Temporal Changes in Retinal Microvascular Caliber and Blood Pressure During Pregnancy

Samantha J. Lupton, Christine L. Chiu, Lauren A.B. Hodgson, Jane Tooher, Sanja Lujic, Robert Ogle, Tien Yin Wong, Annemarie Hennessy, Joanne M. Lind

Abstract—The microvasculature plays an important role in regulating cardiovascular changes in pregnancy, but changes in microvasculature have been difficult to document in vivo. This study objectively quantifies changes in the maternal retinal arteriolar and venular caliber over the course of healthy pregnancy. Healthy pregnant women (n=53) were recruited from Royal Prince Alfred Hospital, Sydney, Australia. Retinal images and mean arterial blood pressures (MAP) were collected at 13, 19, 29, and 38 weeks of gestation and at 6-month postpartum. Retinal vessels were analyzed and summarized as the central retinal arteriolar equivalent and central retinal venular equivalent. Central retinal arteriolar equivalent and central retinal venular equivalent were corrected for MAP. Paired t-tests were performed comparing consecutive time points, with a significance level of P<0.01. There was a decrease in MAP between 13- and 19-week gestation (P=0.001) followed by a return to baseline from 19 weeks to delivery. This was correlated by an increase in vessel caliber between 13- and 19-week gestation (central retinal arteriolar equivalent: P<0.001, central retinal venular equivalent: P=0.007) and a return to baseline from 19 weeks to delivery. There were no differences in the central retinal arteriolar equivalent or central retinal venular equivalent (both uncorrected and corrected for MAP) between nulliparous and parous women. The pattern of dilatation and constriction in the microvasculature mirrored the changes in MAP throughout pregnancy, reflecting changes in peripheral resistance. This study provides insights into physiological changes in the microvasculature throughout a healthy pregnancy. These results can be used as a baseline with which to compare the changes observed in pathological conditions of pregnancy. (Hypertension. 2013;61:XXX-XXX.)

Key Words: blood pressure ■ imaging ■ microcirculation ■ pregnancy ■ retina

Physiological changes to the maternal cardiovascular system during pregnancy include increases in intravascular volume, cardiac output, and heart rate.1 Despite these changes, it has been observed that maternal arterial blood pressure does not increase during normal gestation and may decrease slightly during midpregnancy.2 This has long been thought to be owing to a concomitant reduction in peripheral vascular resistance during normal pregnancy.3 Peripheral vascular resistance is largely determined by the microvasculature, which includes vessels between 10 and 300 μm in caliber, regulating blood pressure through alterations in vascular tone.4 However, this hypothesis cannot be verified because no studies have yet investigated how the physiological cardiovascular changes induced during pregnancy alter the maternal microvasculature in vivo and the degree to which the maternal microvasculature changes throughout the course of pregnancy. In addition, evidence exists that parity is associated with an increased risk of cardiovascular disease;5 however, no studies have investigated whether microvascular changes induced during pregnancy are different between nulliparous and parous women.

The retinal vessels can be noninvasively visualized, providing insights into the microcirculation and thus vascular tone and peripheral resistance. Retinal vessels may be ideally suited as a model to study changes to the microvasculature during pregnancy, as neither the placenta nor the retinal blood vessels are innervated by the autonomic nervous system.7,8 Advances in digital retinal photography and image analysis software have enabled the objective documentation of retinal vascular characteristics, particularly its caliber, which is well described in nonpregnant general populations consisting of healthy9 and diseased10 individuals. Retinal vessel caliber is relatively stable in healthy individuals; the arterioles constricting only subtly for each decade increase in age.11 Overt changes to the retinal arteriolar and venular caliber have been linked to subclinical cardiovascular outcomes and predict incident cardiovascular events, including stroke12 and coronary heart disease.13 Narrowing of retinal arteriolar caliber has consistently been shown to be associated with elevated blood pressure14 and this narrowing precedes the onset of clinically overt hypertension.9,15

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Thus, objective characterization of retinal vessel calibers throughout pregnancy using retinal imaging is a novel approach by which to examine the physiological changes in the cardiovascular system during pregnancy and may be useful in predicting early pathological signs of adverse maternal outcomes. This study aimed to characterize the temporal changes that occur to blood pressure as well as the caliber of the retinal arterioles and venules throughout a healthy pregnancy.

Methods

Participant Recruitment
Ethical approval for this study was obtained from the Sydney South West Area Health Service Human Research Ethics Committee (NSW, Australia) and was ratified by the University of Western Sydney Human Ethics Committee. Recruitment and follow-up were undertaken between July 2010 and January 2012 at Royal Prince Alfred Hospital, a major tertiary referral hospital in Sydney, Australia. This hospital oversaw the delivery of >5000 babies annually.16 Informed consent was obtained at recruitment, which occurred at 13±2 weeks of gestation. A clinical history was provided, including the number of previous pregnancies, previous diagnosis of a hypertensive disorder of pregnancy or gestational diabetes mellitus, smoking history, alcohol intake, and family history of cardiovascular disease. A physical examination was performed at a participant’s first antenatal visit; this included the measurement of height, weight, and blood pressure.

Exclusion Criteria
Postpartum data, including primary clinical diagnosis, were collected from medical records. Women were excluded if they miscarried or delivered elsewhere, were diagnosed with a hypertensive disorder of pregnancy according to the Society of Obstetric Medicine of Australia and New Zealand guidelines,17 were diagnosed with gestational diabetes mellitus according to the Royal Australian and New Zealand College of Obstetricians and Gynaecologists guidelines,18 had a multiple gestation pregnancy, conceived using assisted reproductive technology, had a body mass index <18.5 or >29.9 kg/m² at recruitment, were aged <18 or >40 years, had preexisting cardiovascular disease, or delivered at <36.0 weeks of gestation. Only women with retinal images collected at 2 or more time points were included in the analyses.

Retinal Image Collection and Grading
Retinal imaging was performed using a 45° nonmydriatic fundus camera (Canon CR-1 with a 10D SLR digital camera back; Canon, Tokyo, Japan). Photographs were taken at 13±2, 19±2, 29±2, 38±2 weeks of gestation and at 6-month postpartum. Women rested for 5 minutes in a darkened room before retinal photography to achieve nonpharmacological mydriasis. Retinal vessel characteristics are comparable between the right and left eye,19 as such, one image of the left eye was collected, centered on the optic disc. These images were graded using a semiautomated retinal vascular caliber measurement software program,20 which identified all retinal vessels that passed through an area between ½ and 1 disc diameter from the optic disc margin (zone B) and measured the caliber of the arterioles and venules. Retinal vascular caliber was assessed using a standardized protocol, based on the revised Knudson–Parm–Hubbard formula, as described elsewhere.21 Retinal arteriolar and venular calibers were summarized, using the 6 largest arterioles and the 6 largest venules, as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), respectively. Retinal image graders were blinded to the pregnancy outcome. To determine intergrader reliability, a subset of 40 retinal images was randomly selected and independently measured by 2 graders. One grader repeated these measurements after 2 months to determine intragrader reliability. Overall, intergrader reliability (measured by Chronbach’s Alpha) was 0.96 for CRAE and 0.95 for CRVE and intragrader reliability was 0.96 for CRAE and 0.94 for CRVE.

Blood Pressure Collection
Blood pressure was measured throughout pregnancy as part of the participant’s routine antenatal visits. Blood pressure measurements were collected by a study author or a midwife using an automated Intellisense Digital Blood Pressure Monitor HEM-907 (Omron, Canada). A cuff size appropriate for patient body mass index was chosen,22 and readings were collected from the upper right arm with the patient sitting. Three readings were collected over a period of 5 minutes and the average of these readings was calculated and used in analyses. Blood pressure data were collected at the same time points as the retinal images. Where blood pressure data were unavailable on the day of the collection of the retinal images, a blood pressure reading measured within 1 week of the retinal image was included in the analysis. The mean arterial blood pressure (MAP) was calculated using the formula MAP=DP+1/3(SP – DP), where DP and SP represent diastolic and systolic blood pressure, respectively.

Statistical Analyses
All statistical analyses were performed using SPSS version 20.0 (IBM Corp, New York). Statistical analyses of retinal vascular and MAP data were performed using paired t tests between consecutive time points, with retinal vascular calibers and MAP as continuous variables. As blood pressure has the strongest impact on retinal vascular caliber,23 retinal vascular measures were corrected for MAP by dividing the CRAE and CRVE by MAP to produce corrected CRAE (cCRAE) and corrected CRVE (cCRVE), respectively. Ratios were log-transformed before statistical analysis. Differences between time points or between groups were considered significant for P<0.01 to partially account for multiple testing.24 Data are presented as mean±SE. Women with missing data at one or more time points were excluded from the analysis at the relevant time points. In a subgroup analysis, we compared retinal vascular calibers between nulliparous and parous women and performed independent samples t tests to test differences in retinal vascular caliber between nulliparous and parous women at each of the 5 time points.

Results

Cohort Demographics
A total of 53 women were included in the analysis, of which 26 (49.0%) were nulliparous and 27 (50.9%) were parous. Age at recruitment ranged from 25.0 to 38.0 years (mean, 32.3±0.5 years) and body mass index ranged from 19.6 to 29.6 kg/m² (mean, 23.2±0.3 kg/m²). Gestational age at delivery ranged from 36.0 weeks to 41.9 weeks with birth weights ranging from 2500 to 4745 g. Further characteristics of the study population are described in the Table.

Blood Pressure Throughout Pregnancy
There was a significant decrease in the MAP between 13- and 19-week gestation, followed by a return to the baseline measurement from 19 weeks to delivery. MAP decreased between 13 (79.0±1.3 mm Hg) and 19 weeks (74.6±1.4 mm Hg) of gestation (P=0.001). There was no significant difference between 19- and 29-week gestation (P=0.05) or between 29- and 38-week gestation (P=0.05). There was no significant difference between the 13-week and the 29-week (P=0.56); 38-week (P=0.10) gestation; or the 6-month postpartum time points (P=0.29; Figure 1).

Retinal Vascular Caliber During Pregnancy
Concurrent with MAP changes was a significant increase in vessel caliber between 13- and 19-week gestation and a return to baseline from 19 weeks to delivery. There was a significant difference in the CRAE between 13 (167.0±2.3 μm) and 19
and the 29-week (P=0.92); 38-week (P=0.08); or the 6-month postpartum (P=0.30).

The results were similar for CRAE corrected for MAP (cCRAE), with a significant difference found between 13 (2.13±0.04 μm/mmHg) and 19 (2.35±0.05 μm/mmHg) weeks of gestation (P<0.001). No significant differences were found between 19 and 29 weeks or between 29 and 38 weeks of gestation (Figure 1B). No significant difference was identified between 13-week and the 29-week (P=0.66); 38-week (P=0.04) gestation; or the 6-month postpartum (P=0.45).

A significant difference was identified in the mean retinal venular caliber (CRVE) between 13 (231.2±3.1 μm) and 19 (238.1±3.0 μm) weeks of gestation (P=0.005). No significant differences were found between 19 and 29 weeks or between 29 and 38 weeks of gestation (Figure 2A). No significant difference was identified when comparing the 13-week and the 29-week (P=0.16); 38-week (P=0.72) gestation; or the 6-month postpartum (P=0.75). Results for CRVE corrected for MAP (cCRVE) were largely similar (Figure 2B).

### Relationship Between Blood Pressure, Retinal Vessel Caliber, and Parity

There were no significant differences in MAP between nulliparous and parous women at any gestational time point or postpartum (Figure 3A). There was no significant difference in the retinal arteriolar caliber (CRAE) at any time point between nulliparous and parous women (Figure 3A). Retinal venular caliber (CRVE) was not significantly different between nulliparous and parous women at any time point (Figure 4A). After adjusting for MAP, there were no significant differences between nulliparous and parous women at any time point in either the cCRAE or the cCRVE (Figures 3B and 4B).

### Discussion

This study shows that the microvasculature (retinal arteriolar and venular caliber) changes significantly over the course of a normotensive pregnancy, in concordance with blood pressure changes. Retinal arteriolar caliber (CRAE) increased by an average of 6.5 μm (3.9%) between 13- and 19-week gestation, followed by a slow constriction of the arterioles to the

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### Table. Demographics of Study Population

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*BMl indicates body mass index; CVD, cardiovascular disease; HDP, hypertensive disorder of pregnancy; and LSCS, lower segment cesarean section.

*Among parous women.

(173.6±2.1 μm) weeks of gestation (P<0.001). No significant differences were found between 19 and 29 weeks or between 29 and 38 weeks of gestation (Figure 1A). There was no significant difference between the CRAE at 13-week gestation

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**Figure 1.** Central retinal arteriolar equivalent (CRAE) and mean arterial blood pressure (MAP) throughout pregnancy and 6 months postpartum. A. Uncorrected CRAE (squares) and MAP (circles). B. CRAE corrected for MAP (cCRAE). P: 6 months postpartum. NS indicates not significant. *P<0.01, **P≤0.001.
baseline measurement from 19 weeks to delivery (Figure 2A). Retinal venular caliber (CRVE) also increased by an average of 6.6 μm (2.8%) between 13- and 19-week gestation, followed by a constriction in the caliber of the venules during the remainder of pregnancy (Figure 3A). These changes mirrored the changes in the blood pressure, with MAP decreasing by 4.6 mmHg (5.7%) between 13- and 19-week gestation, then slowly increasing throughout the remainder of pregnancy until delivery (Figures 2A and 3A).

Our study provides the first in vivo demonstration of microvascular changes in pregnancy, providing insights into the key physiological changes seen in the cardiovascular system in pregnancy and confirming a long-standing hypothesis that pregnancy changes that include increases in circulatory volume and cardiac output are tempered by a decrease in peripheral vascular resistance in the microvasculature. An increased release of nitric oxide, a potent vasodilator, has previously been described in pregnancy. The release of other vasodilatory molecules, such as prostacyclin and prostaglandins, adds to the physiological systemic vasodilatation of pregnancy. Angiotensin II, a potent vasoconstrictor, is upregulated in pregnancy; however, there is a marked resistance to the pressor effects of angiotensin II in healthy pregnancy. Therefore, the balance of vasodilatory and constrictive molecules in healthy pregnancy is tipped in favor of vasodilatation. Dilatation of the microvasculature, which makes up the bulk of the systemic vasculature, causes a decrease in peripheral resistance which is disproportional to the raised cardiac output, lowering maternal blood pressure early in the second trimester. We observed the nadir of blood pressure at 19-week gestation, followed by a rise in the MAP throughout the late second and third trimesters to approximately baseline values, in concordance with pressures previously described in pregnancy. This is the first study to document the concurrent temporal changes to the microvasculature throughout pregnancy.

It has previously been reported by some of these authors and their colleagues that blood pressure affects retinal microvasculature calibers at 26-week gestation, independent of pregnancy outcome, a finding that is similar to results in the general population. The study by Li et al measured differences in caliber at one gestational time point only and compared blood pressure in quartiles with retinal vascular caliber in quartiles. In contrast, our study examined 4 gestational time points and 1 postpartum time point, examining changes in blood pressure and caliber (as linear measures) over time. To the best of our knowledge, our study is the first to objectively document temporal changes in the microvasculature throughout pregnancy. We showed an initial dilatation during the second trimester, followed by a slow constriction in the microvasculature during the remainder of pregnancy.

Owing to the reported relationship between retinal caliber and blood pressure, this study shows that correcting the retinal caliber by MAP provides a single measurement with which to compare physiological changes during pregnancy. A relatively high value of cCRAE or cCRVE indicates time points in pregnancy where microvasculature is dilated and blood pressure is lowered, if one of MAP or retinal caliber does not behave as predicted, this would be reflected in the cCRAE or cCRVE. These measurements provide a baseline with which to compare changes that occur in pathological conditions associated with pregnancy, in a single measurement.

Parity status is thought to be associated with changes to the cardiovascular system. However, we identified no differences in the cCRAE or cCRVE between nulliparous and parous women and no significant difference in retinal vasculature between baseline and 6-month postpartum. These data suggest that changes to the microvasculature during a healthy pregnancy are not sustained beyond the course of the pregnancy, or that a short-term return to baseline measurements is experienced by the cardiovascular system.

A limitation of this study was that retinal photographs were not collected for every woman at every time point. We were also unable to collect prepregnancy retinal caliber measurements. However, because CRAE, CRVE, and MAP were not significantly different between 13-week gestation and 6-month postpartum, the 13-week time point can be used as a surrogate for a prepregnancy baseline.

In conclusion, we provide objective documentation of changes in the microvasculature during pregnancy. The pattern of dilatation and constriction which occurred in both the retinal calibers

Figure 2. Central retinal venular equivalent (CRVE) and mean arterial blood pressure (MAP) throughout pregnancy and 6 months postpartum. A, Uncorrected CRVE (squares) and MAP (circles). B, CRVE corrected for MAP (cCRVE). P: 6 months postpartum. NS indicates not significant. *P<0.01, **P≤0.001.
vessels mirrored the fall and rise in MAP throughout gestation, reflecting the degree of peripheral vascular resistance at different times throughout a healthy pregnancy, returning to baseline measures by 6 months postpartum. Retinal imaging is a unique and accurate method of measuring the microvascular changes that occur throughout pregnancy, providing insights into the microvasculature throughout a healthy pregnancy, and a baseline with which to compare the changes observed in pathological conditions of pregnancy.

Perspectives

This study is the first to objectively document changes in the caliber of the retinal microvasculature throughout pregnancy and to describe concurrent retinal vascular and blood pressure changes. The pattern of dilatation and constriction which occurred in the retinal vessels mirrored the fall and rise in MAP throughout gestation, reflecting the degree of maternal peripheral vascular resistance. A return to baseline measurements in the vasculature and blood pressure was observed at 6 months postpartum. Retinal imaging is a unique and accurate method of measuring the microvascular changes that occur throughout pregnancy, providing insights into the microvasculature throughout a healthy pregnancy. These measurements also provide a baseline with which to compare the changes observed in pathological conditions of pregnancy.

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Disclosures

None.

Figure 3. Comparison of the central retinal arteriolar equivalent (CRAE) of nulliparous and parous women throughout pregnancy and 6 months postpartum. A, Uncorrected CRAE (solid squares, nulliparous; open squares, parous) and mean arterial blood pressure (MAP) (solid circles, nulliparous; open circles, parous). B, CRAE corrected for MAP (cCRAE). P: 6 months postpartum. BP indicates blood pressure; and NS, not significant.

Figure 4. Comparison of the central retinal venular equivalent (CRVE) of nulliparous and parous women, throughout pregnancy and 6 months postpartum. A, Uncorrected CRVE (solid squares, nulliparous; open squares, parous) and mean arterial blood pressure (MAP) (solid circles, nulliparous; open circles, parous). B, CRVE corrected for MAP (cCRVE). P: 6 months postpartum. BP indicates blood pressure; and NS, not significant.
References


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