Influence of the Menstrual Cycle, Pregnancy, and Preeclampsia on Arterial Stiffness

Amy O. Robb, Nicholas L. Mills, Jehangir N. Din, Imogen B.J. Smith, Finny Paterson, David E. Newby, Fiona C. Denison

Abstract—Arterial stiffness and compliance are major predictors of adverse cardiovascular events and are influenced by female sex hormones, including estrogen and progesterone. The aim of this longitudinal study was to evaluate the effect of the menstrual cycle, normal pregnancy, and preeclampsia on central and systemic arterial stiffness. Ten healthy nulliparous women with regular menses were studied in the early and midfollicular, periovulatory, and luteal phases of a single menstrual cycle. Twenty-two primigravida pregnant women were studied throughout pregnancy at 16, 24, 32, and 37 weeks gestation and at 7 weeks postpartum. Fifteen primigravida women with preeclampsia were studied at diagnosis and 7 weeks postpartum. Augmentation index and carotid-radial and carotid-femoral pulse wave velocities were measured using applanation tonometry. Augmentation index fell during the luteal phase of the menstrual cycle (luteal phase versus periovulatory phase; P<0.05). In normal pregnancy, pulse wave velocity and augmentation index increased from 24 weeks over the third trimester (P<0.01 for both). All of the measures were increased in women with preeclampsia (P≤0.01), with augmentation index and carotid-femoral pulse wave velocity remaining elevated 7 weeks postpartum (P≤0.02). We conclude that systemic arterial stiffness undergoes major changes during the menstrual cycle and pregnancy and that preeclampsia is associated with greater and more prolonged increases in arterial stiffness. These effects may contribute to adverse cardiovascular outcomes of pregnancy and preeclampsia. (Hypertension. 2009;53:952-958.)

Key Words: hypertension • arterial stiffness • pregnancy • preeclampsia • menstrual cycle

Arterial stiffness is a key determinant of central aortic pressure and is an independent predictor of adverse cardiovascular outcomes and organ damage.1,2 Female sex affects arterial stiffness that is mediated in part via the influence of both estrogen and progesterone on arterial structure and function.3 In the prepubertal and postmenopausal years, when female sex steroids are low, women have stiffer arteries than age-matched men.4,5 During the reproductive years, female sex steroids fluctuate cyclically during the menstrual cycle and increase dramatically in pregnancy. The initial effect of pregnancy reducing arterial stiffness is well documented in both animal and human studies.6–11 However, data are conflicting concerning the effect of sex steroids on arterial stiffness during the menstrual cycle12,13 and the effect of later gestation.14,15

Augmentation index and pulse wave velocity (PWV) are the principal measures of central arterial pressure and stiffness that can be determined noninvasively using applanation tonometry. Within normotensive pregnancy, PWV is more closely associated with birth weight than mean arterial pressure, suggesting that arterial stiffness may represent maternal adaptation to pregnancy better than blood pressure.16 Preeclampsia is a common hypertensive complication of pregnancy, which causes significant maternal and fetal morbidity. Inadequate cardiovascular adaptation in early pregnancy may predate its clinical presentation,17 and it is associated with an increased long-term risk of maternal cardiovascular disease. Understanding the relationship between preeclampsia and arterial stiffness may, therefore, not only inform understanding of its pathogenesis but may also increase our understanding of the association between preeclampsia and later cardiovascular disease. The aim of the current longitudinal study was, therefore, to evaluate the effects of the menstrual cycle, normal pregnancy, and preeclampsia on central and systemic arterial stiffness.

Methods

Subjects
Healthy premenopausal nonsmoking nulliparous women (n=10) with at least a 2-month history of normal regular menstrual cycles were recruited to the study. All of the study group had confirmed ovulation, defined as day 21 serum progesterone >30 nmol/L (please see the online data supplement at http://hyper.ahajournals.org). Exclusion criteria included current or past hypertension, use of hormonal contraception, or use of regular medication. Nonsmoking healthy primigravida women with an uncomplicated singleton pregnancy (n=22) were recruited in the first trimester of pregnancy. Exclusion
criteria included current or past hypertension, the use of regular medication, and the development of complications during pregnancy. Women with a singleton pregnancy who fulfilled the diagnostic criteria for preeclampsia, as defined by the International Society for the Study of Hypertension in Pregnancy, were recruited at diagnosis (n=15). Exclusion criteria included pre-existing hypertension.

All of the subjects gave written informed consent, and the study was approved by the Lothian Research Ethics Committee and undertaken in accordance with the Declaration of Helsinki. All of the procedures followed were in accordance with institutional guidelines.

Visit Schedule
Nonpregnant women attended for 4 visits during a single menstrual cycle: early follicular (days 1 to 3), midfollicular (days 6 to 8), periovulatory (days 13 to 15), and luteal (days 20 to 22) phases. Women with uncomplicated pregnancies attended for 4 visits during pregnancy (16, 24, 32, and 37 weeks) and 1 visit at 7 weeks postpartum. Women with preeclampsia attended after diagnosis and at 7 weeks postpartum.

Study Protocol
At each visit, all of the subjects abstained from alcohol and caffeine for 12 hours and fasted for 4 hours before attendance. All of the subjects had an initial rest period of 30 minutes in a quiet, temperature-controlled room. Nonpregnant subjects rested in the supine position, whereas pregnant subjects rested in the 30° left lateral position to avoid inferior vena cava compression by the gravid uterus. In both groups, all of the subsequent measurements were done in the supine position. Heart rate, blood pressure (recorded in duplicate using an automated sphygmomanometer; Microlife 3BTO-A, validated for use in pregnancy and preeclampsia), and augmentation index were measured on all of the subjects at every visit. PWV was performed at the early follicular visit for nonpregnant subjects and at every visit in women with an uncomplicated pregnancy or with preeclampsia.

Augmentation Index
Applanation tonometry of the radial artery was performed using a micromanometer (Millar Instruments) and the SphygmoCor system (AtCor Medical) in accordance with the manufacturer’s recommendations. An aortic pulse pressure waveform was derived from the radial artery waveform via a mathematical transfer function. From this, the augmentation index (defined as the difference between the second and first systolic peaks, expressed as a percentage of the pulse pressure) was calculated (please see the online data supplement). The SphygmoCor system also reports augmentation index corrected for a heart rate of 75 bpm. The augmentation index is a measure of systemic arterial stiffness and wave reflection. Arterial blood pressure varies with respiration; thus, to cover a complete respiratory cycle, ≥2 independent analyses, incorporating 10 arterial waveforms each, were obtained and averaged from each subject.

Pulse Wave Velocity
Using the same equipment, carotid-femoral PWV and carotid-radial PWV were determined by sequential acquisition of pressure waveforms from the carotid, femoral, and radial arteries. The timing of these waveforms was compared with the R wave on the simultaneously recorded ECG to calculate the time delay. For each subject, a total of 2 consecutive waveform recordings was obtained, and the mean of 2 PWV readings was recorded.

Staff specifically trained in the technique performed all of the vascular measurements. Our interobserver and intraobserver variations have been described previously. Only measurements meeting SphygmoCor quality control criteria were accepted.

Measurement of Soluble Hormones
At each visit, peripheral venous blood was drawn from a large antecubital vein. Serum was prepared from blood collected into serum gel tubes (Sarstedt Monovette) and stored at −80°C until measurement of soluble hormones. Serum was prepared from blood collected into tubes containing heparin and stored at −20°C until measurement of soluble hormones.

Table 1. Baseline Characteristics of Study Population

<table>
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<tr>
<th>Characteristic</th>
<th>Nonpregnant Group (n=10)</th>
<th>Healthy Pregnant Group (n=22)</th>
<th>Preeclampsia Group (n=15)</th>
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<td>30±1</td>
<td>30±4</td>
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<td>66±4</td>
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<td>Gestation at delivery, d</td>
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<td>213±10†</td>
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<td>Gestation at delivery, wk</td>
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<td>30±1.4†</td>
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<tr>
<td>Birth weight, g</td>
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<td>1371±222$</td>
<td>3231±252</td>
</tr>
</tbody>
</table>

Results

Baseline Characteristics
Baseline demographics for the nonpregnant (n=10) and pregnant (women with uncomplicated pregnancy: n=22; women with preeclampsia: n=15) study groups, and longitudinal hemodynamic variables are presented in Table 1. Women with preeclampsia were classified according to gestation at presentation, into preterm (n=7; presented before 34 weeks; mean: 30; range: 24 to 34 weeks) and term (n=8; presented after 34 weeks; mean: 38; range: 36 to 40 weeks) groups, respectively. The mean gestations at delivery for women in the preterm and term groups were 30.3 weeks (range: 24.0 to 35.8) and 38.6 weeks (range: 36.4 to 40.4), respectively. Nonpregnant and pregnant groups were well matched for maternal age and body mass index; however, pregnant women were shorter in height than those in the nonpregnant group. There were no differences in systolic and diastolic blood pressures in the first trimester in women who subsequently had an uncomplicated pregnancy or developed preeclampsia (data not shown). At the postpartum visit, although blood pressure had returned to within the normal range.
range in women who had preeclampsia, it was still higher than in those who had had an uncomplicated pregnancy (P<0.004).

In women with preterm preeclampsia, 5 were taking regular labetalol and nifedipine, 1 was taking regular methyldopa and nifedipine, and 1 was receiving no antihypertensive therapy. Six of these women received antenatal betamethasone. In women with term preeclampsia, 1 was taking regular labetalol, with the remaining 7 women not receiving antihypertensive therapy. None received antenatal betamethasone. Postpartum, of the original 15 women who had developed preeclampsia, only 3 women were taking labetalol, and 1 was taking methyldopa.

**Effect of Menstrual Cycle on Augmentation Index**

Augmentation index varied over the menstrual cycle (P=0.03), with a fall in the luteal phase compared with the periovulatory phase (3.5±1.8% versus 9.9±1.8%; P<0.05; Figure 1A and 1B). There were no changes in any other recorded hemodynamic variables throughout the menstrual cycle. There was no correlation between augmentation index and serum estradiol or progesterone at any time point in the cycle.

**Effect of Normal Pregnancy on Augmentation Index and PWV**

Augmentation index was adjusted for heart rate (calculated at a heart rate of 75 bpm) because of variation in heart rate during pregnancy and postpartum (P<0.0001). Heart rate–corrected augmentation index varied with gestation in normal pregnancy (P<0.0001; Figure 2) rising toward term (16 weeks versus 37 weeks, 24 weeks versus 37 weeks, and 32 weeks versus 37 weeks; all P<0.01). Moreover, heart rate–corrected augmentation index was persistently elevated at 7 weeks postpartum compared with 16 weeks gestation (8.7±1.9% versus −3.0±2.5%; P=0.0002).

Both carotid-femoral and carotid-radial PWVs varied with gestation in normal pregnancy (both P=0.01; Figure 3A and 3B). Carotid-femoral PWV increased from 24 weeks to 7 weeks postpartum (5.0±0.2 m/s versus 5.5±0.2 m/s; P=0.0008). Carotid-radial PWV rose from 16 and 24 weeks to term (16 weeks versus 37 weeks and 24 weeks versus 37 weeks, both 6.4±0.2 m/s versus 7.0±0.2 m/s; P<0.05), and values at 7 weeks postpartum were not different from those at term (postpartum versus 37 weeks, 6.6±0.2 m/s versus 7.0±0.2 m/s; P=0.07). There was no correlation among augmentation index, carotid-femoral PWV, or carotid-radial PWV and serum estradiol or progesterone concentrations at any time point in pregnancy.

**Effect of Preeclampsia on Augmentation Index and Pulse Wave Analysis**

All of the hemodynamic variables differed between the women with preeclampsia and women with uncomplicated pregnancies at similar gestation, apart from heart rate in women with term preeclampsia (Table 2). Augmentation index, carotid-femoral PWV, and carotid-radial PWV were raised in women with both preterm and term preeclampsia compared with gestationally matched women with uncomplicated pregnancies (P<0.001 for both, Figure 2; P=0.01 for both, Figure 3A, and P=0.006 for both, Figure 3B, respectively). There were no differences in augmentation index, carotid-femoral PWV, or carotid-radial PWV between women with preterm or term preeclampsia (all P>0.05). At the postpartum visit, despite blood pressure returning to within the normal range, augmentation index and carotid-
columns, respectively; both preeclamptic groups (preterm and term, white and hatched gestation-matched controls, carotid-femoral PWV was raised in both preeclamptic groups (preterm and term, white and hatched columns, respectively; both $P<0.01$) and remained elevated postpartum (gray column; $P=0.01$). There was no difference in carotid-femoral PWV between the 2 preeclamptic groups. Data are reported as mean $\pm$ SEM. B, Effect of pregnancy, gestation, and preeclampsia on carotid-radial PWV. Carotid-radial PWV varied with gestation during normal pregnancy (circles; $P=0.01$). Compared with gestation-matched controls, carotid-radial PWV was raised in both preeclamptic groups (preterm and term, white and hatched columns, respectively; both $P<0.006$). There was no difference in carotid-radial PWV between the 2 preeclamptic groups in pregnancy. Data are reported as mean $\pm$ SEM.

Figure 3. A, Effect of pregnancy, gestation, and preeclampsia on carotid-femoral PWV. Carotid-femoral PWV varied with gestation in normal pregnancy (circles; $P=0.01$). Compared with gestation-matched controls, carotid-femoral PWV was raised in both preeclamptic groups (preterm and term, white and hatched columns, respectively; both $P<0.01$) and remained elevated postpartum (gray column; $P=0.01$). There was no difference in carotid-femoral PWV between the 2 preeclamptic groups. Data are reported as mean $\pm$ SEM. B, Effect of pregnancy, gestation, and preeclampsia on carotid-radial PWV. Carotid-radial PWV varied with gestation during normal pregnancy (circles; $P=0.01$). Compared with gestation-matched controls, carotid-radial PWV was raised in both preeclamptic groups (preterm and term, white and hatched columns, respectively; both $P<0.006$). There was no difference in carotid-radial PWV between the 2 preeclamptic groups in pregnancy. Data are reported as mean $\pm$ SEM.

femoral PWV remained elevated at 7 weeks compared with women with uncomplicated pregnancies (16.2 $\pm$ 2.5 m/s versus 8.7 $\pm$ 1.9 m/s and 6.5 $\pm$ 0.3 m/s versus 5.5 $\pm$ 0.2 m/s, respectively, $P<0.02$ for both; Figures 2 and 3A). In contrast, there was no difference in carotid-radial PWV by 7 weeks postpartum between women with preeclampsia compared with women with uncomplicated pregnancies (7.5 $\pm$ 0.3 m/s versus 6.8 $\pm$ 0.3 m/s; $P=0.08$; Figure 3B).

Discussion

PWV and augmentation index together provide a comprehensive assessment of arterial function that is highly reproducible and validated in healthy subjects and those with cardiovascular disease.\textsuperscript{20} In this longitudinal study, we have demonstrated that augmentation index decreases during the luteal phase of the menstrual cycle before rising at the beginning of the menstrual cycle. During normal pregnancy, arterial stiffness increases from the midtrimester to term. Preeclampsia is associated with increased arterial stiffness and, despite blood pressure returning to within the normal range, this persists in the immediate postpartum period. Increased arterial stiffness, therefore, seems to be a feature of preeclampsia that extends beyond pregnancy and may contribute to the adverse cardiovascular outcomes associated with preeclampsia.

The present study is the first to use the augmentation index to determine the effect of the menstrual cycle on systemic arterial stiffness. We demonstrated that augmentation index is reduced in the luteal phase of the cycle, indicating decreased systemic arterial stiffness. Previous studies have demonstrated either no change\textsuperscript{22} or an increase in compliance in the ovulatory phase compared with the follicular and luteal phases.\textsuperscript{12,13,23} A variety of factors may account for these seemingly discrepant findings but, in particular, the differing methodologies used, sample population characteristics, and timing of sampling. Our study used the augmentation index as a method of evaluating systemic arterial stiffness, whereas other studies have assessed whole body arterial compliance that combines both central and peripheral measures,\textsuperscript{13} or carotid artery compliance, a surrogate for aortic compliance.\textsuperscript{12} In our longitudinal study, we demonstrated clear differences in the augmentation index depending on the day of study. This variation will be magnified if a broader sampling window is used, as in the study by Giannattasio et al.\textsuperscript{23} Our study group was well characterized, with all of the women ovulating, as indicated by a rise in luteal phase progesterone. Despite this, neither absolute nor change in serum hormone concentrations correlated with change in augmentation index in our study. This perhaps reflects the small numbers of women in our study. Alternatively, it may imply that these hormones do not directly regulate arterial stiffness and that other intermediate factors, eg, the renin-angiotensin\textsuperscript{24} or endothelin\textsuperscript{25} systems, regulate vascular tone and augmentation pressure during the menstrual cycle.

Because of the logistical difficulties in obtaining prepregnancy data for pregnant women, we compared our pregnancy data with those obtained from the same women 7 weeks postpartum. Although many cardiovascular parameters normalize rapidly over the first 2 weeks postdelivery, many require a longer time frame to settle and probably do not fully recover to preconceptual values.\textsuperscript{26} Moreover, Bernstein et al\textsuperscript{27} demonstrated that mean arterial pressure is lower in subsequent normal pregnancies than in first pregnancies and that a shorter interpregnancy interval leads to a greater reduction in mean arterial pressure. Together these studies suggest that structural vascular changes occur in pregnancy and persist beyond the gestational period.\textsuperscript{27}

Our findings of a rise in arterial stiffness from the second trimester to term and postnatally are supportive of previous studies using brachial-ankle PWV as a composite measure of systemic and central stiffness\textsuperscript{28} and augmentation index.\textsuperscript{29} Other studies report no change in PWV with gestation\textsuperscript{30} or a general decrease in PWV and augmentation index during pregnancy.\textsuperscript{6,8,11} All of these studies had limited and wide time points, with the third-trimester visits performed earlier than in our study. These limitations may potentially explain why the subtle rise in PWV and augmentation index in the third trimester went undetected in these studies.
The relative reduction in arterial stiffness during pregnancy compared with postpartum is likely to arise from several factors. Estrogen has favorable effects on the endothelium and vascular smooth muscle cells, with many of the hemodynamic changes observed in normal pregnancy being mimicked in nonpregnant animals chronically exposed to estrogen. Both the endothelium and vascular smooth muscle cells express receptors for estrogen and progesterone through which they can regulate vascular tone. Therefore, they are likely to influence arterial stiffness through effects on mean arterial pressure, as well as structural changes to elastin, collagen, and smooth muscle in the arterial wall. Progesterone has often been thought to have opposing vascular effects to estradiol, although it has favorable vascular effects in vitro and in vivo. However, despite the increased serum estradiol and progesterone concentrations with advanced gestation, we report an increase in arterial stiffness in the third trimester that we postulate is attributed to factors other than sex steroids.

Consistent with previous cross-sectional studies, all of the variables of systemic and central arterial stiffness measured were higher in women with preeclampsia. We cannot exclude the possibility that medication influenced the collected data, because the effect of antihypertensive agents on PWV and augmentation index has not specifically been studied during pregnancy. Given that calcium channel blockers and β-blockers reduce PWV in nonpregnant populations, it seems likely that the increase in arterial stiffness observed in women with preeclampsia would have been even greater if these women were not taking antihypertensive agents.

The present study contrasts with a cross-sectional study that reported no difference in arterial stiffness assessed by augmentation index in women with a history of preeclampsia. However this study was performed on average 5 to 6 years after the index pregnancy, and it is possible that anplation tonometry is not sensitive enough to detect more subtle remote effects. Similarly, Spasojevic et al found no difference in the augmentation index between women with preeclampsia and healthy pregnant women at a 6-week postpartum visit. In our study, we performed a comprehensive assessment of arterial function and demonstrated that augmentation index and carotid-femoral PWV remained elevated at 7 weeks in women with preeclampsia compared with women with uncomplicated pregnancies.

Interestingly, carotid-radial PWV, unlike our other measures of arterial stiffness, had normalized by 7 weeks postpartum. Carotid-radial PWV is partly determined by the muscular brachial artery, whereas carotid-femoral PWV is determined by the more elastic aorta. Carotid-radial PWV is, therefore, susceptible to changes in both vascular smooth muscle tone and smooth muscle remodeling. It is, therefore, plausible that the increase in carotid-radial PWV in preeclampsia and pregnancy is in part attributed to an effect on smooth muscle function that may normalize more rapidly postpartum than any effect on the extracellular elastin-collagen matrix of the aorta. Other conditions, eg, diabetes mellitus and ageing, are known to have preferential effects on central rather than peripheral arteries, and it is, therefore, perhaps not surprising that vascular remodeling in pregnancy similarly does not occur in a uniform manner.

Carotid-femoral PWV is recognized as the gold standard measure of arterial stiffness, as stated in the recent expert consensus document on the measurement of arterial stiffness. In our cohort, carotid-femoral PWV remained elevated at 7 weeks postpartum, suggesting that the effects of pre-
Abnormalities of arterial structure and function were associated with higher postpartum blood pressures, although these women were no longer hypertensive, with blood pressures within the normal range. It is not possible from our studies to determine whether raised blood pressure during preeclampsia is a cause or a consequence of increased arterial stiffness. There is now good evidence to suggest that aortic stiffness is an independent predictor of progression to hypertension even in young nonhypertensive individuals, with endothelial function being inversely related to arterial stiffness in healthy volunteers. We, therefore, believe that in preeclampsia endothelial dysfunction increases aortic stiffness, which, in turn, causes an increase in blood pressure.

Alternative interpretations of our findings are possible, and, in particular, we cannot discount that changes in arterial stiffness occur as a consequence of prolonged hypertension in preeclampsia, nor can we be certain that changes in arterial stiffness at 7 weeks postpartum persist long term. A prospective study in which blood pressure and arterial stiffness were determined in a very large cohort of pregnant women before the onset of preeclampsia with long-term postpartum follow-up would be required to address these issues.

Perspectives
In this longitudinal study, we have demonstrated that the augmentation index decreases during the luteal phase of the menstrual cycle with arterial stiffness rising from the midtrimester of pregnancy to term. The factors regulating these changes in arterial stiffness have yet to be identified. However, preeclampsia is associated with increased arterial stiffness, and this persists into the postpartum period. Increased arterial stiffness, therefore, appears to be a feature of preeclampsia that extends beyond pregnancy and suggests an abnormality of vascular structure and function associated with this condition, perhaps contributing to its adverse cardiovascular outcomes.

Acknowledgment
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Disclosures
None.

References


INFLUENCE OF THE MENSTRUAL CYCLE, PREGNANCY AND PRE-ECLAMPSIA ON ARTERIAL STIFFNESS

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SHORT TITLE:  ARTERIAL STIFFNESS AND FEMALE REPRODUCTION
Augmentation Index
Briefly, the augmentation index derives an aortic pulse pressure waveform from the radial artery wave via a mathematical transfer function. The arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction and a reflected wave generated by peripheral vascular resistance (Figure S1).

The augmentation index, defined as the difference between the second and first systolic peaks expressed as a percentage of the pulse pressure, is a measure of systemic arterial stiffness and wave reflection.

Pulse wave velocity
The distance traveled by the pulse wave between the carotid and femoral arteries was measured using a pair of compasses to reduce the influence of altered body contours due to pregnancy. The proximal distance was measured from the sternal notch to the carotid artery and the distal distance was measured from the sternal notch to the femoral artery. The carotid-to-femoral path length was estimated by subtracting the proximal from the distal distance. The carotid-femoral PWV was then calculated as the quotient of the distance traveled by the pulse wave and the foot-to-foot time delay between the pulse waves. For carotid-radial PWV, the method of calculation was the same; however the distal distance was measured from the sternal notch to the radial artery.
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<th>20-22</th>
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<td>Augmentation Index, %</td>
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<td>9.9 ± 1.8</td>
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<td>LH (U/L)</td>
<td>5 ± 0.4</td>
<td>7 ± 0.4</td>
<td>32 ± 7.2</td>
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<td>FSH (U/L)</td>
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<td>Progesterone (nmol/L)</td>
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Table S1 Haemodynamic and Hormonal Variables of Non-Pregnant Women.

SBP, Systolic blood pressure; DBP, Diastolic blood pressure; PP, Pulse pressure, HR, heart rate; PWV, pulse wave velocity; LH luteinising hormone; FSH, follicle stimulating hormone. Data are reported as mean ± SEM.
Figure S1. An aortic pulse waveform as produced by the SphygmoCor™ system from applanation tonometry of the radial artery. Augmentation pressure is the difference between the systolic peak (forward wave) and first systolic inflection (reflected wave) pressures. This difference divided by the pulse pressure generates the augmentation index. Figure adapted from Mills et al 2008 with permission. ¹