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Reactive oxygen species are involved in lipopolysaccharide-induced intrauterine growth restriction and skeletal development retardation in mice

De-Xiang Xu, MD, PhD,[a,b,*] Yuan-Hua Chen, MS,[a] Lei Zhao, MS,[a] Hua Wang, MS,[a] Wei Wei, MD, PhD[b]

Department of Toxicology, Anhui Medical University[a]; Key Laboratory of Anti-Inflammatory and Immunopharmacology of Anhui Province,[b] Hefei, People's Republic of China

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KEY WORDS
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Objective: Maternal infection is a cause of adverse developmental outcomes including embryonic resorption, intrauterine fetal death, and preterm labor. Lipopolysaccharide-induced developmental toxicity at early gestational stages has been well characterized. The purpose of the present study was to investigate the effects of maternal lipopolysaccharide exposure at late gestational stages on intrauterine fetal growth and skeletal development and to assess the potential role of reactive oxygen species in lipopolysaccharide-induced intrauterine fetal growth restriction and skeletal development retardation.

Study design: The timed pregnant CD-1 mice were intraperitoneally injected with lipopolysaccharide (25 to 75 μg/kg per day) on gestational day 15 to 17. To investigate the role of reactive oxygen species on lipopolysaccharide-induced intrauterine fetal growth restriction and skeletal development retardation, the pregnant mice were injected with alpha-phenyl-N-t-butylnitrone (100 mg/kg, intraperitoneally) at 30 minutes before lipopolysaccharide (75 μg/kg per day, intraperitoneally), followed by an additional dose of alpha-phenyl-N-t-butylnitrone (50 mg/kg, intraperitoneally) at 3 hours after lipopolysaccharide. The number of live fetuses, dead fetuses, and resorption sites was counted on gestational day 18. Live fetuses in each litter were weighed. Crown-rump and tail lengths were examined and skeletal development was evaluated.

Results: Maternal lipopolysaccharide exposure significantly increased fetal mortality, reduced fetal weight and crown-rump and tail lengths of live fetuses, and retarded skeletal ossification in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone in a dose-dependent manner. Alpha-phenyl-N-t-butylnitrone, a free radical spin-trapping agent, almost completely blocked lipopolysaccharide-induced fetal death (63.2% in lipopolysaccharide group versus 6.5% in alpha-phenyl-N-t-butylnitrone + lipopolysaccharide group, $P < .01$). In addition, alpha-phenyl-N-t-butylnitrone significantly reversed lipopolysaccharide-induced intrauterine growth restriction and skeletal development retardation. However, aminoguanidine, a selective inhibitor of inducible nitric oxide synthase, had little effect. Furthermore, lipopolysaccharide-induced intrauterine fetal death, intrauterine fetal growth restriction, and skeletal development retardation were

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* Reprint requests: Professor De-Xiang Xu, Department of Toxicology, Anhui Medical University, Hefei 230032, People's Republic of China.
E-mail: xudex@mail.hf.ah.cn

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associated with lipid peroxidation and glutathione depletion in maternal liver, placenta, and fetal liver. Alpha-phenyl-N-t-butylnitrone significantly attenuated lipopolysaccharide-induced lipid peroxidation and glutathione depletion in maternal liver, placenta, and fetal liver.

Conclusion: Maternal lipopolysaccharide exposure at late gestational stages results in intrauterine fetal growth restriction and skeletal development retardation in mice. Reactive oxygen species might be, at least in part, involved in lipopolysaccharide-induced intrauterine fetal growth restriction and skeletal development retardation.

Lipopolysaccharide (LPS) is a toxic component of cell walls of Gram-negative bacteria and is widely present in the digestive tracts of humans and animals.[1] Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol drinking often increase permeability of LPS from gastrointestinal tract into blood.[2] Maternal LPS exposure has been associated with adverse developmental outcome, including embryonic resorption, intrauterine fetal death (IUFD), and preterm labor in animals.[3,4] However, the exact mechanism of LPS-induced developmental toxicity remains unclear.

Eicosanoids appear to be important mediators of adverse developmental outcomes caused by LPS. Several studies[5-7] indicate that LPS-induced embryo death was associated with cyclooxygenase-2-mediated eicosanoid production. Pretreatment with cyclooxygenase-2 selective inhibitors, indomethacin, SC236, and Celecoxib, significantly attenuated LPS-induced fetal death and preterm delivery. On the other hand, the role of tumor necrosis factor alpha (TNF-α) on LPS-induced developmental toxicity has been demonstrated. First, TNF-α has been associated with preterm labor and delivery caused by Gram-negative bacterial infection in humans.[8] Animal experiments indicate that rapid increases in the maternal serum TNF-α levels contribute to LPS-induced embryo death.[9] Recent studies found that LPS increased TNF-α production in amniotic fluid.[10-12] Our earlier study showed that perinatal LPS exposure upregulated TNF-α messenger ribonucleic acid expression in mouse placenta.[13] Furthermore, pentoxifylline, a TNF-α suppressor, reversed LPS-induced embryonic resorption and abortion.[14] However, a recent study found that LPS-induced IUFD was not blocked by treatment with anti-TNF antibody that inhibited LPS-induced TNF-α production in pregnant females.[15] Another important study by Casado et al,[16] comparing the difference in plasma TNF-α levels between that in mother and fetus, showed that less than 7% of TNF-α was detected in the corresponding fetal serum, although serum from the mother treated with LPS exhibited a significant increase in TNF-α levels, suggesting a restricted permeability of placenta to proinflammatory cytokines. These results indicate that LPS-induced developmental toxicity could not be attributed to mother-derived TNF-α alone.

LPS stimulates macrophages to generate reactive oxygen species (ROS) and increases nitrotyrosine, a marker for superoxide, nitric oxide (NO), and peroxynitrite, formation in macrophage-rich organs.[17] A previous study[18] showed that N-acetylcysteine, a glutathione (GSH) precursor and direct antioxidant, protected against fetal death, preterm delivery, and hepatic GSH depletion in the mother and fetus. In the present study, we investigated the effects of maternal LPS exposure at late gestational stages on intrauterine fetal growth and skeletal development. We also investigated the effects of alpha-phenyl-N-t-butylnitrone (PBN), a free radical spin-trapping agent, and aminoguanidine (AG), a selective inhibitor of inducible NO synthase, on LPS-induced intrauterine fetal growth restriction and skeletal development retardation.

Material and methods

Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8), AG, and PBN were purchased from Sigma Chemical Co (St Louis, MO). All the other reagents were from Sigma or as indicated in the specified methods.

Animals and treatments

The CD-1 mice (8 to 10 weeks old; male mice: 30 to 32 g; female mice: 26 to 28 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. (Wilmington, MA). The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20 to 25°C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, 4 females were housed overnight with 2 males starting at 9:00 P.M. Females were checked by 7:00 A.M. the next morning, and the presence of a vaginal plug was designated as gestational day (gd) 0. The present study included 2 separate experiments.

Experiment 1

To investigate the effects of PBN on LPS-induced IUFD, intrauterine fetal growth restriction and skeletal

development retardation, the timed pregnant mice were divided into several groups randomly. Group A was divided into 3 subgroups, depending on the dose of LPS administration. The pregnant mice were injected daily for 3 days from gd 15 to gd 17 with either the dose of 25 μg/kg per day (group A1, n = 14), 50 μg/kg per day (group A2, n = 14), or 75 μg/kg per day (group A3, n = 12). In group B, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg per day, intraperitoneally), followed by an additional dose of PBN (50 mg/kg, intraperitoneally) at 3 hours after LPS. In group C, the pregnant mice were injected with AG (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg per day, intraperitoneally). The saline-treated pregnant mice served as controls. All dams were killed on gd 18 and gravid uterine weights were recorded. For each litter, the number of live fetuses, dead fetuses, and resorption sites was counted. Live fetuses in each litter were weighed. Crown-rump and tail lengths were measured. All fetuses were then stored in ethanol a minimum of 2 weeks for subsequent skeletal evaluation.

Experiment 2

To investigate the effects of PBN on LPS-induced lipid peroxidation and GSH depletion, the timed pregnant mice were divided into 3 groups randomly. All pregnant mice except controls received an intraperitoneal injection of LPS (75 μg/kg) once on gd 15. In the LPS + PBN group, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg, intraperitoneally), followed by an additional dose of PBN (50 mg/kg) at 3 hours after LPS. The saline-treated pregnant mice served as controls. Dams were killed at 6 hours after LPS treatment. Maternal serum, maternal liver, placenta, amniotic fluid, and fetal liver samples were excised for measurement of thiobarbituric acid-reactive substance (TBARS) and GSH contents.

All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

Skeletal examination and evaluation

The fetuses stored in ethanol were cleared from skin, viscera, and adipose tissue. Fetuses were then incubated in acetone overnight and subsequently macerated and stained with alizarin red S for 2 days. After an overnight incubation in 70% ethanol/glycerol/benzyl alcohol, the fetuses were stored in glycerol until examination. Skeletal evaluation included determination of the degree ossification of the phalanges, metacarpals, vertebrae, sternatrae, and skull. The size of ossification of the supraoccipital was scored (1 = well ossified, 4 = completely unossified).

Determination of GSH content

Proteins of 0.4 mL liver homogenates were precipitated by the addition of 0.4 mL of a metaphosphoric acid solution. After 40 minutes, the protein precipitate was separated from the remaining solution by centrifugation at 5000 rpm at 4°C for 5 minutes. Four hundred microliters of the supernatant were combined with 0.4 mL of 300 mM Na_2HPO_4, and the absorbance at 412 nm was read against a blank consisting of 0.4 mL supernatant plus 0.4 mL H_2O. Then 100 μL 5, 5-a-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.02%, weight/volume; 20 mg DTNB in 100 mL of 1% sodium citrate) was added to the blank and sample. Absorbance of the sample was read against the blank at 412 nm. The GSH content was determined using a calibration curve prepared with an authentic sample. GSH values were expressed as nanomoles of milligram^{-1} protein.

Lipid peroxidation assay

Lipid peroxidation was quantified by measuring TBARS. Tissue was homogenized in 9 volumes of 50 mmol/L Tris-HCl buffer (pH 7.4) containing 180 mmol/L KCl, 10 mmol/L EDTA, and 0.02% butylated hydroxytoluene. To 0.2 mL of the tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid, 1.5 mL of 0.9% thiobarbituric acid, and 0.6 mL of distilled water were added and vortexed. The reaction mixture was placed in a water bath at 95°C for 1 hour. After cooling on ice, 1.0 mL of distilled water and 5.0 mL of butanol/pyridine mixture (15:1, volume/volume) were added and vortexed. After centrifugation at 10,000 g for 10 minutes, absorbance of the resulting lower phase was determined at 532 nm. The TBARS concentration was calculated using 1,1,5,5-tetraethoxypropane as standard.

Statistical analysis

The litter was considered the unit for statistical comparison among different groups. Fetal mortality was calculated per litter and then averaged per group. Fetal mortality was compared using either Fisher's exact or χ^2 test. For fetal weight, crown-rump and tail lengths, and skeletal evaluation, the means were calculated per litter and then averaged per group. Quantified data were expressed as means ± SEM at each point. $P < .05$ was considered statistically significant. Analysis of variance and the Student-Newmann-Keuls post hoc test were used to determine differences between the treated animals and the control and statistical significance.

Results

LPS-induced fetal death

Administration with LPS (25, 50, and 75 μg/kg per day) on gd 15-17 exhibited no obvious maternal side effects

Table I Comparison of fetal outcomes among different groups

Treatments	Litters	Implantation sites per litter ($\bar{x} \pm S$)	Resorptions per litter ($\bar{x} \pm S$)	Live fetuses per litter ($\bar{x} \pm S$)	Dead fetuses per litter ($\bar{x} \pm S$)
Control	19	11.6 ± 2.12	0.58 ± 1.09	11.00 ± 2.10	0.05 ± 1.11
LPS (25 μg/kg)	12	11.0 ± 2.34	0.58 ± 0.87	9.83 ± 1.95	0.58 ± 1.58
LPS (50 μg/kg)	14	11.0 ± 2.09	0.21 ± 0.95	9.57 ± 1.84	1.21 ± 1.75
LPS (75 μg/kg)	12	13.4 ± 1.98	0.50 ± 0.86	4.75 ± 2.25*	8.17 ± 1.95*
PBN	14	12.1 ± 1.85	0.57 ± 0.84	12.1 ± 1.85	0.00 ± 0.00
PBN + LPS (75 μg/kg)	14	11.0 ± 1.97	0.14 ± 0.96	10.14 ± 2.46†	0.71 ± 1.09†
AG	10	12.0 ± 1.95	0.50 ± 0.45	12.0 ± 1.95	0.00 ± 0.00
AG + LPS (75 μg/kg)	10	12.2 ± 2.87	0.58 ± 0.57	4.33 ± 2.84	7.25 ± 2.44

* Significantly different from control, $P < .01$.
† Significantly different from LPS_{75} group, $P < .01$.

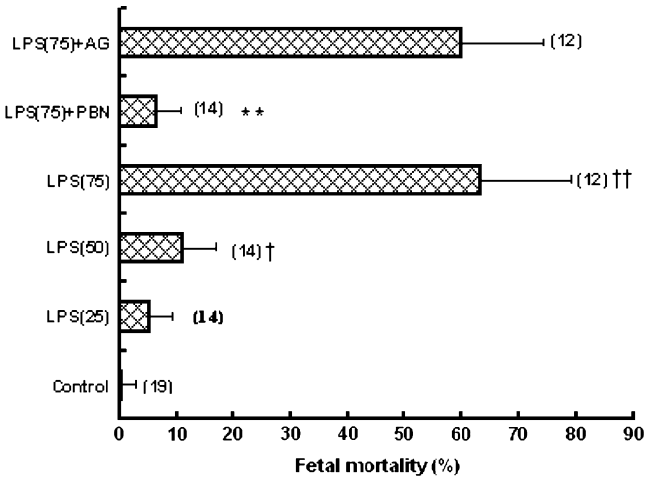

Figure 1 LPS-induced fetal death. In the LPS_{25} group, the pregnant mice received 25 μg/kg per day (intraperitoneally) of LPS on gd 15-17; in the LPS_{50} group, the pregnant mice received 50 μg/kg per day (intraperitoneally) of LPS on gd 15-17; in the LPS_{75} group, the pregnant mice received 75 μg/kg per day (intraperitoneally) of LPS on gd 15-17. In the LPS + PBN group, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg per day, intraperitoneally), followed by an additional dose of PBN (50 mg/kg, intraperitoneally) at 3 hours after LPS. In the LPS + AG group, the pregnant mice were injected with AG (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg per day, intraperitoneally). The pregnant mice were killed on gd 18. The number of live fetuses and dead fetuses were counted. Numbers of litters in each group were presented in *parentheses*. *Asterisk* denotes $P < .05$ as compared with LPS_{75} group. *Dagger* denotes $P < .05$, *double dagger* denotes $P < .01$ as compared with the control group.

(data not shown). Placental size was statistically indistinguishable among different groups (data not shown). The number of litters, implantation sites per litter, resorptions per litter, live fetuses per litter, and dead fetuses per litter are presented in Table I. There were no differences in the number of implantation sites among different groups. No preterm delivery was observed among different groups. As expected, maternal LPS exposure at late gestational stages did not increase embryonic resorptions. The effects of maternal LPS exposure at late gestational stages on fetal death are presented in Figure 1. Results showed that administration with LPS daily on gd 15-17 significantly increased fetal mortality in a dose-dependent manner. In the high-dose group, administration with 75 μg/kg per day of LPS on gd 15-17 resulted in up to 63.2 % fetal death, which was almost completely blocked by PBN ($P < .01$). However, AG had no effect on LPS-induced IUFD. Neither PBN nor AG alone increased fetal mortality (data not shown).

LPS-induced intrauterine fetal growth restriction

The effects of maternal LPS exposure at late gestational stages on fetal weight and crown-rump and tail lengths of live fetuses are presented in Figure 2, A, B, and C. Results showed that administration with LPS (25, 50, and 75 μg/kg per day) on gd 15-17 significantly decreased fetal weight and crown-rump and tail lengths of live fetuses in a dose-dependent manner. PBN treatment returned fetal weight and crown-rump and tail lengths of live fetuses to control levels. However, AG had less effect on LPS-induced intrauterine fetal growth restriction. Neither PBN nor AG alone had effects on fetal weight and crown-rump and tail lengths (data not shown).

LPS-induced on skeletal development retardation

The effects of maternal LPS exposure at late gestational stages on fetal skeletal development are presented in Table II. The skeleton of live fetuses in LPS group exhibited fewer ossification centers in caudal vertebrae and anterior and posterior phalanges as compared with the control group. In addition, LPS also retarded supraoccipital ossification in a dose-dependent manner. PBN restored the number of ossification centers to control level. However, AG treatment had little effect on LPS-induced skeletal development retardation. Neither

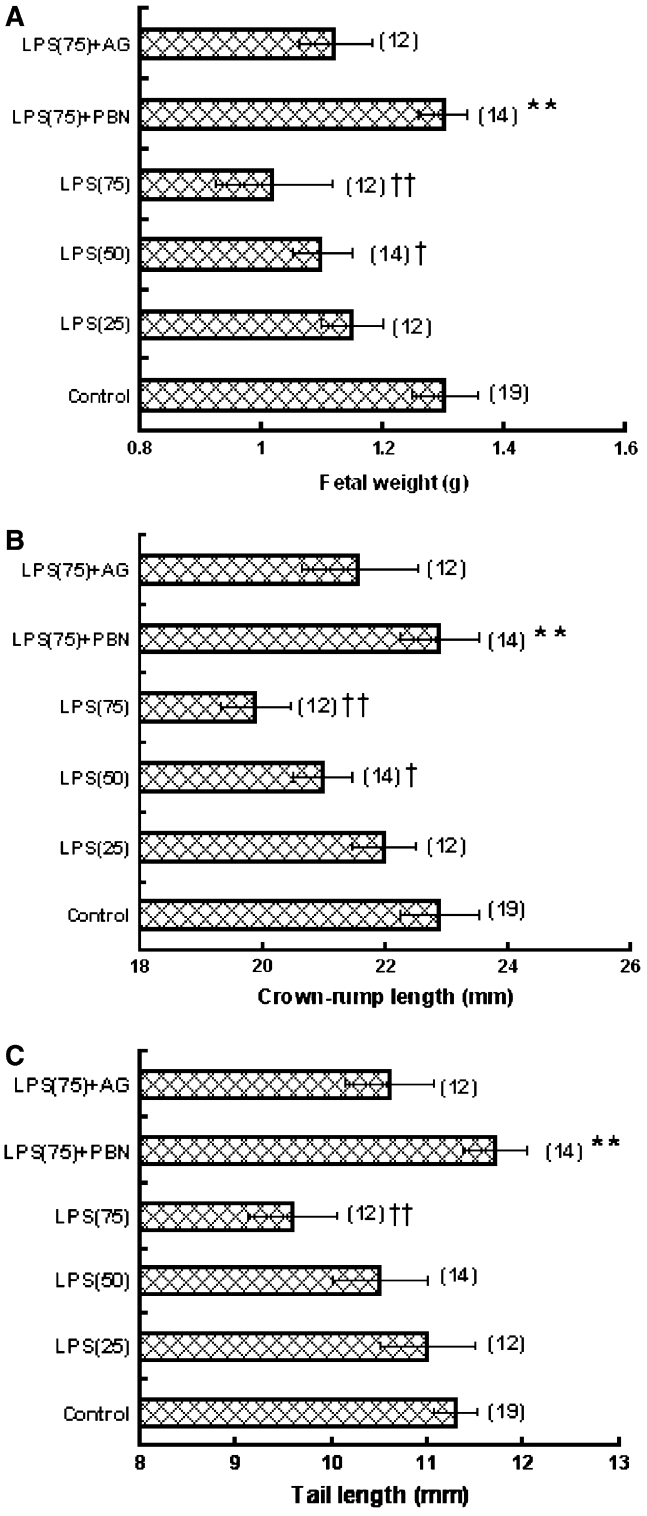

Figure 2 The effects of LPS on fetal growth development. In the LPS_{25} group, the pregnant mice received 25 μg/kg per day (intraperitoneally) of LPS on gd 15-17; in the LPS_{50} group, the pregnant mice received 50 μg/kg per day (intraperitoneally) of LPS on gd 15-17; in the LPS_{75} group, the pregnant mice received 75 μg/kg per day (intraperitoneally) of LPS on gd 15-17. In the LPS + PBN group, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 min before LPS (75 μg/kg per day, intraperitoneally), followed by an additional dose of PBN (50 mg/kg, intraperitoneally) at 3 hours after LPS. In the LPS+AG group, the pregnant mice were injected with AG (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg per day, intraperitoneally). The pregnant mice were killed on gd 18. Fetal weight and crown-rump and tail lengths were measured. Data were expressed as means ± SEM. Numbers of litters in each group were presented in *parentheses*. *A*. The effects of LPS on fetal weight. *B*. The effects of LPS on crown-rump length. *C*. The effects of LPS on tail length. *Asterisk* denotes $P < 0.05$ as compared with LPS_{75} group. *Dagger* $P < 0.05$, *double dagger* $P < 0.01$ as compared with control group.

PBN nor AG alone had effects on fetal skeletal development (data not shown).

LPS-induced lipid peroxidation

Lipid peroxidation was quantified by measuring TBARS. The effects of LPS exposure at late gestational stages on TBARS level are presented in Figure 3. Results showed that administration with LPS once on gd 15 caused a significant increase in TBARS levels in maternal liver and placenta, both of which were attenuated by PBN. Furthermore, administration with a single dose of LPS (75 μg/kg) on gd 15 significantly increased TBARS level in fetal liver, which also was attenuated by PBN treatment.

LPS-induced GSH depletion

The effects of LPS exposure at late gestational stages on GSH content are presented in Figure 4, A, B, and C. Results showed that administration with 75 μg/kg of LPS on gd 15 significantly decreased GSH content in maternal liver, placenta, and fetal liver, all of which were attenuated by PBN treatment.

Comment

The purpose of the present study was to investigate the effects of maternal LPS exposure at late gestational stages on intrauterine fetal growth and skeletal development and to assess the potential role of ROS in LPS-induced intrauterine fetal growth restriction and skeletal development retardation. The present study found that that administration with LPS (25, 50, and 75 μg/kg per day) on gd 15-17 dramatically increased fetal mortality in a dose-dependent manner. In the high-dose group, fetal mortality was up to 63.2%. As expected, maternal LPS exposure at late gestational stages did not increase embryonic resorptions. However, maternal LPS exposure significantly lowered fetal weight and reduced crown-rump and tail lengths of live fetuses in a dose-dependent manner. Furthermore, the

Table II The effects of LPS on fetal skeletal development

	C (n = 21)	LPS25 (n = 12)	LPS50 (n = 14)	LPS75 (n = 12)	LPS+PBN (n = 14)	LPS+AG (n = 12)
Number examined						
Fetuses	231	118	134	57	142	52
Scores						
Supraoccipital bone*	1.02 ± 0.01	1.10 ± 0.03	1.23 ± 0.15†	2.02 ± 0.25‡	1.03 ± 0.01§	1.37 ± 0.20
Number ossified						
Sternum	6.00 ± 0.00	5.94 ± 0.25	5.89 ± 0.26	5.87 ± 0.26	6.00 ± 0.00	5.89 ± 0.14
Metacarpus	4.00 ± 0.00	3.95 ± 0.02	3.92 ± 0.26	3.88 ±0.29	4.00 ± 0.00	3.99 ± 0.02
Anterior phalanx	4.00 ± 0.00	3.95 ± 0.10	3.34 ± 1.15†	3.08 ± 1.42†	4.00 ± 0.00‖	3.94 ± 0.13
Metatarsus	4.98 ± 0.01	4.60 ± 0.30	4.48 ± 0.97	4.56 ± 0.39	4.99 ± 0.01	4.76 ± 0.11
Posterior phalanx	4.95 ± 0.02	3.80 ± 0.74	3.76 ± 1.95†	2.99 ± 2.09‡	4.98 ± 0.05§	4.10 ± 0.82
Caudal vertebrae	6.30 ± 0.21	5.50 ± 0.85	5.27 ± 1.69†	3.67 ± 0.91‡	6.35 ± 0.74§	3.62 ± 0.12

Numbers of litters in each group were presented in *parentheses*. *C,* Control; *L25,* LPS (25 μg/kg); *L50,* LPS (50 μg/kg); *L75,* LPS (75 μg/kg); *LPS+PBN,* LPS (75 μg/kg) + PBN (100 mg/kg); *LPS+AG,* LPS (75 μg/kg) + AG (100 mg/kg).

* Supraoccipital bone scores: 1 = well ossified, 4 = completely unossified.

† Significantly different from control, $P < .05$.

‡ Significantly different from control, $P < .01$.

§ Significantly different from LPS75 group, $P < .01$.

‖ Significantly different from LPS75 group, $P < .05$.

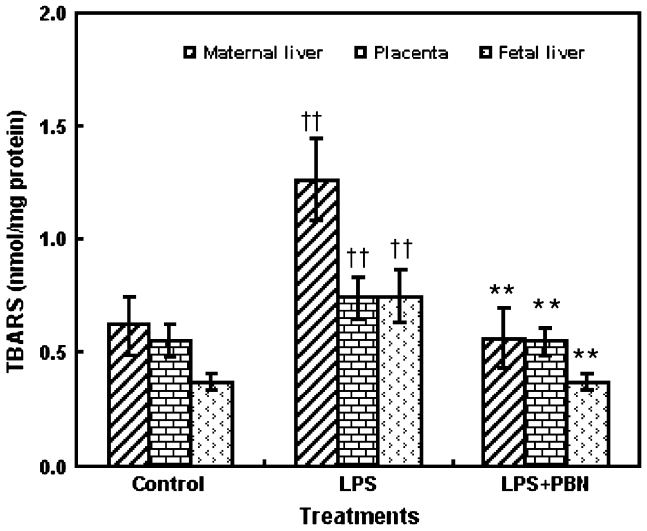

Figure 3 The effects of PBN on LPS-induced lipid peroxidation. All pregnant mice except controls received an intraperitoneal injection of LPS (75 μg/kg) once on gd 15. In the LPS+PBN group, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg, intraperitoneally), followed by an additional dose of PBN (50 mg/kg, intraperitoneally) at 3 hours after LPS. The saline-treated pregnant mice served as controls. Dams were killed at 6 hours after LPS treatment. Maternal liver, placenta, and fetal liver samples were excised. TBARS level was analyzed as described in *Material and methods*. Data were expressed as means ± SEM of 12 mice in each point. *Double daggers*, $P < .01$ as compared with control group. *Double asterisks*, $P < .05$ versus the LPS_{75} group.

present study found for the first time that maternal LPS exposure at late gestational stages significantly retarded skeletal ossification in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone in a dose-dependent manner.

Several studies indicated that LPS stimulates macrophages to generate ROS and increases nitrotyrosine, a marker for superoxide, NO, and peroxynitrite formation, in macrophage-rich organs.[17] Ejima et al[19] reported that LPS enhanced placental expression of 4-hydroxy-2-nonenal–modified proteins, markers of oxidative stress. Our earlier report showed that administration with LPS at late gestational stages increased the levels of TBARS in mouse placenta.[13] In the present study, we investigated the effects of maternal LPS exposure on TBARS and GSH contents in maternal liver and placenta. As expected, administration with LPS once on gd 15 significantly increased TBARS levels in maternal liver and placenta. Correspondingly, a single dose of LPS dramatically decreased the reduced GSH level in maternal liver and placenta.

To determine the role of ROS on LPS-induced intrauterine fetal growth restriction and skeletal development retardation, PBN, a free radical spin-trapping agent, was used to counteract LPS-induced ROS production. Results found that PBN significantly attenuated LPS-induced lipid peroxidation and GSH depletion in maternal liver and placenta. Consistent with its antioxidative effect, PBN almost completely blocked LPS-induced fetal death. Furthermore, PBN treatment protected against LPS-induced intrauterine fetal growth restriction including decreases in fetal weights and crown-rump and tail lengths and reversed LPS-induced fetal skeletal development retardation. Taken together, our results indicate that ROS might be, at least in part, involved in LPS-induced intrauterine fetal growth restriction and skeletal development retardation.

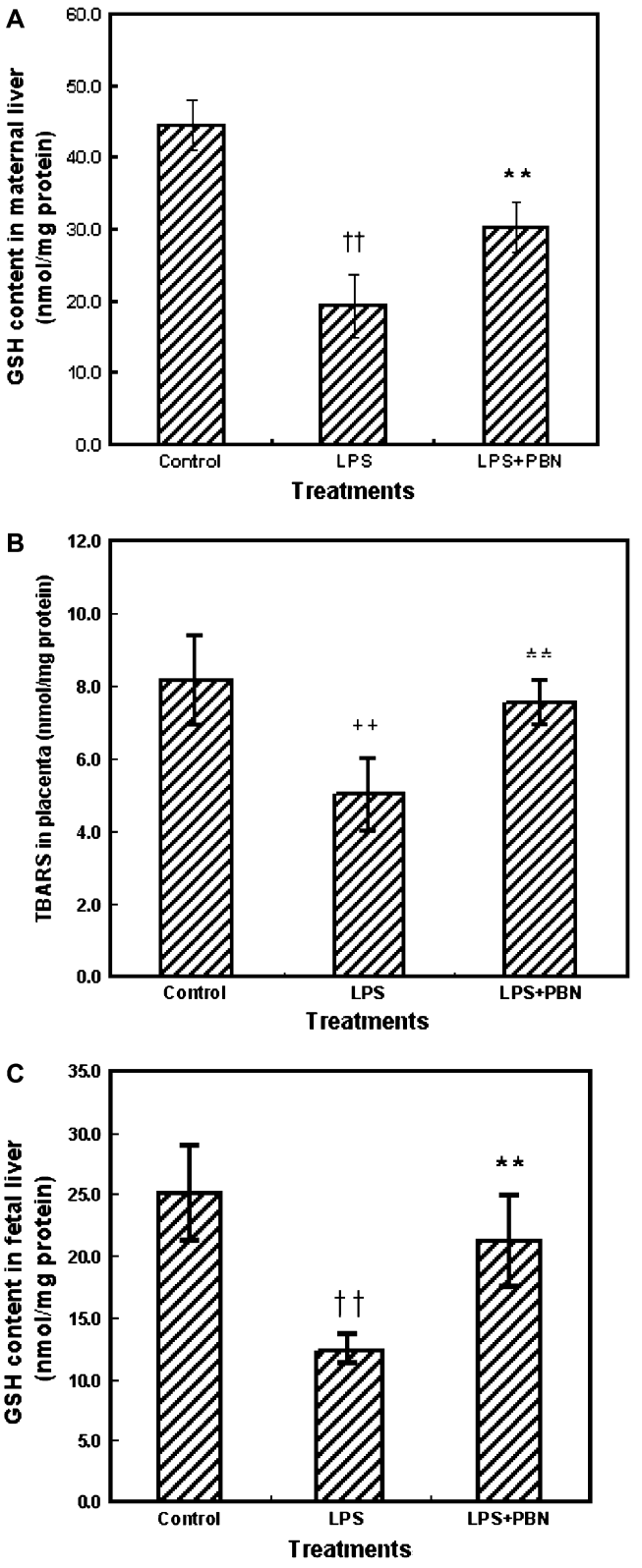

Figure 4 The effects of LPS on GSH content. All pregnant mice except controls received an intraperitoneal injection of LPS (75 μg/kg) once on gd 15. In the LPS+PBN group, the pregnant mice were injected with PBN (100 mg/kg, intraperitoneally) at 30 minutes before LPS (75 μg/kg, intraperitoneally), followed by an additional dose of PBN (50 mg/kg, intraperitoneally) at 3 hours after LPS. Maternal liver, placenta, and fetal liver samples were excised. GSH content was measured as described in *Material and methods*. *A*. GSH content in maternal liver. *B*. GSH content in placenta. *C*. GSH content in fetal liver. Data were expressed as means ± SEM (n = 12). *Double daggers*, $P < .05$ as compared with control group. *Double asterisks*, $P < .05$ as compared with the LPS_{75} group.

NO plays an important role in embryonic implantation, decidualization, vasodilatation, and myometrial relaxation. However, a recent study found that high concentrations of NO contribute to LPS-induced embryonic resorption.[20] AG, an inhibitor of inducible NO synthase, reversed LPS-induced embryonic resorption and abortion.[21] To investigate the potential role of NO in LPS-induced intrauterine fetal growth restriction and skeletal development retardation, the pregnant mice were pretreated with AG before LPS treatment. Surprisingly, AG had little effect on LPS-induced fetal death. Furthermore, pretreatment with AG did not protect against LPS-induced intrauterine fetal growth restriction and skeletal development retardation. These results suggest that maternal LPS exposure at late gestational stages induced fetal death, intrauterine fetal growth restriction, and skeletal development retardation are independent of NO production.

Previous studies thought that LPS-induced embryo lethality is a maternally mediated event.[9] However, a recent study found that maternal LPS exposure at late gestational stages (gd 17) increased the levels of TBARS in fetal liver.[22] In the present study, we investigated the effects of maternally administered LPS on oxidative stress in fetal liver. Our results showed that administration with LPS (75 μg/kg) once on gd 15 significantly increased the levels of TBARS in fetal liver. Furthermore, a single dose of LPS dramatically decreased GSH content in fetal liver, suggesting that maternally administered LPS caused oxidative stress in fetal liver. Indeed, Kupffer cells are also present in fetal liver. Toll-like receptor-4 has been shown to mediate LPS-induced signal transduction.[23] A recent study found that murine fetal liver expresses high levels of toll-like receptor-4 messenger ribonucleic acid, and there were no remarkable changes in the expression during the fetal or postnatal life.[24] In vitro studies indicated that fetal Kupffer cells express mature macrophage function in early gestation.[25] Therefore, our results do not exclude the involvement of other mechanisms. Additional work is required to determine whether LPS can cross the placenta and act directly on fetuses to result in fetal death, intrauterine fetal growth restriction, and skeletal development retardation.

In summary, the present results allow us to reach the following conclusions. First, maternal LPS exposure at late gestational stages results in fetal death, fetal death,

intrauterine fetal growth restriction, and skeletal development retardation in mice; second, LPS-induced fetal death, intrauterine fetal growth restriction, and skeletal development retardation are, at least in part, mediated by oxidative stress.

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