Brachial artery flow-mediated dilation is not affected by pregnancy or regular exercise participation

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ABSTRACT

Whether brachial artery FMD (flow-mediated dilation) is altered in pregnancy by 28–35 weeks compared with non-pregnant women remains controversial. The controversy may be due to limitations of previous studies that include failing to: (i) test non-pregnant controls in the mid-late luteal phase, (ii) account for effects of pregnancy on the dilatory shear stimulus, (iii) account for physical activity or (iv) control for inter-individual variation in the time to peak FMD. In the present study, brachial artery FMD was measured in 17 active and eight sedentary pregnant women (34.1 ± 1.6 weeks of gestation), and in 19 active and 11 sedentary non-pregnant women (mid-late luteal phase). Decreased vascular tone secondary to increased shear stress contributes minimally to pregnancy-induced increases in baseline brachial artery diameter, as shear stress removal during distal cuff inflation in pregnant women did not reduce diameter to baseline levels observed in non-pregnant controls. Neither the shear stimulus nor the percentage FMD was affected by pregnancy or regular exercise. Continuous diameter measurements are required to control for delayed peak dilation during pregnancy (57 ± 15 compared with 46 ± 15 s; P = 0.012), as post-release diameter measured at 60 or 55–65 s post-release underestimated FMD to a greater extent in non-pregnant than in pregnant women.

INTRODUCTION

Pregnancy is characterized by extensive haemodynamic changes, including increases in blood volume, cardiac output, peripheral vascular conductance and peripheral blood flow [1,2]. Increased blood flow may cause chronic elevations in conduit artery shear stress, with both functional and structural consequences. Elevated shear stress as a result of exercise training or sauna exposure can evoke acute up-regulation of eNOS (endothelial nitric oxide synthase) activity and subsequently FMD (flow-mediated dilation) [3]. Shear stress mediates endothelial adaptations to exercise training [4]. Repeated sauna treatment improves vascular endothelial function in chronic heart failure [5]. Thus it may be expected that elevated shear stress would increase FMD during normotensive pregnancy [6]. In actuality, data from existing studies are mixed. Some show that FMD has significantly increased [2,7], while others found no difference [8–10] at 28–35 weeks gestation compared with non-pregnant controls.

However, all available data on FMD in pregnancy were measured using approaches that did not account for four considerations that are critical for valid quantification

Key words: brachial artery, exercise, flow-mediated dilation, shear rate, pregnancy, shear-mediated dilation.

Abbreviations: AUC, area under the curve; BMI, body mass index; FMD, flow-mediated dilation; KPAS, Kaiser Physical Activity Survey; RM, repeated measures; RPE, rating of perceived exertion.

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of FMD [11,12], and the impact thereon of pregnancy. First, no study accounted for potential, pregnancy-induced changes in the ‘relevant shear stimulus’ that causes FMD (the shear stimulus from cuff release to the time of peak diameter [13]). Potential pregnancy-induced changes in FMD may therefore reflect differences in dilatory stimulus strength. Secondly, recently it has been proposed that artery diameter affects the FMD response to shear [14]. Comparison of arteries of different sizes (brachial compared with radial compared with femoral compared with popliteal) suggests that larger arteries dilate less than smaller arteries [14]. In contrast, between-subjects comparisons of the same artery (i.e. brachial compared with brachial) suggest that the effect of artery size on FMD is explained by differences in the shear stimulus [15–17]. Two studies reported that brachial artery diameter was negatively correlated with FMD in pregnant women [9,10]. However, neither study considered whether this effect was mediated by differences in the relevant shear stimulus or may have contributed to potential differences in FMD between non-pregnant and pregnant women. Thirdly, post-release diameter was measured at pre-defined time points [2,7–10], rather than using continuous measurements to identify peak diameter. This approach does not control for inter-individual variability in the timing of peak dilation [18], which could be systematically altered in pregnancy. Fourthly, most previous studies did not control for menstrual cycle phase in non-pregnant controls [2,7,9,10]. FMD varies during the menstrual cycle [19,20], and pregnant women experience this cyclic variation prior to conception. Comparisons with non-pregnant subjects in the phase most similar to pregnancy are required to demonstrate that differences in FMD are attributable to pregnancy, and not to menstrual cycle phase. These limitations must be addressed to ensure that conclusions regarding the effects of pregnancy on FMD are accurate.

In addition to affecting vascular function, elevated shear stress serves as a stimulus for vascular remodelling [21,22]. Brachial artery diameter increases during pregnancy [2,10]. Whether this is a result of vasodilatation due to elevated shear [2] or vascular remodelling is not known.

A further important factor that could influence both functional and structural vascular changes in pregnancy is physical activity. The benefits of physical activity for vascular function and health are well known [22]. Although exercise is recommended for healthy pregnant women, the vascular effects of prenatal exercise have not been examined [23]. This information is urgently needed as researchers have hypothesized that exercise may prevent preeclampsia by correcting endothelial dysfunction [24].

With this as a background, our objectives were to take into account the FMD shear stimulus, baseline artery diameter, variable timing of peak FMD and menstrual cycle effects and to test the following hypotheses: (i) FMD is improved in pregnancy and physical activity status modifies this pregnancy effect, and (ii) pregnancy-induced increases in the baseline shear stimulus explains the increased brachial artery diameter during pregnancy. In addition to providing new mechanistic insight, this approach addresses critical limitations of earlier studies that may have affected the accuracy of conclusions.

MATERIALS AND METHODS

Subjects

Brachial artery FMD was measured in 17 active pregnant, eight inactive pregnant, 19 active non-pregnant and 11 inactive non-pregnant women as part of a larger study examining the effects of pregnancy, and acute and chronic exercise on endothelial function [25,26]. Healthy, non-smoking women, aged 23–40, who were not regularly taking medications, and had no history of hypertension, were recruited using flyers. Pregnant women were tested at 34.1 ± 1.6 (mean ± S.D.) weeks of gestation. Non-pregnant women were not taking hormonal contraception, and were tested in the mid-luteal phase (days 20–28). Progesterone and oestrogen are elevated during this phase [20,27]. Cardiovascular changes are similar to those observed in pregnancy, but are smaller in magnitude [27]. All non-pregnant women reported regular menstrual cycles between 25 and 33 days in length for the past 6 months. Active women had exercised for three or more hours/week for at least 8 months. Inactive women did not exercise regularly. This research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The Health Sciences Research Ethics Board at Queen's University approved the study. All subjects provided written informed consent prior to participation.

Pre-test screening

Non-pregnant women completed a medical screening form (Physical Activity Readiness Questionnaire; http://www.csep.ca/forms.asp) to ensure that they were healthy with no contraindications to exercise. Pregnant women completed a similar form (PARmed-X for Pregnancy; http://www.csep.ca/forms.asp) with their obstetrician or midwife. An obstetric ultrasound confirmed that the subject was having a normal, singleton pregnancy and the fetus was not small for gestational age. An obstetrician provided final approval for testing in pregnancy after examining each screening form and ultrasound result.

Activity indices

Subjects completed a 3-day activity record [28] on consecutive days (one weekend day, two week days)
within 2 weeks of testing to evaluate current activity. Chronic activity was evaluated using the KPAS (Kaiser Physical Activity Survey), which was validated in non-pregnant [29] and pregnant [30] women. Non-pregnant subjects completed the KPAS once, to assess activity during the past year. Pregnant subjects completed the KPAS twice, to assess activity during pregnancy and for 1 year before conception. Mean voluntary physical activity [28] and the sports and exercise index [29,30] were calculated as described previously.

**Reactive hyperaemia protocol**

Subjects avoided caffeine and exercise for 12 and 24 h before the test respectively. Pregnant women need to eat regularly during late gestation; therefore, all subjects consumed a standard meal (Ensure Plus, 350 kcal) at 07:00 h. Women arrived at the laboratory at 08:00 h. Height and weight were measured. Self-reported weight prior to conception for pregnant women was recorded. Women were seated in a semi-darkened room for 20 min. The left arm was positioned on a table at heart level. A blood pressure cuff attached to an automatic inflation device was placed on the forearm distal to the elbow. Beat-to-beat finger arterial pressure was measured continuously using a Finapres (Model 2300, Ohmeda) photo-plethysmographic cuff placed on the right middle finger. Continuous, greyscale brachial artery images were obtained using a 10 MHz probe operating in B-Mode (GE Vingmed System 5, GE Medical Systems). Blood velocity was simultaneously recorded using Doppler ultrasound, with the same probe operating at 4 MHz. A 68° insonation angle was maintained to accurately measure velocity, while optimizing image quality by orienting the ultrasound beam perpendicular to the vessel [31], as has been validated previously [12,32]. The probe was positioned over the brachial artery a few centimetres proximal to the cuff, wherever the clearest image was obtained. Brachial artery diameter and velocity were recorded on video tape for 1 min. The occlusion cuff was inflated to 250 mmHg for 5 min. Diameter and velocity were recorded during the last minute of occlusion, and for 2 min post-release (Figure 1). Recorded images were transferred to a Digital Imaging and Communications in Medicine file at a rate of 25 frames/s for offline analysis [33].

**Exercise test**

Resting heart rate (Polar Vantage Heart Rate Monitor) and breath-by-breath respiratory measurements (VMax II, Cardinal Health or Moxus Modular Metabolic System,
AEI Technologies) were measured for 10 min. After a 3-min warm-up on an upright cycle ergometer (Sensor Medics Model 800S, Cardinal Health), a 90-s ramp work rate increase was performed until the woman reached an RPE (rating of perceived exertion) of 13 on the 6–20 Borg scale. This intensity was maintained for 20 min. RPE was the primary intensity indicator as RPE during weight-supported exercise does not change in pregnancy [34]. Resting heart rate increases by 10–15 beats/min during pregnancy [34]; therefore, intensity was confirmed by steady-state heart rates of 130 (non-pregnant) and 140 (pregnant) beats/min.

Data analysis
Brachial artery diameter was measured as described previously using automated edge-detection software (FMD/blood flow acquisition and analysis) [33]. Pre-inflation and inflation diameter were defined as the median of all measurements obtained during the minute prior to inflation and the minute prior to cuff release respectively. Peak diameter and the time required to reach peak diameter were determined on a smoothed diameter curve generated by an automated algorithm [18] applied to all measurements collected for 2 min post-release. Blood velocity from cuff release to the time of peak diameter was quantified by the same program, using the peak envelope method [18,33]. Time-aligned 2-s averages for velocity and diameter from cuff release until peak dilation were computed. Shear rate for each period was calculated as velocity/diameter. The shear stimulus that contributed to FMD was defined as the portion of reactive hyperaemia between cuff release and peak diameter [11,12], for which shear rate was 50% higher than the pre-inflation level. The shear stimulus was quantified as the AUC (area under the curve) minus the pre-inflation shear rate [13].

FMD was also calculated by the four methods used in previous pregnancy studies [2,7–10]. All methods defined FMD as the percentage increase from the pre-inflation baseline measurement; however, the timing of the post-release diameter measurement was different for each method. The 60 s method [7] measures post-release diameter 60 s post-release. The 55–65 s average method [10] calculates post-release diameter as the average of all measurements between 55 and 65 s post-release. The 40–60–80 s method selects the maximum of diameter measurements obtained at 40, 60 and 80 s post-release [9]. The 45-s method calculates post-release diameter as the average of measurements from the first four cardiac cycles for which clear images were obtained, starting at 45 s post-release [2,8]. Diameter data for the present study were collected continuously, rather than EKG gated. Measured heart rates in our subjects indicated that four cardiac cycles would take approx. 3 s. Therefore we approximated the 45 s method by averaging diameter values obtained between 45 and 48 s post-release.

Statistical analysis
Between-group differences in subject characteristics, activity indices and pregnancy outcomes were assessed using independent-samples Student’s t tests, Wilcoxin’s Rank-Sum tests or 2×2 ANOVA with pregnancy and activity as between-subjects factors.

Image quality was poor in two active non-pregnant subjects, and these subjects were excluded from statistical analyses. Values of FMD and the difference between pre-inflation and inflation diameter in one active pregnant subject were outliers, and were not included in analyses of these variables. Data points from this subject were also removed from regression analyses including FMD (Figures 3C and 3E; data points indicated by dashed circle). High Cook’s distance and centred leverage values indicated that this subject’s values were outliers with disproportionate influence on the regression line slope and correlation strength. One additional active pregnant subject was removed from regression analyses of FMD against shear rate AUC due to high Cook’s distance and centred leverage values (Figure 3E, solid circle).

There were no significant main effects or interactions with activity for any outcome measure; therefore, active and inactive groups were pooled. All variables were normally distributed. The effects of pregnancy on time-to-peak dilation and shear rate AUC were assessed by independent samples t tests. Haemodynamic responses to reactive hyperaemia were assessed using a 3×2 RM (repeated measures) ANOVA (within-subjects factor, time period; between-subjects factor, pregnancy). The effect of the measurement method on FMD was assessed using a 5×2 RM ANOVA (within-subjects factor, method; between-subjects factor, pregnancy). The effects of pregnancy on differences between peak FMD and FMD calculated by each method were evaluated using a 4×2 RM ANOVA (within-subjects factor, method; between-subjects factor, pregnancy). Where significant main effects were present, simple main effects were determined using paired or independent samples Student’s t tests with the Sidak correction for multiple comparisons. Statistical significance was determined by a two-sided P < 0.05. Analyses were performed in SPSS 16.0.

RESULTS
Physical characteristics of subjects
Gestational age did not differ between active and inactive pregnant women (Table 1). Cycle day did not differ between active and inactive non-pregnant women. Age was similar in all groups. BMI (body mass index) was significantly higher in inactive women compared with active women. BMI prior to conception in pregnant women did not differ from BMI in non-pregnant women. Mean finger arterial pressure did not differ.
Subjects characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active non-pregnant (n = 19)</th>
<th>Inactive non-pregnant (n = 11)</th>
<th>Active pregnant (n = 17)</th>
<th>Inactive pregnant (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.5 ± 4.7</td>
<td>33.3 ± 4.8</td>
<td>31.8 ± 3.6</td>
<td>30.3 ± 3.1</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>N/A</td>
<td>N/A</td>
<td>34.1 ± 1.3</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>Menstrual cycle day</td>
<td>25 ± 2</td>
<td>23 ± 2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pre-conception/non-pregnant BMI (kg/m²)</td>
<td>22.5 ± 2.7</td>
<td>25.4 ± 4.4*</td>
<td>23.3 ± 3.0</td>
<td>26.2 ± 4.7</td>
</tr>
<tr>
<td>Gestational weight gain (kg)</td>
<td>N/A</td>
<td>N/A</td>
<td>13.2 ± 4.8</td>
<td>14.0 ± 6.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>83 ± 12</td>
<td>88 ± 10</td>
<td>81 ± 12</td>
<td>84 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± S.D. Non-pregnant BMI was calculated from measured height and mass at the time of testing. Pre-conception BMI was calculated from measured height and self-reported mass. *P < 0.05, significantly different from active group of same reproductive status.

between pregnant and non-pregnant women. Heart rate was significantly higher in pregnant women compared with non-pregnant women (Supplementary Table S1 at http://www.clinsci.org/cs/121/cs1210355add.htm).

Low mean voluntary physical activity and sports and exercise index scores confirm that inactive women did not exercise (Supplementary Table S1). Higher mean voluntary physical activity and sports and exercise index scores in active women compared with inactive women confirm that exercise participation was greater in active women. Two observations suggest that active women were fitter than inactive women. First, work rate during exercise at the same relative intensity (RPE of 13) was higher in active women than in inactive women (non-pregnant, P = 0.020; pregnant, P = 0.004; Supplementary Table S1). Secondly, resting heart rate was lower in active women than in inactive women (non-pregnant, P < 0.000; pregnant, P = 0.047). This is consistent with training bradycardia.

Pregnancy outcome data

Outcome data were not available for one active pregnant subject who delivered at home. One inactive pregnant subject developed gestational hypertension at term. All other women had uncomplicated pregnancies. All pregnant women delivered healthy infants after 37 weeks of gestation. Gestational age and birth weight did not differ between active and inactive women (Supplementary Table S2 available at http://www.clinsci.org/cs/121/cs1210355add.htm).

Heart rate and blood pressure during reactive hyperaemia

Heart rate did not change during reactive hyperaemia (main effect, P = 0.256; results not shown). Mean arterial pressure increased by 3 mmHg during inflation in active non-pregnant, active pregnant and inactive pregnant women (P < 0.01 for main effects; results not shown). Although statistically significant, the small magnitude of this change indicates that haemodynamic parameters were stable during reactive hyperaemia.

Effect of pregnancy, activity and cuff inflation on diameter and shear rate

Activity had no effect on diameter or shear rate responses to cuff inflation (Figure 2); therefore active and inactive women were pooled. Baseline diameter was higher in pregnant than in non-pregnant women (Figure 2A; main effect of pregnancy, P = 0.016). Diameter decreased slightly, but significantly, during inflation only in pregnant women (cuff inflation × pregnancy, P < 0.001). Pre-inflation shear rate was higher in pregnant women than in non-pregnant controls (Figure 2B; main effect of pregnancy, P < 0.001). Inflation reduced shear rate to very low levels that were not different between groups (main effect of inflation, P < 0.001; pregnancy × inflation, P < 0.001).

Effect of pregnancy and activity on brachial artery responses to reactive hyperaemia

Activity did not affect FMD or shear rate responses to reactive hyperaemia; therefore, active and inactive women were pooled. FMD (absolute, P = 0.973; percentage, P = 0.194), shear rate AUC (P = 0.627) and FMD normalized for shear rate AUC (P = 0.511) did not differ between pregnant and non-pregnant women (Table 2). The slight vasoconstriction during cuff inflation in pregnant women (−1.9 ± 3.1%) was not observed in non-pregnant women (0.4 ± 3.6%, P = 0.201). Time-to-peak diameter was longer in pregnant women than in non-pregnant women (P = 0.012).

Relationships between pre-inflation diameter, FMD and shear rate AUC

There was no significant relationship between brachial artery diameter and shear rate AUC in inactive subjects (Figure 3B). Correlations between diameter and shear rate AUC did not reach statistical significance in active non-pregnant (Figure 3A, r² = 0.22, P = 0.056) or active pregnant (r² = 0.19, P = 0.081) women. However, diameter and shear rate AUC were significantly correlated in pooled active women (r² = 0.18, P = 0.014, Figure 3A). There was no relationship between brachial
Figure 2  Effects of occlusion on brachial artery diameter and shear rate in pregnant and non-pregnant women
Solid lines represent active subjects. Dashed lines represent inactive subjects. Grey circles indicate the group mean, pooled for activity. There was no significant effect of activity, therefore active and inactive groups were pooled for analysis. *P ⩽ 0.001, significant difference from non-pregnant group at same timepoint; †P ⩽ 0.004, significant difference from pre-inflation; ‡P = 0.001, significant difference from non-pregnant group pre-inflation.

Table 2  Effect of pregnancy on brachial artery FMD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-pregnant (n = 28)</th>
<th>Pregnant (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter change from pre-inflation to inflation (%)</td>
<td>0.4 ± 3.6</td>
<td>−1.9 ± 3.1*</td>
</tr>
<tr>
<td>FMD (mm)</td>
<td>0.317 ± 0.116</td>
<td>0.327 ± 0.134</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>10.7 ± 3.8</td>
<td>9.6 ± 3.8</td>
</tr>
<tr>
<td>Shear rate AUC</td>
<td>6792 ± 2466</td>
<td>6439 ± 2830</td>
</tr>
<tr>
<td>FMD normalized to AUC</td>
<td>0.0019 ± 0.0010</td>
<td>0.0023 ± 0.0037</td>
</tr>
<tr>
<td>Time to peak diameter (s)</td>
<td>46 ± 16</td>
<td>57 ± 15*</td>
</tr>
</tbody>
</table>

DISCUSSION

Previous studies examining the impact of pregnancy on FMD have provided mixed results. Furthermore, methodological limitations of these studies have limited data interpretation. Our study addresses these limitations by accounting for potential pregnancy-induced changes in the shear stimulus, baseline diameter, time course of peak dilation, menstrual cycle effects in the control group and physical activity levels. This allows us to conclusively identify effects of pregnancy on brachial artery FMD. The results reveal five important physiological findings. First, baseline shear rate is elevated in late pregnancy, but shear-mediated vasodilation contributes minimally to the larger brachial artery baseline diameter in pregnant women. Secondly, our data are consistent with previous reports that within the same artery, vessel size differences between subjects (i.e. brachial artery compared with brachial artery) do not affect FMD [14–17] and extend this to include pregnancy-evoked baseline diameter differences. Thirdly, when menstrual phase effects in non-pregnant women are accounted for, neither brachial artery FMD nor the shear stimulus for FMD is altered in late gestation. However, the timing of peak FMD is significantly delayed. Fourthly, regular exercise does not appear to modify either the reactive hyperaemia-evoked shear stimulus for FMD assessment or brachial artery FMD, in pregnant or non-pregnant women. However, the relationship between FMD and shear rate AUC was stronger in active than in inactive women.

Effect of method on percentage FMD
Peak FMD was greater than FMD determined by all other methods (Table 3; main effect of method, P < 0.001). The 40–60–80 s maximum method gave higher values than the 60 s and 55–65 s average methods in non-pregnant women, and the 45 s method in pregnant women (method × pregnancy, P = 0.005; main effect of pregnancy, P = 0.502). The 60 s and 55–65 s average methods underestimated FMD to a greater extent in non-pregnant than in pregnant women (method × pregnancy, P = 0.009).

Impact of pregnancy on brachial artery diameter
Previous studies hypothesized that pregnancy-induced increases in brachial artery diameter may be caused by shear-mediated vasodilation [2]. In accordance with

artery diameter and percentage FMD in active (Figure 3C) or inactive (Figure 3D) groups. Shear rate AUC was positively correlated with percentage FMD in active pregnant women (Figure 3E, r² = 0.41, P = 0.010), but not in active non-pregnant or inactive groups (Figure 3F).
Figure 3  Relationships between pre-inflation brachial artery diameter, percentage FMD and shear rate increase during reactive hyperaemia

○, Active pregnant; ●, active non-pregnant; □, inactive pregnant; ■, inactive non-pregnant. Dashed regression line represents pregnant group. Solid regression line represents non-pregnant group. Large circles/squares with error bars represent group means and S.D. Circled data points (C and E) show data points that were removed from the regression analysis due to high Cook's distance and centred leverage values, indicating that these points had disproportionate influence on the slope of the regression line and strength of the correlation. Data points circled with a dashed line indicate the subject who was identified as an outlier in FMD analyses.

Table 3  Effect of post-release diameter measurement method in FMD

<table>
<thead>
<tr>
<th>Method</th>
<th>FMD (%)</th>
<th>Difference from true peak FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-pregnant (n = 28)</td>
<td>Pregnant (n = 24)</td>
</tr>
<tr>
<td>Peak FMD</td>
<td>10.7 ± 3.8</td>
<td>9.6 ± 3.8</td>
</tr>
<tr>
<td>40–60–80 s maximum</td>
<td>9.6 ± 3.7</td>
<td>8.6 ± 3.6</td>
</tr>
<tr>
<td>45 s</td>
<td>9.0 ± 3.5</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>60 s</td>
<td>8.0 ± 4.0</td>
<td>8.0 ± 4.2</td>
</tr>
<tr>
<td>55–65 s average</td>
<td>8.1 ± 3.7</td>
<td>8.0 ± 4.3</td>
</tr>
</tbody>
</table>

Values are means ± S.D. *P < 0.001, significant difference from peak FMD; †P < 0.01, significant difference from the 40–60–80 s maximum method; ‡P < 0.05, significant difference from non-pregnant.
this hypothesis, we observed that baseline shear rate was elevated in pregnant women compared with non-pregnant controls. However, substantial reductions in shear rate during 5 min of distal cuff inflation only decreased brachial artery diameter by $-1.9 \pm 3.1\%$ in pregnant women (Figure 2). This means that while baseline diameter under normal shear rate conditions was 0.427 mm greater in pregnant compared with non-pregnant women, virtual abolition of baseline shear stimulus had little impact on diameter, and it remained 0.363 mm greater in pregnant women compared with baseline diameter in non-pregnant women ($P < 0.001$). In other words, 85% of the difference in diameter could not be explained by the elevated shear stimulus in pregnancy.

It is possible that 5 min was insufficient for shear-mediated vasodilatory mechanisms to fully dissipate. The time-course of the off-transition of shear-mediated dilation has not been systematically evaluated. However, graphs of sample [18,35,36] and group mean [37,38] data suggest that more than half of the FMD response in healthy adults has reversed within 60–120 s of peak diameter. A study that performed repeated brachial artery FMD trials in healthy subjects reported that diameter generally returned to baseline levels within 12 min of cuff release [38]. Systematic studies are needed. However, when combined with the observation that 5 min of very low shear eliminated only 15% of the difference in diameter, these observations suggest that shear-mediated vasodilation contributes minimally to pregnancy-induced increases in conduit artery diameter.

Our present results could be explained by an arterial remodelling contribution to increased artery size in response to pregnancy. That such remodelling could take place within the time frame of pregnancy is supported by the following findings. First, intermittent increases in shear stress by upper-body exercise training increase brachial artery diameter in healthy, untrained individuals [39] and athletes [40] within 3 months. Chronic increases in rat mesenteric artery blood flow cause outward remodelling to normalize shear stress within 1 week [41,42]. Studies examining arterial structure throughout gestation are needed to investigate the mechanisms regulating structural remodelling in pregnancy.

**FMD and its stimulus are not enhanced in pregnancy**

Shear rate is a function of blood velocity/vessel diameter. We observed increased brachial artery diameter and increased baseline shear rate in pregnant women. By themselves, these changes would be expected to reduce the reactive hyperaemia-evoked increase in the brachial artery shear stimulus. This would have critical implications for interpretation of FMD responses in pregnancy. The reactive hyperaemia flow was increased with pregnancy, indicating an increase in the forearm resistance vessel dilatory response to occlusion. However, the shear stimulus for FMD was not different in the pregnant group as the brachial artery diameter was larger.

Another important consideration for valid assessment of FMD in pregnancy is the potential confounding effect of increased brachial artery diameter on FMD. Thijssen et al. [14] proposed that artery diameter affects the FMD response to shear, such that larger arteries have smaller FMD. This would suggest that the observation of similar FMD in pregnant compared with non-pregnant women in the present study actually represents improved FMD in pregnancy given that brachial artery diameter was larger in the pregnant group. However, careful consideration of the previous literature argues strongly against this. First, the conclusion by Thijssen et al. [14] is based on comparison of FMD between different arteries of different size (radial compared with brachial compared with popliteal compared with femoral). When their data are analysed across arteries there is a reduced responsiveness to shear in larger compared with smaller arteries. However, when their data are examined in terms of different artery sizes within the same artery (brachial compared with brachial between subjects), greater dilation in the smaller brachial arteries can be accounted for by the greater shear stress they experience in a reactive hyperaemia test. In fact, three other studies support this phenomenon. Silber et al. [16,17] and Pyke et al. [15] found that smaller arteries are more likely to experience a greater shear stimulus with reactive hyperaemia. When shear stimulus is either controlled so that it is the same across artery sizes [15] or when shear stimulus differences are accounted for [15,16,43], the effect of artery size on FMD disappears. In the present study, reactive hyperaemia shear stimulus and diameter were not correlated in individual subject groups, and were only weakly correlated in pooled active subjects (Figure 3). Consistent with the contention that artery size within the same artery does not affect FMD, we found that there was no relationship between baseline diameter and FMD in any subject group or in pooled inactive ($r^2 = 0.09, P = 0.091$) or pooled active ($r^2 = 0.00, P = 0.961$) women. Instead, our results support the hypothesis [15,16,43] that smaller arteries experience greater FMD only if they are subjected to a greater shear stimulus than large arteries. Furthermore, they extend this to comparisons of FMD in pregnant compared with non-pregnant populations where artery diameter differences are the result of a specific physiological condition.

In summary, rigorous quantification of the shear stimulus responsible for FMD, and its interaction with artery size allows us to conclusively interpret FMD responses between our study groups independent of potential confounding effects of altered shear stimulus or artery diameter. This is the first study to do so, and our data establish that pregnancy does not alter endothelial function in terms of FMD responses to elevated shear stimulus.
Importance of accounting for menstrual phase and time course of FMD

Previous studies have shown that FMD is significantly increased [2,7] or is not different [8–10] at 28–35 weeks of gestation compared with non-pregnant controls. These inconsistent results could be an artefact of two aspects of study design, which may have caused previous studies to underestimate FMD in non-pregnant women, and overestimate the effect of pregnancy, by variable amounts. First, previous studies did not control for menstrual cycle phase [2,7,9,10] or tested non-pregnant women in the early follicular phase [8]. Two longitudinal studies demonstrated that FMD varies during the menstrual cycle in healthy young women [19,20]. The first observed increases in FMD during the late follicular (18.2 ± 0.8 %) and luteal (17.5 ± 0.7 %) phases compared with the early follicular (11.2 ± 0.6 %) phase [19]. The second reported significant decreases in FMD during the early luteal phase (4.2 ± 0.6 %) compared with the early follicular (8.8 ± 0.6 %), late follicular (10.0 ± 0.7 %) and late luteal (8.6 ± 0.9 %) phases [20]. These documented effects of menstrual cycle phase on FMD suggest that including women in the early follicular and early luteal phases could decrease the observed FMD response in the non-pregnant group, increasing the magnitude of the difference between pregnant and non-pregnant women. We tested controls in the mid- to late luteal phase to isolate the effect of pregnancy beyond that of acute hormone changes. Our results confirm that pregnancy does not alter FMD.

Secondly, previous studies did not account for delayed peak dilation during pregnancy (57 ±15 s compared with 46 ± 16 s) by using continuous measurements to identify peak diameter. Our study demonstrates that the 60 s and 55–65 s average methods underestimate FMD to a significantly greater extent in non-pregnant than in pregnant women (2.7 % compared with 1.7 %; Table 3) because post-release diameter is measured near peak in pregnant women, but after diameter has declined in non-pregnant women. This systematic bias in some previous studies may have contributed to erroneous conclusions that FMD is increased in pregnancy. In accordance with this hypothesis, the study that used the 60 s method reported the lowest FMD in non-pregnant women, and the largest difference between pregnant and non-pregnant women (non-pregnant: 5.8 ± 2.1 %, 32 weeks: 11.2 ± 5.5 %) [7]. When this bias is eliminated using continuous post-release diameter measurements, FMD does not differ between pregnant and non-pregnant women.

Shear, FMD and activity level

In the present study, regular exercise did not alter the shear stimulus or brachial artery FMD in healthy non-pregnant or pregnant women. Additional studies with larger data sets are warranted to confirm this. FMD and shear rate AUC were positively correlated in active women ($r^2 = 0.13, P = 0.030$), but not in inactive women ($r^2 = 0.02, P = 0.593$). This suggests that regular exercise alters the strength of the relationship between FMD and shear rate AUC. Further research is required to determine whether this reflects a real physiological effect or was due to the smaller sample size in the inactive groups.

Limitations

The present study has two important limitations that should be considered. First, current guidelines recommend the subjects fast for 6 h prior to FMD measurement. Unfortunately, this was not possible as pregnant women need to eat regularly during late gestation. We standardized the content and timing of the pre-test meal to mitigate potential effects. However, it is possible that pregnancy-induced changes in lipid and lipoprotein metabolism may have influenced the effect of the standard meal on FMD.

Secondly, it is possible that small differences in FMD could not be detected given the sample size of the present study. Detection of between-group differences of 50, 40, 35 and 30 % with 80 % power, and $\alpha$ of 0.05, would require 11, 16, 21 and 28 subjects/group respectively. We could have detected differences of <35 % between pregnant and non-pregnant women (pooled for activity), and differences of <40 % between active and inactive women (pooled for reproductive status). Detectable differences for comparisons of the four subgroups range between 40 and 60 %, and are lowest for comparisons between inactive pregnant and inactive non-pregnant women. Larger studies are needed.

Conclusions

Shear-mediated dilation contributes minimally to increased resting brachial artery diameter in pregnancy. Instead, it is likely that structural remodelling is an important contributor, although future studies are required to confirm this.

Brachial artery FMD is not altered by pregnancy at 30–36 weeks of gestation. Furthermore, activity level has no effect on FMD in pregnant or non-pregnant women, although increased activity appears to strengthen the association between reactive hyperaemic shear stimulus and FMD.

Finally, pregnancy leads to a delay in the time-to-peak FMD, and failure to account for this and menstrual cycle effects on FMD could explain previous findings [2,7] of increased FMD in pregnancy.

AUTHOR CONTRIBUTION

Tracey Weissgerber designed and conducted the study, analysed the data, and drafted and edited the paper.
Gregory Davies contributed to the study design, provided medical clearance for each pregnant woman to participate, and edited the paper. Michael Tschakovsky contributed to the study design, data analysis and data interpretation, wrote sections of the paper, and edited the paper.

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Supplementary Online Data

Brachial artery flow-mediated dilation is not affected by pregnancy or regular exercise participation

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Table S1  Subject physical activity characteristics
Values are means ± S.D. Results were available for seven (∥) and nine (***) out of 11 inactive non-pregnant subjects. *P < 0.01 and §P < 0.05, significant difference from non-pregnant group of same activity level; †P < 0.01 and ‡P < 0.05, significant difference from active group of same reproductive status; ¶P < 0.01, significant difference from pre-conception. N/A, not applicable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active non-pregnant</th>
<th>Inactive non-pregnant</th>
<th>Active pregnant</th>
<th>Inactive pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>63 ± 9</td>
<td>78 ± 6†</td>
<td>86 ± 9*</td>
<td>94 ± 11†</td>
</tr>
<tr>
<td>Exercising heart rate (beats/min)</td>
<td>127 ± 15</td>
<td>131 ± 10</td>
<td>142 ± 13*</td>
<td>145 ± 16§</td>
</tr>
<tr>
<td>Work rate during exercise (Watts)</td>
<td>87 ± 34</td>
<td>59 ± 23‡</td>
<td>73 ± 18</td>
<td>52 ± 11†</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Mean voluntary physical activity (kcal))</td>
<td></td>
<td>529 ± 297</td>
<td>15 ± 41‡</td>
<td>264 ± 132</td>
</tr>
<tr>
<td>Sports and Exercise Index</td>
<td>4.3 ± 0.4</td>
<td>1.5 ± 0.6‡</td>
<td>4.4 ± 0.4</td>
<td>2.6 ± 1.3†</td>
</tr>
<tr>
<td>Preconception/non-pregnant**</td>
<td>N/A</td>
<td>N/A</td>
<td>4.1 ± 0.3</td>
<td>1.4 ± 0.5¶</td>
</tr>
</tbody>
</table>

Table S2  Subject physical activity characteristics
Values are means ± S.D. unless otherwise indicated. There were no significant differences between groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active pregnant</th>
<th>Inactive pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.9 ± 1.2</td>
<td>39.5 ± 1.3</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3616 ± 336</td>
<td>3541 ± 292</td>
</tr>
<tr>
<td>Infant sex (% male)</td>
<td>40%</td>
<td>44%</td>
</tr>
</tbody>
</table>

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