Decreased Endothelium-Dependent NO-cGMP Vascular Relaxation and Hypertension in Growth-Restricted Rats on a High-Salt Diet

Jason A. Payne, Barbara T. Alexander, Raouf A. Khalil

Abstract—Low birth weight caused by placental insufficiency increases the risk of hypertension in young adults, particularly while ingesting a high-salt diet; however, the vascular mechanisms involved are unclear. We tested whether intrauterine fetal growth restriction results in salt-sensitive offspring that exhibit impaired endothelium-dependent relaxation, enhanced vascular contraction, and hypertension during high-salt diet feeding. Male offspring of control pregnant rats and pregnant rats with reduced uterine perfusion pressure (intrauterine growth restricted [IUGR]) were fed either a normal-sodium (NS, 1%) or a high-sodium (HS, 8%) diet. Body weight was less in IUGR/NS and IUGR/HS than in NS and HS rats. Arterial pressure was greater in IUGR/NS (144±4 mmHg) than in NS (131±3 mmHg) rats and far greater in IUGR/HS (171±2 mmHg) than in HS (129±2 mmHg) rats. In isolated, endothelium-intact aortic strips, phenylephrine (Phe, 10⁻⁵ mol/L) caused an increase in active stress that was greater in IUGR/NS (13.9±0.9 N/m²) than in NS (8.5±0.6 N/m²) and far greater in IUGR/HS (18.2±1.2 N/m²) than in HS (9.4±0.8×10⁴ N/m²) rats. Acetylcholine caused relaxation of the Phe-mediated contraction and induced vascular nitrite/nitrate production that was less in IUGR/NS than in NS animals and far less in IUGR/HS than in HS rats. N⁶-nitro-L-arginine methyl ester, which inhibits nitric oxide (NO) synthase, or ODQ, which inhibits cGMP production in smooth muscle, inhibited acetylcholine relaxations and enhanced Phe contractions in NS and HS rats but not in IUGR/NS or IUGR/HS rats.

Endothelium removal enhanced Phe-induced stress in NS and HS rats but not in IUGR/NS or IUGR/HS rats. Thus, endothelium-dependent relaxation via the NO-cGMP pathway is inhibited in systemic vessels of IUGR rats, particularly during intake of an HS diet. This might explain the increased vasoconstriction and arterial pressure in low-birth-weight offspring during ingestion of an HS diet. (Hypertension. 2004;43[part 2]:420-427.)

Key Words: arterial pressure ■ endothelium ■ nitric oxide ■ muscle, vascular, smooth ■ constriction ■ hypertension, pregnancy

Low birth weight is a suggested predisposing factor for increased arterial pressure in young adults.¹⁻⁶ According to the “fetal origin” hypothesis, in utero programming of cardiovascular diseases such as hypertension might occur as a result of decreased maternal food intake or a reduction in uteroplacental perfusion.¹,²,⁷ Animal models of an adverse fetal environment induced by maternal malnutrition support a role for in utero programming of hypertension.⁸⁻¹⁰ It has been reported that protein restriction during the last third of gestation in the rat results in offspring with reduced renal function and hypertension.¹¹ Also, undernutrition in early pregnancy is associated with decreased endothelium-dependent relaxation in blood vessels of fetal sheep.¹² Although the majority of low-birth-weight infants in the Western world are the result of a reduction in uteroplacental perfusion rather than maternal malnutrition,¹³ little is known regarding the renal and vascular mechanisms linking intrauterine growth restriction caused by reduced uteroplacental perfusion and hypertension. In recent studies, we have examined renal and vascular functions in a model of intrauterine growth restriction produced in response to reduced uteroplacental perfusion pressure initiated in late pregnancy.¹⁴⁻¹⁸ These studies have suggested possible alterations in the renal and vascular control mechanisms of arterial pressure in growth-restricted offspring of pregnant rats with reduced uteroplacental perfusion.¹⁹,²⁰

A high-salt diet is implicated in the pathogenesis of hypertension, particularly in salt-sensitive individuals,²¹⁻²⁴ and salt moderation is often recommended to protect against excessive increases in blood pressure.²¹,²³,²⁵,²⁶ A high-salt diet is believed to increase the risk of hypertension in young adults with low birth weight, and monitoring of salt intake in

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low-birth-weight infants has been suggested. Also, our preliminary experiments have shown that intrauterine fetal growth restriction caused by placental insufficiency results in low-birth-weight offspring that become severely hypertensive when fed a high-sodium diet. However, the vascular mechanisms linking intrauterine growth restriction in response to decreased uteroplacental perfusion and the increased arterial pressure during intake of a high-salt diet are unclear.

The vascular endothelium plays a major role in the control of vascular tone and has been shown to release endothelium-derived relaxing factors, such as nitric oxide (NO), which diffuse into the smooth muscle, where it stimulates the enzyme guanylate cyclase, leading to increased cGMP production and smooth muscle relaxation. Although previous studies by several groups have shown that a high-salt diet impairs endothelial function, to our knowledge, no previous studies have investigated the relation between a high-salt diet and endothelial cell function in low-birth-weight offspring owing to placental insufficiency.

The purpose of this study was to test the hypothesis that intrauterine fetal growth restriction due to placental insufficiency results in low-birth-weight offspring that are salt-sensitive and exhibit impaired endothelium-dependent vascular relaxation, enhanced vascular contraction, and hypertension, specifically during intake of a high-salt diet. To test this hypothesis, we used the offspring of normal pregnant rats and offspring of a pregnant rat model of reduced uteroplacental perfusion pressure produced by clipping the uterine and ovarian arteries during late pregnancy. After weaning, the offspring were placed on either a normal- or a high-sodium diet. Experiments were designed to investigate (1) whether vascular contraction to the α-adrenergic agonist phenylephrine (Phe) is enhanced in growth-restricted compared with normal rats, particularly during ingestion of a high-salt diet; (2) whether vascular relaxation to acetylcholine (ACh) is reduced in growth-restricted compared with normal rats, particularly during intake of a high-salt diet; and (3) whether the changes in vascular contraction/relaxation in blood vessels of growth-restricted rats, particularly during intake of a high-salt diet, involve alterations in the endothelium-dependent NO-cGMP pathway.

Methods

Pregnant Rats

Female timed-pregnant Sprague-Dawley rats (day 12 of gestation) were purchased from Harlan Sprague-Dawley Inc (Indianapolis, Ind), housed individually per cage in a temperature-controlled (23°C) room, and maintained on ad libitum standard rat chow and tap water under a 12-hour/12-hour light/dark cycle. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center.

Protocol for Reduction of Uteroplacental Perfusion Pressure

On day 14 of pregnancy, pregnant rats were anesthetized with isoflurane, the abdominal cavity was opened by a midline incision, and a silver clip (0.23 mm inner diameter) was placed around the abdominal aorta above the iliac bifurcation. This procedure has been shown to reduce uterine perfusion pressure in the gravid rat by 40%. Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, a silver clip (0.1 mm inner diameter) was also placed on the main uterine branches of the right and left ovarian arteries. Normal pregnant rats were sham-operated.

Offspring Rats

All pregnant rats were allowed to deliver at term. Offspring of normal, sham-operated, pregnant rats were referred to as the normal group. Offspring of pregnant rats that underwent surgical procedures to reduce uteroplacental perfusion were referred to as the intrauterine growth–restricted (IUGR) group. The litter sizes and birth weights of normal and IUGR pups were recorded within 12 hours after delivery. Because the litter size was smaller in pregnant rats with reduced uterine perfusion compared with normal pregnant rats, litter sizes of the normal and IUGR rats were size-matched. All pups were marked for identification by tattooing. Pups were weighed twice a week after birth and were weaned at 3 weeks of age. After weaning, the rats were divided into 4 groups: normal rats on a normal-salt diet (NS, n = 12); normal rats on a high-salt diet (HS, n = 12); IUGR rats on an NS diet (IUGR/NS, n = 9); and IUGR rats on an HS diet (IUGR/HS, n = 10). The NS rats were fed a diet containing 1% NaCl. The HS groups were fed a diet containing 5% NaCl. The rats were kept on their respective diets for 9 weeks and were studied at 12 weeks of age.

Mean Arterial Pressure

Mean arterial pressure (MAP) was determined in conscious, chronically instrumented rats. The rats were anesthetized with 2% isoflurane, and a PE-50 arterial catheter was placed in the carotid artery. After a 2- to 3-day recovery period, the arterial catheter was connected to a pressure transducer (Cobe model CDX III, Sema), and MAP in conscious rats was recorded on a polygraph (Grass model 7D, Astro-Med). Offspring from different litters were chosen at random, and MAP was measured simultaneously in normal and IUGR offspring ingesting NS and HS diets.

Tissue Preparation

On the day of the experiment, the rats were anesthetized by inhalation of isoflurane. The thoracic aorta was excised, placed in oxygenated Krebs solution, cleaned of connective tissue, and cut into 3-mm-wide strips. For endothelium-intact aortic strips, extreme care was taken to avoid injury to the endothelium. For endothelium-denuded aortic strips, the endothelium was removed by gently rubbing the vessel interior with wet filter paper. Removal of the endothelium was verified by the absence of ACh relaxation in tissues precontracted by a submaximal concentration of Phe.

Isometric Contraction

One end of the aortic strip was attached to a glass hook with a thread loop, and the other end was connected to a force transducer (Grass FT03). Aortic strips were stretched to 1.5 times the unloaded initial length. The strips were allowed to equilibrate for 1 hour in a temperature-controlled tissue bath filled with 30 mL Krebs solution continuously bubbled with 95% O2 and 5% CO2 at 37°C. The changes in isometric contraction were recorded on a polygraph (Grass model 7D).

Aortic strips were stimulated with increasing concentrations of Phe, and concentration-contraction curves were constructed. In other tissues, submaximal Phe (3 × 10−7 mol/L) contraction was elicited, increasing concentrations of ACh or sodium nitroprusside (SNP) were added, and vascular relaxation was measured. In other experiments, the tissues were pretreated for 30 minutes with Nω-nitro-L-arginine methyl ester (L-NAME, 10−4 mol/L) to inhibit NO synthase (NOS) or with 1H-1,2,4-oxadiazolo-4,3-quinoxalin-1-one (ODQ, 10−5 mol/L) to inhibit cGMP production in smooth muscle, and the effects on Phe contraction and ACh-induced relaxation of Phe contraction were measured.

NO3 Production

Endothelium-intact aortic strips were placed in test tubes containing 2 mL Krebs solution aerated with 95% O2 and 5% CO2 at 37°C.
Body Weight, MAP, Maximal Phe (10^{-5} mol/L)-Induced Active Stress, and Phe ED_{50} in Vascular Strips of Normal and IUGR Rats on NS and HS Diets

<table>
<thead>
<tr>
<th>Variable</th>
<th>NS</th>
<th>HS</th>
<th>IUGR/NS</th>
<th>IUGR/HS</th>
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<tbody>
<tr>
<td>Weight, g</td>
<td>275±8</td>
<td>281±9</td>
<td>251±6*</td>
<td>244±8†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>131±3</td>
<td>129±2</td>
<td>144±4*</td>
<td>171±12†</td>
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<td>Phe, 10^{-5} mol/L, +endo</td>
<td>8.5±0.6</td>
<td>9.4±0.8</td>
<td>13.9±0.9*</td>
<td>18.2±1.2†</td>
</tr>
<tr>
<td>Active stress, ×10^4 N/m^2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−Endo</td>
<td>10.8±0.6‡</td>
<td>12.9±1.1‡</td>
<td>14.8±1.0*</td>
<td>18.9±1.4†</td>
</tr>
<tr>
<td>l-NAME</td>
<td>11.6±0.8‡</td>
<td>12.9±1.1‡</td>
<td>15.7±1.1*</td>
<td>19.2±1.3†</td>
</tr>
<tr>
<td>ODQ</td>
<td>10.9±0.7‡</td>
<td>13.4±1.2‡</td>
<td>14.7±1.1*</td>
<td>17.4±1.2†</td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>+Endo</td>
<td>7.07±0.06</td>
<td>7.10±0.11</td>
<td>7.52±0.21*</td>
<td>7.80±0.16†</td>
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<tr>
<td>ODQ</td>
<td>7.30±0.09‡</td>
<td>7.43±0.08‡</td>
<td>7.64±0.12*</td>
<td>7.80±0.16†</td>
</tr>
</tbody>
</table>

Endo indicates endothelium. All other abbreviations are as defined in text. Data represent mean±SEM of measurements in 18 to 24 vascular strips from 9 to 12 rats of each group.

*Measurements in IUGR/NS are significantly different (P<0.05) from NS.
†Measurements in IUGR/HS are significantly different (P<0.05) from HS.
‡Significantly different (P<0.05) from corresponding measurements in endothelium-intact vascular strips.

Samples for basal accumulation of nitrite (NO\textsubscript{2}^-) formed from released NO were first taken. The Krebs solution was replaced, and the strips were stimulated with ACh for 5 minutes. The strips were rapidly removed, dabbed dry with filter paper, and weighed. The incubation solutions were assayed for the stable end product of NO, NO\textsubscript{2}^- . In brief, samples of incubation solution (50 μL in triplicate) were mixed in a 96-well microtiter plate with 100 μL of the Griess reagent. The chromophore generated by the reaction with NO\textsubscript{2}^- was detected spectrophotometrically (553 nm) on a microtiter plate reader (THERMOMax, Molecular Devices). The concentration of NO\textsubscript{2}^- was calculated from a calibration curve constructed with known concentrations of NaNO\textsubscript{2}.

Solutions, Drugs, and Chemicals
Normal Krebs solution contained (in mmol/L) NaCl 120, KCl 5.9, NaHCO\textsubscript{3} 25, NaH\textsubscript{2}PO\textsubscript{4} 1.2, dextrose 11.5, MgCl\textsubscript{2} 1.2, and CaCl\textsubscript{2} 2.5 at pH 7.4. Stock solutions of Phe, ACh, SNP, and l-NAME (Sigma) were prepared in distilled water. ODQ (Calbiochem) was dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in solution was <0.1%. All other chemicals were of reagent grade or better.

Statistical Analysis
The developed force was corrected for the cross-sectional area of each aortic strip and expressed as active stress (N/m\textsuperscript{2}) calculated from the equation stress=force/cross-sectional area, where cross-sectional area=wet weight/tissue density×length of the strip), and tissue density=1.055 g/cm\textsuperscript{3}. Data from several vascular strips of the same rat were averaged and presented as the mean±SEM for each group. Data were analyzed by ANOVA with multiple classification criteria, ie, rat type (normal versus IUGR), salt in diet (NS versus HS), condition of endothelium (intact versus denuded), and vascular pretreatment (nontreated versus pretreated with 1-NAME or ODQ), followed by Bonferroni post test to compare selected groups or Dunnet post test to compare all groups with the NS group. Differences were considered statistically significant at P<0.05.

Results
The birth weight of normal rats was 6.6±0.2 g and was significantly reduced (P<0.01) in IUGR rats (5.8±0.1 g). Both normal and IUGR rats showed significant increases in body weight with age. However, the body weight was consistently lower in IUGR compared with normal rats. At 12 weeks of age, the body weight was not significantly different between NS and HS rats (P=0.623) but was significantly reduced in IUGR/NS compared with NS (P=0.036) animals and in IUGR/HS compared with HS (P=0.007) rats (Table). Also, at 12 weeks of age, MAP was not significantly different between NS and HS rats (P=0.585). However, MAP was significantly greater in IUGR/NS than in NS (P=0.016) animals and far greater in IUGR/HS than in HS rats (P=0.001; the Table).

In endothelium-intact aortic strips from all groups of rats, Phe caused concentration-dependent increases in active stress (Figure 1). The maximal Phe (10^{-5} mol/L)-induced vascular contraction in NS rats was not significantly different from that in HS rats (P=0.378; Figure 1A and the Table). The maximal Phe-induced contraction was significantly greater in IUGR/NS than in NS (P<0.001) rats and far greater in IUGR/HS than in HS rats (P<0.0001; Figure 1A and the Table). When the Phe response was presented as the percentage of maximum Phe contraction, the median effective concentration (ED\textsubscript{50}) of Phe in NS rats was not significantly different from that in HS rats (P=0.813; Figure 1B and the Table). Phe was significantly more potent in producing contractions in IUGR/NS than in NS animals (P=0.031) and far more potent in IUGR/HS than in HS rats (P=0.001; Figure 1B and the Table).

Removal of the endothelium significantly enhanced (P<0.05) the maximal Phe-induced contraction in NS rats (Figure 2A) and to an even greater extent in HS rats (Figure 2B and the Table). In contrast, removal of the endothelium did not significantly affect the maximal Phe-induced stress in the IUGR/NS group (Figure 2A) or in IUGR/HS rats (Figure 2B and the Table). Phe was significantly more potent in
causing contractions in endothelium-denuded than in endothelium-intact strips of NS rats (P = 0.036; Figure 2C and the Table). Phe was far more potent in causing contractions in endothelium-denuded than in endothelium-intact strips of HS rats (P = 0.006; the Table). In contrast, the potency of Phe was not significantly different between endothelium-denuded and endothelium-intact strips from IUGR/NS animals (P = 0.842; Figure 2C) or between endothelium-denuded and endothelium-intact strips from IUGR/HS rats (P = 0.817; Figure 2D and the Table).

In endothelium-intact vascular strips, pretreatment with L-NAME (10^(-4) mol/L) for 30 minutes to inhibit NOS significantly enhanced (P<0.05) the maximal Phe-induced active stress in NS rats (Figure 3A and the Table) and to a greater extent in HS rats (Figure 3B and the Table). Also, plotting the Phe response as a percentage of maximum and calculation of the Phe ED50 showed that Phe was more potent in causing contraction in L-NAME-pretreated than nontreated strips of NS rats (P = 0.015; Figure 3C and the Table). Phe was far more potent in causing contraction in L-NAME-pretreated than nontreated vascular strips of HS rats (P = 0.002; Figure 3D and the Table). In contrast, the maximal Phe-induced stress (Figure 3A and 3B) and the Phe ED50 (Figure 3C and 3D) were not significantly different between L-NAME-pretreated and nontreated vascular strips of IUGR/NS and IUGR/HS rats (Table).

Similarly, in endothelium-intact vascular strips, pretreatment with ODQ (10^(-5) mol/L) for 30 minutes to inhibit cGMP production in smooth muscle significantly enhanced the maximal Phe-induced active stress in NS rats (P = 0.016; Figure 3A and the Table) and to a greater extent in HS rats (P = 0.011; Figure 3B and the Table). Also, Phe was more potent in causing contractions in ODQ-pretreated than nontreated strips of NS rats (P = 0.045; Figure 3C and the Table). Phe was far more potent in causing contraction in ODQ-pretreated than nontreated vascular strips of HS rats (P = 0.024; Figure 3D and the Table). In contrast, the maximal Phe-induced stress (Figure 3A and 3B) and the Phe ED50...
Figure 3. Effects of L-NAME and ODQ on Phe-induced contraction in endothelium-intact vascular strips of normal and IUGR rats on NS (A and C) and HS (B and D) diets (all semilog plots). Aortic strips were incubated in the absence or presence of L-NAME (10^{-4} mol/L) or ODQ (10^{-4} mol/L) for 30 minutes and then stimulated with increasing concentrations of Phe. Phe contraction was presented as active stress (A and B) or as percentage of maximum Phe contraction (C and D). Data points represent mean±SEM of measurements in 18 to 24 aortic strips from 9 to 12 rats of each group. *Measurements in IUGR/NS are significantly different (P<0.05) from those in NS rats.

Figure 4. ACh-induced relaxation of Phe contraction in endothelium-intact vascular strips of normal and IUGR rats on NS and HS diets (A and C) and HS (B) and diets (all semilog plots). Aortic strips were incubated in the absence or presence of L-NAME (B) or ODQ (C) (all semilog plots). Aortic strips were incubated in the absence or presence of L-NAME (10^{-4} mol/L) or ODQ (10^{-4} mol/L) for 30 minutes. Submaximal Phe contraction was elicited, ACh was added, and then the percentage of relaxation to Phe contraction was measured. Data points represent mean±SEM of measurements in 18 to 24 aortic strips from 9 to 12 rats of each group. *Measurements in IUGR/NS are significantly different (P<0.05) from those in NS rats. †Measurements in IUGR/HS are significantly different (P<0.05) from those in HS rats.

Pretreatment of endothelium-intact strips with L-NAME (10^{-4} mol/L) to inhibit NOS (Figure 4B) or ODQ (10^{-4} mol/L) to inhibit CGMP production in smooth muscle (Figure 4C) inhibited ACh-induced relaxation significantly in NS and HS and slightly in IUGR/NS animals but not in IUGR/HS rats. Removal of the endothelium completely inhibited the ACh-induced relaxation to Phe contraction in all groups of rats.

In endothelium-intact vascular strips, the basal nitrite/nitrate (NOx) production was 48.4±7.5 pmol/mg tissue weight in NS rats and appeared to be greater in HS rats (67.3±6.9 pmol/mg tissue weight); however, the difference did not reach significant levels (P=0.077; Figure 5). The basal NOx level showed a significant reduction in IUGR/NS compared with NS animals (P=0.041) and a far greater reduction in IUGR/HS compared with HS rats (P<0.001; Figure 5). ACh increased NOx production in vascular strips from all groups of rats, but to variable extents (Figure 4 and the Table). The ACh-induced NOx production was not significantly different between NS and HS rats but showed a significant reduction in IUGR/NS compared with NS groups and a greater reduction in IUGR/HS compared with HS rats (Figure 5).

In endothelium-intact vascular strips of NS rats, ACh caused concentration-dependent relaxation of Phe-mediated (3×10^{-7} mol/L Phe) contraction (Figure 4A). The ACh-induced relaxation of the Phe contraction was not significantly different between NS and HS rats but was significantly less in IUGR/NS than in NS animals and far less in IUGR/HS than in HS rats (Figure 4A).

Because the aortic strips of IUGR/NS and IUGR/HS rats showed greater vascular contraction compared with those from NS and HS rats, control experiments were performed on strips of IUGR/NS and IUGR/HS rats in which the initial Phe concentration was lowered to 1×10^{-7} mol/L to produce a submaximal contraction that was roughly equal in magnitude to the contraction observed in strips of NS and HS rats precontracted with 3×10^{-7} mol/L Phe. These experiments showed that the ED50 of ACh in aortic strips of IUGR/NS precontracted with 1×10^{-7} mol/L Phe (4.8±0.1×10^{-8} mol/L) was not significantly different (P=0.176) from that in strips precontracted with 3×10^{-7} mol/L Phe (4.6±0.1×10^{-8} mol/L).

Data points represent mean±SEM of measurements in 18 to 24 aortic strips from 9 to 12 rats of each group. *Measurements in IUGR/NS are significantly different (P<0.05) from those in NS rats. †Measurements in IUGR/HS are significantly different (P<0.05) from those in HS rats.

(Figure 3C and 3D) were not significantly different between ODQ-pretreated and nontreated vascular strips of IUGR/NS and IUGR/HS rats (the Table).

In endothelium-intact vascular strips of NS rats, ACh caused concentration-dependent relaxation of Phe-mediated (3×10^{-7} mol/L) contraction (Figure 4A). The ACh-induced relaxation of the Phe contraction was not significantly different between NS and HS rats but was significantly less in IUGR/NS than in NS animals and far less in IUGR/HS than in HS rats (Figure 4A). Because the aortic strips of IUGR/NS and IUGR/HS rats showed greater vascular contraction compared with those from NS and HS rats, control experiments were performed on strips of IUGR/NS and IUGR/HS rats in which the initial Phe concentration was lowered to 1×10^{-7} mol/L to produce a submaximal contraction that was roughly equal in magnitude to the contraction observed in strips of NS and HS rats precontracted with 3×10^{-7} mol/L Phe. These experiments showed that the ED50 of ACh in aortic strips of IUGR/NS precontracted with 1×10^{-7} mol/L Phe (4.8±0.1×10^{-8} mol/L) was not significantly different (P=0.176) from that in strips precontracted with 3×10^{-7} mol/L Phe (4.6±0.1×10^{-8} mol/L).
In endothelium-denuded vascular strips of all groups of rats, SNP, an exogenous NO donor and a standard guanylate cyclase activator,11 caused concentration-dependent relaxation of submaximal Phe contractions. The SNP-induced relaxation to Phe contractions was not significantly different in vascular strips from 9 to 12 rats of each group. Measurements in IUGR/NS are significantly less (P<0.05) than those in NS rats. Measurements in IUGR/HS are significantly less (P<0.05) than those in HS rats.

The main findings of the present study are as follows: (1) MAP is elevated in IUGR offspring compared with control offspring, especially during intake of a high-salt diet; (2) Phe-induced contraction is enhanced in vascular strips from IUGR compared with normal rats, particularly in those on a high-salt diet; (3) the endothelium-dependent vascular relaxation is reduced in arteries from IUGR compared with normal rats, particularly during ingestion of a high-salt diet; and (4) the reduced vascular relaxation and enhanced vascular contraction in IUGR rats, particularly those on a high-salt diet, appear to involve the endothelium-dependent NO-cGMP pathway.

Previous studies have suggested a strong relation between low birth weight and the risk of hypertension.1-6,41,42 Also, a high-salt diet is believed to increase the risk of hypertension in low-birth-weight subjects.27 Experimental studies have also shown that maternal protein undernutrition during gestation in the rat results in offspring that are hypertensive.8-10 However, because of good perinatal care, maternal malnutrition represents a small proportion of low-birth-weight neonates in the Western world. Thus, a strong correlation between low birth weight and hypertension in well-nourished populations is more likely caused by fetal undernutrition and IUGR as a result of placental insufficiency rather than maternal malnutrition.13,41,42 When the risk of low birth weight owing to IUGR is combined with another risk factor such as salt sensitivity, severe hypertension can ensue. Therefore, it is important to study the changes in vascular function associated with low birth weight and a high-salt diet in IUGR offspring produced in response to reduced uteroplacental perfusion.

A goal of the present study was to determine whether a model of placental insufficiency in late gestation resulted in hypertensive IUGR offspring that exhibit impaired endothelium-dependent vascular relaxation and enhanced vascular contraction, specifically during ingestion of a high-salt diet. Placental insufficiency was induced by a long-term reduction in uteroplacental perfusion pressure initiated at day 14 of gestation in the pregnant rat.14-18 IUGR offspring of pregnant rats with reduced uterine perfusion pressure had significantly lower birth weights compared with the offspring of normal pregnant rats. To investigate whether low birth weight due to placental insufficiency was associated with salt-sensitive hypertension, offspring of normal pregnant rats and IUGR offspring of pregnant rats with reduced uterine perfusion pressure were placed on either an NS or HS diet from weaning until 12 weeks of age.

The present results show that a high-salt diet alone in otherwise normal rats caused only a modest increase in blood pressure. This is in agreement with other studies, which have shown that feeding Dahl salt-resistant rats a high-salt diet is associated with modest elevation in blood pressure.43 MAP was significantly increased in IUGR/NS compared with NS rats. On the other hand, when low birth weight due to IUGR was combined with an HS diet, significant elevations in blood pressure were observed, to levels even greater than those observed in IUGR/NS rats.

We examined whether the vascular control mechanisms could play a role in mediating the observed increase in arterial pressure in IUGR, especially in response to a high salt intake. We found that the vascular contraction to Phe was greater in IUGR/NS compared with NS rats and was far greater in IUGR/HS than in HS rats. In search of possible mechanisms involved in the enhanced vascular contraction in the IUGR rats, we found that removal of the endothelium enhanced the Phe-induced contraction in NS and HS rats but had minimal effects in IUGR/NS and IUGR/HS rats. Also, ACh-induced relaxation was reduced in IUGR compared with normal rats, particularly those on an HS diet. These results provide evidence that an endothelium-dependent relaxation pathway is active in normal rats and inhibited in IUGR rats, particularly during intake of an HS diet.

The vascular endothelium is known to release relaxing factors such as NO.24-30 The reduced ACh-induced relaxation in IUGR rats, particularly during ingestion of a high-salt diet,
could be due to a decrease in the synthesis/release of NO from endothelial cells, or it might reflect a change in the sensitivity of vascular smooth muscle to relaxation by NO. The sensitivity of vascular smooth muscle to relaxation by NO could be evaluated by its sensitivity to relaxation by exogenous NO donors, such as SNP. The observation that the relaxation of endothelium-denuded vascular strips by SNP was not different between IUGR/NS and NS rats or between IUGR/HS and HS rats suggests that the decreased relaxation in the IUGR rats was not due to decreased vascular smooth muscle sensitivity to NO and therefore, was more likely due to changes in the synthesis/release of NO.

Pretreatment of the vascular strips with l-NAME, an inhibitor of NO synthesis, inhibited ACh-induced vascular relaxation and enhanced Phe-induced contraction in NS rats and to a greater extent in HS rats. However, in vascular strips from IUGR/NS and IUGR/HS rats, l-NAME did not significantly affect ACh-induced vascular relaxation or Phe-induced contraction. These results suggest that NO synthesis by endothelial cells was impaired in IUGR rats, particularly those on an HS diet. This concept is supported by the observation that both basal and ACh-induced NO production were significantly reduced in vascular strips from the IUGR/NS compared with NS rats and showed greater reductions in IUGR/HS compared with HS rats.

NO produced by endothelial cells is known to promote vascular relaxation by activating guanylate cyclase and increasing the production of cGMP in smooth muscle.31,32 ODQ, which inhibits guanylate cyclase and decreases cGMP production in smooth muscle,33-35 inhibited ACh-induced vascular relaxation and enhanced Phe-induced contraction in vascular strips from NS rats and to a greater extent in vascular strips of HS rats. However, ODQ did not significantly affect ACh-induced vascular relaxation or Phe-induced contraction in IUGR rats, particularly those on an HS diet. These results further support the hypothesis that NO synthesis/release by endothelial cells is inhibited in IUGR offspring and thereby, that the activity of the NO-cGMP pathway in smooth muscle is reduced in IUGR rats, particularly those on an HS diet.

It is important to emphasize the following cautionary remarks regarding the aforementioned interpretations. First, although the decreased endothelial cell function and increased vascular contraction in IUGR rats, particularly during intake of a high-salt diet, could contribute to the observed elevation in arterial pressure, the vascular changes might also be secondary to arterial pressure alterations. Further analysis of the time course of the changes in vascular functions and the increase in arterial pressure during the high-salt diet intake in rats <12 weeks of age should help determine whether the relation between these 2 parameters is causal or associative. Second, although the reduction in vascular relaxation and increase in vascular contraction in the IUGR group, particularly during ingestion of the HS diet, 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, the vascular endothelium releases other vasodilators such as prostacyclin and endothelium-derived hyperpolarizing factor.44,45 This might explain why, in the vascular strips from the IUGR rats, some relaxation to ACh was still observed and was not completely inhibited by l-NAME or ODQ. Whether a decrease in other endothelium-derived relaxing factors such as prostacyclin or hyperpolarizing factors contributes to the decreased vascular relaxation associated with IUGR particularly during an HS diet is unclear. Also, the loss of NO function in the arteries of IUGR/HS rats could lead to a compensatory increase in the release of other endothelium-dependent relaxing factors, and that might provide an alternative explanation of the l-NAME- and ODQ-resistant relaxation in IUGR/HS rats. Therefore, analysis of the role of other endothelium-derived relaxing factors in the vasculature and blood pressure changes observed in IUGR rats, particularly during high salt diet feeding, should be carefully examined in future studies.

**Perspectives**

Low birth weight increases the risk of hypertension in young adults. IUGR resulting from complications of pregnancy such as preeclampsia is a major cause of low birth weight in the Western world. Low-birth-weight offspring of pregnant rats with reduced uteroplacental perfusion are good animal models to study the vascular mechanisms of the hypertension associated with IUGR and a high-salt diet. 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 IUGR offspring of pregnant rats with reduced uteroplacental perfusion, specifically during intake of a high-salt diet. The decreased NO-mediated vascular relaxation pathway in IUGR rats might explain the increased vascular contraction and arterial pressure in young adult rats with low birth weight, particularly during intake of a high-salt diet. However, whether the decreased NO-mediated vascular relaxation is caused by a decreased amount or activity of NOS remains to be clarified. Measurement of NOS expression would allow one to determine whether a high salt intake affects NOS expression or acts at some point downstream to decrease NO production or bioactivity/bioavailability. Also, limitations of studying the relation between vascular reactivity in the aorta and blood pressure changes should be considered and should highlight the importance of studying the vascular effects associated with low birth weight and a high-salt diet in the small and more relevant resistance vessels.

The NS and HS diets used in the present study were selected on the basis of previous published reports from our laboratory and others.16,33,40,43 Although we observed significant increases in vascular contraction and arterial pressure in IUGR rats on an HS (8%) diet, whether significant changes in vascular function and blood pressure also occur with modest increases in sodium intake remain to be clarified. In relation to this point, it will be of interest to determine whether the effects of sodium intake on vascular function are dependent on the concentration of sodium or whether they exhibit a threshold effect. Also, although the present results suggest that IUGR rats are salt-sensitive, whether they are uniquely sensitive to a high sodium intake or are also sensitive to other environmental stressors such as stress, a high-fat diet, and obesity is unclear and should represent important areas for future studies. Last, the present data suggest that the endothelial NO-cGMP-mediated vascular relaxation is reduced in male IUGR/HS offspring. Whether endothelial dys-
function also occurs in female IUGR offspring or whether there are sex-dependent differences in sensitivity to a high-salt diet are unclear and should be studied further in future investigations.

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References


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