Pregnancy increases myometrial artery myogenic tone via NOS- or COX-independent mechanisms

Delrae M. Eckman¹,²*, Ridhima Gupta³, Charles R. Rosenfeld⁵, Timothy M. Morgan⁴, Shelton M. Charles³, Heather Mertz³, Lorna G. Moore³,⁶

Departments of ¹Pediatrics, ²Physiology/Pharmacology, ³Obstetrics and Gynecology, ⁴Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157
⁵Department of Pediatrics, Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical School, Dallas, Texas 73590-9063
⁶Wake Forest University Graduate School of Arts & Sciences, Winston-Salem, North Carolina 27157

Running Title: Myometrial arteries and pressure-induced constriction

*Corresponding Author:

Delrae M. Eckman, Ph.D.
Department of Pediatrics
Wake Forest University School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157
Phone: (336) 713-5090
Fax: (336) 716-2525
Email: deckman@wfubmc.edu
Abstract

Myogenic tone (MT) is a primary modulator of blood flow in the resistance vasculature of the brain, kidney, skeletal muscle, and perhaps other high-flow organs such as the pregnant uterus. MT is known to be regulated by endothelial-derived factors, including products of the nitric oxide synthase (NOS) and/or the cyclooxygenase (COX) pathways. We asked if pregnancy influenced MT in myometrial arteries (MA) and if so, whether such an effect could be attributed to alterations in NOS and/or COX. MA, 200-300 µm internal diameter, 2-3 mm length, were isolated from 10 nonpregnant and 12 pregnant women undergoing elective hysterectomy or cesarean section, respectively. In the absence of NOS and/or COX inhibition, pregnancy was associated with increased MT in endothelium-intact MA compared to MA from nonpregnant women (p<0.01). The increase in MT was not due to increased Ca²⁺ entry via voltage-dependent channels since both groups of MA exhibited similar levels of constriction when exposed to 50mM KCl. NOS inhibition (L-NAME) or combined NOS/COX-inhibition (L-NAME/Indomethacin) increased MT in MA from pregnant women (p=0.001 and p=0.042, respectively) but was without effect in arteries from nonpregnant women. Indomethacin alone was without effect on MT in MA from either nonpregnant or pregnant women. We concluded that MT increases in MA during human pregnancy and that this effect was partially opposed by enhanced NOS activity.

Keywords: fetal growth restriction, human myometrium, pressure-induced constriction, preeclampsia, uteroplacental blood flow
Introduction

Human pregnancy is associated with significant cardiovascular changes to meet the metabolic demands of the placenta and fetus as well as the pregnant woman. Heart rate and stroke volume increase ~35% and ~15%, respectively, plasma volume expands ~40%, and unilateral uterine artery blood flow rises as much as 10-fold from ~34 ml/min in nonpregnant women to ~332 ml/min at term pregnancy (15; 16; 36). These changes are accompanied by a pronounced reduction in maternal peripheral vascular resistance that results in a modest lowering of blood pressure near term in healthy pregnancies (5). In spite of these dramatic changes in cardiovascular function, little is known in women about how pregnancy affects the intrauterine vasculature and, in particular, how it responds to changes in pressure and workload. A vessel of particular interest is the myometrial artery (MA) as it is a principal site of uterine vascular resistance and possibly endothelial dysfunction in pregnancies complicated by preeclampsia, and hence may contribute to the regulation of maternal uteroplacental blood flow (21; 33; 34).

One mechanism by which the body regulates blood flow to end organs is by pressure-induced constriction, also known as myogenic tone (MT). MT was first described in the canine carotid artery by Bayliss in 1902 (4) and has been frequently observed in resistance-sized arteries (≤ 300µm) that exist in a partially constricted state from which they can constrict or dilate to meet the metabolic demands and/or perfusion requirements of their end organs. In the uterine circulation, MT has been observed in main uterine arteries in nonpregnant mice and second/third order uterine arteries from nonpregnant sheep, but this MT is attenuated as pregnancy progresses (51; 52). In contrast, second-order radial uterine arteries do not exhibit MT in nonpregnant rats, but MT is present as
pregnancy progresses to term (11; 50). To our knowledge, there are no studies of MT in uterine arteries from nonpregnant women, and most studies in pregnant women are limited by the use of a single physiologic intraluminal pressure (80 mmHg) (21) vs. the broad pressure range used in other species (11; 51; 52). Therefore, the effect of pregnancy on MT in human uterine vessels is unclear.

Increased uteroplacental blood flow during pregnancy is associated with augmented endothelial-derived vasodilator production in experimental animals (11; 12; 18; 25; 47; 51) and women (8; 45; 49), with much of the emphasis placed on the role of increased synthesis of endothelial-derived nitric oxide and/or its downstream signaling. Inhibitors of nitric oxide synthase (NOS) augment MT in uterine vessels from experimental animals (11; 13; 51) and in MA from pregnant women (19-21), but less is known about the contribution of vascular cyclooxygenase (COX) products, which are also up-regulated in pregnancy (14; 19). Furthermore, MA MT and its regulation by NOS and/or COX in nonpregnant women have yet to be studied.

The objectives of this study were to determine 1) whether pregnancy alters MT in human MA, and if so, 2) whether alterations in NOS/COX-signaling are involved. In order to assess MT across the full range of blood pressures, all experiments were performed at intraluminal pressures ranging from 10 to 100 mmHg.

**Materials and Methods**

All experimental protocols were performed in accordance with NIH guidelines for the use of human tissue and followed procedures approved by the Human Research
Protection Program at Wake Forest University School of Medicine for obtaining informed consent.

Subjects. Twelve healthy pregnant women undergoing elective cesarean section and 10 nonpregnant women undergoing elective hysterectomy for benign disorders (see Table 1) were included in the present study. We excluded those women with pre-existing cardiovascular disease (e.g., hypertension, diabetes), a history of current smoking, and those with multiple births. The nonpregnant women included were: 1) premenopausal, 2) undergoing an elective hysterectomy for a benign disorder later confirmed by pathology report (i.e., fibroids, menorrhagia, uterine prolapsed, ovarian teratoma and/or uterine leiomyomata), and 3) without the above cardiovascular risk parameters. The primary anesthetics or analgesics used in the pregnant women were bupicicaine, fentanyl and duramorph. Patients undergoing hysterectomies received general anesthesia with isoflurane or desflurane.

Preparation of myometrial arteries. Full-thickness 1 cm wide x 1 cm deep biopsies were obtained at time of cesarean section from the upper lip of the uterine incision away from the placenta to prevent unwanted hemorrhage. Fundal placentation was observed in all participants. Tissue collection at time of hysterectomy was from the anterior portion of the uterus, which closely approximated the region collected from pregnant women. These methods for tissue collection were similar to those described by others (17; 19-21). The tissue was immediately placed in cooled physiological saline solution (PSS comprised of [in mmol/L]: 118 NaCl, 4.5 KCl, 25 NaHCO3, 1.2 KH2PO4, 1.2 MgCl2, 11 dextrose, 2.5 CaCl2, and 0.26 EDTA at pH 7.4) and brought to the laboratory for isolation of MA.
All tissue samples were maintained in 4°C PSS and aerated with gas containing 21% O₂, 74% N₂ and 5% CO₂ while dissecting MA segments from the outer third (maternal side) of the uterine biopsy in order to isolate similar sized vessels (200 to 300 µm internal diameter, 2 mm to 3 mm length). Arterial segments were transferred to a dissection chamber where they were allowed to equilibrate for 6 – 8 h in oxygenated Krebs maintained at 3°C in order to approximate the same timing interval for all studies. All studies were completed within 24 h of receiving the tissue from the operating room. We observed no differences in the control pressure-response relationship in arteries studied in the first 12 h vs. the last 12 h following tissue collection (pregnant, \( p=0.356 \); nonpregnant, \( p=0.622 \)). After equilibration, vessels were placed into the well of a 2 ml arteriograph (Instrumentation and Model Facility, University of Vermont, Burlington, VT) fitted with a borosilicate (FHC, Bowdoinham, ME) micropipette at both ends. Both the proximal and distal micropipettes were attached to 3-way Luer Stopcocks (Cole-Parmer, Vernon Hills, Illinois). The arteries were cannulated on the proximal pipette, secured with suture, gently flushed free of blood and then cannulated and secured on the distal pipette. The arteriograph containing the cannulated vessel was then transferred to the stage of an inverted microscope (Nikon) where the internal diameter of the artery was viewed, measured, and continuously recorded using video edge detection equipment and data acquisition software, respectively (IonOptix Inc., Milton, MA, USA). After placing the arteriograph on the microscope stage, the proximal cannula was attached to the pressure reservoir and the intraluminal pressure increased to 10 mmHg to allow a gentle continuous flushing of the intraluminal contents from the vessel for 2 min. The 3-way stopcock on the distal cannula was then closed in order to pressurize the artery; all experimental protocols
were conducted under “no-flow” conditions. In all experiments, pressurized arteries were continuously superfused with PSS (8-10 mL/min) aerated with gas containing 21% O2, 74% N2 and 5% CO2 at 37°C.

**Study protocol.** All experiments were conducted in a manner similar to that previously described (7). Briefly, the cannulated arteries were initially pressurized to 20 mmHg and allowed to equilibrate for 20 min at 37°C. The intraluminal pressure was then increased to 60 mmHg for ~90 min during which the vessel was challenged with 50 mM KCl three times to confirm artery viability and assess maximal constriction, defined as smallest diameter recorded over a 2 min period in the presence of 50 mM KCl. Vessels that did not constrict to at least 30% of their passive diameter after exposure to 50 mM KCl were discarded. The constriction responses to 50 mM KCl, for the MA from nonpregnant and pregnant women are shown in Table 2. Endothelial integrity was assessed in vessels using the endothelial-dependent vasodilator, bradykinin (BK, 1 µM). Nonpregnant MA were pressurized at 60 mmHg and, due to the lack of MT, pre-constricted with phenylephrine (PE, 10-100 µM) prior to dilation with BK. MA from pregnant women also were pressurized at 60 mmHg and upon obtaining stable MT, challenged with BK to confirm endothelial function. PE-induced constriction and MT were similar in nonpregnant (n=3) and pregnant (n=5) MA, 40.4±6.0 % vs. 33.3±4.9 % maximal constriction (p=0.4, t-test), respectively. BK reversal of PE-induced tone in nonpregnant MA or MT-induced tone in pregnant MA did not differ (92.9±1.5 % vs. 87.8±3.8 % reversal of constriction, p=0.4 t-test respectively). After affirming smooth muscle viability, vessels were returned to Krebs and intraluminal pressure was set to 10 mmHg. The effects of pressure-induced vasoconstriction (MT) were studied by generating two sequential pressure curves for each arterial segment at pressure
steps from 10 to 100 mmHg intraluminal pressure such that each artery segment acted as its own control (a series experimental design). After the first curve was generated, the tissues were allowed to recover for 10 min at 10 mmHg pressure. The vessels were then re-pressurized to 60 mmHg (to confirm reproducible myogenic tone) and incubated for 30 min in either the non-specific NOS inhibitor N-omega-nitro-L-arginine methyl ester (L-NAME; 200 µmol/L), the non-specific COX inhibitor indomethacin (Indo; 10 µmol/L), or the two inhibitors together prior to starting the second pressure-diameter curve to determine the contribution of NOS-, COX-, or combined NOS/COX inhibition on MT in MA. Tissues were then returned to 10 mmHg, incubated in Ca\textsuperscript{2+}-free PSS with a L-type Ca\textsuperscript{2+}-channel inhibitor diltiazem (80 µmol/L) for 45 min, and, followed by a third pressure-diameter relationship to obtain fully dilated (passive) diameters. MT was expressed as a percent constriction of the passive diameter of the individual MA at the same intravascular pressure using the equation:

$$\frac{DP - DA}{DP} \times 100,$$

where $DP$ is the “passive” diameter of the artery in Ca\textsuperscript{2+}-free PSS with diltiazem and $DA$ is the “active” diameter of the artery in response to the stimulus (change in intraluminal pressure) in Ca\textsuperscript{2+}-containing PSS in the absence/presence of L-NAME, INDO or combined L-NAME/INDO.

**Chemicals.** All chemicals used for PSS were purchased from Fischer Scientific (Pittsburgh, PA) and all other chemicals from Sigma Chemicals (St. Louis, MO). Drug stock solutions were prepared as follows: N-omega-nitro-L-arginine methyl ester (L-NAME, 200 µmol/L),
Indomethacin (INDO, 10 µmol/L). Both phenylephrine (PE, 10µM), and bradykinin (BK, 1µM) were prepared daily from 1.0 mmol/L aliquots made in an aqueous stock solution.

Statistical analyses. Data are expressed as the mean ± standard error of the mean (SEM). In instances where multiple segments from a single MA were used for a particular experimental protocol, the results from all experiments were averaged for a single “n”, and hence, n-values reported in the figures and tables indicate numbers of subjects, not vessels. Comparisons between pregnant and nonpregnant women for the continuous measures in Tables 1 and 2 and between two groups where indicated elsewhere were made using a two-sample (“Student’s”) t-test. Dichotomous measures were compared using Fisher’s exact test. All p-values are based on two-sided comparisons. All analyses of MA MT were performed using analysis of variance (ANOVA) with repeated measures. The between-subject factor was pregnancy status (yes, no) and the within-subject factors were treated as 12 repeated measures in a 2 x 6 doubly-repeated measures design for each woman’s vessel, given that MT was measured in MA on 12 times (i.e., at 6 different pressures and with or without the drug(s) of interest). An unstructured inhibitor-repeated factor and compound-symmetry pressure-repeated factor model provided the best fit for these measures. All repeated measures analyses were performed using SAS PROC MIXED procedures (SAS, Carey, NC). Two approaches were used to test if the shape of the MT pressure curve differed by pregnancy or drug (LNAME, INDO, diltiazem) status. The first was a global test of interaction with pressure treated as a 6-category factor. Since this test has very low power to detect specific types of trends, a second approach was used in which pressure was treated as a continuous factor such that tests of interactions could determine if the first factor affected the general upward or downward trend in MT or a quadratic curvature in
the plots. If there was a significant pressure (categorized) by inhibitor interaction, then pairwise comparisons between groups at individual pressures were analyzed using Bonferroni adjustment for post hoc multiple comparisons. Comparisons between groups were considered statistically significant when the $p < 0.05$. Asterices (*, **, ***) are placed above the symbols to designate comparisons that are significant at specific pressures and are located to the right of curves to designate significant overall main effects at p-values of < 0.05, 0.01, and 0.001 respectively.

**Results**

*Subject characteristics.* Nonpregnant women (n=10) ranged from 28-48 yrs of age and as a group were older than the pregnant women (n=12), who ranged from 21 to 42 yrs (Table 1). Eight of the 10 nonpregnant women fell within the same age range as the pregnant group (≤ 42 yrs). The groups did not differ in blood pressure, height, weight, body mass index (BMI), or parity. Nonpregnant women underwent elective hysterectomy for benign uterine complications (see methods). Women undergoing cesarean section fell within 3 groups: 10 of 12 were repeat cesarean section without complications, one underwent a repeat cesarean delivery due to shoulder dystocia, and the remaining was a first time cesarean delivery due to maternal Herpes infection.

*MT in MA.* Representative responses to stepwise increases in intraluminal pressure from 10 to 100 mmHg in MA are illustrated in Figures 1A and 1B. Although the passive diameters were greater than active diameters in both nonpregnant and pregnant MA ($P<0.001$), those from pregnant women had smaller active diameters compared to nonpregnant (Figure 1C
MT in pregnant MA was greater than in nonpregnant MA (overall p<0.001), with the effect of pregnancy on MT being dependent upon pressure (p<0.001) and the largest differences present at pressures ≥ 40 mmHg (p<0.001, Figure 2). Comparisons within each group also showed that MT in pregnant MA at pressures ≥ 40 mmHg exceeded those observed at ≤ 20 mmHg (p<0.05); there were no effects of pressure in the nonpregnant group (Figure 2).

KCl constriction. MT is closely linked to membrane depolarization, resulting in Ca^{2+} entry via voltage-dependent Ca^{2+} channels. Thus, our next series of experiments were designed to assess whether membrane depolarization using 50 mM KCl was different in MA from nonpregnant and pregnant women. Increasing extracellular KCl from 5 mM to 50 mM resulted in similar decreases in arterial diameter in arteries from both groups, indicating no differences in response to membrane depolarization (Table 2).

Contribution of NO to pressure-induced constriction. To assess the role of nitric oxide (NO), we compared the effects of NOS inhibition on MT in MA from nonpregnant and pregnant women. NOS inhibition with L-NAME (200 µM) did not affect MT in nonpregnant MA (p = 0.8, Figure 3A). In pregnant women, MT rose with increasing pressure in a quadratic fashion, but MT was greater in the presence than the absence of L-NAME (p<0.001, Figure 3B). Therefore, NOS inhibition augmented MT in pregnant arteries and acted in an additive manner to increase MT overall by 8.3 ± 3.2%.

Contribution of COX to pressure-induced constriction. To determine the role of vascular
prostaglandins, we treated the MA from nonpregnant and pregnant women with the COX inhibitor indomethacin (INDO; 10µM). INDO alone did not affect MT in MA from either nonpregnant \( (p=0.26, \text{Figure 4A}) \) or pregnant women \( (p=0.26, \text{Figure 4B}) \).

**Effect of combined NOS and COX inhibition on pressure-induced constriction.** Because of potential redundancy in endothelial-dependent signaling, we also asked if combined NOS/COX inhibition modified the pressure-dependent changes in MA. As with L-NAME alone (Figure 3A), combined NOS/COX inhibition did not have affect MT \( (p=0.28) \) or have any interactive affect with pressure \( (p=0.80) \) in MA from nonpregnant women (Figure 5A). In the pregnant women, MA showed a significant quadratic relationship with pressure \( (p<0.001, \text{Figure 5B}) \), which was augmented 7.6±2.8% by the presence of combined L-NAME/INDO \( (p<0.04, \text{Figure 5B}) \). The effect of combined LNAME/INDO did not differ from that observed by LNAME alone \( (7.6\% \text{ vs. } 8.3\%, p=\text{NS}) \).
Discussion

Increasing evidence suggests that MT contributes to the regulatory mechanisms that control uteroplacental blood flow in pregnancy but the extent of this contribution remains unclear (6; 11; 13; 19; 50). To the best of our knowledge, this is the first study to examine and compare MT in MA from nonpregnant and pregnant women. We observed that arteries from pregnant but not nonpregnant women demonstrated robust pressure-induced constriction and thus evidence of myogenic responses. The attenuated MT in arteries from nonpregnant women was not due to an inability to vasoconstrict since 50mM KCl elicited similar vasoconstriction responses in arteries from both nonpregnant and pregnant women. Likewise, the attenuated MT response in nonpregnant arteries could not be attributed to greater NOS and/or COX activity as their inhibition, alone or in combination, did not alter MT. NOS inhibition enhanced MT in pregnant MA, consistent with the enhanced NOS-activation described in myometrial and uterine arteries during pregnancy (11; 13; 19; 20; 51). In contrast, COX-inhibition was without effect on MT in MA from pregnant women either when INDO was administered alone or combined with NOS inhibition. This suggests that COX inhibition has little influence on MT in MA from pregnant women. Thus, we concluded that myogenic responses are present in MA from pregnant but not nonpregnant women, and suggest that MT may contribute to the regulation of uteroplacental blood flow in pregnancy.

A major strength of our study was our ability to determine if pregnancy altered MT in relevant human uterine arteries, overcoming obstacles faced in studies using animal models (11; 13; 26; 51; 52). The distinctive nature of human pregnancy stemming from the nature of human trophoblast invasion, vascular remodeling, placental architecture and
bipedal posture argues in favor of studying human rather than experimental animal tissues. We studied MA from the outer third of the uterine wall in pregnant women so as to be sampling from the same vascular region and to be studying similarly-sized arteries in the nonpregnant and pregnant groups. In addition, we assessed alterations in MT across the full range of pressures (10 to 100 mmHg) in order to encompass both normal and pathophysiologic pressures, and examined the contributions of NOS and COX in a similar manner using well-established inhibitors separately and in combination. Although the nonpregnant group was nine yrs older than the pregnant women, the age range was similar in the two groups and eight of the 10 nonpregnant women were within the same age range. The uterine vascular bed is progressively remodeled throughout pregnancy in all species (see review by Osol and Mandala (35)), which results in a redistribution of blood flow within the pregnant uterus from the myoendometrial tissues to the uteroplacental vascular bed (i.e., spiral arterioles, intervillous space) (5; 15; 16; 36). Although the spiral arterioles contribute to the regulation of uteroplacental perfusion (27; 31; 37; 38), they are difficult to obtain and study and undergo remodeling that, by definition, is not present in nonpregnant vessels. Therefore, we studied MA of similar size from nonpregnant and pregnant women. We consider that these arteries may play one or more of three roles: 1) be responsible for myoendometrial blood flow, 2) be partially responsible for blood flow through the distal spiral arterioles, and/or 3) representative of the downstream spiral arterioles. It is unclear which is correct but any or all of these possibilities is (are) likely to contribute to the regulation of uteroplacental blood flow. Furthermore, MT is evident in pregnant MA across the full range of pressures (10 to 100 mmHg), which encompasses normal and pathologic perfusion pressures, thus extending the observations of Kublickiene
et al (20), who focused on flow-dependent dilation in human MA and in that report, MT was therefore studied only at a single intraluminal pressure following norepinephrine preconstruction as required to assess flow-dependent dilation (19; 21; 22).

Although MT has been observed in MA from pregnant women (19-22; 48) and various generations of nonpregnant and pregnant UA from other species (11; 51; 52), it is unclear how MT is affected by human pregnancy (19-22). To the best of our knowledge, this is the first comparison of MT in nonpregnant and pregnant human MA. The observation that pregnancy raises MT in MA is somewhat unexpected, given the increased production of endothelial-dependent vasodilators (8; 11; 12; 19) that would be expected to decrease or attenuate MT. Furthermore, the shape of our pressure-response curves in pregnant MA differed from that observed by Kublickiene et al (20) in a study in which MT was examined across a 20 – 120 mmHg pressure range. That study found that MT was greatest at lower intraluminal pressures (i.e., 20 mmHg) and decreased modestly as pressures rose to 100 mmHg) (20), whereas our study found that MT increased from 10 to 60 mmHg and tapered off as pressures approached 100 mmHg. The reasons for the differences in the shape of the pressure dose-response curve are unclear. One possibility is that Kublickiene et al (20) used a HEPES-based buffer while we used a non-HEPES buffer (PSS), since differences in vascular reactivity with different kinds of buffering solutions have been observed (1). A second reason may be the greater diameter of the MA studied by Kublickiene; for example at 20 mmHg, the vessels in that study averaged 295±27 µm whereas those in the present study averaged 229±14 µm. Finally, Kublickiene and colleagues assessed myogenic tone in the absence and presence of NOS inhibition using a parallel experimental design (20), whereas our experiments were performed in series (e.g.,
pressure curves are obtained in the absence/presence of inhibitor in each artery studied). To ensure the myogenic response remained consistent throughout the experiment, we assessed pressures steps from 10mmHg to 60mmHg twice prior to control pressure curve, once at 60 mmHg for the control curve in the absence of the inhibitor, and finally just prior to obtaining the pressure curve in the presence of the inhibitor. We defend our approach on the basis that no difference was observed in MT at 60 mmHg across the four time points in the nonpregnant or pregnant MA (both \( p=NS \)).

In light of our observation that MT was attenuated in MA from nonpregnant women and enhanced in those from pregnant women, we sought to determine what mechanisms contributed to this pregnancy-associated change. Endothelial-derived NO is up-regulated in the uterine vascular bed in pregnancy of most species (8; 44; 46); thus, it might contribute to the regulation of MT in MA in pregnancy (9; 19). Notably, NOS inhibition increased MT in MA from pregnant but not nonpregnant women; thus, nitric oxide appears to modulate myogenic responses in MA during pregnancy. This is consistent with prior observations in pregnant women (19; 22) and radial arteries from pregnant rats (13). They also are consistent with the vasodilating effects of increased nitric oxide during pregnancy in response, for example, to flow (8; 44). It is now evident that the increases in arterial NOS activity that occur in healthy pregnancy are also involved in modulating arterial MT. The contribution of NO-dependent signaling to MT in complicated pregnancies is not well understood. Kublickiene and colleagues demonstrated that attenuated NO-dependent signaling may be responsible for decreased flow-induced vasodilation in MA from preeclamptic women (21), yet in these same studies MA from normal and preeclamptic women had similar levels of MT in the absence and presence of NOS
inhibition (21). Since these were done at a single intraluminal pressure and MT was augmented with norepinephrine (21; 48), it is possible that the contribution of nitric oxide to MT may have been missed. Thus, these studies should be repeated across the full pressure range to capture the relevant pressures present in normotensive and hypertensive states.

Having shown that local vascular NO modifies MT in pregnant human MA, we next sought to determine if local prostaglandin synthesis might also play a role. It is known that prostaglandin synthesis and COX-1 are elevated in ovine uterine artery endothelium during pregnancy (14; 24). Interestingly, COX inhibition had no effect on MT or the pressure-induced increases in contractions in MA from pregnant and nonpregnant women; thus, COX-dependent signaling in the uterine vasculature may differ between species or there is redundancy in endothelial-dependent signaling pathways. When we assessed myogenic reactivity in the presence of combined NOS/COX inhibition, MT in arteries from pregnant women was not different from that seen with NOS inhibition alone and was without effect in arteries from nonpregnant women. These results are similar to those previously obtained in MA from pregnant women (19; 22) and radial and uterine arteries from pregnant mice and rats (13; 51).

Myogenic responses in MA from pregnant women exceeded those observed in nonpregnant MA and appeared to be attenuated by increased NO but not vasodilator prostaglandin production in the pregnant but not the nonpregnant state. Thus, NOS or COX-independent mechanisms are likely involved in increasing myogenic tone during human pregnancy. One possibility is that alterations in the production or activity of endothelial-derived hyperpolarizing factor are involved, as it appears to contribute to endothelial-
dependent dilation in human MA (10; 17; 23) and whose responses are attenuated in pressurized (17) or wire mounted (23) MA from preeclamptic women, suggesting it contributes to overall MA reactivity in normal and pathological conditions. Alternatively, MA may undergo remodeling as in more proximal uterine arteries and therefore, express increasing amounts of contractile proteins (2; 3; 35). This is unlikely since responses to 50 mM KCl did not differ in arteries from nonpregnant and pregnant women. There also may be differences in the expression and/or function of vascular smooth muscle K⁺ channels that contribute to myogenic responses and have been identified in the uteroplacental circulation, e.g., Kv and BKCa (32; 39; 43; 50). These channels have been identified in women (43), sheep (37), and rat (41; 50) uterine arteries and shown to modify maternal uterine blood flow (18; 42) but their expression and function in human MA is not known. Whereas BKCa channels appear to modulate uterine vasodilation and vasoconstriction in women and sheep (39; 41; 43), down-regulation and/or inactivation of Kv channels is associated with increased myogenic responses in small uterine arteries from pregnant rats (50). Although not studied, down-regulation of BKCa channels could result in similar changes. Thus, further studies of these channels in human MA and other uterine vessels are warranted.

**Perspectives and Significance**

Our current understanding of the mechanisms regulating human uteroplacental blood flow in pregnancy remains limited and is primarily derived from various animal models. We and others (19; 39) are now able to obtain and study human arteries from the uterine micro- and macrovasculature. Although endothelial factors such as nitric oxide and
prostaglandins contribute to vascular regulation, increasing evidence suggests that downstream cyclic nucleotides are also essential (18; 32; 40; 41) and that various smooth muscle membrane channels (18; 43; 50) are of major importance and need additional study. Furthermore, it is now evident that myogenic responses associated with these pathways may contribute not only to the regulation of total uterine blood flow, but also its redistribution during the course of pregnancy, accounting for the development of a high resistance (myometrium and endometrium) and low resistance vascular bed on the maternal side of the placenta (28; 28-30). If this is indeed correct, the arteries demonstrating myogenic reactivity might also play an important role in the pathophysiology of preeclampsia and other hypertensive disorders in pregnancy by decreasing perfusion of the maternal placental vascular bed and thus the availability of nutrients required for normal fetal growth and well-being.
**Disclosures:**

No conflicts of interest, financial or otherwise, are declared by the authors.

**Acknowledgements:**

We would like to thank Kristi Lanier, RN and Melissa Swain, RN for their assistance identifying patients and collecting tissue from the operating room, along with Crystal Butler and Mary K. Brewer for their technical assistance conducting experiments. In addition, we would like to thank Drs. Michael O'Shea and Qing Yang for reading of the manuscript and providing editorial comments.

**Grants:**

This study was supported by NIH-HL079647 (LGM), departmental funds from Brenner Children's Hospital (DME), and by the George L. MacGregor Professorship in Pediatrics (CRR).
Figure Legends

Figure 1: Relationship between intraluminal pressure and vascular diameter in myometrial arteries from nonpregnant and pregnant women. Panels A and B demonstrate representative changes in vascular diameter (black line) observed with stepwise increases of intraluminal pressure (gray line) from 10 mmHg to 100 mmHg. Panels C and D summarize changes in diameter with increasing intraluminal pressures in Ca\textsuperscript{2+}-containing Krebs (active) and Ca\textsuperscript{2+}-free Krebs +40µM diltiazem (passive) in arteries from nonpregnant (n=10; ●, active and ○, passive) and pregnant (n=12; ■, active and □, passive) women. Passive diameters were larger at all pressures (10 to 100 mmHg) than active diameters in arteries from both groups of women (**P<0.01; *** P<0.001). Data were analyzed by ANOVA doubly-repeated measures where pregnancy status is the between-subjects factor and the active/passive and pressures are the two repeated factors.

Figure 2: Comparison of myogenic tone in myometrial arteries from pregnant vs. nonpregnant women. Myogenic tone in arteries from pregnant women (■, n=12) was greater than in arteries from nonpregnant women (●, n=10) across all values of intraluminal pressures (p<0.001) and specifically at ≥40 mmHg (** = p<0.001 **=p<0.01). Letters signify values that differ in the MA from pregnant women at the designated pressures at p<0.05. Data were analyzed by ANOVA repeated measures where pregnancy status was the between-subjects factor and pressure was the repeated factor.

Figure 3: Effect of the nitric oxide synthase inhibitor L-NAME (200 µM) on myogenic tone in myometrial arteries from nonpregnant and pregnant women. LNAME had no effect on myogenic tone in nonpregnant women (Panel A: ●, -LNAME vs. ○, +LNAME; n=6, p=0.68,). In contrast, LNAME augmented myogenic tone in pregnant women (Panel B: ■, -LNAME vs. □, +LNAME; n=7, p<0.001). Data were analyzed by ANOVA doubly-repeated
measures analysis where pregnancy status is the between-subjects factor and absence or presence of LNAME and pressure are the within-subject repeated factors. *** = p<0.001.

**Figure 4:** Effect of the cyclooxygenase inhibitor indomethacin (INDO, 10 µM) on myogenic tone in myometrial arteries from nonpregnant and pregnant women. INDO had no effect on myogenic tone in nonpregnant (Panel A: ●, -INDO vs. ○, +INDO; n=5, p=0.66,) and pregnant women (Panel B: ■, -INDO vs. □, +INDO; n=5, p=0.25). Data were analyzed by ANOVA doubly-repeated measures analysis where pregnancy status is the between-subjects factor and the absence or presence of INDO and pressure are the within-subject repeated factors.

**Figure 5:** Effect of combined NOS (L-NAME, 200 µM) and COX (INDO, 10 µM) inhibition on myogenic tone in myometrial arteries from nonpregnant and pregnant women. Combined NOS/COX inhibition with L-NAME/INDO augmented myogenic tone in nonpregnant (Panel A: ●, -L-NAME/INDO vs. ○, +L-NAME/INDO; n=5, NS) and pregnant women (Panel B: ■, -L-NAME/INDO vs. □, +L-NAME/INDO n=6, p=0.040). Data were analyzed by ANOVA doubly-repeated measures analysis where pregnancy status is the between-subjects factor and placebo/inhibitor and pressure are the within-subject repeated factors (* = p<0.05).
References


<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number of patients</strong></td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years) or range (in parentheses)</strong></td>
<td>38.5±1.8 (28 to 48)</td>
<td>29.5±1.6 (21 to 42))</td>
<td>( p&lt;0.001 )</td>
</tr>
<tr>
<td><strong>African American (number)</strong></td>
<td>3</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Caucasian (number)</strong></td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Heart Rate (bpm)</strong></td>
<td>80.6±3.5</td>
<td>92.6±3.5</td>
<td>( p=0.027 )</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>125±6.7</td>
<td>128±2.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>75.4±3.7</td>
<td>75.9±2.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Height (in)</strong></td>
<td>64.7±0.7</td>
<td>64.1±1.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight at time of hyst or pre-pregnancy (lb)</strong></td>
<td>197±16.9</td>
<td>184±14.5</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BMI at time of hyst or pre-pregnancy</strong></td>
<td>32.9±2.6</td>
<td>31.8±2.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight at time of hysterectomy or C/S (lb)</strong></td>
<td>197±16.9</td>
<td>208±16.1</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Gestational age (week)</strong></td>
<td>N/A</td>
<td>39.0±0.2</td>
<td>--</td>
</tr>
<tr>
<td><strong>Birth weight (gm)</strong></td>
<td>N/A</td>
<td>3585±61.2</td>
<td>--</td>
</tr>
<tr>
<td><strong>Gravidity (no. pregnancies)</strong></td>
<td>3.2±0.4</td>
<td>2.9±0.3</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Parity (no. live births)</strong></td>
<td>2.5±0.3</td>
<td>2.3±0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( P \)-values based on two-sample Student’s t tests for continuous data and Fisher’s Exact test for proportions.
Table 2. Comparison of myometrial artery internal diameters in the absence and presence of 50 mM KCl-Krebs.

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant (n=10)</th>
<th>Pregnant (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting diameter (µm)</td>
<td>213.5±15.8</td>
<td>194.1 ± 17.9</td>
<td>NS</td>
</tr>
<tr>
<td>50mM KCl diameter (µm)</td>
<td>114±9.9</td>
<td>128.9 ± 17.5</td>
<td>NS</td>
</tr>
<tr>
<td>Passive diameter (µm)</td>
<td>244.2±15.1</td>
<td>239.1 ± 13.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>% Constriction:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constriction prior to 50mM KCl</td>
<td>12.8±2.6</td>
<td>19.4±4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Constriction with 50mM KCl</td>
<td>52.8±3.8</td>
<td>45.4±6.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Tests for statistical significance were based on two-sample Student’s t tests for continuous data.
Figure 1:
Relationship between intraluminal pressure and vascular diameter in myometrial arteries from nonpregnant and pregnant women.
Figure 2.
Comparison of myogenic tone in myometrial arteries from pregnant vs. nonpregnant women.
Figure 3:
Effect of nitric oxide synthase inhibitor L-NAME (200µM) on myogenic tone in myometrial arteries from nonpregnant and pregnant women.
Figure 4:
Effect of cyclooxygenase inhibitor indomethacin (INDO, 10μM) on myogenic tone in myometrial arteries from nonpregnant and pregnant women.
Figure 5:
Effect of combined NOS (L-NAME, 200μM) and COX (INDO, 10μM) inhibition on myogenic tone in myometrial arteries from nonpregnant and pregnant women.