Assessment of endothelial function in normal twin pregnancy

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ABSTRACT

Objective To assess the maternal endothelial function in normal twin pregnancy.

Design Cross-sectional study.

Subjects Endothelial function was investigated in 74 women with normal twin pregnancy at 11–30 weeks of gestation and the results were compared to previous reported findings in 98 women with normal singleton pregnancy and 19 non-pregnant controls.

Methods Endothelial function was assessed by measuring the changes of the brachial artery diameter in response to reactive hyperemia (flow-mediated dilatation) using external high resolution ultrasound.

Results Flow-mediated dilatation of the brachial artery in both twin and singleton pregnancies was significantly higher than in non-pregnant women ($P = 0.002$ and $P = 0.02$, respectively). However, there was no significant difference in flow-mediated dilatation between women with twin and singleton pregnancy (9.61 ± 4.36 vs. 8.84 ± 3.18, $P = 0.38$). Resting vessel size, baseline flow and reactive hyperemia did not change significantly with gestation in twin pregnancy and were similar to values in singleton pregnancies and controls.

Conclusion Our findings indicate that although in pregnancy endothelial function is enhanced, this change may not be affected by the number of fetoplacental units present.

INTRODUCTION

Pregnancy is associated with profound alterations in maternal physiology. Endothelium-derived nitric oxide is thought to play an important role in pregnancy[1] and impaired production is involved in the pathophysiology of conditions such as pre-eclampsia[2,3]. Recently, a non-invasive method of endothelial function assessment has been introduced which measures the changes of the brachial artery diameter as a response to increased flow (flow-mediated dilatation) using high resolution

ultrasound[4]. This response has been shown to be dependent mainly on the ability of the endothelium to release nitric oxide[5–7] and the technique has proved to be accurate and reproducible[8]. We[9] and others[10] have already used this method to assess endothelial function in normal singleton pregnancy. We have found[9] that flow-mediated dilatation increased from at least 10 weeks of gestation by 38%, compared to the non-pregnant controls. This increase was sustained up to 30 weeks of gestation when it fell to pre-pregnancy levels.

Twin pregnancy is characterized by more prominent cardiovascular adjustments[11–14] than singleton pregnancy and is associated with increased incidence of disorders in which endothelial dysfunction plays a role[15–17]. It is conceivable that altered endothelial function in twin pregnancy may account for these differences. The aim of this study was to investigate whether endothelial function as assessed by flow-mediated dilatation of the brachial artery is altered in normal twin compared to singleton pregnancy.

METHODS

This was a cross-sectional study involving 74 women with twin pregnancies (46 dichorionic and 28 monochorionic). The subjects were recruited from the routine antenatal clinic, they were all healthy, non-smokers, on no medication and did not have a family history of premature heart disease. The findings were compared to those in 98 women with singleton pregnancies and 19 non-pregnant controls[9]. All pregnant women were between 11 and 30 weeks of gestation. In our previous study in singleton pregnancy[9] we found that flow-mediated dilatation was substantially increased until 30 weeks of gestation and decreased thereafter. It was also observed that resting brachial artery diameter increased significantly only after 30 weeks of gestation. Since it is known that vessel diameter can have an effect on flow-mediated dilatation, with larger vessels dilating less than smaller ones[4,18], the increase in resting vessel diameter after 30 weeks could have obscured the assessment of endothelial function during this part of pregnancy. In order to avoid this, we decided to limit the study period to up to 30 weeks of gestation. The study

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was approved by the local ethics committee and all subjects gave written informed consent.

The brachial artery ultrasound scans were performed in a quiet and temperature-controlled (22° to 26°C) room as previously described[4,9]. All examinations were conducted by the same investigator. The diameter of the artery was measured from high-resolution, B-mode images using 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. 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, 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. The brachial artery was imaged continually from 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 (the period of maximum hyperemia). All images were recorded on super VHS tape for later analysis. Brachial artery diameter measurements were performed semiautomatically using commercially available edge detection software (CVI Acquisition and CVI Analysis, Information Integrity Inc., MA, USA)[19].

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. 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, flow–mediated dilatation equaled ((vessel diameter after cuff deflation – resting vessel diameter)/ resting vessel diameter) × 100%. All the scans were analysed by the same experienced observer blinded to the identity of the subjects. As a quality control, 13 scans were analysed by a second independent experienced observer and the inter-observer variability calculated as the mean and the standard deviation of the absolute differences between the two observers for flow-mediated dilatation was 1.01 ± 0.3%.

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 gives an overestimation of blood flow, but relative flows before and after cuff inflation are accurate. Flow change (reactive hyperemia) was calculated as: ((blood flow at 15 s after cuff deflation – resting blood flow)/resting blood flow) × 100%.

Outside the setting of pregnancy, dilatation in response to sublingual glyceryl trinitrate (GTN), which is endothelium-independent, is commonly used as a control. In the current study, a decision was made to avoid the use of GTN during pregnancy but a previous study has shown that GTN-induced dilatation does not alter as a result of pregnancy[10].

Statistical analysis

The effect of gestational age on flow-mediated dilatation, resting vessel diameter, resting blood flow and reactive hyperemia was examined using regression analysis for continuous variables. Regression analysis was used to compare groups after adjusting for the effect of gestational age. One-way analysis of variance followed by the post hoc test of Tukey HSD was conducted to evaluate the relationship of flow-mediated dilatation, resting vessel diameter, resting blood flow and reactive hyperemia between subjects with twin or singleton pregnancies and non-pregnant controls. Normality of the distribution of the data was examined with the Shapiro-Wilk test. For those parameters that were not normally distributed (resting blood flow and reactive hyperemia) logarithmic transformation was performed, which was observed to improve the approximation to normality of the data. Unless otherwise stated, data are expressed as mean ± standard deviation.

RESULTS

Recordings were successfully obtained from all women and they all tolerated the studies well. The median age of the non-pregnant controls was 31 (interquartile range: 27–36) years. The median maternal and gestational age was 35 (interquartile range: 31–37) years and 20 (interquartile range: 14–24) weeks, respectively, for the twin pregnancies and 32 (interquartile range: 28–35) years and 19 (interquartile range: 14–23) weeks for the singleton pregnancies. All subjects had normal systolic (less than 140 mmHg) and diastolic (less than 90 mmHg) blood pressure. The mean heart rate in the non-pregnant controls was 65 ± 6 beats per minute (bpm). There was no significant difference between the regression coefficients for heart rate against gestational age between twins and singletons. Predicted heart rate in twin pregnancy increased by 0.81 bpm for every 1 week increase in gestational age (95% CI: 0.32–1.3, $P = 0.001$). Predicted heart rate in singleton pregnancy increased by 0.51 bpm for every 1 week increase in gestational age (95% CI: 0.11–0.91, $P = 0.01$). The heart rate for twin pregnancy was significantly higher than that for singleton pregnancy at any given gestational age (mean difference: 7.55 bpm, 95% CI: 4.17–10.92, $P < 0.0001$).

In both twin and singleton pregnancies flow-mediated dilatation changed little with time between 10 and 30 weeks of gestation (regression coefficient = 0.04, 95% CI: –0.14–0.22 and regression coefficient: –0.07, 95% CI: –0.19–0.05, respectively). There were also no significant changes with gestation in both groups regarding resting vessel diameter (regression coefficient: 0.01, 95% CI: 0.00–0.03 and regression coefficient: 0.01, 95% CI: –0.01–0.03, respectively), resting blood flow (regression coefficient: 1.01, 95% CI: 0.98–1.04 and regression coefficient: 1.02, 95% CI: 0.99–1.05, respectively) and reactive hyperemia (regression coefficient: 0.99, 95% CI: 0.96–1.02 and regression coefficient: 0.98, 95% CI: 0.95–1, respectively).
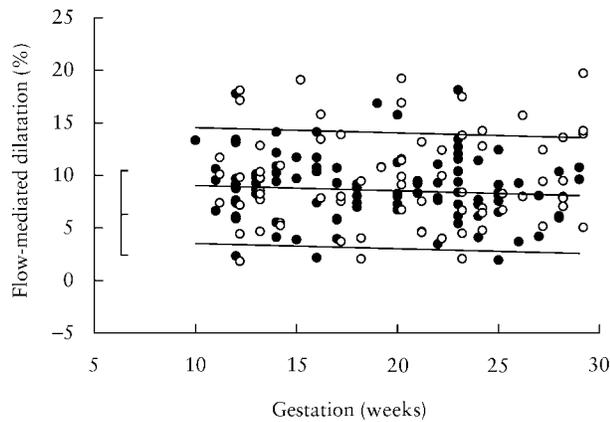
Figure 1 Flow-mediated dilatation of the brachial artery with gestation, illustrating individual values of singletons (●), twins (○) and the regression lines of the mean, 95th and 5th centiles of the singletons. The vertical line illustrates the mean, 95th and 5th centiles of the non-pregnant controls.

Flow-mediated dilatation in both twin and singleton pregnancies were significantly higher than non-pregnant controls (Figure 1, Table 1). However, there were no significant differences in flow-mediated dilatation between twin and singleton pregnancies ($9.61 \pm 4.36\%$ vs. $8.84 \pm 3.18\%$, $P = 0.38$). Resting vessel size, resting blood flow and reactive hyperemia were similar in all three examined groups (Table 1).

DISCUSSION

This study has demonstrated that flow-mediated dilatation of the brachial artery in normal twin pregnancies is similar to that in singleton pregnancies, does not change significantly with gestation at 11–30 weeks and is significantly higher than in non-pregnant controls. These findings indicate that although in pregnancy endothelial function and stimulated nitric oxide production appear enhanced, these changes may be not affected by the number of fetoplacental units present.

Compared to singleton, twin pregnancies present more intense hemodynamic[11–14,20] and hormonal alterations with increased estrogen levels[12,20,21]. There is evidence that estrogens have an effect on nitric oxide production by upregulating nitric oxide synthases[22,23]. The administration of estrogens has been shown to improve arterial endothelium-dependent vasodilation[24]. In postmenopausal women *in vivo*, estrogens have been reported to enhance nitric oxide derivatives and to improve endothelium-dependent vasodilation[25–30]. Thus, it could be expected that twin pregnancy, characterized by increased estrogen levels, would be associated with enhanced nitric oxide activity and flow-mediated dilatation. However, there is evidence that estrogen-dependent increase in flow-mediated dilatation reaches a plateau[25,27]. Previous studies in postmenopausal women have shown that the increase in flow-mediated dilatation after short-term estrogen replacement therapy did not change after the administration of either 1 mg or 2 mg of estradiol[27]. This can offer an explanation for our findings in twin pregnancies, where flow-mediated dilatation was not significantly different from singleton pregnancies. Our findings may indicate that nitric oxide activity is similar in twin and singleton pregnancy. Although flow-mediated dilatation of the brachial artery has been shown to depend mainly on the nitric oxide action[6,7], other vasoactive agents released during reactive hyperemia may also play a role. Thus, it cannot be excluded that nitric oxide activity is increased in twin pregnancy compared to singleton; however, a different behavior of other vasoactive factors in this group may ultimately result in similar flow-mediated dilatation.

Resting blood flow in the brachial artery did not increase significantly in pregnant women compared to controls and was not significantly different in twins compared to singletons. Although it is known that pregnancy is associated with increased cardiac output[31–33] and that cardiac output is even higher in twin pregnancy compared to singleton[13,14], these changes are not necessarily reflected in peripheral flow in the arm[34,35]. Furthermore, the regional blood flow is not determined by dilatation of the conduit arteries, such as the brachial artery, but by the resistance vessels, which cannot be

Table 1 Comparison between twins ($n = 74$), singletons ($n = 98$) and non-pregnant controls ($n = 19$). Grouped comparisons are not adjusted for gestational age

Parameter	Group	Mean*	SD	Overall comparison	Comparison between groups*	
Flow-mediated dilatation (%)	Twins	9.61	4.36	*F = 5,83 P = 0.003*	Twins–singletons	*P* = 0.38 (95% CI: 2.07–0.54)
	Singletons	8.84	3.18	d.f. 2,188	Twins–controls	*P* = 0.002 (95% CI: 5.38 to −0.99)
	Controls	6.42	2.45		Singletons–controls	*P* = 0.02 (95% CI: 4.55 to −0.28)
Resting vessel size (mm)	Twins	3.08	0.34	*F = 0.58 P = 0.5*	Twins–singletons	*P* = 0.55 (95% CI: 0.08–0.2)
	Singletons	3.14	0.45	d.f. 2,188	Twins–controls	*P* = 0.8 (95% CI: 0.18–0.3)
	Controls	3.14	0.26		Singletons–controls	*P* = 1 (95% CI: −0.23–0.23)
Resting blood flow (mL/min)	Twins	125.60	132.28	*F = 0.64 P = 0.53*	Twins–singletons	*P* = 0.59 (95% CI: 0.69–1.1)
	Singletons	112.91	93.87	d.f. 2,188	Twins–controls	*P* = 0.65 (95% CI: 0.55–1.3)
	Controls	106.88	71.03		Singletons–controls	*P* = 0.95 (95% CI: 0.62–1.43)
Reactive hyperemia (%)	Twins	594.91	555.75	*F = 0.19 P = 0.83*	Twins–singletons	*P* = 0.89 (95% CI: 0.74–1.22)
	Singletons	566.80	407.91	d.f. 2,188	Twins–controls	*P* = 0.85 (95% CI: 0.6–1.37)
	Controls	539.92	313.73		Singletons–controls	*P* = 0.95 (95% CI: 0.63–1.43)

*For resting blood flow and hyperemia geometric means are given as well as 95% CI for the ratio of the geometric means.

examined by the use of ultrasound. Hence, the finding that resting diameter of the brachial artery in twin pregnancy is similar to that in singletons and non-pregnant controls is not surprising since resting diameter is mainly regulated by baseline blood flow, which provides the necessary shear stress at the endothelial surface for basal release of nitric oxide[36].

The findings of our study demonstrate that flow-mediated dilatation of the brachial artery is similar in women with normal twin and singleton pregnancy. These results provide evidence that endothelial function and stimulated nitric oxide activity may not be dependent on the number of fetoplacental units present. This knowledge may be valuable in the investigation of pathological pregnancies in which endothelial dysfunction is involved.

ACKNOWLEDGMENT

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