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Non-invasive assessment of endothelial function in normal pregnancy

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ABSTRACT

Objective *To assess endothelial function in normal pregnancy by non-invasive methods.*

Methods *Flow-mediated dilatation of the brachial artery was measured by ultrasonography in 157 women with normal singleton pregnancies between 10 and 40 weeks' gestation and 19 non-pregnant controls.*

Results *Flow-mediated dilatation in the non-pregnant controls was 6.42 ± 2.45%. In pregnant women, between 10 and 30 weeks, the mean flow-mediated dilatation (8.84 ± 3.18%) was significantly higher than the non-pregnant controls ($P = 0.002$), but after 30 weeks of gestation there was a decrease to prepregnancy levels. Resting vessel diameter and blood flow were significantly increased in pregnancy, mainly after 30 weeks' gestation ($P < 0.001$, $P < 0.001$, respectively). Flow-mediated dilatation was significantly correlated to resting vessel diameter and reactive hyperemia.*

Conclusion *Normal pregnancy is associated with enhanced endothelial function which is apparent from at least 10 weeks' gestation.*

INTRODUCTION

Normal pregnancy is associated with an increase in blood volume and cardiac output, of approximately 40%, and a concurrent reduction in arterial blood pressure[1,2]. This decrease in blood pressure may be the consequence of a fall in peripheral vascular resistance, possibly due to increased synthesis of the endothelium-derived relaxing factor, nitric oxide[3].

There is evidence that endothelial dysfunction is involved in the pathophysiology of several conditions in pregnancy such as pre-eclampsia[4,5]. An important functional consequence of endothelial dysfunction is the inability to release nitric oxide[6]. Measuring the changes of the brachial artery diameter as a response to increased flow (flow-mediated dilatation) using high resolution ultrasound is an established non-invasive method for the assessment of endothelial function[7] and has been shown to depend mainly the nitric oxide release[8,9]. In this study we used the above technique to investigate endothelial function in normal pregnancy.

MATERIALS AND METHODS

This was a cross-sectional study involving 157 women with singleton pregnancies between 10 and 40 weeks of gestation and 19 non-pregnant controls. The subjects were recruited from the routine antenatal clinic and hospital staff, respectively. They were all healthy, non-smokers, on no medication and had no family history of premature heart disease. None of the controls was taking hormonal contraception and none of the pregnancies had any complications. The multigravidae had no history of pre-eclampsia or intra-uterine growth restriction in their previous pregnancies. The study was approved by the local ethics committee and all subjects gave written informed consent.

Brachial artery ultrasound scans were performed as described by Celemajer *et al.*[7] in a quiet and temperature-controlled (22° to 26 °C) room. All examinations were conducted by the same investigator. The diameter of the brachial artery was measured from high-resolution, B-mode images obtained with a 7 MHz linear array transducer and an Aspen Acuson system (California, USA). The subjects rested for 10 min before the ultrasound examination. The right brachial artery was scanned over a longitudinal section 2–15 cm above the elbow where the clearest image was obtained. The transmit focus zone was set to the depth of the proximal wall. The depth and gain controls were set to optimize visualization of the lumen-to-arterial wall interfaces. Images were magnified by the

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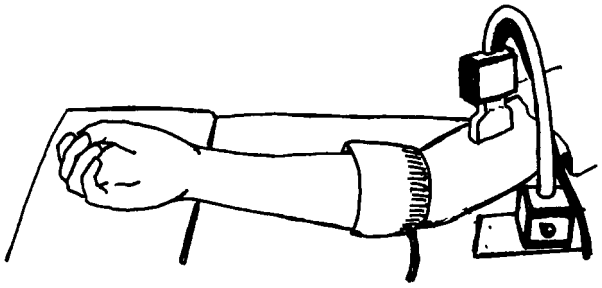

Figure 1 Illustration of the placement of the tourniquet and the ultrasound probe on the arm where the readings were taken.

resolution box function and operating parameters of the machine remained unchanged during each study. Electrocardiographic recordings were made throughout the study. Arterial blood flow velocity was measured by means of a pulsed Doppler signal at a 70° angle to the vessel, placed with a range gate (1.5 mm) in the center of the artery.

Flow-mediated dilatation was assessed by measuring the changes in the diameter of the brachial artery as a response to increased flow. To create increased flow a pneumatic tourniquet, placed on the forearm, was inflated to a pressure of 250–300 mmHg for 5 min (Figure 1). The brachial artery was imaged continually for 1 min before cuff inflation to 5 min after deflation. Flow velocity recordings were taken before cuff inflation and for the first 15 s after cuff deflation corresponding to the period of maximum hyperemia (Figure 2). All images were recorded on super VHS tape for later analysis. The B-mode images were captured on a personal computer at intervals of 3 s throughout the study, via a video frame grabber. Brachial artery diameter measurements were performed semi-automatically using commercially available edge detection software (CVI Acquisition and CVI Analysis, Information Integrity Inc, Boston, MA, USA)[10].

Measurements of the vessel diameter were taken from the leading edge of the anterior wall to the leading edge of the posterior wall of the brachial artery at end diastole, which coincided with the R-wave on the electrocardiogram. Resting (baseline) vessel diameter was calculated as the mean of all the measurements during the first minute of recording. The measurements of the brachial artery diameter between 55 and 65 s following the release of the tourniquet (time of maximum dilatation) were averaged. Changes in diameter were calculated as percentage change relative to the resting (baseline) diameter. Hence, percentage flow-mediated dilatation = [(vessel diameter after cuff deflation − resting vessel diameter)/resting vessel diameter] × 100%. All the scans were analyzed by the same experienced observer blinded to the identity of the subjects or the stage of the examination. As a quality control, 30 scans were analyzed by a second independent experienced observer. The interobserver variability, calculated as the mean and the standard deviation of the absolute differences between the two observers, was 1.02 ± 0.6% for flow-mediated dilatation (95% limits of agreement: −1.7 to 2.4%).

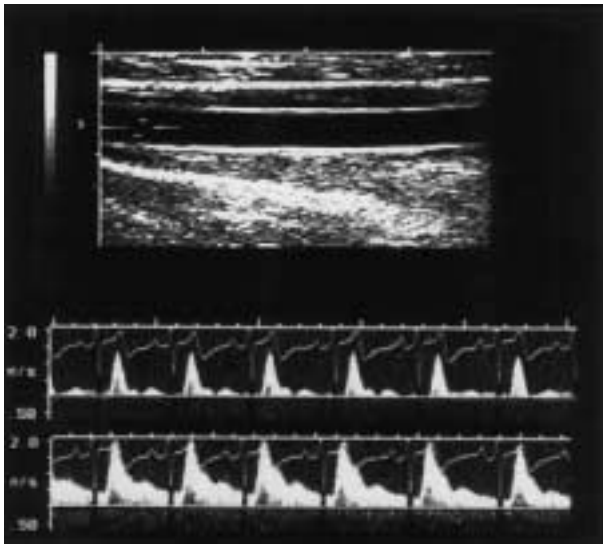

Figure 2 Ultrasound image of the brachial artery (upper panel) and the Doppler waveforms obtained from the brachial artery at rest (lower panel, top) and during reactive hyperemia (lower panel, bottom).

Volumetric flow was calculated for each study by multiplying the angle-corrected velocity time integral of the Doppler flow signal by the heart rate and the vessel cross-sectional area. All measurements used for flow calculations were obtained contemporaneously. The flow velocity was taken from the center of the artery and therefore gave an overestimation of blood flow, but relative flows before and after cuff inflation were accurate.

Flow change (reactive hyperemia) was calculated as: [(blood flow at 15 s after cuff deflation − resting blood flow)/resting blood flow] × 100%.

The statistical program Arcus Quickstat Biomedical (version 1.1, Longman Software Publishing, Cambridge, UK) was used to analyze the data. The effects of gestational age on flow-mediated dilatation, resting vessel diameter, resting blood flow and reactive hyperemia were examined using regression analysis for continuous variables to look for linear or quadratic relationships. Non-pregnant controls were compared with pregnant women between 10 and 30 weeks' gestation using two-sided unpaired *t*-test. Multiple regression analysis was used to examine the relationships between flow-mediated dilatation and gestational age, maternal weight at the time of the study, heart rate, resting vessel diameter and reactive hyperemia. Normality of the distribution of the data was examined with the Shapiro–Wilk test. For those parameters that were not normally distributed logarithmic transformation was performed. Data are expressed as mean ± standard deviation.

RESULTS

Recordings were successfully obtained from all women and all women tolerated the studies well. The mean age of the 157 pregnant women was 30.8 ± 5.3 years, and that of the 19 non-pregnant controls was 31.9 ± 4.8 years ($t = 0.87$, d.f. = 174, $P = 0.38$). All subjects had normal systolic (< 140 mmHg) and diastolic (< 90 mmHg) blood

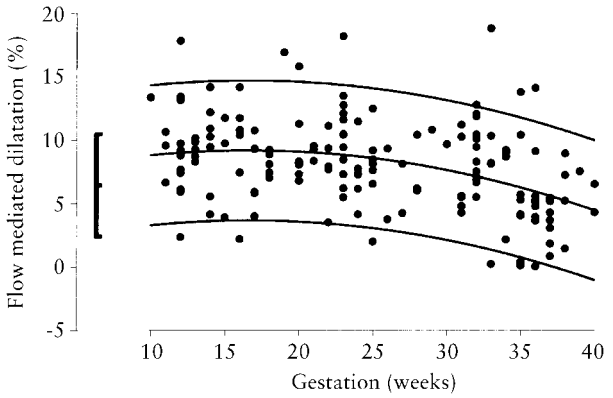

Figure 3 Flow-mediated dilatation of the brachial artery with gestation, illustrating individual values and the regression lines of the mean, 95th and 5th centiles. The vertical line illustrates the mean, 95th and 5th centiles of the non-pregnant controls.

pressure. The mean heart rate in the non-pregnant controls was 65 ± 6 beats per min. In pregnancy mean heart rate increased linearly with gestation ($y = 0.619x + 66.507$, $r = 0.445$, $P < 0.001$) from a mean of 72 ± 3.16 beats per min at 10 weeks to 90 ± 4.24 beats per min at 40 weeks of gestation.

Flow-mediated dilatation in the non-pregnant controls was 6.42 ± 2.45%. In pregnant women, flow-mediated dilatation decreased with gestation but this decrease was only observed after about 30 weeks (Figure 3, $y = 6.84 + 0.28x - 0.008x^2$, $R^2 = 0.13$, $P < 0.001$). Between 10 and 30 weeks the mean flow-mediated dilatation (8.84 ± 3.18%) was significantly higher than the non-pregnant controls ($t = 3.13$, d.f. = 115, $P = 0.002$).

The resting mean vessel diameter of non-pregnant controls was 3.14 ± 0.26 mm. In pregnant women, vessel diameter increased with gestation (Figure 4, $y = 3.56 - 0.06x + 0.002x^2$, $R^2 = 0.21$, $P < 0.001$), but up to 30 weeks of gestation the mean value (3.14 ± 0.43) was not significantly different from that of the non-pregnant controls ($t = 0.08$, d.f. = 115, $P = 0.93$). Flow-mediated dilatation was inversely correlated with the vessel diameter (Figure 5, $y = 17.9 - 3.05x$, $r = -0.42$, $P < 0.001$).

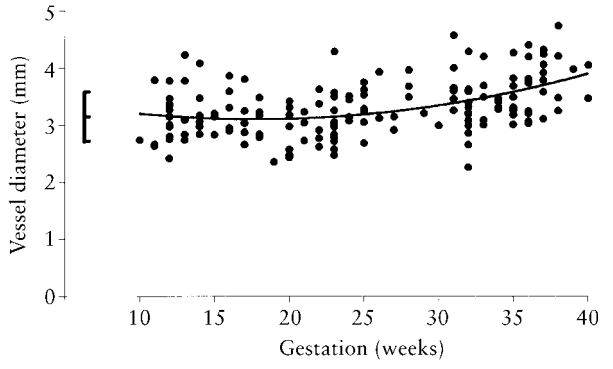

Figure 4 Resting brachial artery diameter with gestation, illustrating individual values and the regression line of the mean. The vertical line illustrates the mean, 95th and 5th centiles of the non-pregnant controls.

Resting blood flow in the brachial artery of the non-pregnant controls was 128.79 ± 70.75 mL/min. In pregnant women resting blood flow increased with gestation (Figure 6, $y = \exp(4.76–0.03x + 0.002x^2)$, $R^2 = 0.35$, $P < 0.001$). The trend of the change was similar to that of the vessel diameter, with no significant difference between controls and pregnant women between 10 and 30 weeks of gestation ($t = 0.86$, d.f. = 115, $P = 0.39$).

Reactive hyperemia in the non-pregnant controls was 620.53 ± 313.73%. In pregnancy, there was a significant decrease with gestation (Figure 7, $y = \exp(7.15 - 0.047x)$, $r = -0.51$, $P < 0.001$).

Univariate regression analysis revealed a significant correlation between flow-mediated dilatation in pregnant women and gestational age ($R^2 = 0.13$, $P < 0.001$), maternal weight at the time of the study ($r = -0.20$, $P = 0.01$), heart rate ($r = -0.16$, $P = 0.03$), resting vessel diameter ($r = -0.42$, $P < 0.001$) and reactive hyperemia ($r = 0.034$, $P < 0.001$). Univariate regression analysis revealed no significant correlation between flow-mediated dilatation in pregnant women and maternal age ($r = 0.08$, $P = 0.31$), height ($r = -0.08$, $P = 0.26$) and parity ($r = 0.02$, $P = 0.74$). Backward stepwise multiple regression analysis demonstrated that flow-mediated dilatation was significantly and independently related to vessel diameter and reactive hyperemia (flow-mediated dilatation = 10.72 + 1.06. ln reactive hyperemia − 2.74 vessel diameter, $r^2 = 0.25$).

DISCUSSION

This study has demonstrated that normal pregnancy is associated with an increase in flow-mediated dilatation of the brachial artery by 38%, and this is apparent from at least 10 weeks of gestation. This increase is sustained until about 30 weeks of gestation after which it subsequently falls to prepregnancy levels. We have also shown that resting vessel diameter and blood flow in the brachial artery do not change significantly in pregnancy until about 30 weeks' gestation, but increase thereafter. A previous study incorporating cross-sectional data from 71 pregnant women, and included longitudinal data from eight pregnant women, which used the same technique also reported that flow-mediated dilatation in the first and second trimesters increased[11]. However, the investigators found that flow-mediated dilatation in the third trimester of pregnancy apparently increased despite a further increase in the vessel diameter[11]. This is contrary to our findings which showed a marked decrease in flow-mediated dilatation towards the end of the third trimester. In the same cross-sectional study, it was also found that the increase in the resting vessel diameter and blood flow were obvious from the second trimester. However, in the longitudinal arm of the study, a significant difference was reported between vessel diameter in the first and third trimesters. Possible explanations for these apparent discrepancies lie in the fact that, firstly, the investigators did

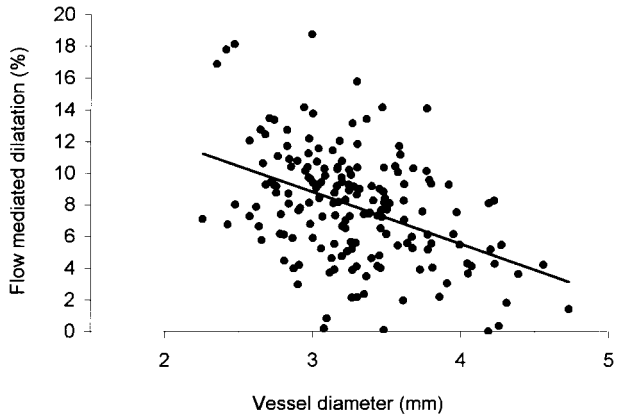

Figure 5 Relation of flow-mediated dilatation of brachial artery with resting vessel diameter illustrating individual values and the regression line of the mean ($r = -0.42$).

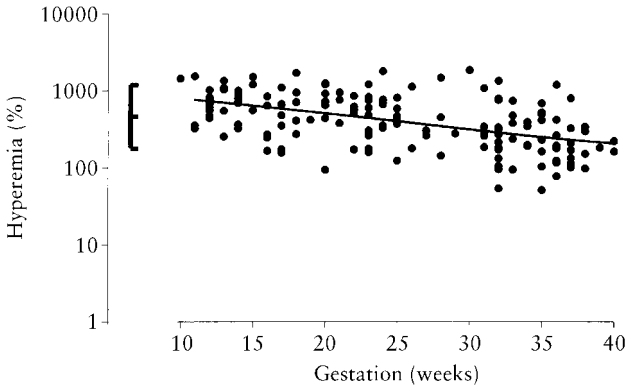

Figure 7 Reactive hyperemia with gestation, illustrating individual values and the regression line of the mean ($r = -0.51$). The vertical line illustrates the mean, 95th and 5th centiles of the non-pregnant controls.

not examine the relationship of the flow-mediated dilatation with gestation as a continuous variable but rather grouped by trimesters and, secondly, the smaller size of their studied population.

In basal conditions, nitric oxide is continuously produced by vascular endothelial cells and this release appears to regulate vascular tone[12]. The trigger for the basal release of nitric oxide is blood flow eliciting a shear stress at the endothelial surface[13]. Flow-mediated dilatation, induced by reactive hyperemia, is also dependent on the endothelial release of nitric oxide, because it can be partially blocked by N^G-monomethyl-L-arginine, a specific inhibitor of nitric oxide synthesis[8,9]. Furthermore, flow-mediated dilatation of the brachial artery reflects systematic endothelial function[14] and is decreased in subjects with endothelial dysfunction[7,15]. The non-invasive method of endothelial assessment used in our study has been shown to be accurate and reproducible[16].

In our study resting blood flow, as assessed by pulsed wave Doppler, was increased only in late pregnancy and this is compatible with findings of previous studies which used plethysmography to assess blood flow in pregnancy[17]. It is likely that this increase in baseline blood flow provides the necessary shear stress at the endothelial surface for increased basal release of nitric oxide and consequent increase in resting vessel diameter, as observed in our study.

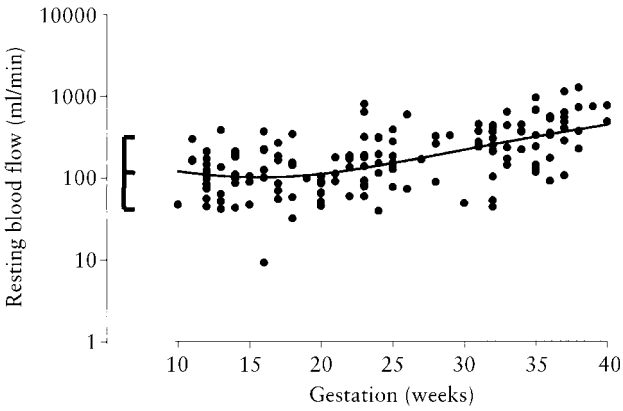

Figure 6 Resting blood flow of brachial artery with gestation, illustrating individual values and the regression line of the mean. The vertical line illustrates the mean, 95th and 5th centiles of the non-pregnant controls.

Flow-mediated dilatation, in contrast to resting blood flow, is increased from at least 10 weeks' gestation. This increase is maintained until the third trimester of pregnancy, when it falls to prepregnancy levels. In vitro studies have shown that flow-induced, nitric oxide-mediated dilatation is increased in isolated small arteries from pregnant women and pregnant rats compared with non-pregnant controls[18,19]. The initial increase in flow-mediated dilatation observed in our study supports the hypothesis that endothelial function and stimulated nitric oxide production are enhanced in normal pregnancy. Pregnancy in rats and possibly in humans is associated with an increase in plasma concentration and urinary excretion of 3′, 5′ -cyclic monophosphate, the second messenger of nitric oxide[20–23]. Nitric oxide synthase activity in platelets has been found to be increased in healthy pregnant women compared with pre-eclamptic and non-pregnant women[24]. The increase of nitric oxide production in pregnancy may be mediated by estrogen. Studies have shown that administration of estrogen improves endothelium-dependent vasodilatation of the coronary arteries[25]. In oophorectomized rabbits, administration of 17β-estradiol enhanced endothelium-dependent dilatation in response to acetylcholine[26]. Additionally, flow-mediated dilatation has been shown to be higher during the follicular and luteal phases of the menstrual cycle than during menstruation[27].

Despite the initial increase, flow-mediated dilatation did not change significantly within the gestational range of 10–30 weeks, whereas the maternal serum concentration of estrogen increases throughout pregnancy. It is possible that the effect of estrogen on endothelial function reaches a plateau within the first trimester of pregnancy. Supportive evidence for this concept is provided by studies in postmenopausal women, where flow-mediated vasodilatation was increased by short-term estrogen replacement therapy. The same increase however, was achieved by the administration of either 1 mg or 2 mg of estradiol[28].

The decrease of flow-mediated dilatation in the third trimester of pregnancy may be a mere consequence of the increase in the resting vessel diameter as flow-mediated dilatation was inversely correlated with the diameter of the brachial artery. Previous studies have shown that larger vessels dilate less than smaller ones[7,15]. It is also conceivable that basal vascular nitric oxide activity is upregulated in late pregnancy, as the increased resting vessel size suggests, and therefore reactive hyperemia may lead to a reduced response[17,29].

Another explanation for our findings is that, in the third trimester, the degree of reactive hyperemia, which represents the stimulus for flow mediated dilatation and decreases with gestation, may not have been sufficiently high to stimulate an adequate response from endothelial cells. The decreased reactive hyperemia in the later phases of pregnancy may suggest that peripheral vascular beds are already prerelaxed as a result of increased baseline blood flow. Finally, although flow-mediated dilatation of the brachial artery has been shown to mainly depend on nitric oxide release[8,9] other vasoactive mediators such as adenosine or prostacyclin may play a role.

This study has shown that in normal pregnancy flow-mediated dilatation is substantially increased from at least 10 weeks' gestation. This increase is sustained until 30 weeks and provides evidence that pregnancy is associated with enhanced endothelial function and stimulated nitric oxide production. Increase in the vessel diameter, upregulation of nitric oxide activity or decreased reactive hyperemia towards term, however, may obscure the assessment of the endothelium during this part of pregnancy. The extent to which this technique will prove to be useful in identifying pathologic pregnancies with altered vascular endothelial function remains to be determined.

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