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Research Article

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Maternal curcumin supplementation ameliorates placental function and fetal growth in mice with intrauterine growth retardation†

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Abstract

Intrauterine growth retardation (IUGR) is a serious reproductive problem in humans. The objective of this study was to investigate the effects of daily maternal curcumin supplementation during pregnancy on placental function and fetal growth in a mouse model of IUGR fed the low-protein (LP) diet. Pregnant mice were divided into four groups: (1) normal protein (19% protein) diet (NP); (2) LP (8% protein) diet; (3) LP diet + 100 mg/kg curcumin (LPL); (4) LP diet +400 mg/kg curcumin (LPH). The results showed that the LP group decreased fetal weight, placental weight, placental efficiency, serum progesterone level, placental glutathione peroxidase activity activity, blood sinusoids area, and antioxidant gene expression of placenta. In addition, in comparison with the NP group, LP diet increased serum corticosterone level, placental malondialdehyde content, and apoptotic index. Daily curcumin administration decreased the placental apoptosis, while it increased placental efficiency, placental redox balance, blood sinusoids area, and antioxidant-related protein expression in fetal liver. The antioxidant gene expression of placenta and fetal liver was normalized to the NP level after curcumin administration. In conclusion, daily curcumin supplementation could improve maternal placental function and fetal growth in mice with IUGR.

Summary Sentence

Daily curcumin supplementation could improve maternal placental function and fetal growth in mice with IUGR.

Key words: intra-uterine growth retardation, placenta, curcumin

Introduction

Intrauterine growth retardation (IUGR) has been characterized by decreased birth weight and/or organ weight of fetus during pregnancy [1, 2]. Intrauterine growth retardation is a common problem in animal reproduction and human pregnancy. Intrauterine growth retardation children often occurs catch-up growth, which could result in type 2 diabetes mellitus, cardiovascular disease, increased fat mass, and obesity [3]. Placental insufficiency is the major cause of IUGR. Oxidative stress occurs during pregnancy due to increased mitochondrial activity in placenta and fetus [4]. Intrauterine growth

retardation has been shown to be associated with increased placental reactive oxygen species and oxidative injury [5, 6].

Placenta is an important place where fetus and mother exchange the nutrition, oxygen, and metabolism transport [7]. Placental transport of nutrients is a key factor of fetal intrauterine growth [8]. Maternal nutrition deficiency or malnutrition has an adverse effect on fetal growth, and has been indicated to induce growth retardation in offspring [9, 10]. A low feed intake could weaken the antioxidant defense system in placenta [11]. A number of studies have reported that protein restriction on maternal diet during pregnancy could result in IUGR on fetus [12–14]. However, more effective nutritional treatments on inefficient placental transfer of nutrients induced by maternal protein malnutrition are yet to be studied.

Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* [15]. It has a broad spectrum of biological activities such as anti-inflammatory, antineoplastic, and antioxidant. Previous studies have indicated that curcumin is able to prevent excessive placental inflammation in lipopolysaccharide-induced preeclampsia [16, 17]. It has also been reported that curcumin can decrease infection and pro-inflammatory cytokines in human placenta [18]. In addition, both in vivo and in vitro studies have shown that curcumin exerts strong antioxidant activity [19–21]. The antioxidant activity of curcumin is associated with the ability of scavenging free radicals and up-regulating various cytoprotective proteins including the nuclear factor erythroid-derived 2 (Nrf2), heme oxygenase1 (HO-1), and NAD(P)H quinone oxidoreductase1 (NQO1) [22, 23]. It has been suggested that curcumin is beneficial in preventing IUGR-induced inflammation and oxidative damage of rat offspring [24]. However, data are lacking about the effects of curcumin on placental function in animals with IUGR.

Thus, we hypothesize that daily curcumin administration could alleviate placental oxidative injury of IUGR. The objective of this study was to investigate the effects of daily maternal curcumin supplementation during pregnancy on placental function and fetal growth in a mouse model of IUGR fed the low-protein (LP) diet. Our results may provide a new prophylactic approach for children with IUGR.

Materials and methods

Reagents

Curcumin (purity >98%) was provided by KeHu Biotechnology Research Center (Guangzhou, China). The purity of curcumin has been confirmed by high performance liquid chromatography (HPLC) analysis as reported by previous studies in our laboratory [24, 25]. The olive oil was bought from Olivoila (Shenzhen, China).

Animals and treatments

All experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (PZ2019032). A total of 64 ICR mice (32 female mice and 32 male mice, 8–10 weeks old; male mice: 32–34 g; female mice: 28–30 g) were purchased from Comparative Medicine Centre of Yangzhou University. Mice were housed under identical conditions and allowed free access to mice chow (Huafukang, Beijing, China) and water ad libitum. The mice were maintained under a 12L:12D photoperiod at 23 ± 2 °C. Mice were allowed to adjust to their surroundings for 7 days.

The protein restriction method of IUGR was based on a previous study [12]. After the acclimation period, female mice were mated overnight with male mice starting at 9:00 pm. Females were checked in the following morning at 7:00 am, and the presence of a vaginal plug was considered as gestational day (GD) 0.5. After confirmation of the vaginal plug, the pregnant mice were housed in individual cages. Mice were randomly assigned to receive either a standard diet (19% protein) or an isoenergetic LP diet (8% protein) (Table 1).

Table 1. Composition of the experimental diets used during the period of gestation.

Components	Normal protein (%)	Low protein (%)
Protein	19	8
Carbohydrate	61	71
Lipids	5	5.6
Fiber	3	3.6
Ash	5.5	4.1
Calcium	1	0.9
Phosphorus	0.78	0.76
Water	4.72	6.04
Total energy (kcal/kg)	3616	3664

From GD 1.5, 32 pregnant mice were randomly divided into 4 groups consisting of 8 mice per group: (1) mice received a standard diet (19% protein) and a daily gavage of olive oil (NP); (2) mice received an isoenergetic LP diet (8% protein) and a daily gavage of olive oil (LP); (3) mice received an isoenergetic LP diet (8% protein) and a daily gavage of 100 mg/kg body weight curcumin dissolved in olive oil (LPL); and (4) mice received an isoenergetic LP diet (8% protein) and a daily gavage of 400 mg/kg body weight curcumin dissolved in olive oil (LPH). Curcumin was dissolved in olive oil to make a suspension as presented in the previous studies [26, 27]. The supplemental curcumin level was optimized according to the previous studies [24]. The body weight of each pregnant mice was measured on GD 1.5, 4.5, 7.5, 10.5, 13.5, 16.5, and 19.5.

Serum and tissue collection

At GD 19.5, 6 pregnant mice per group (a total of 24 mice) were randomly selected and anesthetized with isoflurane and then underwent a cesarean section. The fetal weight, placental weight, and placental diameter were measured. A previous study has reported that IUGR is defined as fetuses with body weight below a certain value (male < 1.229 g and female < 1.186 g) [28]. The maternal blood was centrifuged at 3000*g* for 10 min to collect the serum, and the serum was used for measuring corticosterone, leptin, and progesterone levels. Placentas were collected, rapidly rinsed in PBS, then snap-frozen in liquid nitrogen and stored at −80 °C for further analysis. One placenta of each maternal mouse was fixed in 4% paraformaldehyde for 24 h before being embedded in paraffin. The fetal livers were collected from each fetus, frozen in liquid nitrogen, and stored at −80 °C for gene expression analysis.

Biochemical analysis

The serum concentrations of corticosterone and leptin were determined by enzyme linked immunosorbent assay (ELISA) kits for mice (Shanghai Xinfan Bio Techology Co., Ltd, Shanghai, China). All experimental procedures were performed according to the manufacturer's instructions. The serum progesterone level was detected using a commercial RIA kit (Beijing North Institute of Biological Technology, Beijing, China) by the Shanghai Xinfan Bio Techology Co., Ltd. The sensitivity for progesterone determinations was 0.2 ng/mL, the sensitivity for corticosterone determinations was 0.5 μg/L, the

sensitivity for leptin determinations was 7.5 pg/mL, the intra-assay coefficient of variation (CV) was less than 10%, and the inter-assay coefficient of variation was less than 15%.

Measurement of placenta enzyme activities

The activities of glutathione peroxidase activity (GSH-Px), catalase (CAT), and the concentrations of malondialdehyde (MDA) in placentas were determined using the commercial kits (Beyotime Biotechnology, Shanghai, China). All results were normalized to protein concentration in each sample. The protein concentrations were measured by a Bicinchoninic acid (BCA) Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). All determinations were performed in duplicate and averaged for statistical analysis.

H&E staining

After fixation in 4% paraformaldehyde for 24 h, the placentas were dehydrated through a graded series of ethanol and xylene, embedded in paraffin wax, and serially sectioned at 5 μm with a Lecia RM2235 microtome (Leica Biosystems Inc., Buffalo Grove, IL, USA). The sections were stained with hematoxylin and eosin (H&E), and viewed under an Olympus simon-01 microscope (Olympus Optical Co., Ltd., Beijing, China). The area of blood sinusoids in the placenta was analyzed using Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Washington, DC, USA).

TUNEL assay

Placental apoptosis was determined using a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay with a TUNEL BrightRed Apoptosis Detection Kit (Vazyme Biotech, Nanjing, China) according to the manufacturer's instructions. First, the paraffin sections of placentas were deparaffinized, rehydrated, and then incubated with Proteinase K (20 μg/mL) at room temperature for 20 min. Second, the sections were incubated with the TdT enzyme buffer containing double distilled H_2O, Equilibration Buffer, BrightRed Labeling Mix, and Recombinant TdT Enzyme at 37 °C for 60 min in the dark. Finally, the sections were stained with 4′,6-diamidino-2-phenylindole staining solution (Beyotime Biotechnology, Shanghai, China) for 5 min in the dark. The negative control was performed as above, but without incubation of the TdT enzyme buffer to ensure that no non-specific reaction appeared in the experiment. The images were acquired through a LSM 700 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany). The numbers of apoptotic cells (red color) and total cells (blue color) were counted using the Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Washington, DC, USA). The ratio of apoptotic cells to total cells represents the apoptotic index.

RNA extraction and real-time PCR

Total RNA was extracted from a complete placenta tissue or the fetal livers using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. RNA quality and concentration were determined using a ND-2000 micro spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and then diluted with Diethyl Pyrocarbonate (DEPC) water to 500 ng/μL. Then, the RNA was reverse-transcripted into cDNA using a PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) in a 20 μL reaction system by the procedure of 37 °C for 15 min and 85 °C for 5 s. Real-time PCR analysis was performed using a QuantStudio 5 Real-Time PCR System (Thermo Scientific, Wilmington, DE, USA) and a TB Premix Ex Taq Kit (TaKaRa, Dalian, China). All reaction (total 20 μL) were amplified containing 10 μL of TB Green Premix Ex Taq, 0.4 μL of ROX Reference Dye II, 2 μL of cDNA template, 0.4 μL of each primer (10 μM), and 6.8 μL of double distilled H2O. All genes were assayed for three times. The reaction program was as follows: 95 °C for 30 s, 40 cycles of 95 °C for 10 s, and 60 °C for 30 s. The melting curve was used to verify the amplification of a single product. Relative gene expression levels of placenta and fetal liver were analyzed by the $2^{-\Delta\Delta Ct}$ method after normalization against 18S ribosomal RNA (*18S*) and glyceraldehyde 3-phosphate (*GAPDH*), respectively. The 18S gene was selected to be the housekeeping gene for placenta as described elsewhere [28]. The primers were synthesized by Sangon Biotech (Sangon Biotech Co., Ltd., Shanghai, China), and the primer sequences used in this study were shown in Table 2.

Western blotting

Proteins of fetal liver were extracted using Radio Immunoprecipitation Assay buffer containing phenylmethanesulfonyl fluoride (PMSF), and total protein content was determined using a BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's protocol. Equal amounts of proteins (20 μg) were electrophoresed in 4–20% (w/v) SDS-PAGE, and then transferred on to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were incubated with specific primary antibodies against Nrf2 and HO-1 (Proteintech, 16396-1-AP; Zen-Bioscience, 380753) overnight at 4 °C. β-Actin (Proteintech, 660091-1) was utilized as a loading control. After three washes in TBST for 10 min each, the membranes were incubated with the secondary antibodies (1:3000, ABclonal Biotechnology Co., Ltd., Wuhan, China) for 60 min at room temperature. The antibodies used in the present study were shown in Supplemental Table S1. Finally, the blots were washed in Tris Buffered Saline with Tween (BST) for three times and protein bands were detected using an enhanced chemiluminescence kit (Thermo Scientific, Wilmington, DE, USA). Signals were visualized using Luminescent Image Analyzer LAS4000 (FuJI Film, Tokyo, Japan). The protein expressions were estimated by quantifying the intensities of the bands using ImageJ software.

Statistical analysis

The maternal weight and serum biochemical levels were analyzed using six maternal mice per group as the experimental unit ($n = 6$). The litter was considered as the unit for the statistical comparison of fetal and placental parameters among different groups. For fetal weight, placental weight, and placental diameter, the means were calculated per litter and then averaged per group ($n = 6$). In addition, the fetal livers per litter were mixed to be one sample for analyzing the gene and protein expression level. The gene expression level was analyzed using all six samples per group ($n = 6$). The protein expression level of Nrf2 and HO-1 in the fetal liver was analyzed using three samples randomly selected from different litter per group ($n = 3$). Moreover, the other parameters were analyzed using six placentas randomly selected from different litter per group as the experimental unit ($n = 6$). As shown in previous studies, alterations caused by sex difference on the fetal weight, placental weight, and placental functions may not be present in late gestation [29–31]. Therefore, sex difference was not considered in the present study.

All the data are presented as means ± SEM. The Shapiro–Wilk test was used to assess the normality distribution of the data. Statistical differences were determined by one-way analysis of variance followed by Tukey's test (Graph Pad Software Inc. 7.0. San Diego, CA, USA). $P < 0.05$ was considered to be statistically significant.

Table 2. The primer sequences for real-time PCR.

Genes (GeneBank)	Primer sequences (5′–3′)	Product size (bp)
Nrf2	F: CCATGTGTGGCAGTCCATGAT	183
(NM_006164.5)	R: GCAGGCATACCATTGTGGAT	
HO-1	F: GGAAATCATCCCTTGCACGC	234
(NM_010442.2)	R: TGTTTGAACTTGGTGGGGCT	
IGF1	F: AGAGCCTGCGCAATGGAATA	152
(NM_010512.5)	R: TGCTGATTTTCCCCATCGCT	
IGF2	F: GCCTCGTCACTTCTCCTACG	83
(NM_001122736.2)	R: CAGTGTCCAGTGCGTGTTTG	
VEGF	F: ACATTGGCTCACTTCCAGAAACAC	107
(NM_001110268.1)	R: GGTTGGAACCGGCATCTTTATC	
SNAT1	F: TCAGCCTGGTACGTCGATGG	85
(XM_006520235.4)	R: CCAGGTTCTTCAAGAGACACAG	
SNAT2	F: AGAGCAATTCCAGTATTAGC	76
(NM_175121.4)	R: TTAATCTGAGCAATGCGATTGTG	
SNAT4	F: GGCAGTGGTGTGGAGTACGAAGC	90
(XM_006521351.3)	R: TGGAATCGCGTAGGCCGTG	
SOD1	F: ATTGGCCGTACAATGGTGGT	116
(NM_011434.2)	R: ATCCCAATCACTCCACAGGC	
SOD2	F: GTAGGGCCTGTCCGATGATG	130
(NM_013671.3)	R: CGCTACTGAGAAAGGTGCCA	
CAT	F: AATCCTACACCATGTCGGACA	726
(NM_009804.2)	R: CGGTCTTGTAATGGAACTTGC	
GSH-Px	F: GAAGTGCGAAGTGAATGG	240
(NM_001329528.1)	R: TGTCGATGGTACGAAAGC	
GCLC	F: GGATGATGCCAACGAGTC	180
(NM_010295.2)	R: GTGAGCAGTACCACGAATA	
NQO1	F: CTTTAGGGTCGTCTTGGC	102
(NM_008706.5)	R: CAATCAGGGCTCTTCTCG	
GAPDH	F: TGGAGAAACCTGCCAAGTATGA	135
(NM_008084.3)	R: TGGAAGAATGGGAGTTGCTGT	
18S	F:ACCCCAGCAAGGACACTGAGCAAG	109
(NM_001289726.1)	R: GGCCCCTCCTGTTATTATGGGGGT	

Nrf2, nuclear factor-erythroid 2-related factor 2; *HO-1*, home oxygenase-1; *IGF1*, insulin-like growth factors-1; *IGF2*, insulin-like growth factors-2; *VEGF*, vascular endothelial growth factor; *SNAT1*, sodium-coupled neutral amino acid transporter 1; *SNAT2*, sodium-coupled neutral amino acid transporter 2; *SNAT4*, sodium-coupled neutral amino acid transporter 4; *SOD1*, superoxide dismutase-1; *SOD2*, superoxide dismutase-2; *CAT*, catalase; *GSH-PX*, glutathione peroxidase; *GCLC*, Glutamate-cysteine ligase catalytic; *NQO1*, NAD(P)H quinone dehydrogenase 1; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *18S*, 18S ribosomal RNA.

Results

Placenta and fetal measures

As shown in Figure 1A, the growth curve of the pregnant mice showed no differences ($P > 0.05$). Figure 1B showed that the fetal weight of the LP group significantly decreased ($P < 0.05$) compared with the NP group. The fetal weight of the LPL group significantly increased ($P < 0.05$) compared with the LP group, while it was lower ($P < 0.05$) than the NP and LPH groups. LPH group showed no difference ($P > 0.05$) compared with the NP group on fetal weight. The placental weight of the LP group remarkably decreased ($P < 0.05$) compared with other three groups. Furthermore, the placental diameter showed no differences ($P > 0.05$) among any groups (Figure 1C). The fetal/placental weight ratio significantly decreased ($P < 0.05$) in the LP group, while it increased to the control level in the LPH group.

Maternal serum biochemical index

Compared with the NP group, the LP group showed enhanced ($P < 0.05$) maternal serum corticosterone level, while the LPL and LPH groups exhibited no significant difference (Figure 2A). We observed that the maternal serum leptin level had no difference ($P > 0.05$) among any groups (Figure 2B). Notably reduced maternal serum progesterone levels were observed in all three groups fed the LP diet (Figure 2C).

Placental oxidative status

The effects of maternal curcumin supplementation during gestation on placental GSH-Px activity, CAT activity, and MDA content were analyzed. As shown in Figure 2D, the placental GSH-Px activity was significantly higher ($P < 0.05$) in the LPL group compared with the LP group. The levels of placental MDA content, a marker of lipid peroxidation markedly increased ($P < 0.05$) in the LP group, while treatment with curcumin reduced the concentration of MDA to the control level in the LPL and LPH groups (Figure 2E). The CAT activity showed no differences ($P > 0.05$) among any groups (Figure 2F).

Morphologic observations on placenta

We performed H&E staining on the mice placenta and analyzed the area of blood sinusoids using Image-Pro Plus software. As shown in Figure 3A and B, we found that the LP group had a strikingly

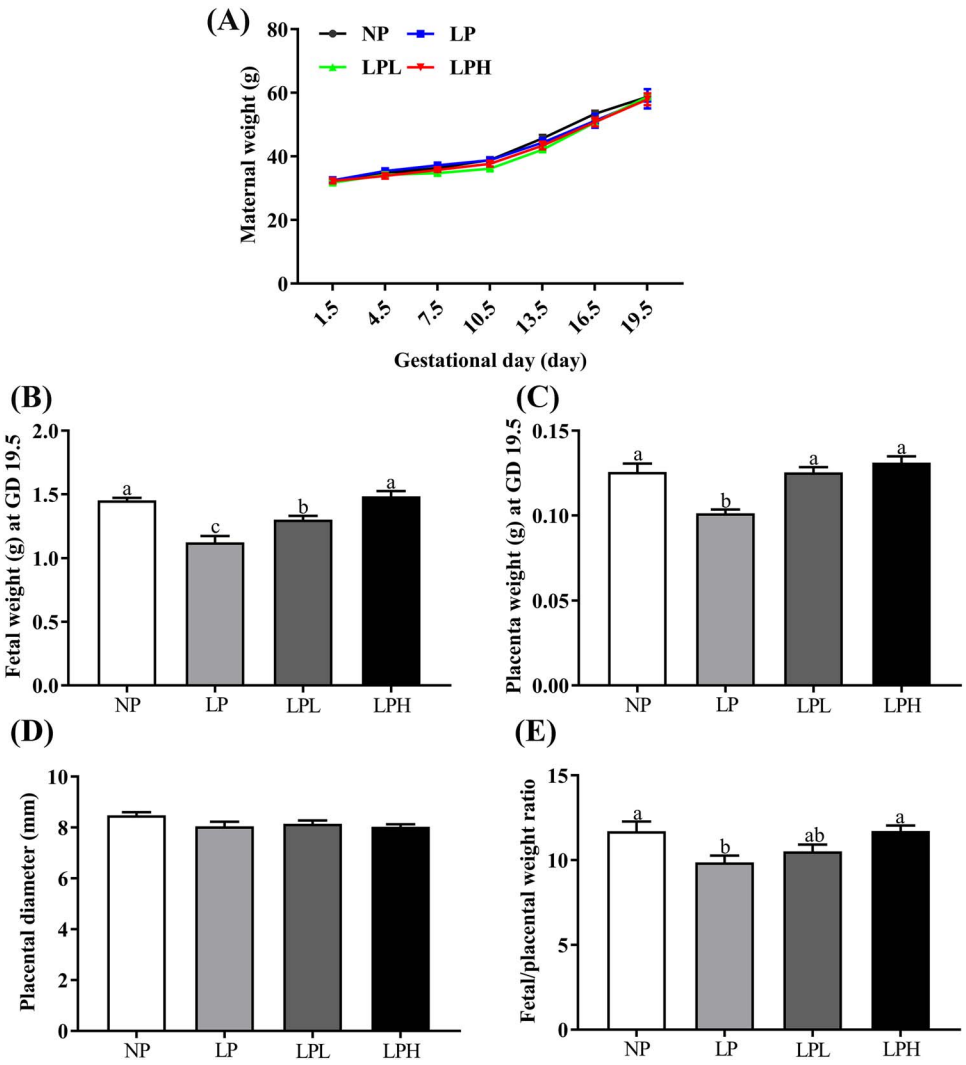

Figure 1. Effects of maternal curcumin supplementation on the maternal weight (A), fetal weight (B), placental weight (C), placental diameter (D) and fetal/placental weight ratio (E) on GD 19.5. Data are represented as means ± SEM ($n = 6$). Values with different letters (a, b, c) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin.

reduced ($P < 0.05$) blood sinusoids area. And the LPH group showed a significantly increased ($P < 0.05$) area compared with the LP group.

TUNEL assay

The apoptotic cells of placenta were presented in red. Figure 3C and D showed that the LP diet enhanced the placental apoptosis in the LP group, while daily maternal curcumin supplementation alleviated the apoptosis in placenta. The LP group exhibited a higher ($P < 0.05$) apoptotic index compared with the other three groups.

Placental gene expression

As shown in Figure 4, the LP group showed significantly decreased ($P < 0.05$) mRNA expression of *Nrf2* compared with the other three groups. The LP group exhibited significantly decreased ($P < 0.05$) *HO-1* expression compared with the NP group, while no differences ($P > 0.05$) were found between the LP group and the curcumin-treated groups. *IGF1* mRNA expression significantly decreased ($P < 0.05$) in the LP and LPL groups, and the LPH group showed no difference compared with the other three groups. *VEGF* mRNA expression significantly elevated ($P < 0.05$) in the LPL group compared with the LP group. *SNAT2* mRNA expression markedly decreased ($P < 0.05$) in all three groups fed the LP diet. In addition, we observed no differences ($P > 0.05$) on the mRNA expressions of *IGF2*, *SNAT1*, and *SNAT4* among any groups.

Gene expression of fetal liver

We tested the mRNA expression of antioxidant genes in the fetal liver of each treatment. The results were showed in Figure 5. Low protein treatment significantly decreased ($P < 0.05$) the mRNA expression of *SOD1*, *SOD2*, *GSH-Px*, and *CAT* compared with the NP group. However, daily maternal curcumin supplementation improved ($P < 0.05$) the expression of *SOD1*, *SOD2*, and *CAT* in the LPL and LPH groups. Furthermore, the mRNA expression of *Nrf2*, *HO-1*, *GCLC*, and *NQO1* significantly decreased ($P < 0.05$) in the LP group. The LPL group increased ($P < 0.05$) the expression of *GCLC* and *NQO1*, and the LPH group enhanced ($P < 0.05$) the expression of *NQO1* compared with the LP group.

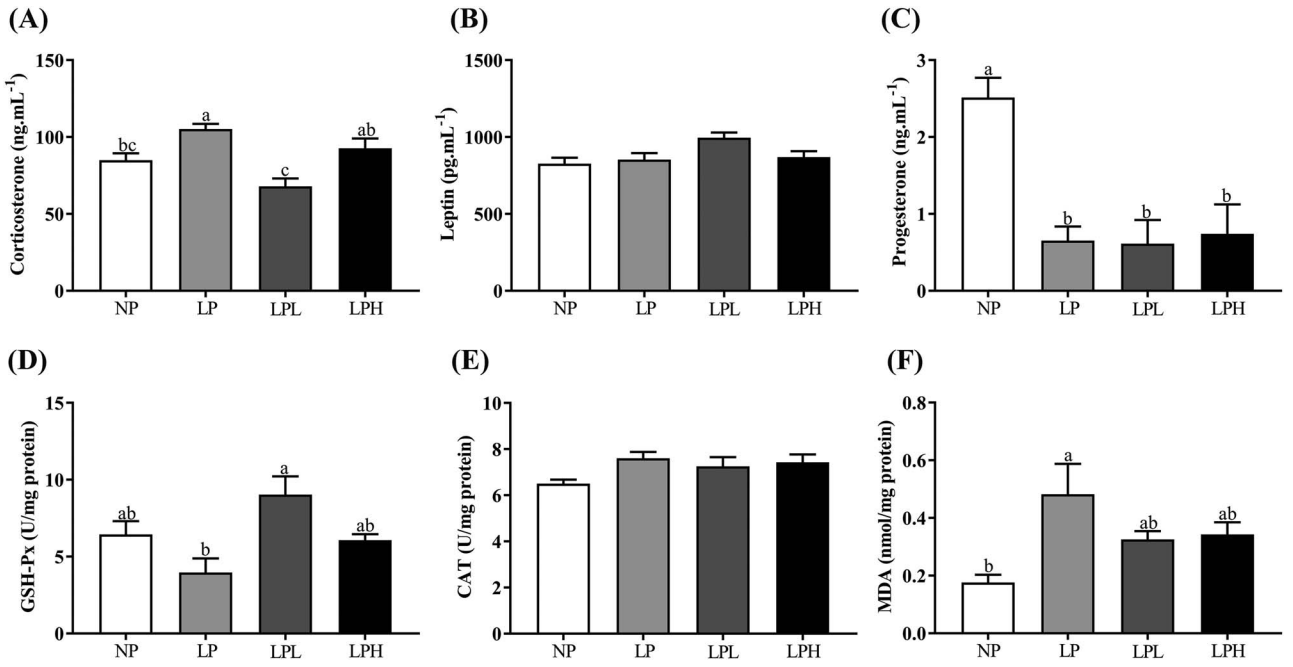

Figure 2. Effects of maternal curcumin supplementation on maternal serum corticosterone (A), leptin (B), progesterone level (C), placental GSH-Px activity (D), CAT activity (E), and MDA content (F). Data are represented as means ± SEM ($n = 6$). Values with different letters (a, b, c) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin.

Protein expression of fetal liver

Figure 6 showed the protein expression of Nrf2 and HO-1 in the fetal liver. The protein expression of Nrf2 significantly increased in the LPL and LPH groups compared with the NP group. In comparison with the LP group, the LPH group exhibited significantly elevated protein expression of HO-1. All uncropped blots from Figure 6 were presented in Supplemental Figure S1.

Discussion

Increasing production of placental oxidants induced by greater maternal and fetal metabolism has been considered as an important factor that leads to IUGR [32, 33]. Curcumin is found to exert antioxidant property, and it has been studied as an additive in animal feed to reduce oxidative stress [34, 35]. Thus we hypothesize that administration of curcumin during maternal pregnancy would alleviate the redox damage on placenta, which, in turn, attenuates the growth retardation of IUGR offspring. In the present study, we used an IUGR model of mice fed a LP diet described elsewhere [12–14]. Daily curcumin treatment in mice fed with the LP diet during pregnancy increased fetal weight, placental weight, fetal weight/placental weight ratio, GSH-Px activity, the blood sinusoids area, antioxidant gene expression of placenta and fetal liver, and antioxidant protein expression of fetal liver. In addition, curcumin decreased placental MDA content and apoptotic index. These results might strengthen the potential effects of curcumin to protect against the oxidative stress of placenta and growth retardation of fetus due to IUGR.

Intrauterine growth retardation is a serious reproductive problem in humans and other mammals. A large number of studies have proven that IUGR induced by maternal nutrition deficiency including protein restriction could cause growth retardation on the fetus [13, 36, 37]. In line with these studies, we observed that the LP diet resulted in IUGR with decreased placental weight, fetal weight, and fetal/placental weight ratio. Fetal/placental weight ratio is widely considered as placental efficiency, which reflects the placental development and function of nutrient transportation [38]. However, daily administration of curcumin increased placental weight, fetal weight, and placental efficiency of mice fed with the LP diet to levels similar with the NP group. Moreover, *IGF* and *VEGF* mRNA expression has been reported to be positively correlated with placental efficiency and function [28, 39]. The results of increased *VEGF* and *IGF1* mRNA expression were in accordance with increased placental efficiency. However, placental *SNAT2* expression reduced in mice fed with the LP diet, even with daily maternal supplementation of curcumin. Low-protein diet did not alter the mRNA expression of *SNAT1* and *SNAT4*. *SNAT* genes are important for supplying neutral amino acids needed for fetal growth [40]. This result might indicate that curcumin could restore the placental efficiency of IUGR without affecting the transportation of amino acids. Therefore, our data suggest that curcumin could improve placental efficiency and growth retardation of IUGR offspring. Since it is still unclear whether sex difference of the fetus would affect the placental function after administration of curcumin during pregnancy, further studies are required to confirm it.

Maternal nutrient deficiency has been reported to increase maternal corticosterone level in mice [41]. We also found that mice fed with the LP diet exhibited an elevated serum corticosterone level. In mice, excessive corticosterone before birth may affect heart maturation, and induce potential long-term adverse consequences [42]. Daily maternal curcumin supplementation inhibited the elevated serum corticosterone level resulting from the LP diet. The exact mechanism of this result requires further investigation. Moreover, the serum progesterone level surprisingly reduced in mice fed with the LP diet.

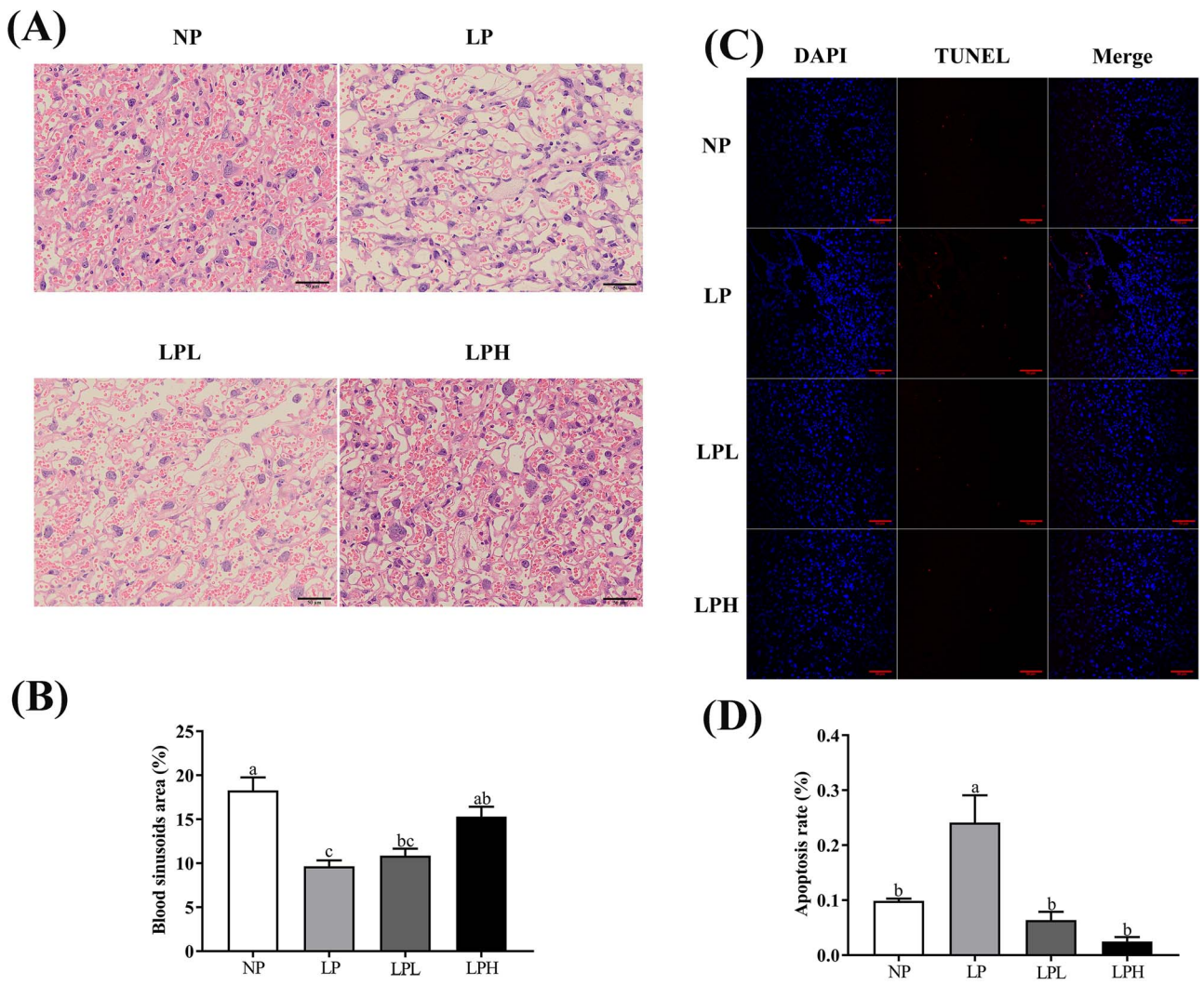

Figure 3. Suggestive images of placenta in each group (A) and statistical analysis of blood sinusoids area percentage (B). TUNEL assay of placenta in each group (C) and statistical analysis of apoptotic index (D). The blue color represents the total cells, and the red color represents the apoptosis cells in placenta. Data are represented as means ± SEM ($n = 6$). Values with different letters (a, b, c) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin. Scale bar = 50 μm.

Progesterone is essential for maintaining the gestation, and is mainly secreted from corpus luteum in the ovary. Lower progesterone level at late pregnancy increases the risk of premature delivery [43]. Nevertheless, curcumin had no effect on the secretion of progesterone in mice fed with the LP diet.

Placenta is extremely sensitive to oxidative stress. During pregnancy, the metabolisms of mother and fetus are enhanced due to higher energy and oxygen requirements [44]. This could result in an increased production of oxidants and eventually oxidative stress. Numerous studies have revealed that maternal protein deficiency could enhance placental oxidative stress, which leads to IUGR in mice [45–47]. Malondialdehyde is a primary marker of lipid peroxidation. In this study, we observed that the LP diet increased the MDA content, while administration of curcumin restored the lipid damage. A considerable amount of evidence has shown that curcumin could inhibit the production of MDA in the oxidative stress of mice [48–50]. Additionally, GSH-Px is able to convert reduced glutathione into oxidized glutathione, which acts as a free radical scavenger to decompose H_2O_2. Consistent with the previous studies that curcumin could enhance the GSH-Px activity [51, 52], we also found that curcumin increased the reduced GSH-Px activity induced by the LP diet. Furthermore, we observed increased mRNA expression of *Nrf2* and *HO-1* in the placenta of LPL and LPH groups. *Nrf2* is an essential transcription factor of antioxidant-related genes, and it controls the transcription of *HO-1*. It is well established that *Nrf2-HO-1* pathway is a critical pathway for maintaining redox balance in the body [53]. Similarly, a previous study also indicated that dietary curcumin increased the mRNA expression of *Nrf2* in the liver of rats with IUGR [24]. Thus, these data indicate that curcumin could protect the placenta of IUGR from oxidative stress.

The growth and development of fetus require nutrient transport from placenta. The placental labyrinth is the main tissue for exchanging nutrients between mother and fetus [54]. Intrauterine growth retardation is closely related to the loss of blood sinusoids area in placental labyrinth, which negatively affects the nutrient

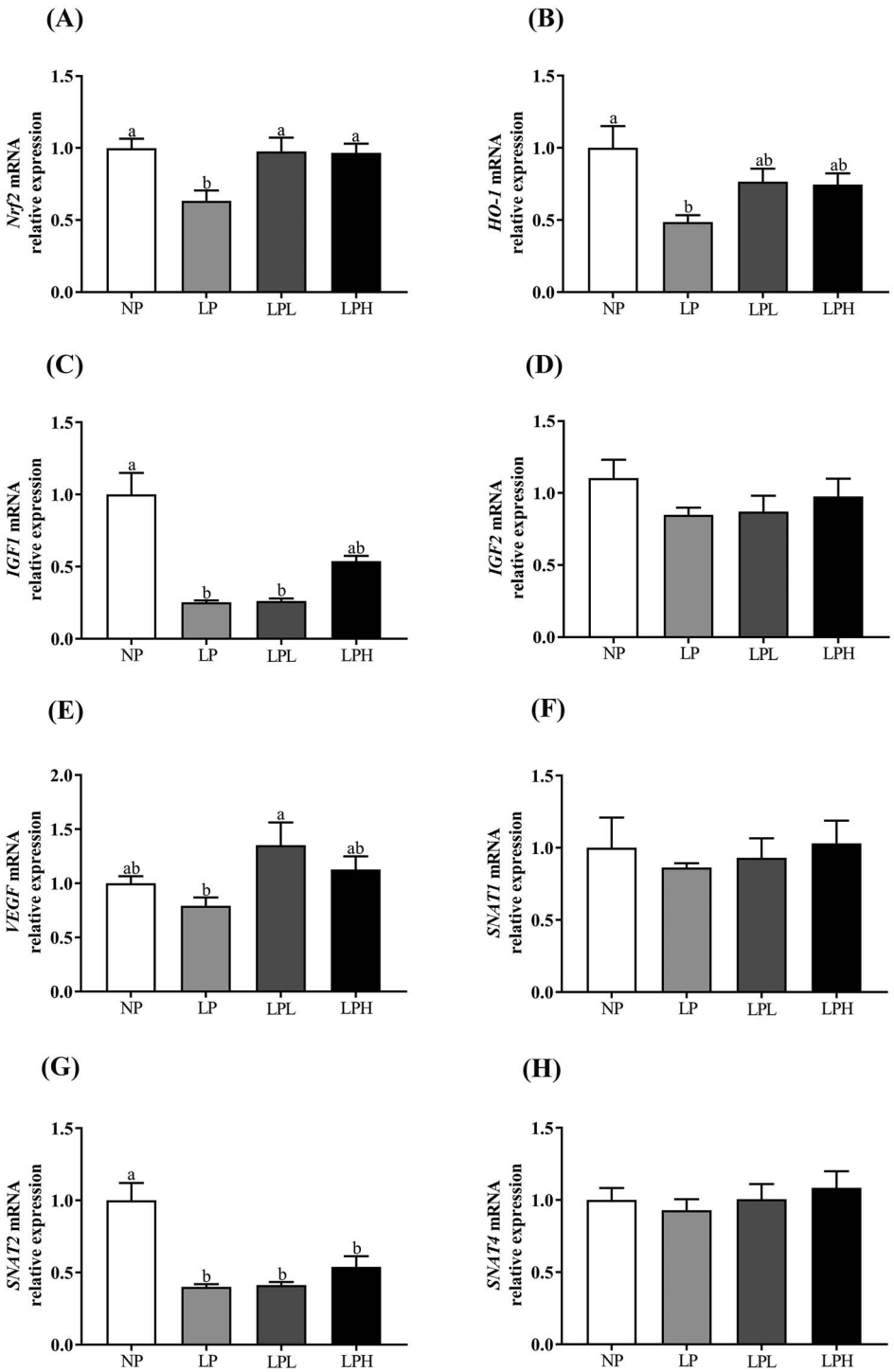

Figure 4. Effects of maternal curcumin supplementation on placental mRNA expression of *Nrf2* (A), *HO-1* (B), *IGF1* (C), *IGF2* (D), *VEGF* (E), *SNAT1* (F), *SNAT2* (G) and *SNAT4* (H). Data are represented as means ± SEM ($n = 6$). Values with different letters (a, b) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin. *Nrf2*, nuclear factor-erythroid 2-related factor 2; *HO-1*, home oxygenase-1; *IGF1*, insulin-like growth factors-1; *IGF2*, insulin-like growth factors-2; *VEGF*, vascular endothelial growth factor; *SNAT1*, sodium-coupled neutral amino acid transporter 1; *SNAT2*, sodium-coupled neutral amino acid transporter 2; *SNAT4*, sodium-coupled neutral amino acid transporter 4.

transportation in placenta [55]. Here, we observed that the LP diet significantly decreased the blood sinusoids area in mice placenta, while daily maternal curcumin supplementation repaired this damage in placental labyrinth. Based on the statistical analysis, we found that high-dose curcumin exerted better prophylactic effect on restoring the loss of blood sinusoids area induced by the LP diet.

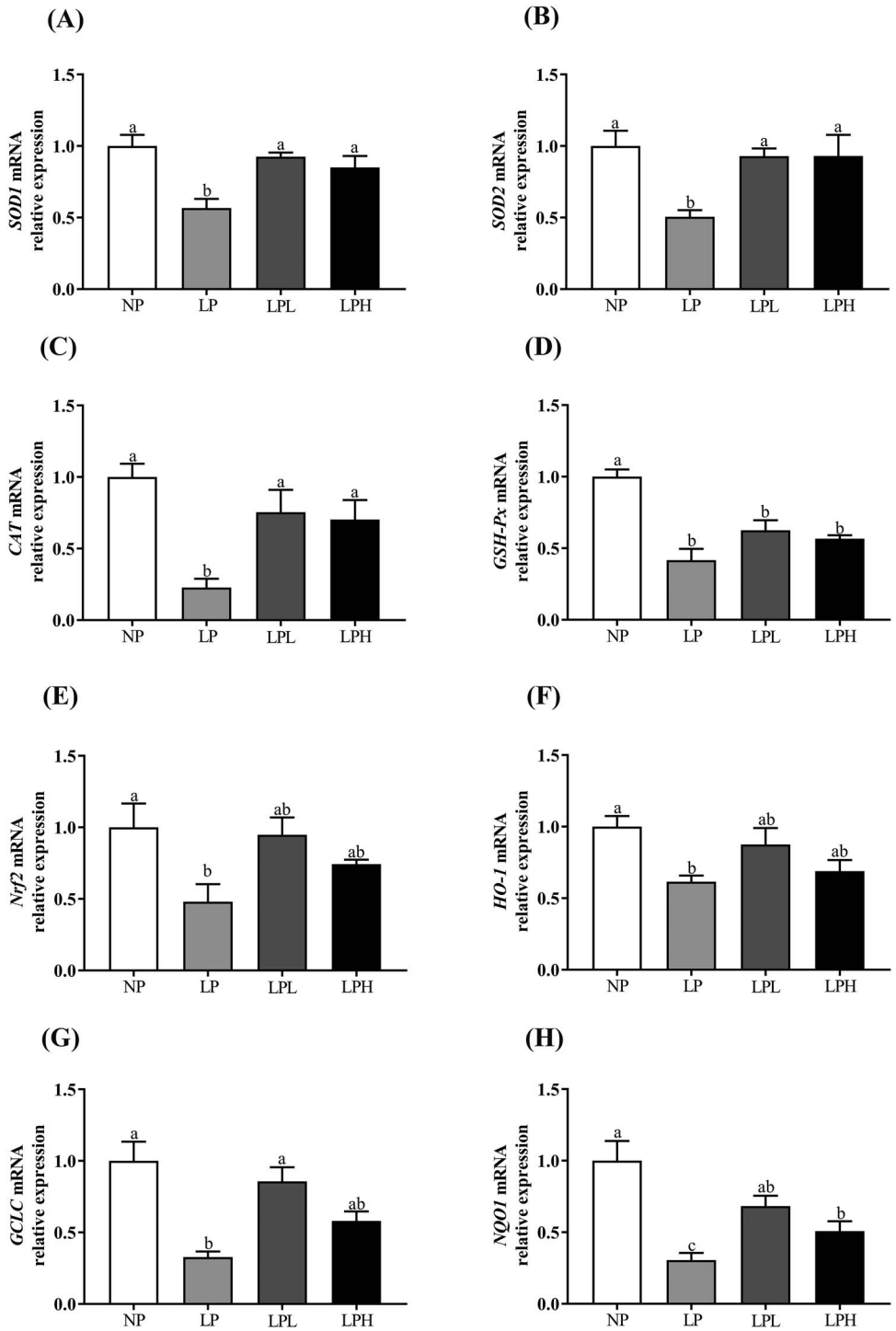

Figure 5. Effects of maternal curcumin supplementation on mRNA expression of *SOD1* (A), *SOD2* (B), *CAT* (C), *GSH-Px* (D), *Nrf2* (E), *HO-1* (F), *GCLC* (G) and *NQO1* (H) in the fetal liver. Data are represented as means ± SEM ($n = 6$). Values with different letters (a, b) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin. *SOD1*, superoxide dismutase-1; *SOD2*, superoxide dismutase-2; *CAT*, catalase; *GSH-PX*, glutathione peroxidase; *Nrf2*, nuclear factor-erythroid 2-related factor 2; *HO-1*, home oxygenase-1; *GCLC*, Glutamate—cysteine ligase catalytic; *NQO1*, NAD(P)H quinone dehydrogenase 1.

Hence, our data show that curcumin could protect against the loss of blood sinusoids area in a dose-effect manner.

Placental trophoblastic cells require apoptosis and proliferation to transport cholesterol in the placenta [56]. Cell apoptosis is involved in the cellular growth and development of placenta, and balance of them is important for sustaining the placental functions. Previous studies have indicated that IUGR could induce excessive apoptosis in placenta of mammals, which is harmful for both mother

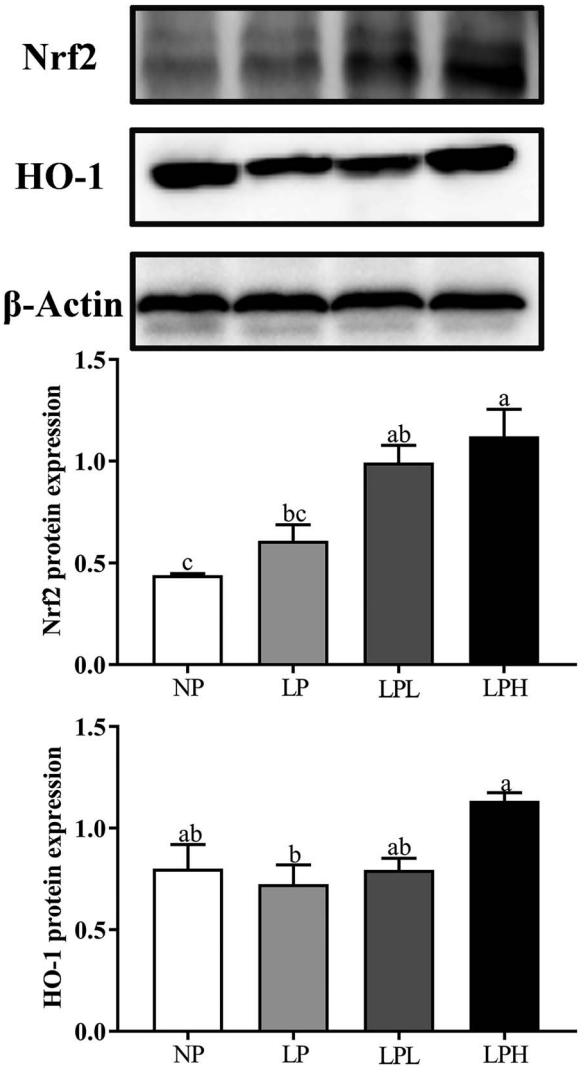

Figure 6. Western blot analysis of Nrf2 and HO-1 protein expression level in the fetal liver. Data are represented as means ± SEM ($n = 3$). Values with different letters (a, b) are significantly different ($P < 0.05$). NP, normal protein (19% protein); LP, low protein (8% protein); LPL, low protein (8% protein) plus daily 100 mg/kg curcumin; LPH, low protein (8% protein) plus daily 400 mg/kg curcumin.

and fetus [57–59]. Oxidative stress also contributes to inducing apoptosis in the placenta of IUGR [60]. The present study corroborated these findings and found an enhanced apoptotic index in the placenta of the LP group. However, increased apoptosis of placenta was alleviated by daily gavage of curcumin. We hypothesize that the potential mechanisms are likely to involve two processes. First, curcumin could activate the Nrf2-ARE (antioxidant response element) pathway, which reduced the reactive oxygen species, and therefore decreased caspase-dependent apoptosis [61, 62]. Second, the transcription of nuclear factor kappaB could be repressed by curcumin, and the placental apoptosis was ultimately inhibited through down-regulation of c-Jun N-terminal kinase pathway [63, 64]. Further molecular studies are required to determine the exact mechanism.

Previous studies have demonstrated that the LP diet could cause liver damage on the offspring of IUGR [14, 65]. In the current study, we collected the liver of unborn fetus. We found that the LP diet induced oxidative stress on the fetal liver during gestation proven by significantly decreased mRNA expression of antioxidant genes. Similar to the maternal placental oxidative status, curcumin reversed redox damage in the fetal liver. Curcumin treatment recovered the mRNA expression of *SOD1*, *SOD2*, and *CAT* in the fetal liver. This might subsequently increase the activity of SOD and CAT, which could convert H_2O_2 into oxygen and water. In addition, the up-regulated expression of *Nrf2-HO-1* pathway induced by daily maternal curcumin supplementation was also observed in both transcription and protein levels. As mentioned above, *Nrf2-HO-1* pathway contributes to controlling the redox homeostasis. Furthermore, the expression of *GCLC* and *NQO1* showed a similar pattern as *Nrf2* and *HO-1* in the fetal liver of IUGR. *GCLC* and *NQO1* are the downstream *Nrf2*-dependent antioxidant defense genes. *GCLC* is responsible for increasing the synthesis of glutathione [66]. *NQO1* is a detoxification enzyme that reduces NADH, and depresses the production of reactive oxygen intermediates by redox cycling [67]. Supportively, curcumin could attenuate liver injury in mice by activating *Nrf2-HO-1* pathway and elevating the downstream *GCLC* and *NQO1* expression [68]. We also found that the protein expression of Nrf2 and HO-1 increased in the fetal liver of the LPH group, which suggesting the activation of antioxidant pathways and an enhanced antioxidant capacity. Consequently, we speculate that curcumin could increase the expression of *Nrf2-HO-1* pathway to improve the redox balance, which protects against the oxidative stress in the fetal liver of mice with IUGR.

Conclusions

In conclusion, daily maternal curcumin supplementation can improve placental function, fetal growth, and redox balance of fetal liver in mice with IUGR induced by the LP diet. Our results demonstrate the nutritional and reproductive importance of curcumin supplementation, and may be helpful for developing a new prophylactic strategy for IUGR.

Supplementary data

Supplementary data is available at *BIOLRE* online.

Conflict of interest

The authors have declared that no conflict of interest exists.

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