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 pulse wave velocity (PWV) 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).18
Exclusion criteria included pre-existing hypertension.

All of the subjects had 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),19 and augmentation index were measured on all of the subjects at every visit. PWV were determined by sequential acquisition of pressure waveforms each, were obtained and averaged from each subject. The SphygmoCor system also reports augmentation index (AtCor Medical) in accordance with the manufacturer’s recommendation. The augmentation index (defined as the difference between the R wave on the simultaneous 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.21 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

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Birth body mass index was recorded during first trimester for pregnant subjects. Data are reported as mean±SEM. NA indicates not applicable.

*P<0.05 vs nonpregnant group.
†P≤0.0009 vs nonpregnant group.
‡P≤0.0003 vs healthy pregnant group.
§P<0.0001 vs healthy pregnant and term groups.

analysis. Estradiol and progesterone were measured using the Siemens Medical Solutions Centaur immunoassay system, and luteinizing hormone and follicle-stimulating hormone concentrations were measured in samples from the nonpregnant women using the Abbott Architect immunoassay system.

Statistical Analysis
Continuous variables were analyzed using the Kolmogorov-Smirnov test for normality and reported as mean±SEM. For comparisons across the menstrual cycle and within healthy pregnancy, analyses were performed using 1-way ANOVA with repeated measures and Bonferroni’s post-tests. Two-tailed paired Student t tests were used when comparing pregnant and postpartum data within a subject group. Two-tailed unpaired Student t tests were used when comparing data between different subject groups. All of the calculations were performed using GraphPad Prism (GraphPad Software). Statistical significance was taken at 5%.

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.21 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 in women who had preeclampsia, it was still higher than in those women 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 \((3.5\pm1.8\%\) versus \(9.9\pm1.8\%;\ P<0.05;\ Figure 1A and 1B)\). 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 \((8.7\pm1.9\%\) versus \(-3.0\pm2.5\%;\ P=0.0002)\).

Both carotid-femoral and carotid-radial PWVs varied with gestation in normal pregnancy \((P=0.01;\ Figure 3A\ and\ 3B)\). Carotid-femoral PWV increased from 24 weeks to 7 weeks postpartum \((5.0\pm0.2\ m/s\ versus\ 5.5\pm0.2\ m/s;\ P=0.0008)\). Carotid-radial PWV rose from 16 and 24 weeks to term \((16\ weeks\ versus\ 37\ weeks\ and\ 24\ weeks\ versus\ 37\ weeks,\ both\ 6.4\pm0.2\ m/s\ versus\ 7.0\pm0.2\ m/s;\ P<0.05)\), and values at 7 weeks postpartum were not different from those at term \((postpartum\ versus\ 37\ weeks,\ 6.6\pm0.2\ m/s\ versus\ 7.0\pm0.2\ m/s;\ P=0.07)\). 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 \((P<0.001\ for\ both,\ Figure 2;\ P\leq0.01\ for\ both,\ Figure 3A,\ and\ P\leq0.006\ for\ both,\ Figure 3B,\ respectively)\). 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-

![Figure 1. A, Augmentation index throughout menstrual cycle. Augmentation index varied during the menstrual cycle \((P=0.03)\) with a fall in the luteal phase compared with the periovulatory phase \((P<0.05)\). Data are reported as mean±SEM. B, Heart rate–adjusted augmentation index throughout menstrual cycle. There was a trend toward a reduction in heart rate adjusted \((at\ 75\ bpm)\) in the luteal compared with the periovulatory phase \((P=0.07)\). Data are reported as mean±SEM.](image1)

![Figure 2. Effect of pregnancy, gestation, and preeclampsia on augmentation index (adjusted for heart rate). Heart rate–adjusted augmentation index varied with gestation in normal pregnancy \((circles;\ P<0.0001)\) rising toward term \((P=0.01)\) and was elevated at 7 weeks postpartum compared with 16 weeks gestation \((P=0.0002)\). Compared with gestation-matched controls, augmentation index was raised in both preeclamptic groups \((preterm\ and\ term,\ white\ and\ hatched\ columns,\ respectively;\ both\ P\leq0.001)\) and remained elevated postpartum \((gray\ column;\ P=0.02)\). There was no difference in the augmentation index between the 2 preeclamptic groups \((P=0.05)\). Data are reported as mean±SEM.](image2)
carotid-femoral PWV remained elevated at 7 weeks compared with women with uncomplicated pregnancies (16.2±2.5% versus 8.7±1.9% and 6.5±0.3 m/s versus 5.5±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±0.3 m/s versus 6.8±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.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 change22 or an increase in compliance in the ovulatory phase compared with the follicular and luteal phases.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,13 or carotid artery compliance, a surrogate for aortic compliance.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.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-angiotensin24 or endothelin25 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.26 Moreover, Bernstein et al.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.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 stiffness28 and augmentation index.29 Other studies report no change in PWV with gestation30 or a general decrease in PWV and augmentation index during pregnancy.6,8,9,11 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,31 with many of the hemodynamic changes observed in normal pregnancy being mimicked in nonpregnant animals chronically exposed to estrogen.32 Both the endothelium and vascular smooth muscle cells express receptors for estrogen and progesterone33 through which they can regulate vascular tone.34 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.3,35 Progesterone has often been thought to have opposing vascular effects to estrogen, although it has favorable vascular effects in vitro3 and in vivo.34 However, despite the increased serum 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.15,16,36 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 blockers57 and β-blockers58 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.39 However this study was performed on average 5 to 6 years after the index pregnancy, and it is possible that applanation tonometry is not sensitive enough to detect more subtle remote effects. Similarly, Spasojevic et al15 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,40 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.1 In our cohort, carotid-femoral PWV remained elevated at 7 weeks postpartum, suggesting that the effects of pre-
eclampsia on vascular structure and function extend beyond pregnancy. If arterial stiffness remains elevated in later life, this may in part contribute to the increased risk of cardiovascular and cerebrovascular diseases.\textsuperscript{41}

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,\textsuperscript{52} with endothelial function being inversely related to arterial stiffness in healthy volunteers.\textsuperscript{43} We, therefore, believe that in preeclampsia endothelial dysfunction increases arterial 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 mid trimester 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**

We thank the Wellcome Trust Clinical Research Facility for their assistance with the conduct of this study.

**Source of Funding**

This work was supported by Action Medical Research Project grant SP4024.

**Disclosures**

None.

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Hypertension. 2009;53:952-958; originally published online April 27, 2009; doi: 10.1161/HYPERTENSIONAHA.109.130898

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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

Amy O Robb¹, Nicholas L Mills², Jehangir N Din², Imogen B J Smith¹, Finny Paterson³, David E Newby², and Fiona C Denison¹*

¹Centre for Reproductive Biology, University of Edinburgh, United Kingdom
²Centre for Cardiovascular Sciences, University of Edinburgh, United Kingdom
³The Wellcome Trust Clinical Research Facility, The Royal Infirmary of Edinburgh, United Kingdom.

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.
Reference:
### 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