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Postnatal delayed growth impacts cognition but rescues programmed impaired pulmonary vascular development in an IUGR rat model

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Abstract *Background and aims:* Intrauterine growth restriction (IUGR) is a state of slower fetal growth usually followed by a catch-up growth. Postnatal catch-up growth in IUGR models increases the incidence of pulmonary arterial hypertension in adulthood. Here, we hypothesize that the adverse pulmonary vascular consequences of IUGR may be improved by slowing down postnatal growth velocity. Meanwhile, cognitive function was also studied.
Methods and results: We established an IUGR rat model by restricting maternal food throughout gestation. After birth, pups were fed a regular or restricted diet during lactation by changing litter size. Thus, there were three experimental groups according to the dam/offspring diet: C/C (gold standard), IUGR with catch-up growth (R/C) and IUGR with delayed growth (R/D). In adulthood (14 weeks of age), we assessed pulmonary vascular development by hemodynamic measurement and immunohistochemistry. Our results showed that adult R/C offspring developed an elevated mean pulmonary arterial pressure (mPAP) and pulmonary arteriolar remodeling accompanied with decreased eNOS mRNA and protein expressions compared to C/C or R/D offspring. This suggested that delayed postnatal growth improved pulmonary circulation compared to postnatal catch-up growth. Conversely, adult R/D offspring performed poorly in cognition. Behavior test and electrophysiology results exhibited a reduced synaptic plasticity. Furthermore, decreased mRNA expression levels of the memory-related gene *zif268* and transcription factor recruitment factor p300 in the hippocampus region were also observed in R/D group.
Conclusion: These findings indicate that delayed postnatal growth results in cognitive impairment, but it reverses elevations in mPAP induced by postnatal catch-up growth following IUGR.

Abbreviations: IUGR, intrauterine growth restriction; SGA, small for gestational age; BPD-PH, bronchopulmonary dysplasia complicated pulmonary hypertension; mPAP, mean pulmonary arterial pressure; PAH, pulmonary arterial hypertension; eNOS, endothelial nitric oxide synthase; LTP, long-term potentiation; HAT, histone acetyltransferase; CBP, CREB-binding protein; p300, E1A-binding protein; ACSF, artificial cerebrospinal fluid; fEPSPs, field excitatory postsynaptic potentials; PS, population spike; GAPDH, glyceraldehyde-3-phosphate dehydrogenase gene; ANOVA, one-way analysis of variance; LSD, least-significant difference test.

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Introduction

Small for gestational age (SGA) refers to the birth weight lower than the 10th percentile of the average gestational age weight [1], partly caused by intrauterine growth restriction (IUGR) [2]. For IUGR individuals, a catch-up growth strategy is usually adopted for obtaining appropriate weight, length, head circumference and neural development [3,4]. However, the mismatch between the prenatal and early postnatal environment may increase the predisposition of adults for adult chronic non-communicable diseases [5]. The adverse outcomes of IUGR rat pups treated with postnatal catch-up growth include cardiovascular diseases, diabetes, dyslipidemia, and shortened lifespan [6–10]. It has been shown that slowing down the postnatal growth velocity by restricting nutritional intake would protect IUGR offspring from obesity [11] and type 2 diabetes [12]. However, negative effects of delayed postnatal growth in IUGR rat model [13] have also been reported. Hence, the management of reasonable postnatal feeding patterns for intrauterine growth restricted individuals remains challenging.

With the increased survival of very low or extremely low birth weight infants, bronchopulmonary dysplasia complicated pulmonary hypertension (BPD-PH) become an increased problem in neonatal intensive care unit [14]. Clinical evidence showed that IUGR was found in 40% of patients with BPD-PH. IUGR is an important risk factor for BPD-PH and a reason for considering BPD-PH screening [14]. Previous studies showed that IUGR rat offspring exhibit higher susceptibility to elevated pulmonary arterial pressure in adulthood compared to their counterparts who grew normally in utero [15–17]. Pulmonary arterial hypertension (PAH) is characterized by abnormal pulmonary hemodynamics, pathologically compensatory hyperplasia of the pulmonary vessel wall and the subsequent right heart failure [18,19]. Mechanistically, it is well recognized that endothelial nitric oxide synthase (eNOS) plays a vital role in the regulation of vasodilation [20]. Down-regulated eNOS results in endothelial dysfunction by enhancing the production of reactive oxygen species in adults with cardiovascular diseases [21]. However, the role and mechanism of the delayed postnatal growth after IUGR on pulmonary circulation remains unclear.

The development of the brain depends on nutrition especially during the perinatal period, a critical time for the “brain growth spurt” [22]. Although thrifty phenotype hypothesis illustrates that the fetus has the ability to prioritize brain growth at the expense of body growth in undesirable environments [23], restricted prenatal nutrition could still interfere with neural development, leading to learning disabilities [24–26]. Many transcriptional factors are involved in the regulation of neuronal activities. One of such factors, is zif268, a transcription factor involved in neuronal plasticity, memory formation and the maintenance of long-term potentiation (LTP) [27–29]. The essential role of the chromatin-modifying enzymes for regulating hippocampal-dependent memory-related gene expression has also been reported recently [30]. Histone acetyltransferases (HAT), including CREB-binding protein (CBP) and E1A-binding protein (p300), have been reported to be involved in the formation of adult neurogenesis [31] and certain types of memory [32]. However, the changes and interaction of these molecules in delayed postnatal growth after IUGR on learning ability remains unclear.

In this study, we hypothesize that the adverse pulmonary arterial physiology of IUGR may be improved by slowing down postnatal growth velocity while evaluating its impact on cognitive function. We have used IUGR rat model and established three experimental groups by varying postnatal growth velocity. Both pulmonary and cognitive functions were assessed and molecular mechanisms were explored.

Methods

Animals and experimental design

All procedures and experiments were permitted by the Ethical Committee of Animal Experiments of Zhejiang University (ethics number: ZJU20160215). The IUGR rat model was established following our previous protocol [33]. Sprague-Dawley rats were obtained and raised at the Zhejiang Chinese Medical University Laboratory Animal Center. Virgin Sprague-Dawley female rats weighing 250–300 g were mated overnight. Once pregnant, they were randomly divided into normal diet and restricted diet groups by offering them normal food and 50% amount of regular food respectively throughout gestation. The weights of the pups were measured at the first day of birth. Offspring of normal dietary dams were labeled as control rats; offspring of nutritional restricted dams (with a birth weight below 5.8 g) were labeled as IUGR rats. After birth, the litter size of normal dietary offspring were 8 pups per litter, however, IUGR rats were divided into two groups by allocating 8 or 16 pups per litter fostered by normal dietary dams. Thus, we generated 3 groups according to the dam/offspring diet: Control/Control (C/C), intrauterine growth restriction with postnatal catch-up growth (R/C) and intrauterine growth restriction with delayed postnatal growth (R/D). The experiments on adult male rats were carried out in offspring from 3 different C/C, R/C and R/D dams. To eliminate variability arising from the hormonal cycle in female rats, only male rats were reserved for this study.

Neurodevelopment analysis

Morris water maze

Morris water maze was performed to test the learning and memorization [34]. Briefly, a round stainless-steel circular tank was put in a dimly-lit room, with a 12-centimeter diameter platform sitting in a random quadrant with four visual cues pasted on the wall. 22 °C water was poured into the pool until the water surface was 1 cm below (the first day) or above (the second to the fifth day) the platform. During reference training, male offspring rats were trained in the maze four times per day for 5 days at

13 weeks of age. On the sixth day, probe trial was conducted by counting the number of times the rats swam to cross the platform area (passing times) within 120 s with the platform being removed. A smart video tracking system recorded the tracks of the rats and the data were analyzed using Smart software (Panlab S.L., Barcelona, Spain). 5–6 rats from each group were used. In particular, after each trial, every rat was dried with a towel before returning to its regular cage.

Electrophysiology

Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) at 14 weeks of age and subsequently perfused with ice-cold sucrose-containing cutting solution (in mM: 234 Sucrose, 5 KCl, 1.25 $NaH2PO_4$, 5 $MgSO_4$, 26 $NaHCO_3$, 25 D-glucose, 1 $CaCl_2$, balanced with 95% O_2/5% CO_2). Brains were then rapidly dissected, and transferred into a chamber filled with ice-cold cutting solution. Transverse slices (400 mm thicknesses) from the middle portion of each hippocampus were cut with a vibroslicer (VT1200 S, Leica Biosystems., Wetzlar). After sectioning, brain slices were incubated in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) (in mM: 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 $KH2PO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose [pH 7.4], 300 mOsm) at 32 °C for 25 min before being incubated in the same ACSF at room temperature for at least 1 h. A single slice was then transferred to the recording chamber and submerged beneath the continuously perfused ACSF that had been saturated with 95% O_2 and 5% CO_2. The slices were allowed to equilibrate in the recording chamber for 10 min before being stimulated at room temperature (~25 °C). A standard procedure was used to record field excitatory postsynaptic potentials (fEPSPs) in the CA1 region of the hippocampus. A bipolar stimulating electrode (FHC Inc., Bowdoin, ME) was placed in the Schaffer collaterals to deliver test and LTP-inducing stimuli. A borosilicate glass recording electrode filled with the ACSF was positioned in the stratum radiatum of the CA1, 200–300 μm from the stimulating electrode. The fEPSPs in the CA1 region were evoked by test stimuli of 30–50% of the intensity required to evoke the maximum population spike (PS) at 0.05 Hz. Baseline responses were recorded for at least 20 min (response variability <10%) before LTP was induced. Two consecutive trains of stimuli (100 Hz, 1 s) separated by 20 s were used to induce LTP. At least seven slices from 3–4 rats for each group were used. The data were recorded, filtered (1 kHz), and sampled (20 kHz) by a Heka EPC 10 amplifier (Harvard Bioscience Inc., Ludwigshafen, HRB).

Pulmonary arterial pressure measurement

Rats were anaesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) at 14 weeks of age following the water maze test and placed on the surgical board in a supine position. A pulmonary artery catheter PE50, connected to a pressure transducer, was inserted into the right external jugular vein. The catheter was rotated leftwards and pushed forward when it passed through the right heart and reached the main pulmonary artery. The hemodynamic values were recorded for at least 2 min by a physiological data acquisition system (BIOPAC MP150; Biopac System Inc., Goleta, California, USA). 5–8 rats from each group were used.

Expression of genes in lung and brain tissues

RNA extraction, reverse transcription and quantitative PCR

Hippocampus and lung tissues were taken after the measurement of pulmonary arterial pressure and kept at −80 °C for use. Total RNA was extracted from the frozen tissues following the isolation RNeasy protocol (Axygen, Union City, USA). RNA was quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific lnc., NanoDrop Technologies, USA) and then reverse transcribed to cDNA with a reverse transcriptase kit (Takara, Kusatsu, Japan). Relative mRNA expression levels were analyzed with the StepOnePlus Real-Time PCR System according to the Takara SYBR-Green protocol (Takara, Kusatsu, Japan). All primers are shown in Table 1. Samples were assayed in triplicate. The PCR conditions were: 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C. $2^{-\Delta\Delta Ct}$ was calculated to quantify the expression of target genes relative to glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH).

Immunohistochemistry

Left lung tissues and right brain hemisphere were isolated and fixed in 10% and 4% formaldehyde solution respectively for later paraffin embedding. The paraffin embedded tissues were sectioned at 4–5 μm, then de-paraffinized with xylene, rehydrated in a graded ethanol concentration to distilled water and followed by antigen retrieval. Then, samples were incubated in methanol with 3% hydrogen peroxide for 25 min to eliminate endogenous peroxidase activity and washed with PBS. Next, the sections were incubated with the primary antibody anti-α smooth muscle actin (anti-α-SMA, GB13044, Servicebio, China, 1:2000) for the lung tissues and anti-zif268 (#4153, Cell Signaling, Danvers, USA, 1:200) for the brain tissues at 4 °C overnight. Then, the sections were blocked with 3% BSA for 30 min at room temperature. The slides were incubated with a secondary antibody (HRP polymer, Dako, Denmark) for 50 min at room temperature. Diaminobenzidine (DAB, Dako, Denmark) was added to the sections and washed away until the appearance of the positive claybank.

Tissue sections were imaged with a digital slices scanner at a magnification of 200× (Axioscan Z1-Zeiss, Jena, Germany) and processed using Image Pro plus 6.0 software (Media Cybernetics Inc., Rockville, USA). Those pulmonary arterioles with an external diameter between 50 μm and 100 μm existing next to bronchioles were selected. Medial thickening (calculated by the ratio of α-SMA_{area}/lumen area) was determined by positive α-SMA staining [33]. The semiquantitative analysis of zif268-positive cells in the CA1 area of the hippocampus was done by counting manually. Each group had at least 6 animals and at least three nonoverlapping fields of view from each section were counted.

Table 1 Primer sequences for genes.

Gene name	Sequences	PCR product
GAPDH	F:5′-CCT-GGA-GAA-ACC-TGC-CAA-G-3′ R:5′-CAC-AGG-AGA-CAA-CCT-GGT—CC—3′	110bp
zif268	F:5′-AAC-AAC-CCT-ACG-AGC-ACC-TG-3′ R:5′-AAA-GGG-GTT-CAG-GCC-ACA-AA-3′	91bp
p300	F:5′-AAG-CAC-CAG-TGT-CTC-AAG-CA-3′ R:5′- CCC-TGG-AGG-CAT-TAT-AGG-AGA-3′	71bp
CBP	F: 5′-AAG-AAT-ATG-GCT-CCG-ATT-GC-3′ R:5′-TGA-GGA-TCT-CAT-GGT-AAA-CAG-C-3′	120bp
eNOS	F:5′-GCA-GCC-CTA-AGA-CCT-ATG-3′ R:5′-GAC-ATC-ACC-GCA-GAC-AAA-C-3′	107bp

Western blot and analysis

Lung tissue samples were homogenized in lysis buffer (RIPA buffer and protease inhibitors) and centrifuged for 20 min at 13000 g. Protein (30 μg per lane) was separated in a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Merk Milipore, Darmstadt, Germany). Membranes were then blocked and probed with the primary antibody anti-eNOS (ab76198; 1:1000, Abcam, Cambridge, UK), anti-β-actin (A2228, 1:20000, Sigma—Aldrich, St. Louis, MO, USA). After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. The blots were captured by using G: BOX gel doc system (Syngene, Frederick, MD, USA). The expression of protein was quantified by densitometry normalized to β-actin using Image J software.

Double-immunofluorescence labeling of hippocampus tissues

Paraffin embedded dorsal hippocampus was sliced into coronal sections at 4—5 μm thickness using a vibratome (Leica, Germany), de-paraffinized with xylene, rehydrated in a graded ethanol concentration to distilled water and followed by antigen retrieval (boiling citric acid/sodium citrate buffer, pH 6.0) and blocking (3% BSA, 30 min). The sections were then incubated with anti-zif268 (#4153, Cell Signaling, Danvers, USA, 1:800) and anti-p300 (Millipore, NA46, Borlington, USA, 2 μg/ml) at 4 °C overnight. Dylight 488- and 594- conjugated secondary antibodies were incubated in a dark room for 1 h. The nuclei were stained with DAPI for 10 min. Fluorescent images were acquired with a Pannoramic Digital Slide Scanner (Pannoramic MIDI, 3DHISTECH Ltd, Budapest, Hungary), and photographed with 3D Histech software (3DHISTECH Ltd, Budapest, Hungary). The number of co-localized nuclei/total nuclei in the CA1 area of the hippocampus was done by counting manually, each group had at least 3 animals and at least three non-overlapping fields of view from each image were counted.

Statistical methods

Statistical analyses were performed using Statistical Program for Social Sciences (SPSS) software (SPSS 20.0, IBM Corporation, NY, USA) and figures were completed in Graphpad Prism 6.0 (Graphpad Software, Lnc., CA, USA). Independent samples between two groups were analyzed using Student's *t* test. Continuous variables between groups were compared using one-way analysis of variance (ANOVA) and multiple comparisons between groups using the Least-significant difference (LSD) test. Values were expressed as mean ± S.E.M (standard error of mean). *P* value$<$0.05 was considered statistically significant.

Results

Establishment of IUGR model and effects of delayed postnatal growth on body weight

The aim of this study was to rescue the impaired pulmonary arterial pressure by delayed postnatal growth in IUGR rat model and evaluated its effects on cognition (Fig. 1). At birth, the weights of IUGR rats were significantly smaller than controls (5.0 ± 0.07 vs 7.0 ± 0.06 g, $P < 0.0001$) (Fig. 2a). At weaning, there was no significant difference in the body weights of C/C and R/C offspring, however, the body weights of R/D pups were significantly lower compared to C/C and R/C pups ($P < 0.001$). After weaning, the body weights of the R/D group were significantly lower compared to C/C and R/C groups until the adulthood, while R/C group also showed a lower body weight than C/C group after 6 weeks of age (Fig. 2b).

Delayed postnatal growth rescues impaired mean pulmonary arterial pressure, pulmonary vascular thickness and vasodilatation factor in IUGR rats

To investigate whether delayed postnatal growth could decrease the risk of elevated pulmonary arterial pressure for IUGR offspring in adulthood, we measured the mean pulmonary arterial pressure (mPAP) and the thickness of the pulmonary smooth muscle layer. At the age of 14 weeks, there was no significant difference in mPAP between R/D (20.56 ± 0.46 mmHg) and C/C (20.73 ± 0.41 mmHg) offspring, however, the mPAP of R/C (22.89 ± 0.67 mmHg) offspring was significantly higher compared to C/C offspring (Fig. 3a, c). A significant increase was seen in the percentage of medial wall area for R/C (57.07 ± 8.11%) rats compared to C/C (40.84 ± 5.01%) and R/D (36.51 ± 2.92%) rats (Fig. 3b, d), illustrating a thicker smooth muscle layer in the R/C rats. This suggested that

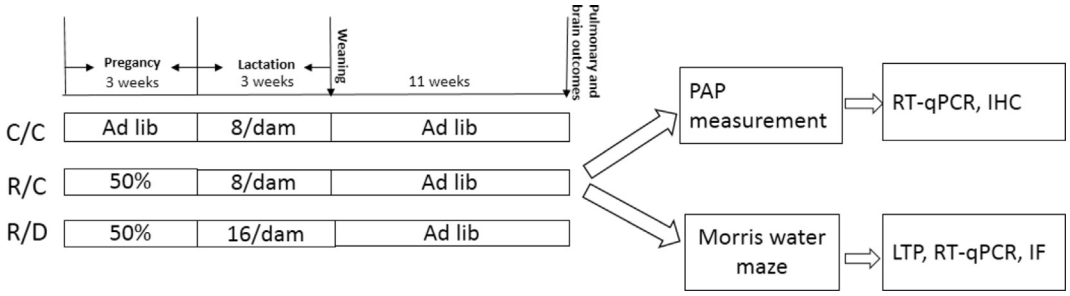

Figure 1 Experimental design. Female Sprague-Dawley rats were divided into normal diet (C) or 50% globe energy restricted diet (R) during gestation. After delivery, the litter size for control group (C/C) was 8 pups/litter, however, restricted dams (R) were divided into two subgroups based on the litter size. The litter size for one group (R/C) was 8 pups/litter whereas it was 16 pups/litter for another group (R/D). The experimental design has been explained in materials and methods section.

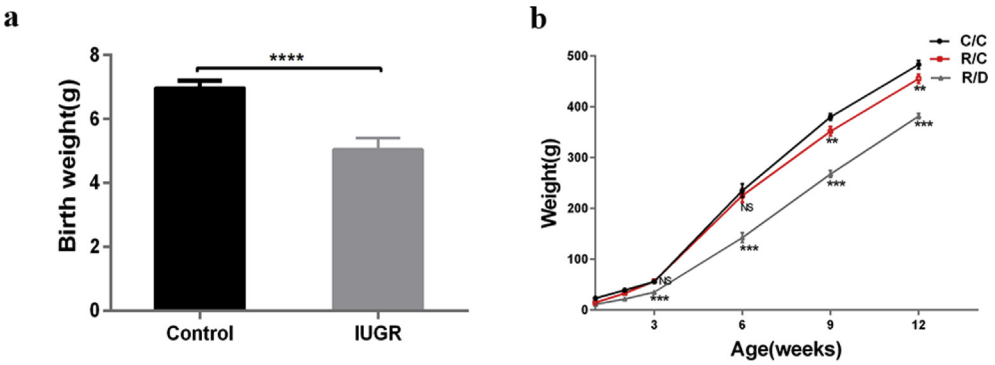

Figure 2 Birth weights of offspring and body weight curves in each group. (a) Birth weights of offspring. Control (n = 13), IUGR (n = 23), values are expressed as mean ± S.E.M and analyzed by Student's two-tailed t-test. (b) Postnatal body weights. n = 9–12 rats per group. Values are expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by LSD test. NS, not significant, **P < 0.01, ***P < 0.001, ****P < 0.0001.

catch-up growth following poor intrauterine growth induces vascular wall hypertrophy, which could be ameliorated by delayed postnatal growth. Furthermore, we analyzed the expression of vasodilatation factor, endothelial cell nitric oxide synthase (eNOS). Quantitative RT-PCR and Western blot results indicated that R/C group had a significantly lower mRNA and protein expression level of eNOS compared to C/C group, however, there was no difference between R/D and C/C groups (Fig. 3e and f). All these results indicated that the delayed postnatal growth mitigated the adverse effects of fetal origins of adult pulmonary hypertension.

Delayed postnatal growth impairs learning and memory ability

Morris water maze was performed in all the three groups to evaluate learning and memorization at 13 weeks of age (Fig. 4a–d). On the first day (D1) and the second day (D2) of training, there was no difference in escape time among the three groups, indicating that energy reduction did not damage their motions and visions. The comparison of the time that each rat spent in finding out the platform during reference training (D3-D5) showed that escape latencies decreased gradually in all groups, however, it was significantly longer in R/D group compared to C/C and R/C groups (Fig. 4b). In the probe trialon the sixth day, the permanence time (%) and the passing times of the R/D group were significantly less compared to R/C group (Fig. 4a, c, d). For further comparison of the difference in memory, the field excitatory postsynaptic potentials (fEPSPs) were obtained following stimulation in the CA1 region of the hippocampus to measure synaptic plasticity. The normal fEPSP amplitude of CA1 PS was significantly reduced in the R/D rats compared to C/C or R/C rats (Fig. 4e and f). The above results indicate that delayed postnatal growth following IUGR negatively affected the cognition in adulthood.

Effects of delayed postnatal growth on the expression levels of zif268, CBP and p300 in hippocampus

To identify the molecular mechanism of the effects of delayed postnatal growth on memory, we analyzed memory-related genes in hippocampus. Our results showed significantly decreased zif268 positive cells in R/D group compared to C/C and R/C groups (Fig. 5b). Furthermore, there was a reduced mRNA expression in both of *zif268* and p300 genes, but no difference in CBP mRNA expression in hippocampus tissues was found among the groups (Fig. 5c). Additionally, representative images of the immunofluorescence in CA1 area indicated less and weaker co-localization of p300 and zif268 in R/D group compared to C/C and R/C groups (Fig. 6a and b).

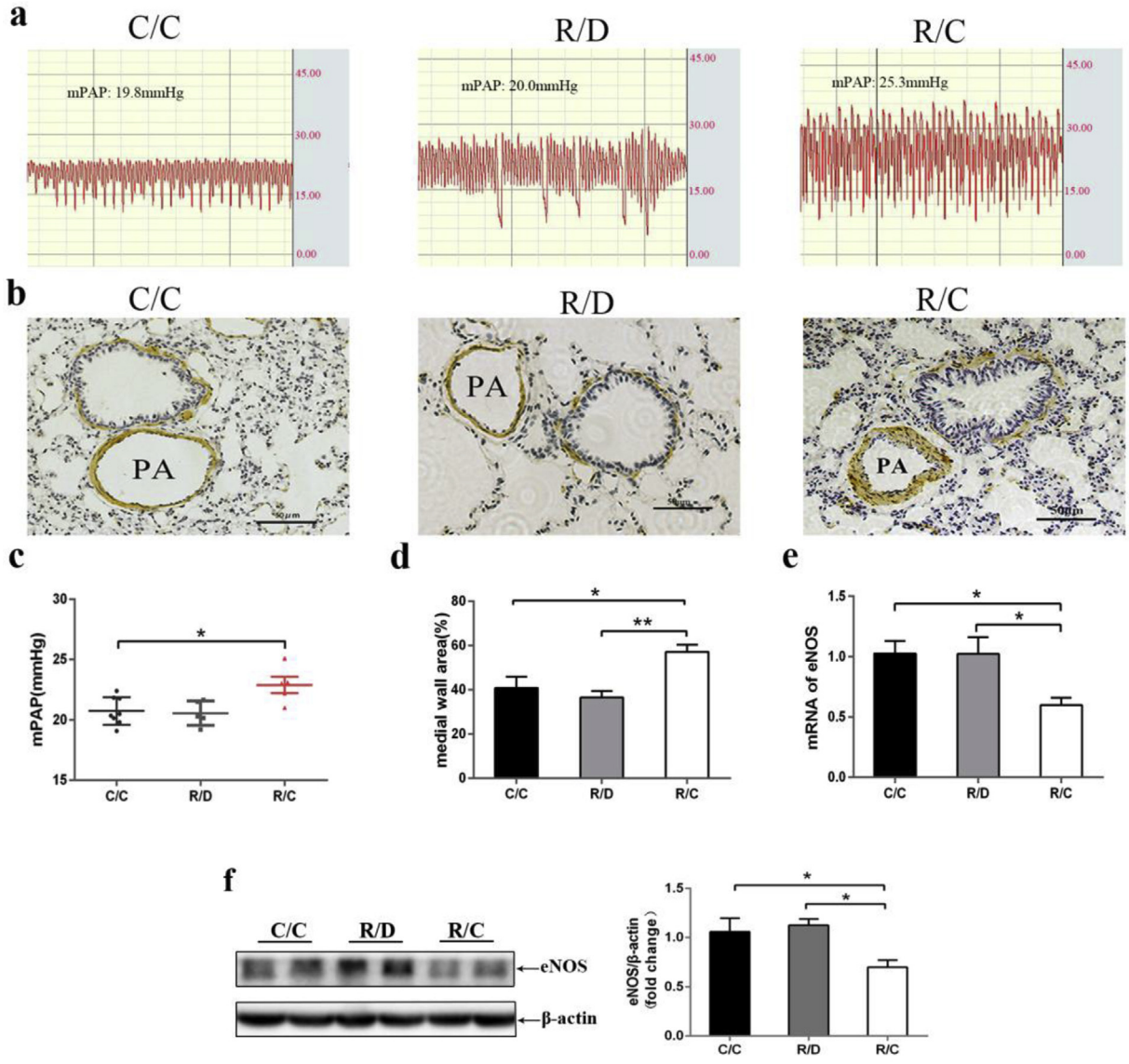

Figure 3 Postnatal delayed growth rescued elevated pulmonary artery pressure. (a) Representative pulmonary arterial pressure measurement of the three groups at 14 weeks of age. (b) Representative lung histological sections of the three groups. PA represents pulmonary artery. Scale bars are 50 μm. (c) Values of mPAP of the three groups at 14 weeks of age. C/C (n = 8), R/C (n = 5), R/D (n = 5). (d) Mean medial wall area. At least 5 pups and at least 5 views per group. (e) The eNOS mRNA expression levels in the lung tissues. n = 5–6 per group. Values are expressed as means ± S.E.M and analyzed by one-way ANOVA followed by LSD test. (f) Representative results of eNOS protein from lung tissues of the three groups detected by Western blot. n = 3 per group, the independent experiment was repeated for three times. Values are expressed as means ± S.E.M and analyzed by one-way ANOVA followed by LSD test. *$P < 0.05$.

Discussion

The present study demonstrates that IUGR rats followed by delayed postnatal growth during the lactation period (R/D) would have a discrepant impact on both pulmonary vascular development and neurocognition in adulthood. More specifically, we found that delayed postnatal growth resulted in cognitive impairment, but it alleviated elevated mPAP induced by postnatal catch-up growth following IUGR. Mechanisms contributing to the improved pulmonary arterial hemodynamics in R/D offspring include a decreased pulmonary arteriolar remodeling and a programmed increase in endothelial nitric oxide synthase compared to postnatal catch-up growth offspring. The impaired cognition in R/D offspring may be associated with the decreased LTP in CA1 area of hippocampus and decreased expression of memory related molecular, zif268, as well as its transcriptional co-activator, p300.

In this study, food restriction during pregnancy had a significant effect on birth weights, as predicted. The R/C

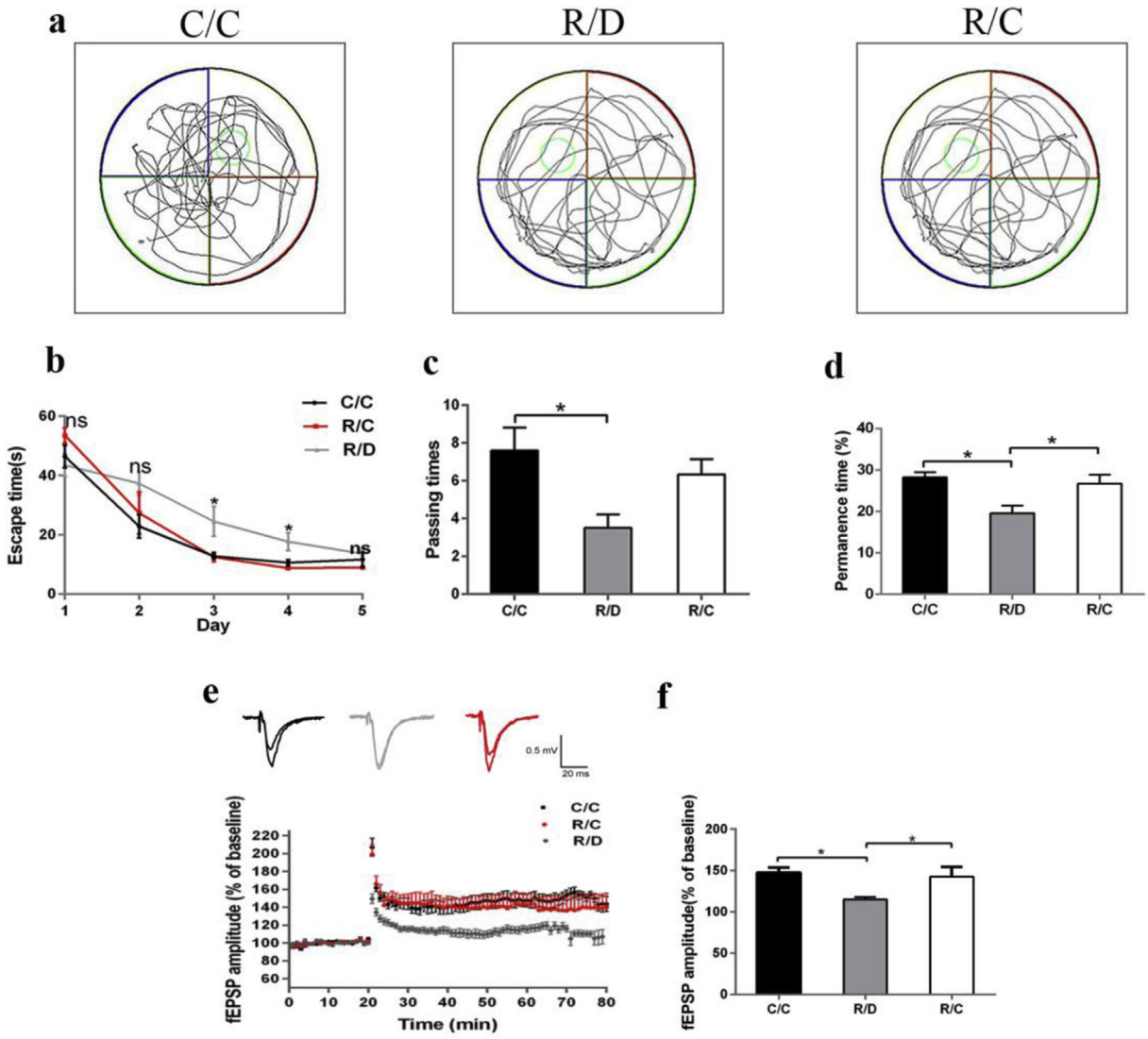

Figure 4 The results of the behavior test and the electrophysiology analysis of the hippocampus slices in vitro in adulthood. (a) The representative track maps of each group. The green circle represents the site of platform and the lines represent the number of times passing through the platform area in the probe trail test. (b) The average escape latency in the hidden platform tests. (c) Numbers of times passing through the platform area. (d) Percentage of time spent in the quarter which the platform stands. C/C (n = 5), R/C (n = 6), R/D (n = 6) (e), (f) the fEPSP amplitude of the three groups. n = 7–9 slices per group. Inset: typical traces of fEPSPs recorded before and after the conditioning stimulation. Scale bars: 0.5 mV, 20 mS. Values are expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by LSD test. $^{*}P < 0.05$.

offspring showed an improved catch-up growth indicated by a continuous weight gain from lactation to 6 weeks of age, although the body weight remained lower than that of C/C group at 14 weeks of age. This growth trajectory was similar to an IUGR rat model established before [35]. We speculate that the delayed growth trajectory after 6 weeks of age may be associated with the undesirable intrauterine hit. In agreement with previous studies, R/D rats had the slowest weight gain compared to C/C and R/C rats [36,37]. It's worth mentioning that the major differences in body weights observed at weaning were probably induced by the quantity but not the ingredients of milk, since they were all fostered by dams with normal perinatal diet.

Epidemiology and animal evidences have illustrated the close relationship between postnatal growth speed and later cardiovascular diseases in offspring with IUGR history [7,38]. However, less has been reported about PAH. In this study, R/C rats showed an elevated mPAP in the normoxic environment compared to control rats at 14 weeks of age. Encouragingly, R/D rats avoided the occurrence of elevated mPAP. Pulmonary vascular remodeling is characterized by hypertrophic media of the distal pulmonary arteries, which contributes to the elevated pulmonary vascular pressure as well [39]. In this study, catch-up growth following IUGR significantly thickened the vessel wall. However, postnatal delayed growth effectively restrained this structural change. This result suggested that a decreased caloric gap between pre- and postnatal period in IUGR rats is responsible for the less significant pulmonary arterial hemodynamic impairment and vascular

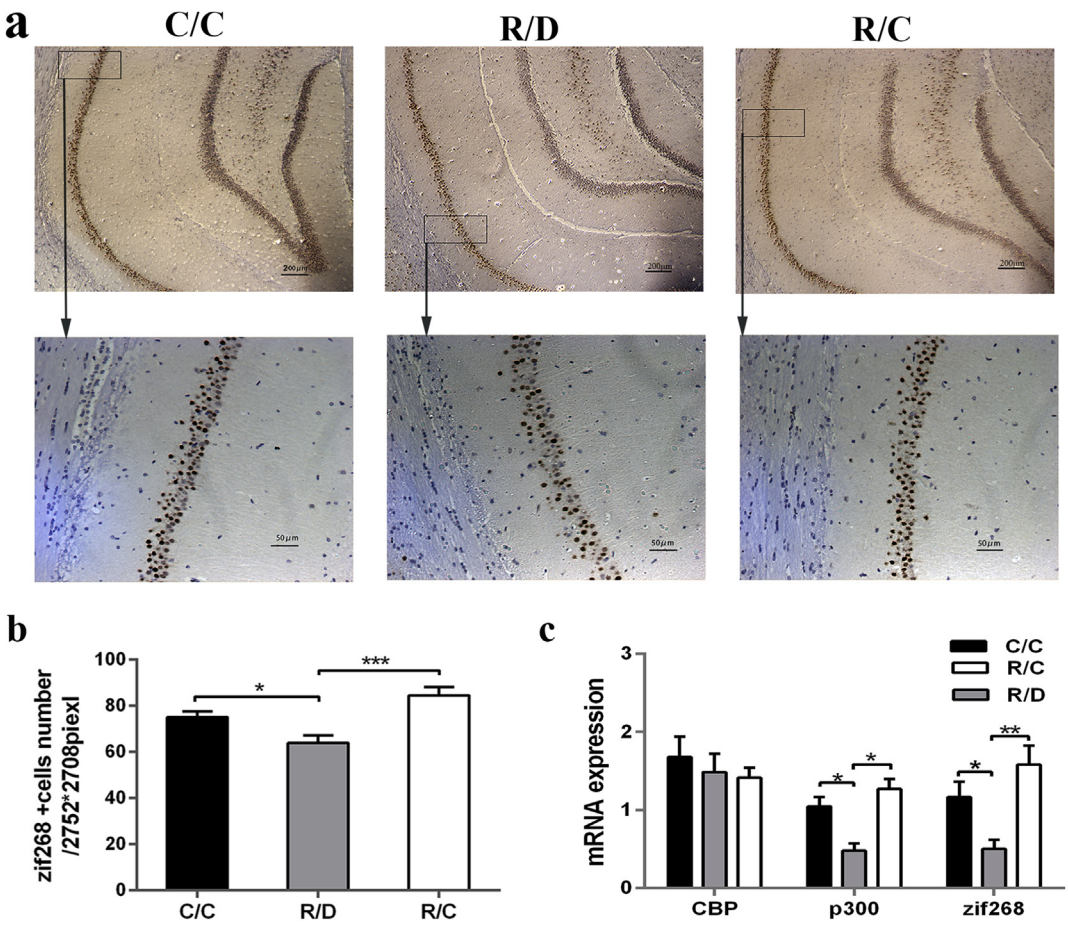

Figure 5 The results of zif268 expression in the hippocampal subfields (CA1) as well as the mRNA expressions of memory-related genes. (a) Representative stained sections of zif268 immunoreactivity in the CA1 area of the hippocampus in the three groups. (b) zif268 + cells number counts in the CA1 area. At least 6 pups and at least three views per group. Values are expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by LSD test. (**c**) The mRNA expression levels of memory-related genes. n = 7–8 per group. Values are expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by LSD test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

remodeling. Endothelial nitric oxide synthase (eNOS) is an endothelium derived relaxing factor that regulates vasodilation by catalyzing the formation of NO and plays a key role in pulmonary arterial pressure [20]. Reduced level of eNOS was observed in the umbilical vein endothelial cell of IUGR individuals and the aorta of IUGR-induced hypertensive rats [40–42]. We found down-regulated eNOS mRNA and protein expression in lung tissues of R/C rats compared to C/C and R/D rats, which may cause elevated pulmonary arterial pressure through unbalanced eNOS-dependent vasodilatation. Collectively, our results suggest that a mismatched feeding pattern during lactation mediates the vascular malprogramming, whereas matched nutrition between gestation and lactation improves it and the resultant pulmonary arterial hypertension.

The structure of the central nervous system forms at the beginning of gestation and is sensitive to the perinatal nutritional environment [43]. Some studies showed an association between IUGR and impaired intellectual outcome [24,25]. However, due to the hippocampal synaptic plasticity during early postnatal life, the cognition of IUGR individuals treated with a catch-up growth could be comparable to adequately grown counterpart in adulthood [44–46]. In this study, while we observed a reduced risk for fetal programming of PAH in IUGR offspring followed by delayed postnatal growth, we found a significant deficit in the acquisition of spatial memory ability, which was consistent with previous animal model findings [47–49]. Some epidemiology researches recently have also shown that SGA children without catch-up growth were at increased risk for behavioral problems between pre-school and school age [45,50]. Two possible reasons would explain why failure of catch-up growth among IUGR offspring leads to negative effects on cognition. First, poor neurodevelopment as a result of in utero food restriction and second is poor neurodevelopment as a result of postnatal food restriction. In our study, postnatal catch-up growth could compensate the poor effects of IUGR on the neurodevelopment in adulthood, which demonstrated that postnatal growth is more important than the uterine growth. In most studies, direct effects of delayed postnatal growth on cognitive function are measured by behavior test phenotype. To this end, LTP, which represents synaptic plasticity in the hippocampus and is a cellular mechanism that explain spatial memory formation [51], has been recorded. We found that the magnitude of the fEPSP slope

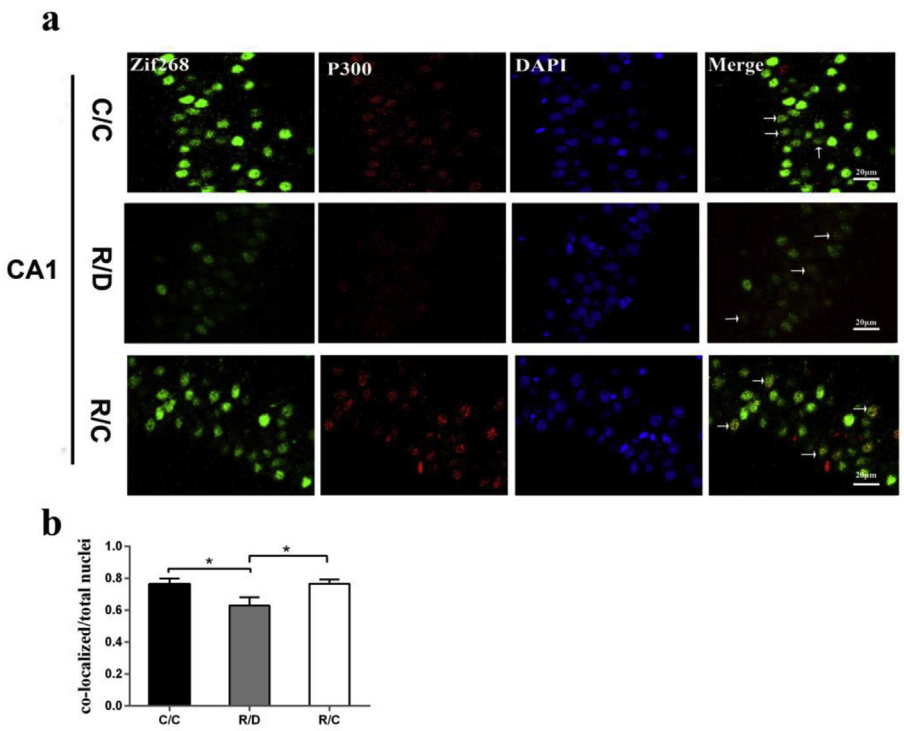

Figure 6 The co-localization expression of zif268 and p300 in hippocampus. (a) The co-localization expression of zif268 and p300 in the CA1 regions of the hippocampus was detected by digital slide scanner. zif268 (green), p300 (red) and DAPI (blue). Multicolor merged fluorescence of zif268 and p300 are marked by white arrows. (b) Quantification of zif268 and p300 based on average labeled number of co-localized/total nuclei in three groups, values are expressed as mean ± S.E.M and analyzed by one-way ANOVA followed by LSD test. *$P < 0.05$.

in the CA1 area was also significantly impaired in IUGR offspring with delayed postnatal growth. This indicated that perinatal undernutrition is probably contribute to the deleterious development in learning and memory by disrupting cellular physiological activity in hippocampus. It is well established that conversion from short-term memory into persistent long-term memory requires large number of new proteins as well as transcriptional co-activators [52,53]. CBP and p300, with an intrinsic histone acetyltransferase activity, are vital transcriptional co-activators that play critical roles in recruiting and integrating multiple memory-related transcriptional factors to enhance memory [54]. Previous studies demonstrated that CBP, but not p300, is necessary for spatial memory in mice [52,55]. We found decreased p300 but a normal CBP mRNA levels in IUGR offspring with delayed postnatal growth. A possible explanation may be the differences in the animal models and species. In addition, we also analyzed mRNA expression of transcriptional factor zif268, which contributes to hippocampus-dependent long-term memory by regulating newborn neurons [56]. Interestingly, its expression was in direct proportion to the expression of p300, suggesting a probable interaction between p300 and the memory-related gene zif268. Bousiges et al. reported that CBP occupancy decreases on the zif268 promoter in learning rats [57]. The decreased co-localization between zif268 and p300 in the IUGR offspring with a delayed postnatal growth suggest that there might be modifications in the promoters of these memory-related genes by different HATs requiring further studies to identify the specific regulation.

This study has limitations. It has been reported that male IUGR offspring have more severe hypertension than female IUGR offspring [40], however, how the sex factor influences adulthood pulmonary arterial pressure in IUGR offspring with a delayed postnatal growth requires further studies. In addition to pulmonary hemodynamics and pulmonary arteriole morphology, right ventricular function should also be determined for the assessment of pulmonary arterial function. Further research is needed to investigate the effects of postnatal dietary which can direct the optimal growth and balance the neurodevelopment and developmental origins of adult pulmonary hypertension in IUGR model offspring. Furthermore, mechanisms mediating the discrepant phenotypes, including genetic regulation and epigenetic modification are needed to be investigated.

In summary, this study demonstrated that delayed postnatal growth of IUGR rats during lactation impairs cognitive function, however, it rescues impaired pulmonary vascular development in adulthood.

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Conflicts of interest

No conflicts of interest are declared by the author(s).

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