Protein Restriction during Pregnancy Induces Hypertension and Impairs Endothelium-Dependent Vascular Function in Adult Female Offspring

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Abstract
Intrauterine undernutrition plays a role in the development of adult hypertension. Most studies are done in male offspring to delineate the mechanisms whereby blood pressure may be raised; however, the vascular mechanisms involved in female offspring are unclear. Female offspring of pregnant Sprague-Dawley rats fed either a control (C; 18%) or a low-protein (LP; 6%) diet during pregnancy were used. Birth weight and later growth were markedly lower in LP than in C offspring. LP offspring exhibited impaired estrous cyclicity with increased mean arterial pressure. Hypotensive response to acetylcholine (ACh) and the hypertensive response to phenylephrine (PE) were greater in LP than in C rats. N-nitro-L-arginine methyl ester (L-NAME) induced greater hypertensive responses in C than in LP rats. Endothelium-intact mesenteric arteries from LP offspring exhibited increased contractile responses to PE and reduced vasodilation in response to ACh. In endothelium-denuded arteries, relaxation responses to sodium nitroprusside were similar in both groups. Basal and ACh-induced increase in vascular nitrite/nitrate production was lower in LP than in C offspring. L-NAME or 1H-1,2,4-oxadiazolo-4,3-quinoxalin-1-one inhibited ACh relaxations and enhanced PE contractions in C offspring, but had minimal effect in LP rats. The decreased NO-mediated vascular response might explain the increased vascular contraction and arterial pressure in female offspring with low birth weight.

Key Words
Mean arterial blood pressure • Endothelium • Nitric oxide • Fetal programming • Pregnancy • Vascular smooth muscle • Relaxation • Constriction • Hypertension

Introduction
Epidemiologic studies have shown that low birth weight is associated with increased risk of cardiovascular (CV) disease in adulthood and have led to the hypothesis that the fetal CV system undergoes programming in response to unbalanced maternal nutrition [1–6]. These human studies are strongly supported by animal experiments showing that a severe but balanced limitation of fetal substrate supply retards fetal growth and induces CV dysfunction and hypertension [7–10]. The hypothesis of developmental origins of disease proposes that maternal undernutrition causes permanent changes in structure and function of tissues and organs, resulting in CV and related disorders in the offspring [11, 12].

The mechanisms involved in the programming of adult hypertension have remained unknown, although a significant correlation between adverse intrauterine environments and alterations of vascular or endothelial function has been reported [10, 13, 14]. In addition, a possible role for the renin-angiotensin system [15, 16], the kidney [17, 18] and stress-induced stimulation of the hy-
is attributed to the presence of sex steroid, e.g. estrogen, during pregnancy. Female offspring having impaired estrous cyclic-
ity or vascular dysfunction induced by a maternal low-pro-
tein diet is more pronounced in males than in females [13, 23, 24]. Most studies have used male animals to explain the potential mechanisms through which hypertension may be initiated by fetal undernutrition. The reasons for the paucity of studies in females may be that females develop moderate and less consistent postnatal hypertension [24]. It is shown that the severity of hypertension in females depends on the extent of maternal protein restriction and the age at which the offspring are examined [25, 26]. Our laboratory has shown that 66% maternal protein restriction consistently results in development of hypertension at ≥6 months of age in female offspring while the males are hypertensive as early as 2 months [27]. The delay in development of hypertension in the females is attributed to the presence of sex steroid, e.g. estrogen [26]. It is well known that adequate levels of estrogen are essential in modulating vascular function through the release of endothelium-derived relaxant factors such as nitric oxide (NO) [28–30]. We and others have shown that there is a reduction in estrogen levels in the hypertensive female offspring [13, 27]. Endothelial dysfunction consequent to decreased estrogen levels or due to other factors may play a role in the development of hypertension in the female offspring of nutritionally restricted dams. Previous studies have reported that endothelium-dependent relaxation was blunted in conduit arteries (aorta) from the adult female offspring of pregnant rat dams fed a globally restricted diet [13]. Also, the endothelial NO synthase (eNOS) activity was shown to be reduced in these animals [13]. However, the functional consequences of low protein on resistance vessels, which are the actual determinants of blood pressure, in female offspring are currently not known. The purpose of this study was to test the hypothesis that intrauterine fetal growth restriction due to protein restriction results in low-birth-weight offspring, female offspring having impaired estrous cyclic-
ity and exhibiting reduced endothelial-dependent ven-
ter relaxation, enhanced vascular contraction and hypo-
tension, specifically during their late adult life. To test this hypothesis, we studied vascular function in vivo and contraction and relaxant (endothelium-dependent and -independent) responses ex vivo in small mesenteric arter-
ties from rat female offspring at 1 year of age.

Methods

Animals
All procedures were approved by the Animal Care and Use Committee at the University of Texas Medical Branch and were in accordance with those published by the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996). Virgin female Sprague-Dawley rats (Harlan Sprague Dawley, Houston, Tex., USA) weighing between 175 and 225 g (4 months old) were mated with male Sprague-Dawley rats; conception was confirmed by observation of a vaginal copulation plug or the presence of sperm in the vagi-
nal flush. Pregnant rats were randomly divided into two dietary groups, housed individually and fed a control (C, 18% casein) or low protein (LP, 6% casein) diet throughout pregnancy. The iso-
caloric low-protein and normal-protein diets were obtained from Harlan Teklad (Madison, Wisc., USA). The composition of the diets for the 2 groups, except for the protein content, was identical as previously described [31]. All pregnant rats were allowed to de-
iver at term. At delivery, all dams were placed on the normal diet and the pups were weaned to the normal diet at 21 days of age and maintained on that diet thereafter. Offspring of C and LP preg-
nant rats were referred to as the C group and LP group, respec-
tively. The litter sizes and birth weights of C and LP pups were recorded within 12 h after delivery and weighed once a week thereafter. The animals were housed in a room with a controlled temperature and a 12-hour:12-hour light-dark cycle.

Estrous Cyclicity

Estrous cyclicity in both groups of rats was assessed by daily vaginal lavages (smears). Monitoring began on postnatal day 150 and continued for 17 days. Smears were taken between 09.00 and 11.00 h each morning, examined unstained, by light microscopy (×20), and assessed for relative abundance of leukocytes, nucle-
ated epithelial cells and cornified epithelial cells. Six animals each from the C and LP groups were assessed for estrous cyclicity. Cy-
clicity was assessed by calculating the average number of estrous cycles observed in each group over the monitoring period (i.e. to-
tal number of estrous cycles divided by the number of rats in that group).

Assessment of Vascular Function in vivo

Mean arterial pressure (MAP) was determined in conscious rats at 1 year of age. Rats were anesthetized with ketamine (45 mg/kg body weight; Burns Veterinary Supply, Westbury, N.Y., USA) and xylazine (5 mg/kg body weight; Burns Veterinary Supply, Westbury, N.Y., USA). The jugular vein and carotid artery were cannulated with polyethylene tubing (PE-50; Becton Dickinson, Sparks, Md., USA). After the animals had recovered from anesthesia, the ca-
rotid cannula that was filled with heparinized saline (50 U·ml−1) was connected to a pressure transducer to record MAP using the DBP001 direct blood pressure system (Kent Scientific, Litchfield, Conn., USA). The jugular vein cannula was used to administer drugs as an intravenous (i.v.) bolus. MAP was monitored continu-
ously for 30 min before administration of any drugs. Dose-depen-
dent hypotensive responses to acetylcholine (ACH; 0.03, 0.1, 0.3, 1, 3 μg·kg−1·i.v.) and hypertensive responses to phenylephrine (PE; 1, 3, 10, 30 μg·kg−1·i.v.) were obtained in both C and LP rats. eNOS was inhibited with the administration of N-nitro-L-arginine methyl ester (L-NAME, 50 mg·kg−1·i.v.) to rats in both groups.
to determine the relative contribution of the endothelium in maintaining the basal MAP. Enough time was allowed between responses for MAP to recover to the resting level.

Preparation of Mesenteric Arteries

The rats were sacrificed by CO₂ inhalation and the mesenteric arcade was excised and immersed in oxygenated Krebs physiological salt solution (KPSS) [6]: NaCl, 119; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.17; NaHCO₃, 25; KH₂PO₄, 1.18; EDTA, 0.026; and d-glucose, 5.5. Small mesenteric arteries were dissected free of connective tissue and mounted using tungsten wires on a wire myograph (Kent Scientific) to record isometric tension. The tissues were incubated for 15 min in KPSS at 37°C, which was gassed with 95% O₂ and 5% CO₂ to maintain pH 7.4. The segments were initially loaded to an optimum stretch, which was previously determined by using high-K⁺ physiological solution (80 mmol/l) as the contracting agent after applying different passive tensions. The initial stretch and the length of the segments (2 mm) were consistently maintained across all arterial rings of either group. For endothelium-intact mesenteric rings, extreme care was taken to avoid injury to the endothelium. For endothelium-denuded mesenteric rings, the endothelium was removed by gently rubbing the ring interior with tungsten wire. Removal of the endothelium was verified by the absence of ACh relaxation in tissues precontracted by a submaximal concentration of PE.

Assessment of Vascular Reactivity in Mesenteric Arteries

Endothelium-intact mesenterial arterial rings were stimulated with increasing concentrations of PE, and concentration-contraction curves were constructed. The PE concentration used for precontraction was that required to produce 80% of the maximal contraction and ACh-induced relaxation of PE contraction were calculated from a calibration curve constructed with known concentrations of NaNO₂.

Half maximal effective concentrations were determined by regression analysis and expressed as negative log-molar concentration. Data analysis was done using GraphPad Prism for Windows (GraphPad Software, San Diego, Calif., USA). Data from several vascular rings of the same rat were averaged and presented as the datum of the PE-induced contraction. Maximal responses and half maximal effective concentration values were then obtained. Half maximal effective concentrations were determined by regression analysis and expressed as negative log-molar concentration. Data analysis was done using GraphPad Prism for Windows (GraphPad Software, San Diego, Calif., USA). Data from several vascular rings of the same rat were averaged and presented as the datum for 1 rat, with the n value representing the number of rats. Differences are considered statistically significant at p < 0.05.

Results

Offspring Birth Weights

The birth weight of C rats was 6.7 ± 0.08 g and was significantly reduced in LP rats (4.7 ± 0.1 g). Both C and LP offspring showed significant increases in body weight
with age. However, the growth curves of the two groups were significantly different and were parallel through adulthood (fig. 1). The body weight at the time of terminal study (1 year) was significantly lower in LP offspring than in C rats ($266.0 \pm 4.2$ vs. $277.1 \pm 2.7$ g). The length of gestation and number of pups per litter were not different among groups (table 1).

**Estrous Cyclicity**

Assessment of estrous cyclicity revealed a significant decrease in the number of observed estrous cycles over a 17-day period in the LP group compared to C rats (table 1). The 6 rats in the C group totaled 18 estrous cycles (average = 3), while in the LP group only a total of 3 estrous cycles were observed (average = 0.5) (table 1). Although few animals showed extended cycle length, most rats had already stopped cycling in the LP group.

**Blood Pressure Measurement**

Baseline MAP measured in 1-year-old rats was significantly higher in LP rats when compared with the C group ($133.9 \pm 4.05$ vs. $104.7 \pm 3.13$ mm Hg). We found that ACh evoked a concentration-dependent decrease in MAP that was attenuated in LP rats (fig. 2a). Administration of PE evoked a concentration-dependent hypertensive response that was more pronounced in LP offspring compared to C rats (fig. 2b). Acute administration of the eNOS inhibitor, L-NAME, induced significantly greater hypertensive responses in C ($60.0 \pm 2.64\%$) than in LP offspring ($30.6 \pm 3.00\%$).

**Contractile Responses**

In endothelium-intact mesenteric rings from both groups of rats, PE caused concentration-dependent increases in contraction. When the PE response was presented as the percentage of maximum PE contraction, the median effective concentration ($ED_{50}$) of PE in C rats was greater than that in LP rats ($5.4 \pm 0.05$ vs. $5.8 \pm 0.30$).

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*Fig. 1.* Body weight gain from birth to 9 weeks of age in offspring of the C and LP groups. Values are expressed as mean ± SEM. *p < 0.05 compared with the C group.

*Fig. 2.* a Dose-response curve for ACh-evoked hypotension in C and LP rats. Conscious female rats were administered increasing concentrations of ACh as intravenous bolus doses. Hypotensive responses were calculated as percent decrease in MAP with respect to baseline MAP before each response. Values are expressed as means ± SEM of 3 or 4 animals. *p < 0.05 compared with the C group. b Dose-response curve for PE-evoked hypertension in C and LP rats. Conscious female rats were administered increasing concentrations of PE as intravenous bolus doses. Hypertensive responses were calculated as percent increase in MAP with respect to baseline MAP before each response. Values are expressed as means ± SEM of 3 or 4 animals. *p < 0.05 compared with the C group.
0.12 mol/l; fig. 3, table 2). This shows that PE was more potent in producing contractions in LP rats than in C animals.

Pretreatment with L-NAME for 30 min to inhibit eNOS activity significantly enhanced the maximal PE-induced contraction in LP rats (116.6 ± 2.54%; fig. 4b, table 2) and to a greater extent in C rats (136.6 ± 5.95%; fig. 4a, table 2). Also, plotting the PE response as a percentage of maximum and calculation of the PE ED50 showed that PE was slightly more potent (but the difference was not statistically significant, p = 0.46) in causing contraction in L-NAME-pretreated than in non-treated arterial rings of LP rats (fig. 4b, table 2). PE was far more potent in causing contraction in L-NAME-treated than non-treated vascular rings of C rats (fig. 4a, table 2).

Similarly, in endothelium-intact vascular rings, pretreatment with ODQ for 30 min to inhibit cGMP production in smooth muscle significantly enhanced the maximal PE-induced contraction in LP rats (119.7 ± 3.18%; fig. 4b, table 2) and to a greater extent in C rats (136.5 ± 5.73%; fig. 4a, table 2). PE was slightly more potent (but the difference is not statistically significant, p = 0.27) in producing contractions in ODQ-treated arterial rings of LP rats than in C animals (fig. 4a, table 2).

**Endothelium-Dependent Relaxant Responses**

In endothelium-intact vascular rings of C rats, ACh caused concentration-dependent relaxation of PE-mediated (3 × 10^{-6} mol/l) contraction. The ACh-induced relaxation of the PE contraction was significantly less in LP rats than in C animals (fig. 5a). ED50 of ACh in mesenteric arterial rings of LP rats (6.1 ± 0.11 mol/l) was significantly different from that in arterial rings of C rats (6.7 ± 0.18 mol/l) (table 2). Because the mesenteric arte-
Table 2. Vascular function in offspring of the C and LP group at 1 year of age

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<tr>
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<th>C group</th>
<th>LP group</th>
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<tr>
<td></td>
<td>pD₂</td>
<td>Eₘₐₓ</td>
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<tr>
<td>PE</td>
<td>5.42 ± 0.054</td>
<td>100</td>
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<tr>
<td>PE + l-NAME</td>
<td>5.98 ± 0.072b</td>
<td>136.63 ± 5.95b</td>
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<tr>
<td>PE + ODQ</td>
<td>5.86 ± 0.028b</td>
<td>136.45 ± 5.73b</td>
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<tr>
<td>ACh</td>
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<td>98.3 ± 1.12</td>
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<tr>
<td>ACh + l-NAME</td>
<td>–</td>
<td>50.8 ± 2.68c</td>
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<tr>
<td>ACh + ODQ</td>
<td>–</td>
<td>48.7 ± 3.15c</td>
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<tr>
<td>SNP</td>
<td>7.72 ± 0.073</td>
<td>98.0 ± 1.06</td>
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Values are expressed as mean ± SEM of 10–12 mesenteric arterial rings from 5–6 rats in each group. ED₅₀ is presented as -log [mol/l] and maximal responses (Eₘₐₓ) are presented as percent of maximal contraction or relaxation. pD₂ = Negative log-molar concentration. *p < 0.05 compared to the C group; b p < 0.05 compared to PE alone in their respective groups; c p < 0.05 compared to ACh alone in their respective groups.

Fig. 5. a ACh-induced relaxation of PE contraction in endothelium-intact mesenteric arteries of C and LP rats. ACh-induced relaxation of PE contraction in the absence or presence of l-NAME (b) or ODQ (c) in C rats and LP rats (all semilog plots). Mesenteric rings were incubated in the absence or presence of l-NAME (10⁻⁴ mol/l) or ODQ (10⁻⁵ mol/l) for 30 min. Submaximal PE contraction was elicited, ACh was added and then the percentage of relaxation to PE contraction was measured. Data points represent means ± SEM of measurements in 10–12 mesenteric arterial rings from 5–6 rats of each group. * p < 0.05 compared to the C group.
rial rings of LP rats showed greater vascular contraction compared to C rats, control experiments were performed on rings from LP rats in which the initial PE concentration was lowered to $1 \times 10^{-6}$ mol/l to produce a submaximal contraction that was roughly equal in magnitude to the contraction observed in rings of C rats precontracted with $3 \times 10^{-6}$ mol/l PE. These experiments showed that the ED$_{50}$ of ACh in mesenteric arterial rings of LP rats precontracted with $3 \times 10^{-6}$ mol/l PE (6.2 ± 0.12 mol/l) was not significantly different from that in arterial rings precontracted with $3 \times 10^{-7}$ mol/l PE (6.1 ± 0.11 mol/l). This shows that ACh relaxation does not vary with the amplitude of PE contractions.

Pretreatment of endothelium-intact rings with L-NAME to inhibit eNOS or ODQ to inhibit cGMP production in smooth muscle inhibited ACh-induced relaxation significantly in LP and C rats (fig. 5b, c, table 2). Removal of the endothelium completely inhibited the ACh-induced relaxation to PE contraction in all groups of rats (data not shown).

**Nitrite Production**

In endothelium-intact vascular rings, the basal nitrite/nitrate ($\text{NO}_x$) production was 83.9 ± 12.51 pmol/mg tissue weight in C rats and this was significantly lower in the LP rats (28.2 ± 6.37 pmol/mg tissue weight) (fig. 6). Similarly, NO$_x$ production in response to ACh was also significantly reduced in the LP group compared with the C group (fig. 6).

**Endothelium-Independent Relaxant Responses**

In endothelium-denuded vascular rings of both LP and C groups, SNP, an exogenous NO donor and a standard guanylate cyclase activator, caused concentration-dependent relaxation of submaximal PE contractions. The SNP-induced relaxation to PE contractions was not significantly different in vascular rings from C and LP rats (7.7 ± 0.07 vs. 7.7 ± 0.09 mol/l; fig. 7, table 2).

**Discussion**

The goal of the present study was to determine whether dietary protein restriction during gestation resulted in hypertensive female offspring that exhibit impaired endothelium-dependent vascular relaxation and enhanced vascular contraction. Terminal studies were done in female offspring at 1 year of age. The main findings are as follows: (1) birth weight and later growth are significantly lower in LP offspring compared to the C group; (2) LP offspring have extended estrous cycle lengths with a reduced number of animals cycling; (3) MAP is elevated in

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Fig. 6. Basal and ACh-induced NO$_x$ productions in endothelium-intact mesenteric arteries of C and LP rats. Data points represent mean ± SEM of measurements in 30–40 mesenteric arterial rings from 3 rats of each group. *p < 0.05 compared with the respective treatment in the C group. b p < 0.05 compared to basal level in the respective group.

Fig. 7. SNP-induced relaxation to PE contraction in endothelium-denuded vascular rings of C and LP rats (semilog plot). Submaximal PE contraction was elicited, increasing concentrations of SNP were added and then the percentage of relaxation to PE contraction was measured. Data points represent means ± SEM of measurements in 10–12 mesenteric arterial rings from 5–6 rats of each group.
weight category, which represented birth weights more resistance of females to the hypertensive effects of peri-
Indeed, in one study in women, the inverse association between birth weight and adult hypertension or cardio-
vascular disease was marked only in the lowest birth weight category, which represented birth weights more than 25% below average [39, 40]. Thus, it appears that the resistance of females to the hypertensive effects of perinatal insults breaks down when the insults are severer.

In order to investigate alterations of vascular function in the female offspring of protein-restricted dams, responses to ACh, an endothelium-dependent vasodilator, to SNP, an endothelium-independent vasodilator, and to PE, a vasoconstrictor whose effects are modulated by the endothelium, were investigated. Hypotensive responses and mesenteric vasorelaxation to ACh were reduced in LP offspring compared to C rats, but no differences could be detected to SNP. These observations suggest that it is not the smooth-muscle vasodilating capability that is reduced in LP offspring but some function related to the endothelium. Reports of impaired endothelial function in female offspring have been shown in previous studies using excess fat [41, 42] and global food restriction during pregnancy [38]. In fact, Goodfellow et al. [43] and Leeson et al. [44] demonstrated that growth restriction in human pregnancy is associated with endothelial dysfunction.

Considering that NO is the main agonist responsible for endothelial-dependent relaxation, we characterized the relative contribution of NO in vasodilation of mesen-
teric arteries isolated from LP and C offspring. Besides NO, ACh also induces vasorelaxation through NO-independent mechanisms [45–48]. This is evident in this study by the observation that L-NAME and ODQ did not completely inhibit ACh-induced relaxation. However, this L-NAME/ODQ-resistant component was shown to contribute equally to vasodilation in both C and LP offspring, indicating that only the NO component may be impaired, thereby contributing to the observed difference in pressor response in these groups. Although endothelium-derived hyperpolarizing factor is known to compensate for loss of NO function in arteries [48], such a response was not observed in this study.

Impaired NO function may be due to decreased NO synthesis and/or bioavailability. The finding that L-NAME induced a greater increase in MAP in C than in LP offspring indicates that NO-mediated control of vascular tone is certainly compromised in female offspring exposed to protein restriction in utero. Consistent with this finding, pretreatment of the isolated mesenteric arteries from C rats with L-NAME inhibited ACh-induced vascular relaxation to a greater extent than in LP offspring. These results indicate that NO synthesis by endothelial cells may be impaired in LP rats. This concept is supported by the observation that both basal and ACh-induced NOx productions were significantly reduced in arteries from the LP compared with C rats. There are similar reports of reduced basal and agonist-induced NO release in pregnant rat dams fed a protein-restricted diet [49] and also in the offspring of patients with essential
hypertension [50]. The decreased levels of NO may be associated with decreased expression/activity of eNOS as previously reported by us and others [13, 51], or due to rapid degradation by superoxide [52]. However, our attempt to determine eNOS activity in mesenteric arteries of LP rats in this study showed varied responses and was less conclusive, necessitating further investigation.

It is well known that the endothelium modulates the vasoconstrictor response to PE, probably by releasing NO. Reduced NO function may lead to increased responses to PE. The fact that the LP offspring were hyper-responsive to PE and that the mesenteric arteries with endothelium from LP rats were more responsive to PE led us to suggest that the modulatory role of the endothelium on the vascular responses has been lost in rats submitted to intrauterine protein restriction. The findings that l-NAME and ODQ pretreatment potentiated PE-induced constriction in mesenteric arteries from C rats but had minimal effects in LP offspring also suggest that the vascular smooth muscle responses to PE have been preserved in LP offspring, reinforcing the hypothesis that protein restriction in utero mainly affects the endothelium.

The factors that contribute to endothelial dysfunction in LP offspring remain unclear. In LP offspring, the reproductive performance was shown to be impaired during adult life (i.e. after 6 months) as evident by increased estrous cycle length and decreased number of cycling animals. Similar findings of reduced reproductive function associated with delayed vaginal opening, onset of first estrous and impaired endocrine function in offspring of nutrient-restricted dams were reported [53]. We and others have reported that, consistent with the impaired reproductive function, the serum estrogen levels in rats exposed to intrauterine undernutrition are only half of those of controls [13, 27, 54]. Estrogen is known to have a vasoprotective effect on endothelium via an NO-mediated mechanism. It was shown that long-term treatment of cultured human and bovine endothelial cells with estrogen upregulates eNOS activity [30, 55]. In addition, deficiency of estrogen (following ovariectomy) in spontaneously hypertensive rats causes a reduction in endothelial function [56]. Since females from dams submitted to protein restriction during pregnancy exhibited reduced estrogen levels, we suggested that the vasoprotective role of the estrogen on the vascular responses may be lost, leading to decreased endothelial function. Not only physiologically relevant concentrations of estrogen are important, but also having active estrogen receptors (ERs; including ERα and ERβ) is essential for both a rapid and long-term positive CV effect [57–60]. We have previously demonstrated a decrease in ERα and ERβ in the thoracic aorta of protein-restricted female offspring [51]. In fact, preliminary studies from our laboratory have shown that ovariectomy exacerbates hypertension in the offspring of protein-restricted dams, indicating a role for estrogen in the normalization of blood pressure. Further studies are under way to delineate the vasoprotective mechanisms of estrogen.

Several previous studies have demonstrated that essential hypertension is associated with impaired endothelium-dependent vascular relaxation [61–63]. However, whether this is a primary or secondary abnormality is unknown. Certain previous investigations have suggested that endothelial dysfunction is a primary defect in essential hypertension, present even before the clinical documentation of elevated blood pressure [64]. On the other hand, certain observations suggest that endothelial dysfunction may occur as a consequence rather than a cause of elevated blood pressure. In particular, human studies and several animal models of induced hypertension, including suprarenal coarctation of the abdominal aorta in rabbits [65], salt-induced hypertension in Dahl salt-sensitive rats [66], and pressure increases in cat cerebral [67] and dog coronary arteries [68], have all shown a selective impairment of endothelium-dependent vasodilation after elevations in blood pressure. It is important to emphasize the following cautionary remarks regarding the aforementioned interpretations. First, although we hypothesized that the decreased endothelial cell function and increased vascular contraction in LP rats could contribute to the observed elevation in arterial pressure, the reverse is also plausible. Further analysis of the time course of the changes in vascular functions and the increase in arterial pressure in rats <1 year of age should help determine whether the relation between these two parameters is causal or associative. Second, although the reduction in vascular relaxation and increase in vascular contraction in the LP group could explain in part the increase in arterial pressure, other factors such as renal, neural and hormonal control mechanisms of arterial pressure could also be involved. Third, as the vascular endothelium also releases contracting factors such as thromboxane and endothelin, we cannot exclude a role for these factors in the endothelial dysfunction induced by intrauterine undernutrition. Therefore, analysis of the role of other endothelium-derived relaxing factors in the vasculature and blood pressure changes observed in LP rats should be carefully examined in future studies.

In summary, the present study has shown that an endothelium-dependent vascular relaxation pathway involving the release of NO from endothelial cells, but not
the smooth muscle response to NO, is inhibited in systemic vessels of LP offspring of pregnant rats with reduced protein intake. The decreased NO-mediated vasomotor dysfunction in LP rats might explain the increased vascular contraction and arterial pressure in young adult rats with low birth weight.

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