Original Article

Pulsatile Stress, Inflammation and Change in Arterial Stiffness

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Aim: Understanding factors that contribute to changes in arterial stiffness over time is important as this may lead to therapies that can abrogate cardiovascular risk. We compared the contribution of pulsatile stress and inflammation to changes in arterial stiffness in middle-aged men using a 1-year follow-up study design.

Methods: Arterial stiffness was derived from brachial-ankle pulse wave velocity (baPWV) in 107 men (mean age 53 ± 6 yrs) on two separate occasions. The changes in outcome variables were calculated as the difference between the first and second examinations (mean interval 403 ± 122 days). Pulsatile stress was calculated as the product of heart rate and brachial pulse pressure. C-reactive protein (CRP), white blood cell count (WBC) and fibrinogen were measured as inflammatory markers.

Results: At baseline, baPWV was significantly correlated with pulsatile stress ($r=0.37$, $p<0.01$), WBC ($r=0.19$, $p<0.05$), HbA1c ($r=0.39$, $p<0.01$), HDL-C ($r=-0.20$, $p<0.05$), but not CRP ($r=0.06$, $p=0.56$), or fibrinogen ($r=0.12$, $p=0.21$). The change in baPWV over 1 year was associated with the change in pulsatile stress ($r=0.26$, $p<0.01$) and HbA1c ($r=0.19$, $p<0.05$) over that same time period. Change in baPWV was not associated with the change in WBC ($r=0.18$, $p=0.06$) or CRP ($r=0.05$, $p=0.62$).

Conclusions: These results demonstrate that both pulsatile stress and inflammation may be associated with arterial stiffness at any given moment in time, but change in pulsatile stress is a better predictor of change in arterial stiffness over time.

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Key words; Arterial stiffness, Pulse wave velocity, Pulsatile stress, Inflammation

Pulse wave velocity (PWV), an index of arterial stiffness, is associated with coronary atherosclerosis¹⁾ and increased risk of cardiovascular (CV) events and mortality²⁻⁴⁾, and lowering PWV with therapeutic intervention reduces CV risk⁵⁾. Despite the clinical significance of PWV as a measure of CV risk, the mechanisms that contribute to changes in PWV remain poorly understood. It is often suggested that

arterial stiffness is the integration of CV risk factors on the vessel wall over time⁶⁾. Yet while numerous cross-sectional studies have noted that age and blood pressure are strong predictors of PWV^{7, 8)}, other traditional CVD risk factors do not seem to play an integral role in modulating arterial stiffness⁹⁾; that is, the association of factors such as lipids, diabetes, sex, smoking and BMI with PWV is weak or insignificant, suggesting the dissociation of traditional risk factors and PWV⁹⁾. Individual risk factors can fluctuate over time and their values, recorded at the time of risk assessment, may not reflect their true impact on the arterial wall¹⁰⁾.

Non-traditional CV risk factors may offer insight

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into the mechanisms that govern arterial modulation over time. Recently, it has been suggested that both pulsatile stress and inflammation are associated with arterial stiffness over a 20-year span¹¹. Chronically increased pulsatile stress (assessed as the product of heart rate and pulse pressure) introduces a repetitive cyclic load to the vascular wall which may contribute to fatigue fracture of elastic wall elements and consequently increase PWV¹¹. Inflammation may affect arterial stiffness via altering endothelial function and vascular tone^{12, 13}; however, failure to assess arterial stiffness at baseline did not allow previous investigators to examine whether these factors are important contributors to the change in arterial stiffness over time¹¹. Whether changes in pulsatile stress and/or inflammation affect short-term changes in arterial stiffness (i.e. over 1-year) remains unknown. Using a prospective design with a 1-year follow-up, we compared the contribution of pulsatile stress and systemic inflammation to changes in arterial stiffness over a 1-year period in middle-aged men.

Methods

Subjects consisted of 107 apparently healthy men (mean age 53 ± 6 yrs) who had at least two measures of baPWV over an approximate 1-year time course. The changes in outcome variables were calculated as the difference between the first and second examinations (mean interval 403 ± 122 days). Subjects did not have any cardiovascular, metabolic, or inflammatory disease, and none were using cardiovascular medications of any kind. Written informed consent was obtained from all men and the study was approved by the medical center institutional review board.

Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Resting systolic and diastolic blood pressures (SBP/DBP) were measured in the sitting position using an automated blood pressure monitor (PRO 100; Dinamap, Milwaukee, WI) during quiet rest. Pulse pressure was calculated as SBP minus DBP. Resting heart rate (HR) was measured in the supine position using 12-lead electrocardiography (ECG M 1700A; Hewlett-Packard, Palo Alto, CA). Pulsatile stress was calculated as the product of resting heart rate and brachial pulse pressure (HR x PP). Rate pressure product as a measure of peak systolic stress was calculated as HR x SBP.

Arterial stiffness was measured using brachial-ankle pulse wave velocity (baPWV; VP-1000; Colin Co. Ltd, Komaki, Japan). Measurement of baPWV was performed in the supine position following the method previously described. In short, a microphone

overlying the left edge of the sternum recorded the heart sounds while ECG leads, attached to both arms, recorded heart rate. The time interval (T) between the wave front of the brachial waveform and that of the ankle waveform was measured. The distances between the suprasternal notch and brachial sampling point (Lb) and between the suprasternal notch and ankle sampling point (La) were estimated from the patient's height. The baPWV was then calculated as: $baPWV = (La - Lb) / T$.

Blood samples were collected in the morning following a 12-hour overnight fast. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were analyzed enzymatically using a Hitachi-7600 (Tokyo, Japan) analyzer. High-sensitivity C-reactive protein (CRP) was measured using a CRP (II) Latax X2 turbidimetric method (Hitachi Corporation, Tokyo, Japan). White blood cell (WBC) count was determined using a quantitative automated hematology analyzer (Sysmex Corporation, Japan). Fibrinogen was measured with the Clauss method using a CA-500 analyzer (Sysmex). Plasminogen activator inhibitor 1 (PAI-1) activity was measured by enzyme-linked immunoassay kits (BEP III). Fasting glucose levels were determined using the glucose oxidase method (747; Hitachi, Tokyo, Japan). Insulin levels were determined by IRMA (Pipetting system, GammaCounter). Lipoprotein (a) (LPa) was determined by immunoturbidity (Roche Modular PEE). Inter and intra-assay coefficients of variation were <5% for all blood variables.

Data are expressed as the mean $\pm$ SD for continuous variables and as proportions for categorical variables. Because the distribution of CRP was highly skewed, we used the log-transformation of these variables before descriptive statistics. Subject's characteristics and risk factors were compared between the baseline examination and follow-up examination using the paired *t*-test for continuous variables and the χ^2 test for categorical variables. Pearson's correlations was used to determine the association between baPWV and pulsatile stress, inflammatory markers and risk factors at the baseline and the association between the change in baPWV and change in pulsatile stress, inflammatory markers and risk factors over time. Variable changes were calculated by post-exam values minus pre-exam values. Multivariable regression analysis was assessed by the change in baPWV as the dependent variable and change in inflammatory markers, pulsatile stress and other selected risk factors (those that significantly changed over time or demonstrated a univariate association with baPWV $p=0.01$)

Table 1. Subjects characteristics at baseline and follow up ($n = 107$)

Variables	Baseline	Follow- up	<i>p</i> -values
Age, years	52.4 ± 6	53.5 ± 6	0.001
BMI, kg/m ²	25.0 ± 2.3	24.8 ± 2.3	0.002
Waist girth, cm	88.2 ± 5.8	87.5 ± 5.9	0.160
Current smokers, %	15.9	6.5	0.001
Heart rate, bpm	62.6 ± 8.2	61.8 ± 7.5	0.217
Brachial SBP, mmHg	118.9 ± 14	119.2 ± 12.6	0.843
Brachial DBP, mmHg	77.9 ± 9.1	77.1 ± 9.1	0.334
Brachial PP, mmHg	40.98 ± 9.92	42.06 ± 9.58	0.277
Brachial MAP, mmHg	91.6 ± 9.94	91.16 ± 9.4	0.606
Total cholesterol, mg/dL	198.9 ± 32.9	192.4 ± 31.8	0.039
HDL-C, mg/dL	52.2 ± 12.2	50.6 ± 12.8	0.024
LDL-C, mg/dL	125.8 ± 27.4	119.4 ± 28.9	0.024
TG, mg/dL	128.1 ± 63.6	132.7 ± 70.3	0.353
Glucose, mg/dL	100.8 ± 18.5	102.2 ± 20.4	0.335
Insulin, μ units/mL	9.35 ± 4.2	9.37 ± 5.4	0.962
HbA1c, %	5.7 ± 0.8	5.8 ± 0.7	0.042
HOMA-IR, aU	2.34 ± 1.17	2.36 ± 1.42	0.856
PAI-1, ng/mL	34.6 ± 24.8	38.3 ± 31.9	0.215
LP(a), mg/dL	28.5 ± 23.5	28.8 ± 23.6	0.652
Uric acid, mg/dL	6.07 ± 1.25	6.06 ± 1.18	0.923
hsCRP, mg/dL	0.09 (0.03-0.09)	0.16 (0.03-0.08)	0.759
WBC, $\times 10^9$ cells/L	5.67 ± 1.68	5.81 ± 1.58	0.321
Fibrinogen, mg/dL	261.9 ± 50.3	261.4 ± 60.8	0.911
baPWV, cm/s	1379 ± 159	1389 ± 163	0.282
Rate pressure product, aU	7451 ± 1344	7369 ± 1220	0.462
Pulsatile stress, aU	2565 ± 711	2590 ± 641	0.698

as independent variables. A separate model was created to examine the contribution of change in SBP $\times$ PP as a predictor of change in baPWV. Given the high correlation between PP $\times$ HR and SBP $\times$ HR ($r = 0.73$, $p < 0.05$), both variables could not be entered into the same model due to issues of co-linearity. The Mann-Whitney U test was used to compare changes in baPWV by increased or decreased pulsatile stress and inflammatory markers over time. Statistical significance was set at $p < 0.05$ for all data. Statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL).

Results

Table 1 shows the comparison of subjects' characteristics and risk factors at baseline and follow up. Body mass index, current smoking status, lipid profiles, and HbA1C were significantly different at follow up compared to baseline ($p < 0.05$, each). In the group as a whole, baPWV, inflammatory markers and pulsatile stress did not change over 1 year (**Table 1**). At baseline, baPWV was significantly correlated with

pulsatile stress ($r = 0.37$, $p < 0.01$), rate pressure product ($r = 0.41$, $p < 0.01$), WBC ($r = 0.19$, $p < 0.05$), HbA1c ($r = 0.39$, $p < 0.01$), HDL-C ($r = -0.20$, $p < 0.05$), but not CRP ($r = 0.06$, $p = 0.56$), fibrinogen ($r = 0.12$, $p = 0.21$) and age ($r = 0.11$, $p = 0.86$) (**Table 2**). The change in baPWV was associated with the change in pulsatile stress ($r = 0.26$, $p < 0.01$), change in rate pressure product ($r = 0.39$, $p < 0.05$) and change in HbA1c ($r = 0.19$, $p < 0.05$). Change in baPWV was not associated with the change in WBC ($r = 0.18$, $p = 0.06$), fibrinogen ($r = 0.07$, $p = 0.46$) or CRP ($r = 0.05$, $p = 0.62$) (**Table 2**). Multivariable linear regression models adjusted for follow-up days revealed that the change in pulsatile stress was positively associated with the change in baPWV (**Table 3**, $\beta = 0.21$, $p = 0.04$). A separate multivariable linear regression model revealed that the change in rate pressure product was also positively associated with the change in baPWV (**Table 4**, $\beta = 0.45$, $p = 0.04$). Changes in inflammatory markers, HbA1c and BMI were not predictors of change in baPWV. Comparisons of the change in baPWV by increased or decreased pulsatile stress and

Table 2. Association between baPWV and inflammatory markers and CVD risk factors at baseline (left) and association between change* in baPWV and change in inflammatory markers and CVD risk factors over time (right)

Variables	Baseline <i>r</i> (<i>p</i> -values)	Δ 1-Year <i>r</i> (<i>p</i> -values)
Age, years	0.11 (0.26)	0.18 (0.07)
BMI, kg/m ²	-0.04 (0.97)	0.05 (0.59)
Waist girth, cm	0.13 (0.19)	0.19 (0.08)
Heart rate, bpm	0.23 (0.02)	0.33 (0.01)
Brachial SBP, mmHg	0.36 (0.01)	0.22 (0.03)
Brachial DBP, mmHg	0.23 (0.02)	0.17 (0.09)
Brachial PP, mmHg	0.30 (0.01)	0.14 (0.16)
Brachial MAP, mmHg	0.31 (0.01)	0.21 (0.03)
Total cholesterol, mg/dL	-0.04 (0.67)	0.04 (0.72)
HDL-C, mg/dL	-0.20 (0.04)	0.16 (0.09)
LDL-C, mg/dL	0.02 (0.86)	0.03 (0.76)
TG, mg/dL	0.14 (0.17)	-0.05 (0.63)
Glucose, mg/dL	0.17 (0.08)	0.01 (0.89)
Insulin, μ units/mL	-0.03 (0.80)	0.11 (0.29)
HbA1c, %	0.39 (0.01)	0.19 (0.05)
HOMA-IR, aU	0.04 (0.68)	0.08 (0.43)
PAI-1, ng/mL	0.07 (0.47)	0.01 (0.94)
LP(a), mg/dL	-0.04 (0.66)	0.01 (0.93)
Uric acid, mg/dL	-0.02 (0.88)	0.04 (0.67)
Log hsCRP, mg/dL	0.06 (0.56)	0.05 (0.62)
WBC, ×10 ⁹ cells/L	0.19 (0.05)	0.18 (0.06)
Fibrinogen, mg/dL	0.12 (0.21)	0.07 (0.46)
Rate pressure product, aU	0.41 (0.01)	0.39 (0.01)
Pulsatile stress, aU	0.37 (0.01)	0.26 (0.01)

*change was calculated by post-measurement minus pre-measurement.

inflammatory markers over time are shown in **Fig. 1**, which demonstrates that men who had increased pulsatile stress over time had a significant increase in baPWV compared to men who had decreased pulsatile stress over time ($p < 0.05$). However, baPWV was not significantly different between the two groups in terms of increased or decreased inflammatory markers over time (**Fig. 1**); that is, an increase or decrease in inflammation over time was not associated with a commensurate increase or decrease in baPWV over time.

Discussion

Pulse wave velocity is associated with an increased risk of cardiovascular disease events and a better understanding of the risk factors for increased arterial stiffness over time might help in the determi-

nation of appropriate therapeutic strategies in clinical practice. The main findings from the present study are that both increased pulsatile stress (assessed as the product of heart rate and pulse pressure), peak systolic stress (assessed as the product of heart rate and systolic blood pressure) and inflammation are associated with increased arterial stiffness cross-sectionally, but changes in pulsatile stress (and peak systolic stress) are better predictors of changes in arterial stiffness over time in free-living middle-aged men without any intervention. Moreover, when participants were separated into those that experienced an increase in pulsatile stress over 1 year versus those that experienced a decrease, there were commensurate increases and decreases in PWV, respectively. When participants were separated according to those that experienced an increase in inflammation versus a decrease in inflammation over 1 year, there was no difference in PWV between groups. These observations suggest that pulsatile stress and peak systolic stress may contribute to short-term (1-year) modulation of arterial stiffness more so than inflammation. Therefore, reducing this hemodynamic stress via appropriate pharmaceutical interventions (ACE inhibition) and/or non-pharmaceutical interventions (exercise) may be important to prevent age-associated arterial stiffening.

Our results are consistent with previous studies and note that changes in pulsatile stress are associated with changes in arterial stiffness over time¹¹. Pulsatile stress as quantified in the present study (HR × PP) is believed to reflect cumulative/repetitive mechanical stress on the vascular wall¹¹. Over time, this cyclic stress will contribute to elastin fatigue-fracture with subsequent smooth muscle hypertrophy and/or fibrosis, target organ damage¹⁴ and ultimately mortality¹⁵. Higher systolic pressure may impose greater wall stress and induce greater vascular remodeling, offering insight into our finding of an association between peak systolic stress and baPWV. Tomiyama *et al.* reported that changes in heart rate, but not changes in C-reactive protein were associated with increases in PWV in middle-aged Japanese adults over a 5-6-year time period¹⁶. Similarly, Benetos *et al.* noted that changes in SBP and HR were determinants of change in PWV over 6 years in normotensive and treated hypertensive adults¹⁷, while van Bussel *et al.* recently noted no association of the change in inflammation with the change in PWV over a 6-year period¹⁸. Taken together, these findings suggest that pulsatile stress and peak wall stress (i.e. systolic stress), but not inflammation, are important modulators of change in arterial stiffness over time. Our results add to previous findings by noting for the first time that the chronic-

Table 3. Multivariable regression analysis with change in baPWV as the dependent variable and change in inflammatory markers, pulsatile stress and other selected risk factors as independent variables

Variables	Non-standardized β	95% CI	Standardized β	<i>p</i> -values
Pulsatile stress	0.04	0.02-0.09	0.21	0.04
hsCRP	-16.9	-86.9-53.1	-0.05	0.63
WBC	10.2	-11.5-31.9	0.10	0.35
Fibrinogen	0.19	-0.43-0.82	0.07	0.54
BMI	16.7	-24.0-57.7	0.08	0.42
HbA1c	48.3	-15.6-112.3	0.14	0.14
MAP	1.6	-1.6-4.7	0.10	0.32

Adjusted for F/U days

CRP, C-reactive protein; WBC, white blood cell count; BMI, body mass index; HbA1c, hemoglobin A1c; MAP, mean arterial pressure

Table 4. Multivariable regression analysis with change in baPWV as the dependent variable and change in inflammatory markers, RPP and other selected risk factors as independent variables

Variables	Non-standardized β	95% CI	Standardized β	<i>p</i> -values
RPP	0.06	0.03-0.09	0.45	0.01
hsCRP	-22.6	-89.9-44.7	-0.07	0.51
WBC	8.43	-12.4-29.3	0.08	0.42
Fibrinogen	0.12	-0.48-0.72	0.05	0.69
BMI	12.7	-26.6-52.0	0.06	0.52
HbA1c	46.7	-14.8-108.2	0.14	0.14
MAP	-1.88	-5.67-1.91	-0.12	0.33

Adjusted for F/U days

RPP, rate pressure product; CRP, C-reactive protein; WBC, white blood cell count; BMI, body mass index; HbA1c, hemoglobin A1c; MAP, mean arterial pressure

ity of the pulsatile signal does not need to be excessive (i.e. 6-20 years as previously reported) to alter arterial stiffness. It would appear that hemodynamic stress can affect baPWV in as little as 1 year, underscoring the importance of early intervention to reduce arterial stiffness.

It is well known that inflammation is associated with increased CVD morbidity and mortality. In chronic hemodialysis patients, C-reactive protein is a better predictor of mortality than even PWV¹⁹. It has been suggested that inflammation contributes to endothelial dysfunction and consequently decreases small and large artery elasticity²⁰. Indeed, several studies have noted associations between endothelial function and PWV²¹. Previous acute experimental and cross-sectional observational studies have reported that inflammation is associated with arterial stiffness^{12, 22}; however, there is limited evidence supporting a causal relationship between inflammation and arterial stiffness over time. Recent work from van Bussel noted that both endothelial dysfunction and inflammation were associated with peripheral arterial stiffness over a

6-year period²³. Although McEniery *et al.* noted an association between central PWV and change in C-reactive protein using a 20-year long-term follow-up study design, investigators did not measure PWV at baseline (thus, the change in PWV over time could not be ascertained). Therefore, it is hard to understand whether the aforementioned relationships were indeed causal. Our results, along with those of others^{16, 18}, suggest that inflammation may not influence arterial stiffness over time.

Even though changes were well within clinical norms, changes in PWV and the risk for CV events occur on a continuous scale. Vlachopoulos *et al.* has previously reported in a meta-analysis of 15,877 subjects that an increase in aortic PWV by 1 m/s corresponds to an age-, sex-, and risk factor-adjusted risk increase of 14%, 15%, and 15% in total CV events, CV mortality, and all-cause mortality, respectively⁶. Matsuoka *et al.* reported that a 200 cm/s increase in baPWV is associated with a 30.2% increase in CV mortality risk (independent of traditional risk factors and sympatho-vagal balance)⁴. Conversely, a lack of

Fig. 1. Box plot of changes in baPWV by increased or decreased pulsatile stress ($p < 0.05$) and inflammatory markers (NS) over time.

decrease in baPWV over time is associated with a 4 times greater risk of having a major adverse cardiac event⁹); therefore, small changes in arterial stiffness may still have a profound impact on CV risk over time.

We did not control for the resting autonomic state at the time of PWV assessment and acute changes in HR from autonomic arousal may affect the calculation of pulsatile stress²⁴). Measures of sympatho-vagal balance have been shown to be associated with baPWV²⁵). Increased sympathetic strain may increase arterial stiffness via increasing vascular smooth muscle tone. Conversely, increased arterial stiffness may increase sympathetic tone via altering baroreflex modulation of vascular sympathetic outflow²⁶). Thus there is a bidirectional association between autonomic tone and arterial stiffness. Future research should control for sympatho-vagal balance when assessing pulsatile stress. It is interesting to note that increases in

arterial stiffness stemming from an increase in HR have been shown to be independent of sympathetic strain. Using an animal model, Mircoli *et al.* previously demonstrated that increasing HR via atrial pacing decreases elastic artery distensibility even in sympathectomized rats²⁷). Moreover, not all studies in humans note an association between sympathetic tone and large artery stiffness²⁸) and those that do report that the association is independent of HR²⁹). More research is needed to disentangle autonomic-dependent and -independent mechanisms related to pulsatile stress and arterial stiffness. This may provide insight into the numerous epidemiological studies that note associations between elevated resting HR and CV mortality independent of traditional risk factors³⁰⁻³⁷) and inflammation³⁸).

Other limitations to this study should be noted. Although baPWV is associated with aortic PWV^{39, 40})

it is not strictly a measure of central arterial stiffness and can be influenced by peripheral muscular artery stiffness/vascular tone³⁹⁾. It is important to note that baPWV and aortic PWV exhibit similar associations with CVD risk and clinical events⁴⁰⁾. The sample size used was relatively small and consisted entirely of men. Thus additional research is needed to examine the contributions of pulsatile stress and inflammation to changes in arterial stiffness in women. In summary, these results demonstrate that change in pulsatile stress and peak systolic stress are important predictors of arterial stiffness regression/progression over time in free-living middle-aged men. Inflammation may correlate with arterial stiffness cross-sectionally, but does not appear to be a significant modulator of arterial stiffness over time.

Conflict of Interest

No.

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