Dual Impedance Cardiography: An Inexpensive and Reliable Method to Assess Arterial Stiffness

Mark R. Scudder¹, J. Richard Jennings^{1,2}, Caitlin M. DuPont¹, Kimberly G. Lockwood³, Shrenik H. Gadagkar¹, Belen Best¹, Swetha P. Jasti¹, & Peter J. Gianaros¹

¹Department of Psychology, University of Pittsburgh, Pittsburgh, PA

²Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

³Center for Health and Community, University of California San Francisco, San Francisco, CA

Corresponding author:

Mark R. Scudder
3943 O'Hara Street
506 Old Engineering Hall
Pittsburgh, PA 15260
412-648-4512
mas721@pitt.edu

Abstract

Pulse wave velocity (PWV) is a common measure of arterial stiffness. Non-invasive methods to measure PWV are widely used in biomedical studies of aging and cardiovascular disease, but they are rarely used in psychophysiology. Barriers to wider use include the prohibitive costs of specialized equipment and need for trained technicians (e.g., ultrasonographers). Here, we describe an impedance cardiography method to measure PWV. By this method, impedance signals are dually collected from the thorax and calf. Combined with ensemble averaging of vascular signals, this dual impedance cardiography (d-ICG) method allows for the measurement of aortic flow onset and the arrival time of peripheral pulse waveforms to compute PWV. In a community sample of adults (aged 19–78 years), PWV measured with d-ICG exhibited a strong positive correlation with age. Moreover, age-specific mean PWV values were within the normative reference intervals established by large scale studies using other techniques. PWV derived from d-ICG exhibited high test-retest reliability across several days, as well as excellent inter-rater reliability. Lastly, PWV exhibited expected associations with known cardiovascular disease risk factors (i.e., blood pressure) and indicators of autonomic cardiovascular control. d-ICG is an inexpensive and reliable method to assess arterial stiffness.

1. Introduction

Vascular function in health and disease is a prominent phenomenon of interest in behavioral medicine and psychophysiology. A canonical indicator of vascular function is pulse wave velocity (PWV). PWV is the speed (in meters per second) with which a pulse waveform propagates along an arterial path with each beat of the heart. PWV is chiefly determined by arterial stiffness, which is the compliance of the arterial wall to distending pressure (Vlachopoulos, Nichols, & O'Rourke, 2011). The stiffness of an artery influences its ability to dampen the pulsatile force of ejected blood against the vascular wall. This force is generated with each ventricular contraction of the heart. Arteries progressively lose their dampening capacity and stiffen with both advancing age and an accumulation of plaque in the vessel wall due to exposure to the pathogenic effects of risk factors for cardiovascular disease (CVD) (Hallock, 1934; Rogers et al., 2001). The arterial stiffening that results from advancing age and risk factor exposure leads to a faster PWV. On this basis, faster PWV confers risk for premature death, myocardial infarction, and stroke in both clinical and non-clinical samples (Bérard, Bongard, Ruidavets, Amar, & Ferrières, 2013; Boutouyrie et al., 2002; Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Likewise, greater arterial stiffness – represented by faster PWV – is widely interpreted as reflecting the cumulative impact of biological, behavioral, social, psychological, and environmental risk factors for CVD across the lifespan (Laurent, Marais, & Boutouyrie, 2016).

The PWV index of greater central arterial stiffness can be non-invasively measured by diverse methods and devices, which each exhibit strengths and weakness (Laurent et al., 2016; Pereira, Correia, & Cardoso, 2015). For instance, magnetic resonance imaging (MRI) enables the targeting of specific aortic segments and provides a more precise measurement of arterial path length; however, the (a) prohibitive cost of MRI for most researchers, (b) relatively long protocol times, and (c) confined bore of the MRI device preclude its broad use by non-specialists and its application among certain populations (e.g., individuals with large bodies; Mohiaddin, Firmin, & Longmore, 1993). Other PWV methods involve placing various pulse-sensing devices at two locations on the body to record pulse waveforms. These waveforms can then be used to derive the pulse transit time (PTT). The most common pulse-sensing

approaches can be heuristically categorized into either mechanical (e.g., tonometry) or ultrasound methods. These methods are less expensive than MRI and have broader applicability, yet they are prone to recording artifacts caused by the angulation and physical stability of probes, sensors, and transducers. As such, these methods require expert technicians who have undergone extensive training or certification (Boutouyrie, Briet, Collin, Vermeersch, & Pannier, 2009; Kelly et al., 1989). These methods also engender problematic issues with respect to reliability and intra- and inter-operator agreement in scoring PWV data to compute arterial stiffness metrics. In addition, signals derived from mechanical and ultrasound methods are recorded for just a few heart beats. Under such brief assessment periods, recording conditions must be ideal to reduce measurement error – characterized by an absence of (a) participant movement, (b) sensor placement instability, and (c) change in transducer/probe angle. Indeed, pulse-wave recording locations on the body are of crucial importance when assessing arterial stiffness, regardless of the particular method or measurement device.

To elaborate, sites of pulse-wave recording on the body constrain the conditions under which different methods can be implemented. At present, positioning ultrasound or other sensors over the carotid and femoral arteries is a ‘gold standard’ approach to assess PWV. This placement yields a measure of transit time in the aortic artery and robust evidence indicates reliable and independent associations between carotid-to-femoral measures of PWV and CVD outcomes (see, The Reference Values for Arterial Stiffness’ Collaboration, 2010). Positioning of participants for carotid-to-femoral monitoring, however, is restrictive. Measures of PWV derived from other vessels and recording sites on the body (e.g., arms and ankles) may be less restrictive in terms of participant positioning; however, these measures are often less interpretable because they can be confounded by peripheral influences and other tissues (e.g., muscular) beyond the aorta (Laurent et al., 2016). Ultimately, the type of PWV method used should not only depend on its practicality, but also the rigor of physiological interpretations that are afforded by a given method to address a particular research question.

Because of the increasing use of PWV in studies of health, aging, and disease, and because of the practical and financial limitations of many existing techniques, there is a need for additional PWV

assessment methods. Ideally, such methods should have most or all of the following characteristics. First, they should yield accurate and reliable measures of PWV obtained for more than just a few heartbeats, ideally reflecting central arterial stiffness. Second, they should pose few or no restrictions to their use (a) in diverse research settings, (b) by research assistants without advanced training or certification (e.g., undergraduate research assistants), and (c) with diverse participants of varying body sizes. Third, they should exhibit cost efficiency. Lastly, if PWV assessment methods are to be used more broadly, they should not pose barriers to the simultaneous and non-invasive assessment of other fundamental indices of autonomic and cardiovascular function, such as heart rate variability, systolic time intervals, and baroreflex sensitivity. Next, we describe a PWV assessment method based on dual impedance cardiography (ICG) that has all of these characteristics.

ICG itself is a relatively low-cost and widely used non-invasive method for measuring changes in blood flow (Sherwood et al., 1990). Changes in blood flow result in corresponding electrical impedance changes within a defined region (e.g., the thoracic cavity). ICG is commonly used to measure systolic time intervals, cardiac contractility, and peripheral vascular resistance. Far less common is the use of ICG to measure peripheral blood flow outside the thoracic cavity, although this use has been previously reviewed (Jennings, Tahmouh, & Redmond, 1980). When peripheral and thoracic ICG assessments are combined simultaneously, however, they permit the beat-to-beat measurement of pulse waveforms that originate centrally from the aorta and arrive in the periphery. In this way PWV, and hence arterial stiffness, can be assessed in the primary systemic arteries.

In this study, we implemented a dual ICG setup using a spot electrode configuration applied to the thorax and calf of the leg to derive PWV. Validity of this measure can be established by its relation to known health determinants, such as age, blood pressure, adiposity, and other risk factors, as well as concurrently measured indicators of cardiovascular autonomic control that are often used in behavioral medicine and psychophysiology (i.e., high-frequency heart rate variability [HF-HRV] and ‘spontaneous’ baroreflex sensitivity [BRS]). We also tested for associations between PWV, total peripheral resistance (TPR), and a non-invasive measure of endothelium dependent vasodilation (EDV) induced by reactive

hyperemia (de Berrazueta et al., 2010). Lastly, we assessed the test-retest reliability of PWV (over ~1 week) and inter-rater agreement using standard ICG data reduction methods involving ensemble averaging (Árbol et al., 2017; Jennings & Choi, 1983). We hypothesized that PWV values would correspond to established norms and correlate positively with age, blood pressure, TPR, and adiposity, but negatively with HF-HRV, BRS, and EDV. We also expected that PWV would exhibit high test-retest reliability and that there would be strong inter-rater agreement among laboratory research assistants already trained in ICG. If PWV derived by dual ICG exhibits acceptable reliability and expected associations with known cardiovascular and autonomic measures, then this would support its utility because of its minimal participant burden and training needs, non-invasiveness, and low cost.

2. Methods

2.1 Participants

Participants were 78 community dwelling adults (54 female), aged 19 – 78 years. Exclusion criteria included current pregnancy, current (active) treatment for cardiac arrhythmias (including implanted defibrillators), prior heart surgery, and prior myocardial infarction or stroke. Recruitment methods included print advertisements (fliers) posted in the Pittsburgh, PA, USA area and the University of Pittsburgh Research Participant Registry (<https://pittplusme.org/>). All participants provided informed consent and were paid \$10.00 US per visit (plus parking and/or public transportation reimbursement). Study approval was granted by the University of Pittsburgh Human Research Protection Office. The period of data collection began in September, 2017 and ended in December, 2018.

2.2 Overview of study procedures

Participants were instructed to refrain from exercising, eating a heavy meal, and consuming caffeine or nicotine for 3 hours prior to arrival at the laboratory. They were also asked to refrain from consuming alcohol on the day of the visit. Participants reporting symptoms of an acute illness prior to or at the time of the visit were rescheduled. After participants arrived at the laboratory, trained research assistants re-verified study eligibility, described the study protocol, and obtained informed consent. Participants then completed self-report surveys to assess demographic information, medical histories, and

medication use. Tobacco use (coded: 0 - never; 1 - former or current user) was assessed in a subsample of 42 participants at this time or by phone or electronic surveys administered following the visit. Next, participants sat quietly for 5 minutes before measures of resting blood pressure (BP) and heart rate (HR) were recorded using an Omron IntelliSense[®] BP Monitor (model HEM-907XL, Omron Healthcare Inc.). While remaining seated, participants were prepped for the first assessment protocol involving venous occlusion plethysmography to derive a measure of EDV. Thereafter, participants were escorted to an adjacent room in the laboratory to measure height and weight, and to complete the final assessment protocol involving dual ICG (d-ICG), electrocardiography (ECG), and beat-to-beat BP. After applying the recording sensors, participants sat quietly for 5 minutes prior to completing a 6-minute recording at rest. Following the completion of both protocols, participants were paid and debriefed. The test-retest reliability subsample consisted of 14 individuals (aged 20-77 years; 12 female) who returned to the laboratory 3 to 23 (median = 7) days later to repeat the venous occlusion and d-ICG protocols. Considering the primary focus of the current study was to assess the reliability of the new d-ICG method, PWV was the only variable entered in test-retest analyses. Each visit lasted ~1 - 2 hours.

2.3 Assessment of anthropometric measures

Participants' height was measured using a stadiometer with shoes removed. The height of the left coracoid process was also estimated by palpation of the area below the clavicle and medial to the anterior deltoid, which was used to approximate the height of the aortic arch. This measurement (to the nearest 0.1 cm) was collected for later computation of path length and PWV. Weight (kg) was measured using a Tanita Body Composition Analyzer (TBF-410, Tanita Corporation of America Inc., Arlington Heights, IL), and body mass index (BMI) was calculated as kg/m^2 .

2.4 Assessment of self-rated health and illness burden

Using a self-report inventory, participants were asked about whether they previously experienced or were diagnosed with diseases of the heart and blood vessels (e.g., atrial fibrillation, hypertension, etc.), brain and cerebrovasculature (e.g., stroke), or other systems (e.g., diabetes, kidney, lungs, etc.). A composite health burden score was computed by summing the total number of illnesses and diagnoses

endorsed. These included use of anti-hypertensive medication or measured stage 1 hypertension (i.e., resting systolic BP ≥ 130 or diastolic BP ≥ 80 mmHg [n=10]; Whelton et al., 2017), angina (n=1), heart valve problems (n=1), rheumatic fever (n=0), abnormal heart rhythm (n=2), diabetes (n=1), lung problems (n=2), thyroid (n=5), or liver (n=1) illness diagnoses. Overall, 71.0% of participants reported no burden, while 25.8% had a burden score of 1, 1.6% had a burden score of 2, and 1.6% had a burden score of 4.

Self-rated health was also assessed using the question, “Would you say your health in general is excellent, very good, good, fair, or poor?” (coded 1-5, respectively). This single-item measure has been shown to predict cardiovascular and all-cause mortality independent of other established CVD risk factors (Barger, Cribbet, & Muldoon, 2016). Overall, 28.1% of participants rated their health as excellent, 38.6% as very good, 29.8% as good, and 3.5% as fair. No participants rated their health as poor.

2.5 Measurement of ECG and d-ICG signals

BioLab software (version 3.3.1) and a BioNex-8 slot chassis (MindWare Technologies LTD, Westerville, OH), specially outfitted with two ICG signal acquisition modules for the thorax and calf, were used to obtain and digitize the ECG and ICG signals. ECG signals were obtained from 3 disposable and pre-gelled Ag/AgCl electrodes that were placed on participants using a modified Lead-II configuration to minimize the influence of limb movement artifacts and maximize the detection of R-spikes (Berntson, Quigley, Norman, & Lozano, 2017). By this configuration, the positive electrode was placed over the left lowest rib and the negative electrode was placed below the right clavicle, just medial to the anterior deltoid. The following ICG signals were obtained from disposable and pre-gelled Ag/AgCl electrodes positioned on the thorax in a spot configuration: basal thoracic impedance (Z_0), and the 1st derivative of the pulsatile change in basal thoracic impedance (dZ/dt). Two inner recording electrodes were affixed to the front of the thorax, one on the suprasternal notch and one over the approximate location of the xiphisternal junction. Two outer source electrodes were affixed to the back of the thorax so that they were ~4 cm above (suprasternal) and below (xiphisternal) the recording electrodes (Sherwood et al., 1990). The source electrodes of the thorax passed a 1mA, 100kHz AC current. Another spot electrode

configuration was used to obtain ICG signals from the calf (see Figure 1). Here, two *outer* source electrodes were affixed to the lateral side of the left calf, one directly above the lateral malleolus (ankle) and the other just below the tibial tuberosity. The two *inner* recording electrodes were placed ~4 cm above/below the source electrodes on the medial side of the left calf. The source electrodes passed a 1mA, 102kHz AC current, which permitted the simultaneous measurement of thorax and calf ICG signals without interference. The lack of interference was attributable to the 2kHz offset between thoracic and calf sources. However, it should be noted that initial testing of the calf configuration incorporated band electrodes, which yielded an unusable/noisy signal in 11/16 participants. The source of interference was likely attributable to the recorded impedance values being too high and oversaturating the amplifier, but switching to spot electrodes alleviated this issue. The 11 excluded participants (5 females) missing a calf impedance signal did not differ from the rest of the sample on measures of BMI, BP, and resting HR, yet participants without a calf signal tended to be younger (likely due to the large proportion of younger adults in the study). Further, the overall pattern of results was unchanged when the 5 individuals with a useable calf band signal were included in the final analyses.

Next, two distance measurements were recorded for later scoring and computation of ICG and PWV measures. First, while the participant was standing, the distance between the inside edges of the two inner recording electrodes on the thorax was measured (to the nearest 0.1 cm). This distance was required for the computation of stroke volume (SV; Kubicek, Karnegis, Patterson, Witsoe, & Mattson, 1966), which was then used to compute cardiac output (CO) by multiplying SV and HR. Second, while participants were seated with their shoes off and feet flat on the floor, calf height was measured as the distance (to the nearest 0.1 cm) from the floor to the mid-point between the two inner recording electrodes (Figure 1). This was the second distance measurement needed to compute path length for PWV (see next section for further details).

2.6 ECG/ICG signal processing and PWV calculation

All ECG and ICG signals were visually inspected, corrected for artifacts, and processed to compute primary study variables using the Mindware Heart Rate Variability (version 3.1.5) and Cardiac

Impedance (version 3.1.6) software packages. To calculate HF-HRV, interbeat intervals (IBI's) were first examined and edited using the artifact detection steps developed by Berntson, Quigley, Jang, and Boysen (1990). IBI's were inspected and analyzed in 1-minute segments across the 6-minute recording epoch, and the resulting values for each segment were averaged to compute a grand mean value. A minimum of three, 1-minute segments was required, segments could not contain more than 3 ectopic beats, and less than 5% of the IBI's were corrected in each segment. The IBI series was then (1) converted to a time-based representation (i.e., heart period time series), (2) linearly detrended to minimize non-stationarity, (3) tapered using a Hamming window, and (4) passed through a Fast Fourier Transform to derive the HF spectral power (ms^2/Hz) in the 0.15–0.40 Hz bandwidth. Mean respiratory rates were derived from the integrated Z0 signal (i.e., impedance pneumogram; Ernst, Litvack, Lozano, Cacioppo, & Berntson, 1999), which was submitted to a fast Fourier transform for each minute of the recording epoch. Each segment was visually inspected to ensure the peak respiration frequency fell within the 0.15-0.40 Hz bandwidth. HF-HRV values were adjusted for the prevailing heart period using the formula: $100 * [\ln(\text{HFpower}) / \ln(\text{IBI}^2)]$ by current recommendations (de Geus, Gianaros, Brindle, Jennings, & Berntson, 2019).

ICG signals from the thorax and calf were also inspected/analyzed in 1-minute segments, and later averaged to compute a grand mean value. For each segment, ensemble average waveforms of the ECG and thorax/calf dZ/dt signals (see Figure 2) were used to identify key landmarks that are required for computing cardiac impedance and PWV measures. Given that a continuous IBI time series is not required for calculations, any artifact in the ECG or ICG signals was corrected by simply removing that portion of data. A minimum of three, 1-minute segments was required. Any segments with more than 10% of the cardiac cycles removed or those containing more than 3 ectopic beats were not included in the grand mean. Ensemble averages were visually inspected to ensure that key landmarks were correctly placed on each waveform, namely the Q point of the ECG, the B point of the thorax dZ/dt signal, and the B point of the calf dZ/dt signal (Figure 2). When determining the correct location of the B point on the *thorax*, scorers followed the guidelines recommended by Árbol et al. (2017), which provides a decision tree for

visual B point detection based on waveform morphology. However, to complement this procedure, a max slope method (block size 30) was applied by the software to aid in B point placement when the waveform exhibited a steep gradient or rise, in which case the B point was automatically placed at the onset of the waveform. Similarly, this max slope method was automatically applied to the *calf* dZ/dt signal to place the B point at the onset of the rise in the impedance waveform. Next, we describe how these landmarks were used to compute the primary cardiac impedance and PWV variables.

The first measure of interest was the systolic time interval commonly referred to as pre-ejection period (PEP), calculated as the time difference (in ms) between the Q and thorax B points. PEP represents the time from ventricular activation (Q) to the onset of ventricular ejection (B) when intraventricular pressure overcomes aortic pressure and the aortic valve opens (Berntson et al., 2017). PEP is often used as an index of beta-adrenergic influences on the heart (Sherwood et al., 1990), with several studies indicating that PEP is inversely related to myocardial contractility (i.e., longer PEP represents less contractility). Next, the time interval between the Q and calf B points was calculated, which reflects the time (in ms) from ventricular activation to the arrival of the pulse waveform in the calf (calf PTT). Arterial PTT was then computed as the difference between these two intervals (calf PTT - PEP). While this approach is similar to other non-invasive PWV techniques that derive PTT from the difference in time intervals between two distant arterial pathways (e.g., carotid-femoral), d-ICG is unique in that the PTT is directly related to the onset of blood flow in the *aorta*. Studies have suggested that the PTT is not only influenced by propagation through the arterial tree, but also contractility of the heart (i.e., PEP; Shapiro et al., 1996). As such, d-ICG may provide an accurate method for estimating PTT while accounting for such factors. Lastly, a path length measurement for determining PWV was estimated by subtracting the calf height from the height of the left coracoid process. PWV (in meters per second) was then computed using the formula: $PWV = \text{path length (meters)} / \text{PTT (seconds)}$.

2.7 Measurement of beat-to-beat BP

Concurrent with d-ICG and ECG monitoring, beat-to-beat (continuous) BP was monitored non-invasively using a CNAP® 500 Monitor (CNSystems, Medizintechnik, AG, Austria). Beat-to-beat signals

were obtained by positioning appropriately-sized finger cuffs on the middle and index fingers of the left hand. These signals were passed to BioLab for digitization via a MindWare BioNex chassis 4-channel transducer module, and were calibrated to an oscillometric BP recording obtained from the brachial artery of the right arm. Participants' arms were situated comfortably at heart level by having them rest both arms on foam padding positioned on a tray table, which helped to minimize movement. Beat-to-beat BP was visually inspected and analyzed with Mindware Blood Pressure Variability software (version 3.1.5). The steps for artifact detection and computing grand mean values were identical to those used for HRV (see previous section), however, the use of beat-to-beat BP provided three additional primary outcomes: mean arterial pressure (MAP), TPR, and BRS. First, using grand mean values, TPR (dyne-seconds/cm⁵) was calculated as: $TPR = [MAP \text{ (mmHg)} / CO \text{ (liters/min)}] \times 80$ (Sherwood et al., 1990). Additionally, BRS (ms/mmHg) was measured using the sequence method (Berntson et al., 2017). This method identifies “up” sequences of 3 to 6 consecutive cardiac cycles where increases in systolic blood pressure (SBP) coincide with increases in IBI (i.e., slower HR), as well as “down” sequences where decreases in SBP are accompanied by decreases in IBI (i.e., faster HR). Minimum criteria for inclusion in a sequence was an increase/decrease of 1 mmHg in SBP, and a corresponding change of 1 ms in IBI (Reyes del Paso, de la Coba, Martín-Vázquez, & Thayer, 2017). BRS values were also adjusted for the prevailing heart period using the coefficient of variation method: $100 \times (BRS/IBI)$ (de Geus et al., 2019).

2.8 Measurement of endothelium dependent vasodilation (EDV)

Venous occlusion strain-gauge plethysmography was used to measure forearm blood flow, both at rest and following EDV induced by post-occlusive reactive hyperemia. EDV assessed by this method is reliable (Thijssen, Bleeker, Smits, & Hopman, 2005), predicts CVD outcomes (de Berrazueta et al., 2010), and associates with CVD risk factors (Higashi & Yoshizumi, 2003). EDV was evaluated in the present study using a modified Sivertsson protocol (Sivertsson, 1970), as described previously (Cheng, Daskalakis, & Falkner, 2013). Specifically, an indium-gallium strain gauge was applied to an area of the right forearm that corresponded to its maximum (widest) circumference, and these signals were fed to a Hokanson EC5R plethysmograph monitor (D. E. Hokanson Inc., Bellevue, WA). A proximal occlusion

cuff was positioned over the brachial artery of the same arm, which was attached to a Hokanson E20-AG101 rapid cuff inflator and Hokanson AG101 air source. The forearm was suspended approximately 3 cm above the atrium, with the hand angled slightly upward above the elbow using foam padding. A second, distal occlusion cuff was attached to the wrist, which was inflated to 40 mmHg above resting SBP during all venous occlusion assessments.

First, baseline readings (EDVbase) were made by rapidly inflating the upper proximal cuff to ~50 mmHg for 7 seconds (to occlude venous return) while arterial inflow (in %/min) was recorded via plethysmographic signals from the strain-gauge. The proximal cuff pressure was then released for 8 seconds in between inflations (each reading was 15 seconds long), and this process was repeated twelve times (i.e., 3 minutes). The flow rates for each reading were then averaged to calculate EDVbase (a minimum of 6 clean readings was required). Next, maximal vasodilation (EDVmax) induced by post-occlusive reactive hyperemia was assessed after a 3-minute recovery period from baseline readings. Following recovery, the proximal brachial occlusion cuff was inflated for 5 minutes with the pressure set to ~40 mmHg above resting SBP. After 5 minutes and the rapid deflation of the proximal occluding cuff, arterial inflow was measured once again using the same procedures outlined for EDVbase. Participants were then given another recovery period lasting 5 minutes before completing the EDVmax protocol a second time. To compute EDVmax the flow rates from the first reading in each max protocol were averaged together when possible; however, 8 participants had only one max reading and one did not have any due to movement artifact. Lastly, the primary variable reflecting EDV was the percent increase in post-hyperemia forearm blood flow (de Berrazueta et al., 2010; Tousoulis, Antoniades, & Stefanadis, 2005), which was calculated as: $EDV \% \text{ increase} = ((EDV_{\text{max}} - EDV_{\text{base}}) / EDV_{\text{base}}) \times 100$. The latter served as the primary indicator variable of endothelial function.

2.9 Statistical analysis

All analyses were completed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Continuous variables were visually inspected for normality, and four outliers were excluded for having values 3SD above the mean (one outlier each for BMI, BP, BRS, and TPR).

Additionally, one participant was excluded for having too many ectopic beats per segment in their ECG signal, leaving a total of 62 participants in the final analyses. Bivariate Pearson correlations (r) were used to examine the relationships between d-ICG PWV, cardiovascular disease risk factors, and other measures of cardiovascular autonomic control and vascular health. Five participants were missing data for TPR, BRS, and EDV because they completed their visits prior to operational availability of the venous occlusion and beat-to-beat BP equipment. Additionally, three participants were missing TPR and BRS because of equipment (CNAP) failure, one participant was missing EDVmax due to motion artifact, five participants did not complete the self-rated health questionnaire, and one participant was missing BMI (refused to have weight measured). Nonparametric Spearman's correlations (ρ) were used to test the relationship between PWV and measures of self-rated health and illness burden.

The test-retest reliability of PWV was calculated among 14 participants using the within-subject coefficient of variation, as well as intraclass correlations (ICC) stemming from a two-way mixed effects model with absolute agreement. Similarly, among 32 participants whose data were scored by two trained raters, inter-rater reliability was tested using a two-way mixed effects ICC model with absolute agreement. Lastly, exploratory analyses using Welch's t-test were carried out to compare PWV mean values according to sex and tobacco use.

3. Results

Participant characteristics and mean values for each measure are presented in Table 1. The mean age of participants was 36.6 years (SD = 18.5), with a larger percentage of participants identifying as female ($n=45$; 72.6%), Caucasian ($n=43$; 69.4%), and never having used tobacco ($n=34$; 81.0%). The average PWV in the present study was 8.35 m/s (SD = 2.17), which exhibited a strong positive association with age, $r(62) = .80$, as depicted in Figure 3. Age-specific median values of PWV (grouped by decade) are provided in Table 2. Pearson correlations between PWV and other cardiovascular health measures are illustrated in Figure 4 as a correlogram, and values are reported in Supplement Table S1. In addition to age, PWV demonstrated significant positive associations with BP and self-reported illness burden, indicating that individuals with higher BP and greater burden had faster PWV. Also observed

were significant negative associations between PWV and two canonical indicators of autonomic cardiovascular control: HF-HRV and BRS. Thus, faster PWV was related to lower HF-HRV and BRS, both before and after adjustment for the prevailing heart period (i.e., IBI). PWV was not significantly associated with self-rated health, height, BMI, resting HR, or TPR. Although PWV was negatively correlated with EDV, as reflected by the percent increase in post-hyperemia forearm blood flow (EDV % increase), the relation between faster PWV and lower EDV did not reach conventional statistical significance, $r(56) = -0.19, p = .16$.

In a subsample of 14 participants who completed the d-ICG protocol on two separate visits, PWV exhibited strong test-retest reliability. High consistency across the two measurement occasions was indicated by an intraclass correlation (ICC) of 0.952 (95% CI = 0.856, 0.984), as well as an average within-subject coefficient of variation of 3.4% (range of 0.8-10.8%). Further, among 32 participants whose data were scored by two trained raters, strong inter-rater agreement in PWV values was reflected by an ICC of 0.993 (95% CI = 0.985, 0.997).

Lastly, exploratory analyses revealed that PWV did not statistically differ between males ($M = 9.07$ m/s, $SD = 2.55$) and females ($M = 8.08$ m/s, $SD = 1.98$; $t[23.7] = -1.4, p = .16$). PWV mean values were also not statistically different among participants reporting current/former ($M = 8.40$ m/s, $SD = 1.69$) versus no prior tobacco use ($M = 7.95$ m/s, $SD = 2.07, t[12.5] = -0.6, p = .53$).

4. Discussion

Here, we tested whether a measure of PWV derived from dual ICG signals on the thorax and calf (d-ICG) was related to age, known risk factors for cardiovascular disease, and common indicators of cardiovascular autonomic activity. Also, at issue was determining whether age-related values of d-ICG PWV approximated those of published normative values, as well as comparing the test-retest reliability and inter-rater agreement with other techniques. As expected, PWV correlated robustly with age (Figure 3) and moderately with BP and two canonical indicators of cardiovascular autonomic activity: HF-HRV and spontaneous BRS. Participants reporting a greater number of chronic health conditions also exhibited faster PWV. The latter findings are compatible with the interpretation that faster PWV – presumably

reflecting greater arterial stiffness – relates to poorer cardiovascular health and cardiovascular autonomic control. However, several indicators of cardiovascular health and function were not statistically related to PWV, including self-rated health, adiposity (BMI), vascular resistance (TPR), and endothelial function (EDV % Increase). Notably, we observed general comparability between PWV and population normative values as a function of age (Table 2), despite salient methodological differences and algorithms used to derived normative values and our measure of d-ICG PWV (see below). The test-retest reliability of PWV (over ~1 week) was strong, as was its inter-rater agreement among research assistants trained in standard ICG data reduction methods and ensemble averaging. Collectively, the present findings support the utility and broader use of d-ICG to measure PWV in psychophysiology, behavioral medicine, and related fields.

As reflected in Figure 2 and Table 2, values of PWV observed in this community sample increased with age and approximated those of age-specific normative values. Specifically, an interpretive comparison can be made with respect to PWV values and ranges reported by Khoshdel and colleagues (2006), who derived normative PWV estimates from 25 studies of over 8000 people. Our PWV estimates agree closely with these normative values despite the much smaller sample sizes and greater variability among the older age groups in the current study. We should note the importance of differences between instrumentation and scoring methodologies that are used to derive d-ICG PWV, while still agreeing with normative references and ranges. As mentioned previously, most current methods used to measure PWV rely on proprietary medical devices (e.g., the Compilor instrument), trained technicians, and very brief recording periods (i.e., a few heart beats) among recumbent participants. In the existing literature, moreover, measurements of arterial distance are often ‘adjusted’ to normalize estimates across laboratories and corrected with mathematical formulae to reduce bias (e.g., The Reference Values for Arterial Stiffness’ Collaboration, 2010). In addition to these methodological variations in the literature are conceptual issues pertaining to the scoring of pulse waveforms. For example, existing measures of PWV are often derived by identifying the foot of obtained waveforms for carotid and femoral artery sites (or other distal sites), and not those originating from the aorta itself. Of clear importance, inter-rater agreement for d-ICG PWV was comparable or exceeded what has been previously reported between

sonographers/technicians and trained raters using other techniques (Sigrist, Chiarelli, Levin, Romann, & Weber, 2010; Sutton-Tyrrell, Mackey, Holubkov, Vaitkevicius, Spurgeon, & Lakatta, 2001). PWV derived from d-ICG also exhibited high test-retest reliability over ~1 week with a coefficient of variation and ICC comparable to other methods (Grillo et al., 2018; Salvi et al., 2008); however, repeated measurements have typically been collected minutes apart as opposed to days as in the present study. Indeed, the repeatability of these other method tends to be lower when measured weeks apart (Meyer et al., 2016). In sum, the strong reliability and comparability of d-ICG PWV metrics to established reference values supports the validity of this measure.

d-ICG has been validated in comparison with accepted ultrasound measures of PWV in a pilot study by our research group (Jennings, Scudder, Matthews, Owens, & Gianaros, 2017), and in a study using a commercially available variant of the impedance technique referred to as “whole-body ICG” (CircMon, JR Medical Ltd, Estonia) (Kööbi, Kähönen, Iivainen, & Turjanmaa, 2003). Studies adopting these impedance techniques have demonstrated the utility of PWV to track with other related health factors including childhood socioeconomic status and cardiometabolic risk (Koivisto et al., 2011; Puolakka et al., 2017), changes in ideal cardiovascular health behaviors (e.g., smoking, physical activity, etc.; Aatola et al., 2014), as well as cross-sectional associations with markers of inflammation (i.e., interleukin-6 and C-reactive protein; Lockwood, Jennings, & Matthews, 2017). However, Kööbi and colleagues (2003) have noted that the whole-body ICG method is partially limited by the fact it does not reflect the beginning of pulse transmission in the aortic arch precisely enough, resulting in a slight overestimation of PWV. Although the majority of non-invasive methods derive PWV by calculating the difference in PTT between two distal arterial sites, d-ICG is advantageous in that the onset of each pulse waveform is time-locked to the moment the aortic valve opens and blood is ejected from the heart, which also provides a useful index of myocardial contractility (i.e., PEP). As such, d-ICG may provide a unique estimate of pulse transmission through an arterial pathway that can simultaneously account for individual differences or changes in inotropy.

The validity of d-ICG PWV is supported to some degree by its relationship to other techniques, but more so by the appropriate correlation of the measure with other biological indices. Therefore, we added an additional set of physiological measures (i.e., cardiovascular autonomic function and vasodilative recovery) to see if expected relationships with PWV were observed. To date, studies examining the relationship between arterial stiffness and aspects of autonomic function have largely focused on their roles in the short-term regulation of BP. Arterial baroreceptors located in the aortic arch and carotid sinuses play a crucial role in this regulation by sensing acute changes in BP with each pulse. Given that baroreceptor sensitivity is dependent on the ability to *stretch* and respond to the mechanical force of blood flow against the arterial wall, increased stiffening of these segments (i.e., greater PWV) is believed to contribute to baroreflex dysfunction (Lu et al., 2014; Mattace-Raso et al., 2007; Monahan, 2007). The current findings also align with previous PWV studies that have revealed an inverse relation between arterial stiffness and HF-HRV (Lu et al., 2014; Perkins, Owen, Swaine, & Wiles, 2006), which likely stems from impaired BRS and the associated alteration in vagal motor neuron outflow (Berntson, Cacioppo, & Quigley, 1993). However, despite d-ICG PWV exhibiting moderately strong associations with indices of autonomic function, only a relationship of small effect size ($r = -0.19$) was observed between arterial stiffness and vasodilative recovery (i.e., EDV % Increase). Much like PWV, endothelial function can be assessed non-invasively using several different techniques, which all tap into the ability of the endothelium to release vasodilating factors in response to reactive hyperemia-induced stress in the peripheral vasculature (Stoner, Young, & Fryer, 2012). Endothelial dysfunction has been implicated in the progression of arterial stiffness and other atherogenic changes, which are collectively integrated under the concept of ‘vascular failure’ (Tanaka et al., 2018). Previous work using ultrasound has revealed a negative correlation between endothelial function (using flow-mediated dilation) and PWV (McEniery et al., 2006; Nigam, Mitchell, Lambert, & Tardif, 2003). However, the findings reported by Koivisto and colleagues (2012) using their whole-body ICG method indicated that a weak inverse relation between endothelial function and PWV was attenuated when controlling for age and sex. While there appears to be a consistent trend with studies reporting a link between poorer endothelial function and greater arterial

stiffness, the strength of this association appears variable and likely fluctuates across studies due to differences in methodology, sample size, and the specific participant population.

While d-ICG holds promise for simultaneously assessing PWV and other indices of cardiovascular health/function, there are study limitations of which to be aware. First, the current sample was small and primarily included younger, healthy adults. As such, we were potentially unable to detect differences in PWV as it relates to factors such as endothelial function, BMI, sex, and smoking. The latter have demonstrated somewhat inconsistent associations with PWV in the literature (Cecelja & Chowienczyk, 2009). Second, as with most non-invasive measures of PWV, d-ICG cannot provide a direct measurement of *central aortic* stiffness due to the longer pulse trajectory through distal arteries (e.g., popliteal and tibial). Alpha and, to a lesser extent, beta sympathetic receptors are present in the vasculature and regulate the vascular smooth muscle tone that can influence vessel diameter and stiffness (Vlachopoulos et al., 2011). Third, our initial decision to use band electrodes for the calf impedance channel resulted in an unstable signal for a portion of the participants due to impedance levels falling outside the bounds of the amplifier. Although the amplitude of the waveform remains trivial, when adopting the ICG technique it is necessary to capture the full range of the signal from which the first derivative can be used for timing characteristics, so certain hardware settings may need to be adjusted. Also, it has yet to be determined if a single set of source/current electrodes could be combined with the two sets of sensing electrode pairs to simplify this technique even further.

Minor limitations aside, perhaps the greatest strength of the d-ICG PWV method is its ease of use across diverse research settings. The technique requires minimal expertise (saving on technician costs) and can be reliably collected for extended periods of time as participants perform tasks or rest quietly. It also alleviates much of the difficulty obtaining measurements in participants with greater levels of adiposity, given that the impedance waveform does not rely on the detection of small pressure changes from transducers on the surface of the skin. Indeed, d-ICG PWV was collected successfully in one participant with a BMI of 60; however, this individual was excluded from analyses for being 3SD above the mean. d-ICG PWV is also advantageous because it does not involve the use of proprietary algorithms

that may require additional health information (e.g., sex, HR, BP, etc.) to generate an estimate of arterial stiffness.

In conclusion, d-ICG provides an accurate and reliable means of assessing arterial stiffness, with several advantages compared to other available methods. Its relative ease of implementation further enables the simultaneous measurement of common psychophysiological indices of cardiovascular health and autonomic function.

References

- Aatola, H., Hutri-Kähönen, N., Juonala, M., Laitinen, T. T., Pahkala, K., Mikkilä, V... & Kähönen, M. (2014). Prospective relationship of change in ideal cardiovascular health status and arterial stiffness: The Cardiovascular Risk in Young Finns Study. *Journal of the American Heart Association*, 3(2), e000532. <https://www.doi.org/10.1161/JAHA.113.000532>
- Árbol, J. R., Perakakis, P., Garrido, A., Mata, J. L., Fernández-Santaella, M. C., & Vila, J. (2017). Mathematical detection of aortic valve opening (B point) in impedance cardiography: A comparison of three popular algorithms. *Psychophysiology*, 54, 350-357. <https://www.doi.org/10.1111/psyp.12799>
- Barger, S. D., Cribbet, M. R., & Muldoon, M. F. (2016). Participant-reported health status predicts cardiovascular and all-cause mortality independent of established and nontraditional biomarkers: Evidence from a representative US sample. *Journal of the American Heart Association*, 5(9), e003741. <https://www.doi.org/10.1161/JAHA.116.003741>
- Bérard, E., Bongard, V., Ruidavets, J., Amar, J., & Ferrières, J. (2013). Pulse wave velocity, pulse pressure and number of carotid or femoral plaques improve prediction of cardiovascular death in a population at low risk. *Journal of Human Hypertension*, 27, 529–534. <https://www.doi.org/10.1038/jhh.2013.8>
- Berntson, G. G., Caccioppo, J. T., & Quigley, K. S. (1993). Respiratory sinus arrhythmia: Autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology*, 30(2), 183-196. <https://www.doi.org/10.1111/j.1469-8986.1993.tb01731.x>
- Berntson, G. G., Quigley, K. S., Jang, J. F., & Boyson, S.T. (1990). An approach to artifact identification: Application to heart period data. *Psychophysiology*, 27(5), 586–598. <https://www.doi.org/10.1111/j.1469-8986.1990.tb01982.x>
- Berntson, G. G., Quigley, K. S., Norman, G. J., & Lozano, D. L. (2017). Cardiovascular psychophysiology. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Cambridge*

- handbooks in psychology. Handbook of psychophysiology* (p. 183–216). Cambridge University Press.
- Boutouyrie, P., Tropeano, A. I., Asmar, R., Gautier, I., Benetos, A., Lacolley, P., & Laurent, S. (2002). Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: A longitudinal study. *Hypertension*, 39(1), 10-15. <https://www.doi.org/10.1161/hy0102.099031>
- Boutouyrie, P., Briet, M., Collin, C., Vermeersch, S., & Pannier, B. (2009). Assessment of pulse wave velocity. *Artery Research*, 3, 3-8. <https://www.doi.org/10.1016/j.artres.2008.11.002>
- Cecelja, M., & Chowienczyk, P. (2009). Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: A systematic review. *Hypertension*, 54(6), 1328-1336. <https://www.doi.org/10.1161/HYPERTENSIONAHA.109.137653>
- Cheng, C., Daskalakis, C., & Falkner, B. (2013). Non-invasive assessment of microvascular and endothelial function. *JoVE (Journal of Visualized Experiments)*, (71), e50008. <https://www.doi.org/10.3791/50008>.
- de Berrazueta, J. R., Guerra-Ruiz, A., García-Unzueta, M. T., Toca, G. M., Laso, R. S., de Adana, M. S... & LLorca, J. (2010). Endothelial dysfunction, measured by reactive hyperaemia using strain-gauge plethysmography, is an independent predictor of adverse outcome in heart failure. *European Journal of Heart Failure*, 12(5), 477-483. <https://www.doi.org/10.1093/eurjhf/hfq036>
- de Geus, E. J. C., Gianaros, P. J., Brindle, R. C., Jennings, J. R., & Berntson, G. G. (2019). Should heart rate variability be “corrected” for heart rate? Biological, quantitative, and interpretive considerations. *Psychophysiology, SPR Meeting Issue 2019*, e13287. <https://www.doi.org/10.1111/psyp.13287>
- Ernst, J. M., Litvack, D. A., Lozano, D. L., Cacioppo, J. T., Berntson, G. G. (1999). Impedance cardiography: Noise as a signal in impedance cardiography. *Psychophysiology*, 36, 333-338. <https://www.doi.org/10.1017/S0048577299981003>
- Grillo, A., Parati, G., Rovina, M., Moretti, F., Salvi, L., Gao, L... & Salvi, P. (2018). Short-term repeatability of noninvasive aortic pulse wave velocity assessment: Comparison between methods

- and devices. *American Journal of Hypertension*, 31(1), 80-88.
<https://www.doi.org/10.1093/ajh/hpx140>
- Hallock, P. (1934). Arterial elasticity in man in relation to age as evaluated by the pulse wave velocity method. *Archives of Internal Medicine*, 54(5), 770–798.
<https://www.doi.org/10.1001/archinte.1934.00160170113010>
- Higashi, Y., & Yoshizumi, M. (2003). New methods to evaluate endothelial function: Method for assessing endothelial function in humans using a strain-gauge plethysmography: Nitric oxide-dependent and -independent vasodilation. *Journal of Pharmacological Sciences*, 93(4), 399-404.
<https://www.doi.org/10.1254/jphs.93.399>
- Jennings, J. R., & Choi, S. (1983). An arterial to peripheral pulse wave velocity measure. *Psychophysiology*, 20(4), 410-418. <https://www.doi.org/10.1111/j.1469-8986.1983.tb00922.x>
- Jennings, J. R., Scudder, M., Matthews, K., Owens, J., & Gianaros, P. (2017). Arterial pulse wave velocity derived from impedance plethysmography. *Psychophysiology*, 54(S1), S76.
<https://www.doi.org/10.1111/psyp.12950>
- Jennings, J. R., Tahmouh, A. J., and Redmond, D. P. (1980). Non-invasive measurement of peripheral vascular activity. In I. Martin and P. H. Venables (Eds.), *Techniques in psychophysiology* (pp. 69-137). Chichester, England: Wiley.
- Kelly, R., Hayward, C., Ganis, J., Daley, J., Avolio, A., & O'Rourke, M. (1989). Noninvasive registration of the arterial pressure pulse waveform using high-fidelity applanation tonometry. *Journal of Vascular Medicine and Biology*, 1(3), 142-149.
- Khoshdel, A. R., Thakkestian, A., Carney, S. L., & Attia, J. (2006). Estimation of an age-specific reference interval for pulse wave velocity: A meta-analysis. *Journal of Hypertension*, 24(7), 1231-1237. <https://www.doi.org/10.1097/01.hjh.0000234098.85497.31>
- Koivisto, T., Hutri-Kähönen, N., Juonala, M., Aatola, H., Kööbi, T., Lehtimäki, T... & Kähönen, M. (2011). Metabolic syndrome in childhood and increased arterial stiffness in adulthood - The

- Cardiovascular Risk in Young Finns Study. *Annals of Medicine*, 43(4), 312-319.
<https://www.doi.org/10.3109/07853890.2010.549145>
- Koivistoinen, T., Virtanen, M., Hutri-Kähönen, N., Lehtimäki, T., Jula, A., Juonala, M... & Kähönen, M. (2012). Arterial pulse wave velocity in relation to carotid intima-media thickness, brachial flow-mediated dilation and carotid artery distensibility: The Cardiovascular Risk in Young Finns Study and the Health 2000 Survey. *Atherosclerosis*, 220(2), 387-393.
<https://www.doi.org/10.1016/j.atherosclerosis.2011.08.007>
- Kööbi, T., Kähönen, M., Iivainen, T., & Turjanmaa, V. (2003). Simultaneous non-invasive assessment of arterial stiffness and haemodynamics - a validation study. *Clinical Physiology and Functional Imaging*, 23(1), 31-36. <https://www.doi.org/10.1046/j.1475-097X.2003.00465.x>
- Kubicek, W. G., Karnegis, J. N., Patterson, R. P., Witsoe, D. A. & Mattson, R. H. (1966). Development and evaluation of an impedance cardiac output system. *Aerospace Medicine*, 37, 1208-1212.
- Laurent, S., Marais, L., & Boutouyrie, P. (2016). The noninvasive assessment of vascular aging. *Canadian Journal of Cardiology*, 32(5), 669-679.
<https://www.doi.org/10.1016/j.cjca.2016.01.039>
- Lockwood, K. G., Jennings, J. R., & Matthews, K. A. (2017). Psychophysiological correlates of systemic inflammation in black and white men. *Brain, Behavior, and Immunity*, 59, 93-102.
<https://www.doi.org/10.1016/j.bbi.2016.08.013>
- Lu, D. -Y., Sung, S. -H., Yu, W. -C., Cheng, H. -M., Chuang, S. -Y., & Chen, C. -H. (2014). Wave reflections, arterial stiffness, heart rate variability and orthostatic hypotension. *Hypertension Research*, 37, 1056-1061. <https://www.doi.org/10.1038/hr.2014.127>
- McEniery, C. M., Wallace, S., Mackenzie, I. S., McDonnell, B., Yasmin, Newby, D. E... & Wilkinson, I. B. (2006). Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension*, 48(4), 602-608.
<https://www.doi.org/10.1161/01.HYP.0000239206.64270.5f>

- Meyer, M. L., Tanaka, H., Palta, P., Patel, M. D., Camplain, R., Couper, D... & Heiss, G. (2016). Repeatability of central and peripheral pulse wave velocity measures: The Atherosclerosis Risk in Communities (ARIC) Study. *American Journal of Hypertension*, 29(4), 470-475.
<https://www.doi.org/10.1093/ajh/hpv127>
- Mohiaddin, R. H., Firmin, D. N., & Longmore, D. B. (1993). Age-related changes of human aortic flow wave velocity measured noninvasively by magnetic resonance imaging. *Journal of Applied Physiology*, 74(1), 492-497. <https://www.doi.org/10.1152/jappl.1993.74.1.492>
- Monahan, K. D. (2007). Effect of aging on baroreflex function in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 293(1), R3-R12.
<https://www.doi.org/10.1152/ajpregu.00031.2007>
- Nigam, A., Mitchell, G. F., Lambert, J., & Tardif, J. (2003). Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. *The American Journal of Cardiology*, 92(4), 395-399. [https://www.doi.org/10.1016/S0002-9149\(03\)00656-8](https://www.doi.org/10.1016/S0002-9149(03)00656-8)
- Pereira, T., Correia, C. & Cardoso, J. (2015). Novel methods for pulse wave velocity measurement. *Journal of Medical and Biological Engineering*, 35, 555–565.
<https://www.doi.org/10.1007/s40846-015-0086-8>
- Perkins, G. M., Owen, A., Swaine, I. L., & Wiles, J. D. (2006). Relationships between pulse wave velocity and heart rate variability in healthy men with a range of moderate-to-vigorous physical activity levels. *European Journal of Applied Physiology*, 98, 516-523.
<https://www.doi.org/10.1007/s00421-006-0303-9>
- Puolakka, E., Pahkala, K., Laitinen, T. T., Magnussen, C. G., Hutri-Kähönen, N., Kähönen, M... & Juonala, M. (2017). Childhood socioeconomic status and arterial stiffness in adulthood. *Hypertension*, 70(4), 729-735. <https://www.doi.org/10.1161/HYPERTENSIONAHA.117.09718>
- Reyes del Paso, G. A., de la Coba, P., Martín-Vázquez, M., & Thayer, J. F. (2017). Time domain measurement of the vascular and myocardial branches of the baroreflex: A study in physically

- active versus sedentary individuals. *Psychophysiology*, 54(10), 1528-1540.
<https://www.doi.org/10.1111/psyp.12898>
- Rogers, W., Hu, Y-L., Coast, D., Vido, D., Kramer, C., Pyeritz, R., & Reichek, N. (2001). Age-associated changes in regional aortic pulse wave velocity. *Journal of the American College of Cardiology*, 38(4), 1123-1129. [https://www.doi.org/10.1016/S0735-1097\(01\)01504-2](https://www.doi.org/10.1016/S0735-1097(01)01504-2)
- Salvi, P., Magnani, E., Valbusa, F., Agnoletti, D., Alecu, C., Joly, L., & Benetos, A. (2008). Comparative study of methodologies for pulse wave velocity estimation. *Journal of Human Hypertension*, 22, 669-677. <https://www.doi.org/10.1038/jhh.2008.42>
- Shapiro, D., Jamner, L. D., Lane, J. D., Light, K. C., Myrtek, M., Sawada, Y., & Steptoe, A. (1996). Blood pressure publication guidelines. *Psychophysiology*, 33(1), 1-12.
<https://www.doi.org/10.1111/j.1469-8986.1996.tb02103.x>
- Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Livallo, W. R., & van Doornen, L. J.P. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology*, 27(1), 1-23.
<https://www.doi.org/10.1111/j.1469-8986.1990.tb02171.x>
- Sigrist, M. K., Chiarelli, G., Levin, A., Romann, A., & Weber, C. (2010). Pulse wave velocity measurements are reproducible in multiple trained observers: A short report. *Nephron Clinical Practice*, 116, c60-c64. <https://www.doi.org/10.1159/000314664>
- Sivertsson, R. (1970). The hemodynamic importance of structural vascular changes in essential hypertension. *Acta Physiologica Scandinavica*, 343(suppl), 3-56.
- Sutton-Tyrrell, K., Mackey, R. H., Holubkov, R., Vaitkevicius, P. V., Spurgeon, H. A., & Lakatta, E. G. (2001). Measurement variation of aortic pulse wave velocity in the elderly. *American Journal of Hypertension*, 14(5), 463-468. [https://www.doi.org/10.1016/S0895-7061\(00\)01289-9](https://www.doi.org/10.1016/S0895-7061(00)01289-9)
- Stoner, L., Young, J. M., & Fryer, S. (2012). Assessments of arterial stiffness and endothelial function using pulse wave analysis. *International Journal of Vascular Medicine*, 2012, 903107.
<https://www.doi.org/10.1155/2012/903107>

- Tanaka, A., Tomiyama, H., Maruhashi, T., Matsuzawa, Y., Miyoshi, T., Kabutoya, T... & Node, K. (2018). Physiological diagnostic criteria for vascular failure. *Hypertension*, 72(5), 1060-1071. <https://www.doi.org/10.1161/HYPERTENSIONAHA.118.11554>
- The Reference Values for Arterial Stiffness' Collaboration. (2010). Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values. *European Heart Journal*, 31, 2338-2350. <https://www.doi.org/10.1093/eurheartj/ehq165>
- Thijssen, D. H. J., Bleeker, M. W. P., Smits, P., & Hopman, M T. E. (2005). Reproducibility of blood flow and post-occlusive reactive hyperaemia as measured by venous occlusion plethysmography. *Clinical Science (Lond)*, 108(2), 151-157. <https://www.doi.org/10.1042/CS20040177>
- Tousoulis, D., Antoniades, C., & Stefanadis, C. (2005). Evaluating endothelial function in humans: A guide to invasive and non-invasive techniques. *Heart*, 91, 553-558. <https://www.doi.org/10.1136/hrt.2003.032847>
- Vlachopoulos, C., Aznaouridis, K., & Stefanadis, C. (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: A systematic review and meta-analysis. *Journal of the American College of Cardiology*, 55(13), 1318-1327. <https://www.doi.org/10.1016/j.jacc.2009.10.061>
- Vlachopoulos, C., Nichols, W., & O'Rourke, M. (2011). *McDonald's blood flow in arteries: Theoretical, experimental and clinical principles* (6th ed.). Boca Raton, FL: CRC Press.
- Whelton, P. K., Carey, R. M., Aronow, W. S., Casey Jr., D. E., Collins, K. J., Himmelfarb, C. D... & Wright Jr., J. T. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults. *Journal of the American College of Cardiology*, 71(19), e13-115. <https://www.doi.org/10.1016/j.jacc.2017.11.006>

Author Notes

Grant Funding: Research reported in this publication was partly supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number P01-HL040962. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgments: We would first like to extend our gratitude posthumously to Dave Lozano for his contributions in psychophysiology, and for supporting our endeavor to create a dual impedance PWV measure using a MindWare platform. We would also like to thank Eric Morgan for consulting with us on the technique, most specifically for his help in understanding the bioelectric difficulties and challenges imposed by band electrodes, and for his assistance creating Figure 2 to display dual impedance ensemble average waveforms.

Conflict of Interest: Due to their interest in the technique, MindWare upgraded three software licenses for signal analysis free of charge. None of the authors have any financial or other direct interest in MindWare, and costs related to equipment modification and supplies were borne by the authors.

Address correspondence to Mark Scudder at mas721@pitt.edu

Data and code are available at: https://osf.io/tp83x/?view_only=772cc8ffd8c343afa7c6c27762760c97

Table 1*Mean Values of Participant Cardiovascular Health Measures*

Measure	Mean	SD	Range
<i>N</i>	62 (45 female)		
Age (years)	36.6	18.5	19 - 78
BMI (kg/m ²)	25.0	4.3	17.9 - 37.4
SBP (mmHg)	114.4	14.0	91.5 - 153.5
DBP (mmHg)	67.5	8.9	55.5 - 94.5
MAP (mmHg)	85.3	10.4	70.0 - 116.5
Resting HR (bpm)	70.0	10.0	50.5 - 94.5
TPR (dyne-seconds/cm ⁵)	820.3	291.2	424.1 - 1555.5
BRS	2.57	1.08	0.86 - 5.45
EDV % Increase	1000.8	467.5	199.5 - 2203.6
HF-HRV	41.71	9.99	15.25 - 61.98
PEP (ms)	111.0	16.6	60.8 - 151.5
Calf PTT (ms)	246.4	22.0	187.0 - 279.3
PWV (m/s)	8.35	2.17	5.99 - 16.40

Note. BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; MAP - mean arterial pressure; HR - heart rate; TPR - total peripheral resistance; BRS - baroreflex sensitivity (heart period corrected); EDV - endothelium dependent vasodilation; HF-HRV - high frequency heart rate variability (heart period corrected); PEP - pre-ejection period; PTT - pulse transit time; PWV - pulse wave velocity.

Table 2

Comparison of Age-Specific PWV Values with Published Reference Intervals

Age Group (years)	N	Mean (SD)	Median	5 th - 95 th Reference Intervals
< 30	32	7.26 (0.67)	7.27	6.31 - 10.94
30 - 39	8	7.31 (0.58)	7.46	6.15 - 11.33
40 - 49	6	8.44 (0.96)	8.12	6.07 - 11.86
50 - 59	6	9.50 (1.53)	9.92	6.10 - 12.43
60 - 69	6	10.61 (2.04)	10.38	6.27 - 13.18
70 - 80	4	13.96 (2.77)	14.15	6.63 - 14.15

Note. Reference intervals are those found in Khoshdel, Thakkestian, Carney, & Attia (2006).

Pulse wave velocity (PWV) values are reported in meters per second.

Supplement Table S1

Pearson Correlations between PWV and other Cardiovascular Health Measures

Measure	1	2	3	4	5	6	7	8	9	10	11	12	13
1. PWV	-												
2. Age	.80**	-											
3. BMI	.08	.23	-										
4. MAP	.55**	.47**	.35*	-									
5. Resting HR	.00	-.01	.17	-.02	-								
6. TPR	.03	-.04	-.03	.14	-.16	-							
7. BRS	-.45**	-.54**	-.17	-.41**	-.24	.19	-						
8. EDV % Increase	-.19	-.26*	-.30*	-.22	-.07	-.30*	.10	-					
9. HF-HRV	-.43**	-.58**	-.23	-.33**	-.41**	.24	.62**	.03	-				
10. PEP	.51**	.28*	.01	.40**	-.06	.12	-.22	-.04	-.13	-			
11. Calf PTT	-.68**	-.74**	-.04	-.36**	-.06	.04	.27*	.21	.45**	.18	-		
12. Self-Rated Health	-.04	.10	.32*	.32*	.39*	-.14	.06	-.06	-.13	-.01	-.11	-	
13. Illness Burden	.41**	.59**	.13	.42**	-.12	.03	-.36**	-.20	-.37**	.13	-.51**	.13	-

Note. Spearman's rho is reported in the bottom two rows for self-report measures (higher values reflect poorer health and greater burden). PWV - pulse wave velocity; BMI - body mass index; MAP - mean arterial pressure; HR - heart rate; TPR - total peripheral resistance; BRS - baroreflex sensitivity; EDV - endothelium dependent vasodilation; HF-HRV - high frequency heart rate variability; PEP - pre-ejection period; PTT - pulse transit time.

* $p < .05$; ** $p < .01$

Figure Captions

Figure 1. To measure calf impedance, two *outer* source electrodes (red) were affixed to the lateral side of the left calf, one above the lateral malleolus (ankle) and the other below the tibial tuberosity. Two *inner* recording electrodes (white) were placed ~4 cm above/below the source electrodes on the medial side of the calf. Calf height was measured from the floor to the mid-point of the recording electrodes.

Figure 2. Pulse transit time (PTT) was measured using two time intervals: 1) the Q point of the electrocardiogram to the B point of the thorax impedance dZ/dt waveform, also known as pre-ejection period (PEP), and 2) the Q point to the B point of the calf dZ/dt waveform (calf PTT). Arterial PTT was calculated as the difference between these intervals (calf PTT - PEP), and used in later computation of pulse wave velocity.

Figure 3. As expected, faster pulse wave velocity (i.e., greater arterial stiffness) measured with dual impedance cardiography was strongly related to increased age.

Figure 4. A correlogram depicting the strength of associations between pulse wave velocity (PWV) and other included measures. The color and size of each circle correspond to the direction and strength of each relationship, as indexed by the scale on the horizontal axis. BMI - body mass index; MAP - mean arterial pressure; HR - heart rate; TPR - total peripheral resistance; BRS - baroreflex sensitivity; EDV - endothelium dependent vasodilation; HF-HRV - high frequency heart rate variability; PEP - pre-ejection period; PTT - pulse transit time.

* $p < .05$; ** $p < .01$; *** $p < .001$

Figure 1

Calf Electrode Placement for Dual Impedance Cardiography

Figure 2

Ensemble Average Waveforms Derived from Dual Impedance Cardiography

Figure 3

The Positive Association between Pulse Wave Velocity and Age

Figure 4

Correlogram of Pulse Wave Velocity and other Study Variables