Inhibition of Nitric Oxide Synthases Abrogates Pregnancy-Induced Uterine Vascular Expansive Remodeling

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Key Words

Hydralazine • Hypertension • Nitric oxide • Uterine circulation • Vascular remodeling during pregnancy

Abstract

Background/Aims: It was the aim of this study to test the hypothesis that hypertension and/or inhibition of nitric oxide (NO) synthases alters uterine vascular remodeling during pregnancy. **Methods:** Using a model of hypertension (NO synthase inhibition with L-NAME) in nonpregnant and pregnant rats, comparisons were made with age-matched controls, as well as with animals receiving hydralazine along with L-NAME to maintain normotension in the presence of NO synthase inhibition. Circumferential and axial remodeling of large (main uterine, MUA) and small (premyometrial radial) arteries were quantified and compared. **Results:** L-NAME treatment prevented expansive circumferential remodeling of the MUA; cotreatment with hydralazine was without effect. Circumferential remodeling of smaller premyometrial radial arteries was also significantly attenuated in hypertensive pregnant animals, while premyometrial radial arteries from rats receiving hydralazine with L-NAME were of intermediate diameter. Neither hypertension nor NO synthase inhibition had any effect on the substantial (200–300%) axial growth of MUA or premyometrial radial arteries. **Conclusion:** NO plays a major role in facilitating pregnancy-induced expansive remodeling in the uterine circulation, particularly in larger arteries. Some beneficial effects of

hydralazine on expansive circumferential remodeling were noted in smaller radial vessels, and these may be linked to its prevention of systemic hypertension and/or to local effects on the arterial wall. Neither NO synthase inhibition nor hypertension had any effect on arterial longitudinal growth.

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Introduction

Preeclampsia, a complex human disease that occurs in approximately 5% of pregnancies, most often in the third trimester, is defined by 2 distinct criteria: repeatedly elevated blood pressure (>140/90 mm Hg) and proteinuria (>300 mg/24 h). Although its epidemiology remains elusive, there is a strong association with reduced uterine perfusion and abnormal placentation [1, 2].

Unfortunately, there are no animal models that replicate the human condition. Increased production of nitric oxide (NO) by the endothelium contributes to the hemodynamic changes associated with normal pregnancy [3–6]; conversely, a reduction in NO signaling has been observed in preeclampsia [7] and in several forms of chronic hypertension [8, 9]. Therefore, chemical inhibition of NO synthases has become an accepted experimental approach for inducing hypertension in pregnant animals [10–16]. Rats subjected to chronic NO synthase inhibition during gestation develop the principal pathologic condi-

tions associated with preeclampsia, e.g., hypertension, proteinuria, renal damage and fetal intrauterine growth retardation [10, 14, 16].

Using this model, most published studies to date have focused on understanding the functional implications of hypertension/NO synthase inhibition and have documented enhanced vascular pressor responses in vivo [10], heightened vasoconstrictor responses in isolated vessels [6, 17, 18], impaired endothelium-dependent relaxation [18, 19], enhanced calcium signaling [20] and overactivation of calcium-sensitizing enzymes such as protein kinase C and RhoA [11, 21].

Less is known about the effects of hypertension and/or NO synthase inhibition on vascular structure. Two published studies [22, 23] reported a significant attenuation of uterine vascular growth, although neither distinguished the effects of NO synthase inhibition from those of hypertension, which quickly develops secondary to chemical (L-NAME) or genetic (NOS-3 knockout) inhibition of NO signaling. This distinction is relevant, since hypertension-induced remodeling of the vascular wall has been documented in numerous studies, with the general pattern being opposite to that of pregnancy, i.e. inward remodeling (narrowing of the lumen) with increased wall thickness. Conversely, at least in the uterine circulation, pregnancy is associated with outward eutrophic or hypertrophic remodeling [22–27].

The objective of this study was to test the hypothesis that hypertension and/or NO synthase inhibition would reduce the normal remodeling of uterine arteries during gestation. Corollary hypotheses were that (1) the effects may be more pronounced in smaller resistance vessels, (2) both axial and circumferential remodeling (i.e. arterial widening and lengthening) would be affected, and (3) elevated blood pressure may have effects that are different from those of NO synthase inhibition. To make the latter distinction, we included a group of animals that received hydralazine in addition to L-NAME to prevent the development of systemic hypertension in the continued presence of NO synthase inhibition.

Methods

Animals, Experimental Treatments and Preparation of Tissues

Adult (12–14 weeks old) virgin and timed pregnant female Sprague-Dawley rats ($n = 69$) were purchased from Charles River Laboratories (Kingston, Calif., USA) and housed in the Small Animal Facility at the University of Vermont which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Feed and water were provided ad libitum.

All experimental protocols were approved by the Institutional Animal Care and Use Committee.

The 6 treatment groups were: (1) nonpregnant and (2) late pregnant (day 20 of a 22-day gestation) controls (NP-C, $n = 14$; LP-C, $n = 17$), (3) nonpregnant and (4) late pregnant hypertensives (0.5 g/l of L-NAME in drinking water; NP-L, $n = 10$; LP-L, $n = 15$), and (5) nonpregnant and (6) late pregnant NO synthase-inhibited normotensive animals (NP-L+H, $n = 6$; LP-L+H, $n = 7$) that received a mixture of L-NAME (0.5 g/l) and hydralazine (0.27 g/l), also in drinking water. In pregnant animals, treatment was started on day 10 of pregnancy and continued until day 20; both age-matched NP-L and NP-L+H animals were also treated for a 10-day duration prior to use.

Blood pressures were measured noninvasively by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail cuff (CODA System, Kent Scientific, Torrington, Conn., USA). Pressures were taken at the same time of day (late morning), 10 readings per animal, and averaged during the week prior to experimentation. Mean arterial pressures (diastolic + $1/3$ of pulse pressure) were derived and expressed in mm Hg.

On the morning of an experiment, each animal was euthanized with an intraperitoneal injection of Nembutal (pentobarbital sodium, 50 mg/kg; Ovation Pharmaceuticals, Deerfield, Ill., USA) and, once a surgical plane of anesthesia was attained (toe pinch test), decapitated in a small animal guillotine. The abdomen was opened and the uterus and its contents were removed en bloc and pinned in a Petri dish filled with oxygenated (bubbled with a mixture of 5% CO₂, 10% O₂ and 85% N₂) buffered saline (PBS) solution. Care was taken to pin out the tissue in a planar manner without imposing any stretch or compression on the mesometrial arcade or uterine horns.

Experimental Measurements

In pregnant animals, the number of fetuses in each horn was recorded, as were individual fetal and placental weights. The length of the main uterine artery (MUA, mm) was measured along its curvature from the cervix to the end of the uterine horn. The inner and outer diameter of the MUA at the approximate midpoint of each horn was measured with a stereomicroscope (Zeiss, Germany) using a calibrated reticule and the difference between the inner and outer diameter divided by 2, as a measure of wall thickness. We previously verified that, under these experimental conditions, the MUA does not possess any tone, and measurements correspond to the fully relaxed state. The inner and outer diameter values were then used to derive the wall to lumen ratio (dimensionless) and the cross-sectional area (μm^2) by calculating the area defined by the outer diameter and subtracting the area of the lumen (based on the inner diameter) using the standard formula for the area of a circle (πr^2). All measurements were performed in PBS at room temperature.

In rodents, the uterus is supplied by a manifold arrangement of segmental vessels that emanate from the MUA, which runs parallel to and at some distance from the uterine wall. Segmental artery (SA) length was determined by measuring the distance between the MUA and the uterine wall at 2 randomly chosen points in each uterine horn and averaged to provide 1 value per animal.

Although the number of implantation sites in each horn was generally comparable (6–9, on average), some animals did have a

Table 1. Maternal and fetal characteristics of all treatment groups

Treatment group	Maternal characteristics		Fetal characteristics			
	Maternal weight, g	MAP, mm Hg	Litter size, n	Resorptions	Fetal weight, g	Placental weight, mg
NP-C	295 ± 2.4 ^a (n = 14)	106 ± 1.4 ^a (n = 6)	–	–	–	–
NP-L	294 ± 6.6 ^a (n = 10)	136 ± 0.2 ^b (n = 6)	–	–	–	–
NP-L+H	273 ± 4.0 ^a (n = 6)	110 ± 2.6 ^a (n = 6)	–	–	–	–
LP-C	380 ± 6.4 ^b (n = 17)	93 ± 2.1 ^a (n = 13)	14.5 ± 0.41 (n = 11)	3/159 [1.9] (n = 11)	2.19 ± 0.040 ^b (n = 11)	427 ± 6.0 ^{a,b} (n = 11)
LP-L	358 ± 4.8 ^b (n = 15)	125 ± 1.9 ^b (n = 15)	14.4 ± 0.51 (n = 13)	10/188 [5.3] (n = 13)	1.98 ± 0.039 ^a (n = 13)	414 ± 7.6 ^a (n = 13)
LP-L+H	362 ± 7.7 ^b (n = 7)	98 ± 1.5 ^a (n = 12)	15.1 ± 0.77 (n = 7)	5/110 [4.5] (n = 7)	2.14 ± 0.032 ^b (n = 7)	450 ± 10.6 ^b (n = 7)

Values are the mean ± SE. Numbers in brackets are percentages. MAP = Mean arterial pressure. Different letters denote statistical significance ($p < 0.05$).

more disparate number of fetoplacental units in the 2 horns. To determine the relationship between the number of fetoplacental units and the extent of vascular remodeling, regression analysis was performed on measurements of MUA and SA length to determine the nature and strength of the correlation. In view of the positive correlation observed, measurements of MUA length were restricted to horns containing 6–9 pups (± 1 SD of 7.5, the mean) in all pregnant treatment groups.

Earlier studies have established that the radial arteries are a primary site of vascular resistance in the rodent [26]. These vessels were too small to be measured accurately with the stereomicroscope, so we dissected second-order radial vessels from the midpoint of the uterine arcade, taking care to sample a vessel that was premyometrial rather than preplacental. As differences in structure and remodeling have been noted before [23, 28, 29], premyometrial arteries afforded the best comparison with the non-pregnant state, in which all segmental vessels feed the uterine wall. Furthermore, preplacental arteries lose their contractile properties and widen as they approach the placenta, making the determination of lumen diameter and wall thickness more difficult to standardize [28].

Once isolated, the end of each vessel was cannulated in the chamber of an arteriograph (Living Systems Instrumentation (LSI), Burlington, Vt., USA) and pressurized to 5 mm Hg using an LSI servo system. The preparation was positioned on the stage of an inverted microscope (Zeiss, Germany) equipped with a video camera. Following warming to 37°C at pH 7.4 for 40–60 min, vessel dimensions were determined at a pressure of 5 mm Hg in a relaxing solution containing diltiazem (10 μ M) and papaverine (100 μ M) by using a calibrated card to measure the inner and outer diameter from the screen of a monochrome television monitor at a magnification of $\times 260$. Under cannulated conditions, the opening pressure of small vessels is on the order of 3–4 mm Hg, hence, 5 mm Hg was the lowest pressure under which consistent dimensional measurements could be made under minimally stressed conditions, as in the MUA.

Solutions

PBS composition (in mM): 119 NaCl, 4.7 KCl, 24.0 NaHCO₃, 1.2 KH₂PO₄, 1.6 CaCl₂, 1.2 MgSO₄, 0.023 EDTA and 11.0 glucose, pH 7.4. All buffer reagents, as well as papaverine and diltiazem, were purchased from Sigma Chemical Co. (St. Louis, Mo., USA)

or Fisher Scientific Co. (Pittsburgh, Pa., USA) and prepared on the day of an experiment.

Statistical Analysis

Statistical differences between treatment groups were determined from individual vessel data (SigmaPlot 9.0; Systat Software Inc., San Jose, Calif., USA) by analysis of variance (ANOVA) followed by multiple comparison tests to evaluate differences between treatment means. Statistical differences ($p < 0.05$) are denoted by different letters (e.g., 'a' is different from 'b', while 'b, c' is different from 'a', but not different from 'b' or 'c'). Data are expressed as mean values $\pm$ standard error of the mean, with n values representing the number of animals. In some cases, to isolate the effects of gestation alone (NP-C vs. LP-C) without the confounding effects of variability due to treatment, unpaired t tests were used to assess differences between mean values. Regression analysis was performed followed by the calculation of the coefficient of determination (r^2) to determine the relationship between arterial (MUA and SA) length and the number of implantation sites.

Results

Effects of L-NAME and Hydralazine Treatment on Blood Pressure and Reproductive Status

At the concentration (0.5 g/l) and duration (10 days) used, L-NAME treatment did not affect the body weight of nonpregnant rats. All LP animals were significantly heavier than age-matched NP-C, and body weights of pregnant animals receiving L-NAME with or without hydralazine (LP-L, LP-L+H) were approximately 5% lower than those of LP-C.

Relative to controls, while mean arterial pressures were significantly increased (by 28 and 34%, on average) in the NP-L and LP-L groups, respectively (table 1), the mean arterial pressure of LP-L+H animals was similar to that of LP-C, as well as NP-L+H and NP-C.

Fig. 1. a Summary showing changes in the length of the MUA in vessels from the 6 treatment groups. Note the significant elongation (approximate doubling) of vessel length in all of the pregnant versus nonpregnant groups. Different letters above each bar denote significant statistical differences ($p < 0.05$). **b** Regression

analysis showing the positive relationship between the number of pups present in the uterine horn and the length of the MUA in the LP-C animals. The coefficient of determination (r^2) was calculated to be 0.76. ($n = 27$; i.e. measurements were made in 27 horns from 14 animals.)

Fecundity was not affected, as there were no statistical differences in the total number of pups per litter (combined average 14.7) or resorption rate (combined average 18/457, or 3.9%). Fetal weights were significantly reduced in LP-L versus LP-C animals, with no difference between LP-C and LP-L+H. Placental weights were smaller in the LP-L group – significantly so relative to LP-L+H ($p = 0.02$), but not to LP-C ($p = 0.08$).

Effects of L-NAME and Hydralazine Co-Treatment on Arterial Elongation and Mesometrial Dimensions

In nonpregnant animals, treatment with L-NAME did not have any measurable effect on MUA length. The growth of the mesometrial arcade during pregnancy was considerable and resulted in an approximate doubling of MUA length in all 3 LP treatment groups, without any significant intertreatment differences (fig. 1a).

Although the average number of fetuses was similar between treatment groups (table 1), the number of pups in a particular horn ranged from 3 to 11. Reasoning that the overall uterine length would be influenced by the number of pups, regression analysis between MUA length and number of pups per individual horn was performed. A positive linear correlation was noted in the LP-C group, with an r^2 value of 0.76, showing that the number of implantation sites is a major determinant of MUA longitudi-

dinal growth (fig. 1b). Correlations between MUA length and number of pups in the LP-L and LP-L+H groups were also positive, but weaker (r^2 values = 0.50 and 0.35, respectively; data not shown).

Relative to nonpregnant animals, the SA length increased approximately 3 fold, with no differences between LP-C, LP-L or LP-L+H groups (fig. 2a). The relationship between the number of pups and LP-C SA length was also positive, with an r^2 value of 0.35 (fig. 2b), and of 0.43 and 0.51 in LP-L and LP-L+H, respectively (data not shown). The combined effect of increased MUA and SA length was to increase the mesometrial area approximately 5 fold, and to a similar extent in all treatment groups, with an average value of 261 ± 9.3 versus $1,350 \pm 37.5 \text{ mm}^2$ in the nonpregnant versus the pregnant group ($p < 0.001$).

Effects of L-NAME and Hydralazine Co-Treatment on Circumferential Dimensional Changes in the MUA

Significant differences were noted in the unstressed lumen diameters of the MUA (fig. 3a). In nonpregnant animals, treatment with L-NAME induced a narrowing of the lumen by approximately 25% ($p < 0.05$). Pregnancy-induced circumferential remodeling resulted in a 50% increase in MUA lumen diameter (from 105 ± 6.7 to $158 \pm 11.5 \mu\text{m}$ in NP-C vs. LP-C animals). There was no

Fig. 2. a Effects of treatment and pregnancy on SA length. Differences noted were identical to those in the MUA length, illustrating the significant (approximately tripling) of vessel length in all of the pregnant versus nonpregnant groups. Different letters above each bar denote significant statistical differences ($p < 0.05$).

b Regression analysis showing the relationship between the number of pups in a uterine horn and SA length in the LP-C animals. A positive relationship was noted, with an r^2 value of 0.35. ($n = 27$; i.e. measurements were made in 27 horns from 14 animals.)

Table 2. MUA measurements of all treatment groups

Treatment group	Wall thickness, μm	Wall:lumen	Wall CSA μm^2
NP-C (n = 12)	58 ± 3.67	$1.01 \pm 0.07^{a,b}$	$29,042 \pm 2,272$
NP-L (n = 10)	62 ± 3.61	1.73 ± 0.12^b	$29,156 \pm 3,337$
NP-L+H (n = 6)	61 ± 3.29	$1.51 \pm 0.24^{a,b}$	$29,447 \pm 2,389$
LP-C (n = 17)	56 ± 3.66	0.83 ± 0.11^a	$37,768 \pm 3,299$
LP-L (n = 15)	56 ± 3.28	$1.42 \pm 0.15^{a,b}$	$26,585 \pm 2,612$
LP-L+H (n = 7)	67 ± 4.51	1.42 ± 0.10^b	$35,237 \pm 4,090$

Values are mean $\pm$ SE. CSA = Cross-sectional area. Different letters denote statistical significance ($p < 0.05$).

measurable remodeling in LP-L or LP-L+H animals (lumen diameters were not statistically different from the NP-C or NP-L groups) indicating that NO synthase inhibition rather than hypertension was primarily responsible for the observed effect.

MUA wall thickness was similar in all treatment groups (table 2); however, in view of the differences in lumen diameter, wall-to-lumen ratios were significantly greater in L-NAME-treated animals (NP-L, LP-L) and in LP-L+H animals. The cross-sectional area did not

change with L-NAME treatment in nonpregnant animals and, due to within-treatment variability, there were also no significant differences among the 6 groups of animals.

Effects of L-NAME and Hydralazine Co-Treatment on Circumferential Dimensional Changes of Premyometrial Radial Arteries

As with the MUA, there were no differences between treatment groups in the extent of SA elongation, which was considerable in all pregnant animals (approximately 300%; fig. 2a). As shown in figure 3b, lumen diameters of radial premyometrial arteries increased by 33%, on average, in LP-C versus NP-C animals. There were no treatment differences in nonpregnant animals. Vessels from LP-L rats were significantly smaller than those from LP-C, while LP-L+H arteries were between those from the LP-C and LP-L animals.

The radial artery (RA) NP-C wall thickness values were larger than those of any pregnant group, while those from the NP-L+H group were smaller than those of NP-C, with intermediate values in the LP-L group. Due to expansive remodeling, the wall-to-lumen ratio was significantly reduced in vessels from pregnant animals, with no differences among the treatment groups. In view of the combination of a larger lumen and a some-

Fig. 3. a Unstressed MUA lumen diameter in all treatment groups. Different letters denote statistical significance ($p < 0.05$). Note significant enlargement of vessels from LP-C versus LP-L or LP-L+H-treated animals. **b** Preplacental RA lumen diameter in all

treatment groups. Different letters denote statistical significance ($p < 0.05$). Note significant enlargement of vessels from LP-C versus LP-L animals. Diameter of vessels from the LP-L+H group were intermediate.

Table 3. Radial artery measurements of all treatment groups

Treatment group	Wall thickness, μm	Wall:lumen ratio	Wall CSA μm^2
NP-C (n = 12)	15.3 ± 0.78^c	0.56 ± 0.06^b	$3,562 \pm 282$
NP-L (n = 10)	$14.5 \pm 0.94^{b, c}$	0.52 ± 0.03^b	$3,366 \pm 338$
NP-L+H (n = 6)	$12.4 \pm 0.94^{a, b}$	$0.42 \pm 0.02^{a, b}$	$2,931 \pm 481$
LP-C (n = 6)	$11.8 \pm 0.95^{a, b}$	0.30 ± 0.03^a	$3,392 \pm 321$
LP-L (n = 14)	11.5 ± 0.70^a	$0.39 \pm 0.03^{a, b}$	$2,866 \pm 242$
LP-L+H (n = 14)	$12.7 \pm 0.81^{a, b}$	0.35 ± 0.03^a	$3,531 \pm 425$

Values are mean $\pm$ SE. CSA = Cross-sectional area. Different letters denote statistical significance ($p < 0.05$).

what thinner wall in pregnant animals, the cross-sectional area was similar among all 6 treatment groups (table 3).

Discussion

The principal findings of this study are that (1) NO is an important regulator of MUA remodeling during pregnancy, as treatment with L-NAME completely prevented the circumferential growth of the MUA; co-treatment with hydralazine was ineffective in restoring this process, suggesting the principal underlying mechanism to be

chemical (NO synthase inhibition) rather than physical (elevation in intravascular pressure). (2) In the smaller resistance (radial) vessels, both hypertension and NO synthase inhibition appear to influence the circumferential remodeling process, since co-treatment with hydralazine resulted in diameters that were intermediate between those of control and L-NAME-treated animals. (3) In addition to circumferential expansion, significant gestational axial growth (elongation) occurs in the MUA, as well as in the smaller segmental (radial) vessels.

Pregnancy-induced uterine vascular growth is clinically relevant, as its attenuation is associated with placental underperfusion, preeclampsia and intrauterine growth restriction. Uteroplacental underperfusion may occur for any number of reasons, including attenuated placental growth, insufficient vascular remodeling, or excessive tone due to an imbalance of vasoactive influences favoring vasoconstriction. Although systemic hypertension induced by L-NAME would increase uterine perfusion pressure accordingly and may therefore partly obviate the need for expansive remodeling, the significant reduction in fetal weights in this and other studies utilizing the L-NAME model [10, 11, 16] suggests that a perfusion deficit does indeed exist, since fetal growth has long been known to be dependent on sufficient uteroplacental flow [30], although other mechanisms may also be contributory. In a recent preliminary report [31] using the eNOS knockout mouse model, a 55% reduction in uterine blood flow was measured (normalized to the weight of the uter-

us and its contents). Spiral and intraplacental labyrinthine artery remodeling was also compromised, based on corrosion casting, possibly due to inhibition of NO secretion by invasive trophoblast cells. The resulting increase in distal resistance would reduce blood flow velocity and shear stress upstream, and likely have some additional effect on any residual NO production and secretion by the endothelium due to the reduction in shear stress, which is considered to be the most potent physiological stimulus for endothelial NO release and an important influence on expansive circumferential remodeling.

Treatment with L-NAME has been associated with a reduction in outward remodeling in response to augmented flow in other settings, such as the carotid or coronary circulation of male rats [32]. Therefore, shear stress-induced NO upregulation is a plausible mechanism for facilitating circumferential remodeling in the uterine circulation due to (1) the almost certain increases in blood velocity that would occur as a result of the decreased resistance afforded by hemochorial placentation, (2) evidence from earlier studies showing an increase in NO synthase activity due to elevated shear stress [33, 34], and (3) the well-established linkage between elevated shear stress and expansive circumferential remodeling [35, 36].

Two earlier studies identified NO as an important influence on uterine vascular remodeling during pregnancy, but did not attempt to distinguish between the effects of altered molecular signaling versus elevated intravascular pressure in the observed effects. In a corrosion cast study by Mayr et al. [37], intraplacental maternal arterial channels that supply the labyrinthine system were found to be 50% narrower in L-NAME-treated rats. Since infusion pressure and time were not specified, differences in vessel size may have affected the rate of filling with a viscous medium, thus exaggerating actual differences in size at a fixed pressure. As already noted, aside from the effect of inhibiting arterial NO production, if this effect exerts a hemodynamic influence *in vivo*, the reduction in downstream resistance would be limited and, consequently, the increase in upstream shear stress would be reduced. Thus, NO synthase inhibition may have both direct and indirect effects on the vascular wall of maternal uterine arteries that supply myometrial and placental tissues.

In a different study, Van der Heijden et al. [38] utilized a NOS-3 (eNOS) knockout mouse and found a significantly reduced rate of growth of the MUA during pregnancy, as evidenced by a reduction in vessel radius, wall thickness and cross-sectional area relative to wild-type

controls. Our findings are consistent with these previous observations and further substantiate the importance of NO in gestational uterine vascular remodeling. In view of the results of Van der Heijden et al. [38], the effects we observed are likely due to the suppression of endothelial NO production, although L-NAME is a nonspecific inhibitor of NOS and may therefore affect other isoforms of this enzyme (e.g., NOS-1 and NOS-2).

Another study, focused on the effects of L-NAME treatment on the coronary circulation in male rats, reported inward remodeling with an increase in wall thickness and wall-to-lumen ratio [39]. Some of these changes, particularly the latter, may be secondary to elevated blood pressure rather than NO synthase inhibition, as a similar pattern of remodeling (inward hyper- or eutrophic) has been reported in nonpregnant, chronically hypertensive rats in other circulations, and has been prevented or reversed by the normalization of blood pressure [35].

The results of this study also reveal another aspect of gestational uterine vascular remodeling that has been largely overlooked thus far – the significant structural elongation of both large (MUA) and small (segmental) mesometrial arteries. Although the measurement of veins was not included in this study, venous enlargement and elongation occur to a comparable extent since the arrangement of arteries and veins in the uterine circulation is generally parallel and in close apposition.

The process of arterial axial growth was completely unaffected by NO synthase inhibition or hypertension and appears to be mechanistically distinct from expansive circumferential remodeling and due to other mechanisms. Measurements of axial length were also made in the unstressed state in order to allow intertreatment comparisons, as differences in axial distensibility as a function of transmural pressure exist in main uterine arteries from pregnant versus nonpregnant animals (unpublished data). Although uterine arterial narrowing and vasoconstriction have been noted in preeclampsia [40], to our knowledge, changes in vessel length have not been examined in humans. In view of the 20- to 25-fold increase in uterine mass during pregnancy (discounting fetal tissues), it is difficult to imagine that significant vessel elongation and angiogenesis do not occur, although some accommodation in length may be facilitated by uncoiling of vessels that display tortuosity, as does the MUA in women.

Although circumferential remodeling was outward eutrophic in both large and small vessels (as evidenced by similar cross-sectional areas in all treatment groups), taking both axial and circumferential growth into con-

sideration, reveals the extensive hypertrophic remodeling of the vessel wall in all arteries studied, with increases in mass on the order of 200–300%. This also highlights the fact that measures of the cross-sectional area alone, without consideration of axial changes, may underestimate the extent and lead to a misinterpretation of the true pattern of vascular remodeling. Because vessel elasticity is altered in pregnancy, measurements were intentionally made on unstressed (MUA) or minimally stressed (radial arteries) vessels as, under pressurized conditions, altered distensibility may introduce a bias in the results and lead to misinterpretation of remodeling data.

In summary, our results demonstrate an important role for NO signaling in the expansive circumferential gestational remodeling of the uterine circulation. This observation provides an interesting link to the theory that preeclampsia results from elevated levels of sFlt-1, a soluble receptor for vascular endothelial growth factor and placenta growth factor, in preeclamptic women [41]. An excess of soluble receptor would reduce the availabil-

ity of these ligands to the vascular wall. Since both placenta growth factor and vascular endothelial growth factor stimulate endothelial NO release, a reduction in their signaling would create a vasoconstrictor imbalance and increase peripheral resistance and blood pressure. Our findings add a new dimension to this thinking, as they show that a reduction in NO signaling also impacts vessel remodeling in a way that would further increase uterine vascular resistance. This effect on structure, combined with loss of function (vasodilation), would further mitigate the increases in uterine blood flow required for normal placental perfusion and successful pregnancy outcome.

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