Normal pregnancy is associated with enhanced endothelium-dependent flow-mediated vasodilation

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Normal pregnancy is associated with enhanced endothelium-dependent flow-mediated vasodilation. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H821–H825, 1999.—Normal pregnancy is characterized by reduced systemic vascular resistance, which may be mediated by nitric oxide (NO). We compared endothelial vasomotor function in 71 normal pregnant women (13 in first, 29 in middle, and 29 in last trimester) to 37 healthy age-matched controls. With external ultrasound, brachial artery diameter was measured at rest, during reactive hyperemia [with increased flow causing endothelium-dependent dilation (FMD)], and after sublingual nitroglycerin (causing endothelium-independent dilation). Compared with controls, resting flow and brachial artery diameter were significantly higher during the middle and last trimesters. Reactive hyperemia was reduced in all pregnant groups. FMD increased from the first trimester (by 26%), reaching the highest value in the last trimester (to 47% above nonpregnant values). FMD was significantly correlated to pregnancy status (nonpregnant or pregnant) and to vessel size. Nitroglycerin-induced dilation was similar in pregnant and nonpregnant women. A longitudinal study of eight women evaluated in the first, middle, and last trimesters confirmed an increase in FMD throughout pregnancy. The study supports the idea that basal and stimulated NO activity is enhanced in normal pregnancy and may contribute to the decrease in peripheral resistance.

PREGNANCY IS ASSOCIATED with profound hemodynamic changes. Thus systemic vascular resistance remains lower throughout pregnancy and arterial blood pressure shows a progressive fall in the first and middle trimesters, whereas cardiac output and heart rate gradually increase to a plateau by the end of the second trimester (21, 23). The mechanisms responsible for the pregnancy-associated vasodilatation are not yet fully understood, but recent data suggest endothelium-derived relaxing factor or nitric oxide (NO) as a prime mediator for the fall in vascular resistance (7, 20, 32). In animal models, NO synthesis is increased in pregnancy (1, 8, 9, 19, 26). In humans, however, the role of NO as a mediator of the reduction in systemic vascular resistance remains controversial (26). In vitro, small subcutaneous arteries from pregnant women show increased relaxation in response to shear stress, a known stimulus for NO release (7). In vivo, Nω-nomonomethyl-L-arginine (L-NMMA), an NO synthase inhibitor, produces greater reduction in hand blood flow in pregnant than in nonpregnant women, suggesting a higher NO activity during pregnancy (32).

In preeclampsia, blood pressure is elevated, systemic vascular resistance is increased, and the response to vasoconstrictors is maintained and not attenuated as otherwise seen in normal pregnancy (20). It has therefore been hypothesized that endothelial dysfunction including failure to increase NO production is a key event in preeclampsia (7, 20). In an animal study, NO synthase inhibition induced a preeclampsia-like syndrome with hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation (19). Likewise, isolated small arteries from preeclamptic women showed attenuated relaxation to shear stress, supporting the hypothesis that endothelial dysfunction and a functional deficiency of NO are crucial in this syndrome (7).

Various methods have been used to study endothelial function in the normal or pathological human pregnancy. Most studies, however, have been restricted to in vitro experiments on isolated endothelial cells or vessels from maternal or fetal tissues.

In vivo assessment of endothelial vasomotor function has been performed for more than a decade (18). With the use of vasoactive substances such as acetylcholine and nitroglycerin (NTG), vascular responses have been studied extensively in the coronary and in the forearm circulation (3, 18). Because of the invasive nature of these tests, they are not suitable for the study of pregnant women. A strictly noninvasive technique for assessment of endothelial function was described in 1992 (6). With this technique, endothelium-dependent and-independent vascular reactivity can be studied accurately and reproducibly in systemic arteries using high-resolution ultrasound (27). We have used this approach to study vascular physiology in the first, middle, and last trimesters of normal pregnant women.

METHODS

Subjects

Cross-sectional study. One hundred eight women (71 pregnant and 37 nonpregnant controls) were studied. All were healthy, normotensive lifelong nonsmokers without a family history of premature vascular disease. None was taking any regular medications, and none of the controls received oral contraception.

The pregnant women were recruited either from hospital staff or from midwife-based maternal care clinics. The mean age was 30.4 ± 4.0 yr (mean ± SD; range 24–44 yr) (Table 1). Thirteen women were studied in the first trimester (gestational weeks 9–14, median 12 wk), 29 in the middle trimester (gestational weeks 22–27, median 24 wk), and 29 during the
last trimester (gestational weeks 31–38, median 35 wk). All pregnancies were uncomplicated, i.e., not accompanied by hypertension, preeclampsia, or intrauterine growth retardation. Thirty-seven nonpregnant women aged 29.7 ± 4.7 yr (range 19–41 yr) were recruited among hospital staff.

Longitudinal study. The longitudinal study comprised a total of 29 vascular scans obtained in eight women studied on several occasions during the course of their pregnancy. Five of these women also had a vascular scan within the last 1–5 mo (median 1.5 mo) before pregnancy.

Ethics

All subjects gave written informed consent, and the study was approved by the local Ethical Committee.

Vascular Study

Endothelial vasomotor function was assessed as described by Celermajer et al. (6). Using 7.0-MHz ultrasound imaging (Acuson 128 XP 10, Acuson, Mountain View, CA), we measured changes in the brachial artery diameter in response to reactive hyperemia (an endothelium-dependent stimulus) and compared it with the vasodilatory response to NTG (an endothelium-independent stimulus).

The subject rested at least 10 min before a baseline scan was acquired. The brachial artery was identified above the elbow and scanned in longitudinal section. Scans were taken at rest, during reactive hyperemia, again at rest, and after NTG. Flow increase in the artery was achieved by placing a blood pressure cuff around the forearm distal to the brachial artery and inflating to 250–300 mmHg, followed by release of the cuff after 4 min. The artery was scanned continuously from 30 s before to 90 s after cuff deflation. The vessel then recovered for 10 min, after which a second resting scan was recorded. Finally, 400 µg NTG were administered sublingually and the artery was rescanned 3 min later. Of the 71 pregnant women, 19 (27%) declined the NTG challenge (4 in first, 8 in middle, and 7 in last trimester).

Images were recorded on videotape, and a minimum of four cardiac cycles from each scan sequence was subsequently analyzed by two observers blinded to the identity of the subject and the sequence of the scan protocol. Vessel diameters were measured with ultrasonic calipers, from the anterior to the posterior interface between media and adventitia (the "m line") at a fixed distance from an anatomic marker such as a vein or a fascial plane. Measurements were made at end diastole, incident with the R wave on the simultaneously recorded electrocardiogram. The mean values obtained by the two observers were used for analysis. Flow-mediated dilation (FMD) and NTG-induced dilation were derived relative to the baseline scan (100%).

Baseline blood flow and the peak flow increase induced by transient forearm cuff occlusion were estimated from the pulsed Doppler recordings of the resting and the immediate postocclusive brachial artery flow velocities. These were obtained at a scan angle deviating 70° from the flow direction. Flow (ml/min) was estimated using the following equation

\[ \text{Flow} = \text{VTI} \times \text{HR} \times (0.5 \times d) \times \pi \times (1/\cos 70°) \]

where VTI is the velocity time index, i.e., the area under the flow velocity curve, HR is heart rate, d is vessel diameter, and 1/\cos 70° is the angle correction.

Statistics

Descriptive data are expressed as means ± SD or SE. The significance of difference was assessed by two-tailed t-test for groups of nonpaired or paired observations and by one-way analysis of variance when more than two groups were compared.

Univariate analysis and stepwise linear multiple regression analysis were performed to assess the possible determinants of FMD (the dependent variable). Independent variables included age, vessel size, and pregnancy status (nonpregnant or pregnant).

RESULTS

Compared with that in nonpregnant controls, heart rate was significantly increased from the first trimester and throughout pregnancy. Systolic and diastolic blood pressures were lower in the first and middle trimesters but returned to nonpregnant values in the last trimester (Table 1).

Cross-Sectional Study

The brachial artery diameter was larger in the middle and last trimesters although not significantly larger in the last trimester (P = 0.08, Table 2). If a circular vessel shape is assumed, this corresponds to a 12% increase in cross-sectional vessel area. Baseline flow was not significantly different from controls in the first trimester but increased by 56 and 83% during the middle and last trimesters (both P < 0.001), respectively. The degree of reactive hyperemia decreased during the course of the pregnancy, reaching a nadir in the last trimester, when it was reduced by 41% (from 563 to 332%, P < 0.001; Table 2).

Table 1. Clinical characteristics of 108 women according to pregnancy status

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant Controls (n = 37)</th>
<th>First Trimester (n = 13)</th>
<th>Middle Trimester (n = 29)</th>
<th>Last Trimester (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>29.7 ± 4.7</td>
<td>30.0 ± 3.7</td>
<td>30.6 ± 3.3</td>
<td>30.3 ± 4.8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>66 ± 11</td>
<td>75 ± 13†</td>
<td>79 ± 11‡</td>
<td>79 ± 13‡</td>
</tr>
<tr>
<td>BP systolic, mmHg</td>
<td>121 ± 11</td>
<td>111 ± 7†</td>
<td>115 ± 8*</td>
<td>121 ± 12</td>
</tr>
<tr>
<td>BP diastolic, mmHg</td>
<td>72 ± 6</td>
<td>66 ± 5†</td>
<td>68 ± 7*</td>
<td>74 ± 9</td>
</tr>
</tbody>
</table>

Data are means ± SD. BP, blood pressure. *P < 0.02, †P < 0.005, ‡P < 0.001, pregnant group vs. nonpregnant controls.

Table 2. Vascular data in 108 women according to pregnancy status

<table>
<thead>
<tr>
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<th>Last Trimester (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel size, mm</td>
<td>3.17 ± 0.28</td>
<td>3.08 ± 0.34</td>
<td>3.34 ± 0.28*</td>
<td>3.35 ± 0.47</td>
</tr>
<tr>
<td>Baseline flow, ml/min</td>
<td>118 ± 71</td>
<td>125 ± 52</td>
<td>188 ± 68‡</td>
<td>219 ± 136‡</td>
</tr>
<tr>
<td>Reactive hyperemia, %</td>
<td>580 ± 28</td>
<td>446 ± 138*</td>
<td>365 ± 117‡</td>
<td>332 ± 156‡</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.2 ± 2.8</td>
<td>9.1 ± 4.0‡</td>
<td>9.1 ± 3.7*</td>
<td>10.6 ± 4.4‡</td>
</tr>
</tbody>
</table>

Data are means ± SD. Reactive hyperemia, peak blood flow after cuff release as% of resting flow; FMD, flow-mediated dilation. *P < 0.05, †P < 0.02, ‡P < 0.001, pregnant group vs. nonpregnant controls.

Table 3. Clinical characteristics of 108 women according to pregnancy status

<table>
<thead>
<tr>
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Data are means ± SD. BP, blood pressure. *P < 0.02, †P < 0.005, ‡P < 0.001, pregnant group vs. nonpregnant controls.
FMD was markedly increased in all three pregnancy groups (Fig. 1A, Table 2), reaching the highest value in the last trimester. FMD was 26% higher in the first two trimesters and 47% higher in the last trimester than in the controls (P < 0.001). On multivariate regression analysis, FMD was significantly related to pregnancy status (nonpregnant or pregnant; r = 0.38, P < 0.0001) and inversely related to vessel size (r = −0.35, P = 0.0001). As shown in Fig. 1B, NTG-induced dilation was not significantly different from nonpregnant controls for any of the pregnancy groups.

**Longitudinal Study**

In the eight women who were studied prospectively during pregnancy, the brachial artery size tended to be larger in the second and third trimesters, with a significant difference between vessel size in the first and last trimesters (3.1 ± 0.4 vs. 3.47 ± 0.4 mm, P = 0.02). Baseline flow showed a graded increase during the course of pregnancy, and among the pregnant women, a significant correlation between pregnancy duration (weeks) and flow could be established (r = 0.30, P < 0.01; results not shown).

Figure 2 shows the time course of FMD in the longitudinal study. The pre preganant FMD for the women who had a vascular scan before pregnancy was similar to the values in the nonpregnant control group in the cross-sectional study (7.4 ± 3.5 vs. 7.2 ± 2.8%, P > 0.5). As in the cross-sectional study, there was a tendency toward a gradual increase in FMD during pregnancy, which in the first, middle, and last trimesters amounted to 9.9 ± 5.0, 11.1 ± 1.9, and 12.4 ± 5.2%, respectively. Furthermore, there was a significant difference between FMD measured before pregnancy compared with that in the last trimester (P = 0.025, n = 5).

**DISCUSSION**

During normal pregnancy significant vascular and hemodynamic adjustments occur. These include a fall in peripheral vascular resistance, a reduction in blood pressure, an increased cardiac output, and subsequently increased flow in the systemic and pulmonary circulations (23).

Vascular tone is determined by the instantaneous balance between numerous vasoactive substances of which NO plays a key vasodilatory role (28). Animal and human studies support the hypothesis that increased NO activity plays a major role for the pregnancy-associated drop in systemic resistance (1, 8, 19, 20, 32). The gestational reduction in blood pressure seen in spontaneously hypertensive rats has been shown to depend on the L-arginine-NO pathway (1), whereas chronic inhibition of NO synthesis induces sustained hypertension in pregnant rats (19). The increased plasma and urinary concentrations of cGMP, the second messenger of NO, observed in pregnant humans and animals also suggest an important vasoregulatory role.
of NO during normal pregnancy (8, 9, 15). Finally, Williams et al. (32) used venous occlusion plethysmography to study vascular physiology in human pregnancy. Basal hand blood flow was higher during pregnancy, and L-NMMA caused a significantly greater reduction in hand blood flow in pregnant versus nonpregnant women. These studies all suggest that normal pregnancy represents a state of increased vascular NO activity.

Our data, from a strictly noninvasive technique in intact humans, confirmed that resting blood flow in large systemic arteries is higher in the late phases of normal pregnancy, undoubtedly secondary to a reduction in vascular resistance downstream to the target artery. Because resting flow is a major stimulus for endothelial NO release, the observation that resting brachial artery diameter is significantly larger during the second and third trimesters may indicate that the vessel is continuously stimulated by increased shear stress.

FMD is a marker of stimulated endothelial vasomotor function with attenuated dilatory responses seen in subjects with early vascular damage or risk factors for atherosclerosis (5, 6). It depends highly on resting vessel size, with large arteries responding less than small arteries (5, 6). Despite the larger resting vessel diameter we found FMD markedly enhanced through all phases of normal pregnancy, with the highest dilation in the last trimester. This was evident despite the fact that the degree of reactive hyperemia, i.e., the stimulus for FMD, was significantly lower (up to 41%) during all gestational periods.

The reduced degree of reactive hyperemia observed during pregnancy may suggest either that the resistance vessels are less reactive in pregnancy or, perhaps more likely, that the microvasculature is already pre-relaxed, as suggested by the increased basal flow. Our flow estimates should, however, be interpreted with caution, because the values were derived with the assumption not only that the brachial artery is a strictly circular structure throughout the cardiac cycle but also that the flow profile in this vessel is flat and that the scan angle is constant (70°).

In contrast to the increase in FMD observed in pregnancy, the response to NTG was not different from nonpregnant controls. Although the basal NO availability and the flow-stimulated NO release may be increased in pregnancy, the normal response to NTG is preserved.

The noninvasive methodology used to assess vascular physiology and endothelial vasomotor function has been widely validated (4, 10, 27). Although the measured differences in vessel diameter are small, the technique has proven accurate and reproducible (27). Furthermore, FMD of the brachial artery is considered to reflect systemic endothelial vasomotor function, because a close correlation between endothelial vasomotor reactivity in the brachial and coronary arteries has been demonstrated (2). Finally, FMD has been shown to rely on the ability of the intact endothelium to release NO, because dilation to reactive hyperemia can be blocked by L-NMMA (13, 16).

The demonstration of increased brachial artery FMD in pregnancy therefore supports the hypothesis that basal and stimulated NO activity is enhanced during normal pregnancy and that this may contribute to the fall in the peripheral vascular resistance. This is in accordance with the recent observation that isolated, small subcutaneous arteries from pregnant women showed greater flow-associated relaxation than those from nonpregnant women (7). This vasodilatory response was largely mediated by NO, because it could be blocked by the NO synthase inhibitor Nω-nitro-L-arginine.

Pregnancy-associated activation of the L-arginine-NO pathway could be mediated through several mechanisms. Cardiac output is increased very early in pregnancy as a result of increased heart rate and increased stroke volume (21, 23). Increased shear stress secondary to augmented flow in both systemic and resistance arteries may increase NO release, resulting in enlarged basal vessel size.

Like others, however, we could not detect increased flow (32) or increased brachial artery size in early pregnancy. This does not exclude that this mechanism is responsible, because only one particular vascular bed was studied, in which an activation may not be detectable very early.

Increased plasma volume may also cause NO-dependent vasodilation (3). In pregnancy, however, the reduction in peripheral vascular resistance and the increase in cardiac output seem to precede blood volume expansion (21).

Circulating estrogens increase early and progressively during pregnancy and may stimulate vascular function directly or indirectly by various means including increased NO availability. In vitro, estrogen exerts an acute vasodilatory effect by relaxing vascular smooth muscle directly, possibly by blocking cell membrane voltage-dependent Ca2+ channels (11).

In animals, estrogens have been shown to upregulate NO synthases (30, 31), and in oophorectomized ewes, estrogen-induced uterine vasodilation could be antagonized by an NO synthase inhibitor (29).

During the normal human menstrual cycle, FMD increases significantly during the follicular and luteal phases, when serum estradiol levels are high (12). In postmenopausal women, estrogen elicits both acute and chronic vascular effects, which are largely endothelium dependent (17, 22). Infusion of estrogen acutely improved endothelium-dependent vasodilation of the coronary arteries (22), and with the use of external ultrasound 9-wk estrogen replacement therapy was shown to improve FMD in the brachial artery (17). The finding that circulating nitrite/nitrate levels are increased in postmenopausal women substituted with estradiol further supports the hypothesis that estrogens increase NO synthesis (24).

Finally, the antioxidant property of estrogen may increase NO availability. In a cholesterol-fed animal model, estrogen therapy was associated with enhanced endothelium-dependent dilation (14). Furthermore, this effect correlated with diminished in vitro oxidation of low-density lipoprotein (LDL) derived from the animals.
In conclusion, ultrasound-based noninvasive assessment of endothelial function is feasible, and using this technique we have demonstrated enhanced endothelial function in normal pregnancy. Because endothelial dysfunction is believed to be a key early event in pathological pregnancies such as pregnancy-induced hypertension and preeclampsia, this technique may be a valuable tool in the future evaluation of vascular physiology in such pregnancies.

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