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Research Article

Intrauterine Growth Retardation Enhances Intestinal Autophagy and Proliferation in Rat Pups Responding to Colostrum

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Abstract

This study aimed to investigate responsible mechanisms for rapid intestinal catch-up growth in intrauterine growth restriction (IUGR) pups via analysis of autophagy, apoptosis and proliferation in a rat model. Twenty primiparous dams were assigned into two groups as 1) dams with feed ad libitum (Adlib) and 2) dams with 50% feed restriction from gestational day 10 to 21 to achieve normal birth weight (NBW) and IUGR pups, respectively. Litter size and pup weight were recorded at parturition and 8 pups were kept in each litter to have sufficient colostrum for 24 h. Subsequently, 2 pups from each litter were decapitated. Results indicated that feed restriction dams had similar litter size with rats in Adlib group although produced IUGR pups. Histological analysis indicated that IUGR rats had decreased villus height and surface area in jejunum. There was an accumulation of autophagosomes in jejunal mucosa of IUGR pups, however, the mitochondria and microvilli were unaffected. mRNA expressions of *WIPI1*, *MAP1LC3B, Atg13, ULK1* and *Beclin1* were increased, and *mTOR* expression was decreased in jejunum of IUGR, which also had lower *Bcl-2* mRNA expression, increased *caspase* 9 and relative increased *ki67* mRNA expression. Results suggested that after feeding colostrum, IUGR pups had impaired jejunum with unaffected mitochondrial histology. Enhanced intestinal autophagy under low-stress conditions might improve intestinal proliferation, which may be contributed to the rapid intestinal catch-up growth.

Keywords: *Autophagy, Colostrum, Intrauterine growth restriction, Intestinal proliferation, Apoptosis*

Kolostruma Cevap Veren Rat Yavrularında İntrauterin Büyüme Geriliği İntestinal Otofaji ve Proliferayonu Artırır

Özet

Bu çalışmanın amacı, rat modeli üzerinde otofaji, apoptozis ve proliferasyonu incelemek suretiyle intrauterin büyümesi kısıtlanan (IUGR) yavrularda hızlı intestinal büyümeden sorumlu mekanizmaları araştırmaktır. Yirmi adet bir doğum yapmış anneler iki gruba ayrıldı; 1) ad libitum beslenen (Adlib) anneler ve 2) Gestasyonun 10 ile 21. günü arasında yemi %50 kısıtlanan anneler. Böylece normal doğum ağırlığı (NBW) olan yavrular ve IUGR yavrular elde edildi. Doğumda yavru sayısı ve ağırlıkları kaydedildi ve her batında 8 yavru 24 saat süresince yeterli kolostrum alması için annesi ile birlikte tutuldu. Sonrasında, her batından 2 yavruya dekapitasyon uygulandı. Elde edilen sonuçlar yemi kısıtlanan annelerin yavru sayıları ile Adlib grubun yavru sayılarının benzer olduğunu ancak IUGR yavrular ürettiğini gösterdi. Histolojik incelemede IUGR ratların jejunumda azalmış villus yüksekliğine ve yüzey alanına sahip olduğu belirlendi. IUGR yavruların jejunum mukozasında otofagozomların oluştuğu ancak mitokondri ve mikrovillusların etkilenmediği gözlemlendi. IUGR yavruların jejunumlarında *WIPI1*, *MAP1LC3B, Atg13, ULK1* ve *Beclin1* mRNA ekspresyonlarının arttığı ve *mTOR* ekspresyonunun azaldığı belirlendi. Bu hayvanlarda daha düşük *Bcl-2* mRNA ekspresyonu, artmış *caspase* 9 ve orantısal olarak artmış *ki67* mRNA ekspresyonu tespit edildi. Elde edilen sonuçlar kolostrum ile besleme sonrası IUGR yavrularda histolojik olarak mitokondrilerde bir değişim olmaksızın jejunumda hasarın oluştuğunu gösterdi. Düşük stres altında gelişmiş intestinal otofaji, intestinal proliferasyonu iyileştirebilir ve bu durum hızlı intestinal büyümeye katkı sağlayabilir.

Anahtar sözcükler: *Anahtar sözcükler: Otofaji, Kolostrum, İntrauterin büyüme kısıtlaması, İntestinal proliferasyon, Apoptozis*

INTRODUCTION

Intrauterine growth restriction (IUGR) refers to the birth weight <10th percentile in a given population, which is a common and severe problem in both humans and livestock [1,2]. In America, IUGR affects more than 8% of newborns and is intimately related to metabolic disorder in adults, such as increased risk of obesity and hypertension [3,4].

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In swine industry, between 15 and 20% of neonatal piglets are affected by the growth restriction [1]. Maternal nutrition is a critical factor, which plays important roles in fetal development since both the composition of nutrients and biologically active substances through maternal placenta could affect fetal growth and are closely associated with IUGR occurrence, such as glucose and amino acids [1,5]. Therefore, the maternal feed restriction has commonly been used to construct IUGR animal models. For example, Alexandre-Gouabau et al.[6] used maternal protein restriction from the day of conception to obtain IUGR rat offspring and investigate their metabolomic responses. Gupta et al.[7] reported that maternal magnesium deficiency could alter maternal metabolism and leads fetal growth restriction.

IUGR is responsible for the relatively high rates of morbidity and mortality in neonates or fetuses, which may lead to various organ dysfunctions, and affect immune and metabolic systems [4,8]. The small intestine plays critical roles in immunity, nutrient digestion and absorption. Previous studies have documented that IUGR inhibits small intestinal development, impairs the intestinal integrity, and changes bacterial colonization [9-11]. Similarly, D'Inca et al.[12] found IUGR altered the intestinal structure of piglets with a longer and thinner small intestine and reduced villus size, while the preterm IUGR piglets provided with sufficient colostrum showed rapid intestinal catch-up growth during post-parturition period. However, the underlying mechanism of intestinal catch-up growth is still largely unclear.

Autophagy is an important process involved in cytoplasmic component degradation by the lysosome, such as damaged organelles and long-lived proteins [13]. Recently, emerging evidences have verified that autophagy is responsible for multiple biological functions, such as proliferation and apoptosis [14,15]. It has been documented that the development of early brain injury could be prevented by initiating autophagy under low-stress conditions [16,17]. The homeostasis roles of autophagy, as "remodeling" and "refreshment" functions, are critical for the differentiated cells, which has been well investigated in the neurons [13,18]. Based on these findings, we hypothesized that the intestinal catch-up growth of IUGR in response to colostrum during post-parturition period might be concerned with the intestinal autophagy. To test this hypothesis, a rat model of IUGR with catch-up growth was constructed by maternal 50% feed restriction as reported by Anderson et al.[19] and Desai et al.[20]. The intestinal structure, autophagy, apoptosis and proliferation were determined in this study. These results might be helpful to understand the responsible mechanism for intestinal catch-up growth of IUGR neonates in response to colostrum.

MATERIAL and METHODS

Ethical Procedures

Experiments were conducted under the supervision of the Institutional Animal Care and Use Committee of Nanjing Agricultural University, China.

Animal and Experimental Design

IUGR rats were obtained from the rat model of IUGR as described by Anderson et al.[19] and Desai et al.[20]. Briefly, 20 primiparous Sprague Dawley rat dams, purchased from the experimental animal center of Soochow University (Jiangsu, China), were housed in the facilities with the relatively constant temperature of 20±2°C and a light-darkness cycle (12h:12h). From the gestational day 10 to 21, 20 dams were randomly assigned into two groups, which were provided with a commercial diet (crud protein: 20.19%; metabolic energy: 2.90 Mcal/kg) *ad libitum* (Adlib) and 50% feed restriction (FR) to achieve normal birth weight (NBW) and IUGR rat pups, respectively (n=10). The pups were weighed and litter size was recorded immediately following parturition. Eight pups were then kept in each litter to make sure that pups could have sufficient colostrum for 24 h. Subsequently, 2 pups (fast for 2 h) from each litter size were chosen and decapitated. The tail-vein blood glucose was analyzed with a glucometer (Bayer HealthCare, USA). The organs were weighed, including heart, liver, spleen, kidney, brain, and lung.

Analysis of Intestinal Histology

For the intestinal morphological evaluation, 1.5 cm-long jejunum samples (about 5 cm distance from pyloric sphincter) were obtained and analyzed as described by Dong et al.[8]. Briefly, the intestinal samples were fixed in 4% paraformaldehyde solution, dehydrated with graded series of ethanol and embedded in paraffin. The intestinal cross sections with 4 μm thickness were cut and stained with hematoxylin and eosin. Images of each section were obtained with an optical microscope (Olympus BX5, Olympus Optical Co. Ltd., Japan). Intestinal villus height, crypt depth and villus width were analyzed with an Image-Pro Plus 6.0 software. The villus height to crypt depth ratio (villi/crypt ratio) and villus surface area were calculated.

For the histological examinations with transmission electron microscope (TEM), 0.2 cm-long jejunum samples were fixed in 2.5% glutaraldehyde solution at 4°C for at least 48 h, post-fixed in 1% osmium tetroxide for 2h, dehydrated with series of ethanol, and finally embedded in EPOK. The TEM (Hitachi H-7500, Japan) was operated at 80 kV to observe the 70 nm thin sections, which were stained with uranyl acetate and Sato's lead staining solution [8].

Analysis of mRNA Expression

The analysis of mRNA expression in the jejunum of rat pups was conducted as described by Dong et al.[8] and Bustin et al.[21]. Briefly, approximate 8 cm jejunum samples (about 6.5 cm distance from pyloric sphincter) were quickly collected on ice, immediately frozen in liquid nitrogen and stored at -80°C. The total RNA was extracted with Trizol-based

procedure as recommended by the company (Invitrogen, USA). RNA quality and integrity was verified by agarose gel electrophoresis and by the determination of the absorption ratios of 260/280 nm and 260/230 nm (between 1.90 and 2.05) with Nano-drop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Subsequently, 1 µg RNA was used to obtain the cDNA with the Primer-Script™ reagent kit, which was purchased from TakaRa Biotechnology Co. Ltd. (Dalian, China).

The reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) assay was performed on an ABI 7500 instrument (Applied Biosystems, Foster City, CA) using a SYBR Premix Ex Taq™ Kit (TakaRa Biotechnology Co. Ltd., Dalian, China) and specific primer sequences for the target genes, including *WIPI1, MAP1LC3B, Atg5, Atg14, Atg13, ULK1, mTOR, Beclin1, Bcl-2, Bax, caspase3, caspase9* and *ki67*. The primers for genes mentioned above were prepared by Invitrogen Biotech Co. Ltd. (Shanghai, China) and are listed in *Table 1*. After the primer specificity was checked with the tool of BLAST, the specificity was confirmed again by the experimental evidences (melting profile and electrophoresis gel). In addition, the efficiency of amplification was about 100% (95%-105%). The threshold values were determined with the ABI software and mRNA expressions were normalized to the reference-gene expression (*GAPDH*), which was stably expressed in rat jejunum as confirmed in present experiment. The relative mRNA expression was calculated with the $2^{-\Delta\Delta Ct}$ as described previously [22] and normorlized to the NBW pups.

Statistical Analysis

Data were analyzed by the student *t*-test of SPSS statistical package for Windows (IBM SPSS, version 20.0, Chicago) and expressed as mean ± standard error (SE). The litter was used as the experimental unit for the analysis of pup's body weight and litter size. For the other parameters, the individual rat pup was used as an experimental unit. The *P* value below 0.05 was considered as statistically significant level, and between 0.05 and 0.10 was considered as a tendency towards statistical difference.

Table 1. *Primers sequences used for real time PCR assays*

Genes	Accession No.	Primers	Sequences(5'--3')	bp
GAPDH	NM_017008.4	Forward	CAGGGCTGCCTTCTCTTGTG	170
		Reverse	TGGTGATGGGTTTCCCGTTG	
WIPI1	NM_001127297.1	Forward	CCAAGACTGCACATCCCTAGC	162
		Reverse	TGACTGACCACCACAACCAG	
MAP1LC3B	NM_022867.2	Forward	TCCTGAACCCCAGCCATTTC	141
		Reverse	GGCATGGACCAGAGAAGTCC	
Atg5	NM_001014250.1	Forward	CAGAAGCTGTTCCGTCCTGT	128
		Reverse	CCGTGAATCATCACCTGGCT	
Atg13	NM_001271212.1	Forward	AGGCTTCCAGACAGTTCGTG	118
		Reverse	TGGGGTCCTCTCAAATTGCC	
Atg14	NM_001107258.1	Forward	GGCTAACAGATCAGTTGCGATG	247
		Reverse	TGTTCCCTCAGGTCACTGGT	
mTOR	NM_019906.1	Forward	GCAATGGGCACGAGTTTGTT	94
		Reverse	AGTGTGTTCACCAGGCCAAA	
Beclin1	NM_053739.2	Forward	GCCTCTGAAACTGGACACGA	113
		Reverse	CTTCCTCCTGGCTCTCTCCT	
ULK1	NM_001108341.1	Forward	CATCCGAAGGTCAGGTAGCA	148
		Reverse	GATGGTTCCCACTTGGGGAGA	
Bcl-2	NM_016993.1	Forward	TCGCGACTTTGCAGAGATGT	116
		Reverse	CAATCCTCCCCCAGTTCACC	
Bax	NM_017059.2	Forward	GGGCCTTTTTGCTACAGGGT	106
		Reverse	TTCTTGGTGGATGCGTCCTG	
Caspase 3	NM_012922.2	Forward	GAGCTTGGAACGCGAAGAAA	221
		Reverse	TTGCGAGCTGACATTCCAGT	
Caspase 9	NM_031632.1	Forward	AGCATCACTGCTTCCCAGAC	328
		Reverse	CAGGTGTCCCCACTAGGGTA	
Ki67	NM_001108341.1	Forward	CATCCGAAGGTCAGGTAGCA	148
		Reverse	GATGGTTCCCACTTGGGGAGA	

RESULTS

Litter Size and Pup's Body Weight

Table 2 illustrates that maternal 50% feed restriction from gestation day 10 to 21 did not affect the litter size (P=0.68), however, it produced IUGR offsprings with decreased body weight (more than 10%) as compared with the Adlib dams (P<0.001).

Blood Glucose and Selected Organ Weights

Results indicated that there was no significant difference in blood glucose concentration and weights of spleen and brain between IUGR and NBW rats (P>0.05), as shown in *Table 3*. However, IUGR rats had significantly lower weights of heart, liver, kidney and lung as compared with the NBW rats (P<0.01).

Histological Observation of Jejunum

As shown in *Table 4*, compared to the NBW rats, IUGR rats had significantly reduced villus height (P<0.01) and villus surface area (P=0.012) and tended to have lower crypt depth (P=0.07). However, there were no significant differences in villi/crypt ratio, and villus width between IUGR and NBW pups (P>0.05). Moreover, IUGR pups showed accumulated autophagosomes in the jejunal mucosa *(Fig. 1A2, Fig. 1B2)*, however, the microvilli and mitochondrial histology were similar to the NBW pups *(Fig. 1A1, Fig. 1B1)*.

Autophagy Related Gene Expression

Effects of IUGR on the autophagy-related gene expression in the jejunum are presented in *Fig. 2*. Results showed that IUGR significantly enhanced mRNA expressions of *WIPI1*, *MAP1LC3B*, and *Atg13* (P<0.01), while expressions of *Atg5* and *Atg14* were unaffected in the jejunum (P>0.05). Compared with the NBW pups, IUGR pups had increased mRNA expressions of *ULK1* and *Beclin1*, and decreased *mTOR* mRNA expression (P<0.05).

Apoptosis and Proliferation Related Gene Expression

Effects of IUGR on expression of apoptosis and proliferation related genes in the jejunum are shown in *Fig. 3*. Results indicated that IUGR pups had significantly lower mRNA expression of *Bcl-2*, and higher *caspase 9* mRNA expression in the jejunum as compared to NBW pups (P<0.05). However, IUGR did not affect mRNA expressions of *Bax* and *caspase 3* (P>0.05), while it tended to increase *ki67* mRNA expression in the jejunum as compared with NBW pups (P=0.06).

Table 2. *The body weight of neonatal pups and litter size of rat dams*

Item[1]	NBW	IUGR	*P*
Litter size	13.90±1.79	13.50±2.37	0.68
Body weight (g)	6.42±0.08	5.77±0.08**	<0.001

*[1] Data were expressed as mean ± SE (n=10); ** P<0.01 means differences between IUGR and NW pups*

Table 3. *The blood glucose and selected organ weights of IUGR and NBW pups*

Item[1]	NBW	IUGR	*P*
Blood glucose (mmol/L)	6.52±0.37	5.69±0.34	0.11
Heart (g)	0.034±0.001	0.027±0.001**	<0.001
Liver (g)	0.317±0.010	0.253±0.013**	<0.001
Spleen(g)	0.010±0.001	0.010±0.001	0.451
Kidney (g)	0.064±0.002	0.055±0.002**	0.001
Brain (g)	0.214±0.005	0.221±0.005	0.276
Lung (g)	0.126±0.004	0.105±0.005**	0.001

*[1] Data were expressed as mean ± SE (n=20); ** P<0.01 means differences between IUGR and NW pups*

Table 4. *The intestinal morphology of IUGR and NBW pups*

Item[1]	NBW	IUGR	*P*
Villus height (μm)	260.49±6.95	227.63±6.55**	<0.01
Crypt depth (μm)	61.33±2.49	55.25±2.05	0.07
Villi/Cryptratio	4.41±0.17	4.20±0.13	0.34
Villus Width (μm)	58.36±1.28	57.32±1.12	0.55
Villus surface area (mm^2)	0.024±0.001	0.021±0.001*	0.012

*[1] Data were expressed as mean ± SE (n=20); * P<0.05 and ** P<0.01 means differences between IUGR and NW pups*

DISCUSSION

Maternal nutrition plays important roles in the development of fetuses as both the quality and quantity of maternal nutrients affect fetal development. Maternal malnutrition commonly causes low birth weight offsprings, those having birth weight less than 10th percentile are considered as IUGR. They commonly exert long-term effects on the health as termed "programming" [23-25]. Animals, such as pigs, sheep, and rodents, are widely used to construct IUGR models via maternal feed restriction [7,26,27]. Recently, it has been confirmed that rapid intestinal catch-up growth occurs in IUGR neonates which were provided with sufficient colostrum [12]. However, the underlying mechanism for catch-up growth is still largely unknown. Therefore, in this study, a rat model of IUGR with postnatal catch-up growth was constructed by maternal 50% feed restriction as previous reports [19,20].

In present study, maternal 50% feed restriction from gestational day 10 to 21 reduced neonatal body weight by more than 10%, which indicated that these pups were IUGR. These results were consistent with previous results reported by Desai et al.[20], who found that maternal feed restriction in late gestation decreased the body weights of offsprings, which showed rapid postnatal catch-up growth. Likely, Woodall et al.[28] reported that maternal

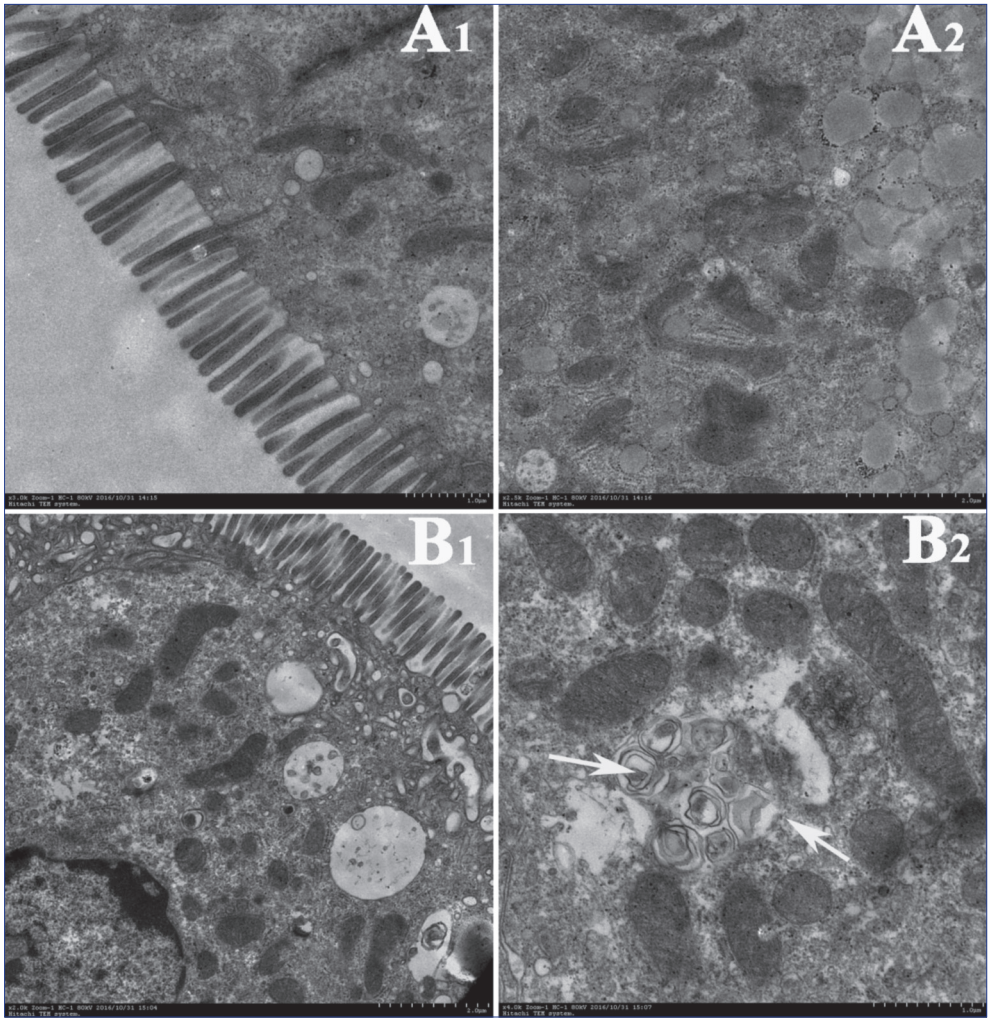
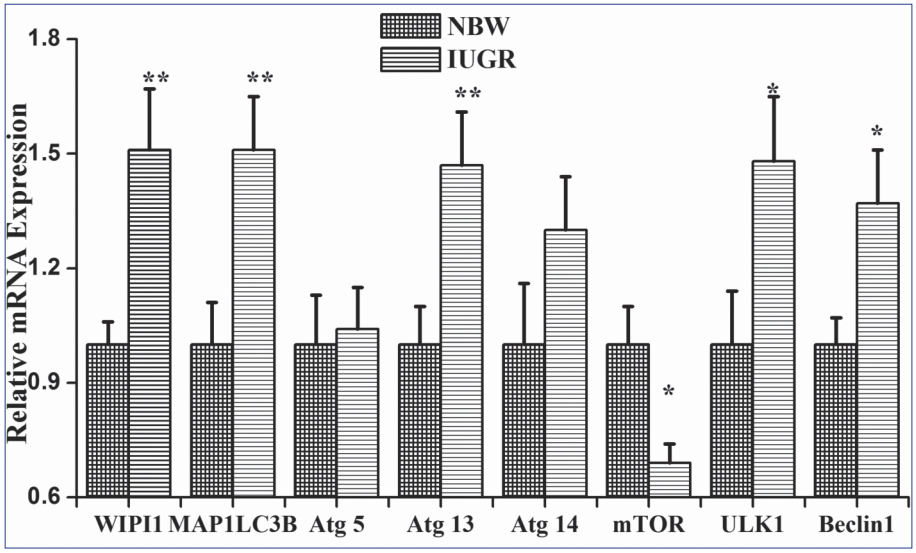


Fig 1. Effects of IUGR on the interior structure of the jejunum in pups. The microvilli of jejunum and its interior structure of NBW pups (A_1 and A_2) and IUGR pups (B_1 and B_2)

was not affected, which is in line with our present results that litter size was not affected by maternal 50% feed restriction from gestational day 10 to 21. Desai et al.[29] also found that maternal protein restriction throughout the pregnancy did not affect the litter size, but had selectively decreased organ growth. At the age of 21 days, the IUGR offsprings from protein restricted dams exhibited slight decreases in lung and brain weights, but a greater reduction in weights of pancreas, spleen, muscle and liver. In present study, IUGR pups had reduced organ weights, including heart, kidney and lung. However, Meyer et al.[30] and Anderson et al.[19] found that nutrient restriction during early to midgestation did not affect fetal organ weights, although the total and net maternal body weights were significantly decreased. The author suggested that dams could compartmentalize all the available nutrients to

Fig 2. Effects of IUGR on the autophagy related gene expression. No. for the expression was 10, data were normalized to the NBW pups and were expressed as mean± SE. * P<0.05 and ** P<0.01 means for the same parameter between IUGR and NBW pups were significantly different



feed restriction throughout the gestation produced IUGR pups, and inhibited the postnatal growth of the IUGR pups from birth to 90 days of age, while the litter size prevent serious fetal damages during maternal malnutrition [19], and sufficient maternal nutrients during the period of rapid fetal growth (the later gestation)

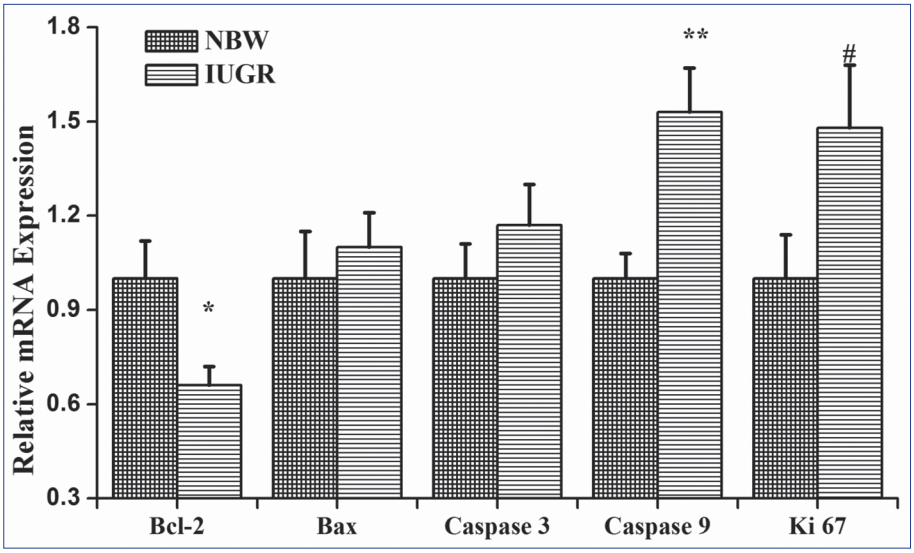


Fig 3. Effects of IUGR on expression of apoptosis and proliferation related genes in the jejunum. No. for the expression was 10, data were normalized to the NBW pups and were expressed as mean± SE. * P<0.05 and ** P<0.01 means for the same parameter between IUGR and NBW pups were significantly different, while # means a tendency towards significant difference (*P*=0.06)

are very important for the development of organs.

The small intestine plays a critical role in immunity, nutrient digestion and absorption. Various studies have focused on physiology and functions of small intestine of IUGR animals. For example, IUGR significantly lowered the small intestinal mucosa weight, decreased the length of the small intestine and colon in neonatal piglets [9], which was in line with previous reports [31]. Similarly, Dong et al.[8] observed that newborn IUGR piglets without suckling colostrum after birth had damaged and shorter intestinal villi with accumulated autophagosomes and swelled mitochondria in small intestine. Wang et al.[32] proved that the impaired small intestine of newborn IUGR piglets was accompanied with the altered intestinal proteomes. In agreement with previous reports, we found that IUGR pups showed decreased villus height and surface area, and tended to have lower crypt depth. However, microvilli and mitochondrial histology was unaffected in IUGR pups, indicating that sufficient colostrum could decrease the intestinal stress in IUGR pups, which is consistent with the previous report [12].

An accumulation of autophagosomes is an important indicator for the evaluation of autophagy [33]. In the current study, accumulated autophagosomes in the jejunum of IUGR pups were observed. Moreover, the IUGR pups also showed increased mRNA expressions of *WIPI1*, *MAP1LC3B*, and *Atg13* in the jejunum. It has been documented that the increase in mRNA expressions of *WIPI1* and *MAP1LC3B* is prior to the accumulation of autophagy marker protein *MAP1LC3* in a wide range of cells, suggests that analysis of their mRNA expression is one convenient method for monitoring autophagy [34]. Atg13 is in a complex with *Atg1*, *Atg101* and *ULK1*, which is essential for the induction of autophagy. Once the mRNA expression is decreased, the autophagy (in the cells of HEK293) is also reduced, which is similar to the *ULK1*depletion [35]. Therefore, these results indicated that IUGR enhanced the autophagy in the jejunum. To further elucidate the related molecular mechanism, the expression of *mTOR*, *Beclin1*, and *ULK1* in jejunum was determined in present study. It has been documented that *mTOR-Beclin1-ULK1* signal pathway plays critical roles in the regulation of autophagy [36-38]. As a sensor of nutritional status, stress and growth factor signals, *mTOR* can regulate autophagy through direct phosphorylation of *ULK1*, which further induces autophagy by phosphorylating *Beclin1* and activating *VPS34* lipid kinase [39,40]. Moreover, the up-regulated autophagy is commonly accompanied by the increased mRNA levels of *ULK1* and *Beclin1*, which plays critical role in embryonic development [34,41]. Results of present study showed that mRNA expressions of *Beclin1* and *ULK1* were increased, and *mTOR* gene expression was decreased in the jejunum of IUGR pups, which were in agreement with our results that the autophagy was enhanced in the jejunum, suggesting that the enhanced autophagy in jejunum of IUGR pups should be related with *mTOR-Beclin1-ULK1* signal pathway. However, the mechanism for the decreased *mTOR* mRNA expression in response to colostrum should be further studied as there was no significant difference in blood glucose, which is a primary energy source for intestinal development, between IUGR and NBW pups.

There is a complicated interaction among autophagy, apoptosis and proliferation. Despite the enhanced intestinal autophagy, IUGR pups still had higher mRNA expression of *caspase 9* and lower level of *Bcl-2* mRNA expression in present study, which indicated that intestinal apoptosis has been up-regulated [42,43]. The over-induced apoptosis might lead to further organ damage [44]. For example, Xia et al.[44] proposed that hypoxia induced renal autophagy via *Beclin1* signal pathway, enhanced apoptosis and affected the renal development in IUGR rat fetuses. We also observed the similar phenomenon in small intestine of IUGR fetuses (data not published) and newborn IUGR piglets (without feeding colostrum) that over-enhanced autophagy and apoptosis might

further impair the intestinal morphology [8]. However, after feeding with sufficient colostrum for 24 h, IUGR pups were under low-stress conditions as discussed above, but still exhibited increased intestinal autophagy in this study, which might contribute to the rapid catch-up intestinal growth [12,16]. It has been documented that autophagy regulates differentiation via notch signaling pathway [45]. Therefore, enhanced intestinal autophagy under low-stress conditions after feeding with sufficient colostrum may enhance the proliferation of intestinal crypt base columnar stem cells and improve the crypt regeneration [46,47]. In consistence with these results, the *ki67* mRNA expression tended to increase in the jejunum of IUGR pups fed with colostrum in present study. The mRNA expression of *ki67* is a sensitive indicator for the proliferative status. Once the cell exits from the active cell cycles, the reduced *ki67* mRNA expression can be easily detected [48,49]. Specific reduction of *ki67* mRNA inhibits the proliferation and increases apoptotic cell death [49]. Therefore, the tendency towards increased mRNA expression of *ki67* in present study suggested that the intestinal proliferation in IUGR pups fed with colostrum tended to be enhanced, which may contribute positively to the rapid intestinal catch-up growth. In accordance with our present results, we also found that IUGR pups with sufficient colostrum/milk had similar villus height and crypt depth, and tended to increase the villus width and surface area as compared to the NBW rat pups at the age of 7 days (data not published). Similarly, previous studies also verified that under low-stress conditions enhanced autophagy could prevent early brain injury [16,17].

In summary, our present results indicated that maternal 50% feed restriction from gestational day 10 to 21 did not affect the litter size, but produced IUGR pups. After feeding with sufficient colostrum for 24h, IUGR rat pups still had impaired intestinal morphology with enhanced apoptosis and increased autophagy via *mTOR-Beclin1-ULK1* signalling pathway. However, the small intestine of IUGR pups with sufficient colostrum was under low-stress conditions (unaffected intestinal mucosal mitochondrial histology). Combination with previous reports that autophagy (under low-stress conditions) could enhance proliferation and our results (the tendency to increase *ki67* mRNA and the catch-up growth in intestinal morphology at day 7), it can be concluded that the rapid intestinal catch-up growth of IUGR pups in response to sufficient colostrum should be related to the enhanced intestinal autophagy (under low-stress conditions) and intestinal proliferation. These results may be beneficial for the development of IUGR neonates during post-parturition periods. However, the long-term effects of catch-up growth on the health should be investigated precisely in future.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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