Original Article

Measurement of the Brachial-Ankle Pulse Wave Velocity and Flow-Mediated Dilatation in Young, Healthy Smokers

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The brachial-ankle pulse wave velocity (PWV) is a quick test which adequately estimates arterial stiffness. Because flow-mediated dilatation (FMD) of the brachial artery assesses an essential endothelial function, we tested the hypothesis that the brachial-ankle PWV could reflect the early stages of endothelial dysfunction caused by smoking in young, healthy subjects. Fifty-seven healthy subjects (13 females and 44 males; mean 29.9±5.6 years) were enrolled. Twenty-six of the subjects (30.4±5.7 years) were active smokers, with a mean cumulative nicotine consumption of 10.0±8.6 pack/years, and thus were assigned to the smoking group. Thirty-one subjects without a history of smoking (29.5±5.5 years) were assigned to the non-smoking group. The brachial-ankle PWV and arterial blood pressure were simultaneously measured using a recently established, non-invasive automatic device (model BP-203RPE; Nihon Colin, Tokyo, Japan). Endothelium-dependent FMD was induced by reactive hyperemia, while endothelium-independent vasodilation of the brachial artery was induced by administration of sublingual nitroglycerin spray. The FMD was lower in the smoking group than in the non-smoking group ($p<0.05$). There was no significant difference between the two groups with respect to the brachial-ankle PWV. In the non-smoking group, multiple stepwise regression analysis revealed that FMD was predicted by the systolic blood pressure ($F=16.351$). In the smoking group, statistical analysis revealed that FMD was independently predicted by either the brachial-ankle PWV ($F=8.108$) or the subject's age ($F=4.381$). Our results suggest that a reduction in FMD is closely associated with the early stages of endothelial dysfunction caused by cigarette smoking in young, healthy subjects, which is at least partly reflected by the PWV value. (*Hypertens Res* 2007; 30: 607–612)

***Key Words*: flow-mediated dilatation, pulse wave velocity, arterial stiffness, endothelial function**

Introduction

Endothelial dysfunction is a disease process that occurs throughout the vascular system and results in abnormal regulation of blood vessel tone and the loss of the atheroprotective properties of normal endothelium (*1*, *2*). Endothelial dysfunction is, therefore, evolving as an important pathogenic mechanism for atherosclerosis and may be one of the earliest manifestations of certain cardiovascular diseases (*3*). Flow-mediated dilatation (FMD) of the brachial artery, as induced by reactive hyperemia, has been convincingly demonstrated to reflect endothelium-dependent vasodilation, which is mediated by nitric oxide (NO) (*4*–*8*).

Recently, a non-invasive automatic device has been developed which measures the pulse wave velocity (PWV) (*9*). This device monitors arterial pressure waves in the arm and ankle using a volume-based plethysmographic method. It has been demonstrated that the aortic PWV, when measured invasively, accurately estimates arterial stiffness (*10*). Subse-

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quently, it was shown that the brachial-ankle PWV correlates well with the aortic PWV as determined using an invasive method (*10*). The measurement of the brachial-ankle PWV by a non-invasive automatic device is notable for the associated time-savings and the lack of a requirement for specialized skills on the part of the operator. However, the validity of the brachial-ankle PWV in assessing peripheral vascular endothelial function has not been fully evaluated.

Cigarette smoking causes a reduction in FMD in asymptomatic, young adults, consistent with an early stage of endothelial dysfunction (*11*). In the present study, therefore, we tested the hypothesis that the measurement of the brachial-ankle PWV could detect the early stages of endothelial dysfunction caused by cigarette smoking, as characterized by a reduction of FMD in young, healthy subjects.

Methods

Study Population

Fifty-seven young, healthy subjects, 20–40 years of age (mean 29.9±5.6 years), were enrolled in the study. The study group was comprised of 13 females and 44 males. None of the participants had a history of hypertension, a pulse pressure >60 mmHg, hypercholesterolemia, dyslipidemia, thyroid disease, or diabetes mellitus. Further, none of the participants took anti-oxidants or cardiovascular medications before or during this study. Of the 57 subjects, 26 (30.4±5.7 years) were active cigarette smokers with a mean cumulative nicotine consumption of 10.0±8.6 pack/years, and were thus assigned to the smoking group. The remaining 31 subjects (29.5±5.5 years) did not have a history of cigarette smoking and were assigned to the non-smoking group. One pack/year was defined as smoking 20 cigarettes per day for 1 year (*11*). The study conformed to the principles outlined in the Declaration of Helsinki. The study was approved by the ethics review board of our institution and written informed consent was obtained from all subjects.

Measurement of the Brachial-Ankle PWV

The brachial-ankle PWV was determined using a non-invasive automatic waveform analyzer (model BP-203RPE; Nihon Colin, Tokyo, Japan). Three pulse waves, detected as the displacement by wide-band pressure transducers with a cuff, were recorded by the oscillometric method from the spots where pulses of the right brachial artery and both tibial arteries were palpated. The arterial elastic properties were calculated as the mean of consecutive cardiac cycles for 30 s. During the recordings of the pulse waves, the pressure of the cuff was maintained at 60 mmHg. The pulse wave was maintained <60 mmHg if the diastolic blood pressure (DBP) of the patient was below 60 mmHg. A phonocardiogram was recorded on the right sternal border in the second intercostal space at the same time and on the same recorder. An electrocardiogram, the blood pressure, and the pulse volume were recorded simultaneously. The pressure waveforms obtained at two different sites were simultaneously recorded to determine the time interval between the initial rise in the right brachial and tibial pressure waveforms (ΔT_a). The path length from the suprasternal notch to the elbow (ΔD_a) was obtained from superficial measurements and was expressed using the following formula:

$$\Delta D_a = 0.2195 \times H - 2.0734,$$

where H is the height of the patient in cm. The path length from the suprasternal notch to the femur and then to the ankle (ΔD_b) was calculated as follows:

$$\Delta D_b = (0.5643 \times H - 18.381) + (0.2486 \times H + 30.709).$$

The following formula was used for the PWV from the elbow to the ankle (brachial-ankle PWV): $(\Delta D_b - \Delta D_a)/\Delta T_a$. The average of the brachial-ankle PWV values obtained in each ankle was used in the data analysis. The coefficient of variation of the brachial-ankle PWV for repeated within-subject measurement was 0.7%.

Assessment of FMD and Nitroglycerin-Induced Dilatation

Endothelium-dependent FMD in response to reactive hyperemia and endothelium-independent nitroglycerin-induced dilatation (NMD) were examined in the brachial artery according to the method described by Celermajor *et al*. (*4*). Using a 7.5 MHz linear array transducer (model SSH-160A; Toshiba, Tokyo, Japan) longitudinal B-mode ultrasound images of the right brachial artery above the antecubital fossa were taken after 10 min of rest. The ultrasound images were recorded on a Super-VHS videocassette recorder (model BR-S601M; Victor, Tokyo, Japan). The arterial diameter was measured at a fixed distance from an anatomical marker. The measurements were taken from the anterior to the posterior interface between the media and adventitia (*i.e.*, the "m" line) at end-diastole, coincident with the R wave on the continuously recorded electrocardiogram. After measurements of the brachial-ankle PWV, the subjects rested in a supine position for 10 min. Thereafter, a blood pressure cuff was placed proximal to the imaging transducer. The cuff was inflated to at least 50 mmHg above systolic blood pressure (SBP) for exactly 5 min. The diameters during three cardiac cycles were analyzed for each scan, and the measurements were averaged. The diameter measurements for the reactive hyperemia were obtained 45–90 s after deflation of the cuff to measure the peak diameter. The baseline resting brachial artery dimension was again obtained 10 min later. Thereafter, subjects were given 0.3 mg sublingual nitroglycerin spray, and the brachial artery was imaged 3–5 min later to determine the peak diameter. The FMD and NMD were calculated as the percent change in diameter compared with the baseline. The ultrasounds scans were analyzed by one observer, who was

Table 1. Clinical Characteristics of Studied Patients

	Non-smoking group (n=31)	Smoking group (n=26)	p value
Age (years)	29.5±5.5	30.4±5.7	0.5163
Gender (women/men)	8/23	5/21	0.781
Pack years	0	10.0±8.6	<0.0001
BMI (kg/m^2)	21.6±2.6	22.5±2.9	0.2137
SBP (mmHg)	116.7±10.1	116.8±9.3	0.9719
DBP (mmHg)	70.4±8.0	70.3±7.0	0.9686
MBP (mmHg)	86.2±9.0	86.2±9.0	0.9868
PP (mmHg)	46.3±5.4	46.5±4.8	0.9009
HR (beats/min)	66.8±11.0	66.5±10.4	0.9061
PWV (cm/s)	1,201±161	1,232±160	0.4738
FMD (%)	16.1±6.6	12.4±5.8	0.03
NMD (%)	20.9±9.2	21.2±5.7	0.8964

Values are means±SD. BMI, body mass index; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; HR, heart rate; MBP, mean blood pressure; NMD, nitroglycerin-induced dilatation; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

blinded to the identity of the volunteer and the study phase. The coefficients of variation of FMD and NMD for repeated within-subject measurements were 2.7% and 3.0%, respectively.

Statistical Analysis

Data are presented as the mean±SD. Statistical analysis was performed using Stat View statistical software, version 5.0. Pearson's correlation analysis was performed to assess potential relationships between the brachial-ankle PWV and other clinical variables. A value of $p<0.05$ was considered statistically significant. Stepwise multiple regression analysis was performed to determine the independent variables for FMD. In our multivariate analysis, F values ≥4 were considered significant.

Results

Study Population

The clinical characteristics of the non-smoking and smoking groups are summarized in Table 1. No significant differences existed between the two groups with respect to age, gender, body mass index, SBP or DBP, mean blood pressure, pulse pressure, or heart rate. FMD was lower in the smoking group than in the non-smoking group ($p<0.05$); however, there was no significant differences between the two groups regarding brachial-ankle PWV and NMD.

Correlation of FMD with Clinical Variables

Table 2 shows the Pearson's correlation of FMD with various clinical variables in each group. In the non-smoking group, the FMD was correlated with SBP ($p<0.0005$), DBP ($p<0.05$), mean blood pressure ($p<0.005$), pulse pressure ($p<0.01$), and brachial-ankle PWV ($p<0.01$). In the smoking group, the FMD was correlated with the brachial-ankle PWV ($p<0.005$) and the subject's age ($p<0.05$). In the non-smoking group, stepwise regression analysis carried out using variables, including SBP, pulse pressure, and the brachial-ankle PWV, revealed that FMD was best-predicted by the SBP ($F=16.351$). On the other hand, stepwise regression analysis in the smoking group revealed that FMD was independently predicted by the brachial-ankle PWV ($F=8.108$) and subject age ($F=4.381$; Table 3).

Discussion

The core findings of the present study were as follows: 1) FMD was lower in the smoking group than in the non-smoking group and 2) FMD was correlated with the brachial-ankle PWV and subject age in the smoking group. Multiple stepwise regression analysis also revealed that FMD was independently predicted by either the PWV or the subject's age. Based on our observations, it is suggested that cigarette smoking induces endothelial dysfunction in young, healthy subjects, which is characterized by a reduction in FMD and is at least in part reflected by the PWV. The PWV has been established as a non-invasive measure of arterial stiffness and sclerosis (*12–14*). The ability of the brachial-ankle PWV to assess peripheral vascular endothelial function has been incompletely investigated. To the best of our knowledge, this is the first report that has demonstrated that the brachial-ankle PWV is closely associated with endothelial function in young, healthy subjects who smoke cigarettes.

Several previous studies have demonstrated that cigarette smoking alone is sufficient to impair FMD in healthy, young

Table 2. Correlation of Flow-Mediated Dilatation to Measures of Variables in Non-Smoking Group and Smoking Group

	Non-smoking group		Smoking group	
	r	*p* value	*r*	*p* value
Age	0.123	0.5091	−0.446	0.0224
Pack years			−0.386	0.0516
BMI	−0.028	0.8791	−0.372	0.0612
SBP	−0.6	0.0004	−0.135	0.5112
DBP	−0.434	0.0148	−0.377	0.0579
MBP	−0.502	0.004	−0.18	0.3801
PP	−0.482	0.0061	0.286	0.5563
HR	−0.001	0.9976	0.207	0.3093
PWV	−0.475	0.0069	−0.543	0.0041

BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; MBP, mean blood pressure; NMD, nitroglycerin-induced dilatation; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

Table 3. Stepwise Regression Analysis between Flow-Mediated Dilatation and Measures of Variables in Non-Smoking Group and Smoking Group

	Independent variables			
	Regression coefficient	SEM	Standard regression coefficient	*F*-value
Non-smoking group: to flow-mediated dilatation				
SBP	−0.389	0.096	−0.6	16.351
Smoking group: to flow-mediated dilatation				
Age	−0.354	0.169	−0.344	4.381
Brachial-ankle PWV	−0.017	0.006	−0.468	8.108

PWV, pulse wave velocity; SBP, systolic blood pressure.

adults (*11*, *15*). The consequences of long-term cigarette smoking in young adults have been investigated by using external ultrasound imaging with analysis of vessel diameter changes due to hyperemia and after nitrate application (*11*). In a study investigating 200 subjects, Celermajer *et al*. (*11*) demonstrated an inverse relationship between FMD and lifetime cigarette exposure, findings which are in agreement with the findings presented herein. Oxidative stress may largely account for the nicotine-induced endothelial dysfunction in humans. A recent experimental study demonstrated that chronic exposure to nicotine and an acute infusion of nicotine causes an impairment of endothelium-dependent arteriolar dilatation by increasing oxidative stress (*16*). Cigarette smoking has also been reported to inhibit the reduction in PWV associated with antihypertensive therapy. Matsui *et al*. (*17*) demonstrated that amlodipine administration effectively reduced blood pressure in both smokers and non-smokers, while PWV reduction was only observed in non-smokers.

Although our study did not include subjects with hypertension, it is noteworthy that FMD had a negative correlation with the blood pressure in the non-smoking group. In previous studies, hypertension increased large artery dilatation by as much as 15% (*18*) and retinal artery dilatation by as much as 35% (*19*), indicating that systemic hypertension increases vascular stretch. The vessel endothelium is continuously subjected to mechanical stretch and shear forces *in vivo*. Mechanical stretch can initiate intracellular signaling, regulate protein synthesis, and alter secretion of numerous factors, including NO (*20*), endothelin-1, platelet-derived growth factor, fibroblast growth factor, and angiotensin II. In addition, mechanical stretch induces hypertrophy and/or hyperplasia in vascular smooth muscle cells (*21*). Although the pathogenesis of the association between endothelial dysfunction and elevated blood pressure in our young, healthy, non-smoking subjects remains to be determined, impairment in NO production is known to precede the onset of essential hypertension in offspring (*22*). Specifically, when subjects were divided into two groups according to the presence or absence of a family history of essential hypertension, subjects with a family history were characterized by a reduced response to acetylcholine (*22*). Accordingly, it was suggested that vasodilatation in response to acetylcholine was blunted in the offspring of hypertensive patients before the clinical manifestation of hypertension compared to that in normotensive subjects (*22*).

One unknown that may be pertinent to the present study is the question of whether the PWV is closely associated with FMD. Generally, factors that impair endothelial function are believed to be associated with increased arterial stiffness. The

large conduit vessels possess a significant muscular component, which is partially under the control of the endothelium. Thus, changes in endothelial function alter the mechanical properties of the large arteries and result in increased stiffness (*15*). It has been shown that intra-arterial infusion of an NO synthase inhibitor, N^G-monomethyl-L-arginine, increased PWV in the human brachial artery (*23*) and sheep common iliac artery (*24*), explaining why conditions that exhibit endothelial dysfunction are also associated with increased arterial stiffness. It has been reported that the PWV is one of the independent determinants of the urinary albumin excretion rate, which reflects increased vascular permeability regulated by the endothelium (*25*). These findings suggest that increased aortic stiffness may play a role in the progression of systemic vascular injuries, probably *via* universal endothelial dysfunction, and may also explain the close relationship between the brachial-ankle PWV and FMD observed in the subjects who smoked cigarettes in the present study.

There are several limitations in the present study. First, our study included several young female subjects. Previous studies (*26*, *27*) have shown that during menstrual cycles, FMD is higher in healthy, young women during the follicular phase than in either the luteal or menstrual phase. Large and small vessel endothelial function increase during the follicular phase, decline after ovulation, and then rise again during the luteal phase, whereas the central PWV does not vary significantly during menstrual cycles, but whole body arterial compliance increases during follicular development and falls after ovulation (*27*). Thus the FMD and PWV measurements in young female subjects should be interpreted with caution, which was not clarified in the present study. Second, our FMD and NMD values appear to be higher than those of previous reports (*28*, *29*). This may be explained by our enrollment of only young and healthy subjects. In fact, as in the present study, FMD and NMD have previously been reported to be 14.6±7.6% and 16.7±5.8%, respectively, in normotensive patients (mean age, 59±15 years) (*30*) and 15.7±3.9% and 20.4±6.9%, respectively, in healthy, young subjects (mean age, 26±5 years) (*31*). Finally, although a close association between endothelial dysfunction and cigarette smoking was demonstrated, the cumulative nicotine consumption, as assessed in the present study by pack years, did not correlate with FMD or PWV. This finding may be explained by the large deviation in pack years, the inclusion of only young subjects with a relatively small cumulative nicotine consumption, or the small number of subjects studied.

Conclusions

Our results suggest that a reduction in FMD is closely associated with the early stages of endothelial dysfunction caused by smoking in young, healthy subjects, which is at least in part reflected by the PWV. A future, large-scale, prospective study will be required to assess the significance of measuring the brachial-ankle PWV for the progression of atherosclerosis and the development of cardiovascular diseases.

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