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Vascular Aging: Endothelial Function and Arterial Stiffness

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ABSTRACT:

VASCULAR AGING: AN ASSOCIATION BETWEEN ENDOTHELIAL FUNCTION AND ARTERIAL STIFFNESS

BACKGROUND: Endothelial dysfunction and arterial stiffness, two hallmarks of vascular aging, are both related to a number of cardiovascular risk factors and diseases. However, the relationship between these two vascular parameters is not well understood.
OBJECTIVE: The purpose of this study was to test the hypothesis that 1) endothelial dysfunction is associated with an increase in arterial stiffness and 2) endothelial dysfunction and arterial stiffness are associated with ageing.
METHODS: 48 healthy subjects, divided into three age groups of young, middle-aged and older subjects, participated in this study. Endothelial function was measured by brachial artery flow mediated dilation (FMD). Central and peripheral arterial stiffness were assessed by aortic pulse wave velocity (aPWV) and brachial pulse wave velocity (bPWV), respectively.
RESULTS: Flow mediated dilation, measured as the percentage change from baseline to maximal dilation, was inversely correlated with age ($r=-0.751$, $p<0.001$). No relation was found between changes in shear rate and advancing age. Notably, after normalizing FMD for shear rate, we observed a significant correlation between FMD and age ($r=-0.577$, $p=<0.001$). In addition, aPWV showed a positive relationship with age ($r=0.632$, $P<0.001$) and with FMD ($r=-0.404$, $p=0.004$). No relationship was found between bPWV and age.
CONCLUSION: Our findings suggest that in healthy individuals , declines in endothelial function and increases in arterial stiffness with age are correlated and may be mediated by common underlying mechanisms.

WHAT IS KNOWN: There is evidence linking nitric oxide (NO) to the regulation of arterial stiffness. But research on the relationship between endothelial function and arterial stiffness is highly limited to studies involving subjects with cardiovascular risk factors or diseases and regarding this, the evidence is controversial.

WHAT IS NEW: In this study we emphasized on healthy and normal ageing patients to investigate the relationship between endothelial function and arterial stiffness. The results indicate that, even in healthy individuals, a decline in flow mediated endothelial function is associated with an increase in central arterial stiffness with age.

KEYWORDS: Endothelial function, Nitric Oxide, Arterial Stiffness, FMD, PWV, Vascular aging

Introduction

A reduced bio-availability of nitric oxide (NO) in resistance and conduit vessels, known as endothelial dysfunction, is associated with advancing age[1-4]. Since NO is an anti-atherogenic molecule, this leads to a vascular phenotype more prone to atherogenesis.[5]. Indeed, endothelial dysfunction has been demonstrated to be a key event in the development of atherosclerotic cardiovascular disease,[6] the leading cause of morbidity and mortality in the USA, and a predictor of clinically obvious vascular pathology. Over the past twenty years clinical and experimental studies have demonstrated that endothelial dysfunction is associated with cardiovascular risk factors such as diabetes,[7] smoking,[8] hypertension,[9] hypercholesterolemia,[10, 11] ageing,[2] and with cardiovascular diseases such as coronary artery disease.[12]
Besides endothelial dysfunction, increased arterial stiffening, another parameter for vascular aging, is a coexisting event in patients with these cardiovascular risk factors and outcomes.[13-15] Both animal and human studies have investigated the relationship between these two vascular parameters and this has given evidence linking NO to the regulation of arterial stiffness. In a recent study, inhibition of NO production by administration of the NO-synthase inhibitor L-NAME in spontaneously hypertensive rats induced aortic stiffening.[16] Moreover it is demonstrated that a number of therapeutic interventions that improve endothelial function also reduce arterial stiffness.[17] These observations have led to the hypothesis that endothelium derived NO is an important regulator of arterial stiffness.
Endothelial dysfunction is a highly investigated area, but to date the research investigating the relationship between endothelial function and arterial stiffness is mostly limited to studies involving subjects with cardiovascular risk factors or diseases[18-23] and regarding this, the evidence is controversial.[24] In healthy, normotensive and normal ageing patients, the relationship is not adequately defined. Therefore, the present study was performed to investigate this association between arterial stiffness and endothelial function and the relationship of these vascular parameters with ageing in a group of healthy individuals. To our knowledge there is only one study describing the relationship between endothelial function and arterial stiffening in a group of healthy subjects, however this study did not focus on the effects of ageing on either of these parameters.[25]
Arterial stiffness was assessed by measuring aortic/central (carotid-femoral) pulse wave velocity (PWV), considered as the "gold standard" method to evaluate arterial stiffness, and brachial/peripheral (carotid-radial) PWV (bPWV). Endothelial function was obtained by using flow-mediated dilation (FMD), the most widely used non-invasive test

to measure this vascular parameter. This describes the vasodilatory response of a vessel to elevations in blood-flow associated with increases in shear stress, induced by a 5-minute period of reactive ischeamia caused by temporary arterial occlusion.[26] Because of the suggestion that shear stress can differ with age,[27, 28] and in this way can influence the extent of the vasodilatory response of the brachial artery, we also compared the association between shear rate, an adequate surrogate measure of shear stress, and age. By doing this we normalized the effect of change in shear stress on FMD outcomes and were able to perform a more reliable comparison between endothelial function and aging.
First we aimed to compare FMD and PWV with age. Then, we investigated the relationship between FMD and PWV. We hypothesized that endothelial dysfunction is associated with increases in arterial stiffness and that both these parameters are related with an increase in age.

Methods

Subjects

All patients included in this cross-sectional study were participants of the Arterial aging, Brain Perfusion and Exercise (ABC) study, an ongoing, single-center, cohort study. The objectives of the ABC-study are to determine the effects of exercise and aging on vascular parameters, brain blood flow and cognitive function.
The subject characteristics are shown in table 1. A total of forty-eight healthy, sedentary volunteers were included in this sub-study and were stratified based on age into three different groups: young adults (n=13, 4 males and 9 females, 20-39 yr), middle-aged adults (n=21, 12 males and 9 females, 40-59 yr), and older adults (n=14, 3 males and 11 females, 60-80 yr). All subjects were non-smokers, non-obese, normotensive(<140/90 mmHg) and free of cardiovascular, pulmonary and metabolic disease. All subjects were sedentary, defined as <90 minutes of exercise a week. All procedures were approved by Texas Health Presbyterian Hospital Institutional Review Boards, IRB at UT Southwestern. Written informed consent was obtained from all subjects prior to participation in the study.

Study design

Measurements for brachial artery FMD and aortic and brachial PWV were performed during one visit under standardized conditions, in a quiet, dimly-lit, and temperature controlled room, after a resting period of at least 20 min and at least 24 hours of abstinence of caffeine and alcohol.

Study procedure

Asessment of endothelial function and arterial stiffness
Subjects were in supine position and asked to extend the left arm, in an abduction of approximately 80°. The arm was immobilized using foam. Heart rate was continuously monitored using a three-lead ECG system (Hewlett Packard, USA) and brachial blood pressures were taken on the contralateral arm with an automated sphygmomanometer (Sun Tech Medical Inc, Morrisville, NC). A rapid inflation/deflation pneumatic cuff was positioned on the left arm, distal to the elbow to provide a stimulus for forearm ischemia, following established guidelines for assessing FMD.[6] To obtain high resolution B-mode ultrasound images of the brachial artery a 3-12 MHz linear array transducer of a colour coded ultrasonography system (CX-50, Philips, Healthcare, The Netherlands) was used. The probe was placed 10-20 cm above the antecubital fossa and stabilized using a clamp. Probe placement was carefully adjusted to maintain optimal imaging. Continuous Doppler blood flow velocity signals were recorded at the lowest insonation angle possible (always <60°) and were recorded during baseline and during the first 15 seconds after release of the cuff.
After a period of at least 20 minutes of rest, baseline images and velocity measurements were collected for 3 minutes. Then the pneumatic arm cuff was inflated to a suprasystolic pressure (>225 mm Hg), for 5 minutes. At least 30 seconds before deflation, brachial diameter and velocity recordings were restarted. The increase in blood flow (reactive hyperaemia) was recorded during the first 30 seconds after cuff deflation. The change in vessel diameter was recorded from 30-210 seconds, in serial 20 seconds segments at approximately 21 Hz.
After the measurement of FMD, pulse wave velocity (PWV) was measured to assess arterial stiffness using applanation tonometry with a Sphygmocor instrument (SphygmoCor Px, AtCor Medical, Australia) with methods previously established.[29] Brachial and aortic PWV were measured between the right common carotid artery and the right radial and left femoral artery, respectively. PWV index was calculated as the ratio between the distance and the time needed for the pressure wave to travel between the measurement sites and was expressed in meters per second.

Data analysis
The echo-Doppler signal was taken directly from the ultrasound machine and encoded and stored as a digital DICOM file on the PC. Subsequent data analysis of the brachial artery diameter was performed using edge-detection and wall-tracking, commercially available software(Brachial Analyzer 5.9.0, Medical Imaging Applications, LLC, Coralville, IA). In short, the software allows the user to identify a region of interest (ROI) on the portion of the image where the vessel walls are most clear. The arterial wall borders were detected by an optimal graph search-based segmentation that uses a combination of pixel density and image gradient as an objective function. Each sequence of images was reviewed and interactively edited when needed to ensure that diameter measurements were always calculated from the intima-lumen interface at the distal and proximal vessel wall, during the diastolic phase of the cardiac cycle. All measurements were performed by a single investigator (MH). Analyses from the brachial analyser software were transversed to sigma-plot and peak changes in vessel diameter and time to peak changes in diameter during flow mediated vessel dilation were determined using a smoothing function. The time course of diameters and velocities were determined using a 3-sec moving average. The peak dilation of post-occlusion was determined as the highest 3-sec average and was presented as a percent change from baseline diameter (peak FMD; %).
FMD was calculated as the absolute change in millimetre from baseline to peak diameter and as the percentage rise of the peak diameter from the preceding baseline diameter. The time to peak diameter was calculated from the point of cuff deflation to the maximum post-deflation diameter.
Using Xcelera 1.2 L4 SP2 2007 software (Philips, Best, The Netherlands) peak systolic flow velocity and mean flow velocity integrated over a cardiac cycle in baseline and deflation were determined by taking the average of 10 consecutive cardiac cycles. For deflation, the data segment(10-15 cardiac cycles) representing the maximum velocity during hyperaemia were considered, excluding the first cardiac cycle. From the blood flow velocity estimates and diameter measurements, local shear rate was calculated, using the following equation.
Shear rate (s^{1}) = 4 x mean blood velocity (cm/s) / diameter (cm)
Shear rate values are reported as the mean±SD from 0-15 seconds

after cuff deflation.

Statistics

Statistics were performed with the use of commercially available software (SigmaPlot 12.0, Point Richmond, CA). One-way ANOVA was used to examine the significance of differences between age-groups. When a significant main effect was present, the Holm-Sidak method was used for post hoc testing.
A linear regression model was used to examine the relation between FMD, PWV, SR and age on the one hand and between FMD and PWV on the other. Pearson's product-moment correlation analyses were used to further calculate these correlations. Data are expressed as means±SD. P<0.05 was considered to be statistically significant.

Results

Brachial artery FMD and shear rate
The mean values of FMD and PWV measurements are shown in table 2. Mean FMD, presented as the absolute change from baseline, was significantly higher (p<0.001, 95-CI: 0.19-0.25) in the young (0.29±0.10 mm) and middle-aged subjects (0.23±0.16 mm) than in old subjects (0.14±0.06 mm). There was a declining trend in absolute FMD between young and middle-aged subjects, but the difference between these groups was not significant (p=0.161, 95-CI 0.21-0.28). Mean percentage FMD (%FMD) differed significantly (p<0.001, 95-CI: 5.29-6.79) between all groups (young: 9.04±2.11%, middle-aged: 5.62±2.09%, old: 4.07±1.33%). Mean time till peak diameter was significantly higher (p=0.011, 95-CI: 58.17-74.12) in the old(82.1±42.8s) than in the middle-aged(73.5±25.6s) and young subjects(48.7±9.4s). There was no post-hoc significant difference (p=0.343, 95-CI: 9.03-10.16) in shear rate between the different age groups (young: 10.3±2.71, middle-aged: 8.55±1.79, old: 9.93±1.46).
Using stepwise linear regression models, we found a significant negative relationship between age and absolute FMD (r=-0.544, p<0.001) and age and %FMD (r=-0.751, p=<0.001). Concerning the correlation between age and time till peak deflation, Pearsons product showed a weak (r=0.391), but significant (P=0.00603) positive correlation.
With ageing, shear rate did not decline significantly(r=-0.108, p=0.476). When we corrected %FMD for shear rate and investigated the correlation of this corrected %FMD with age we found a significant inverse correlation coefficient of -0.577. These results are illustrated in figure 1A-D.

Aortic and brachial pulse wave velocity
Aortic PWV was significantly higher (p<0.001, 95-CI: 6.8-7.63) in the old age group than in the young and middle-aged age group. (young: 6.01±0.91, middle-aged: 7.58±1.41, old: 7.88±1.31 m/s). Our analysis did not show any significant difference (p=0.985, 95-CI: 7.77-8.42) in bPWV across the different age groups (young:8.07±0.9 , middle-aged: 8.12±1.46, old: 8.15±0.81 m/s)With a positive Pearson correlation coefficient of 0.623 our analysis revealed a significant(p=<0.001) relationship between age and aPWV. There was no significant correlation between bPWV and age (r=0.126, p=0.393). This is illustrated in figure 2A and 2B.

Correlations between FMD and PWV
Our analysis revealed a significant and inverse correlation between %FMD and aPWV, with a correlation coefficient of -0.404(p<0.05). There was no significant correlation between %FMD and bPWV (r=0.036). This is illustrated in figure 3A and 3B.

Discussion

Arterial stiffness is not only regulated by the structural elements of the vessel wall and related to arterial distending pressure but also by the smooth muscle tone, regulated by nitric oxide (NO). It has been shown that removal of the vascular endothelium in animals alters arterial stiffness,[30, 31] leading to the suggestion that endothelial derived mediators play an important role in the regulation of arterial stiffness in vivo. Nitric oxide is one of these endothelium derived mediators that possibly contribute to the regulation of arterial stiffness. The bioavailability of NO in both the resistance and conduit vessels is known as a key determinant of endothelial function and declines with advancing age. This study was designed to examine the effects of age on brachial artery endothelial function, measured by flow mediated dilation (FMD), and on central and peripheral arterial stiffness, measured by aortic pulse wave velocity (aPWV) and brachial pulse wave velocity (bPWV), respectively. Furthermore, an important aim was to examine the relationship between endothelial function and central and peripheral arterial stiffness in a group of healthy, sedentary individuals.
The most important finding of the present study is that aortic PWV was significantly and inversely correlated with flow-mediated dilation in the brachial artery. In addition we found a significant, age-related decline in both absolute and percentage FMD. We also found an inverse increasing relationship between aPWV and age. When we studied the flow mediated dilation parameters in detail we found a significant difference in the time to peak diameter index after deflation between the age groups. In older subjects this parameter was significantly longer than in middle-aged and younger subjects. When we measured shear rate, a surrogate measure for shear stress that causes the vessel to dilate, we did not see a significant difference between the three age groups. After normalization of %FMD for this shear rate the correlation between age and FMD changed from -0.751 to -0.577, which were both significant.
Together, these data show that an advance in age is related to a decline in endothelial function. Concerning arterial stiffness there is a relationship between advancing age and an increase in central arterial stiffness, but for peripheral stiffness this is not the case. Furthermore, our data suggest that endothelial function plays an important role in the process of arterial stiffness in vivo and this emphasizes the importance of NO as a regulator of this process.
Decline in endothelial function with advancing age is a consistent finding in prior studies.[1-4] The mechanisms are still uncertain, but may be related to the age-related turnover of endothelial cells. With ageing the endothelial cells detach from the vessel wall which may result in uncovered areas. Endothelial cell genesis may repair the uncovered area but they exhibit a selective loss in responses that involve cell membrane receptors linked to the activation of nitric oxide synthase (NOS). The result is a reduced bioavailability of NO that causes endothelial dysfunction.[32] The turnover of cells is stimulated by risk factors such as high cholesterol, smoking, hypertension and diabetes. These risk factors may work together with the negative impact of ageing setting up the scene for development of vascular disease.
There is an ongoing debate about whether or not to normalize FMD for shear stress, or its surrogate measure of shear rate. Several studies have demonstrated that exposure to shear stress leads to diameter increases in a dose-dependent fashion.[33, 34] When distinct FMD responses are observed between groups or individuals, it still is unclear whether this is attributable to different biological variability

in endothelial function or a difference in the magnitude of reactive hyperaemia induced shear stress. Since shear rate did not change significantly with an advance in age in our study, and a significant correlation between FMD and age still existed after normalization for shear rate, we excluded the possibility that differences in the magnitude of reactive hyperaemia would be interpreted as changes in FMD with age.

To explore the relationship between arterial stiffness and age we assessed aortic and brachial PWV, which provides an accurate measure of arterial stiffness. The age associated augmentation was much stronger in aortic pulse wave velocity than in brachial pulse wave velocity. Such different effects of ageing on the stiffness of central versus peripheral arteries may be related to their distinct roles in hemodynamic regulation. The cushion functions that damp fluctuations in blood flow that are present in central arteries are not present in the peripheral arteries. Because of this, the peripheral arteries do not exhibit the same extent of pulsatile changes in diameter and as such they may not undergo the adaption (or wear and tear stress) leading to a loss of elasticity.[35]

When determining the relationship between endothelial function and arterial stiffness, flow mediated dilation emerged as a significant determinant of aortic PWV, suggesting endothelial function declines as central arterial stiffness increases. A number of other studies have extensively investigated the relationship between endothelial function and various indices of arterial stiffness in selected patient groups with cardiovascular risk factors and diseases.[18-22]. To our knowledge this is the only study focused on investigating the age-related correlation between endothelial function and arterial stiffness in healthy individuals. The other study describing this relationship in healthy individuals was performed by McEniery et al. and published in 2006.[25] Their observations also showed a significant correlation between flow mediated dilation and aortic PWV in a group of 89 healthy individuals(age of 41±16 years, r=-0,39, p<0.001). Our data confirm these findings, although in a smaller group of participants.

Strengths and Limitations

A limitation of our study is that our cohort largely consisted of Caucasian individuals. Results may not be applicable to other racial or ethnic groups. However, a prior study suggests that flow mediated dilation is comparable in white and black populations.[36] The size of our study cohort also forms a limitation, whereas the other study by McEniery et al. included 89 subjects to compare %FMD with aPWV,[25] our study only included 48 participants. Besides this a limitation can be found in our gender distribution, in both the young and the old age-group, the number of female participants exceeded the number of male participants. This may have influenced our outcomes, because our results show that females have a smaller FMD response and a lower aPWV in comparison to males. It is shown that smooth muscle sensitivity to nitric oxide is modulated by the hormonal patterns of the menstrual cycle,[37], however we did not track the menstrual cycle in the female participants.

Notwithstanding the above limitations, the present study has several strengths, the most important is the inclusion of elderly participants. Ageing exerts a marked effect on arterial stiffness and endothelial function and by dividing our subjects in a young, middle-aged and old age group we were able to make an association between an advance in age with both vascular parameters. Moreover, this study only contained healthy individuals, whereas previous studies describing the relationship between endothelial function and arterial stiffness mainly included subjects with cardiovascular risk factors and diseases.

Conclusion

Since NO is a potent anti-atherogenic molecule it plays a major role in the development of cardiovascular diseases and it is associated with cardiovascular risk factors.[2, 5, 7, 8, 10-12] Arterial stiffness is also associated with these cardiovascular diseases and risk factors,[13-15] leading to the suggestion that impaired NO-bioavailability may be the link between these two vascular parameters. The results of the present study indicate that, even in healthy individuals, a decline in flow mediated endothelial function is associated with an increase in central arterial stiffness. This describes the importance of NO as the common underlying mechanism in the regulation of endothelial function and arterial stiffness in vivo.

This evidence, linking NO to the regulation of arterial stiffness provides a novel therapeutic target for drugs. Particularly, drugs that can restore vascular NO-bioavailability may attenuate or prevent age-related increases in arterial stiffness and its clinical consequences. In this regard, the mechanisms behind the association of NO and arterial stiffness need to be fully studied and understood. Mechanistic study of the role of NO on arterial stiffness is likely to have an significant impact on both health and disease.

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Tables

Table 1: Subject characteristics of the participants divided into a group of young, middle-aged and older adults.

Age categorie	20-39	40-59	60-80	Anova
n	13	21	14	
Gender male/female	4/9	12/9	3/11	
Age, yr	28.5±6.2	50.7±5.7	66.6±5.7	<0.001
Heart rate at rest	65.5±6.8	63.3±8.8	61.6±9.2	0.491
Systolic blood pressure, mmHg	107.9±9.3	113.2±12.5	115±10.2	0.236
Diastolic blood pressure, mmHg	67.5±8.7	72.9±8.7	68.7±9.6	0.197
Mean blood pressure, mmHg	81.0±8.0	86.3±9.6	84.1±8.4	0.246
Height, cm	165.1±8.1	170.8±9.7	164.0±5.6	0.041
Weight, kg	61.3±11.2	79.5±14.1	67.7±10.2	<0.001
BMI, kg/m²	22.4±3.1	27.1±3.8	25.1±3.3	0.002

BMI, body mass index
Values are presented as means± SD, n is number of participants, P<0.05 is significant.

Table 2: Brachial artery characteristics and arterial stiffness parameters

Age categorie	20-39			40-59			60-80			Anova*ºǂ
Gender	man	female	overall	man	female	overall	man	female	overall	overall
n	4	9	13	12	9	21	3	11	14	
Baseline diameter, mm	3.92±0.19	2.77±0.50	3.16±0.67º	4.42±0.46	3.38±0.62	3.98±0.73º	3.61±0.87	3.23±0.17	3.31±0.41º	0.001
Peak diameter, mm	4.30±0.22	3.07±0.55	3.45±0.75º	4.71±0.49	3.52±0.62	4.20±0.81º	3.82±0.98	3.35±0.18	3.45±0.46º	0.003
Absolute change from baseline, FMD mm	0.38±0.03	0.25±0.09	0.29±0.10º	0.30±0.07	0.14±0.06	0.23±0.16º	0.21±0.12	0.12±0.03	0.14±0.06º	<0.001
% change from baseline, %FMD	9.74±0.44	8.73±2.50	9.04±2.11*	6.71±1.61	4.17±1.80	5.62±2.09*	5.5±1.97	3.68±0.86	4.07±1.33*	<0.001
time to peak dilation, sec	48.3±12.7	48.0±8.5	48.7±9.4º	74.2±24.1	72.7±29.0	73.5±25.6º	79.3±73.2	82.9±36.2	82.1±42.8º	0.011
Shear rate 0-15 sec, s^{-1}, 10^2	8.15±1.59	11.3±2.56	10.3±2.71ǂ	7.76±1.29	9.38±2.04	8.55±1.79ǂ	8.55±1.83	10.2±1.37	9.93±1.46ǂ	0.043
Aortic pulse wave velocity m/s	6.68±1.21	5.71±0.62	6.01±0.91º	7.64±1.33	7.49±1.60	7.58±1.41º	8.53±1.29	7.71±1.34	7.88±1.33º	<0.001
Brachial pulse wave velocity m/s	8.41±1.37	7.92±0.80	8.07±0.97	8.35±1.51	7.81±1.43	8.12±1.46	9.02±0.95	7.91±0.61	8.15±0.81	0.985

**post hoc significance between all groups.*
ºpost hog significance between young and old and middle-aged an old,
ǂno post hoc significance between groups
Values are presented as means± SD, n is number of participants, P<0.05 is significant.

Figures

Figure 1: The relationship between age and: %FMD(A), time till peak dilation(B) and shear rate(C) and %FMD normalized for Shear Rate(D).

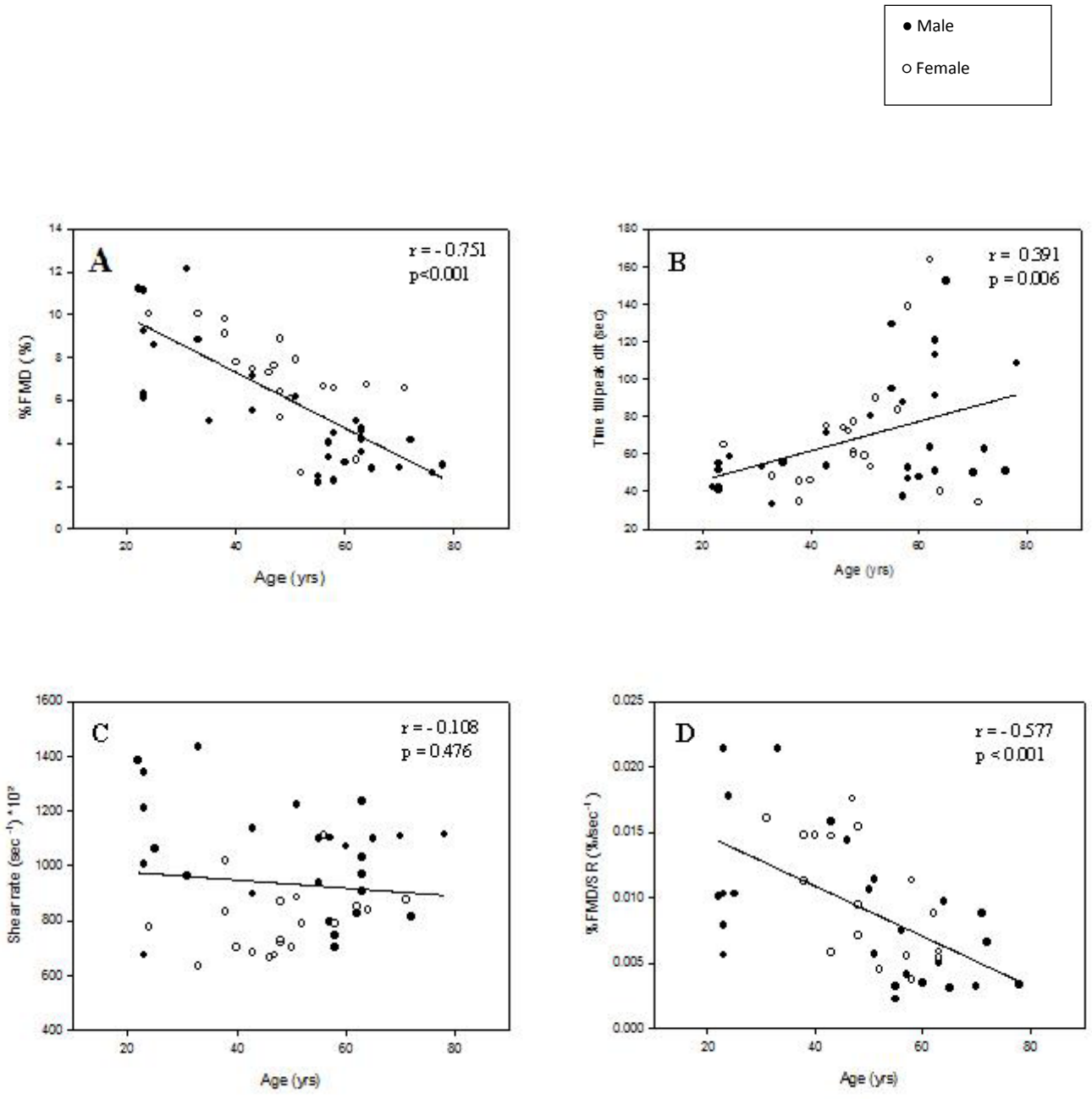

Figure 2: The relationship between age and aPWV (A) and bPWV (B)

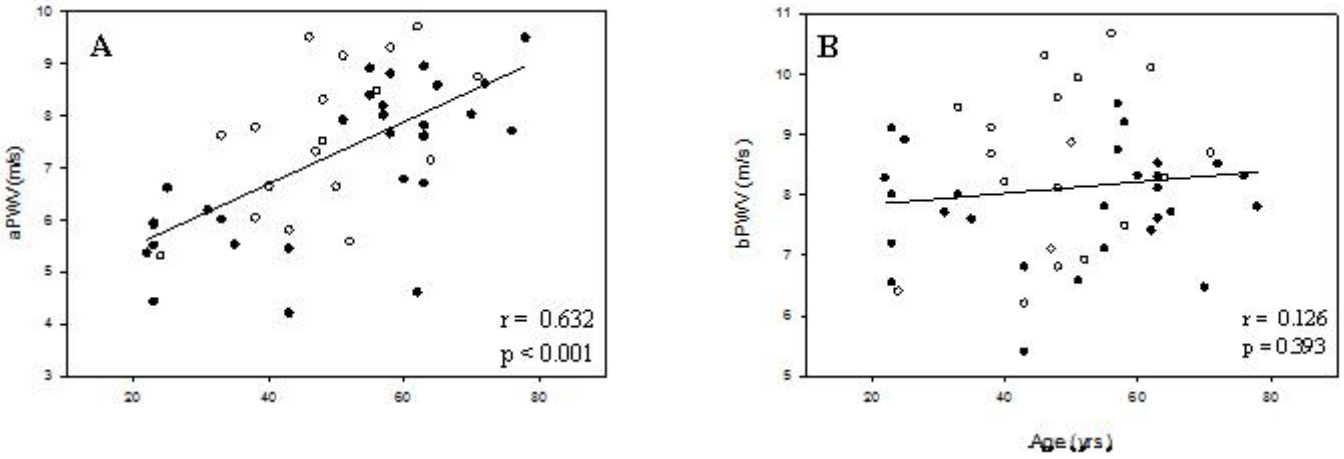

Figure 3: The relationship between %FMD and aPWV(A) and bPWV(B)