Carotid-Femoral Pulse Wave Velocity Acquisition Methods and Their Associations with Cardiovascular Risk Factors and Subclinical Biomarkers of Vascular Health

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ABSTRACT:

Different methods to measure carotid-femoral pulse wave velocity (CFPWV) may affect the measurements obtained and influence the association between CFPWV, cardiovascular risk factors and biomarkers of subclinical vascular health. The estimation of distance between the carotid and femoral artery measurement sites (the arterial path-length) is particularly problematic. We determined if CFPWV and equation-based estimates of CFPWV were influenced by arterial path-length and if this affected the association of CFPWV with cardiovascular risk factors and subclinical vascular biomarkers. The CFPWV derived from the measurement of surface-distance (CFPWV-D), arterial path-length formula (CFPWV-F), and estimated CFPWV (ePWV) were obtained from 489 older adults (67.2±8.8 yrs). Macrovascular [carotid artery: lumen diameter (LD), inter-adventitial diameter (IAD), intima-media thickness (IMT) and total plaque area (TPA)] and microvascular [reactive hyperaemia index and urinary albumin-creatinine ratio (UACR)] biomarkers were also measured. CFPWV-D was significantly greater than CFPWV-F [9.6 (8.0-11.2) vs 8.9 (7.6-10.5) m/s, $p<0.001$], due to estimated path-length being longer in CFPWV-D than CFPWV-F (495.4±44.8 vs 465.3±20.6 mm, $p<0.001$). ePWV was significantly greater than both CFPWV-F and CFPWV-D [11.0 (10.0-12.2) m/s, $p<0.001$]. The three CFPWV methods were similarly associated with LD, IAD, IMT, TPA and UACR, but not with cardiovascular risk factors. In conclusion, different methods to measure CFPWV affect the derived measurement values and the association with cardiovascular risk factors, but not the association with subclinical biomarkers of vascular health. These hitherto unreported observations are important considerations in experimental design, data interpretation and, of particular importance, comparison between studies where CFPWV is measured.

Key Words: Ageing, Arteriosclerosis, Atherosclerosis, Cardiovascular, Ultrasound.

INTRODUCTION:

Elevated central artery stiffness adversely affects cardiovascular haemodynamics and increases cardiovascular risk. For instance, a widening of pulse pressure in large conduit arteries increases transmission of pulsatile force into the microcirculation [1] and is detrimental to target organs like the brain, heart and kidneys. The ability to measure arterial stiffness has thus added to an understanding of cardiovascular risk beyond that provided by conventional risk factors [2]. Consequently, non-invasive assessment of arterial stiffness is a promising tool to identify, stratify and manage individuals who may benefit from early intervention to reduce cardiovascular risk, where intervention may otherwise be overlooked on the basis of conventional risk factors alone. However, there are several reasons why measurement of arterial stiffness remains under-used in a clinical setting.

Carotid-femoral pulse wave velocity (CFPWV) is the most widely used technique to evaluate central artery stiffness and can be performed non-invasively, has reference values, and possesses evidence-based prognostic utility [3]. However, a well-recognised problem with CFPWV is the estimation of distance between the carotid- and femoral artery measurement sites – the arterial path-length. This is usually done by measuring the surface distance between the two measurements sites. Different approaches have been taken to measure this distance [4-7], and guidelines published [1,7], but uncertainties remain because all are estimates of the distance travelled by tortuous arteries within the body, but without directly measuring their length. Furthermore, because arterial path-length is used in the calculation of CFPWV, small errors translate into much larger errors in the resultant CFPWV, with errors up to ~30% being reported by some [1,8].

Recently, Weir-McCall and colleagues [9] developed a population-derived simple path-length formula to calculate CFPWV (CFPWV-F) using routine clinical data that negated the need for actual distance measurements. The formula removed inter-centre CFPWV measurement variability without influencing the diagnostic utility of CFPWV and also weakened the association between CFPWV and obesity-related risk factors [body mass index (BMI) and waist circumference] [9]. Adoption of this formula could therefore reduce error, improve CFPWV accuracy and increase the feasibility of using CFPWV in clinical settings.

Another approach to quantifying central artery stiffness has come from estimated pulse wave velocity (ePWV), derived solely from equations that include age and mean arterial pressure (MAP) [10]. This approach overcomes limitations of time, equipment and specialist training. The method has been reported to predict cardiovascular events independently of risk score models in apparently healthy populations, in a hypertensive population and in patients with suspected coronary artery disease [11-14]. Again, this method promises to deliver a clinically useful estimate of central artery stiffness that can easily be implemented in the clinic.

These two new approaches to estimating central artery stiffness are promising, but to gain traction, a number of issues need to be addressed. Here, we wanted to determine if ePWV and CFPWV-F are similar to those obtained by the conventional measurement using surface-distance measurement (CFPWV-D) in a relatively large cohort of older men and women. Also, we wanted to know if the three methods are similarly associated with cardiovascular risk factors and subclinical biomarkers of vascular health. Finally, we wanted to know which

estimate of arterial stiffness had the strongest associations with cardiovascular risk factors and subclinical biomarkers of vascular health. These are important considerations for the use of these new methods in experimental and clinical settings, as well as being helpful for the comparison and interpretation of data from studies that use different methods to estimate central artery stiffness.

MATERIALS AND METHODS:

Participants were recruited from our existing research volunteer database [Exeter 10000, National Institute for Health Research (NIHR) Exeter Clinical Research Facility] as well as from the daily stroke clinic, the acute stroke unit and the cardiology clinic at the Royal Devon and Exeter Hospital (Exeter, UK). UK National Research Ethics Service South West Committee approved all study procedures and written informed consent was obtained from all participants.

In the morning of the study visit, participants arrived at our temperature-controlled laboratory following an overnight fast, had blood samples taken for biochemical analysis, and ate a standardised meal. Participants rested in the supine position for 10 min before study procedures were initiated. Brachial blood pressure (BP) and heart rate were measured by trained personnel using an automated oscillometric device (M5-I, Omron, Japan) three times, and the average of last two measurements was used for statistical analysis [15].

Cardiovascular risk factors

Following information were collected from each study participant: age, BMI, waist circumference, total cholesterol, high-density lipoprotein (HDL) cholesterol, haemoglobin

A1c (HbA1c), systolic BP, diastolic BP, pulse pressure (PP), mean arterial pressure (MAP), heart rate [16,17]. BMI (calculated from height and weight) and waist circumference were obtained using a standard protocol. Total and HDL cholesterol, and HbA1c were measured from participants' fasting blood samples by the Exeter Pathology Services (Royal Devon and Exeter NHS Foundation Trust, Exeter, UK) [18]. Systolic and diastolic BP, PP, MAP and heart rate were measured from the brachial BP assessment as described above.

Determination of path-length and calculation of carotid-femoral pulse wave velocity

The path-length for CFPWV-D was obtained by the 4-point method – that is, the sum of the distances 1) between the suprasternal notch and the umbilicus and 2) between the umbilicus and the femoral site, with the distance between the carotid artery and suprasternal notch being subtracted [16]. The path-length for CFPWV-F was obtained using the right carotid-femoral path-length formula [9]:

$$\text{Path-length (mm)} = 100.36 + [0.70\times\text{age (yrs)}] + [137.81\times\text{height (m)}] + [0.51\times\text{weight (kg)}] - [0.18\times\text{heart rate (bpm)}] + (46.25 \text{ if female, } 53.89 \text{ if male})$$

The ECG-gated carotid and femoral pulses were recorded sequentially by trained personnel using a SphygmoCor® device (ver 8.2, AtCor Medical, Australia), as described previously [16]. The recording was repeated three times and the average of those recordings was used for statistical analysis [16,19]. The time difference (in msec) between the R-wave of the ECG and foot of the carotid and femoral pulse waves were calculated using the intersecting tangent algorithm. CFPWV-D and CFPWV-F were then calculated as:

$$\text{CFPWV-D (m/s)} = (\text{SD path-length} \times 10^{-3}) / (\text{carotid–femoral time interval} \times 10^{-3})$$

$$\text{CFPWV-F (m/s)} = (\text{DF path-length} \times 10^{-3}) / (\text{carotid–femoral time interval} \times 10^{-3})$$

Estimated pulse wave velocity

As previously described by Greve et al [11] and Vlachopoulos et al [12], the equations to calculate ePWV were derived from The Reference Values for Arterial Stiffness Collaboration [10]. In this study, as in previous studies [12,13], two different equations were used to calculate ePWV for those who did not have cardiovascular risk factors (ePWV1, n=83) and for the rest of the cohort (ePWV2, n=406):

$$\text{ePWV1 (m/s)} = 7.84 - (0.33 \times \text{age}) + (3.80 \times 10^{-3} \times \text{age}^2) - (1.97 \times 10^{-5} \times \text{age}^2 \times \text{MAP}) + (2.50 \times 10^{-3} \times \text{age} \times \text{MAP}) - (1.90 \times 10^{-3} \times \text{MAP})$$

$$\text{ePWV2 (m/s)} = 9.587 - (0.402 \times \text{age}) + (4.560 \times 10^{-3} \times \text{age}^2) - (2.621 \times 10^{-5} \times \text{age}^2 \times \text{MAP}) + (3.176 \times 10^{-3} \times \text{age} \times \text{MAP}) - (1.832 \times 10^{-2} \times \text{MAP})$$

where MAP is mean arterial pressure and was calculated as: diastolic BP + 0.4 (systolic BP–diastolic BP).

Macrovascular assessments

The details of our non-invasive carotid artery assessments have been described previously [15]. Briefly, a Doppler ultrasound machine with a high-resolution linear array transducer (SSD-5500 SV, Aloka, Japan) was used to acquire B-mode images of the common carotid artery taken at the R-wave of the ECG, while participants lay supine on an examination bed

with the head turned ~45° away from the examined side. Semi-automated edge-detection software (Artery Measurement System: ver 2.02, Gothenburg, Sweden) was used to analyse stored B-mode images for intima-media thickness (IMT) [20]. The software automatically identified a 10 mm-long segment (starting from the beginning of the bulb) of the near wall-lumen boundary and the far wall IMT. Plaque was defined as a >50% increase in IMT compared with adjacent IMT, and each plaque was manually traced to obtain a plaque area using the same software. Lumen diameter (LD) was defined as the distance between the lumen-intima borders of the near and far walls. Inter-adventitial diameter (IAD) was defined as the distance between the media-adventitia borders of the near and far walls, and calculated as IAD=LD+(2×IMT). IMT was defined as the height between the lumen-intima and the media-adventitia borders at the far wall. Total plaque area (TPA) was defined as the sum of all plaque areas identified in the right and left sides of the common, internal, and external carotid arteries [17]. For all the carotid artery parameters except TPA, data from the right and left sides of the common carotid arteries were averaged, and mean values were used for statistical analysis.

Microvascular assessments

Microvascular endothelial function was assessed by means of reactive hyperaemia using an EndoPAT device (Itamar Medical, Caesarea, Israel) as described previously [16]. Reactive hyperaemia index (RHI) was calculated as the ratio of post-occlusion to pre-occlusion signal amplitudes as a marker of endothelial-dependent vasodilation. Urinary albumin to creatinine ratio (UACR), a marker of endothelial dysfunction, was obtained by a random spot urine sample obtained during the study visit with a detection limit for albumin of 3.0 mg/l [18].

Statistical analysis

Data are presented as means±SD, median (interquartile range) or number (%). A paired sample t-tests and a Kruskal-Wallis test, as appropriate, was used to examine the differences in variables. Pearson's correlation coefficients were used to examine bivariate associations between CFPWV and cardiovascular risk factors as well as between CFPWV and macro- and microvascular parameters. Skewed data were appropriately transformed for a bivariate association analysis. Regression slopes derived from the bivariate analysis were compared using Prism 8 (GraphPad Software, San Diego, USA) after each CFPWV was normalised. The rest of the statistical analysis was conducted using IBM SPSS Statistics 25 (IBM, Armonk, NY). Significance was set *a priori* at $p<0.05$.

RESULTS:

Data were obtained from 489 older individuals (67.2±8.8 yrs), of which 154 were female. **Table 1** shows the selected characteristics of the study participants. This cohort of participants included 244 (49.9%) individuals who had a previous history of cardiovascular disease, and 233 (47.7%) individuals with type 2 diabetes.

The left panel of **Figure 1** shows arterial path-length measured by surface distance and by the distance formula. The arterial path-length measured by surface distance (495±45 mm) was significantly longer than that calculated by the distance formula (465±21 mm, $p<0.001$). As a result, CFPWV-D was significantly greater than CFPWV-F [**Figure 1** right panel: 9.6 (8.0-11.2) vs 8.9 (7.6-10.5) m/s, $p<0.001$]. ePWV was significantly greater than both CFPWV-F and CFPWV-D [**Figure 1** right panel: 11.0 (10.0-12.2) m/s, $p<0.001$].

There was a strong bivariate association (r=0.949, p<0.001) between CFPWV-D and CFPWV-F (**Figure 2,** left panel). CFPWV-D and CFPWV-F were significantly associated with ePWV (**Figure 2,** centre and right panels; r=0.562 and r=0.616, respectively, both p<0.001), and there were no differences in the regression slopes (**Supplemental Digital Content 1**).

Association with cardiovascular risk factors

Table 2 shows the bivariate associations of CFPWV-D, CFPWV-F, and ePWV with cardiovascular risk factors. CFPWV-D was significantly associated with age, BMI, waist circumference, total and HDL cholesterol, HbA1c, systolic BP, PP, MAP and heart rate. CFPWV-F was also significantly associated with age, waist circumference, total cholesterol, HbA1c, systolic BP, PP, MAP and heart rate. ePWV was significantly associated with age, HbA1c, systolic BP, diastolic BP, PP and MAP.

Association with subclinical vascular biomarkers

The bivariate associations between CFPWV-D and subclinical vascular biomarkers are shown in **Figures 3 and 4 (left columns).** CFPWV-D was associated with LD (A1:r=0.347), IAD (B1:r=0.440), IMT (C1:r=0.364), TPA (D1:r=0.207) and UACR (F1:r=0.259). CFPWV-D was not associated with RHI (E1:r=0.000).

Figures 3 and 4 (middle columns) shows the bivariate associations between CFPWV-F and subclinical vascular biomarkers. CFPWV-F was associated with LD (A2:r=0.352), IAD (B2:r=0.460), IMT (C2:r=0.400), TPA (D2:r=0.215) and UACR (F2:r=0.248). CFPWV-F was not associated with RHI (E2:r=0.048).

The bivariate associations of ePWV with subclinical vascular biomarkers is presented in **Figures 3 and 4 (right columns)**. Similar to CFPWV-D and CFPWV-F, ePWV was associated with LD (A3:*r*=0.254), IAD (B3:*r*=0.376), IMT (C3:*r*=0.412), TPA (D4:*r*=0.201) and UACR (F3:*r*=0.247). ePWV was not associated with RHI (E3:*r*=-0.001).

DISCUSSIONS:

The salient findings of this cross-sectional study are as follows. First, within a cohort of 489 older adults, different acquisition methods produced different values of CFPWV (i.e., CFPWV-F < CFPWV-D < ePWV). Thus, our results suggest that when comparing absolute values of CFPWV between different studies, the method used to determine arterial path-length (e.g., by actual measurement or by equation) will influence the derived CFPWV value and should be taken into consideration. Second, the association between CFPWV and subclinical vascular biomarkers was similar, irrespective of the method used to measure arterial path-length (surface-distance or using the path-length formula). We note here that ePWV (with no path-length measurement) was associated with subclinical vascular biomarkers, and the magnitude of association was similar to those of CFPWV-D and CFPWV-F. Third, the well-established association of CFPWV with age, systolic BP and MAP [21] was confirmed, regardless of the acquired methods. That is, there is sufficient information in all three CFPWV markers of central artery stiffness to find associations with age, BP and subclinical vascular biomarkers in older adults. However, our final finding was that the association between CFPWV and some traditional cardiovascular risk factors differed between the three different methods of acquisition. Notably, BMI was not associated with CFPWV-F and ePWV, and ePWV was not associated with waist circumference or blood

cholesterol measurements. Thus, caution should be used when interpreting data in this regard.

Comparison between CFPWV-D, CFPWV-F and ePWV

The time-interval used for calculating CFPWV-D and CFPWV-F was the same, so the differences between CFPWV-D and CFPWV-F reflect the difference between surface distance measurement and formula-derived arterial path-lengths. The longer path-length measured by the surface distance probably indicates an overestimation of true arterial path-length, which is expected to diverge further in the presence of abdominal obesity. This inherent source of error has previously been documented and resulted in a greater CFPWV in comparison with CFPWV calculated from the actual arterial path-length [22,23]. Weir-McCall et al have recently reported that the use of the arterial path-length formula reduces the error associated with BMI and waist circumference, both of which are known to critically affect surface path-length measurements [9]. Wider adoption of the path-length formula could help to standardise the measurement of CFPWV worldwide, reduce error and variability, and improve patients' cardiovascular risk stratification, although further research is warranted about the applicability of the formula outside of the current study cohorts (e.g., age and ethnicity).

ePWV was significantly greater than both CFPWV-D and CFPWV-F, and the magnitude of overestimation became greater with increasing values of both CFPWV-D and CFPWV-F (**Figure 2**). Greve and colleagues have previously reported discrepancies between ePWV and CFPWV, but in this case ePWV underestimated CFPWV to a greater extent as values increased [11]. Taken together, these observations suggest that ePWV may not reflect

actual CFPWV, especially at higher values of CFPWV. These shortcomings of ePWV preclude the interchangeable use of ePWV and CFPWV and the comparisons of absolute values obtained by ePWV and CFPWV.

Association between CFPWV and cardiovascular risk factors

Age and BP are well-established risk factors that influence CFPWV [21] and were significantly associated with all CFPWV in this study. We note that ePWV is derived from age and BP, and thus its strong associations were expected.

HbA1c is the only risk factor that was positively associated with all CFPWV in a step-wise manner (CFPWV-D > CFPWV-F > ePWV). People with higher HbA1c concentrations are likely to be overweight/obese, and a stronger association with CFPWV-D may be explained by the overestimation of arterial path-length with surface distance measurement with a more corpulent body shape. Nevertheless, CFPWV derived using the arterial path-length formula was also associated with HbA1c, as was ePWV. A note of caution with ePWV is that it uses age in the ePWV equation, and age alone may partly contribute to the association with HbA1c. A positive association between age and HbA1c (r=0.09, p=0.049) found in this study support this speculation.

In this study, obesity-related risk factors (i.e., BMI and waist circumference) showed a significant positive association with CFPWV-D, a diminished (or null) association with CFPWV-F, and no association with ePWV. The positive association of obesity-related risk factors with CFPWV-D may result from the overestimation of arterial path-length by surface distance measurements [9], creating a bias with increasing BMI and waist circumference in

our cohort. Consistent with this notion, the increase in waist circumference has been demonstrated to be positively associated with overestimation of arterial path-length that results in the overestimation of CFPWV in a community-dwelling cohort [22]. On the other hand, the lack of association between CFPWV-F and BMI, and the weakened association between CFPWV-F and waist circumference in this study imply that the use of distance formula may have minimised the error and variability associated with surface distance measurement. Of note, the weakened, but still significant association between CFPWV-F and waist circumference could explain a deleterious effect of visceral fat accumulation on central artery stiffness rather than the presence of obesity itself [22].

Association between CFPWV-D, CFPWV-F and subclinical vascular biomarkers

We found that most subclinical vascular biomarkers associated with CFPWV, irrespective of the method used to determine arterial path-length. Associations were, in general, slightly stronger for CFPWV-F than CFPWV-D, perhaps reflecting reduced error in the arterial path-length formula. The overall comparable degree of associations between CFPWV and subclinical vascular biomarkers regardless of the path-length determination method may have been caused by the downward shift of each data point as a whole. This looks plausible because 1) CFPWV-F was slower than CFPWV-D, 2) the regression slopes from CFPWV-D and CFPWV-F were found to be similar, and 3) the y-intercepts of the slopes from CFPWV-F were generally lower than those from CFPWV-D. Alternatively, CFPWV itself, irrespective of the path-length determination method, may simply contain sufficient physiological/biomechanical information to find the association with subclinical vascular biomarkers. Overall though, this finding provides evidence that the arterial path-length determination method does not meaningfully influence the association between CFPWV

and subclinical vascular biomarkers at least in older adults. We also found that reactive hyperaemia index did not correlate with any CFPWV measurement.

Association between ePWV and subclinical vascular biomarkers

ePWV has attracted growing interest in recent years owing to the practicality of its measures in the area where CFPWV has been struggling over the years. Despite its greater dependence on only two parameters (age and MAP) in the equation, ePWV has recently demonstrated prognostic utility independently of cardiovascular risk score models [11-14]. An interesting finding of this study was that ePWV was associated with subclinical vascular biomarkers, and that the magnitude of associations observed were mostly comparable with those between CFPWV-D, CFPWV-F and subclinical vascular biomarkers. These observations extend the current understanding of ePWV and may also facilitate its use for studying not only cardiovascular outcomes but also its relationships with intermediate biomarkers when the assessment of CFPWV is not feasible/impractical.

Limitations

At the outset of this study, the surface-distance measurement employed was the 4-point subtraction method rather than 80% of the direct distance measurement between carotid and femoral sites that the professional societies in the field have subsequently suggested [7]. Given the comparable associations between CFPWV-D, CFPWV-F and subclinical vascular biomarkers observed in this study, however, we do not envisage that the use of recommended distance measurement would be likely to cause a meaningful influence on our outcomes. Additionally, our participants were older and nearly a half of them had

concomitant cardiovascular disease and/or type 2 diabetes. For that reason, our observations may not be applicable to other populations.

Conclusions

We conclude that, within the same cohort, different acquisition methods produce significantly different CFPWV values. The association between CFPWV and traditional cardiovascular risk factors differed with different methods of CFPWV acquisition, but acquisition method bore almost no influence on the association with subclinical biomarkers of vascular health. Adoption of a formula to calculate arterial path-length for CFPWV may overcome some of the problems of surface distance measurement in people with disparate body morphology. These hitherto unreported observations are important considerations in experimental design, data interpretation and, of particular importance, comparison between studies where CFPWV is measured.

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CONFLICT OF INTERESTS:

Nothing to disclose.

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Table 1. Selected characteristics of the study participants.

Parameter	Values (n=489)
Age (yrs)	67.2±8.8
Female [n (%)]	154 (31.5)
CVD [n (%)]	244 (49.9)
Type 2 Diabetes [n (%)]	233 (47.7)
Current Smoking [n (%)]	27 (5.5)
Body Mass Index (kg/m^2)	27.9 (25.2-31.0)
Waist circumference (cm)	100.8±12.1
Total CHOL (mmol/l)	4.3 (3.6-5.2)
HDL CHOL (mmol/l)	1.3 (1.1-1.6)
HbA1c (mmol/mol)	45.0 (40.0-56.0)
Systolic BP (mmHg)	138.6±16.4
Diastolic BP (mmHg)	77.3±8.6
Heart Rate (bpm)	63.4±10.6
eGFR (ml/min/1.73m^2)	79.0 (67.0-88.0)
UACR (mg/mmol)	0.9 (0.5-2.2)

Data are means±SD, median (interquartile range), or numbers (%). CVD, cardiovascular disease; CHOL, cholesterol; HDL, high-density lipoprotein; HbA1c, Haemoglobin A1c; BP,

blood pressure; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-creatinine ratio.

Table 2. Bivariate association between each CFPWV method and cardiovascular risk factors.

	CFPWV-D[#]	CFPWV-F[#]	ePWV
Age (yrs)	***r*=0.522, *p*<0.001**	***r*=0.590, *p*<0.001**	***r*=0.900, *p*<0.001**
Body mass index[#] (kg/m^2)	***r*=0.144, *p*=0.001**	***r*=0.000, *p*=0.994**	***r*=-0.073, *p*=0.105**
Waist circumference (cm)	***r*=0.264, *p*<0.001**	***r*=0.149, *p*=0.001**	***r*=0.053, *p*=0.239**
Total CHOL[#] (mmol/l)	***r*=-0.134, *p*=0.003**	***r*=-0.092, *p*=0.046**	***r*=-0.070, *p*=0.129**
HDL CHOL[#] (mmol/l)	***r*=-0.130, *p*=0.005**	***r*=-0.042, *p*=0.370**	***r*=0.055, *p*=0.243**
HbA1c[#] (mmol/mol)	***r*=0.257, *p*<0.001**	***r*=0.155, *p*=0.001**	***r*=0.110, *p*=0.016**
Systolic BP (mmHg)	***r*=0.365, *p*<0.001**	***r*=0.386, *p*<0.001**	***r*=0.645, *p*<0.001**
Diastolic BP (mmHg)	***r*=0.069, *p*=0.126**	***r*=0.052, *p*=0.249**	***r*=0.254, *p*<0.001**
Pulse pressure (mmHg)	***r*=0.400, *p*<0.001**	***r*=0.437, *p*<0.001**	***r*=0.623, *p*<0.001**
Mean arterial pressure (mmHg)	***r*=0.242, *p*<0.001**	***r*=0.240, *p*<0.001**	***r*=0.501, *p*<0.001**
Heart rate (beat/min)	***r*=0.233, *p*<0.001**	***r*=0.186, *p*<0.001**	***r*=0.082, *p*=0.069**

[#] Log-transformed for statistical analysis. CFPWV-D, carotid-femoral pulse wave velocity calculated from surface-distance measurement; CFPWV-F, carotid-femoral pulse wave velocity calculated from distance formula; ePWV, estimated pulse wave velocity; CHOL, cholesterol; HDL, high-density lipoprotein; HbA1c, haemoglobin A1c; BP, blood pressure.

FIGURE LEGENDS:

Figure 1. Left panel: Path-length derived from the standard surface distance measurement and from the distance formula. * Significantly different from surface distance (p<0.05). **Right panel**: Carotid-femoral pulse wave velocity calculated from surface distance (CFPWV-D) and from distance formula (CFPWV-F), and estimated pulse wave velocity (ePWV). *significantly different from CFPWV-D (p<0.05). †significantly different from CFPWV-F (p<0.05).

Figure 2. Left panel: Bivariate association between CFPWV-D and CFPWV-F. **Mid panel**: Bivariate association between CFPWV-D and ePWV. **Right panel:** Bivariate association between CFPWV-F and ePWV. The regression slopes in the middle panel and right panel were similar to each other (p=0.365). LN, natural logarithm.

Figure 3. Bivariate associations of CFPWV-D (**left column**), CFPWV-F (**mid column**) and ePWV (**right column**) with A) carotid lumen diameter, B) carotid inter-adventitial diameter, C) carotid intima-media thickness, D) carotid total plaque area, E) reactive hyperaemia index, and F) urinary albumin to creatinine ratio. Carotid total plaque area (TPA) is expressed as $\sqrt[3]{\text{TPA}}$.

Figure 4. Bivariate associations of CFPWV-D (**left column**), CFPWV-F (**mid column**) and ePWV (**right column**) with E) reactive hyperaemia index, and F) urinary albumin to creatinine ratio. Urinary albumin to creatinine ratio (ACR) is expressed as the inverse of (-√ACR). LN, natural logarithm.

FIGURE 1.

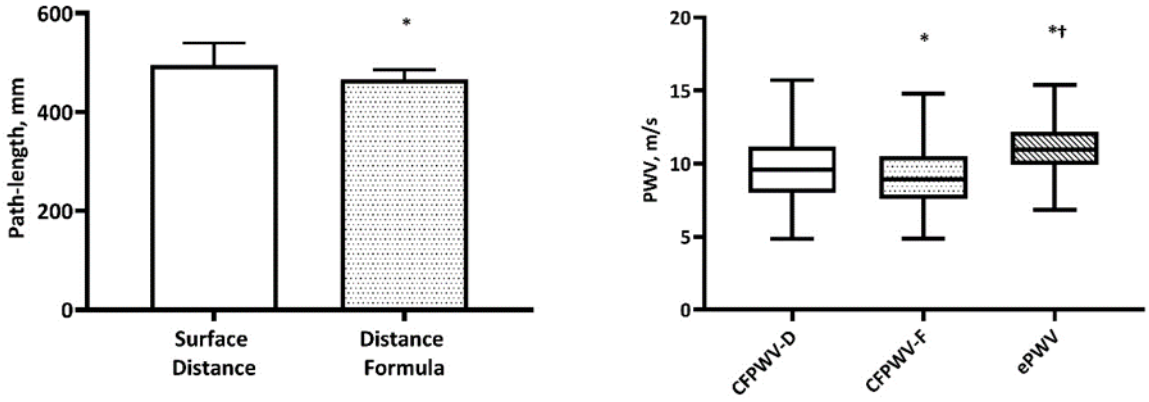

600
400
200
0
Path-length, mm
*
Surface Distance
Distance Formula
20
15
10
5
0
PWV, m/s
*
*†
CFPWV-D
CFPWV-F
ePWV

FIGURE 2.

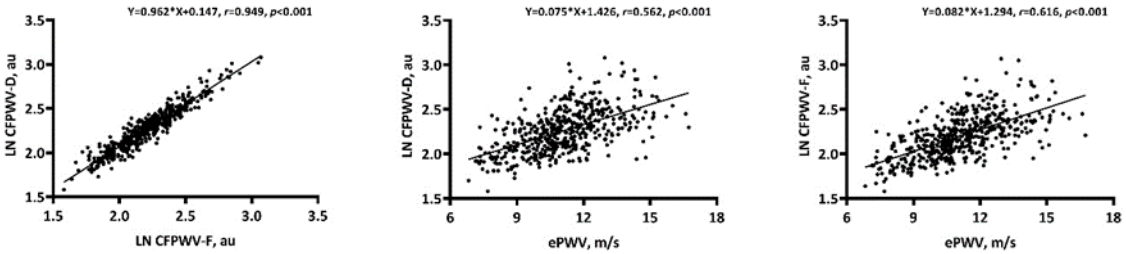

Y=0.962*X+0.147, r=0.949, p<0.001
LN CFPWV-D, au
LN CFPWV-F, au
Y=0.075*X+1.426, r=0.562, p<0.001
LN CFPWV-D, au
ePWV, m/s
Y=0.082*X+1.294, r=0.616, p<0.001
LN CFPWV-F, au
ePWV, m/s

FIGURE 3.

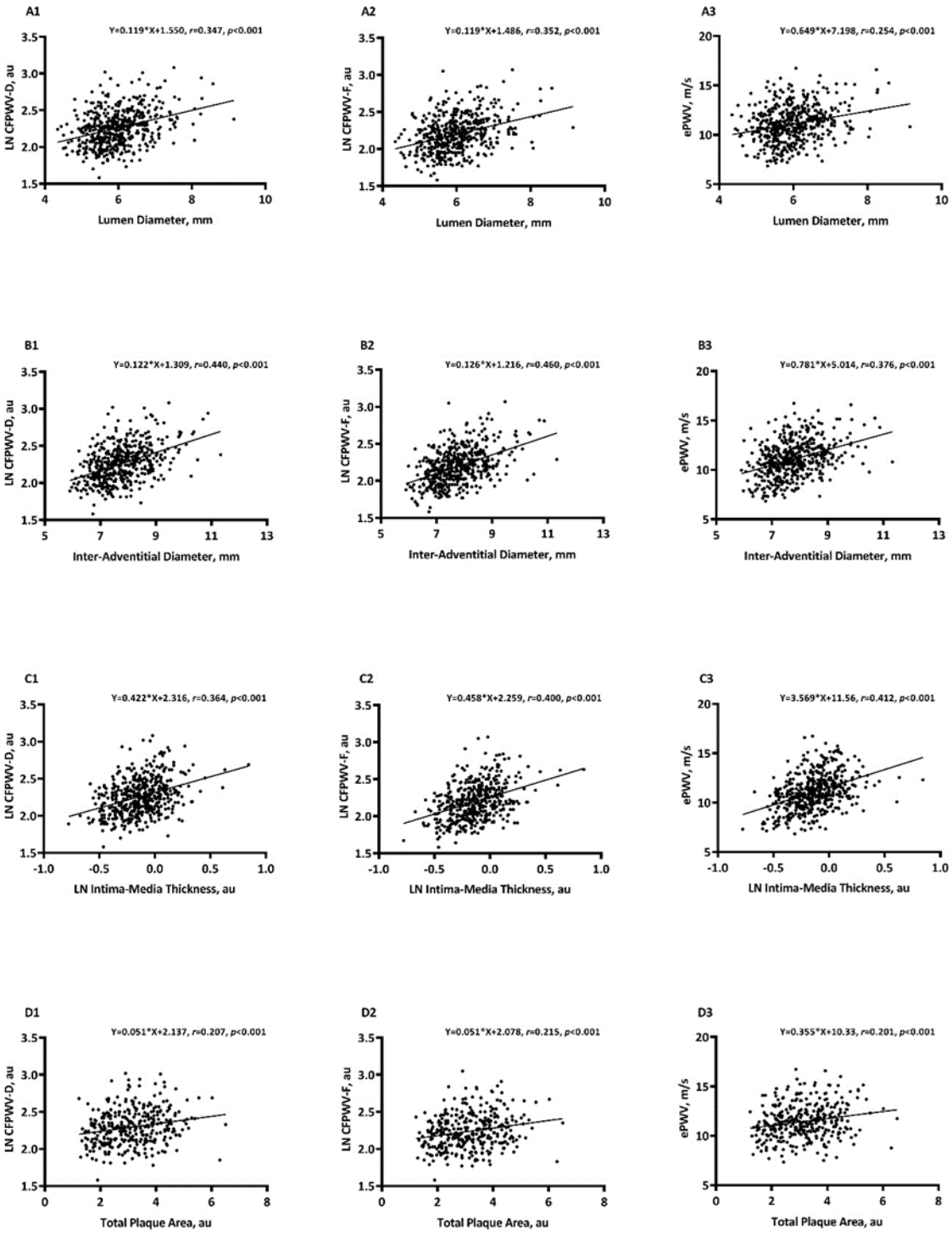
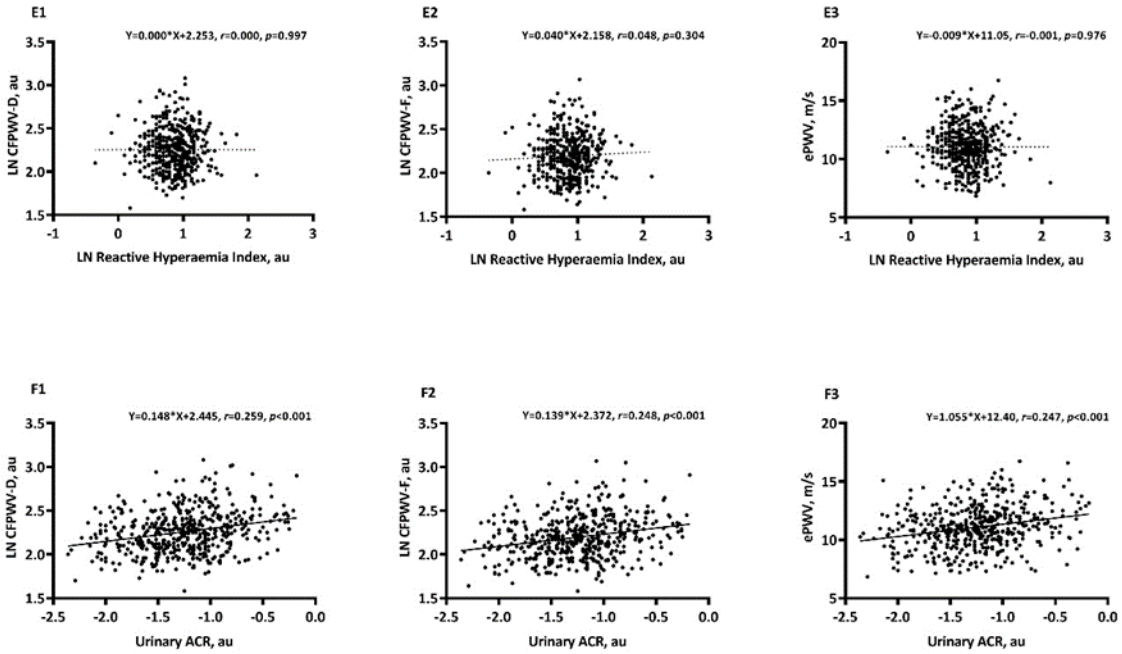

A1
Y=0.119*X+1.550, r=0.347, p<0.001
LN CFPWV-D, au
Lumen Diameter, mm
A2
Y=0.119*X+1.486, r=0.352, p<0.001
LN CFPWV-F, au
Lumen Diameter, mm
A3
Y=0.649*X+7.198, r=0.254, p<0.001
ePWV, m/s
Lumen Diameter, mm
B1
Y=0.122*X+1.309, r=0.440, p<0.001
LN CFPWV-D, au
Inter-Adventitial Diameter, mm
B2
Y=0.126*X+1.216, r=0.460, p<0.001
LN CFPWV-F, au
Inter-Adventitial Diameter, mm
B3
Y=0.781*X+5.014, r=0.376, p<0.001
ePWV, m/s
Inter-Adventitial Diameter, mm
C1
Y=0.422*X+2.316, r=0.364, p<0.001
LN CFPWV-D, au
LN Intima-Media Thickness, au
C2
Y=0.458*X+2.259, r=0.400, p<0.001
LN CFPWV-F, au
LN Intima-Media Thickness, au
C3
Y=3.569*X+11.56, r=0.412, p<0.001
ePWV, m/s
LN Intima-Media Thickness, au
D1
Y=0.051*X+2.137, r=0.207, p<0.001
LN CFPWV-D, au
Total Plaque Area, au
D2
Y=0.051*X+2.078, r=0.215, p<0.001
LN CFPWV-F, au
Total Plaque Area, au
D3
Y=0.355*X+10.33, r=0.201, p<0.001
ePWV, m/s
Total Plaque Area, au

FIGURE 4.


E1
Y=0.000*X+2.253, r=0.000, p=0.997
LN CFPWV-D, au
LN Reactive Hyperaemia Index, au
E2
Y=0.040*X+2.158, r=0.048, p=0.304
LN CFPWV-F, au
LN Reactive Hyperaemia Index, au
E3
Y=-0.009*X+11.05, r=-0.001, p=0.976
ePWV, m/s
LN Reactive Hyperaemia Index, au
F1
Y=0.148*X+2.445, r=0.259, p<0.001
LN CFPWV-D, au
Urinary ACR, au
F2
Y=0.139*X+2.372, r=0.248, p<0.001
LN CFPWV-F, au
Urinary ACR, au
F3
Y=1.055*X+12.40, r=0.247, p<0.001
ePWV, m/s
Urinary ACR, au