Nitric oxide-mediated vasodilation in human pregnancy

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During pregnancy, blood vessels dilate and cardiac output increases (27). These changes precede activation of the renin-angiotensin-aldosterone system and an expansion of blood volume (23). The mechanism for the primary reduction in total peripheral vascular resistance is unclear, but one possibility is that there is increased production of a vasodilator substance.

Peripheral arterial vasculature is maintained in a state of active vasodilatation by continuous synthesis of endothelium-derived nitric oxide (NO) from L-arginine (31). Inhibition of NO synthesis leads to vasoconstriction and a reduction in blood flow, whereas activation leads to further vasodilatation and an increase in blood flow (31). The response to \( N^\circ \)-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthase (NOS) (16), has been widely used as an index of the contribution made by NO to resting vascular tone (6). The constrictor action of this agent is mediated by loss of endothelium-derived NO, not to a direct constrictor action of the drug (25).

Studies in animals have shown a role for increased NO-mediated dilatation in the cardiovascular adaptation to pregnancy (9, 10) and increased expression of NOS in blood vessels of pregnant animals (33). Preliminary data from human studies during pregnancy indicate increased urinary concentrations of the second messenger for NO, guanosine 3',5'-cyclic monophosphate (cGMP), providing indirect evidence consistent with a gestational rise in NOS activity (17). However, attempts to correlate urinary NO breakdown products, nitrite and nitrate, with NO production during pregnancy have led to conflicting results (8, 18). Furthermore, no functional studies have been done during human pregnancy.

In this study, we used venous occlusion plethysmography to measure changes in hand blood flow during pregnancy. We studied hand blood flow, since it is representative of skin blood flow and, unlike the predominantly muscular forearm vascular bed, flow in the hand increases two- to sixfold by late pregnancy (1, 13). We compared the response to the NOS inhibitor L-NMMA on hand blood flow with the response to an endothelium-independent vasoconstrictor, norepinephrine.

**METHODS**

This study was approved by the Local Committee on Ethics. Three groups of volunteers were studied after giving informed consent: women in early pregnancy (9–15 wk gestation) following completion of arrangements for a therapeutic termination of pregnancy (n = 10); women in late pregnancy (36–41 wk) 24 h before an elective caesarean section or induction of labor for nonmedical reasons (n = 10); and healthy nonpregnant women (n = 10). Only healthy normotensive subjects who were taking no medication were recruited.

**Venous occlusion plethysmography.** Subjects lay semirecumbent, and blood flow (in ml·100 ml hand \(^{-1}\)·min \(^{-1}\)) was measured throughout the study in both hands simultaneously, using water-filled plethysmographs placed above the level of the heart (4). Wrist cuffs were inflated above venous pressure (40 mmHg) for 10 s in each 20-s cycle. Ambient temperature was kept constant during each study [23.2 ± 1.9°C (SD)]. The temperature of the water in the plethysmographs was kept constant within and between experiments (31.6 ± 0.38°C). Blood pressure was measured in all subjects before the study and in some subjects from each group during infusion of the maximum doses of norepinephrine and L-NMMA.

Drugs or physiological saline was infused continuously (0.5 ml/min) through a 27-gauge needle inserted into the brachial artery of the nondominant arm (31). One percent lidocaine solution was used to anesthetize the skin before insertion of the needle. After we established resting control values of blood flow for 15 min during an infusion of saline, four doses of norepinephrine were infused to produce a dose-response curve (60, 120, 240, and 480 pmol/min; each dose for 5 min). After a further 15 min of saline infusion and when blood flows had returned to baseline, four doses of L-NMMA were infused to produce a second dose-response curve (1, 2, 4, and 8...
RESULTS

The characteristics of each group of women are shown in Table 1. There were no changes in uterine activity or fetal heart rate during the studies.

**Basal blood flow**: In all groups, blood flow in the noninfused hand remained stable throughout each experiment. Basal blood flows in nonpregnant women 9.4 ± 2.3 (SE) ml·100 ml hand⁻¹·min⁻¹ were similar to flows in early pregnancy 7.7 ± 2.1 ml·100 ml hand⁻¹·min⁻¹. However, basal blood flows were significantly higher in late pregnancy 20.6 ± 3.5 ml·100 ml hand⁻¹·min⁻¹ compared with the other two groups (P = 0.007; Fig. 1).

**Response to L-NMMA and norepinephrine**: In all three groups of subjects, both L-NMMA and norepinephrine produced a dose-dependent reduction in hand flow ratio (infused arm to control arm) (Fig. 2). Women in both pregnant groups had an increased response to L-NMMA compared with nonpregnant women (P = 0.0003; Fig. 2A). In contrast, the response to norepinephrine in late pregnancy was blunted when compared with nonpregnant and early pregnant subjects (P = 0.0029; Fig. 2B). Comparison between drugs within each group demonstrates the following: 1) women in late pregnancy had a greater response to L-NMMA than to norepinephrine (P = 0.0002; Fig. 3A); 2) women in early pregnancy had almost identical responses to both norepinephrine and L-NMMA (P = 0.99; Fig. 3B); and 3) although nonpregnant women appeared to have a greater reduction in hand blood flow with norepinephrine compared with L-NMMA, this did not reach statistical significance (P = 0.16; Fig. 3C).

When the AUC for norepinephrine was subtracted from the AUC for L-NMMA for each individual. The average (mean) difference between drug responses for each group was compared between groups using a series of t-tests.

**DISCUSSION**

The results of this study indicate that inhibition of NOS with L-NMMA reduces hand blood flow more during pregnancy than in the nongravid state. This was apparent even though basal blood flow was higher in late pregnancy, and therefore, the concentration of L-NMMA reaching the resistance vessels would have been lower. Indeed, in contrast to the responses seen in the nonpregnant and early pregnant groups, the response to the highest dose of L-NMMA (8 μmol/min) in late pregnancy was not at the top of the dose-response
late pregnant women did not reach a maximal reduction in hand blood flow after 8 μmol/min L-NMMA, it is possible that an even higher dose would have reduced blood flow even further, to levels found in nonpregnant
women post-L-NMMA (i.e., 5.3 ml·100 ml⁻¹·min⁻¹). Under these circumstances, increased NO-mediated vasodilatation would be solely responsible for the gestational increase in hand blood flow. However, we were unwilling to pursue this point by using higher doses of L-NMMA in this group of patients.

When the response to L-NMMA is compared with the response to norepinephrine within each group (a situation in which both drugs would be exposed to the same conditions of basal blood flow and pressure), the L-NMMA response increased relative to that of norepinephrine as pregnancy progressed. This is particularly important, since venous occlusion plethysmography is at its most powerful when used to compare relative potencies to different drugs given sequentially in the same experiment (26). Together these findings indicate an enhanced response to L-NMMA in pregnancy and suggest that basal nitric oxide-mediated dilatation is increased. Our findings support animal studies that suggest increased NO activity mediates vascular adaptation in pregnancy (9, 10). The response to L-NMMA was enhanced in both early and late pregnancy, suggesting that increased basal NO-mediated dilatation in the skin occurs early in pregnancy, at a time when cardiovascular changes are starting to occur. The augmented response to L-NMMA in early pregnancy is particularly striking, since 9 of 10 subjects in this group were smokers, a habit normally associated with endothelial cell damage and a reduced response to L-NMMA (15).

The response to norepinephrine in late pregnancy was reduced compared with that recorded in early pregnancy and nonpregnant controls. Although a blunted response to direct-acting vasoconstrictors has not been a universal finding in studies during human pregnancy (19, 24), similar results have been reported in studies on animals in vivo and in vitro (9, 21). The pattern of hyporesponsiveness to constrictor agents with exaggerated vasoconstriction to L-NMMA or other NOS inhibitors is similar to that reported in sepsis (22) and other vasodilated hypotensive states associated with enhanced generation of NO (2). It remains to be determined whether the enhanced generation of NO is responsible for a generalized hyporesponsiveness to vasoconstrictors in pregnancy.

Our study has explored the functional effects of NO in maternal vasculature. Increased NOS activity has been found in platelets from healthy pregnant compared with nonpregnant and preeclamptic women (11). Indirect biochemical assays also support the finding of a gestational increase in NO activity. For instance, concentrations of cGMP, the second messenger for NO, are increased in plasma and urine from pregnant animals (10) and humans (17), and a stable oxidation product of NO, nitrate, is found in elevated concentrations in the urine and plasma of pregnant rats (10) and possibly in humans (18). However, interpretation of such measures is not straightforward, since cGMP can be elevated by atrial natriuretic peptide and nitrate present in the diet (3, 8).

There are several mechanisms by which NO-mediated vasodilatation could be activated in pregnancy. Shear stress increases NO release (29), and it is possible that the elevated blood flow in the hand itself stimulates NO activity. However, because we found an increased response to L-NMMA in early pregnancy, before hand blood flow increased, this seems an unlikely explanation of our findings. Indeed, we might have underestimated the typical response to L-NMMA in early pregnancy, since a large number of this group were smokers. Further studies would be required to address this issue. Volume expansion or the increase in hand temperature during pregnancy could enhance NO generation (5, 34). Estrogens upregulate NOS in animals (32), and therefore, the huge rise in circulating estradiol concentration during early pregnancy (30) could stimulate increased NO synthesis. Consistent with this suggestion, postmenopausal women given transdermal 17β-estradiol show enhanced serum levels of nitrite and nitrate (28). Furthermore, hand vasculature contains multiple arteriovenous anastomoses (14). Such vascular networks, which occasionally exist as arteriovenous malformations, enlarge during pregnancy and regress postpartum (12).

This is the first study to examine the effect of L-NMMA in human pregnancy, and we examined for systemic effects in mother and fetus. After local infusion of both L-NMMA or norepinephrine, we found the following: 1) there was no change in uterine activity or fetal heart rate in the late pregnant group, and all babies were born healthy; 2) there was no significant overall change in basal blood flow in the noninfused hand throughout the study in all groups; and 3) in those three or four subjects from each group in whom blood pressure was measured at the end of the study, there was no change in blood pressure. These observations confirm previous studies (7, 31) indicating that local infusion of L-NMMA in these doses into the brachial artery produces a local effect only and is a safe investigatory procedure to explore basal NO activity.

In conclusion, this study demonstrates that norepinephrine (an adrenoreceptor agonist) and L-NMMA (a competitive inhibitor of NOS) produce dose-dependent falls in hand blood flow in pregnant and nonpregnant women. These results suggest that catecholamines and endogenous NOS activity are able to alter skin blood flow in healthy pregnant and nonpregnant women. Furthermore, we have demonstrated that the vascular response to blocking NOS increases in pregnancy, the degree of inhibition appearing to increase further toward term. These results suggest that there is enhanced NO-mediated vasodilatation, at least in the skin of the hand. The observation that L-NMMA returned basal blood flow back to normal in late pregnancy is consistent with the proposal that NO might mediate the gestational increase in blood flow and contribute to the hyporesponsiveness to vasoconstrictors that occurs in pregnancy. It remains to be determined whether the changes we have seen also occur in other vascular beds and account for the widespread cardiovascular changes of normal pregnancy. It would also be important to determine whether failure to increase NO
generation contributes to the development of pregnancy-induced hypertension or preeclampsia.

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