Hypoxia during pregnancy in rats leads to early morphological changes of atherosclerosis in adult offspring

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Wang Z, Huang Z, Lu G, Lin L, Ferrari M. Hypoxia during pregnancy in rats leads to early morphological changes of atherosclerosis in adult offspring. Am J Physiol Heart Circ Physiol 296: H1321–H1328, 2009. First published March 20, 2009; doi:10.1152/ajpheart.00440.2008.—Exposure to an adverse intrauterine environment increases the risk of cardiovascular disease later in adult life. However, the time course relationship between prenatal hypoxia and the onset of atherosclerosis in offspring remains unknown. The purpose of this study is to evaluate the role of reduced fetal oxygen supply on early development of atherogenesis in the thoracic aortas of Sprague-Dawley rat offspring. Intrauterine growth restriction, altered body shape at birth, and accelerated postnatal weight gain occurred in the maternal hypoxia group but did not occur in the control group. In 16-mo-old maternal hypoxia offspring, the thoracic aortas exhibited lesions similar to early events in atherosclerosis that involved impaired endothelial cells, thickening and fibrosis of intimas, infiltration of inflammatory cells to the subendothelial space, and migration and proliferation of vascular smooth muscle cells to the intima. In contrast, no detectable pathological changes were observed in the offspring without maternal hypoxia exposure. Morphometric analysis further demonstrated that prenatal hypoxia caused a significant thickening of intima (P < 0.001) with a main effect of 5.5 μm, an approximately twofold increase compared with controls. In addition, there was a positive additive relationship between prenatal hypoxia and hyperlipidemia on the intimal thickness (P < 0.05). There were no other main effects or interaction among these four factors. In summary, our results indicate that maternal hypoxia during pregnancy leads to early pathological appearances of atherogenesis in adult offspring. This effect was enhanced with hyperlipemia but was unaffected by postnatal hypoxia or sex.

fetal programming; intimal thickness; sex; hyperlipidemia; postnatal hypoxemia

RECENT EPIDEMIOLOGICAL STUDIES indicate that intrauterine growth-restricted infants are at increased risk of developing atherosclerosis and other cardiovascular diseases in adult life (2, 3, 12, 32). Fetal adaptations to an adverse intrauterine environment may include altered cellular differentiation and tissue growth to ensure short-term survival but may also lead to impaired cardiovascular structure and function later in adult life. Experimental investigations have demonstrated that impaired fetal growth may predispose individuals to the development of hypertension, cardiac remodeling, and vascular dysfunction and to the impairment of postischemic cardiac recovery in adult offspring (4, 8, 9, 14, 15, 20, 21, 23, 34, 46, 48). However, the impact of intrauterine growth restriction on the development of atherosclerosis is still unclear.

Intrauterine growth restriction, in many cases, is thought to occur as a result of placental dysfunction leading to impaired oxygen and/or nutrient transfer to the fetus. Experimentally, considerable studies have primarily been focused on maternal malnutrition during pregnancy (4, 8, 9, 15, 20, 21, 34) or fetal exposure to elevated glucocorticoid levels (26, 27, 30), which results in vascular dysfunction in adult offspring. To our knowledge, only a few studies describing the persistence and long-term consequences of these changes in prenatal hypoxia offspring have been reported. Exposure to chronic hypoxia in vivo results in reduced nitric oxide modulation of vascular responses in arteries from both embryonic and adult chickens (40, 41). In mammals, the vascular effects of chronic prenatal hypoxia have been examined primarily in the fetus (24, 43) or the neonate (45). Two recent studies demonstrated increased myogenic tone and impaired endothelial function in the peripheral artery from adult rat offspring after prolonged intrauterine hypoxia (14, 46). Since endothelial dysfunction is thought to also play a prominent role in the development of atherosclerosis, these results suggest that prenatal hypoxia might induce vascular changes that predispose to atherosclerotic diseases in adult offspring. To date, however, the specific effects of chronic hypoxia in utero on vascular structure in mammalian adult offspring remain to be elucidated. Likewise, the time course relationship between prenatal hypoxia and the onset of atherosclerosis in offspring remains unknown.

Given the above considerations, we hypothesized that prenatal hypoxia would lead to morphological changes of atherogenesis in the aorta from adult offspring. In addition, it has been demonstrated that prenatal hypoxia decreases adult heart tolerance to ischemia-reperfusion injury (23), suggesting that prenatal hypoxia may heighten adult injury to high fat diet or hypoxic stress. Moreover, it is well known that sex, hyperlipidemia, and hypoxemia are associated with increased risk of atherosclerotic diseases and that hyperlipidemia and hypoxemia are critically impaired stresses to the adult. Therefore, we also hypothesized that postnatal risk factors of sex, hyperlipidemia, and hypoxemia might modify the programming effects of prenatal O2 deficiency.

In the present study, we have used three animal models of maternal hypoxia, high-fat diet feeding, and offspring hypoxemia by a 4 × 2 full factorial design to determine the impact of prenatal hypoxia on morphological changes in the thoracic aorta from adult offspring and further assessed its susceptibility to sex-, hyperlipidemia-, and hypoxemia-related differences. Given that aging may contribute to the development of athero-
sclerosis, we chose the age of 16 mo (equivalent to human cases of 40–50 years old) so that the rats may mimic the human case within the atherosclerosis-prone age.

MATERIALS AND METHODS

Animals. All experimental procedures were in accordance with National Institutes of Health guidelines with approval by the Standing Committee on Ethics and Animal Experimentation at the Fujian Medical University (China). Female Sprague-Dawley rats (Shanghai Experimental Animal Center, Shanghai, China) were mated at 3 mo of age. A vaginal smear obtained the following morning was examined for the presence of sperm, which signified day 0 of pregnancy (term = 22 days). During pregnancy and lactation, all rats were fed standard lab rat chow ad libitum. On day 7, rats were randomly assigned to control and maternal hypoxia groups.

Maternal hypoxia. From day 7 to day 21 of pregnancy, rats (n=10) were put into a Plexiglas chamber (volume, 140 l) that could hold a maximum of three pregnant, individually housed rats at any one time. Maternal oxygen supply was then reduced to 10 ± 1% oxygen by continuous infusion of a nitrogen gas and compressed air mixture. The expired CO₂ was eliminated by circulating the atmosphere through soda lime, and the water contained in the expired gas was trapped in a chilled glass tank. A portable oxygen analyzer (S-450; IST-AIM) was calibrated daily and used to monitor the oxygen concentration of the chamber. For the measurement of blood gas and pH, arterial blood samples were withdrawn from cannulated left femoral artery after 1 h hypoxia and measured by a blood gas analyzer (Rapidlab 850; Bayer) in three randomly selected rats. After 3 h of hypoxia, pregnant rats were removed from the chamber and housed in room air. Control animals (n = 10) were put into an identical Plexiglas chamber, but into which compressed air was continuously infused. They then underwent the same procedures as the animals exposed to hypoxia.

Offspring. One randomly selected pup per litter and its brain, heart, lung, liver, and kidney were weighed within 3–12 h after birth. The litter size was then reduced to eight pups to ensure equal nutrient access for all of the offspring. Another randomly culled pup per litter was weighed into which compressed air was continuously infused. They then undertook the same procedures as the animals exposed to hypoxia.

High-fat diet feeding in offspring. At 10 mo of age, feeding of a high-fat diet (92.3% standard lab rat chow supplemented with 2% cholesterol, 5% lard, 0.5% sodium cholate, and 0.2% propylthiouracil) commenced in rats assigned to hyperlipidemia. High-fat diet feeding continued for 4 mo. To determine whether hyperlipidemia was induced in rats, blood samples (300–500 μl) were collected in the fed state at 9 AM on days 1, 4, 8, 11, 15, 18, 22, and 25 of high-fat diet feeding. After an overnight fast from 8 PM to 8 AM the following day, blood samples were taken from the jugular vein of rats at 8 AM. Blood was centrifuged at 3,000 rpm (1,500 g) for 15 min, and plasma was removed and stored at −20°C for the determination of serum lipids.

Postnatal hypoxia in offspring. At 12 mo of age, offspring rats assigned to hypoxemia were exposed to hypoxia twice per day, 4 h in the morning and 4 h afternoon, for 4 wk with 1 day of interruption at every 6-day period. The rats underwent the same procedures as the animals exposed to maternal hypoxia, which were described previously in the current study.

Histopathology. At 16 mo of age, all of the offspring rats were euthanized and immediately perfusion-fixed with 10% neutral-buffered formalin (0.1 mol/l phosphate-buffered saline) through the left ventricle at a controlled pressure of 100 mmHg for 30 min in an attempt to preserve normal structural configurations. After perfusion, portions of the descending aorta were immediately excised for histopathological evaluation. For light microscopic analysis, one cross-sectional ring, about 1 cm distal from the bifurcation of the left subclavian artery, was further fixed with 10% formalin overnight. Tissues were processed by standard procedures in graded alcohols and xylene, paraffin-embedded, and stored at room temperature. Sections were cut at 5 μm thickness and performed by hematoxylin-eosin staining, Masson’s trichrome staining, Weigert’s staining, Oil red O staining, and CD68 immunohistochemical staining, respectively. For transmission electron microscopic studies, adjacent segments were cut longitudinally, placed in 3% glutaraldehyde/1.5% paraformaldehyde solution buffered at pH 7.2 with 0.1 mol/l phosphate-buffered saline, and refrigerated at 4°C until processing. Samples were prepared for transmission electron microscopy by standard methodology. Briefly, samples were postfixed at 4°C for 1.5 h in 1% osmium tetroxide/1.5% potassium ferrocyanide solution. They were then washed in 0.1 mol/l phosphate-buffered saline, dehydrated in graded concentrations of ethanol, and embedded in Epon 618. The epoxy blocks were sliced on a LKB-V ultratome at 70 to 80 nm thickness, stained with uranylacetate and lead citrate, and examined with a Hitachi Hu-12A transmission electron microscope. High-resolution digital images were acquired directly to a computer.

Morphometric analysis. The image of a cross-sectional section of thoracic aorta stained with Weigert’s method was captured on a microcomputer equipped with a digital camera through a Leica microscope (LAS AF-TCS SP5; Leica), and an automated computer-based image analysis system (Motic Images Advanced 3.0; Motic) was applied to measure the aortic intimal thickness by a single investigator who was blinded to the treatment protocol. The tunica intima thickness measurement was conducted in the thickest area of the tunica intima. Mean value was obtained from the three slices. The reproducibility of the measurement by the same observer was assessed in 10 randomly selected slices by repeating the measurements on two occasions (1–7 days apart) under the same basal conditions.

![Fig. 1. Comparison of arterial blood gas between control and maternal hypoxia pregnant rats after 1 h hypoxia. All data are expressed as means ± SD for control (n = 6) and hypoxia (n = 6) rats. * P < 0.05 compared with control. PaO₂, arterial O₂ partial pressure; PaCO₂, arterial CO₂ partial pressure.](http://ajpheart.physiology.org/ by 10.220.33.6 on November 10, 2017)
Statistical analysis. Results were presented as grouped means ± SD. The unpaired Student’s two-tailed t-test was used to compare differences between maternal hypoxia and control groups. A general linear model of univariate process was performed to analyze the main effects and the interaction effect of prenatal hypoxia, sex, hyperlipidemia, and postnatal hypoxemia on morphological changes of aorta in a completely randomized factorial design experiment. Reproducibility was analyzed by an Intraclass correlation coefficient (IntraCC) test. There was a good consistency with an IntraCC value of 0.89 (95% CI, 0.54 – 0.97) between the two occasions in the same observer’s measurements. Probability values of \( P < 0.05 \) were considered statistically significant. All data analyses were performed with commercially available statistical analysis software packages (SPSS 11.5; SPSS, Chicago, IL).

RESULTS

Arterial blood gas analysis results of chronic hypoxia in gestational rats and their offspring. After 1 h hypoxia, significantly decreased blood \( \text{PaO}_2 \) and \( \text{SaO}_2 \) were found in gestational rats and their offspring compared with the control (\( P < 0.05 \)). However, there were no significant differences in mean \( \text{PaCO}_2 \) and pH value between them (Fig. 1). These results showed that chronic hypoxia induced significant hypoxemia without \( \text{CO}_2 \) retention and acidosis, suggesting that it can be used to study the effects of hypoxia alone by the current chronic hypoxia animal model.

Characteristics of offspring. There was no significant difference in the number of pups between maternal hypoxia and control group (13.1 ± 2.1 vs. 13.2 ± 2.3). The birth weights of maternal hypoxia offspring were significantly reduced compared with control offspring (4.87 ± 0.51 g vs. 5.86 ± 0.31; \( P < 0.01 \)). However, by 28 days of age there were no differences in body weight between them (72.8 ± 6.0 g vs. 75.1 ± 4.5). In addition, at birth, the brain weight-to-body weight and heart weight-to-body weight ratio were significantly higher, and the liver weight-to-body weight ratio was prominently lower in maternal hypoxia offspring compared with control. But there were no obvious differences in mean weight ratios of the lung and kidney (Fig. 2). These results indicate that maternal exposure to hypoxia resulted in intrauterine growth restriction with a disproportion in neonatal organ size of offspring who exhibited a postnatal catch-up growth.

Serum lipids profiles of offspring with high-fat diet. The baseline of serum total cholesterol and triglyceride concentrations in rat offspring were 1.41 ± 0.54 and 1.06 ± 0.39 mmol/l, respectively. Serum total cholesterol began to elevate by day 4 of high-fat diet feeding and triglyceride elevated by day 8. Both of them were then gradually increased with a stable platform of 12.14 ± 3.58 and 2.05 ± 0.49 mmol/l, respectively, at day 15 of high-fat diet feeding. When compared with the baseline, serum total cholesterol and triglyceride levels at day 15 were significantly increased (all \( P < 0.05 \)). This elevation of serum concentration was more prominent in total cholesterol than in triglyceride (Fig. 3). Persistently increased serum total cholesterol and triglyceride levels suggested the offspring suffer from hyperlipidemia with the exposure to high-fat diet.
Morphological changes in the thoracic aorta from adult offspring. By light microscopy, no detectable pathological changes in the thoracic aorta were observed in offspring without all treatments of maternal hypoxia, hyperlipidemia, and postnatal hypoxemia or with a single exposure to either hyperlipidemia or hypoxemia. The aortic lumen was smooth and round. The vessel wall was composed of three layers with clear boundaries among them. The thin innermost layer, the tunica intima, bordered on the lumen of the vessel and was lined by a single integrity layer of flattened endothelial cells. The thick tunica media consisted mainly of large sheets of regular parallel-arranged elastic membranes and spindle-shaped vascular smooth muscle cells (SMCs). The tunica adventitia was a thin layer of connective tissue containing small blood vessel (Fig. 4, A–C). However, the thoracic aorta of maternal hypoxia offspring exhibited lesions that showed an irregular lumen, impairment and desquamation of endothelial cells, thickening and fibrosis of the intima, attenuation of tunica media, disarranged atrophic SMCs, proliferative collagen fibers, and unclear elastic fiber layers (Fig. 4, D–F). Infiltrating macrophages were also seen in the subendothelial space by CD68 immunohistochemical staining, which were not detectable in the offspring without maternal hypoxia (Fig. 4, G and H). The lesions, which were independent of sex, were more prominent with superimposed hyperlipidemia but not with postnatal hypoxemia. Thoracic aortas from high-fat diet rats or from normal diet rats were negative for Oil red O staining (Fig. 4I).

When transmission electron microscopy was used, no detectable pathological changes in the thoracic aorta were observed in control offspring without maternal hypoxia, hyperlipidemia, and hypoxemia exposure. All of the three layers in the vessel wall were intact, clear, and regular parallel arrayed (Fig. 5, A and B). However, the thoracic aorta of maternal hypoxia offspring exhibited lesions that showed an early event

Fig. 4. Representative light micrographs of thoracic aortas from 16-mo-old rat offspring. No detectable pathological changes are observed in (A–C) offspring without all treatments of maternal hypoxia, hyperlipidemia, postnatal hypoxemia, or with a single exposure to either hyperlipidemia or hypoxemia. Lesions that show an irregular lumen, impairment and desquamation of endothelial cells, thickening and fibration of the intima, attenuation of tunica media, disarranged atrophic smooth muscle cells (SMCs), proliferative collagen fibers, and unclear elastic fiber layers are seen in maternal hypoxia offspring (D and E). Infiltrating macrophages (arrowhead) were also seen in the subendothelial space by CD68 immunohistochemical staining in (G) maternal hypoxia offspring but not detectable in (H) the offspring without maternal hypoxia. Oil red O staining was negative in either high-fat diet or normal diet (I) rats. A and D, hematoxylin-eosin staining; B and E, Masson’s trichrome staining; C and F, Weigert’s staining; G and H, CD68 immunohistochemical staining; I, oil red O staining. Magnification for all photomicrographs is ×400.
in atherogenesis. Some of the endothelial cells were seen to be edematous, necrotic, detached from the basement membrane, or even desquamated. Local platelet adhesion and aggregation and microthrombosis were occasionally observed in the surface of aortic lumen. Edema and thickening of subendothelial layer and fragmentation of the internal elastic lamina were also observed (Fig. 5, C and D). Lymphocytes, macrophages with many lysosomes in the cytoplasm and SMCs, were often observed in the subendothelial space (Fig. 5, E–G). SMCs migrating from the media into the intima, breaking through the internal elastic lamina, were often observed. These SMCs exhibited fewer intracytoplasmic filaments and more developed intracellular organelles, such as rough endoplasmic reticulum and mitochondria. Namely, these SMCs in the intima showed the morphological characteristics of proliferative-type SMCs rather than of contractile-type SMCs in the media. Fibrillar or granular materials were seen to accumulate in the extracellular space around these cells (Fig. 5, F and G). Attenuation of tunica media were also seen, in which disarranged atrophic SMCs with migration trends, mitochondrial swelling in some of SMCs, proliferation of collagen fibers, and unclear elastic fiber layers were observed (Fig. 5, H and I). In addition, the above pathological appearances were even more prominent when it was complicated with hyperlipemia but not found in the offspring with a single exposure to either hyperlipidemia or hypoxemia. There were no significant differences between female and male offspring rats.

The thickness of tunica intima in the thoracic aorta from offspring. The thickness of tunica intima in the thoracic aorta from offspring was presented in Table 1. A general linear model of univariate process was used to analyze the main effects and the interaction effects of prenatal hypoxia, sex, hyperlipidemia, and hypoxemia on the thickness of intima. The model performed in the analysis had statistical significance.
Table 1. Thickness of intima in thoracic aorta from offspring at 16 mo of age

<table>
<thead>
<tr>
<th>Intima, μm</th>
<th>M0G0L0H0</th>
<th>M0G0L1H0</th>
<th>M0G1L0H0</th>
<th>M0G1L1H0</th>
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<tr>
<td>8.3 ± 1.3</td>
<td>10.7 ± 1.6</td>
<td>10.3 ± 2.2</td>
<td>9.5 ± 1.4</td>
<td>5.6 ± 1.7</td>
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Values are means ± SD. M0G0L0H0, normoxia female; M0G0L1H0, normoxia male; M0G1L0H0, normoxia female hyperlipidemia; M0G1L1H0, normoxia male hyperlipidemia; M1G0L0H0, maternal hypoxia female; M1G0L1H0, maternal hypoxia male; M1G1L0H0, maternal hypoxia female hyperlipidemia; M1G1L1H0, maternal hypoxia male hyperlipidemia; M1G1L0H1, maternal hypoxia female hyperlipidemia; M1G1L1H1, maternal hypoxia male hyperlipidemia; M1G0L0H1, maternal hypoxia female hyperlipidemia; M1G0L1H1, maternal hypoxia male hyperlipidemia; M1G0L1H0, maternal hypoxia female hyperlipidemia; M1G0L0H1, maternal hypoxia male hyperlipidemia; M1G0L1H1, maternal hypoxia male hyperlipidemia.

(F = 5.053; P < 0.001; R² = 0.703). Prenatal hypoxia caused a significant thickening of intima (P < 0.001), with a main effect of 5.5 μm. In addition, there was a positive interaction effect between prenatal hypoxia and hyperlipidemia on the thickness of intima (P < 0.05), with a thickness of 3.8 μm for hypoxia and 7.1 μm for hypoxia with hyperlipidemia. Other factors were not found to have an effect on intimal thickness. These results revealed that hyperlipidemia and prenatal hypoxia exerted a synergistic effect on the thickness of intima. However, there were no other main effects and no other interaction among these four factors. The two unrelated factors of sex and hypoxemia were then excluded, and further, a general linear model of univariate process was performed to determine the effects of prenatal hypoxia and hyperlipidemia with exclusion of unrelated factors of sex and postnatal hypoxia. Similarly, the analysis model on intima had statistical significance (F = 31.045; P < 0.001; R² = 0.679). A similar effect has also been observed that prenatal hypoxia caused a significant thickening of intima (P < 0.001), which exhibited a positive interaction to hyperlipidemia (P < 0.01). However, there was no significant effect of hyperlipidemia alone on the thickness of intima.

**Discussion**

This study is the first to demonstrate that prenatal hypoxia produces early morphological changes of atherosclerosis in 16-mo-old offspring rats. These pathological changes occur more prominently in conjunction with postnatal hyperlipidemia but were indifferent to sex and postnatal hypoxia.

Several modifications have been made in the present study compared with previous work (14, 45, 46). First, oxygen was reduced from 12% to 10%. Moreover, to reduce the minimum impact of hypoxic treatment on food intake, hypoxic duration was shortened to 3 h/day in the pregnant rats. Finally, considering that different systems in the developing embryo are particularly vulnerable to impaired stress during their periods of maximum growth and differentiation, which is most apparent about halfway through gestation (between approximately gestational day 9 and day 13) for the developing cardiovascular system in rats, hypobaric hypoxia treatment was advanced to day 7 of pregnancy and persisted to day 21 to cover this critical window of development in cardiovascular system.

Chronic maternal hypoxia imposed with different severity and gestational ages in pregnant rats reduces fetal or neonatal body weight and alters fetal organ growth (5, 11, 18, 23, 25, 33, 35, 36, 44, 45, 47). Consistent with these findings, our data demonstrated that a reduction in maternal oxygen supply during days 7 to 21 of gestation resulted in reduced neonatal size and perturbations of neonatal organ weight and proportion, the latter suggesting an altered body shape at birth. Our data also demonstrated that pups with hypoxia-induced growth restriction displayed postnatal catch up growth. By 21 days of age, there was no difference in body weight between maternal hypoxia offspring and control. These results are comparable with a previous study that has also shown accelerated postnatal weight gain following intrauterine growth restriction attributed to maternal hypoxia (18). Recent epidemiological studies indicate that intrauterine growth retardation, low birth weight, altered body shape at birth, and rapid childhood weight gain are associated with increased risk of developing atheros!erotic disease in adult life (2, 3, 6, 7, 12, 32, 37). The alternations in our data of rats were coincident with these epidemiological studies and together presumably may contribute to the impaired morphological changes in the aorta from adult offspring that are reported here.

Injury of the arterial endothelium is an early event in the development of an atherosclerotic lesion. An intimal thickening response to this injury that involved infiltration of inflammatory cells, migration and proliferation of SMCs, and accumulation of fibrillar and granular materials in the subendothelial space occurs. These morphological changes are considered as an early event in atherogenesis and a distinctive feature in atherosclerosis-prone sites (13, 29, 38, 39). In this study, we observed the above pathological changes in prenatal hypoxia offspring rats, which were comparable with others that have also shown atherosclerosis-like lesions in rats (17, 28, 29, 42), but not in control animals, indicating that maternal exposure to hypoxia leads to early pathological appearances of atherogenesis in adult offspring. Besides the intima injury, significant changes were recognized at the tunica media in prenatal hypoxia offspring as well. Moreover, the above pathological changes occur more prominently in conjunction with postnatal hyperlipidemia. It has been shown that although high-fat diet cannot induce detectable atherosclerotic lesions in aortas from rats, it promotes the adhesion of circulatory monocytes to the endothelium, which is the first step of subendothelial infiltration of inflammatory cells (22). This may be partly responsible for the enhancement of the effect of prenatal hypoxia on the thoracic aorta by hyperlipidemia. Again, consistent with previous studies (22, 28), there were no detectable morphological appearances of the lesions in the offspring with a single exposure to either hyperlipidemia or hypoxemia, suggesting that impaired stress of hyperlipidemia or hypoxemia alone in the adult is not sufficient to induce such atherosclerosis-like lesions in rats.
Changes in the tunica intima represent the first step in atherosclerosis development. Along with the progress of atherosclerosis lesions, thickness of intima is increased, which in turn enable intimal thickness to be used to represent the severity of the lesions. In addition, the thickness of the intima can be used as a quantitative measure of vascular pathology to complement the qualitative changes in histology. Our data demonstrated that prenatal hypoxia caused a significant thickening of intima with a main effect of 5.5 μm, which is considerably increased to approximately twofold of the intimal thickness in control. However, the other factors of sex, hyperlipidemia, and hypoxemia did not exhibit any main effects on it. These results were consistent with our histopathological findings that early morphological changes of atherogenesis in offspring from maternal hypoxia were not seen in rats exposed to the other factors except maternal hypoxia treatment and together presumably suggested that prenatal hypoxia might serve as a novel factor that can increase the risk of atherosclerotic diseases in adult offspring. Consistent with our findings in histopathology that have shown enhanced pathological appearances in the aortas from the prenatal hypoxia offspring complicating with hyperlipemia, there was a positive interaction effect between prenatal hypoxia and hyperlipidemia on the thickness of intima. These results indicated that thickened intima induced by prenatal hypoxia was sensitized to hyperlipemia stress in adult offspring. In contrast, the two factors of prenatal hypoxia and postnatal hypoxemia did not exert an interaction effect on the thickness of intima, which suggested that the stress of hypoxemia in adult offspring did not contribute to this sensitization of prenatal hypoxia. Likewise, prenatal hypoxia did not show an interaction with sex either, which confirmed that the pathological alterations of atherogenesis induced by prenatal hypoxia did not exhibit any sex differences. This finding is in contrast with previous studies on vascular endothelial function that vascular myogenic responses exhibit sex differences (10, 16, 19, 31) and that vascular responses in adult offspring from maternal hypoxia during pregnancy are impaired in a sex-specific manner with males more profoundly affected (14).

In conclusion, the results of this study highlight the impact of reduced maternal oxygen during pregnancy on the morphological changes in the thoracic aorta from adult offspring. We have demonstrated that maternal exposure to hypoxia during days 7 to 21 of gestation restricted fetal growth and led to early pathological appearances of atherogenesis in 16-mo-old adult offspring rats. These results indicate that the impact of prenatal hypoxia can persist postnatally and predispose the individual to atherosclerotic disease in later life and, therefore, might serve as a novel risk factor for atherosclerotic diseases in adult. This study further demonstrates that this effect of prenatal hypoxia is enhanced when it is complicated with hyperlipemia, suggesting that this effect was sensitized toward hyperlipemia stress in adult offspring, whereas the impact of prenatal hypoxia does not exhibit significant interaction to the impaired stress of hypoxemia in adult offspring and does not show specific sex difference.

GRANTS
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