Reduced Arterial Elasticity Is Associated With Endothelial Dysfunction in Persons of Advancing Age

Comparative Study of Noninvasive Pulse Wave Analysis and Laser Doppler Blood Flow Measurement

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Background: Endothelial dysfunction is the earliest marker for age-related abnormalities in vascular function, and examination of endothelial function has important clinical relevance. The present study was performed to evaluate effects of aging on arterial elasticity by using pulse waveform analysis and to investigate whether the changes in arterial elasticity might be used as a noninvasive measure for endothelial dysfunction.

Methods: A total of 24 healthy male volunteers were divided into young (n = 12) and elderly (n = 12) groups. Endothelial function was evaluated by delivering acetylcholine (Ach) and sodium nitroprusside (SNP) to the forearm vessels using iontophoresis, respectively, and measured blood flow using laser Doppler fluximetry. Large and small artery elasticity indices were noninvasively assessed using pulse wave analysis.

Results: Basal blood flow was similar between the young and elderly groups (14.58 ± 3.4 vs 13.52 ± 3.41 PU, P = NS). Peak blood flow induced by Ach was significantly reduced in the elderly group compared with the young group (83.4 ± 11.9 vs 93.75 ± 10.87 PU, P < .05). However, peak blood flow induced by SNP was similar in the two groups (119.17 ± 16.76 vs 128.33 ± 21.29 PU, P = NS). In parallel, C1 large artery elasticity and C2 small artery elasticity indices were significantly reduced in the elderly group compared with the young group (11.42 ± 1.67 vs 16.75 ± 2.09 mL/mm Hg × 10, P < .001; and 7.67 ± 1.56 vs 10.75 ± 1.86 mL/mm Hg × 100, P < .001, respectively). The Ach-induced peak blood flow correlated with C1 large and C2 small artery elasticity indices.

Conclusions: Advancing age is associated with endothelial dysfunction and reduced arterial elasticity. Reduced arterial elasticity parallels changes in impaired endothelial function. It appears that reduced arterial elasticity may be used as a noninvasive measure for the determination of endothelial function.

Key Words: Arterial elasticity, endothelium, aging, laser Doppler, endothelial dysfunction.

Advancing age is one of the major risk factors for the pathogenesis of vascular diseases and is associated with changes in vascular structure and function.1-3 Age-related structural and functional alterations of the arterial wall precede apparently obstructive atherosclerosis and cardiovascular events. It is generally accepted that structural and functional changes associated with advancing age also impair vascular endothelium.4-6 Endothelial cells control vascular tone through the release of different substances that determine the contractile activity of the underlying smooth muscle.7,8 Endothelial dysfunction appears to be the earliest marker for these structural and functional changes that make the vasculature sensitive to the adverse effects of blood pressure, lipids, diabetes, smoking, and other risk factors, leading to the development of atherosclerotic vascular diseases.

Endothelial function can be measured in coronary arteries and in the periphery by measuring vasomotor function after intra-arterial infusion of pharmacologic substances that enhance the release of endothelial nitric oxide (NO).4-6 The disadvantage of these methods is their invasive nature, which generally makes them unsuitable.
for studies involving asymptomatic subjects. For this rea-
son, noninvasive tests of endothelial function have been
developed. Iontophoretic administration of acetylcholine
(Ach) to measure cutaneous microvascular endothelial
function is one approach for noninvasive endothelial func-
tion testing and can provide valuable insights into early
atherogenesis.9

It has also been demonstrated that reduced arterial
elasticity provides a marker for the structural and func-
tional abnormalities associated with aging by using pres-
sure pulse contour analysis.3 One recent study showed
that, in humans, NO production is involved in arterial
waveform alteration10. Whether reduced arterial elasticity
or compliance is also associated with cutaneous microvas-
cular endothelial dysfunction in humans with advancing
age, however, has not yet been reported.

We hypothesized that reduced arterial elasticity is as-
associated with early endothelial dysfunction and provides
evidence for abnormalities in vascular structure and func-
tion in humans with aging. To test this assumption, the
present study was designed to evaluate endothelial de-
pendent vasodilation and arterial elasticity in both young
and elderly normal subjects, by using noninvasive pulse
wave analysis and laser Doppler blood flow measurement.

Methods
Subjects
A total of 24 healthy male workers from our hospital
volunteered to be the subjects for this study and were
divided into groups based on young age (n = 12, aged 20
to 30 years) and older age (n = 12, aged 60 to 70 years).
All subjects underwent a full medical history and exami-
nation including laboratory tests and electrocardiography;
subjects with hypertension, diabetes mellitus, hypercho-
lesterolemia, and other cardiovascular abnormalities were
excluded. No subjects had been taking any drugs for at
least 2 weeks before starting this study. This clinical
investigation was approved by the Ethical Committee of
our hospital.

Procedures
The investigation was performed in a controlled environ-
ment maintained at 23°C after the participants had rested
for at least 15 min in a supine position. The subjects
refrained from consuming alcohol or caffeine and did not
smoke for 12 h before the study. Radial artery pressure
pulse waves and laser Doppler forearm cutaneous micro-
vascular blood flow were measured.

Radial Arterial Pulse
Waveform Measurement
Radial arterial pulse waves were recorded with an acoustic
transducer using the CVProfiler DO-2020 CardioVascular
System (Hypertension Diagnostics, Eagan, MN) as previ-
ously reported.3,11 A wrist stabilizer was positioned on the
right wrist using two hook and loop straps to gently
immobilize the wrist and to stabilize the radial artery,
making it readily accessible for placement of the Arterial
Pulsewave Sensor (Perimed, Järfalla, Sweden). The Sen-
or electronically adjusts itself automatically to obtain an
acceptable waveform. A 30-sec collection of the radial
artery waveform, digitized at 200 samples/sec, is then
stored in an onboard computer for analysis. To obtain
arterial elasticity data, a model was used that divides the
total systemic arterial compliance into C1 large artery and
C2 small artery elasticity indices. The model describes
diastolic pressure contours by the following equation:

\[
P_2(t) = A_1e^{-A_3t} + A_2e^{-A_4t}\cos(A_5t + A_6)
\]

where \(P_2(t)\) is the diastolic pressure at time \(t\) relative to
aortic value closure. A parameter estimating algorithm
was applied for determination of the best set of \(A_i\) values
for matching the diastolic portion of the measured beats to
this equation. These \(A_i\) parameters, together with an esti-
mate of systemic vascular resistance, determine the large
or capacitive compliance and the small or oscillatory com-
pliance. The compliance values for each beat were
weighted inversely with respect to an estimate of error and
then averaged. The estimate of error was the predicted
variance in the compliance divided by a measure of the
goodness-of-fit of the model to the data. This approach
ensures that individual compliance values with high esti-
mated variance will contribute proportionally less to the
overall compliance value. In addition, end-diastolic distor-
tions were eliminated by defining end-diastole as the point
at which diastolic pressure is no longer monotonically
decreasing.

Laser Doppler Perfusion Imaging
and Iontophoretic Drug Delivery
Iontophoresis allows the noninvasive delivery of drugs to
a relatively small area of skin without perturbing the site
or inducing systemic effects.12 The method used in the
present study was slightly modified according to previous
report.9 Acetylcholine (Ach) (Sigma Chemical, St. Louis,
MO), an endothelium dependent vasodilator, and sodium
nitroprusside (SNP), an endothelium independent vasodi-
lator, were dissolved in 5% NaH2PO4 and physiologic
saline to an appropriate concentration, respectively. We
chose 5% NaH2PO4 as an Ach dilutive solution because
this solution did not cause pain at the measurement site or
accumulation of local black elements compared with the
effects observed with deionized water. We delivered Ach
and SNP onto the volar surface of the forearm after clean-
ing the site with distilled water. The iontophoresis cham-
ber (Perimed) was modified with an internal diameter of 6
mm suitable for a wire electrode positioned around its
inner surface. This chamber was fixed to the skin with
adhesive tape and filled with 1 mL of solution. For the Ach
delivery, the positive lead of a current source was con-
ected to the iontophoresis electrode and the negative lead
was attached to a conductive hydrogel pad on the subject’s wrist, which serves as a reference. For the SNP delivery, the procedure was opposite to that for Ach.

When an electrical potential difference is established, ions of the drugs migrate across the skin, and the dose delivered is therefore a product of the magnitude and duration of the current. We used a current of 100 μA, which, in our experience, is not high enough to cause nonspecific electrical effects with the electrode. The dose delivered was defined by the duration of current and therefore equivalent to the delivery of a particular electrical charge (current × time).

We measured cutaneous microvascular perfusion at the delivery site using laser Doppler imaging with a standard probe PF413 (Periflux4001, Perimed). A laser scans the surface of the skin and light, back-scattered from moving erythrocytes, is shifted in frequency by an amount proportional to their velocity, according to the Doppler principle. These Doppler shifts are collected with a computer and automatically processed (PCL-818HG; Advantech Ltd., Taipei, Taiwan). For each scan, the computer builds up a color-coded image representing skin perfusion in two dimensions. The relative measure of volume flow is called the laser Doppler flux, and measurement of cutaneous microcirculation is expressed in arbitrary perfusion units (PU).

Before administering the drug, we recorded baseline images for 60 sec. The Ach at a dose of 0.1% was iontophoretically delivered into the right forearm using 100 μA current lasting for 60 sec, and images were then recorded for 5 min. The SNP at a dose of 0.1% was iontophoretically delivered into the left forearm using 100 μA current lasting for 60 sec, and images were then recorded for 5 min. The peak blood perfusion is expressed as arbitrary PU.

### Statistical Analysis

Results were expressed as means ± SD. Data were analyzed with the unpaired Student t-test between the two means. Single linear regression was used to correlate the association between the arterial elasticity indices and endothelium dependent or independent vasodilation.

### Results

Baseline characteristics for both the young and elderly normal subjects are shown in Table 1. Apart from age, there was no significant difference in other parameters according to enrollment criteria. As shown in Fig. 1, C1 large artery and C2 small artery elasticity indices were significantly reduced in the elderly group compared with the young group (P < .001). Cutaneous basal blood flow was similar between the young and the elderly groups. Peak blood flow induced by Ach was significantly reduced in the elderly group compared with the young group (P < .05), but peak blood flow induced by SNP was similar in the two groups (P = NS; Fig. 2). Figure 3 shows the association between cutaneous microvessel peak blood flow induced by Ach and SNP and both C1 large artery (panels A and B) and C2 small artery (panels C and D) elasticity indices. There was a significant linear regression relationship between Ach-induced peak blood flow and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young Group (n = 12)</th>
<th>Elderly Group (n = 12)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>24.25 ± 3.52</td>
<td>64.75 ± 3.62*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.30 ± 0.32</td>
<td>23.50 ± 0.38</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>106.32 ± 12.18</td>
<td>110.83 ± 10.70</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>69.42 ± 6.19</td>
<td>72.50 ± 7.14</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>81.58 ± 7.49</td>
<td>85.58 ± 8.01</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>65.20 ± 5.50</td>
<td>62.41 ± 6.30</td>
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</table>

* P < .01.

BMI = body mass index; DBP = diastolic blood pressure; HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure.

**FIG. 1.** Difference in C1 large artery and C2 small artery elasticity indices between young and elderly subject groups. *P < .001.
both C1 large artery and C2 small artery elasticity indices. There is no linear regression relationship between SNP-induced peak blood flow and both C1 large artery and C2 small artery elasticity indices.

**Discussion**

The major findings of the present studies are that, in humans with advancing age, the large and small artery elasticity indices are both reduced, and, in parallel, the endothelium dependent cutaneous peak blood flow is also diminished. There was a significant linear regression relationship between endothelium dependent vasodilation and arterial elasticity. The data reported here suggest that endothelial function is progressively impaired with aging and that reduced arterial elasticity is, at least in part, related to endothelial dysfunction.

The mechanism underlying the reduced arterial elasticity with advancing age is not completely clear. McVeigh et al hypothesized that impaired endothelial NO production could be related to arterial compliance reduction with aging. Accumulating evidence demonstrates that aging impairs endothelial function. Experimental data indicate that, independent of the presence of other, pathologic conditions, aging alters endothelium dependent relaxation in both the peripheral and small resistance arteries in rats.\textsuperscript{13–15} Taddei et al\textsuperscript{6} used strain gauge plethysmography to evaluate age-related forearm blood flow modification in humans induced by intrabrachial infusion of acetylcholine and sodium nitroprusside. They found that acetylcholine-induced vasodilation was reduced with aging but that vasodilation to sodium nitroprusside was similar, suggesting that aging as an independent factor impairs endothelial function in humans.

More recently, McVeigh et al\textsuperscript{10} investigated the association between NO production and arterial compliance measured by arterial waveform analysis. A total of 15 healthy subjects were infused with N\textsuperscript{\text{-}6}-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, and then administered L-arginine or D-arginine. They found that arterial compliance decreased when L-NAME and L-arginine were infused, but that L-arginine restored arterial compliance to pretreatment levels. These investigators concluded that endothelium derived NO production is involved in the modulation of arterial compliance as identified by arterial waveform analysis.

Iontophoretic laser Doppler blood flow measurement is a method for assessing microvascular endothelial function by noninvasive means.\textsuperscript{9} In endothelial dysfunction conditions such as essential hypertension, it has been demonstrated that cutaneous blood flow increase induced by Ach is significantly reduced,\textsuperscript{16} indicating that laser Doppler blood flow measurement can reflect the alteration of endothelial function. To investigate further the potential association between endothelial function and arterial compliance in humans with advancing age, we, in parallel, studied the arterial elasticity and cutaneous blood flow by using arterial waveform analysis and iontophoretic laser Doppler blood flow measurement. The present studies demonstrated that arterial elasticity is decreased with aging and that the vasodilating response to acetylcholine, an endothelium dependent relaxing agent, is also reduced with advancing age, whereas the vasodilating response to sodium nitroprusside, an endothelium independent vasodilator, was not affected by aging. There was a significant linear regression relationship between endothelium dependent vasodilation and arterial elasticity. However, there was no linear regression relationship between endothelium independent vasodilation and arterial elasticity. Our data, taken together with previous observations, confirm that advancing age impairs endothelial function and that reduced arterial elasticity as determined by pulse waveform analysis may be used as an index of endothelial dysfunction in humans with aging.

The age-dependent reduction of arterial elasticity could reflect either functional or structural alterations in the artery wall. Endothelial dysfunction may result in an increase in tone of the small arteries that would reduce oscillatory compliance or C2 elasticity index. In the large arteries, however, endothelial dysfunction may more likely facilitate the development of structural abnormali-
ties that reduce large artery compliance or C1 elasticity index.

Concerning the mechanisms responsible for the age-related impairment of endothelial function, several possibilities have been proposed. First, advancing age is associated with derangement of endothelial cells leading to a decrease in NO production. Second, aging is also accompanied by increased superoxide anion production with subsequently increased inactivation of NO, which is involved in the endothelial dysfunction with aging. Third, age dependent impairment of endothelial function is related to enhanced cyclooxygenase dependent, endothelium derived constricting factor. The exact mechanism remains to be studied further.

In summary, the present study provides data by using arterial waveform analysis and iontophoretic laser Doppler blood flow measurement to demonstrate that advancing age impairs arterial elasticity and endothelial function, independent of other, pathologic factors. The study also indicates the positive association between arterial elasticity and endothelial dysfunction. Thus, it is suggested that reduced arterial elasticity may be used as a noninvasive surrogate for the clinical evaluation of endothelial dysfunction.

References