Fetal origins of adult vascular dysfunction in mice lacking endothelial nitric oxide synthase

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Fetal Physiological Programming

IN 1986, BARKER AND OSMOND (6) proposed that the intrauterine environment impacts a fetus well beyond the perinatal period. Stimuli or insults to the fetus during the critical period of development lead to fetal programming and produce adaptive changes in the fetal anatomy, physiology, and metabolism that have long-term consequences (4, 5). In support of this hypothesis, Barker (4) and others (35a) have found an association between low birth weight and the occurrence of hypertension, cardiovascular disease, and diabetes later in life. Although several observational studies have implicated uterine environment as being causative in various disease processes, direct evidence linking the effects of pathogenic factors during fetal development and disease in later life is lacking.

Although epidemiological studies have eloquently demonstrated the role of the “in utero” environment in the fetal origins of adult diseases, a role for genetic factors has not been completely excluded (19). The maternal and paternal gene pools collectively determine the fetal genotype, which is a major determinant of fetal phenotype. The parental source of certain genes can also alter their expression and by extension the offspring’s phenotype, a phenomenon referred to as genomic imprinting (18, 33).

Endothelium-derived relaxing factor, first discovered by Furchgott and Zawadzki (15), was later identified as nitric oxide (NO) (15, 20, 28, 32). Defects in the NO pathway have been implicated in the causation of various vascular pathological states, such as atherosclerosis, hypertension, and intrauterine growth restriction (22, 34). NO is a potent smooth muscle relaxant and is believed to be one of the primary modulators of vascular tone (15, 20, 28, 32). It is also an inhibitor of smooth muscle proliferation, thus playing an important role in vascular remodeling (16, 30, 35). NO synthase (NOS) is the enzyme responsible for the production of NO from its substrate L-arginine (9). Three NOS isoforms, i.e., inducible NOS (iNOS or NOS2), endothelial NOS (eNOS or NOS3), and neuronal NOS (nNOS or NOS1), have been described (1, 14). In the vasculature, NOS3 is the main isoform responsible for the production of NO (1, 14). NOS3-mediated NO production is believed to play a significant role in vascular adaptations of pregnancy, including maintenance of adequate uteroplacental perfusion (8, 27, 39). Chronic treatment with nitro-L-arginine methyl ester in rats is associated with the high blood pressure believed to be due to nonspecific inhibition of NOS isoforms, mainly NOS3 (10, 40). Infusion of an inhibitor of NO synthesis during pregnancy causes hypertension and fetal growth restriction (40). Transgenic mice lacking the expression of NOS3 are hypertensive and lack the endothelium-dependent response to acetylcholine (17, 36). Mice with NOS3 overexpression dem-

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onstrate low blood pressure (31). From evidence linking the intrauterine environment with adult cardiovascular function in later life, we hypothesized that fetuses born to mothers lacking NOS3 expression, and in effect exposed to a maternal/uterine vascular environment deficient in NO production, will exhibit abnormal vascular reactivity in later life. To test this hypothesis, transgenic mice lacking a functional endothelial NOS were crossbred with wild-type mice to produce litters with mutant and normal NOS3 genes in various combinations. In addition to the homozygous NOS3-knockout and wild-type litters, the crossbreeding produced two types of heterozygous litters, both with one normal and one mutant NOS3 allele. However, one of the heterozygous litters, i.e., with the maternally derived mutation, developed in a NOS3-deficient environment, whereas the other, i.e., with the paternally derived mutation, developed in a uterine environment similar to that of the wild-type mice. The objective was to compare the vascular reactivity of genotypically similar offspring after development in a normal vs. abnormal (NOS3 deficient) maternal/uterine environment. Because fetuses adapt to uteroplacental dysfunction by redistributing blood flow to vital organs such as the brain at the expense of nonvital organs such as abdominal viscera (3), we chose the carotid artery (representing blood supply to the brain) and the mesenteric artery (representing blood supply to the intestines) for our study to represent these two diverse circulations. In vitro reactivity to vasoactive agents was studied in vessels from adult offspring in each of the four litters.

METHODS

Animals. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch. Female and male mice homozygous for disruption of the endothelial NOS gene [NOS3+/-/KO] and their age-matched wild-type controls (NOS3+/+WT) and their age-matched wild-type controls (NOS3+/+WT) were bred to obtain NOS3+/-/mat and paternally derived (NOS3+/+mat) heterozygous NOS3+/- litters. There were three or four litters in each group. One or two female offspring were taken from each litter for the in vitro vascular studies, with a total of six offspring in each experimental group. Except for the parental origin of the single defective allele, both groups of heterozygous pups were genotypically identical and had one functional NOS3 allele. The maternal environment, however, was different between the two heterozygous litters. In the NOS3+/-/mat litters, the female parent was the NOS3+/- Ko, thus the pups matured in a maternal environment completely lacking the contribution of NOS3. In the NOS3+/-/mat litters, the male parent was the NOS3+/- Ko and the pups developed in a maternal environment expressing the NOS3 gene. In addition, female mice lacking NOS3+/-/mat and their NOS3+/+ WT controls were bred to obtain NOS3+/-/KO and NOS3+/-/WT litters. The number of pups in each litter was determined at the time of delivery. In addition, birth weight and weight at age 6 wk was determined for each pup. Mature cycling female mice (7–8 wk old) from the four litter groups were used for the in vitro experiments. The animals were killed by CO2 inhalation.

Segments of carotid and mesenteric arteries were isolated for evaluation of in vitro responses. The carotid arteries were taken from the segment just proximal to their bifurcation into internal and external carotid arteries. The mesenteric arteries were the second-order branches of the superior mesenteric artery. The internal diameters of both vessels were ~100 μm.

Drugs and solutions. The drugs used in the in vitro experiments were acetylcholine hydrochloride (ACh), phenylephrine hydrochloride (PE), and isoproterenol (Sigma, St. Louis, MO). Stock solutions of all of the drugs (10⁻² mol/l) were prepared in deionized water and stored at -20°C. The composition of physiological salt solution was as follows (in mmol/l): 115 NaCl, 5 KCl, 1.2 NaH2PO4, 25 NaHCO3, 1.2 MgCl2, 2.5 CaCl2, 0.026 EDTA, and 11 glucose. In the experiments with high-K+ (0.9 mol/l) and Ca²⁺ free physiological salt solution (80 mmol/l K+), Ca²⁺ was omitted from the physiological salt solution and KCl replaced NaCl to maintain osmolality of the solution. In vitro experiments. Two-millimeter segments of the carotid and mesenteric arteries from the four groups of mice were mounted in a wire myograph (model 410A, J. P. Trading, Aarhus, Denmark) over 25-μm tungsten wires (29). The preparations were bathed in a physiological salt solution maintained at 37°C, pH ~7.4, and a mixture of 95% O2 and 5% CO2, was bubbled continuously through the solution. The force was recorded continuously by an isometric force transducer and analyzed with Windaq data acquisition and playback software (Dataq Instruments, Akron, OH). The relationship between the internal circumference vs. the wall tension was determined to characterize the viscoelastic properties of the vascular rings. The vessels were stretched in 10- to 30-μm steps every 5 min, and Myosight software (J. P. Trading) was used to generate the length-tension (L-T) relationship (L-T curve) and calculate its slope (2, 23, 25, 29). The software uses the Laplace equation to calculate the equivalent transmural pressure generated in the mounted vessel based on the vessel circumference (distance between the two myograph jaws) and wall tension (measured by the force transducer). The slope obtained from the L-T curve is a measure of the elasticity of the vessel wall; the greater the slope, the more rigid is the vessel wall. We calculated the optimal diameter (OD, μm) of the vessels under relaxed conditions at a transmural pressure of 100 mmHg using the Laplace equation (2, 23, 25, 29). OD is an estimate of the vascular diameter in situ at a specific transmural pressure.

Further experiments were performed at the ring diameter equal to 0.9 OD. After stabilization of the tone, the rings were contracted twice with 60 mmol/l KCl (30 min each) at 30-min intervals to stabilize the vascular responsiveness. The second response was used as a reference contraction for data analysis. After equilibration in physiological salt solution, contractile responses to α-adrenergic agonist PE (10⁻⁹–10⁻⁵ mol/l) were assessed. In addition, relaxant responses to the β-adrenergic agonist isoproterenol (10⁻⁶–10⁻³ mol/l) and to the endothelium-dependent relaxant ACh (10⁻⁹–10⁻⁵ mol/l) were examined in carotid and mesenteric vessels precontracted with PE (10⁻⁷–10⁻⁵ mol/l to match the amplitude of contractions in the different groups). After the vessels were equilibrated in high-K+, Ca²⁺-free solution, contractile responses to cumulative concentrations of Ca²⁺ (0.05–5 mmol/l) were studied to evaluate the Ca²⁺ responsiveness of the vascular smooth muscle.

Data analysis. Data are expressed as means ± SE; n represents the number of female mice used in each experiment. For the experiments that examined vascular structural changes, the data are expressed as the OD and slope of the L-T curve. For the vascular reactivity studies, the area under the concentration-response curve (AUC) and logarithm of the concentration producing 50% of the maximal effect (log IC₅₀), were calculated. The Kolmogorov-Smirnov test was used to check for normality of the data, and one-way ANOVA followed by Newman-Keuls multiple comparison tests were used for statistical analysis. A P value of <0.05 was considered significant.

RESULTS

Litter characteristics. The number of fetuses in each litter was significantly smaller in NOS3+/-mat and NOS3+/-KO...
birth weight of the pups (in g) was not significantly different between the four mouse groups (NOS3+/+WT = 1.52 ± 0.02, NOS3+/+pat = 1.51 ± 0.06, NOS3+/+mat = 1.36 ± 0.02, and NOS3−/−KO = 1.41 ± 0.04). In addition, the mean weight of the pups at age 6 wk (in g) was not significantly different between the four groups of mice (NOS3+/+WT = 18.70 ± 0.27, NOS3+/+pat = 18.34 ± 0.56, NOS3+/+mat = 18.88 ± 0.18, and NOS3−/−KO = 17.89 ± 0.48). The sex ratios (male-to-female) in the four groups were not significantly different (NOS3+/+WT = 1.40 ± 0.55, NOS3+/+pat = 1.33 ± 0.33, NOS3+/+mat = 1.47 ± 0.28, and NOS3−/−KO = 1.25 ± 0.59).

Vessel wall characteristics. The L-T curves generated from the carotid artery rings of NOS3+/+mat and NOS3−/−KO mice were shifted to the left (Fig. 1), and their slopes were significantly greater (Fig. 2A) than those from NOS3+/+WT and NOS3+/+pat mice. In addition, the OD of the carotid artery rings of the NOS3+/+mat and NOS3−/−KO mice were significantly smaller than those of the NOS3+/+WT and NOS3+/+pat mice (Fig. 3A). Similar differences in L-T curves (Fig. 2B) and OD (Fig. 3B) were noted in the mesenteric artery.

In vivo vascular reactivity. The K+ (60 mmol/l)-induced contraction in the carotid artery rings was significantly greater in the NOS3+/−mat and NOS3−/−KO mice compared with the NOS3+/+pat and NOS3+/+WT mice (NOS3+/+WT = 0.87 ± 0.05 mN, NOS3+/+pat = 0.72 ± 0.08 mN, NOS3+/+mat = 1.56 ± 0.16 mN, and NOS3−/−KO = 1.48 ± 0.11 mN; P < 0.05). A similar increase in the responses to potassium chloride was seen in the mesenteric artery of NOS3+/+mat and NOS3−/−KO mice (NOS3+/+WT = 0.44 ± 0.09 mN, NOS3+/+pat = 0.37 ± 0.10 mN, NOS3+/+mat = 1.01 ± 0.12 mN, and NOS3−/−KO = 1.15 ± 0.17 mN; P < 0.05). In addition, the contractile responses to cumulative concentrations of Ca2+ were significantly increased in carotid and mesenteric arteries from NOS3+/+mat and NOS3−/−KO mice compared with the NOS3+/+pat and NOS3+/+WT mice (Fig. 4). The AUCs for the Ca2+ concentration-response curves were also significantly different between the groups in the carotid (NOS3+/+WT = 3.96 ± 0.29, NOS3+/+pat = 3.70 ± 0.19, NOS3+/+mat = 7.77 ± 0.56, and NOS3−/−KO = 7.30 ± 0.40; P < 0.05) and mesenteric arteries (NOS3+/+WT = 1.77 ± 0.37, NOS3+/+pat = 1.49 ± 0.36, NOS3+/+mat = 3.87 ± 0.44, and NOS3−/−KO = 4.24 ± 0.84; P < 0.05).

The responses to PE were significantly greater in the NOS3+/+mat and NOS3−/−KO mice compared with the NOS3+/+pat and NOS3+/+WT mice in the carotid (maximal effect: NOS3+/+WT = 1.20 ± 0.26 mN, NOS3+/+pat = 0.88 ± 0.27 mN, NOS3+/+mat = 2.99 ± 0.51 mN, and NOS3−/−KO = 2.61 ± 0.28 mN; P < 0.05) and mesenteric arteries (maximal effect: NOS3+/+WT = 0.87 ± 0.33 mN, NOS3+/+pat = 0.56 ± 0.41 mN, NOS3+/+mat = 3.92 ± 0.81 mN, and NOS3−/−KO = 3.60 ± 0.46 mN; P < 0.05). Because the potassium chloride responses were also increased in the NOS3+/+mat and NOS3−/−KO mice, and to exclude the possibility that the difference in PE responses resulted from an increase in the contractile potential of the vessels, the responses were normalized to
The AUCs and the log IC50 values were significantly different (AUC: NOS3+/−/WT = 279.35 ± 16.5, NOS3+/−/pat = 257.75 ± 15.16, NOS3+/−/mat = 195.22 ± 25.39, and NOS3−/−KO = 91.13 ± 26.55, P < 0.05; log IC50: NOS3+/−/WT = −8.0 ± 0.12, NOS3+/−/pat = −7.78 ± 0.11, NOS3+/−/mat = −7.28 ± 0.10, and NOS3−/−KO = −7.31 ± 0.05; P < 0.05).

The vasorelaxation induced by the β-adrenoceptor agonist isoproterenol was lower in the NOS3+/−/mat and NOS3−/−KO mice compared with NOS3+/−/WT and NOS3+/−/pat mice (Fig. 6B). The AUC and IC50 results were significantly different (AUC: NOS3+/−/WT = 279.35 ± 16.5, NOS3+/−/pat = 257.75 ± 15.16, NOS3+/−/mat = 195.22 ± 25.39, and NOS3−/−KO = 91.13 ± 26.55, P < 0.05; log IC50: NOS3+/−/WT = −8.0 ± 0.12, NOS3+/−/pat = −7.78 ± 0.11, NOS3+/−/mat = −7.28 ± 0.10, and NOS3−/−KO = −7.31 ± 0.05; P < 0.05).

Fig. 4. Ca2+ concentration-response curves in the carotid (A) and mesenteric (B) arteries of NOS3+/−/WT, NOS3+/−/pat, NOS3+/−/mat, and NOS3−/−KO mice (n = 6 in each group). One-way ANOVA followed by Newman-Keuls tests were used. *P < 0.05 vs. NOS3+/−/WT and NOS3+/−/pat.

The carotid arteries from the NOS3+/−/WT and NOS3+/−/pat mice relaxed in response to ACh, indicating normal endothelium-dependent relaxation, whereas those from the NOS3+/−/mat and NOS3−/−/KO mice did not (or even contracted), indicating the complete absence of the endothelium-dependent relaxation (Fig. 4A). The AUCs were significantly different (AUC: NOS3+/−/WT = 264.3 ± 12.15, NOS3+/−/pat = 279.35 ± 16.5, NOS3+/−/mat = −76.17 ± 12.3, and NOS3−/−KO = −74.8 ± 17.1; P < 0.05). Similarly, relaxant responses to ACh in the mesenteric arteries were significantly decreased in NOS3+/−/mat and NOS3−/−KO compared with NOS3+/−/WT and NOS3+/−/pat mice (Fig. 6B). The AUC and IC50 results were significantly different (AUC: NOS3+/−/WT = 279.35 ± 16.5, NOS3+/−/pat = 257.75 ± 15.16, NOS3+/−/mat = 195.22 ± 25.39, and NOS3−/−KO = 91.13 ± 26.55, P < 0.05; log IC50: NOS3+/−/WT = −8.0 ± 0.12, NOS3+/−/pat = −7.78 ± 0.11, NOS3+/−/mat = −7.28 ± 0.10, and NOS3−/−KO = −7.31 ± 0.05; P < 0.05).

Fig. 3. Optimal diameters of the carotid (A) and mesenteric (B) arteries of NOS3+/−/WT, NOS3+/−/pat, NOS3+/−/mat, and NOS3−/−KO mice. Each bar represents means ± SE (n = 6 in each group). One-way ANOVA followed by Newman-Keuls tests were used. *P < 0.05 vs. NOS3+/−/WT and NOS3+/−/pat.

The K+ (60 mmol/l)-induced reference contraction. Even after normalization, the responses remained significantly different (Fig. 5). The AUCs and the log IC50 values were significantly different between the groups for the carotid artery (AUC: NOS3+/−/WT = 349.73 ± 25.43, NOS3+/−/pat = 326.17 ± 41.49, NOS3+/−/mat = 473.43 ± 52.6, and NOS3−/−KO = 519.24 ± 35.74, P < 0.05; log IC50: NOS3+/−/WT = −7.5 ± 0.10, NOS3+/−/pat = −7.4 ± 0.2, NOS3+/−/mat = −7.45 ± 0.12, and NOS3−/−KO = −8.4 ± 0.30, P < 0.05), whereas only the AUCs were significantly different for the mesenteric artery (AUC: NOS3+/−/WT = 525.71 ± 39.33, NOS3+/−/pat = 448.85 ± 69.62, NOS3+/−/mat = 766.65 ± 57.39, and NOS3−/−KO = 985.52 ± 75.00, P < 0.05; log IC50: NOS3+/−/WT = −7.73 ± 0.12, NOS3+/−/pat = −7.69 ± 0.08, NOS3+/−/mat = −7.64 ± 0.16, and NOS3−/−KO = −7.41 ± 0.05; P > 0.05).

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mice compared with the NOS3+/−pat and NOS3+/−WT mice, both in the carotid as well as in the mesenteric arteries (Fig. 7). The AUC but not log IC50 results were significantly different between the groups in the carotid artery (AUC: NOS3+/−WT = 166.46 ± 24.37, NOS3+/−pat = 162.51 ± 34.32, NOS3+/−mat = 73.0 ± 14.87, and NOS3+/−KO = 83.90 ± 20.66, P < 0.05; log IC50: NOS3+/−WT = −6.2 ± 0.50, NOS3+/−pat = −6.7 ± 0.49, NOS3+/−mat = −6.8 ± 0.19, and NOS3+/−KO = −7.0 ± 0.2, P > 0.05), whereas both the AUC and log IC50 results were significantly different in the mesenteric artery (AUC: NOS3+/−WT = 177.34 ± 18.85, NOS3+/−pat = 160.04 ± 19.36, NOS3+/−mat = 42.26 ± 13.11, and NOS3+/−KO = 73.97 ± 5.39, P < 0.05; log IC50: NOS3+/−WT = −8.39 ± 0.12, NOS3+/−pat = −7.85 ± 0.13, NOS3+/−mat = −7.30 ± 0.02, and NOS3+/−KO = −7.01 ± 0.03, P < 0.05).

Fig. 6. Acetylcholine (ACh) concentration-response curves in the carotid (A) and mesenteric (B) arteries of NOS3+/−WT, NOS3+/−pat, NOS3+/−mat, and NOS3+/−KO mice (n = 6 in each group). Responses are presented as percent relaxation of the PE contraction. One-way ANOVA followed by Newman-Keuls tests were used. *P < 0.05 vs. NOS3+/−WT and NOS3+/−mat, #P < 0.05 vs. NOS3+/−WT, KO.

**DISCUSSION**

The purpose of this study was to compare the mechanical and contractile properties of carotid and mesenteric arteries between mice completely lacking NOS3 function, wild-type mice, mice heterozygous for the NOS3 gene disruption that developed in a normal environment, and mice heterozygous for the NOS3 gene disruption that developed in a maternal/uterine environment lacking NOS3-mediated NO production. In NOS3+/−KO mice, lack of expression of a functional NOS3 was associated with changes in the tensile properties and in vitro reactivity of the arteries compared with the wild-type mice. Interestingly, NOS3+/−mat mice demonstrated changes in vascular mechanical and contractile properties similar to those observed in NOS3+/−KO mice, whereas the characteristics of the NOS3+/−pat mice were not different from those of the NOS3+/−WT mice.
and NOS3+/−/mat mice (increased slopes of L-T curves and smaller ODs) compared with the NOS3+/+pat and wild-type mice. The changes in vascular elastic properties noted not only in NOS3−/−/KO but also in the NOS3+/−/mat mice are consistent with vascular remodeling in the absence of NO, which may include increased smooth muscle proliferation in the vascular media and changes in the extracellular matrix. Because a vessel with a more rigid wall reaches the target transmural pressure after being stretched to a smaller diameter in vitro compared with a vessel with a less rigid wall (increased slope and smaller ODs in NOS3−/−/KO and NOS3+/−/mat mice), the more rigid vessel would likely have a smaller luminal diameter at the same blood pressure in vivo. Therefore, the passive properties of the vessels in the NOS3−/−/KO and NOS3+/−/mat mice, i.e., greater vessel wall rigidity, may contribute to the increased vascular resistance caused by their increased responsiveness to vasoconstrictors and decreased responses to vasodilators. We acknowledge that we have studied conduit vessels and have not examined the downstream vasculature. However, these vessels are likely to contribute to the resistance in these vascular beds due to their small caliber (24). It is known that there are differences in vascular reactivity when comparing conduit with resistance vessels, e.g., lower NOS3 expression in pig coronary resistance vs. conduit vessels (24). However, these differences are mostly quantitative (magnitude of effect) and not qualitative (nature of response).

The nonspecific depolarizing agent K+ contracts a variety of isolated vascular tissues. In our experiments, arterial rings from NOS3−/−/KO and NOS3+/−/mat mice responded to K+ with greater contraction than the rings from NOS3−/+WT and NOS3+/−/pat mice. Increasing concentrations of Ca2+ in a Ca2+-free, high-K+ depolarizing solution also induced a greater increase in tension in the rings from NOS3−/−/KO and NOS3+/−/mat mice than in the NOS3−/+WT and NOS3+/−/pat counterparts. The increased responsiveness to both K+ and Ca2+ may result, at least in part, from increased smooth muscle in the medium layer of the arteries from NOS3−/−/KO and NOS3+/−/mat mice, although increased sensitization of the contractile apparatus of the vascular smooth cell to Ca2+ cannot be ruled out.

Contractile response to the α-adrenoceptor agonist PE was increased in arterial rings from NOS3−/−/KO and NOS3+/−/mat mice compared with the NOS3+/+WT and NOS3+/−/pat mice. This difference may be explained by the absence of a direct effect of NO on α-adrenoceptor agonist-mediated contraction and/or to α-adrenoceptor upregulation in the NOS3−/−/KO and NOS3+/−/mat mice (11, 21). In support of the former, our group (26) has previously shown that the difference in the contractile responses to PE between NOS3−/−/KO and NOS3+/−/WT mice is abolished by the NOS inhibitor nitro-l-arginine methyl ester. Less pronounced relaxation of PE-contracted arterial rings from NOS3−/−/KO and NOS3+/−/mat mice by the β-adrenoceptor agonist isoproterenol compared with the NOS3+/−/WT and NOS3+/−/pat mice confirms the previously reported involvement of endothelial NO in the relaxation induced by β-adrenoceptor activation (12, 13). Despite being genomically identical, and supposedly having similarly decreased NOS3 expression compared with NOS3+/+WT offspring, vascular responses in the heterozygous offspring that developed in a normal maternal environment were similar to the wild-type offspring that also developed in a normal environment, whereas those in

![Fig. 7. Isoproterenol concentration-response curves in the carotid (A) and mesenteric (B) arteries of NOS3+/+WT, NOS3+/−/pat, NOS3+/−/mat, and NOS3−/−/KO mice (n = 6 in each group). Responses are presented as percent relaxation of the PE contraction. One-way ANOVA followed by Newman-Keuls tests were used. *P < 0.05 vs. NOS3+/+WT and NOS3+/−/pat; #P < 0.05 vs. NOS3+/−/mat; δP < 0.05 vs. NOS3−/−/KO.](http://ajpregu.physiology.org/)

In addition to its effect on vascular smooth muscle contractility, endogenous NO may function as a modulator of vascular smooth muscle cell proliferation through a cGMP-mediated mechanism (16). It was demonstrated that, in response to remodeling stimulus (ligation of external carotid artery in mice), there is a decrease in the lumen diameter of the carotid artery in mice homozygous for eNOS locus (+/+), whereas no such luminal remodeling is seen in the vessels of mice with homozygous disruption of the eNOS locus (−/−) (35). On the contrary, the medial thickness is increased in carotid arteries from mice with (−/−) eNOS genotype (35). Endothelial NO also regulates extracellular matrix turnover in articular chondrocytes and cartilage (30). In rabbits, reduction in arterial diameter produced by chronic decreases in blood flow is dependent on the vascular endothelium (23). These studies support a role for NO in vascular remodeling. Our data demonstrate greater rigidity of the vessel wall in the NOS3−/−/KO
heterozygous offspring that developed in a maternal environment lacking a functional NOS3 were similar to the knockout offspring that also developed in a similar maternal environment. The apparent discrepancy in NOS3 function between the heterozygous offspring is likely due to differences at the vascular structural level. However, differences in NOS3 expression and NO production need to be investigated.

NOS3 is the main isoform producing NO in the vascular endothelium under normal physiological conditions (1). NO is the predominant endothelium-dependent vasodilator that may be released by “chemical activation,” as in the case of the neurotransmitter ACh, or by “mechanical activation” through flow-mediated shear stress (37). ACh induced a nearly complete relaxation of PE-contracted arterial rings in NOS3+/+WT mice, whereas this endothelium-dependent relaxation was minimal or absent in NOS3+/−KO mice, confirming that NO generated by NOS3 is the main factor mediating endothelium-dependent relaxation in these vessels. In addition, the similarity in the responses of arteries between NOS3+/−pat mice and NOS3+/+WT mice indicates the presence of endothelium-derived NO in these mice, whereas the lack of ACh-induced relaxation in NOS3+/−KO and NOS3+/−mat mice suggests lack of significant endothelial NO production.

Despite their allelic similarities, the vascular responses in the heterozygous mice depended on the parental origin of the mutated allele. The maternally derived heterozygous mice had abnormal vascular reactivity that was similar to the homozgyous knockout mice completely lacking a functional NOS3, whereas the paternally derived heterozygous mice had normal vascular reactivity that was not different from the wild-type control mice with normal NOS3 function. One possible explanation for the discrepancy between the phenotypic expression of the heterozygous mice is genetic imprinting (18, 33, 38). However, this is less likely because no imprinted regions have been described on the mouse chromosome 5 where the NOS3 gene is located (7). The other more likely explanation for the observed differences in the two heterozygous litters is the differences in the maternal/uterine environment, as proposed by Barker and colleagues (4–6). The maternally derived heterozygous mice develop in a female completely lacking NOS3 expression, and their phenotype resembles that of the NOS3 knockout mice. In contrast, the paternally derived heterozygous mice develop in a female with completely normal NOS3 expression, and their phenotype resembles that of the wild type mice. Although we did not examine the uterine blood flow in the pregnant NOS3-knockout mice, it is likely that a decrease in the uteroplacental perfusion may be the mechanism responsible for creating the deleterious maternal environment in this model (8, 27, 39). Another mechanism that may be operative is a more generalized change in maternal cardiovascular function caused by vascular NO deficiency as evidenced by the hypertension reported in these mice (17, 36). In either case, mice lacking NOS3 function are likely to have an abnormal uterine environment as evidenced by a smaller litter size. Further studies are needed to confirm similar expression of NOS3 in the maternally and paternally derived heterozygous offspring. Also, it would be interesting to see whether the noted effects are gender specific or whether they occur in male offspring too.

In summary, we present an animal model that provides direct evidence in support of the hypothesis that vascular responses in adulthood are influenced by the maternal/uterine environment during in utero development. This animal model was not produced by nutritional or surgical interventions and therefore may represent the contribution of a genetically determined abnormality in the maternal environment to fetal vascular programming. Additional studies are needed in this novel animal model to further elucidate the mechanisms that mediate vascular programming in utero and the long-term effects of the uterine environment on future vascular function in the adult.

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