Low flow-mediated constriction occurs in the radial but not the brachial artery in healthy pregnant and nonpregnant women

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The objectives of this study were therefore 1) to determine whether L-FMC is artery independent in healthy women and whether this is impacted by pregnancy or physical activity levels, 2) to determine the shear rate sensitivity of L-FMC and whether this is impacted by pregnancy or physical activity levels, and 3) to determine if the magnitude of L-FMC is related to the magnitude of FMD.
METHODS

Participants were 15 active pregnant (AP), 8 inactive pregnant (IP), 17 active nonpregnant (ANP), and 10 inactive nonpregnant (INP) women who completed a larger study examining the effects of pregnancy exercise on endothelial function. All women were healthy, nonsmokers, between the ages of 20 and 40 yr old, with no history of pregnancy-induced hypertension, who were not taking hormonal contraception or other medications. Subjects were not born following a pregnancy complicated by preclampsia. Pregnant women were between 30 and 36 wk gestation. Nonpregnant women had regular menstrual cycles and were in the mid-luteal phase (days 20–28).

Active women had exercised for three or more hours per week for at least 6 mo, while inactive women did not regularly exercise at a sufficient intensity to break into a sweat. The Health Sciences Research Ethics Board at Queen’s University approved the study, and all subjects provided written informed consent before participating.

Pretest screening. Nonpregnant 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 standardized form (PARmed-X for Pregnancy, http://www.csep.ca/forms.asp) with their obstetrician or midwife to obtain medical clearance. An abdominal ultrasound was performed to ensure that the subject was having a normal, healthy pregnancy and the fetus was not small for gestational age. An obstetrician (G. A. L. Davies) provided final approval for testing in pregnancy after reviewing each screening form and ultrasound report.

Activity index. Women completed a 3-day physical activity record (3) on consecutive days (2 weekdays, 1 weekend day) within 2 wk of the test to evaluate current physical activity. Mean voluntary physical activity (MVPA) was calculated as described previously (3).

Endothelial function assessment. Women avoided caffeine and exercise for 12 h before the test and consumed a standard meal (350 kcal) at 7 AM. After arriving at the laboratory at 8 AM, women were seated in a semidarkened room with the right arm resting on a table at the level of the heart. A child-sized blood pressure cuff attached to an automatic inflation device was wrapped around the extended right wrist and hand. Beat-to-beat blood pressure measurements were obtained using a Finapres (model 2300, Ohmeda) photoplethysmographic cuff placed around the left middle finger of the opposite hand. Continuous, gray-scale images of the radial artery were obtained using a 10-MHz probe operating in B-Mode (GE Vingmed System 5, GE Medical Systems). Simultaneous blood flow velocity measurements were recorded using Doppler ultrasound, with the same probe operating at 4 MHz. An insonation angle of 68° was maintained throughout each trial, to allow for accurate velocity measurements while optimizing image quality by ensuring that the ultrasound beam was perpendicular to the vessel (19). The probe was positioned over the radial artery between 10 and 15 cm proximal to the cuff, wherever the clearest image was obtained. Baseline data were recorded for 1 min, after which the occlusion cuff was inflated to 250 mmHg for 5 min. Measurements were collected for 2 min postrelease.

The left arm was then positioned on a table at heart level. A large blood pressure cuff attached to an automatic inflation device was placed on the forearm distal to the elbow. The ultrasound probe was positioned over the brachial artery between 2 and 8 cm proximal to the cuff, wherever the clearest image was obtained. Resting brachial artery diameter was determined during a 1-min scan, after which the occlusion cuff was inflated to 250 mmHg for 5 min. Measurements were collected for 2 min postrelease. The right arm was then repositioned on the table at the level of the heart, and the radial artery reactive hyperemia protocol was repeated.

During each trial, artery diameter and blood flow velocity measurements were collected during 1 min before cuff inflation, the last minute before cuff release, and for 2 min following release (Fig. 1). Ultrasound images of brachial artery diameter and velocity were recorded on videotape, then transferred to a Digital Imaging and Communications in Medicine file at a rate of 25 frames/s for offline analysis (43).

Hematocrit (Hct) was determined in a subset of five active pregnant, five active nonpregnant, and one inactive nonpregnant women. A venous blood sample was collected in an EDTA-coated tube. Hct was measured at the Kingston General Hospital Core Laboratory. Viscosity was calculated using the formula for high shear rates (208 s–1), viscosity = 0.12 × Hct (%) + 0.17 × (p – 2.07), where p is plasma protein (in g/dl) (6, 40). Published plasma protein values from our laboratory [luteal phase, 6.6 g/dl (29); third trimester, 6.2 g/dl (14, 17)] were used. Shear stress was calculated as 4 × viscosity × shear rate (32).

Exercise test. Women sat quietly for 10 min while 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 collected. After a 3-min warm-up on the cycle ergometer (Sensor Medics model 800S, Cardinal Health), women completed a 90-s ramp work rate increase until a rating of perceived exertion (RPE) of 13 on the 6–20 Borg scale was reached. This intensity was maintained for 20 min. RPE was the primary indicator of intensity as RPE during weight-supported exercise is not effected by pregnancy (42). Intensity was confirmed by a steady-state heart rate of ~130 beats/min in nonpregnant subjects, and 140 beats/min in pregnant subjects, to account for the pregnancy-induced 10–15 beat/min increase in resting heart rate (42).

Data analysis. Artery diameter was measured as described previously using automated edge-detection software (FMD/blood flow...
acquisition and analysis) (43), which acquires continuous diameter measurements within a user-defined region of interest. Preinflation and inflation, artery diameters were defined as the median of all measurements obtained during the minute before cuff inflation, and the minute before cuff release, respectively. Peak diameter, and the time required to reach peak diameter, were determined by applying an automated algorithm (2) to all measurements collected within 2 min of cuff release. The algorithm identifies peak diameter on a smoothed postrelease diameter curve generated by calculating the median diameter during each time period from 100 data points, with an overlap of 20 data points for consecutive time periods (2). Blood flow velocity from the time of cuff release to the time of peak diameter was quantified using the same automated analysis program, which tracks the peak envelope of the velocity waveform within a user-defined region of interest (2, 43). Time aligned, 2-s averages for velocity and diameter were computed for all time periods of interest (baseline, last minute of inflation, time of cuff release until the time of peak dilation). Shear rate (a measure of the shear stimulus without accounting for viscosity) was calculated as velocity/diameter for each 2-s period.

Statistical analysis. Change in mean, typical error (the within-subjects SD, after accounting for changes in the mean), and intraclass correlation coefficients of radial artery diameter and L-FMC measurements between trials 1 and 2 were calculated using a spreadsheet program (Reliability spreadsheet, www.sportststats.org). A one-sample t-test was used to determine whether the change in the mean diameter and L-FMC between trials was significantly different from 0.

Differences between active and inactive groups in menstrual cycle day and gestational age at the time of the study were determined using independent-samples t-tests. Comparisons of MVPA in active and inactive subgroups were performed using Wilcoxon’s rank-sum test. The effects of pregnancy and activity on all remaining subject characteristics and physical activity indexes were assessed using a two × two ANOVA with pregnancy (nonpregnant vs. pregnant) and activity (active vs. inactive) as between-subjects factors.

Two active nonpregnant subjects were removed from calculations of means, SDs, and ANOVAs for comparisons between the brachial and radial arteries due to poor image quality in the brachial artery during occlusion and postcuff release. Data from these subjects was displayed on graphs. All variables were normally distributed. The effects of pregnancy on subject characteristics, radial artery L-FMC, shear rate AUC, and time to peak diameter were assessed using independent-samples t-tests. Radial artery measurements were the average of values obtained in reactive hyperemia trials 1 and 2. The effects of pregnancy, activity, artery (brachial vs. radial), and cuff inflation (preinflation vs. inflation) were assessed using repeated-measures ANOVA, with artery and cuff inflation as within-subjects factors, and activity and pregnancy as between-subjects factors. In cases where comparisons between active and inactive subgroups revealed no significant main effects of activity or interactions with activity, active and inactive groups were pooled. Where significant main effects were present, simple main effects were determined using paired- or independent-samples t-tests with the Sidak correction for multiple comparisons. Relationships between L-FMC, FMD, and changes in shear rate in the brachial and radial arteries were assessed using Pearson correlation coefficients. High Cook’s distance and centered leverage values were used to identify outliers that had a disproportionate influence on the slope of the regression line, and strength of the correlation. These points were excluded from regression analyses but are shown on graphs. Statistical significance for all analyses was determined by a two-sided P value < 0.05. All analyses were performed in SPSS 16.0 (SPSS, Chicago, IL).

RESULTS

Physical characteristics and exercise participation. Age did not differ between groups (Table 1). Menstrual cycle day of testing did not differ between active and inactive nonpregnant women. Gestational age and weight gain during pregnancy were similar in active and inactive pregnant women. Body mass index (BMI) in nonpregnant women was similar to preconception BMI in pregnant women of the same activity status. BMI was significantly greater among inactive nonpregnant women than among active nonpregnant women (P = 0.006). Although BMI also tended to be greater in inactive pregnant women than in active pregnant women, this difference did not reach statistical significance (P = 0.067). Resting heart rate was increased in pregnant women compared with nonpregnant women (main effect: P = 0.012); however, these differences only reached statistical significance in active women. Systolic and diastolic finger arterial pressure were not affected by pregnancy or activity.

Low mean voluntary physical activity demonstrated that inactive women did not exercise. Higher mean voluntary physical activity in active women compared with inactive women confirmed that exercise participation was greater in active women (Table 1). Two observations indicate that active women were also more fit than inactive women. First, the lower resting heart rate in active women compared with inactive women (main effect of activity: P = 0.003) is consistent with training bradycardia. Second, during 20 min of steady-state exercise an identical RPE of 13 represented a significantly greater work rate in active women than in inactive women of the same reproductive status.

Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active Nonpregnant (n = 17)</th>
<th>Inactive Nonpregnant (n = 10)</th>
<th>Active Pregnant (n = 15)</th>
<th>Inactive Pregnant (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>32.9 ± 4.6</td>
<td>32.7 ± 4.7</td>
<td>31.8 ± 3.6</td>
<td>30.3 ± 3.1</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>N/A</td>
<td>N/A</td>
<td>34.1 ± 1.2</td>
<td>34.2 ± 2.2</td>
</tr>
<tr>
<td>Menstrual cycle day</td>
<td>25 ± 2</td>
<td>23 ± 3</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>BMI (nonpregnant or preconception), kg/m²</td>
<td>22.5 ± 2.8</td>
<td>25.8 ± 4.7†</td>
<td>23.3 ± 3.0</td>
<td>26.2 ± 4.7</td>
</tr>
<tr>
<td>Weight gain during pregnancy, 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>Systolic finger arterial pressure, mmHg</td>
<td>122 ± 17</td>
<td>121 ± 17</td>
<td>117 ± 17</td>
<td>122 ± 22</td>
</tr>
<tr>
<td>Diastolic finger arterial pressure, mmHg</td>
<td>64 ± 10</td>
<td>68 ± 10</td>
<td>62 ± 9</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63 ± 9</td>
<td>78 ± 6†</td>
<td>86 ± 9†</td>
<td>94 ± 11†§</td>
</tr>
<tr>
<td>Mean voluntary physical activity, kcal</td>
<td>497 ± 276</td>
<td>18 ± 44†</td>
<td>264 ± 131</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td>Work rate during exercise, W</td>
<td>85 ± 34</td>
<td>57 ± 24‡</td>
<td>73 ± 18</td>
<td>52 ± 11†</td>
</tr>
<tr>
<td>RPE during exercise</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>

Data are means ± SD. Heart rate and blood pressure were measured during the preinflation baseline period of the brachial artery low flow-mediated vasoconstriction (L-FMC) trial. BMI, body mass index; RPE, rate of perceived exertion; N/A, not applicable. Significantly different from nonpregnant group: *P < 0.01. Significantly different from active group: †P < 0.01, ‡P < 0.05. Trend toward significant difference from active group: §P = 0.054.
Hemodynamic responses to reactive hyperemia. Heart rate and finger arterial pressure did not differ between brachial and radial artery trials (data not shown). Heart rate, and finger arterial pressure, increased by 0–4 beats/min (or mmHg) during cuff inflation ($P \leq 0.007$ for main effects), Although statistically significant, these results indicate that hemodynamic changes during reactive hyperemia were physiologically minimal.

Repeatability of radial artery diameter and L-FMC. High intraclass correlation coefficients (0.91–0.95) and the absence of significant changes in diameter between trials in pregnant and nonpregnant subgroups indicated good repeatability for diameter measurements (data not shown). Lower intraclass correlation coefficients indicated that L-FMC was less repeatable (nonpregnant, 0.56; pregnant, 0.86), suggesting that pooling trials may be an important strategy to reduce error.

Effects of artery and pregnancy on L-FMC. Artery diameter was significantly increased in pregnant women compared with nonpregnant controls (Fig. 2; main effect, $P < 0.001$). Radial artery diameter decreased during cuff inflation in 84% and 96% of trials in nonpregnant and pregnant subjects, did not change in 4% of trials in pregnant subjects, and increased slightly in 16% of trials in nonpregnant subjects. In contrast, brachial artery diameter decreased during occlusion in 44% of nonpregnant and 71% of pregnant subjects, remained unchanged in 4% of nonpregnant and pregnant subjects, and increased in 52% of nonpregnant and 25% of pregnant subjects.

Radial artery diameter decreased significantly during cuff inflation in all groups ($P < 0.001$); however, brachial artery diameter did not change during inflation in active and inactive nonpregnant, and inactive pregnant women (main effect of cuff inflation: $P < 0.001$; cuff inflation $\times$ artery: $P < 0.001$). There was a small (1.9%, $P = 0.014$), but statistically significant, decrease in brachial artery diameter during inflation in active pregnant women (cuff inflation $\times$ pregnancy: $P = 0.005$; artery $\times$ activity: $P = 0.035$). These results indicate that L-FMC occurred in the radial artery but did not occur in the brachial artery. Further analyses of brachial artery L-FMC were not performed. Active and inactive women were pooled for analysis of radial artery L-FMC, as there were no effects of activity. Radial artery L-FMC did not differ between pregnant and nonpregnant women ($P = 0.082$).

Effects of pregnancy and artery on shear rate. Activity had no effect on shear rate; therefore active and inactive women were pooled. Preinflation shear rate was significantly greater in pregnant women than in nonpregnant women (Fig. 3A). Shear rate during inflation did not differ between pregnant and nonpregnant women (main effect of pregnancy: $P < 0.001$; pregnancy $\times$ cuff inflation: $P < 0.001$). The reduction in shear rate during occlusion was not significantly correlated with radial artery L-FMC in individual subject groups, or among pooled pregnant, or pooled nonpregnant subjects (Fig. 3).

![Fig. 2. Brachial and radial artery diameter during distal occlusion in women. Lines represent individual subjects. Circles represent group means. A: black circles, solid lines represent brachial artery data. Open circles, dashed lines represent radial artery data. Significant difference from: preinflation, $^aP < 0.01$, $^bP < 0.05$; nonpregnant group of same activity level, $^cP < 0.01$, $^dP < 0.05$; Trend toward significant difference from nonpregnant group of same activity level, $^e0.057 \leq P \leq 0.060$. B: black circles, solid lines represent active groups. Open circles, dashed lines represent inactive groups. There was no effect of activity; therefore active and inactive groups were pooled. Significant difference from: nonpregnant, $^gP < 0.01$; brachial artery, $^hP < 0.05$. L-FMC, low flow-mediated vasoconstriction.](http://jap.physiology.org/doi/abs/10.1152/jappl.00968.2009)
Effect of pregnancy on viscosity and resting shear stress. Analyses were performed in a subset of five pregnant and seven nonpregnant women. Viscosity was lower in pregnant women than in nonpregnant women; however, this difference was not statistically significant (5.52 ± 0.33 vs. 5.10 ± 0.36, \( P = 0.064 \)). In the radial artery, baseline shear stress (\( P = 0.032 \)) and the decrease in shear stress following occlusion (\( P = 0.022 \)) were significantly greater in pregnant women than in nonpregnant women (Table 2). These effects were not observed in the brachial artery.

Effects of pregnancy and artery on FMD, and the combined index. Activity had no effect on FMD; therefore active and inactive women were pooled. Brachial and radial artery FMD did not differ between pregnant and nonpregnant women (Fig. 4; main effect of pregnancy, \( P = 0.556 \)). There was no effect of activity on the radial artery combined index (L-FMC + FMD), and values did not differ significantly between pregnant and nonpregnant women (\( P = 0.084 \)).

Relationship between radial artery L-FMC and FMD. There was a significant positive correlation between L-FMC (negative number) and FMD (positive number) in inactive nonpregnant women (\( r = 0.86, \ P = 0.002 \)), but not in active nonpregnant (\( r = 0.35, \ P = 0.167 \)), active pregnant (\( r = -0.12, \ P = 0.666 \)), and inactive pregnant (\( r = 0.48, \ P = 0.233 \)) women (Fig. 4). Radial artery L-FMC and FMD were significantly correlated when nonpregnant (\( r = 0.69, \ P = 0.001 \)) and all subject groups (\( r = 0.45, \ P = 0.001 \)) were pooled. These correlations suggest that FMD may be reduced among women who experience greater L-FMC.

![Fig. 3. Relationship between shear rate and L-FMC. Lines (A and B) and small circles (C and D) represent individual subjects. Large circles represent group means. Error bars represent SD. Black circles and solid lines represent active women. Open circles and dashed lines represent inactive women. Significant difference from: *preinflation, \( P < 0.01 \); nonpregnant, †\( P < 0.01 \); brachial artery at same time point, ‡\( P < 0.01 \).](http://jap.physiology.org/)

Table 2. Effects of pregnancy on shear stress

<table>
<thead>
<tr>
<th>Artery</th>
<th>Nonpregnant (n = 7)</th>
<th>Pregnant (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Occlusion</td>
</tr>
<tr>
<td>Brachial</td>
<td>20.8 ± 5.4</td>
<td>5.6 ± 1.4*</td>
</tr>
<tr>
<td>Radial</td>
<td>17.8 ± 5.5</td>
<td>6.4 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are mean ± SD in s\(^{-1}\). Significant difference from baseline, *\( P < 0.01 \). Significant difference from nonpregnant, †\( P < 0.05 \). Significant difference from brachial artery, ‡\( P < 0.05 \).
DISCUSSION

This is the first study to address the issue of artery specificity and shear sensitivity of L-FMC. The key findings are as follows. First, L-FMC is an artery-specific response in healthy women, occurring in the radial but not the brachial artery. Second, we were unable to detect a relationship between the magnitude of L-FMC and the magnitude of shear stimulus reduction during occlusion. Third, pregnancy and activity had no effect on L-FMC artery specificity, in relation to shear reduction. Finally, weak correlations between radial artery L-FMC and FMD suggest that FMD may be reduced among women who experience greater L-FMC.

Artery specificity of L-FMC. The present study extends the results of previous studies (11, 26, 38) by demonstrating that radial artery L-FMC also occurs in healthy pregnant and nonpregnant women, regardless of physical activity status. Mean values are within the 4 to 7% range reported for men (38) and men and women (11, 26). In contrast, brachial artery diameter did not change during forearm occlusion among active nonpregnant, inactive nonpregnant, or inactive pregnant women. Small, but statistically significant, decreases were observed in active pregnant women (−1.9%, P = 0.014).

These data are consistent with two studies reporting no change in brachial artery diameter during occlusion in healthy men and women (10, 39). The clustering of changes in mean brachial artery diameter around zero [2.4% (41), −1.1% (33), −1.7% (28)] in several other studies further supports the hypothesis that L-FMC does not occur in the brachial artery. One study reported 15% decreases in brachial artery diameter during occlusion (10). The reason for this divergent result is unclear. The absence of L-FMC in the brachial artery could reflect diminished stimulus strength, reduced responsiveness, or the activation of autoregulatory mechanisms that prevent vasoconstriction. Differences in production, receptor concentration, or smooth muscle responsiveness to endothelin-1, prostaglandins, and EDHF could also contribute to the differing brachial and radial artery responses.

The artery specificity of L-FMC demonstrates that vascular responsiveness/function observed in one artery is not necessarily reflective of that in other arteries in women, and this is not impacted by pregnancy or physical activity status. Furthermore, the manner in which dysfunction is manifested may also be artery specific. Among healthy subjects, L-FMC occurs in the radial (11, 26, 38) but not the brachial (10, 33, 39, 41).

Fig. 4. Relationship between radial artery L-FMC and flow-mediated dilation (FMD). Small symbols represent individual subjects. Large symbols with error bars represent group means and SDs. Black symbols and solid regression line represents active group; open symbol and dashed regression line represents inactive group. AP, active pregnant; ANP, active nonpregnant; IP, inactive pregnant; INP, inactive nonpregnant. *P < 0.05.
artery. The opposite results are observed in clinical patients, in whom L-FMC occurs in the brachial artery (10, 24, 39) but is attenuated in the radial artery (11). Radial artery L-FMC appears significantly reduced in hypertensive patients compared with healthy controls, and by acute smoking in patients with cardiovascular disease and hypertension (8). In contrast, brachial artery L-FMC occurred in smokers (39) and hypercholesterolemic patients (10), but not in healthy controls (10, 39). Brachial artery L-FMC was correlated with total cholesterol when healthy and hypercholesterolemic patients were pooled \((r = 0.72, P < 0.001)\) (10). Three months of lipolowering therapy attenuated brachial artery L-FMC in hypercholesterolemic patients; however, placebo administration had no effect (24). The reason for disparate direction of change of L-FMC magnitude dependent on artery in these studies is unclear, but suggests a complexity and potential localized specificity of mechanism. Future studies should test the hypothesis that vascular dysfunction is manifested as enhanced brachial artery L-FMC, but attenuated radial artery L-FMC.

Role of shear stimulus reduction in L-FMC. It has been proposed that L-FMC is caused by reduced shear stress (11). A key question then is, if L-FMC is a result of reduced shear stress, does L-FMC have a dose-response dependency as is observed for FMD (27, 30, 31). Three observations from our data argue against this. First, occlusion reduced shear rate in both the brachial and radial arteries, yet L-FMC only occurred in the radial artery. Second, radial artery L-FMC did not correlate with the reduction in shear rate in any subject group, or in pooled pregnant or pooled nonpregnant women. Third, radial artery L-FMC was not elevated in pregnancy, even though pregnant women had substantially higher baseline shear rates and therefore much greater reductions in shear rate than nonpregnant women (see Fig. 3). However, the second and third observations have limitations that should be considered. Our ability to detect a correlation between the shear stimulus and radial artery L-FMC may have been limited by the small sample size, error associated with Doppler velocity measurements, and lack of measured viscosity values to calculate shear stress. No effect of pregnancy might also be explained if pregnancy reduces artery responsiveness to low shear. But this must be stated in the context of pregnancy having been shown to enhance (7, 9), or at least not impair (34–36) FMD. Regardless, unlike FMD, in which increases in endothelially mediated vasodilator release are proportional to the increase in shear stimulus (27, 30, 31), our observations are consistent with L-FMC being an ‘all or none’ response of either reduced dilator release or increased constrictor release, or both.

These data also provide insight into the nature of the relationship between FMD and L-FMC as a function of shear. Gori et al. (12) have postulated that the magnitude of FMD observed may be a function of the level of resting shear stress-dependent vessel tone. They recently observed that following handgrip exercise, radial artery L-FMC was increased, while FMD was blunted. Previous work by McGowan et al. (22) interpreted a blunted FMD following acute exercise as an indication of endothelial dysfunction. However, Gori et al. (12) postulated that the blunted FMD is due to a more dilated “basal” state following exercise, such that there is more recruitment possible for L-FMC, and less recruitment possible for FMD. Our results demonstrating that basal shear has no impact on L-FMC or on FMD (no effect of pregnancy on FMD) argue against this. Replication of these findings in other subject groups would be beneficial to rule out a potential effect of pregnancy on shear responsiveness. However, the results of this study are not consistent with the hypothesis that basal shear and resting tone determine the reciprocal magnitude of L-FMC vs. FMD.

Effect of pregnancy and activity on L-FMC. Neither pregnancy nor regular exercise significantly affected artery specificity of L-FMC, or its magnitude. Fifty-five subjects per group would be required to detect a significant effect of pregnancy with 80% power, given the means and SDs observed in the present sample \((-4.4 \pm 4.2\% vs. -6.4 \pm 3.2\%, P = 0.082)\). Studies evaluating the clinical significance of L-FMC, and the mechanisms regulating L-FMC, are needed to determine the clinical implications of these results. However, the diminished L-FMC response that occurs in clinical populations (11) was not observed in any subject group in the present study. This suggests that this aspect of vascular function is preserved in pregnancy, and is not enhanced by exercise, or diminished by inactivity.

Muscle sympathetic nerve activity is elevated in pregnancy (13), which might influence vascular tone and function. While early studies reported decreased FMD following sympathetic activation (15), recent evidence suggests that changes in FMD depend on the method used to invoke sympathetic activation (8). The effect of sympathetic activation on L-FMC is not known. However, sympathetic activation is a systemic response, which would be expected to impact the brachial and radial arteries. The observation that L-FMC differs between the brachial and radial arteries, and that these differences are preserved in pregnancy, suggests that sympathetic activation does not cause L-FMC.

Relationship between L-FMC and FMD. A previous study suggested that L-FMC and FMD were independent (11), as single blockade of endothelin-1 type A receptors (38), prostaglandins, or EDHF (11) diminished L-FMC without affecting FMD (11, 38). Furthermore, the authors found no relationship between L-FMC and FMD in healthy or clinical subpopulations, or when all subjects were pooled (11). The present study questions these results. The weak positive correlation between L-FMC and FMD suggests that FMD is reduced among women who experience greater L-FMC. These divergent results may be due to several factors. First, the small sample sizes in the present and previous \((n = 13–20)\) (11) studies may limit the ability to detect relationships in individual subpopulations. Second, the relationship between L-FMC and FMD may differ between clinical and healthy subjects, resulting in no correlation when both populations were pooled (11). Third, two trials were averaged in the present study to derive each subject’s L-FMC and FMD, which may have reduced response variability and improved sensitivity for detection of a correlation. The mechanistic evidence supporting the independence of L-FMC and FMD has significant limitations. Single blockade studies do not account for interactions, and may not have included all factors that contribute to L-FMC. Our observations were characteristic of greater L-FMC resulting in lower FMD. One explanation for such a relationship is that the L-FMC response mechanisms are still active when peak FMD occurs. Unless they are completely reversed before this, then the magnitude of L-FMC must negatively impact FMD. The duration of L-FMC needs to be determined to understand how these mechanisms may impact FMD. Comparisons of FMD between subjects that...
do, and do not, experience L-FMC are only meaningful if these tests are independent.

Limitations. There are four limitations to the existing study. First, this study focused on women, and different results may be observed in men. However, mean radial artery L-FMC values are within the -4 to -7% range reported for men (38) and men and women (11, 26). Similarly, mean brachial artery “L-FMC” values in the present study were similar to values reported in previous studies in men (10, 33) and men and women (10, 33, 39, 41). This suggests that similar results will be observed in men. Second, L-FMC may have occurred in the brachial artery, but been below the detection limit of the ultrasound equipment and analysis software used in this study. Functional MRI can detect smaller changes (37), but is less accessible and requires more technical expertise. Third, brachial and radial artery L-FMC were measured in different arms. Brachial artery FMD does not differ between the dominant and nondominant arms in healthy men (1) and medicated hypertensive patients (23). Unilateral handgrip training improves FMD in the trained arm, but not the untrained arm (1, 23). No subject in the present study participated in handgrip training, or sports which train one arm to a greater extent than the other (i.e., tennis) during the past year. However, different results could occur in the radial artery, in pregnant and nonpregnant women, or with L-FMC. Fourth, pregnancy-induced decreases in viscosity (16) could cause shear rate to systematically overestimate shear stress in pregnant women, relative to nonpregnant controls. Shear stress is the stimulus for FMD, however, shear rate is often used as a surrogate index because viscosity is unlikely to change during a reactive hyperemia trial (32). To investigate this potential limitation, shear stress was determined using viscosity calculated from measured hematomicrit in a subset of women. In the radial artery, both preinflation shear stress and the decrease in shear stress following occlusion were significantly greater in pregnant women than in nonpregnant women. Therefore, differences in viscosity did not effect the conclusion that the radial artery shear stimulus was greater in pregnancy.

Conclusions. This is the first study to use paired measurements to demonstrate that L-FMC is artery specific, occurring in the radial but not brachial artery of healthy nonpregnant and pregnant women. This highlights the need for caution in assuming that local measures of vascular function reflect systemic function. The finding that the magnitude of shear reduction did not influence L-FMC magnitude suggests that unlike FMD, L-FMC is not graded to shear stimulus magnitude, and that enhanced basal shear likely does not reciprocally impact L-FMC and FMD. Finally, weak correlations between L-FMC and FMD suggest that there may be an interdependence of these responses, which could be due to FMD peak occurring while L-FMC mechanisms are still active.

DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES


