Influence of hyperglycemia during and after pregnancy on postpartum vascular function

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1R. Samuel McLaughlin Foundation Exercise and Pregnancy Lab, 2Neurovascular Research Lab, 3School of Kinesiology, 4Child Health Research Institute, and 5Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, Canada

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Davenport MH, Goswami R, Shoemaker JK, Mottola MF. Influence of hyperglycemia during and after pregnancy on postpartum vascular function. Am J Physiol Regul Integr Comp Physiol 302: R768–R775, 2012. First published January 25, 2012; doi:10.1152/ajpregu.00115.2011.—Endothelial dysfunction is commonly observed in women with a previous diagnosis of gestational diabetes mellitus (GDM). Whether arterial stiffness is also related to pregnancy and/or postpartum glucose intolerance has not been determined. We examined the influence of GDM during pregnancy and hyperglycemia in the postpartum period on arterial function. Thirty postpartum women were stratified into one of three groups: 1) normoglycemic pregnancy, normoglycemic postpartum (NORM), 2) GDM during pregnancy, normoglycemic postpartum (GDM-N); and 3) GDM during pregnancy, hyperglycemic postpartum (GDM-H). Ten never-pregnant controls were also recruited (Control). All measures were made at 2 mo postpartum or in the early follicular phase in Control women. Arterial stiffness was assessed by pulse wave velocity (PWV) and brachial and carotid artery distensibility. Endothelial function was determined by flow-mediated dilation (FMD). PWV was not different between the four groups. Distensibility of the brachial and carotid arteries was lower in GDM-N women (brachial: 1.1 × 10^{-3} mmHg^{-1} ± 3.6 × 10^{-3}; carotid: 2.0 × 10^{-3} ± 3.3 × 10^{-4}) and GDM-H (brachial: 1.4 × 10^{-3} mmHg^{-1} ± 4.1 × 10^{-4}; carotid: 1.8 × 10^{-3} mmHg^{-1} ± 5.0 × 10^{-4}) compared with NORM women (brachial: 3.4 × 10^{-3} mmHg^{-1} ± 7.0 × 10^{-4}; carotid: 3.9 × 10^{-3} ± 7.4 × 10^{-4}). However, only brachial artery distensibility returned to control levels by 2 mo postpartum in the NORM women. FMD was lower in previously GDM women (GDM-N: 4.1% ± 2.3%; GDM-H: 4.4% ± 0.9%) compared with NORM women (10.8% ± 1.3%; P < 0.01). These findings suggest that the vascular function of women in the early postpartum period is influenced by GDM during pregnancy and the persistence of clinical and/or subclinical hyperglycemia after delivery.

arterial stiffness; endothelial function; gestational diabetes; postpartum

type 2 diabetes (T2D) is a well-established risk factor for the development of cardiovascular disease (17). However, impaired glucose tolerance (IGT), which is characterized by less severe hyperglycemia, also elevates the risk of developing cardiovascular disease and subsequent morbidity and mortality (14). Several mechanisms have been suggested that link altered glucose metabolism and the development of cardiovascular disease. IGT and T2D increase the stiffness of both muscular (brachial and femoral) and elastic (carotid) arteries (25), in part as a result of remodeling of the vasculature that leads to the development of cardiovascular disease (43). Elevated levels of low-density lipoprotein (LDL)-cholesterol, homocysteine, and oxidative stress may also play a role in the development of cardiovascular disease (CVD) in the presence of hyperglycemia (4, 9, 38).

Alternatively, pregnancy is associated with positive cardiovascular adaptations such as increased stroke volume (SV), heart rate (HR), and cardiac output (Q) and decreased total peripheral resistance (TPR) designed to promote the convective delivery of oxygen and nutrients to the developing fetus (7, 11). As part of this adaptation, muscular (brachial) artery stiffness, as measured by carotid-radial pulse wave velocity (PWV), is decreased with pregnancy (28, 39). In contrast to the peripheral vasculature, the carotid artery has been found to stiffen during pregnancy (30). Several studies have indicated that some of these pregnancy-induced adaptations persist past one year after delivery (7, 11). A longitudinal study by Clapp and Capeless (11) demonstrated that left ventricular volume, Q, and TPR remain altered 1 year postdelivery. Similarly, in a cross-sectional study, Hart et al. (23) found that aortic diameter and compliance are higher in parous women, suggesting that these changes also persist past the first postpartum year.

Despite these positive adaptations during and after pregnancy, the development of glucose intolerance or gestational diabetes mellitus (GDM) has been found to be associated with decreased endothelial function and increased arterial stiffness (33, 40). GDM affects up to 18% of all pregnancies, depending on ethnicity (5). Although glycemic levels generally return to normal following delivery, women with a previous history of GDM have an increased risk of developing T2D and subsequent cardiovascular disease later in life (15). Anastasiou et al. (2) demonstrated that endothelial dysfunction remains in the early postpartum period of previously GDM women, indicating the potential for long-term consequences stemming from short durations of hyperglycemia. Although there is evidence of vascular dysfunction in women with a history of GDM, including endothelial dysfunction and arterial stiffness, despite normal glycemic status (22, 24, 26), it is unknown whether this vascular dysfunction is directly related to GDM during pregnancy or additional influences in the years following a GDM pregnancy.

An examination of vascular function immediately after delivery of a GDM pregnancy has not been conducted to determine whether there is a carryover effect into the early postpartum period. Thus the objectives of this study were to examine arterial stiffness (carotid and brachial artery distensibility, PWV) and endothelial function of J women in the early postpartum period who had either normal pregnancies or pregnancies complicated by GDM; and 2) women who had never been pregnant. It was hypothesized that indices of arterial
stiffness and endothelial function would be decreased as a function of glucose intolerance. Furthermore, we hypothesized that women who experienced a normal pregnancy would have enhanced vascular function compared with women who had never been pregnant.

MATERIALS AND METHODS

Participants

Thirty postpartum nonsmoking women were recruited into the study at 7–9 wk after delivery through physician and midwife referrals. The women were stratified into one of three groups: 1) normoglycemic pregnancy, normoglycemic postpartum (NORM; \(n = 10\)), 2) GDM during pregnancy, normoglycemic postpartum (GDM-N; \(n = 10\)), and 3) GDM during pregnancy, hyperglycemic postpartum (GDM-H; \(n = 10\)). All women were screened for GDM using a fasted 2-h oral glucose tolerance (OGTT) test during pregnancy and diagnosed if they had elevated glucose values at 2 of 3 time points (fasted \(\geq 5.3\) mmol/l, 1 h \(\geq 10.6\) mmol/l or 2 h \(\geq 8.9\) mmol/l) according to the guidelines set by the Canadian Diabetes Association (5, 6). A control group of 10 women were matched to the NORM group for current body mass index (BMI) and age, had never been pregnant, and had regular menstrual cycles.

Postpartum participants visited the laboratory at 2 mo postpartum on two separate occasions. On visit 1, participants underwent a fasted 2-h OGTT and assessment of body composition via dual-energy X-ray absorptiometry (DEXA, Lunar iDXA, GE Healthcare, Madison, WI). Age, height, current body mass, waist circumference, hip circumference, prepregnancy body mass, and pregnancy weight gain were recorded. Waist-to-hip ratio was also calculated. On visit 2, participants reported to the laboratory for their vascular assessment. They were asked to refrain from eating or drinking for 4 h before testing and refrained from caffeine or alcohol consumption and exercise for 12 h before the test. The control group reported to the laboratory once during the early follicular phase of the menstrual cycle for collection of demographic data, body composition, and vascular assessment. The protocol for this study was approved by the Human Ethics Committee of The University of Western Ontario, and all women gave written informed consent.

Assessment of Postpartum Hyperglycemia

Postpartum glucose status was assessed via a fasted oral glucose tolerance test between 7 and 9 wk postpartum. Briefly, participants reported to the laboratory after a 12-h overnight fast. After 20 min of quiet sitting, blood samples were drawn from an indwelling catheter. After the fasted sample was drawn, each participant was given a 75-gram glucose load. Samples were taken 30, 60, 90, and 120 min following the ingestion of the glucose drink. All samples were placed on ice and then immediately centrifuged at 4°C for 10 min at 3,000 rpm. Before the glucose analysis, a YSI 2300Stat plus dual analyzer (Interscience) was calibrated to stabilize the membrane current. A glucose standard of 50 ± 2 mmol/l was used as an internal standard. Potassium ferrocyanide (FeCN) was used to assess membrane permeability. A standard fasted plasma sample of known glucose concentration was run in duplicate at the beginning and end of each test to ensure quality control. Each sample (fasted, 30, 60, 90, and 120 min after glucose ingestion) was run in duplicate, with concentrations being rounded off to the nearest 0.01 mmol/l. For samples in which two consecutive analyses produced variations greater than 0.1 mmol/l, the sample was run in triplicate, and the three analyses were averaged together (all values were reported in mmol/l). The area under the curve for glucose (AUC) was calculated using the trapezoid method (1). Classification of postpartum hyperglycemia (IGT or T2D) were made based on guidelines set by the Canadian Diabetes Association (5, 6). Insulin values at each time point were analyzed in duplicate using a standard insulin radioimmunoassay kit (Coat-a-Count; Intermedico) and counted using a gamma radiation counter (Gamma 5500 counting system, Beckman Instruments). A standard was run at the start of the analyses to ensure that the proper standard curve was followed. Insulin sensitivity was determined using the Matsuda Equation (29).

The high-density lipoprotein (HDL), triglyceride (TG), and total cholesterol (TC) samples were analyzed at a large medical laboratory. The validity of these values was based on standards (samples of a known concentration) that were analyzed each time the experimental samples were tested. Samples were analyzed using a Roche Modular analyzer (Roche Diagnostics, Indianapolis, IN). Plasma LDL concentrations were calculated using the Friedewald calculation (LDL = TC − HDL) (19). The interassay variability was 1.8, 1.7, and 1.3%, for TG, TC, and HDL, respectively.

Hemodynamic Measures

Heart rate was measured using a standard three-lead ECG. The arterial blood pressure waveform was measured continuously from the right middle finger (Photoplethysmography, Finometer; Finapres Medical Systems BV) from which beat-by-beat mean, systolic and diastolic blood pressures were determined. Validation of blood pressure was made by manual sphygmomanometer measurements at the brachial artery. Cardiac output (Q) was calculated on a beat-by-beat basis using the Model Flow Method (47).

Vascular Assessment

The participant lay in the supine position for 20 min of rest before the vascular measurements. At the end of the 20-min rest period, duplicate B-mode images of the right brachial artery were recorded. Based on the ECG cycle, an average of three arterial diameters at both diastole and systole (10 MHz; B-Mode ultrasound; GE System Five; GE Healthcare) were analyzed per image. B-mode images were also obtained of the right carotid artery 1–2 cm proximal to the carotid sinus. Continuous carotid blood pressure waveforms were collected using a hand-held tonometer on the left carotid artery 1–2 cm proximal to the carotid bifurcation for approximately 3 min (Millar Instruments). All images were recorded and stored for off-line analysis.

After collection of the baseline data, brachial artery endothelial function was assessed using flow-mediated dilation (FMD). In short, brachial artery blood flow velocity was collected continuously using Doppler ultrasound (10 MHz; GE System Five; GE Healthcare) from the right arm proximal to the cubital fossa for 5 min of baseline. At the end of baseline blood flow velocity collection, the right brachial artery blood flow was occluded by rapid inflation of a blood pressure cuff placed proximal to the right elbow to 200 mmHg (at least 50 mmHg above systolic blood pressure) (13). Occlusion was maintained for 5 min. After 5 min of occlusion, the blood pressure cuff was rapidly deflated, and the reactive hyperemia was measured for 3 min. Blood flow velocity and images were collected throughout the FMD and stored offline for further analysis.

Data Analysis

Baseline hemodynamics. At the end of the 20 min of rest, a 1-min average of HR, Q, mean arterial pressure (MAP), systolic pressure (SBP), and diastolic pressure (DBP) were calculated. Further calculations included pulse pressure (PP = SBP − DBP), total peripheral resistance (TPR = MAP/Q), and systemic vascular conductance (SVC = Q/MAP).

Arterial distensibility. Brachial and carotid artery distensibility were calculated using simultaneous B-mode diameter and PP (brachial PP from the Finometer; carotid PP from Millar) measurements. The duplicate systolic and diastolic pressure and diameter values of three consecutive cardiac cycles were averaged together. Arterial distensibility was calculated according to the following equation (44):
where $CSA_i$ is systolic cross-sectional area, $CSA_d$ is diastolic cross-sectional area, $P_s$ is systolic pressure, and $P_d$ is diastolic pressure.

*Pulse wave velocity,* PWV was used as a measure of total arterial segmental stiffness (31). Upper limb PWV was determined by simultaneous ECG and finger blood pressure waveforms (ECG-finger). We assume that the R-spike of the ECG represents the initiation of the pulse wave at the heart with ventricular contraction. Transit time was measured from the R-spike of the ECG to the foot of the finger blood pressure wave from the Finometer fitted to the intermediate phalange of the middle finger in the same cardiac cycle. The distance between the sternal notch to the center of the intermediate phalange was measured along the surface of the body and PWV was calculated as distance/transit time (20, 44).

*Flow-mediated dilation,* FMD was used as a noninvasive measurement of endothelial function. An average of three arterial diameters was measured along the surface of the body and PWV was calculated between the sternal notch to the center of the intermediate phalange according to the equation (31):

\[
FMD = [(D_m - D_o)/D_o] \times 100
\]

where $D_m$ is maximal diastolic diameter, and $D_o$ is baseline diastolic diameter.

To account for the effect of shear stress, FMD was normalized to shear stress (35, 36):

\[
\text{Normalized FMD} = \frac{\text{peak FMD}}{\text{AUC}_{SR}}
\]

where AUCSR is the area under the curve for shear stress from cuff release until peak dilation of the brachial artery, and peak FMD is the maximal dilation of the brachial artery after cuff release.

**Statistical Analysis**

All data were expressed as means ± SE unless otherwise stated. Descriptive values between groups were performed using a one-way ANOVA. Tukey’s post hoc analysis was used to compare means when main effects were found to be significant using ANOVA. The strength of the association measure of arterial stiffness, endothelial function, and glucose tolerance were determined using the Pearson product-moment correlation coefficient. In addition, the association between glucose AUC and other metabolic variables were assessed.

The effect of group on vascular health (FMD, PWV, and carotid/brachial distensibility) was performed by ANCOVA where the vascular health markers were the dependent variables, group was the fixed factor, and BMI, MAP, insulin sensitivity, glucose AUC, TG, HDL, LDL, or TC were covariates. Bonferroni post hoc analysis was used to compare main effects found to be significant using ANCOVA. Statistical analyses were performed using SPSS Version 16.0 (SPSS, Chicago, IL). Statistical significance was assumed at $P < 0.05$.

**RESULTS**

Participant characteristics and baseline hemodynamic parameters are presented in Table 1. Participant characteristics and baseline hemodynamic variables were not different between groups (Table 1).

Glucose values were higher in the GDM-H at rest, 60, 90, and 120 min compared with NORM (Fig. 1). In addition, the GDM-H glucose value was significantly higher at 120 min than for GDM-N. Insulin sensitivity and TG were elevated in GDM-H and GDM-N compared with NORM (Table 2). Insulin sensitivity and TG were correlated with glucose AUC ($R^2 = 0.53, P < 0.01$ and $R^2 = 0.41, P < 0.01$, respectively) as well as with each other ($R^2 = 0.47, P < 0.01$).

Postpartum carotid artery distensibility was lower for the two groups affected by GDM during pregnancy (GDM-N and GDM-H) compared with the two groups not affected by GDM (Control and NORM, Fig. 2A). Furthermore, NORM had a significantly lower carotid artery distensibility than the control group ($P = 0.03$, Fig. 2A). However, the difference in distensibility was subsequently accounted for by controlling for insulin sensitivity ($F = 2.610, P = 0.118$), glucose AUC ($F = 3.723, P = 0.055$), or TG ($F = 3.655, P = 0.053$).

### Table 1. Participant characteristics and hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NORM</th>
<th>GDM-N</th>
<th>GDM-H</th>
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<tr>
<td><strong>Age, y</strong></td>
<td>33 ± 2</td>
<td>35 ± 1</td>
<td>35 ± 2</td>
<td>32 ± 2</td>
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<tr>
<td><strong>Height, m</strong></td>
<td>1.65 ± 0.02</td>
<td>1.69 ± 0.02</td>
<td>1.63 ± 0.02</td>
<td>1.64 ± 0.03</td>
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<td>Insulin use during pregnancy, %</td>
<td>0</td>
<td>50</td>
<td>60</td>
<td></td>
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<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>26.0 ± 1.1</td>
<td>27.6 ± 2.5</td>
<td>31.6 ± 4.2</td>
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</tr>
<tr>
<td>Pregnancy Weight Gain, kg</td>
<td>17.1 ± 1.1</td>
<td>14.1 ± 2.6</td>
<td>18.9 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>BMI at testing, kg/m²</td>
<td>27.9 ± 1.6</td>
<td>28.0 ± 1.5</td>
<td>29.5 ± 1.7</td>
<td>34.0 ± 3.9</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>37.7 ± 2.5</td>
<td>36.8 ± 2.1</td>
<td>39.6 ± 1.4</td>
<td>44.2 ± 3.1</td>
</tr>
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<td>Waist circumference, cm</td>
<td>95.4 ± 12.1</td>
<td>98.0 ± 13.6</td>
<td>110.1 ± 28.9</td>
<td></td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>109.2 ± 9.7</td>
<td>112.0 ± 9.3</td>
<td>122.6 ± 24.0</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.87 ± 0.06</td>
<td>0.87 ± 0.06</td>
<td>0.89 ± 0.08</td>
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<tr>
<td>HR, beats/min</td>
<td>67 ± 2</td>
<td>65 ± 3</td>
<td>69 ± 2</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Q, l/min</td>
<td>5.6 ± 0.2</td>
<td>5.5 ± 0.4</td>
<td>6.4 ± 0.3</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>128 ± 5</td>
<td>125 ± 3</td>
<td>127 ± 4</td>
<td>136 ± 6</td>
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<tr>
<td>DBP, mmHg</td>
<td>70 ± 3</td>
<td>70 ± 3</td>
<td>72 ± 2</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>90 ± 3</td>
<td>89 ± 3</td>
<td>90 ± 2</td>
<td>97.0 ± 5</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>57 ± 3</td>
<td>56 ± 3</td>
<td>55 ± 5</td>
<td>58 ± 3</td>
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<tr>
<td>TPR, mmHg/min⁻¹</td>
<td>16.4 ± 0.9</td>
<td>16.7 ± 1.3</td>
<td>14.4 ± 0.9</td>
<td>14.0 ± 1.2</td>
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<tr>
<td>SVC, l/min⁻¹/mmHg⁻¹</td>
<td>0.0622 ± 0.003</td>
<td>0.0641 ± 0.005</td>
<td>0.0714 ± 0.004</td>
<td>0.0747 ± 0.006</td>
</tr>
<tr>
<td>Normalized FMD</td>
<td>0.0042 ± 0.0018</td>
<td>0.0044 ± 0.0027</td>
<td>0.0027 ± 0.0021†</td>
<td>0.0024 ± 0.0011†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 for all groups. NORM, normoglycemic pregnancy, normoglycemic postpartum; GDM-N, gestational diabetes mellitus pregnancy, normoglycemic postpartum; GDM-H, gestational diabetes mellitus pregnancy, hyperglycemic postpartum; BMI, body mass index; GDM, gestational diabetes; HR, heart rate; Q, cardiac output; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; TPR, total peripheral resistance; SVC, systemic vascular conductance; FMD, flow mediated dilation. *Different from NORM, P < 0.05; †Different from Control, P < 0.05.
Brachial artery distensibility was also lower for the two groups affected by GDM during pregnancy (GDM-N and GDM-H; Fig. 2B) compared with the two groups not affected by GDM (control and NORM). Similar to carotid distensibility, brachial distensibility was no longer different between groups after controlling for insulin sensitivity ($F_{11005} = 0.628, P_{11005} = 0.550$), glucose AUC ($F_{11005} = 0.658, P_{11005} = 0.534$), and TG ($F_{11005} = 1.903, P_{11005} = 0.183$). Brachial distensibility was also correlated with carotid distensibility ($R_{11005}^2 = 0.172, P_{11005} = 0.02$).

Upper limb PWV was not different between the four groups (see Fig. 2C). After controlling for BMI, MAP, insulin sensitivity, glucose AUC, TG, HDL, LDL, and cholesterol the difference between groups remained nonsignificant. Endothelial function, as measured by FMD, was impaired in the GDM-H and GDM-N compared with the NORM and Control women (Fig. 3). Similar to carotid distensibility, this difference could be accounted for such that after controlling for glucose AUC, FMD was not significant between groups ($F_{11005} = 3.432, P_{11005} = 0.066$). Furthermore, the findings were not altered after normalizing FMD to AUCSR (see Table 1). FMD was positively correlated to brachial artery distensibility ($R_{11005}^2 = 0.184, P_{11005} = 0.01$).

**Discussion**

The data from the current study suggest that the vascular function of women in the early postpartum period is directly influenced by the development of GDM during pregnancy and the persistence of clinical and/or subclinical hyperglycemia after delivery. This is supported by the findings that at 8 wk postdelivery 1) distensibility of the brachial and carotid arteries was lower in previously GDM women compared with non-GDM women, 2) FMD was lower in previously GDM women.
and returned to control levels in non-GDM women, and 3) the differences between groups could be accounted for by controlling for glucose AUC and insulin sensitivity.

Glucose AUC, insulin sensitivity, and TG were found to be the three metabolic variables that were associated with arterial stiffness. However, a recent systematic review has suggested that triglycerides may not be independently related to arterial stiffness (8). TG and insulin resistance are typically found to increase simultaneously with increasing obesity and/or metabolic syndrome (21) and were related in the present study. The observed finding of a relationship between TG and arterial stiffness may simply be a result of the parallel relationship between TG and glucose AUC and insulin sensitivity rather than arterial stiffness, per se.

PWV is a measure of the speed at which a pulse wave moves through the arterial system and is inversely related to arterial distensibility (14, 31). During a normal pregnancy, aortic PWV has been found to increase (18, 30, 32) or remain unchanged (24) with increasing gestation. Women affected by GDM exhibit a marginally increased PWV compared with non-GDM women in the third trimester (40). After delivery, aortic PWV has been found to increase but has not been compared with nonpregnant women to determine whether these values return to prepregnant/nonpregnant values (18, 30, 32). In contrast, upper limb PWV appears unaffected by increasing gestation, suggesting a differential effect on central elastic (aorta) and peripheral muscular (brachial) arteries (24). The present study indicates that upper limb PWV is similar between postpartum and nonpregnant women 8 wk after delivery. Surprisingly, there were no differences between the GDM and non-GDM groups. In a previous study in nonpregnant populations, PWV increased progressively from normoglycemic to impaired glucose tolerance to T2D (41). The authors suggested that this continuum may be explained, in part, by the duration of hyperglycemia. In contrast to the PWV data, the present study found a decreased brachial artery distensibility in previously GDM women. The between-group differences were no longer apparent after controlling for glucose AUC and insulin sensitivity, suggesting that brachial distensibility is related to postpartum glycemic status persisting from development of GDM during pregnancy. These disparate data present two possibilities. The first is that the duration of the hyperglycemia in the GDM women of the present study was not sufficient to affect arterial stiffness as measured by PWV. Second, although PWV and distensibility are both measures of arterial stiffness, PWV measures total segmental stiffness including both conduit and resistance vessels, whereas distensibility measures the local (i.e., conduit) arterial stiffness. Contrasting the results of PWV and distensibility suggest that development of GDM during pregnancy and persistent clinical/subclinical postpartum hyperglycemia has a larger influence on conduit artery stiffness.

A recent longitudinal study by Mersich et al. indicated that pregnancy is associated with an increase in carotid artery stiffness that reverses in the postpartum period (30). Whether the reversal in carotid artery stiffness resulted in a return to prepregnancy levels could not be determined as pre-pregnancy and/or control measures were not done (30). This carotid artery stiffening during normal pregnancy resulted from an increase in diameter that was offset by decreased distension and increased pulse pressure (30). Visontai et al. (46) proposed that carotid artery stiffening during pregnancy is related to plasma levels of estrogen (a vasodilator) and angiotensin II (a vasoconstrictor). Arterial smooth muscle cells express receptors in varying densities to both hormones in the brachial and carotid artery. When the density of the angiotensin II receptors was increased, there was a resulting stiffening of the artery due to contraction of the smooth muscle cell (31). The present study suggests that carotid artery distensibility of women not affected by GDM is lower than that of nonpregnant women at 8 wk postpartum. These results are somewhat surprising and require further investigation as estrogen levels return to normal in the postpartum. Furthermore, previous GDM women demonstrated further increased arterial stiffness compared with non-GDM women. The underlying mechanism resulting in increased arterial stiffness in previously GDM women, regardless of current glycemic status, is not well understood. The generation of advanced glycation end products in response to hyperglycemia has been suggested to create cross bridges between macromolecules in the arterial wall, which can result in arterial stiffening (3, 17). In addition, homocysteine has been shown to increase smooth muscle cell proliferation (9) and is associated with the development of atherosclerosis in patients with diabetes (42). It is well known that women with GDM are at increased risk of developing hypertensive disorders (gestational hypertension and preeclampsia) during pregnancy. The elevated arterial stiffness present in both the carotid and brachial arteries of previous GDM women suggests a link between increased glycemic levels during pregnancy and hypertensive disorders during pregnancy. Indeed, after controlling for insulin sensitivity or glucose AUC, the between-group differences for both brachial and carotid artery distensibility were no longer evident suggesting decreased insulin sensitivity plays an important role.

FMD increases progressively during pregnancy from the first to third trimester and returns to control levels by 6 wk postpartum (37, 48). This pregnancy-induced alteration coincides with the rise and fall of estrogen during pregnancy and postpartum. Estrogen is believed to promote vasodilation through an endothelial nitric oxide (NO) synthase (eNOS)-dependent mechanism (16) that is responsible for maintaining
the vasodilatory properties of the endothelium and opposing the effects of vasoconstrictors (45, 48). Studies in animals have demonstrated that there is an increased expression of NOS in the blood vessels of pregnant animals and that cardiovascular adaptations during pregnancy increasingly rely on NO-mediated dilation throughout gestation (10, 12). When hyperglycemia is present, such as during GDM, there is an impairment in the endothelial function (33). This impairment is believed to be the result of a decrease in the bioavailability of NO (49). Several studies have suggested that there may be long-term vascular consequences to pregnancies affected by gestational diabetes (2, 22, 24, 26); however, each of these were conducted after menstruation had resumed (6 mo to 20 years after delivery) and do not provide a strong link to hyperglycemia during pregnancy. The results of the present study indicated that FMD in non-GDM women was similar to nonpregnant values at 8 wk postpartum, as would be expected because of significant drops in estrogen levels after delivery. Furthermore, women who developed GDM during pregnancy demonstrated a persistent impairment in FMD 8 wk after delivery, regardless of current glycemic status. After controlling for glucose AUC, the between-group differences in FMD were no longer evident, suggesting that both clinical and subclinical hyperglycemia continues to play a role in endothelial dysfunction of postpartum women. Indeed, Paradisi et al. (33) also demonstrated that glucose AUC but not insulin AUC accounted for 35% of the variance in the FMD of GDM and non-GDM women during pregnancy. Our data are internally consistent as brachial artery distensibility was positively correlated to FMD and is also depressed in previously GDM women. This positive correlation may suggest that the decreased ability of the arteries of previously GDM women to dilate in response to cuff occlusion may be influenced by the decreased distensibility of the brachial artery. These findings of vascular dysfunction in the brachial arteries of previously GDM women just 8 wk after delivery provide a link between pregnancy and late postpartum studies.

There are considerations that must be taken into account when interpreting these data. The first consideration is the use of a cross-sectional study design. Longitudinal assessment from prepregnancy, pregnancy, to postpartum could potentially provide a causal pathway between glycemic status, arterial stiffness, and endothelial function. However, as GDM is not usually diagnosed until the third trimester and affects 3–18% of all pregnancies (5), it is therefore logistically difficult to conduct a longitudinal study from prepregnancy until the early postpartum period in GDM women. A second consideration was the placement of the cuff during the assessment of FMD. Reactive hyperemia with the cuff placed below the elbow causes dilation that is almost entirely due to endothelial-dependent vasodilation (13). FMD assessment with the cuff placed above the elbow may include both endothelial-dependent mechanisms as well as ischemia and myogenic mechanisms (31). However, the data from the current study are similar to Anastasiou et al. (2), who measured FMD with the cuff on the forearm, thereby only measuring endothelial-mediated dilation. The authors demonstrated a FMD for the control group of 10.3%, similar to the results of the present study. In contrast, there was only a 1.6% increase for the GDM group, whereas the present study had a 4.3% increase. Therefore, our data would only underestimate the decrease in FMD associated with GDM, but the interpretation remains the same. This disparity could be due to alterations in ischemia or myogenic mechanisms during GDM. Further investigation is warranted. A final consideration is our use of the ECG R-wave as a reference for calculating upper limb PWV. If the prejection period (delay between R-wave and aortic valve opening) differs between groups, this may have affected our measurement and interpretation of these data. To address this concern, we performed a post hoc assessment of upper limb PWV calculated from the opening of the aortic valve, as determined from the upstroke of stroke volume velocity (SVV) and the foot of the Finometer waveform in a subset of women (Control: 7/10; NORM: 8/10; GDM-N: 5/10; GDM-H: 5/10). SVV was measured from the suprasternal notch by a hand-held 2 MHz Doppler ultrasound probe (Multigun, New York, NY). The PWV values calculated using the R-wave to finger PWV and SVV to finger PWV were significantly correlated $R^2 = 0.62$, and there remained no differences in the SVV to finger PWV between the four groups. Therefore, we are confident that the R-wave based PWV values are a reliable measure of upper limb PWV and our interpretation of the data remains the same.

**Perspectives and Significance**

The findings of the present study indicate that women who developed GDM have increased arterial stiffness and decreased endothelial function in the early postpartum period compared with non-GDM postpartum women resulting, at least in part, from clinical/subclinical hyperglycemia and decreased insulin sensitivity that persists in the postpartum period. As the development of cardiovascular disease is mediated, in part, through arterial stiffness and endothelial dysfunction (27, 34), it is important to determine whether the vascular dysfunction observed in this population is pathological. If so, interventions in the early postpartum period to prevent the long-term development of cardiovascular disease may be warranted.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: M.H.D., J.K.S., and M.F.M. conception and design of manuscript; M.H.D. and R.G. interpreted results of experiments; M.H.D. prepared figures; M.H.D., J.K.S., and M.F.M. performed experiments; M.H.D. analyzed data; M.H.D. and R.G. interpreted results of experiments; M.H.D. prepared figures; M.H.D. drafted manuscript; M.H.D., R.G., J.K.S., and M.F.M. edited and revised manuscript; M.H.D., R.G., J.K.S., and M.F.M. approved final version of manuscript.

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