expressed relative to the housekeeping gene GAPDH, normalized to the NBW group and represent means ± SE (n = 6); *P <0.05
NBW: normal birth weight; IUGR: intrauterine growth retardation

Discussion

Most essential minerals, such as Mg, as components of enzymes, are involved in major metabolic pathways in tissues. IUGR neonates are more susceptible to serum mineral disturbance and need long-term mineral supplement as they were fed on their own mother's milk (Raupp *et al*., 1990; Blaga *et al*., 2008). It was further hypothesized that IUGR might impair tissue mineralization and physiological functions of tissues.

The results of this study indicated that IUGR pigs had decreased bodyweight, lower ADGs, reduced ADFI (14–35 days), and higher F : G (14–35 days) than NBW pigs. These findings coincide with those of previous studies (Poore & Fowden, 2004; Attig *et al*., 2008; Zhang *et al*., 2014; Li *et al*., 2015; Zhang *et al*., 2016).

Many factors can lead to the lower ADGs of IUGR and high nutrient density is encouraged for IUGR infants to alter the long-term adverse effects on restricted growth, such as high protein and energy (Desai *et al*., 2005; Desai *et al*., 2007; Senterre & Rigo, 2011; Senterre & Rigo, 2012). However, the safety of early nutrient enhancement of IUGR needs to be further evaluated, as it may cause adult obesity (Desai *et al*., 2005; Desai *et al*., 2007) and more severe mineral disturbance (Moltu *et al*., 2013).

It was reported that IUGR infants had reduced serum P concentration, and showed hypophosphatemia (Moltu *et al*., 2013) and that bone mineralization was affected (Abrams *et al*., 1988; Li *et al*., 2016). In the present study, IUGR pigs had lower P level in the LDM than NBW pigs. However, there was no difference in blood P content between IUGR and NBW pigs. The time of collecting blood may partially account for the lack of change in blood P level, as the blood samples were collected two hours after the last meal to evaluate the absorption of minerals.

Furthermore, the current results showed that IUGR and NBW pigs had similar Mg concentrations in the blood, heart, and kidneys. Moltu *et al*. (2013) reported that enhanced feeding in IUGR infants might cause electrolyte disturbances without affecting the serum Mg level. Dauncey *et al*. (1977) suggested that breast milk supplies enough Mg for IUGR infants by determining the serum Mg. However, IUGR pigs showed lower Mg concentrations in the liver and LDM, and higher Mg concentrations in the lungs than NBW pigs in the present study, suggesting that Mg metabolism was disturbed.

Since the liver is an important organ that distributes Mg in tissues, the expression of Mg metabolism-related genes in the liver was further determined. The results indicated that IUGR and NBW pigs had similar relative mRNA expression of TRPM7 and MagT1. TRPM7 is ubiquitous and emphasizes the role in control of Mg influx in individual cells (Romani, 2011). MagT1 is highly expressed in the liver and appears to be highly specific for Mg ion influx (Sontia & Touyz, 2007; Romani, 2011). No differences in relative mRNA expression of TRPM7 and MagT1 suggested that IUGR may not affect the influx of Mg in the liver. However, the expression of HNF1B and Mrs2 were decreased in the liver of IUGR pigs. The HNF1B was first identified in the liver (Cereghini *et al*., 1988), could bind to the FXYD2 gene, encode the γ-subunit of the Na/K-ATPase, and is an important molecular player in the renal Mg reabsorption (Ferre *et al*., 2011). The lack of Mg is often associated with HNF1B deficiency (Adalat *et al*., 2009; Heidet *et al*., 2010), while dietary Mg restriction could enhance HNF1B expression (Van Angelen *et al*., 2013). Reduced relative mRNA expression of HNF1B may increase the efflux of Mg and decrease the Mg content in the liver of IUGR pigs. The Mrs2 plays an essential role in regulating mitochondrial Mg homeostasis (Romani, 2011). A deficiency in Mrs2 leads to a decrease in total mitochondrial Mg level, a decrease in matrix-free Mg ion concentration, and the dysfunction of mitochondria respiration (Martin *et al*., 2003). In contrast, over-expression of Mrs2 markedly increased Mg content in mitochondria (Martin *et al*., 2003). Decreased expression of Mrs2 indicated Mg influx in mitochondria was inhibited and the dysfunction of liver mitochondria of IUGR pigs (Zhang *et al*., 2016; Zhang *et al*., 2017), although the mitochondria function was not determined here.

Mg is one of the most abundant intracellular cations and is involved in over 300 enzyme activities, which play important roles in many functions of tissues (Swaminathan, 2003; Das, 2016). Recently, Mg deficiency was linked closely with incidence of IUGR, growth performance and hepatic lipid metabolic disorder in the rat model (Roman *et al*., 2013; Roman *et al*., 2015). Since the hepatic Mg deficiency of IUGR pigs was proved in the present study, the authors determined the hepatic lipid content and autophagy, which is critical in hepatic lipid metabolism (Liu & Czaja, 2013). In the present study, hepatic TG was increased in IUGR pigs, which was partly in agreement with previous reports (Gupta *et al*., 2014; He *et al*., 2015). The mRNA of MAP1LC3B was synthesized before the MAP1LC3 protein, which is a common indicator of autophagy. Determining the MAP1LC3B mRNA expression is regarded as a convenient method to monitor the autophagosome formation (Tsuyuki *et al*., 2014). In this study, the relative mRNA expressions of MAP1LC3B and MAP1LC3B : MAP1LC3A ratios were decreased, suggesting that the autophagy was inhibited in the liver of IUGR pigs. Inhibited hepatic autophagy could reduce lipolysis, lead to lipid droplet accumulation and elevate hepatic TG content (Singh *et al*., 2009a; Singh *et al*., 2009b; Liu & Czaja, 2013). These results suggested that reduced hepatic autophagy might be related to increased hepatic lipids. However, in future, more in-depth studies are required to investigate the possible pathways behind reduced

hepatic autophagy. Controlling hepatic autophagy may be a novel way to regulate hepatic lipids in IUGR pigs.

Conclusions

In summary, the current results revealed that IUGR still showed reduced growth performance at the age of 35 days, with some disturbances in tissue mineralization, especially for Mg. The possible mechanism for hepatic Mg deficiency might be attributed to the increased Mg efflux through reducing HNF1B expression. Hepatic Mg deficiency might be related to increased hepatic autophagy and lipid contents. This study will help researchers to uncover the critical areas of tissue mineral disturbances and their related biological functions in IUGR pigs that have not been explored before. However, further studies are required to investigate possible ways to regulate hepatic Mg metabolism and autophagy in IUGR nursery pigs.

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Authors' contributions

CW and TW conceived and designed the experiments CW, XCZ, FAS, KC, WX and JTH performed the experiments. CW analysed the data. CW and LLZ contributed reagents, materials and analysis tools. CW and XZ wrote the paper. DB and TA edited it.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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