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The pressure-dependency of local measures of arterial stiffness

Running Title: Pressure-dependency of arterial stiffness

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ABSTRACT

Objective: To determine which ultrasound-based, single-point arterial stiffness estimate is least dependent on blood pressure to improve assessment of local vascular function. Methods: Ultrasound was used to assess blood flow and diameters at the left brachial artery of twenty healthy adults [55% F, 27.9 y (5.2), 24.2 (2.8) kg/m²]. Blood pressure of both arms was measured simultaneously. Experimental (left) arm blood pressure was then systematically manipulated by adjusting its position ABOVE (+30°) and BELOW (-30°) heart level in a randomized order following measurement at heart level (0°). The control (right) arm remained at heart level. Six stiffness measurements were calculated: compliance, distensibility, beta-stiffness, and three estimates of pulse wave velocity (Bramwell Hill, blood flow, and Beta-stiffness). We considered the measurement technique with the least significant change across positions to be the least pressure-dependent. Results: There was a large effect change in mean arterial pressure (n²p = 0.75, p < 0.001) in the experimental arm when it was ABOVE (∆-4.4 mmHg) and BELOW (∆10.4 mmHg) heart level. There was a main effect (p < 0.05) of arm position on all arterial stiffness measures. From least to most pressure-dependent, the arterial stiffness measurements were: pulse wave velocity (blood flow method), compliance coefficient, beta-stiffness, distensibility coefficient, pulse wave velocity (Bramwell-Hill method), and pulse wave velocity (beta-stiffness index method). Conclusions: All single-point measures assessed are pressure-dependent. The pulse wave velocity (blood flow method) may be the least pressure-dependent single-point measure, and may be the most suitable single-point measure to assess local vascular function.

KEY WORDS: blood pressure, hydrostatic pressure, posture; vascular stiffness; atherosclerosis
Introduction

Arterial stiffness (AS) measures are widely used to investigate regional and local vascular health.[1–3] There are multiple means of estimating AS, including the conventional two-point Pulse Wave Velocity (PWV)[4], and various calculations based on less conventional single-point, ultrasound (US)-based measurements[5]. While two-point measures such as carotid-femoral PWV are more commonly used to prognose cardiovascular disease risk[1], single-point measures have also been shown to provide some insight into subclinical cardiovascular risk[6]. Further, single-point measures provide important complimentary mechanistic information including local compliance and distensibility[7]. However, measures of AS, including single-point estimates derived from US, are confounded by blood pressure (BP)[8][9], which is known to be affected by a range of physiological, mechanical, and psychological factors[10–12]. Indeed, the American Heart Association has recommended that BP be measured at the time and site of AS measures, underscoring the importance of the conflating influence of BP on AS[13]. Confounding by BP makes it challenging to compare AS-related outcomes between individuals, as well as to track changes over time for a given individual.

There are several well-reputed single-point US measure of AS, including distensibility coefficient (DC), compliance coefficient (CC), beta-stiffness index (β), and PWV. DC and CC are the relative and absolute changes in lumen area during systole for a given pressure change respectively. The β is based on logarithm-transformed changes in BP and changes in lumen diameter across the cardiac cycle and is purportedly pressure-independent[14]. Additionally, several methods are available to estimate PWV, including a derivative of β (PWVβ), the Bramwell-Hill equation (PWV_{BH}), as well as an estimation based on local changes in blood-flow
No known study has directly compared the dependency of these single-point AS estimates on BP.

One way to assess the dependency of AS on BP is to manipulate BP at the AS assessment site. However, it is challenging to manipulate local BP at central sites, such as the carotid artery, without inducing systemic changes in hemodynamics. For example, use of pharmacological interventions or other laboratory-based perturbations (i.e. lower body negative pressure, cold pressor testing, head-up tilt, isometric handgrip exercise, etc.) result in robust changes in the neural and hormonal profile, markedly affecting central hemodynamics[9][16]. Alternatively, BP can be passively manipulated in the leg or arm by inducing a change in hydrostatic pressure. For example, arm elevation decreases the transmural pressure and induces an unloading effect as the artery dilates, whereas lowering the arm causes an opposing effect[8]. This mild hydrostatic manipulation has previously been shown to introduce a regional transmural pressure gradient without altering systemic BP, sympathetic activity, heart rate (HR), venoarteriolar reflexes, or vascular resistance[10][17].

The purpose of this study was to determine which of six US-based, single-point AS estimates is least dependent on BP. The current study surveyed the brachial artery and used gravity to induce local changes in BP[10]. Identifying the least BP-dependent measure of AS may lead to more accurate assessments of local vascular function, particularly in longitudinal studies in which BP may change, or in studies comparing groups with differing BP.

**Methods**

This observation study is reported in accordance with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines[18]. Institutional Review Board (IRB) approval was obtained from the University of North Carolina at Chapel Hill.
Subjects

Following IRB approval, 20 healthy young adults (55% F) were recruited to participate in this study. For this preliminary study, a healthy population sample was recruited to minimize the influence of age- or disease-states on BP and AS. Participants were excluded if they smoked, had any known cardiometabolic disorders, or were taking any vasoactive medication. All participants provided written informed consent prior to participating in the study.

Experimental Design

Prior to their single visit, participants were familiarized with the experimental procedures. They were also instructed to abstain from food and drink, with the exception of water, for four hours, and caffeine and alcohol for 12 hours prior to the study. Participants were also asked not to participate in vigorous exercise 24 hours prior to testing. Lastly, participants were randomly allocated to the order of arm positions (after measurements were taken at heart level) for the experimental arm: i) ABOVE, or, ii) BELOW, using online randomization software (ww.randomizer.org), where 2 sets of 10 unique numbers were generated from a number range of 1-20. Figure 1 depicts the order and timing of testing for a given participant.

For the single experimental visit, anthropometric measurements were made and then the participant rested quietly for 20 minutes in a supine position. Both arms were placed at a right angle from the torso, at heart level, half the distance between the mid-sternum and exam table. The left ‘experimental arm’ was supported by a table with an adjustable height and tilting surface, permitting passive adjustment and full support during all arm positions. The right “control” arm was supported at heart level throughout experimentation. Participants were asked to avoid leg crossing or other body movements, and abstain from speaking throughout the procedure.
A duplex Doppler US device (GE P6) equipped with a 12 MHz linear array probe was used to collect the continuous arterial diameter and blood flow measures necessary for the stiffness calculations on the experimental arm. Immediately following US measurements, BP was simultaneously measured using an oscillometric device on the experimental (SphygmoCor, XCEL, Australia) and control (Oscar, SunTech, NC, USA) arms.

After measurements with the experimental arm at heart level, all measurements were repeated with the experimental arm positioned 30° ABOVE or BELOW heart level in a randomized order. The subject rested for five minutes at each new arm position prior to repetition of all measurements. All measures were taken in triplicate at each position. The average of the closest two readings were recorded.

**Peripheral and Central Blood Pressure**

Systolic (SBP), diastolic (DBP), central systolic (cSBP) and mean arterial pressure (MAP) were measured using pulse wave analysis (PWA) following standard manufacturer guidelines[19]. Each measurement cycle lasted approximately one minute, consisting of a brachial BP recording followed by a 10-second sub-systolic recording. Brachial waveforms were calibrated using cuff-measured brachial systolic and diastolic pressures, and a corresponding aortic pressure waveform was generated using a validated transfer function to derive cSBP.[20] Between-day, within-subject reliability of BP measures across arm positions were assessed in six people, on two days under identical conditions to the main protocol. Relative standard error of the means (SEM%) at heart level, ABOVE, and BELOW respectively were calculated for SBP (2.64, 2.75, 2.04), DBP (3.68, 5.31, 4.54), and MAP (2.82, 4.11, 3.44).

**Ultrasound**

*Capture of Ultrasound*
A single trained US operator collected the measurements necessary for calculations of arterial stiffness on the experimental arm. The US probe was placed on the brachial artery, 5-10 cm proximal to the antecubital fossa and marked for subsequent assessments. The US beam was placed at mid-vessel and was angled at $\leq 60^\circ$ relative to the longitudinal axis of the artery to permit simultaneous measurement of brachial diameter and blood velocity. An effort was made to ensure that the vessel clearly extended across the entire (unzoomed) imaging plane to minimize the risk of skewing the vessel walls. Ultrasound global (acoustic output, gain, dynamic range, gamma and rejection) and probe-dependent (zoom factor, edge enhancement, frame averaging and target frame rate) settings were standardized. Three 10-second videos of the US and gated ECG readings were recorded at each position using external video capturing software (AV.io HD Frame Grabber, Epiphan Video, CA). A fourth brightness-mode-only recording was made in which the isonation angle was perpendicular to the vessel wall to ensure an optimal diameter measurement. Participants were instructed to hold their breath (without a large initial inhale) during each 10-second recording period.

**Data Analysis**

The captured videos were analyzed using automated edge-detecting software (FMD Studio, Quipu, Italy). Custom written Excel Visual Basic code was used to fit peaks and troughs to the diameter waveforms in order to calculate diastolic, systolic, and mean diameters, and to automate calculation of study outputs [21][22]. Blood flow was calculated from continuous diameter and mean blood velocity recordings using the following equation: $3.14 \times (\text{radius})^2 \times \text{mean blood velocity} \times 60$. Between-day, within-subject reliability of the US measures across arm positions were assessed. SEM% was calculated at heart level, ABOVE and BELOW respectively for
systolic diameter (1.59, 1.04, 2.62), diastolic diameter (1.76, 0.80, 2.57), distention (12.41, 7.31, 8.37), and blood velocity change (10.21, 10.93, 5.68).

**Calculations of Arterial Stiffness**

The stiffness measures included DC, CC, $\beta$, $PWV_\beta$, $PWV_{BH}$, and $PWV_{BF}$. Calculations are shown below.

(1) Distensibility Coefficient (DC) is the relative change in lumen area during systole for a given pressure change:

$$\frac{(2\Delta D \cdot D + D^2)}{(\Delta P \cdot D^2)}$$

where $D$ is the lumen diameter and $\Delta P$ is the pulse pressure (SBP-DBP)[3].

(2) Compliance coefficient (CC), is the absolute change in lumen area during systole for a given pressure:

$$CC = \frac{(2D \cdot \Delta D + D^2)}{(4 \cdot \Delta P)}$$

where $D$ is the lumen diameter and $\Delta P$ is the pulse pressure (SBP-DBP)[3].

(3) The $\beta$-stiffness index ($\beta$) is a method of measuring arterial stiffness that is purportedly independent of BP:

$$\beta = \frac{\ln(SBP/DBP)}{[(Ds-Dd)/Dd]}$$

where $\ln$ is the natural logarithm, SBP is systolic blood pressure, DBP is diastolic blood pressure, $Ds$ is the lumen diameter during systole, and $Dd$ is the lumen diameter during diastole[14].

(4) The BH equation theoretically relates PWV, distensibility and pulse pressure using the following mathematical model:

$$PWV_{BH} = \sqrt{\frac{A_p}{(1/C_C)}}$$
Where A is the lumen area, p is the blood density (1059 kg/m³), and CC is the compliance coefficient[3].

(5) The $\beta$-stiffness derivative method utilizes the $\beta$-stiffness index to estimate PWV. The $\beta$-stiffness index is based on changes in pressure and diameter and can be described as:

$$PWV_{\beta} = \sqrt{(\beta \cdot DBP)/(2p)}$$

Where $\beta$ is the $\beta$-stiffness index, DBP is the diastolic blood pressure, and p is the blood density (1059 kg/m³)[23].

(6) With the BF method, PWV is estimated as the ratio between the change in blood flow and the change in cross-sectional area during the reflection-free (early systolic wave) period of the cardiac cycle:

$$PWV_{BF} = (\Delta V/\Delta A)$$

Where V is blood volume and A is the lumen area[24].

Between-day, within-subject reliability of the AS measures across arm positions were assessed. SEM% was calculated at heart level, ABOVE and BELOW respectively for $\beta$ (14.11, 7.86, 13.60), CC (13.91, 8.19, 10.59), DC (16.88, 9.17, 10.40), $PWV_{\beta}$ (6.98, 4.46, 5.29), $PWV_{BH}$ (7.35, 4.49, 5.45), and $PWV_{BF}$ (14.74, 10.87, 8.00).

**Statistical Analysis**

Statistical analyses were performed using Statistical Package for Social Sciences version 21 (SPSS, Inc., Chicago, Illinois, USA). All data are reported as means (X) and standard deviation (SD), unless specified. The alpha level was set at $p < 0.05$ *a priori*. The effects of arm position on study outcomes were examined using repeated measures analysis of variance (ANOVA) with one within-subject factor (arm position: ABOVE, equal to and BELOW heart level). Contrasts against the heart level position were performed, and are reported as 95%
confidence intervals. Effect sizes (ES) are reported using partial eta-squared ($\eta^2_p$) where 0.01, 0.06, and 0.14 represent a small, medium, and large effects, respectively[25]. The AS measurement with the smallest ES was presumed to be the least pressure-dependent. Lastly, the Pearson product-moment correlation coefficient was used to determine associations between the stiffness variables.

Results

Data was successfully collected from all 20 participants [age: 27.9 years (SD: 5.2); BMI: 24.2 (SD: 2.8); 55% female (n=11)].

AS Variables: Arterial Diameter, Blood Pressure and Blood Flow

AS variables are reported in Table 1. While positional change had a large effect ($\eta^2_p = 0.18, p = 0.026$) on mean vessel diameter, the changes were not significant in either position. Blood flow decreased when the arm was lowered ($p < 0.001$), but did not change when raised.

BP variables are shown in Table 2. For the experimental arm, there was a large effect change in SBP ($\eta^2_p = 0.75, p < 0.001$), DBP ($\eta^2_p = 0.80, p < 0.001$), and MAP ($\eta^2_p = 0.82, p < 0.001$) when the arm was ABOVE (SBP $\Delta -4.4$ mmHg, DBP $\Delta -4.7$ mmHg, MAP $\Delta -4.6$ mmHg) and BELOW (SBP $\Delta 10.4$ mmHg, DBP $\Delta 13.3$ mmHg, MAP $\Delta 9.8$ mmHg) heart level. In the control arm, no change occurred in SBP or cSBP, but there was a change in DBP ($p = 0.002$) and MAP ($p = 0.002$).

HR did not change across positions, which was measured with the oscillometric devices on the experimental and control arms.

Effects of Arm Position on Measures of AS

The effects of arm position on each AS measure are shown in Table 1 and are ranked in order of ES. There was a significant main effect of arm position on all AS measures, with a large
ES ($n^2p = 0.297 – 0.511$) for all measures except $\text{PWV}_{\text{BF}}$, which was medium ($n^2p = 0.068$). There were non-significant changes in all AS measures when the arm was elevated ABOVE the heart. In contrast, each AS measure, except CC and $\text{PWV}_{\text{BF}}$ changed significantly when the arm was positioned BELOW the heart. When the arm was positioned BELOW the heart, and when compared to heart level, the contrast indicated a non-significant 23% decrease in CC, and 12% non-significant increase in $\text{PWV}_{\text{BF}}$.

Correlations between the AS measures when the arm was positioned ABOVE and BELOW are shown in Tables 3 and 4 respectively. There were significant, moderate-strong correlations ($r = \pm 0.474$ to $0.998$) among the six AS measures at each position.

*Sensitivity Analysis: Effect of Sex*

While this study was not powered to determine a sex effect, we specified sex as a between-subjects factor for each model. No sex interactions were found for $\beta$ ($p = 0.435$), DC ($p = 0.985$), $\text{PWV}_{\beta}$ ($p = 0.751$), $\text{PWV}_{\text{BH}}$ ($p = 0.681$), or $\text{PWV}_{\text{BF}}$ ($0.727$).

**Discussion**

Arterial stiffness is an important marker of vascular structure and function[13]. However, AS measures may be confounded by BP. In the current study we investigated the pressure-dependence of six single-point, US-based AS measures. From least to most pressure-dependent, our findings showed the following order of AS measures: $\text{PWV}_{\text{BF}}$, CC, $\beta$, DC, $\text{PWV}_{\text{BH}}$, and $\text{PWV}_{\beta}$. $\text{PWV}_{\text{BF}}$ was the least pressure-dependent single-point, US-based AS measurement, and thus may be the most suitable single-point method to assess local vascular function.

*Limitations and Strengths*

To better contextualize the discussion, several limitations of the current study must be noted. First, blood density was part of the $\text{PWV}_{\text{BH}}$ and $\text{PWV}_{\beta}$ calculations, and we assumed a
constant density of 1059 kg/m³. Because that only young, healthy subjects were tested, the constant is likely to be an accurate representation. Further, this constant is commonly used. Second, single-point, US-based methods for measuring local AS assume that early systole is unidirectional and reflection-free, which is important because the pressure and flow waves are presumably congruous during this period[13]. There is strong evidence to supporting this assumption[23][26]. Third, we acknowledge that different devices were used to assess local BP in experimental and control arms. However, the devices incorporate identical technologies and calculations for measuring BP and subsequently determining central pressure.

Despite these limitations, we are confident that this study’s strengths – in particular, the robust research design[10] - off-set the potential sources of error. Additionally, the sole US operator has extensive experience obtaining flow and diameter measurements using US[21][22]. Second, all measurements were taken in triplicate, with the two closest values averaged and confirmed against a fourth reading. Finally, all measurements were performed with the participant resting in a supine position following 20 minutes of rest, in a noise and temperature-controlled environment.

Comparisons with Other Studies

Few studies have examined the pressure-dependence of the single-point AS methods assessed in the current study and no studies that we are aware of have measured AS and BP simultaneously. The finding that $\text{PWV}_{BF}$ was the least BP-dependent measure of local AS is not surprising given the non-use of BP with the $\text{PWV}_{BF}$ calculation. This method has previously been shown to be similar to a well-established MRI method which measured transit time across the aortic arch[24]. In the same study, $\text{PWV}_{BH}$ was not strongly correlated to MRI-based $\text{PWV}_{BF}$[24]. This is in line with our results as $\text{PWV}_{BF}$ was a more robust measure of AS than
PWV$_{BH}$, while PWV$_{BH}$ was the second-most pressure-dependent method in the current study.

Our finding that CC was the second-most pressure-independent AS measure is surprising given that it is a major component of the PWV$_{BH}$ equation, which seemed to be very pressure-dependent. One possible, albeit unlikely explanation for the apparently lesser pressure dependency of CC is its omission of an assumed constant for blood density (viscosity). Our study also indicated that CC had a similar, if not slightly less, sensitivity to pressure perturbations than $\beta$ which is purportedly a pressure-dependent measure[9]. It must also be noted that despite the fact that $\beta$ is often claimed to be pressure-independent, other researchers have demonstrated pressure-dependency of this measure, which, again, is in line with our findings[15][27]. It is unclear if the small differences in pressure-dependency of $\beta$ and CC are due to their respective calculations representing slightly different physiological phenomena, or if they are simply circumstantial. Importantly, however, $\beta$ and CC are associated with cardiovascular disease risk such as hypercholesterolemia[28] and hypertension[29], as well as cardiovascular events such as stroke and myocardial infarction[30][31].

The pressure dependency of AS has also been demonstrated when central and systemic measures of PWV have been used. Increased aortic-radial PWV and changes in the shape of the pressure waveform have been associated with a more upright body position.[16] Carotid PWV has also been innately linked to BP-lowering medication.[32] Further, Schroeder et al.[27] reported that changes in body position from a supine to seated position increased arterial stiffness measures including $\beta$, carotid-femoral PWV, and cardio-ankle vascular index (CAVI). Interestingly, while carotid $\beta$ seemed to be pressure-dependent in this last study, the researchers suggested that the increases observed in PWV and CAVI occurred as a result of position-mediated alterations in compliance rather than BP per se[27]. Yet, some caution should be taken
when comparing position-mediated effects of stiffness between a large elastic artery such as the carotid and a muscular artery such as the brachial. Nevertheless, it may be reasonably assumed that both peripheral and central measures of hemodynamics and AS are altered in response to changes in limb position and body posture. Such effects are likely due to the fundamental pressure-dependency of AS as well as other related or unrelated functional adjustments in the vasculature.

Implications and Further Direction

This study adds to the evidence that there is no completely pressure-independent measure of AS. Specifically, the current study indicates that, in terms of single-point US-based methods, $PWV_{BF}$ may be the most pressure-independent single-point, US-based AS measurement. This measure may be superior when making between-subject comparisons, as well as for longitudinal assessments when BP may be subject to change. However, it is unknown if, under standardized conditions, this method would also be the most valid in terms of conferring disease prediction, particularly when compared to the non-invasive gold-standard propagation-based models. Additionally, the current results imply that researchers and clinicians must be mindful of, and account for confounders such as body position during AS measurement.

In terms of how this study should guide further research, we posit several suggestions. First, pressure-dependency and body position are necessary variables to consider during any AS measurement, regardless of whether a single- or two-point method is utilized.

Second, future research should seek to determine if the $PWV_{BF}$ is also the most predictive of cardiovascular outcomes. Third, though we investigated the linear relationship of AS and BP, there may be a non-linear relationship that exists - which may change over time with aging and in particular, the transition from health to the early stages of cardiovascular risk. Future
longitudinal studies that assess the potential non-linear relationship and predictive values of AS and BP are needed. Longitudinal studies will also help to discern the extent to which the predictive value of AS mirrors or exceeds the predictive value of BP alone.

Conclusion

AS is an important marker of vascular health. However, AS measures are confounded by changes in BP. This is the first study that we are aware of, that has assessed the relative pressure dependence of multiple single-point US-based AS measures. This study demonstrated that the six US-based, single-point methods for assessing AS are all impacted by BP with $\text{PWV}_{BF}$ and $\text{PWV}_\beta$ being the least and most BP-dependent AS measures respectively. $\text{PWV}_{BF}$ may be the least pressure-dependent single-point, US-based AS measurement. This AS estimate offers promising potential as a sensitive measure to compare local vascular health between individuals and across time, while minimizing the confounding effects of BP.
References


FIGURES and TABLES

Figure 1. Order and timing of measurements on the experimental arm. Four duplex Doppler ultrasound (US) measurements and three pulse wave analysis measurements were completed with the experimental arm at heart level (0°). Following measurements at heart level (0°), the arm was positioned ABOVE (+30°) and BELOW (-30°) heart level in a randomized order. The subject rested for 5 minutes at each new arm position prior to measurements. The control arm remained fixed at heart level and PWA measures were taken at the same time as experimental arm measures. PWA, pulse wave analysis; US, ultrasound; ’, minute.

Table 1. Arterial stiffness variables at each arm position. Variables are ranked in order of effect size. Effects of arm position on arterial stiffness variables were examined using repeated measures analysis of variance with one within-subject factor (arm position: ABOVE, equal to and BELOW heart level). Contrasts against the heart level position are reported as 95% confidence intervals. Effect sizes are reported using partial eta-squared. X, mean; SD, standard deviation; sig, significance; P, p-value; n²p, partial eta squared effect size; Cont, contrast; LCI, lower confidence interval; UPI, upper confidence interval; Dmean, mean diameter; mm, millimeter; Dist, distension; ∆, change; BF, blood flow; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill.

Table 2. Blood pressure measurements on experimental and control arms at each arm position. Effects of arm position blood pressure measures were examined using repeated measures analysis of variance with one within-subject factor (arm position: ABOVE, equal to and BELOW heart level). Contrasts against the heart level position are reported as 95% confidence intervals. Effect sizes are reported using partial eta-squared. X, mean; SD, standard deviation; Sig, significance; P, p-value; n²p, partial eta squared effect size; Cont, contrast; LCI, lower confidence interval; UPI, upper confidence interval; MAP, mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; cSBP, central aortic blood pressure.

Table 3. Correlations between AS Measures when arm positioned ABOVE heart level. Dmean, mean diameter; mm, millimeter; Dist, distension; ∆, change; BF, blood flow; ml, milliliters; min, minute; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill; r, correlation coefficient; p, p-value.

Table 4. Correlations between AS Measures when arm positioned BELOW heart level. Dmean, mean diameter; mm, millimeter; Dist, distension; ∆, change; BF, blood flow; ml, milliliters; min, minute; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill; r, correlation coefficient; p, p-value.
Figure 1. Order and timing of measurements on the experimental arm. *Four duplex Doppler ultrasound measurements and three pulse wave analysis measurements were completed with the experimental arm at heart level (0°). Following measurements at heart level (0°), the arm was positioned ABOVE (+30°) and BELOW (-30°) heart level in a randomized order. The subject rested for 5 minutes at each new arm position prior to measurements. The control arm remained fixed at heart level, and PWA measures were taken at the same time as experimental arm measures.*
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**Table legend:** Variables are ranked in order of effect size. SD, standard deviation; sig, significance; P, p-value; n²p, partial eta squared effect size; Cont, contrast; LCI, lower confidence interval; UCI, upper confidence interval; Dmean, mean diameter; mm, millimeter; Dist, distension; Δ, change; BF, blood flow; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill.
Table 2. Blood pressure measurements on experimental and control arms at each arm position.

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Table legend: $X$, mean; $SD$, standard deviation; Sig, significance; $P$, $p$-value; $\eta^2p$, partial eta squared effect size; Cont, contrast; LCI, lower confidence interval; UCI, upper confidence interval; MAP, mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; cSBP, central aortic blood pressure; HR, heart rate.
Table 3. Correlations between AS Measures when arm positioned ABOVE heart level.

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<th>β</th>
<th>CC</th>
<th>DC</th>
<th>PWVβ (m/s)</th>
<th>PWVBH (m/s)</th>
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Table legend: Dmean, mean diameter; mm, millimeter; Dist, distension; ∆, change; BF, blood flow; ml, milliliters; min, minute; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta-stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill; r, correlation coefficient; p, p-value.
Table 4. Correlations between AS Measures when arm positioned BELOW heart level.

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<th>PWV_BH (m/s)</th>
<th>PWV_BF (m/s)</th>
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<td>0.010</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>PWV_BF (m/s)</td>
<td>-0.198</td>
<td>-0.786</td>
<td>-0.067</td>
<td>0.976</td>
<td>-0.575</td>
<td>-0.817</td>
<td>0.996</td>
<td>1.000</td>
</tr>
<tr>
<td>PWV_BF</td>
<td>0.417</td>
<td>0.000</td>
<td>0.786</td>
<td>0.000</td>
<td>0.010</td>
<td>0.000</td>
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<td>0.000</td>
</tr>
<tr>
<td>PWV_BF</td>
<td>-0.214</td>
<td>-0.686</td>
<td>0.353</td>
<td>0.801</td>
<td>-0.285</td>
<td>-0.659</td>
<td>0.790</td>
<td>0.770</td>
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<tr>
<td>PWV_BF</td>
<td>0.380</td>
<td>0.001</td>
<td>0.138</td>
<td>0.000</td>
<td>0.237</td>
<td>0.002</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table legend: Dmean, mean diameter; mm, millimeter; Dist, distension; ∆, change; BF, blood flow; ml, milliliters; min, minute; PWV, pulse wave velocity; m/s, meters per second; CC, compliance coefficient; β, Beta-stiffness index; DC, distensibility coefficient; BH, Bramwell-Hill; r, correlation coefficient; p, p-value.