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# Chronic hypoxia opposes pregnancy-induced increase in uterine artery vasodilator response to flow

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**Mateev, Stephanie, A. Hugo Sillau, Rhonda Mouser, Robert E. McCullough, Margueritte M. White, David A. Young, and Lorna G. Moore.** Chronic hypoxia opposes pregnancy-induced increase in uterine artery vasodilator response to flow. *Am J Physiol Heart Circ Physiol* 284: H820–H829, 2003. First published November 14, 2002; 10.1152/ajpheart.00701.2002.—We tested the hypotheses that pregnancy increases the uterine artery (UA) vasodilator response to flow and that this increase is impaired under conditions of chronic hypoxia (30 days, simulated elevation 3,960 m). UA were isolated from 24 normoxic or chronically hypoxic midpregnant guinea pigs and studied with the use of pressure myography. Normoxic pregnancy increased UA flow vasodilator response and protected against a rise in wall shear stress (WSS). Chronic hypoxia opposed these effects, prompting vasoconstriction at high flow and increasing WSS above levels seen in normoxic pregnant UA. The nitric oxide synthase inhibitor $N^G$-nitro-L-arginine (L-NNA) eliminated the pregnancy-associated increase in flow vasodilation in normoxic UA, suggesting that increased nitric oxide production was responsible. The considerable residual vasodilation after nitric oxide synthase and cyclooxygenase inhibition implicated endothelial-derived hyperpolarizing factor (EDHF) as an additional contributor to flow vasodilation. L-NNA increased flow vasodilation in UA from chronically hypoxic animals, suggesting that chronic hypoxia may have lowered EDHF or elevated peroxynitrite production. In conclusion, flow is an important physiological vasodilator for the acute and more chronic UA dimensional changes required to increase uteroplacental blood flow during normal pregnancy. Chronic hypoxia may be a mechanism that opposes the pregnancy-associated rise in UA flow vasodilation, thereby increasing the incidence of preeclampsia and intrauterine growth restriction at a high altitude.

acetylcholine; endothelium-derived hyperpolarizing factor; nitric oxide; peroxynitrites; preeclampsia

CARDIAC OUTPUT INCREASES during pregnancy as a result of decreases in afterload due to lower blood pressure and systemic vascular resistance and increased preload due to blood volume expansion. Much of the increase in blood flow is directed toward the uteroplacental circulation as a result of alterations in vasoreactivity and vascular growth and remodeling. Such changes include the fall in vascular resistance with the establishment of the placental circulation and the enlargement of the uterine artery (UA), mesometrial, and other uterine vessels. UA diameter increases as the result of enhanced vasodilator response, decreased vasoconstrictor response, alterations in active and passive mechanical properties of the UA wall, and UA outward hypertrophic growth and remodeling (18, 24, 28, 29, 39–41). We considered that the factors regulating UA diameter were important insofar as UA diameter is a major determinant of uterine blood flow. Furthermore, because UA enlargement occurs well before the highest flows are achieved (18), factors enlarging UA diameter appear required to help initiate this rise.

We considered that an increased vasodilator response to flow in the UA might be an important early stimulus for prompting both acute dimensional changes as well as more chronic outward hypertrophic growth and remodeling. Flow is a potent physiological vasodilator. Moreover, flow is also likely to be an important signal involved in vascular growth and remodeling insofar as outward hypertrophic growth occurs with increased flow, whereas inward hypertrophic growth with a resultant reduction in luminal diameter typifies elevated pressure conditions (25, 31). Pregnancy increases the vasodilator response to flow in myometrial (19) as well as mesenteric and subcutaneous arteries (2, 3, 22), but it is unknown whether flow responsiveness is increased in UA. Myometrial arteries from women whose pregnancies are complicated by preeclampsia fail to show a pregnancy-associated increase in vasodilator response to flow (20). These human studies are limited to term pregnancy, the time when tissues can be obtained from women under-

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going cesarean section. Hence, it is unknown whether an increased vasodilator response to flow occurs earlier in pregnancy when it could contribute to the normal vascular adaptation to pregnancy. Also, its absence might be important in the promotion of endothelial injury and uteroplacental ischemia characteristic of preeclampsia.

We therefore asked whether pregnancy increases the UA vasodilator response to flow and whether such a response is impaired in pregnancies complicated by chronic hypoxia. We (45) have reported lower near-term UA blood flows in pregnant women residing at a high altitude (3,100 m) compared with lower altitudes in Colorado. Residence at a high altitude increases the incidence of intrauterine growth restriction and preeclampsia (15, 30), both conditions in which uteroplacental blood flow is reduced. Furthermore, we (32) have shown that the pregnancy-associated rise in UA DNA synthesis is only one-half as great in guinea pigs exposed to chronic hypoxia versus normoxia throughout gestation, suggesting that the flow-mediated growth stimulus might be impaired. Because chronic hypoxia in the guinea pig decreases the contribution of UA nitric oxide (NO) production to acetylcholine (ACh)-induced vasodilation (40) and the levels of UA endothelial NO synthase (eNOS) protein (42), we hypothesized that reduced vasodilator response to flow under conditions of chronic hypoxia was due to decreased NO production.

We isolated the main UA from pregnant or nonpregnant guinea pigs that had been exposed to either normoxia or hypoxia throughout gestation or an equivalent period in the nonpregnant state. The dimensional changes in response to increasing flow were measured with pressure myography. Vessels from midterm pregnant animals were used because this is at the onset of maximal growth in these vessels (18). Previous studies (2, 19) have shown that increased NO production is largely responsible for the pregnancy-associated increase in vasodilator response to flow in myometrial arteries in normal pregnancies, whereas it makes little or no contribution in vessels from nonpregnant or preeclamptic women. To determine the contribution of increased NO production to flow vasodilation in the UA, we examined the effect of flow before and after the administration of a NOS inhibitor. Because cyclooxygenase products have been implicated in vasodilator responsiveness in the uterine vascular bed (4, 14), the combined effects of cyclooxygenase and NOS blockade were evaluated in a subset of animals. The use of the combined inhibitors also gave us a preliminary assessment of the contribution of the third endothelial-dependent vasodilator, endothelium-derived hyperpolarizing factor (EDHF), to the UA vasodilator response to flow.

## METHODS

*Animals.* For these experiments, 12 guinea pigs (7 nonpregnant, 5 pregnant) were kept at the laboratory altitude of 1,600 m (normoxia, $P_{I_{O_2}}$ = 125 Torr) and 12 animals (6 nonpregnant, 6 pregnant) exposed to a simulated altitude of 3,960 m for 30 days (chronic hypoxia, $P_{I_{O_2}}$ = 90 Torr). In all 24 animals, the contractile response to phenyephrine (PE) and dilator responses to ACh and flow studies were determined before and after administration of the NOS inhibitor $N^G$-nitro-L-arginine (L-NNA). The combined effects of NOS and cyclooxygenase inhibition were evaluated in 21 of these 24 animals.

Animals were placed in a hypobaric chamber within 3 days of conception or in the nonpregnant state and remained there for 30 days (term = 65 days). All animal handling and study procedures were approved by the University of Colorado Animal Care and Use Committee. Total body weights were greater in the normoxic or chronically hypoxic pregnant groups than in their nonpregnant counterparts at the time of study (normoxia: 776 ± 27 vs. 639 ± 11 g, hypoxia: 780 ± 21 vs. 728 ± 20 g, both $P < 0.05$), but there were no differences between pregnant normoxic versus hypoxic animals nor did maternal body weights (total body weight − uterine contents) differ among the four groups.

*Isolated vessel preparation.* Animals were euthanized with pentobarbital sodium (6.5%), and the UA was removed. Several ~4-mm-long segments without collaterals were carefully dissected to avoid mechanical damage. These were from the middle segment of the UA, equidistant between the cervical and ovarian ends. Both ends of the vessel were cannulated with the use of a dual-pipette system described previously (11, 13). Vessels were mounted by the insertion of an inner pipette into each end of the vessel. The inner pipette was then advanced into an outer holding pipette connected to the microscope stage with the use of four-axis micromanipulators (Spiderwort Design; Colorado Springs, CO). The vessels were visualized using an inverted binocular light microscope (model CK, Olympus; Tokyo, Japan), and signals were recorded by videocamera (model JE 7442, Javelin Electronics; Los Angeles, CA) and displayed on a monochrome monitor (model BWM 12A, Javelin Electronics). Approximately 2 mm of the vessel were visible under the microscope. The mounted vessel assembly was submerged in a 2-ml perfusion bath maintained at 37°C by a thermal control circuit (model 16060, Love Controls; Wheeling, IL). The vessels were perfused externally with a modified physiological salt solution containing (in mM) 145 NaCl, 5.0 glucose, 4.7 KCl, 2.75 NaOH, 2.0 MOPS, 2.0 $CaCl_2$, 2.0 pyruvate, 1.17 $MgSO_4$, 1.2 $NaH_2PO_4$, and 0.02 EDTA maintained at pH 7.4 ± 0.04 as previously described (6). The vessel lumen was perfused with the same solution with the addition of 1% bovine albumin (A-2934, Sigma; St. Louis, MO) and 5% Dextran 40 (Braun; Irvine, CA).

The system was equipped with upstream and downstream pressure monitors and a servo-controlled pump (Living System Instrumentation; Burlington, VT). The pipettes used for a given study were carefully matched for resistance. Because the transducers monitoring upstream and downstream pressure were equidistant from the vessel, intraluminal pressure was simply the mean of the upstream and downstream value. As described by Halpern and Kelley (10), vessels can be studied in the absence of flow by equalizing the upstream and downstream pressures or in the presence of flow without altering midvessel intraluminal pressure by raising upstream pressure and lowering downstream pressure in the same amount.

*Protocol.* The mounted vessels were pressurized to 20 mmHg, which we had previously determined as the optimal pressure or that at which the greatest contractile responses to 40 mM KCl and PE ($10^{-8}$ to $10^{-5}$ M) were observed. After a 30-min equilibration period, vessels were contracted with 40 mM KCl, rinsed, and allowed to return to the initial

diameter. Initial test doses of PE ($10^{-6}$ M) and ACh ($3 \times 10^{-5}$ M) were administered, followed by a complete contractile dose response to PE ($10^{-8}$ to $10^{-5}$ M) and ACh ($3 \times 10^{-7}$ to $3 \times 10^{-4}$ M). Vessels were considered viable and accepted for study if they exhibited an at least 30% reduction in inner diameter (ID) to PE and dilated at least 40% to ACh.

To evaluate the vasodilator response to flow, flow through the vessel lumen was increased progressively from 0 to 4 μl/s. ID became stable within 1–2 min at a given level of flow and remained constant for the 4 min when flow was maintained. No transient fluctuations in vessel diameter were observed. Flow was then adjusted to the next level, and the measurements were repeated. This flow range corresponds to that used in previous studies (2, 3, 19). It was also the greatest flow that could be maintained with this system while the midvessel intraluminal pressure was held constant. The response to flow was first determined in vessels without prior vasoconstriction and then in vessels that had been 50% maximally preconstricted with PE. The PE dosages required to produce 50% maximal contraction did not differ in the pregnant compared with the nonpregnant groups ($9 \times 10^{-5}$ vs. $3 \times 10^{-6}$ M, respectively, $P = 0.12$). Vessels were then treated with L-NNA (200 μM), an inhibitor of NOS, and the contractile responses to PE and dilator responses to ACh and flow were repeated. In vessels from 21 of 24 animals, the cyclooxygenase inhibitor meclofenamate (3 μM) was also added to the vessel bath, the vessels were again preconstricted with PE, and the flow dose response was repeated. A separate series of animals ($n = 4$) was studied to evaluate the reproducibility of the flow-mediated responses. In PE-preconstricted vessels, there were no differences in the vasodilator responses to three successive flow challenges, similar to previous reports (20).

*Calculations.* Changes in ID in response to chemicals added to the bath or increasing flow were considered to represent the contractile or dilator responses. Maximum contractile response to PE ($C_{max}$) was calculated as the difference between the initial resting diameter and the minimum diameter observed for each vessel. The best-fitting nonlinear regression curve was fit to the percent maximum contraction values at each PE dose for each subject and used to determine the negative log of the half-maximal contractile response ($pD_2$) as a measure of contractile sensitivity.

The percent maximal vasodilation at each level of flow was calculated as $(ID_{pe} - ID_{flow})/(ID_{rest} - ID_{pe})$, where $ID_{pe}$ is the ID after 50% maximal PE constriction, $ID_{flow}$ is the ID at the given flow rate, and $ID_{rest}$ is the ID before preconstriction with PE. Similarly, the percent maximal vasodilation at each dosage of ACh was calculated as $(ID_{pe} - ID_{ACh})/(ID_{rest} - ID_{pe})$, where ACh is the ID at the given ACh dosage. The maximal ($R_{max}$) change in ID was calculated as ($ID_{pe}$ − the largest ID observed) in response to ACh or flow.

Wall shear stress (WSS) was calculated as $(4 \times p \times Q \times 10^9)/(\pi \times r^3)$, where $p$ is viscosity (in dynes per square centimeter), $Q$ is flow, $\pi$ is the constant 3.14, and $r$ is the artery radius (in micrometers). The viscosity of the perfusate was 0.025 poise, which was somewhat greater than the viscosity of water (0.007) as the result of adding dextran to the perfusate. The factor $10^9$ was used to correct for the use of both microliters per second for flow and micrometers for arterial radius (1 μl = $10^9$ cubic micrometers expressed in dyn/cm). The resultant values for WSS were similar to those achieved in other studies (2, 3, 19, 20, 22) and likely within the range of midpregnancy values present in sheep (16).

*Statistical analyses.* Data are reported as means ± SE. Vessel dimensions, $C_{max}$, $pD_2$, and $R_{max}$ in response to flow or ACh were compared among all four groups (normoxic nonpregnant, normoxic pregnant, chronically hypoxic nonpregnant, and chronically hypoxic pregnant) using one-way ANOVA with Student-Newman-Keuls multiple comparisons. Effects of L-NNA, a NOS inhibitor, and meclofenamate, a cyclooxygenase inhibitor, on $C_{max}$ or $pD_2$ were compared within groups with paired $t$-tests. The vasodilator response to flow as the percentage of maximum dilation was analyzed with a linear mixed-effect modeling approach, which accounts for variability between animals as well as between multiple measurements on the same animal (21, 23). The process consisted of fitting polynomials of increasing order to the data for all groups and then selecting, visually inspecting, and validating the best-fit lines with likelihood ratio tests using the MIXED program (SAS/STAT version 8.1, SAS Institute; Cary, NC). The regression lines predicted by the best-fit lines were superimposed on plots of the means ± SE to inspect fit and contrast results. All four groups were compared over the range of flow levels using Scheffé's-type simultaneous contrasts (44). A similar approach was used for comparing the WSS response to flow among all four groups and for identifying the within-group effects of L-NNA alone or together with meclofenamate on the vasodilator response to flow. Comparisons were considered significant when $P < 0.05$.

## RESULTS

*Vessel dimensions and contractile response to PE.* Vessel inner and outer diameters were greater in the pregnant than nonpregnant animals but similar in the normoxic and chronically hypoxia groups when controlled for pregnancy status (Table 1). Wall thickness was similar in all four groups.

Contractile sensitivity to PE was diminished in the UA from pregnant compared with nonpregnant animals at each altitude (Table 1). $C_{max}$ was similar in all four groups. $pD_2$ was modestly but not significantly altered by the administration of the NOS inhibitor L-NNA in any group (Table 1).

*Effect of pregnancy under normoxic conditions on vasodilator response to flow.* Without preconstriction, the guinea pig UA had little vasodilator response to flow (Table 1). PE-preconstricted vessels showed a brisk vasodilator response to flow (Fig. 1 and Table 1). Pregnancy increased the UA vasodilator response across all flows and particularly at low flow (<1 μl/s) in normoxic animals (Fig. 1*A*). WSS rose with increasing flow (Fig. 2*A*). The initial rise in WSS at low flow in the nonpregnant UA was absent in vessels from the pregnant animals. Across all flows, WSS values were lower in the UA from the pregnant compared with the nonpregnant normoxic group.

Administration of the NOS synthesis inhibitor L-NNA reduced the UA vasodilator response to flow in the normoxic nonpregnant group (Fig. 3*A*). An even more marked reduction occurred in the pregnant animals such that the vasodilator response to flow no longer differed in the UA from the pregnant versus the nonpregnant groups (Fig. 3*B*). At the highest flows, L-NNA eliminated flow-induced vasodilation in the pregnant vessels. The addition of the cyclooxygenase inhibitor meclofenamate had no additional inhibitory effect on the vasodilator response to flow in UA from

Table 1. *Uterine artery characteristics*

| Variable | Normoxic Nonpregnant | Normoxic Pregnant | Hypoxic Nonpregnant | Hypoxic Pregnant |
|---|---|---|---|---|
| Inner diameter, μm | 439 ± 27 | 644 ± 36* | 458 ± 23 | 767 ± 55* |
| Outer diameter, μm | 560 ± 22 | 759 ± 38* | 588 ± 28 | 895 ± 54* |
| Wall thickness, μm | 121 ± 8 | 115 ± 10 | 130 ± 9 | 128 ± 5 |
| | | *Phenylephrine* | | |
| Without L-NNA | | | | |
| $pD_2$ | 5.9 ± 0.05 | 5.6 ± 0.09* | 5.8 ± 0.15 | 5.5 ± 0.09* |
| $C_{max}$, μm | 366 ± 31 | 425 ± 57 | 352 ± 47 | 515 ± 79 |
| With L-NNA | | | | |
| $pD_2$ | 6.1 ± 0.09 | 5.9 ± 0.21 | 6.1 ± 0.16 | 5.7 ± 0.07 |
| $C_{max}$, μm | 417 ± 16 | 516 ± 29 | 451 ± 38 | 635 ± 38 |
| | | *Acetylcholine* | | |
| Without L-NNA | | | | |
| $R_{max}$, μm | 166 ± 23 | 149 ± 20 | 134 ± 27 | 267 ± 41* |
| With L-NNA | | | | |
| $R_{max}$, μm | 79 ± 36† | 74 ± 25† | 84 ± 19† | 110 ± 25† |
| | | *Flow* | | |
| Without PE preconstriction | | | | |
| $R_{max}$, μm | 57 ± 10 | 19 ± 3 | 33 ± 11 | 41 ± 12 |
| With PE preconstriction | | | | |
| $R_{max}$, μm | 186 ± 39 | 260 ± 62 | 201 ± 42 | 331 ± 77 |

Values are means ± SE. L-NNA, $N^G$-nitro-L-arginine; $pD_2$, one-half maximal contractile response (−log mol/l); $C_{max}$, maximum contractile response; $R_{max}$, maximal vasodilator response (see METHODS); PE, phenylephrine. *$P < 0.05$, nonpregnant vs. pregnant guinea pigs; †$P < 0.05$, without vs. with L-NNA.

either the nonpregnant or the pregnant group, but rather restored the dilator response to flow.

*Effect of chronic hypoxia and pregnancy on vasodilator response to flow.* UA from chronically hypoxic nonpregnant animals vasodilated in response to flow in a manner that was similar to that observed in the nonpregnant normoxic vessels (Fig. 1, $P$ = not significant). At very low flow (0.1 μl/s), pregnancy increased the UA vasodilator response to flow in the chronically hypoxic animals. But vasodilation was not maintained and resulted in net vasoconstriction at higher flows (Fig. 1*B*). WSS rose with increasing flow. Unlike the UA from normoxic animals, WSS at a given flow was not reduced in the pregnant versus nonpregnant chronically hypoxic groups (Fig. 2*B*). Compared with their normoxic counterparts, the chronically hypoxic pregnant vessels vasodilated less in response to flow and exhibited greater WSS (both $P < 0.05$).

Administration of the NOS inhibitor L-NNA increased the vasodilator response to flow in the chronically hypoxic nonpregnant group (Fig. 3*C*). In the pregnant group, L-NNA decreased the vasodilator response at flows ≤0.25 μl/s but increased vasodilation at flows ≥2 μl/s (both comparisons $P < 0.05$, Fig. 3*D*). Thus whereas L-NNA reduced flow vasodilation in the UA from the normoxic nonpregnant and pregnant groups, L-NNA increased the vasodilator response to higher flows in the chronically hypoxic nonpregnant and pregnant groups. The addition of the cyclooxygenase inhibitor meclofenamate did not lower flow vasodilation in either group but rather raised the contractile response to flow in the nonpregnant and pregnant UA (Fig. 3, *C* and *D*).

*Effect of pregnancy and chronic hypoxia on vasodilator response to ACh.* Pregnancy augmented the dilator response to ACh in UA from normoxic and chronically hypoxic guinea pigs (Fig. 4, *A* and *B*). The maximal change in diameter was unaffected by pregnancy or chronic hypoxia (Table 1). Administration of L-NNA decreased the dilator response to ACh, achieving complete inhibition at lower dosages of ACh ($<10^{-5}$ M) in all four groups (Fig. 5). L-NNA reduced the

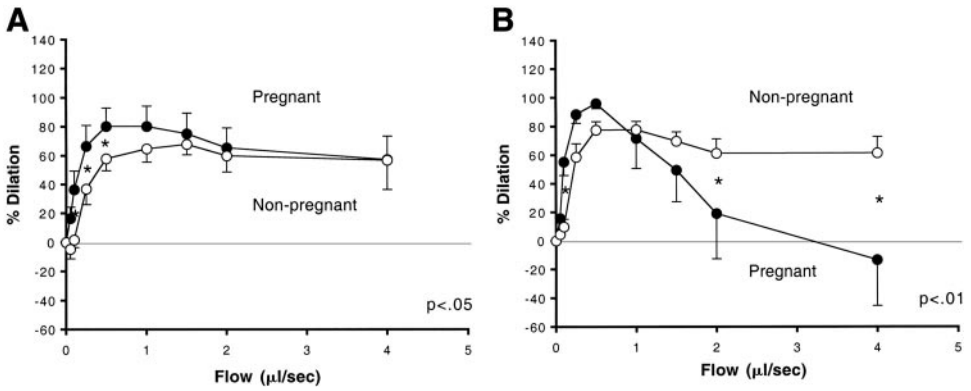


Fig. 1. The percent maximal vasodilation at each level of flow was measured in 50% maximally preconstricted uterine arteries (UA) from pregnant (●) and nonpregnant (○) guinea pigs that had been housed under normoxic (*A*) or chronically hypoxic (*B*) conditions. Pregnancy increased UA flow vasodilation in the normoxic animals, but the vasodilator response to flow was not sustained in the vessels from chronically hypoxic animals, resulting in net vasoconstriction at high flow. $P$ values refer to comparisons between groups (see METHODS). *$P < 0.05$ for specified comparisons.

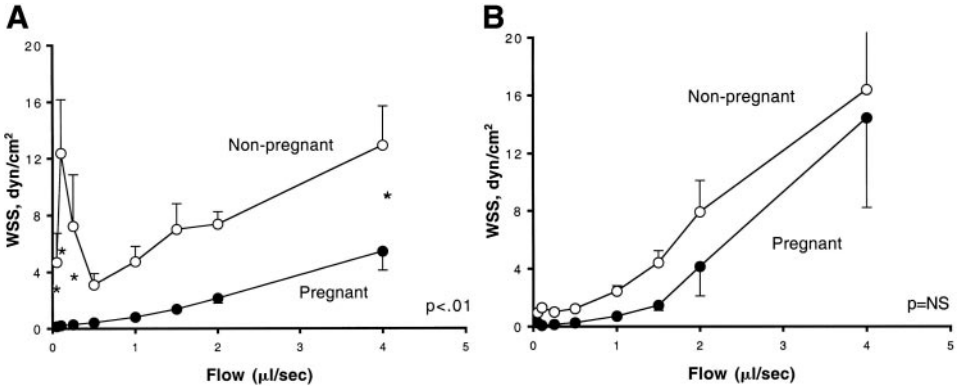


Fig. 2. Wall shear stress (WSS) was calculated as described in the methods for UA from normoxic or chronically hypoxic pregnant (●) or nonpregnant (○) guinea pigs. Pregnancy lowered WSS in response to flow in UA from normoxic (*A*) but not chronically hypoxic (*B*) animals. NS, not significant. *P* values refer to comparisons between groups (see METHODS). $^*P < 0.05$ for specified comparisons.

maximal change in vessel diameter in the normoxic nonpregnant and chronically hypoxic pregnant groups (Table 1). There was no further reduction in vasodilator response after the administration of meclofenamate (data not shown).

## DISCUSSION

Our results indicated that pregnancy increased the vasodilator response to flow and protected against a flow-induced rise in WSS in preconstricted UA from

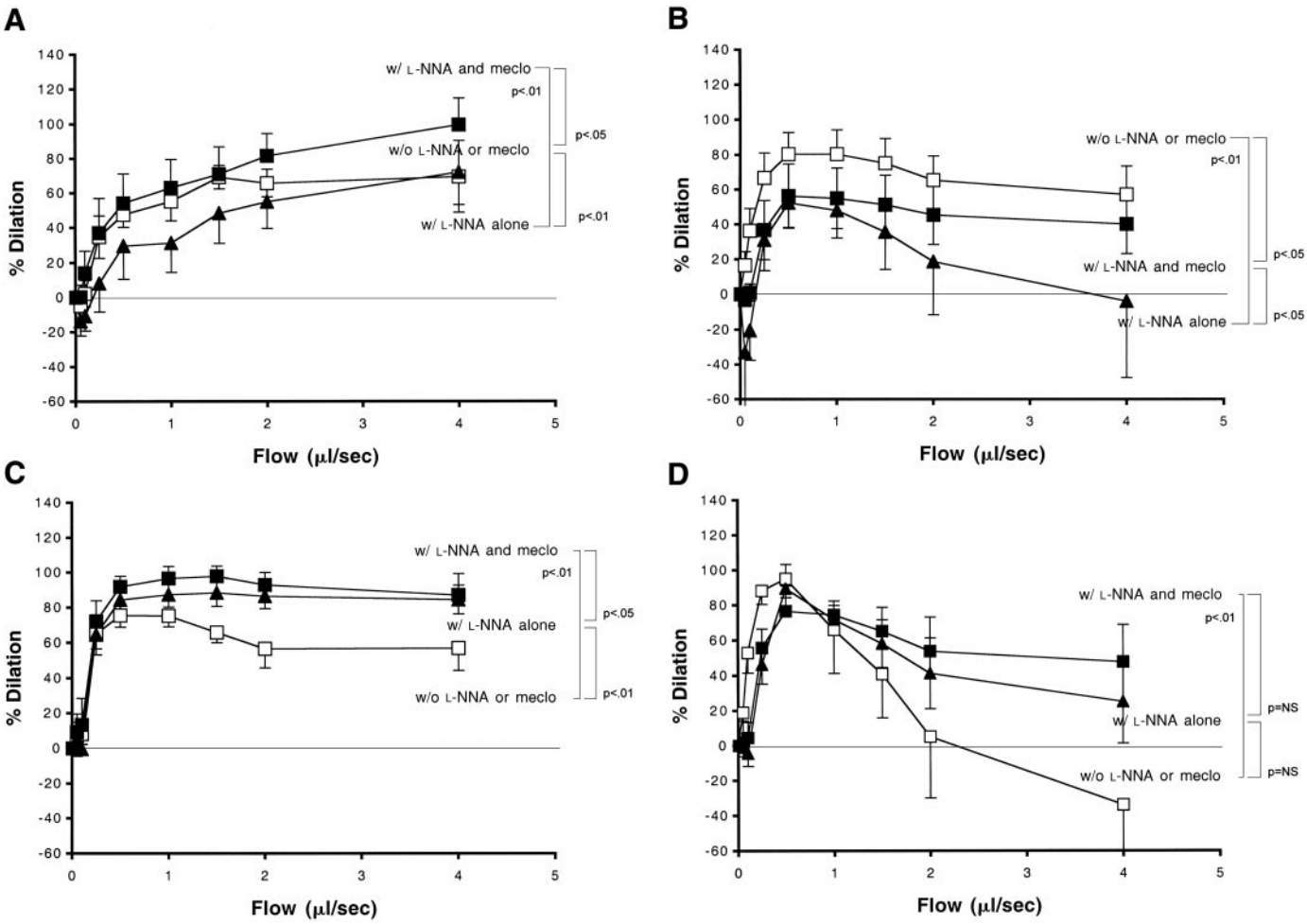


Fig. 3. The percent maximal vasodilation at each level of flow was measured in 50% maximally preconstricted UA before the administration of the nitric oxide synthase (NOS) inhibitor $N^G$-nitro-L-arginine (without L-NNA; □), after NOS inhibition (with L-NNA; ▲), or after NOS and cyclooxygenase inhibition [with L-NNA and meclofenamate (Meclo), ■]. L-NNA decreased the vasodilator response to flow in the UA from normoxic nonpregnant (*A*) and pregnant (*B*) animals but increased it in the chronically hypoxic nonpregnant (*C*) and pregnant (*D*) groups. $P < 0.01$ and $P < 0.05$, comparisons between designated groups.

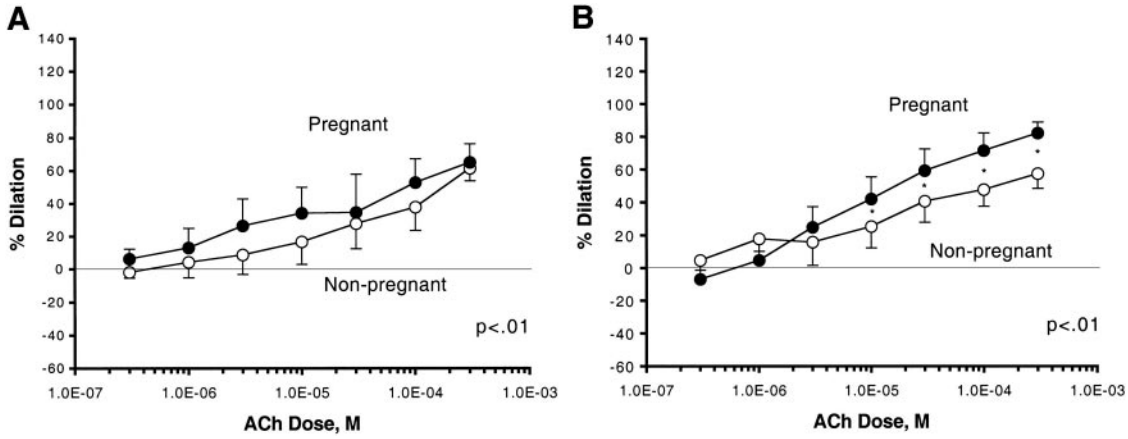


Fig. 4. The percent maximal vasodilation at each dosage of ACh was measured in 50% maximally preconstricted UA from pregnant (■) and nonpregnant (□) guinea pigs. Pregnancy increased the vasodilator response to ACh in UA from both the normoxic (*A*) and chronically hypoxic (*B*) animals. *P* values refer to comparisons between groups (see METHODS). $*P < 0.05$ for specified comparisons.

guinea pigs housed under normoxic conditions. Chronic hypoxia opposed these effects, prompting vasoconstriction at higher flows and increasing WSS above levels seen in UA from normoxic pregnant animals. The ability of NOS inhibition to eliminate differences between the nonpregnant and pregnant normoxic groups suggested that increased NO production and/or activity could account for the normal pregnancy-associated increase in vasodilator response to flow. However, in UA from chronically hypoxic animals, NOS inhibition increased the vasodilator response to flow, suggesting that some factor other than a hypoxia-associated decrease in NO production was responsible. The effects of chronic hypoxia were confined to the vasodilator response to flow as pregnancy increased vasodilator responsiveness to ACh equally in the normoxic and chronically hypoxic groups.

We considered the possibility that the effects of pregnancy and/or chronic hypoxia observed were artifacts of the methodology employed. This appeared unlikely because the same methods were used in all four groups and repetitive flow challenges alone did not alter the vasodilator responsiveness observed. We chose pressure myography because this permitted control of flow through the vessel lumen. The additional advantages of this preparation were that it better mimics physiological conditions and minimizes the possibility of endothelial damage, given that nothing is passed through the vessel lumen. As a further insurance against endothelial damage, we required that each vessel demonstrate vasodilation to ACh, a known endothelium-dependent vasodilator. The range of flows and WSS generated was similar to that which has been used in other perfused vessel preparations (2, 3, 19, 20, 22). While the maximal flow was lower than levels present in the guinea pig uterine artery in vivo, the WSS values were intermediate between luteal and late pregnancy levels reported for sheep (16). We chose to study animals at midpregnancy (30 days) because this is at the onset of the maximal rise in UA DNA synthesis and hence, we reasoned, when increased flow vasodilation could serve as an early growth stimulus (18, 32). Pregnant vessels had larger intraluminal diameters than nonpregnant vessels but because diameters under our study conditions were equally large in normoxic versus chronically hypoxic animals, the lack of vasodilator response to flow in the chronically hypoxic versus normoxic pregnant vessels was not due to differences in vessel dimensions.

As in previous studies (2, 19, 20), preconstriction was required to observe a vasodilator response to flow. Other studies have used norepinephrine whereas we used PE to preconstrict the vessel, but similar molar concentrations were used and both exert their vasoconstrictor effects through α-adrenergic-dependent mechanisms. Consistent with previous reports (41), pregnancy modestly reduced the UA vasoconstrictor response to PE. However, the differences were sufficiently small so as not to affect the amount of PE required to achieve 50% maximal preconstriction in pregnant versus the nonpregnant groups. Hence differences in absolute PE concentrations or amounts of relative preconstriction did not explain the effects of pregnancy and/or chronic hypoxia observed. In support of the likelihood that the concentrations of L-NNA and meclofenamate chosen were effective, levels were similar to those that have been shown previously to inhibit NOS or cyclooxygenase (37, 38). Furthermore, the amount of L-NNA administered inhibited the vasodilator response to ACh in all study groups. We therefore concluded that pregnancy increased the vasodilator response to flow and that chronic hypoxia opposed this effect.

Several important studies (2, 3, 19, 20, 22) have demonstrated that normal pregnancy increases the vasodilator response to flow in isolated near-term myometrial arteries and other vessels. The response to flow has not been previously studied at midpregnancy or in the UA but previous studies have shown that pregnancy increases the vasodilator response to ACh in isolated UA rings from near-term guinea pigs (39, 40) and the in situ perfused rat uterine vascular bed (4, 8). Our finding of a greater vasodilator response to flow in

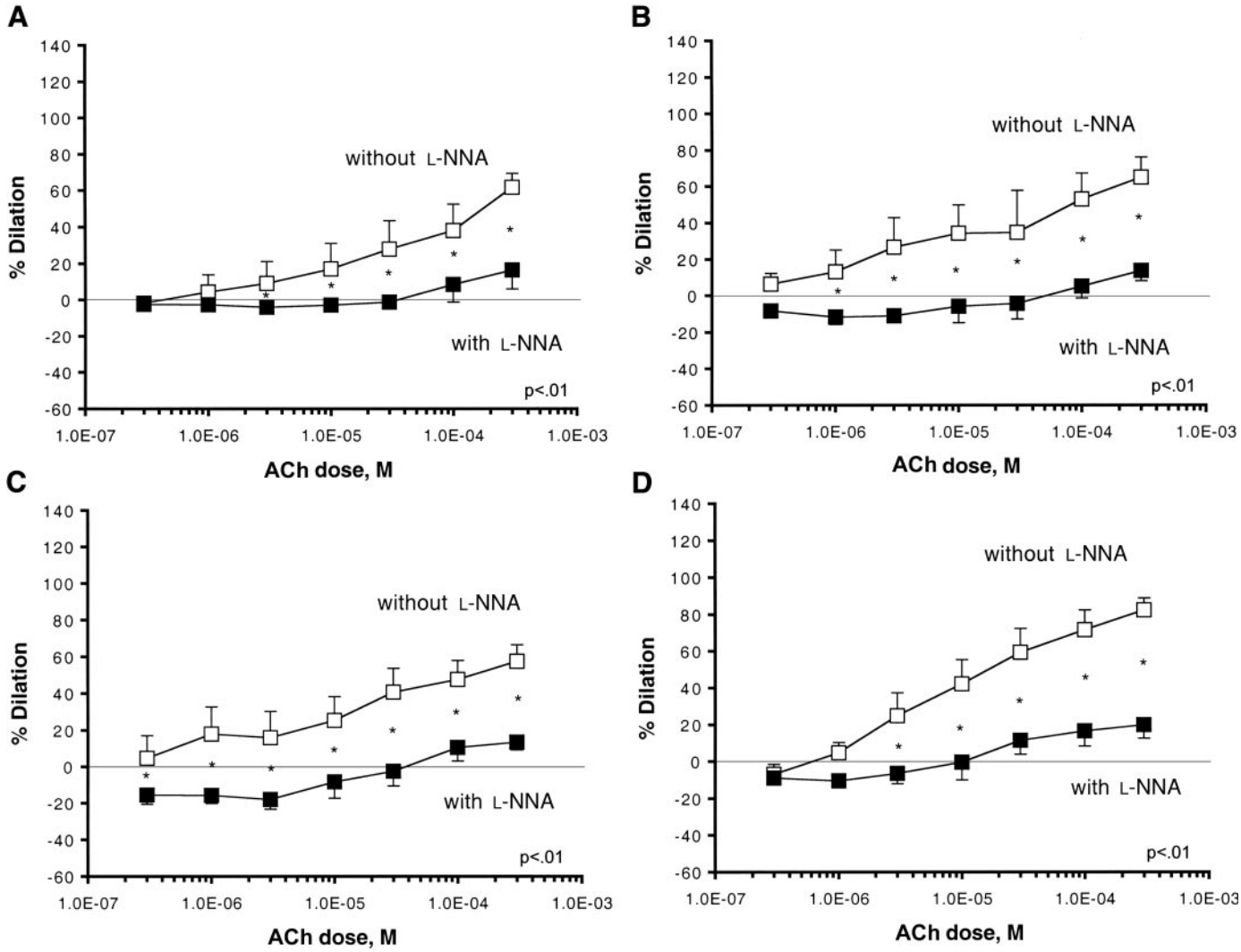


Fig. 5. Percent maximal vasodilation in response to ACh was measured before administration of the NOS inhibitor L-NNA (without L-NNA) or after NOS inhibition (with L-NNA) in preconstricted UA from pregnant (■) and nonpregnant (□) guinea pigs. *A*: normoxic nonpregnant; *B*: normoxic pregnant; *C*: chronically hypoxic nonpregnant; *D*: chronically hypoxic pregnant. L-NNA reduced the vasodilator response to ACh in each group with similar, complete levels of inhibition being achieved at ACh levels $\leq 10^{-5}$ M in all four groups. *P* values refer to comparisons between groups (see METHODS). $^{*}P < 0.05$ for specified comparisons.

the UA from normoxic animals was consistent with these findings and suggested that increased flow vasodilation was established by midpregnancy. One difference, however, was that we observed the greatest increase in flow vasodilation at low to moderate flow whereas previous studies found the greatest effect at high flow. Whether this was due to the period of pregnancy being studied or to differences between vessels and/or species is unknown. As in previous studies, we found that the increased vasodilator response to flow helped maintain lower WSS in the pregnant compared with the nonpregnant vessels (2, 3). This was evident at all flows but especially at low flows where the greater vasodilator response eliminated any rise in WSS. The lower WSS in the pregnant compared with the nonpregnant vessels likely protects against shear-induced damage to the endothelium and, hence, may contribute to the maintenance of endothelial vasodilator production during pregnancy.

At least three classes of endothelial-dependent vasodilators–NO, cyclooxygenase products, and EDHF–have been implicated as potential mediators of flow-induced vasodilation. The present study as well as previous studies (2, 3, 19, 20, 22) support the concept that increased NO production can account for the pregnancy-associated increase in flow vasodilation in uteroplacental as well as nonuteroplacental arteries from uncomplicated pregnancies. This is consistent with finding higher circulating nitrite-nitrate levels and increased amounts of eNOS protein in endothelial cell and whole vessel homogenates from normal pregnant compared with nonpregnant animals or humans (16, 27, 42). However, we found that considerable flow-induced vasodilation remained after NOS inhibition in the UA, whereas

Kublickiene and co-workers (19) found that it abolished flow-induced vasodilation altogether in myometrial vessels. Our results are consistent with others who found that NOS inhibition did not fully inhibit the vasodilator response to flow or the pregnancy-associated increase in ACh relaxation (1, 9). Our study and previous reports also indicate that cyclooxygenase products make little contribution to flow-induced vasodilation as judged by no further reduction in vasodilator response after cyclooxygenase inhibition (19). However, cyclooxygenase products contribute to ACh relaxation in the perfused rat uterine vascular bed (4). The third endothelial vasodilator, EDHF, is difficult to study in the absence of specific inhibitors and uncertainty as to the nature of this substance(s). One criterion for its involvement is the retention of vasodilator responsiveness after NOS and cyclooxygenase inhibition (8). Thus the considerable residual vasodilation after NOS and cyclooxygenase inhibition implicates EDHF as an important contributor to UA flow vasodilation in the guinea pig. EDHF has been shown to be important in flow-induced vasodilation in several vascular beds (34) as well as in the pregnant uterine circulation where low concentrations of KCl and $K^+$ channel inhibitors eliminated residual vasodilation in response to ACh (4, 8). We therefore concluded that both NO and EDHF likely contribute to the UA vasodilator response to flow, with increased NO production being responsible for the normal pregnancy-associated rise in flow vasodilation observed.

Both chronic hypoxia and preeclampsia interfere with the effect of normal pregnancy on enhancing the vasodilator response to flow, although their effects differ somewhat with preeclamptic myometrial vessels failing to show a vasodilator response throughout the flow range (2, 20), whereas chronic hypoxia prevents UA flow vasodilation only at higher flows. It is unknown whether such differences in flow sensitivity were due to the different periods of pregnancy being sampled, vessels, and/or species studied. Endothelial injury, likely due to toxic compounds and/or reactive oxygen species released from the uteroplacental circulation, is thought to play a central role in preeclampsia (reviewed in Ref. 36). An attractive hypothesis, therefore, to account for the lack of an enhanced vasodilator response to flow in pregnancies complicated by preeclampsia and/or chronic hypoxia is reduced endothelial vasodilator production. Consistent with this, NOS inhibition had no effect on flow responsiveness in myometrial arteries from preeclamptic women whereas it abolished flow vasodilation in vessels from normal pregnant women (20). Also consistent are our previous findings of less reduction in ACh relaxation after NOS inhibition in UA rings and lower eNOS protein levels in UA homogenates from chronically hypoxic versus normoxic guinea pigs (40, 42). These findings differ from those of Xiao and co-workers (43), who found a greater effect of NOS inhibition on A-23187-induced relaxation in isolated UA rings, higher basal and stimulated UA nitrite-nitrate release, and higher levels of eNOS protein in UA endothelial cell homogenates from pregnant sheep exposed to 3,820 m throughout pregnancy compared with their normoxic counterparts. It is unknown whether the slightly lower altitudes, protocol, or species differences are responsible; however, the level of hypoxia employed in the sheep studies was not sufficient to produce fetal growth restriction. However, under our study conditions, near-term fetal guinea pigs weighed less than their normoxic counterparts (M. M. White, personal communications). Also, unlike the findings in preeclamptic myometrial vessels, we found that NOS inhibition with L-NNA increased the UA vasodilator response to higher flows in the nonpregnant and pregnant vessels. The addition of meclofenamate further raised, not lowered, flow vasodilation, supporting the likelihood that decreased vasodilator prostaglandin production was not responsible for the lack of flow vasodilation observed (2, 19). We considered the possibility that decreased production of the third endothelial vasodilator EDHF was responsible for the reduced vasodilator response to flow in chronic hypoxia. Consistent with a role for EDHF are previous reports implicating EDHF as an important contributor to flow vasodilation (34) and the residual vasodilation after NOS and cyclooxygenase inhibition seen in the UA in the present studies. Furthermore, the rise in UA flow vasodilation after NOS inhibition may have been due to restoration of EDHF production, given the ability of NOS inhibition to prompt a compensatory increase in EDHF (17). Thus further studies are warranted of the role of EDHF in the control of flow-induced vasodilation in both normal and complicated pregnancies.

We also considered the possibility that rather than simply a lack of a pregnancy-associated increase in vasodilators, chronic hypoxia increased vasoconstrictor relative to vasodilator production. Chronic hypoxia raises the levels of the potent vasoconstrictor endothelin-1 (ET-1) and potentiates ET-1-induced vasoconstriction (reviewed in Ref. 33). A role for increased ET-1 in opposing flow-mediated vasodilation has been suggested in mesenteric arteries from spontaneously hypertensive rats and myometrial arteries from normal pregnant women (14, 20). While further studies are required, against the possibility that increased Et-1 production was responsible for the lack of flow vasodilation in the chronically hypoxic pregnant UA was the failure of NOS inhibition to further reduce the vasodilator response. This would have been expected because, in addition to its vasoconstrictor effects, ET-1 stimulates NO production via endothelial cell EtB receptors (12). The increase in flow vasodilation following NOS inhibition also suggests that chronic hypoxia could have increased the production of a NOS-inhibitible vasoconstrictor. Peroxynitrite is a potent vasoconstrictor formed by NO and the superoxide radical whose production is increased under conditions of chronic hypoxia and preeclampsia (5, 26). NOS inhibition lowers peroxynitrite levels (7) and thus could have prompted the rise in flow vasodilation observed. Finally, greater myogenic tone may have contributed to the decreased vasodilator response to flow in the UA from the chronically hypoxic pregnant animals. High

levels of tone have been shown to cause transient dilation, followed by constriction in response to flow in rabbit posterior cerebral arteries (35), and we have observed greater myogenic tone in UA from pregnant chronically hypoxic versus normoxic guinea pigs (24). Therefore, whereas the explanation for the reduced vasodilator response to flow in the UA from the chronically hypoxic pregnant animals compared with their normoxic counterparts remains unclear, existing data are consistent with the possibility that reduced EDHF levels, increased peroxynitrite formation, and/or increased myogenic tone, may have played important roles.

Overall, these studies demonstrate the presence of a brisk vasodilator response to flow in the UA at midpregnancy. In normal pregnancy, this serves to lower WSS and may therefore aid in preventing endothelial injury under the high-flow conditions present in the UA. We speculate that it may also serve as a feedforward mechanism for prompting the UA outward hypertrophic growth and remodeling required for increasing intraluminal diameter, thereby permitting the rise in UA blood flow that occurs later in pregnancy. The pregnancy-associated increase in UA vasodilator response to flow appears able to be accounted for by increased NO production. But other factors, most notably EDHF, also contribute to flow vasodilation in nonpregnant as well as pregnant UA. In pregnancies complicated by chronic hypoxia, the vasodilator response to flow is absent or reduced, perhaps as the result of reduced EDHF production, increased levels of peroxynitrites and/or myogenic tone. The resultant rise in WSS at a given flow could cause endothelial injury and thereby impair endothelial vasodilator and/or increase vasoconstrictor production. Thus chronic hypoxia may oppose the normal UA vascular adjustments to pregnancy that may, in turn, be among the mechanisms that serve to increase the incidence of preeclampsia as well as intrauterine growth restriction at high altitude (15, 30).

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