A novel apparatus for the multifaceted evaluation of arterial function through transmural pressure manipulation

Abbreviated title: Artery function measured using external pressure change

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Abstract

A novel apparatus for the multifaceted evaluation of artery function was developed. It measures endothelial and smooth muscle functions and the pressure–strain elastic modulus (E_p). A rigid airtight chamber with an ultrasound probe was attached to the upper arm to manipulate the transmural pressure (Tp) of the brachial artery. Endothelial function was measured via a standard flow-mediated dilation (FMD) protocol. Smooth muscle function was evaluated via a myogenic contraction of the artery following the application of negative pressure to the chamber and was named pressure-mediated contraction (PMC). E_p was obtained by measuring the instantaneous increase in the artery diameter following the negative pressure application. The PMC and FMD values had a significant negative correlation with age, indicating that the age-related decrease in FMD is caused by the decay of endothelial and smooth muscle function. A consideration of PMC may help improve the accuracy of artery function measurement. E_p in subjects aged >40 years was found to be significantly higher in the supra-physiological pressure range than in the physiological one (P = 0.02); this did not occur in younger subjects. Artery stiffening may begin in the supra-physiological range, and this stiffness may also be used for the diagnosis of atherosclerosis.

Keywords: Atherosclerosis, Flow-mediated dilation, Endothelial cell, Smooth muscle cell, Bayliss effect

INTRODUCTION

In association with the economic growth of developing countries and aging in developed countries, the rate of cardiovascular disease (CVD) is increasing rapidly[24]. Atherosclerosis, one of the most well-known types of CVD, is a major cause of myocardial infarction and stroke. The progression of atherosclerosis begins from endothelial dysfunction caused mainly by hypertension and hypercholesterolemia, by which low-density lipoprotein (LDL) in blood plasma invades the sub-endothelial space and becomes oxidized LDL. Macrophages then ingest this oxidized LDL, become foam cells, and accumulate in the sub-endothelial space, causing intimal hyperplasia and atheromatous plaques. Subsequently, the arterial wall shows irreversible stiffening because of fibrosis and calcification of the intima and consequently becomes stenotic and/or occluded. Because these processes progress without subjective symptoms, detection of the early stage of atherosclerosis is not easy, and once a subjective symptom has developed, the disease is already at an advanced stage and it is often too late to restore the artery wall to a healthy condition. On the other hand, if atherosclerosis stays at an early stage, the health of the artery can be recovered by a change in lifestyle. Thus, the diagnosing atherosclerosis at an early stage is very important.

Therefore, the flow-mediated dilation (FMD) test is widely used to evaluate vascular endothelial cell (EC) function[4, 7]. In this test, the brachial artery is initially occluded for 5 min and then reperfused to measure its dilation. An increase in shear stress following reperfusion stimulates EC production of nitric oxide (NO), a relaxant of smooth muscle cells (SMCs) in the medium[6], thus causing arterial diameter to increase. The amount of dilation is used as an index of the health of ECs. It has been reported that the FMD of patients at a high risk of atherosclerosis, such as those with diabetes and hyperlipidemia and those who smoke, is significantly lower than that of healthy controls[7, 15, 17, 22]. However, it has been highlighted that FMD values tend to vary widely, which sometimes makes an accurate diagnosis difficult[5, 20, 28].

We speculated that such variation in FMD measurement is caused at least in part by a change in smooth muscle contractility. FMD is decreased not only by the decrease in NO production by ECs but also by the decrease in the vasomotor activity of SMCs[1]. We thus need to evaluate FMD while paying attention to vasomotor activity. Vasomotor activity has been evaluated using smooth muscle relaxation after the administration of nitroglycerin (endothelium-independent vasodilation, EIVD) [6, 21]. Many studies have reported that EIVD is stable compared with FMD, and a change in FMD is attributable to a change in EC function. However, this difference between EIVD and FMD might be caused by the difference in the stimulation used to measure their function; in other words, although endothelial function is measured as a response to mechanical stimulation (fluid shear stress), smooth muscle function is measured as a response to pharmaceutical stimulation (nitroglycerin), which

might be more potent than mechanical stimulation. We thus need to employ a new method that can evaluate SMC function as a response to mechanical stimulation to improve the precision of FMD measurement, i.e., to detect atherosclerosis at an early stage.

Artery stiffness is also an important factor for diagnosing atherosclerosis. It has been reported that atherosclerotic arteries do not become stiff until calcification occurs[14, 23]. However, we recently reanalyzed previous data obtained by one of us[14] and observed that the stiffness of the rabbit thoracic aorta increased in the early stage of atherosclerosis in high-pressure ranges (Fig. A1 in the supplement). Similar results have been obtained in human aortas[26], in which compliance in a high-pressure range (>150 mmHg) decreased monotonously with the progression of the disease, whereas that in the physiological pressure range (80–120 mmHg) did not change significantly at an early stage of atherosclerosis and then decreased with the progression of the disease. Thus, the early stage of atherosclerosis might be evaluated by measuring artery stiffness in a high-pressure range.

In this study, we have thus established a new method in which the transmural pressure (T_p) of the brachial artery is manipulated to evaluate the vasomotor activity of the SMCs and to determine the pressure-strain elastic modulus (E_p) of the artery in a high-pressure range. A rigid airtight chamber is attached to the upper arm to manipulate the T_p of the brachial artery by changing the pressure in the chamber. As the hydrostatic pressure around the brachial artery (P_u) is expected to be equal to the chamber pressure (P_o), the T_p of the brachial artery increases by an amount equal to the negative pressure loaded into the chamber, i.e., $T_P = P_a - P_o$ (Fig. 1). The diameter of the artery is measured with an ultrasound (US) probe during T_p manipulation at the end-diastole. Smooth muscle function is evaluated by its myogenic contraction, the Bayliss effect[3], i.e., the spontaneous contraction of the artery following its stepwise passive dilation caused by the stepwise application of negative pressure to the chamber. We call this response a pressure-mediated contraction (PMC). Furthermore, the E_p of the artery is obtained by measuring the diameter change immediately after the stepwise application of negative pressure inside the chamber. By integrating these functions with conventional FMD measurement, we have developed a novel apparatus for the multifaceted evaluation of artery function.

MATERIALS AND METHODS

Multifaceted Evaluation System for Artery Function

System configuration

The multifaceted evaluation system is mainly composed of a rigid airtight chamber to manipulate T_p of the brachial artery, a pressure control unit to manipulate the chamber pressure, and a US unit to measure the arterial diameter (Fig. 2). The airtight chamber is equipped with a US probe and is attached to the upper arm. The position and angle of the

US probe are controlled by a five-axis actuator during pressure loading and unloading. Gaps between the chamber and the arm are sealed with rubber tubes attached inside and outside of the chamber (Fig. A2). To accommodate patients with different upper arm diameters, we provided six sizes of sealing flanges with rubber tubes having diameters of 65, 80, 85, 90, 95 and 100 mm to fit upper arms with diameters in the range of 65–100 mm. A valve attached to the rubber tube inside the chamber opens when negative pressure is applied to the chamber and closes when positive pressure is applied to it. When negative pressure is applied to the chamber, the rubber tubes attached outside the chamber adhere firmly to the upper arm and seal the gaps (Fig. A2 A). When positive pressure is applied to the chamber, the arm is pressurized by that pressure via the rubber tube (Fig. A2 B).

The pressure control unit is composed of a compressor and a vacuum pump, electro-pneumatic regulators for adjusting negative and positive pressures, an electronic pressure gauge to validate pressure inside chamber, reservoirs to stabilize the pressure, solenoid valves, and an air filter (Fig. 2). The unit is controlled by a PC included in the US unit and can apply stepwise negative or positive pressure controlled by the solenoid valves as well as temporal pressure change controlled by the electro-pneumatic regulators. The air tightness of the chamber was confirmed by monitoring the pressure inside the chamber during pressure loading, which was found to change by less than 3 mmHg in 3 min of pressure loading. The US unit is a slightly modified version of a conventional system for FMD measurement (UNEX EF-18G; UNEX Corp., Japan)[30], consisting of a US probe, its controller, an electrocardiogram (ECG) unit, and a PC. The US probe has three ultrasonic vibrator arrays arranged in the shape of the letter H and can measure two cross-sectional images of the brachial artery and a longitudinal image between the two. The system can record US images of the brachial artery in each heartbeat at the end-diastole, arterial diameter measured from the image, and pressure inside the chamber, as well as heartbeat intervals.

Measurement protocols

Endothelial function is measured with a conventional FMD protocol. Smooth muscle motility can be measured with active contraction of the artery following its passive expansion caused by the stepwise application of negative pressure to the chamber (PMC) as well as active dilation following passive contraction caused by stepwise positive pressure, namely, pressure-mediated dilation (PMD). Actually, our preliminary experiment revealed that the brachial artery shows both PMC and PMD responses (Fig. A3, ***Preliminary examination for measurement protocols*** in the supplement). However, the stepwise application of positive pressure results in vein collapse, while the artery remains patent. If this lasts for a couple of minutes, it raises capillary pressure and, finally, might cause internal hemorrhage, as observed in the tourniquet test. We thus decided to measure PMC alone. Based on a preliminary investigation, we decided to apply −50 mmHg for 120 s to induce spontaneous contraction of the artery (Fig. A4 in the supplement). We also checked the

repeatability of the PMC measurements, and found that a 10-min interval was sufficient to obtain reproducible results (see the supplement).

The mechanical properties of an artery can be evaluated from the pressure-diameter relationship of the brachial artery, which can be obtained by changing the chamber pressure gradually from negative to positive. In reality, however, active contraction of the artery during pressurization was frequently observed, possibly because of the Bayliss effect[3] (Fig. A6, ***Trial to measure pressure-diameter relationship*** in the supplement). We thus decided to measure the E_p [25] from the change in diameter immediately after the stepwise application of negative pressure.

Multifaceted Evaluation of Artery Function

Subjects

Sixty-one healthy volunteers aged 21–67 years (44 men and 17 women) were recruited after obtaining written informed consent from them. This study was approved by the Nagoya Institute of Technology Human Research Committee (Approval No. 23-004). Paying attention to FMD measurement guidelines[6], subjects were instructed to fast and refrain from consuming caffeine for at least 3 h before the measurement and to avoid physical exercise for a day prior to the testing.

Measurement of FMD, PMC, and E_p

Each subject lay in a supine position, and the rigid airtight chamber was attached to the right upper arm. US gel was applied between the US probe and the skin. The position of the US probe in the chamber was adjusted with the five-axis actuator to obtain clear US images of the brachial artery. A cuff was attached to the left upper arm to measure blood pressure. Clip-type electrodes were attached to the left wrist and the right ankle for ECG measurement. The subject remained at rest for at least 10 min. Blood pressure was measured during this resting period. The conventional FMD test was then performed with UNEX EF-18G following the standard protocol to assess endothelial function[30], while the airtight chamber was exposed to the atmosphere (Fig. 3A). Briefly, a cuff was wrapped around the forearm and was pressurized to systolic pressure plus 50 mmHg for 5 min to occlude the arteries in the forearm. The diameter of the brachial artery was then measured for 3 min after reperfusion. The %FMD was defined as follows:

$$\%\mathrm{FMD} = \frac{D_2 - D_1}{D_1} \times 100 \ (\%) \tag{1}$$

where D_1 is the base diameter, i.e., the mean diameter during five cardiac cycles just before cuff occlusion, and D_2 is the peak diameter after reperfusion.

PMC measurement was then performed following a resting period (>10 min) to minimize the effect of the previous measurement. We confirmed in a preliminary study that 10 min is sufficient to obtain reproducible results (See ***Preliminary examination for***

measurement protocols in the supplement for details). The change in artery diameter was measured following the stepwise application of negative pressure of −50 mmHg for 2 min (Fig. 4A). The %PMC was defined as follows:

$$\%\mathrm{PMC} = \frac{D_3 - D_4}{D_3} \times 100\ (\%) \tag{2}$$

where D_3 is the peak diameter just after loading of the negative pressure and D_4 is the minimum diameter during the loading of the negative pressure.

As an index of the mechanical properties of the artery, the E_p was calculated from the increase in the brachial artery diameter (ΔD) in response to the stepwise increase in T_p (ΔP):

$$E\mathrm{p} = \frac{\Delta P}{(\Delta D/D_0)} \tag{3}$$

where D_0 is the diameter at diastole before pressure loading. To compare the mechanical properties in the normal- and high-pressure ranges, E_p was obtained for two conditions: ΔP = 50 and 100 mmHg, denoted as $E_{p\Delta 50}$ and $E_{p\Delta 100}$, respectively. $E_{p\Delta 50}$ was calculated from the data obtained during the PMC measurement, while $E_{p\Delta 100}$ was measured separately after the PMC measurement by applying a negative pressure of −100 mmHg for 10 s. A resting period of at least 10 min was provided between the measurements. Because our preliminary study indicated that E_p does not increase significantly until subjects are >40 years, we measured $E_{p\Delta 100}$ only for subjects aged >30 years.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences were analyzed using paired and unpaired Student's t-tests unless otherwise indicated and were considered significant when $p < 0.05$. Changes in the FMD, PMC, and E_p with age were evaluated using Pearson's correlation constants. The level of significant correlation was also taken at $p < 0.05$.

RESULTS

Fig. 3A shows a typical change in the brachial diameter during FMD measurement obtained with the present apparatus. The artery diameter began to increase within 30 s after the release of the forearm cuff occlusion and peaked at 40–60 s. The mean %FMD value from 61 subjects was 5.4% ± 2.0%. Fig. 3B shows the correlation between age and %FMD; a statistically significant correlation was observed for those aged >40 years ($R = -0.525$, $P = 0.005$, $n = 27$).

A typical result of PMC measurement is shown in Fig. 4A. The brachial artery expanded passively within 10 s after negative pressure loading and then shrank gradually because of active contraction of smooth muscle during negative pressure loading. The mean PMC value was 6.5% ± 3.0% (n = 61). Fig. 4B shows a summary of the average PMC response obtained

from 45 subjects. Of 61 subjects, 16 were omitted because tracking of the artery failed upon unloading. Following stepwise negative pressure loading, the diameter abruptly increased by 8% in 5 s and then gradually decreased by 7% in 90 s. Subsequently, the diameter gradually increased by 2% and then decreased in a stepwise manner by 4% in 6 s upon pressure unloading. Fig. 4C shows the correlation between age and %PMC. In subjects aged >40 years, a significant correlation was observed (R = −0.439, P = 0.022) between age and %PMC, as observed between age and %FMD.

The pressure-strain elastic moduli at the physiological pressure range, $E_{p\Delta 50}$, had a significant correlation with age (R = 0.594, P < 0.001, n = 41; Fig. 5A). The moduli at a high-pressure range, $E_{p\Delta 100}$, were sometimes difficult to obtain because the brachial artery moved too much to track it when a high negative pressure (100 mmHg) was applied. The number of values for $E_{p\Delta 100}$ was thus reduced to 21. $E_{p\Delta 50}$ and $E_{p\Delta 100}$ were 85.8 ± 82.9 kPa and 165.8 ± 98.8 kPa, respectively, for all subjects aged between 30 and 67 years. These values were similar to a reported value of 139 ± 24 kPa obtained for 10 subjects (5 men and 5 women) aged 22 ± 2 years[27]. There was no significant difference between $E_{p\Delta 50}$ and $E_{p\Delta 100}$ when comparing subjects of all ages. These two values also did not differ significantly in the subjects younger than 40 years. In contrast, $E_{p\Delta 100}$ was significantly higher than $E_{p\Delta 50}$ in the subjects aged ≥40 years, indicating that stiffening of the brachial artery occurs from the high-pressure region (Fig. 5B).

DISCUSSION

In the present study, we developed a multifaceted evaluation system for artery function, in which endothelial function was evaluated with a conventional FMD test, smooth muscle function with a novel PMC test, and mechanical properties with the E_p at various pressure levels. In this system, the patients have to insert their upper arm into a rigid airtight chamber. The position of the system's US probe is adjusted by a five-axis actuator, not by hand. Despite this inconvenience, the FMD values measured with this system were similar to previously reported values[21], and a significant negative correlation was observed between age and %FMD, as reported in the literature[10, 31]. Thus, we confirmed that we can obtain reasonable FMD values with this new system.

In the PMC measurement, the brachial artery diameter increased in a stepwise manner in response to negative pressure loading and then decreased gradually to a level slightly higher than that before negative pressure loading (Fig. 4). Thus, we successfully induced active contraction of SMCs in the brachial artery. The PMC method might be a promising approach to evaluate smooth muscle function. PMC values showed a significant negative correlation with age as FMD values did for subjects over 40. This is a very important observation because this could indicate that the decrease in FMD with aging is caused by the decrease in vasomotor activity but not the decrease in endothelial function. In fact, a study

has reported that the decrease in FMD can be explained in part by the decrease in the vasomotor activity of the smooth muscle, evaluated with sublingual nitroglycerin[1]. Thus, we believe that it is important to consider the vasomotor activity when evaluating the FMD response to improve its accuracy. As an index of vasomotor activity, we measured smooth muscle contraction (PMC). However, it would be more appropriate to take smooth muscle relaxation as a reference for FMD. Smooth muscle relaxation could be induced by applying positive pressure inside the airtight chamber, as shown in the supplement (Fig. A3). However, we did not adopt this method because it might cause internal hemorrhage from the capillaries as discussed in the "Measurement protocols" section. We are currently developing a new method to induce smooth muscle relaxation without increasing the capillary pressure by intermittently applying chamber pressure. Through the application of chopper pressure synchronized with an electrocardiogram in the chamber, the pulsation of the artery can be changed. In fact, we have confirmed that positive and negative chopper pressures cause myogenic dilatation and contraction, respectively[18]. We also found that chopper pressure application significantly reduces artery movement. Details of the chopper pressure method will shortly be reported elsewhere.

It has often been reported that FMD values tend to vary widely [5, 20, 28]. This might be caused by changes in autonomic nerve activities. It is well known that vasomotor tone changes depending on the level of mental stress. If smooth muscle contractility is increased for some reason, the arteries may easily shrink and hardly dilate when external stimulation is applied; thus, PMC may increase and FMD may decrease. As shown in Fig. A7, we observed that stressful events increased PMC and decreased FMD responses. The effects of mental stress on FMD response have been investigated by several groups[8, 12, 13, 19, 29]. Some groups reported a decrease in FMD[12, 29] in response to mental stress, while others reported no change[8, 19] or even an increase[13]. Ghiadoni *et al.*[12] found that mental stress decreased FMD, while it had no effect on EIVD (denoted as GTN in their paper) induced by 50 μg of sublingual glyceryl trinitrate; they thus concluded that mental stress may cause transient endothelial dysfunction. However, their EIVD values (~11%) were more than double the FMD values (~5%). This may indicate that the dilation caused by glyceryl trinitrate is too large to detect subtle changes caused by mental stress. Thus, the method chosen to evaluate vasomotor activity with physiological stress is important to study the reason for the wide variation of FMD. We believe that the present PMC method is promising for this purpose.

Our results on arterial stiffness showed that $E_{p\Delta 100}$ was significantly larger than $E_{p\Delta 50}$ in the older group, while these values were similar in the younger group (Fig. 5B). These results indicate that the arteries become stiffer in a high-pressure region with aging. This has also been reported by Gao *et al.*[11]. The results of Richter and Mittermayer[26] and our findings shown in Fig. A1 indicate that aortic stiffening during atherogenesis preferentially occurs in high-pressure regions. Stiffness in high-pressure regions might thus be a good index to

detect atherosclerosis. The mechanical properties of the arteries have mostly been measured in the physiological pressure range as well as in the sub-physiological pressure range in some studies[2, 9, 16], but to the best of our knowledge, no studies have yet been conducted on the supra-physiological pressure range. The present T_p manipulation method appears to be promising to determine the mechanical properties of the brachial artery in a high-pressure range.

There are several features that could have been improved in the present study. One of the most important features is that we need to measure smooth muscle relaxation (PMD), as discussed previously. Another point is the displacement of the blood vessel during pressure loading. When negative pressure is applied in the chamber, part of the arm tissue is sucked into the chamber, which sometimes causes large displacement of the brachial artery. We thus used the cross section of the artery to measure its diameter. However, it is preferable to use a longitudinal section to improve the accuracy of diameter measurement. Furthermore, the chopper pressure application method discussed previously may assist to this end. The proposed device requires an airtight chamber and a 5-axis actuator, which may be somewhat stressful for patients. Also, it may be challenging for some elderly patients to insert their arm into the chamber while in their spine position. As such, the design of the device must be modified to accommodate patients in a sitting position, similar to conventional blood pressure measurements. A comparison between PMC or PMD response and EIVD response would also be very interesting. Unfortunately, our team did not include an individual able to perform pharmaceutical intervention. We are planning to include a medical doctor in our team to conduct an EIVD experiment as a next step and to develop a new method to improve the precision of the FMD measurement through integration of the new parameters introduced in the current study.

In conclusion, we have developed a multifaceted evaluation system that is capable of measuring the endothelial function with conventional FMD and smooth muscle function and artery stiffness with T_p manipulation for the impaired arterial function. The results obtained using the developed system showed that the PMC values had a significant negative correlation with age as FMD did, indicating that the age-related decrease in FMD is caused by the decay of not only endothelial function but also smooth muscle function. A consideration of PMC as an index of vasomotor activity may help improve the accuracy of artery function measurements. Additionally, artery stiffness (E_p) was significantly higher in a high-pressure range than in the physiological pressure range in subjects aged >40 years but not in subjects <40 years. This indicates that artery stiffening begins in the supra-physiological range, and the measurement of artery stiffness in a high-pressure range could be a sensitive index for atherosclerosis. Thus, we conclude that the present system is promising for diagnosing atherosclerosis at an early stage.

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CONFRICT OF INTEREST

The authors have no conflict of interest to declare.

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Figures

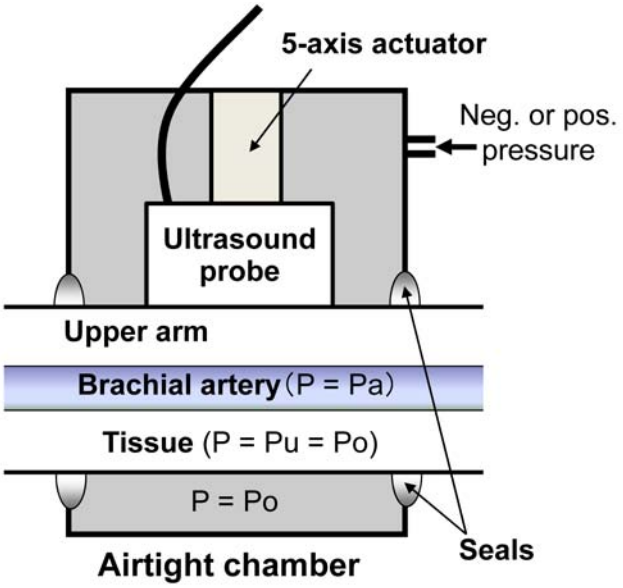


Fig. 1 Schema of the transmural pressure manipulation method and rigid airtight chamber. A rigid airtight chamber equipped with an ultrasound (US) probe is attached to the upper arm. Hydrostatic pressure around the brachial artery (*P*u) is expected to be equal to the chamber pressure (*P*o). The transmural pressure (*T*p) of the brachial artery changes inversely to the change in the chamber pressure, i.e., *T*p = *P*a–*P*o.

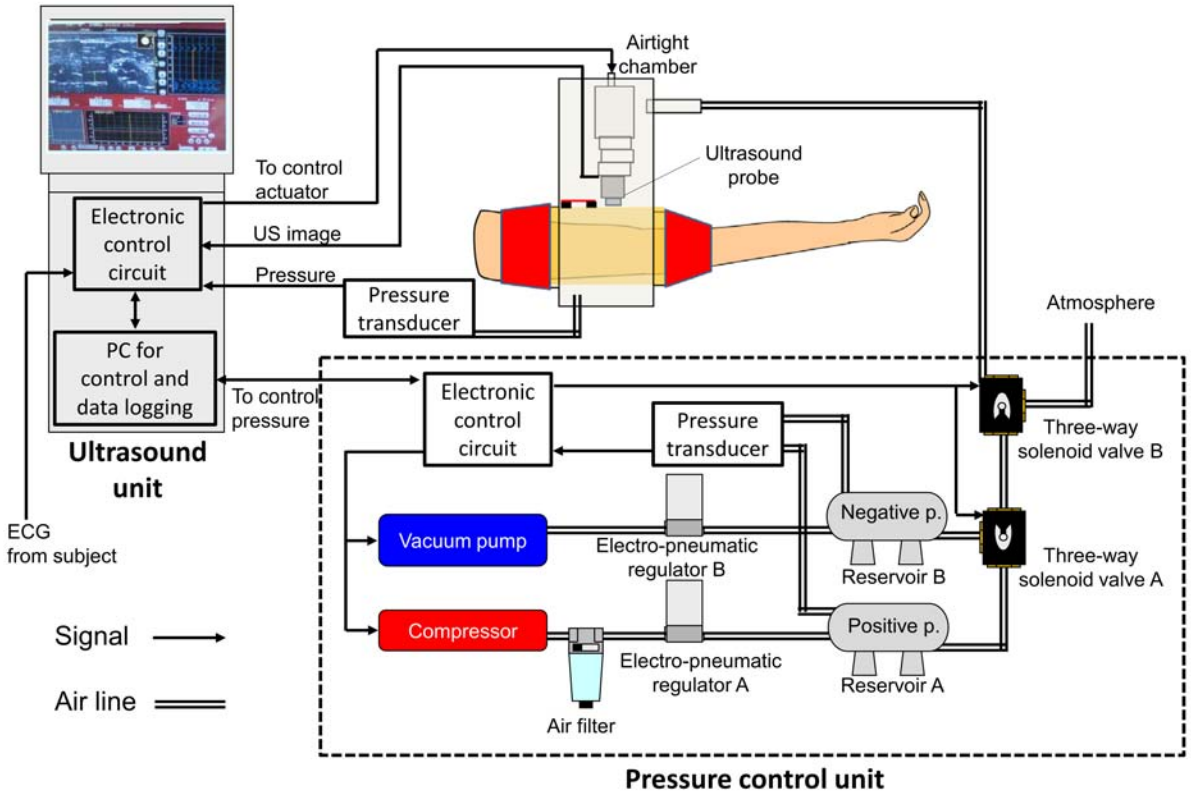
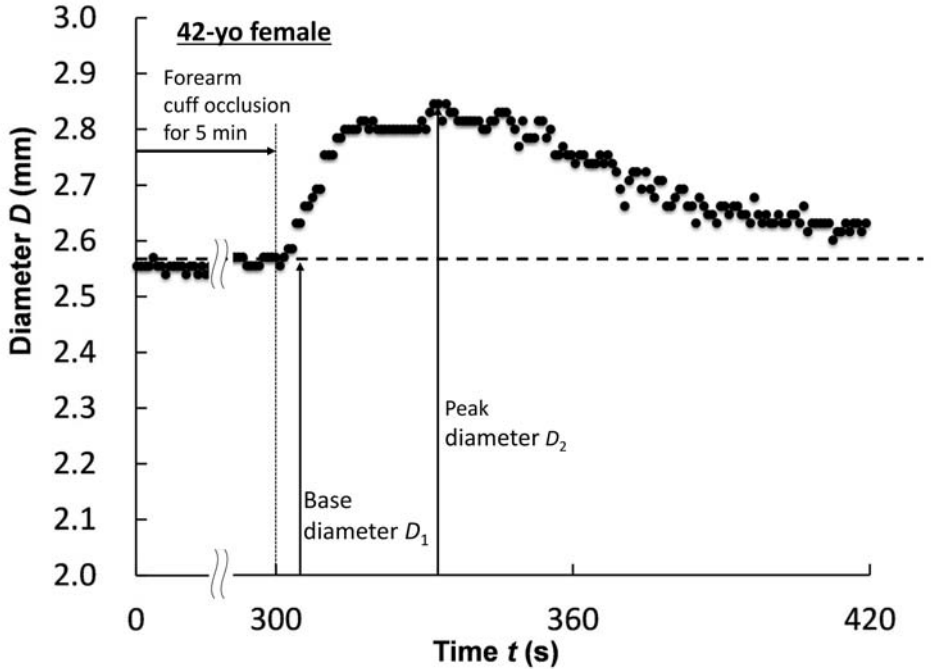
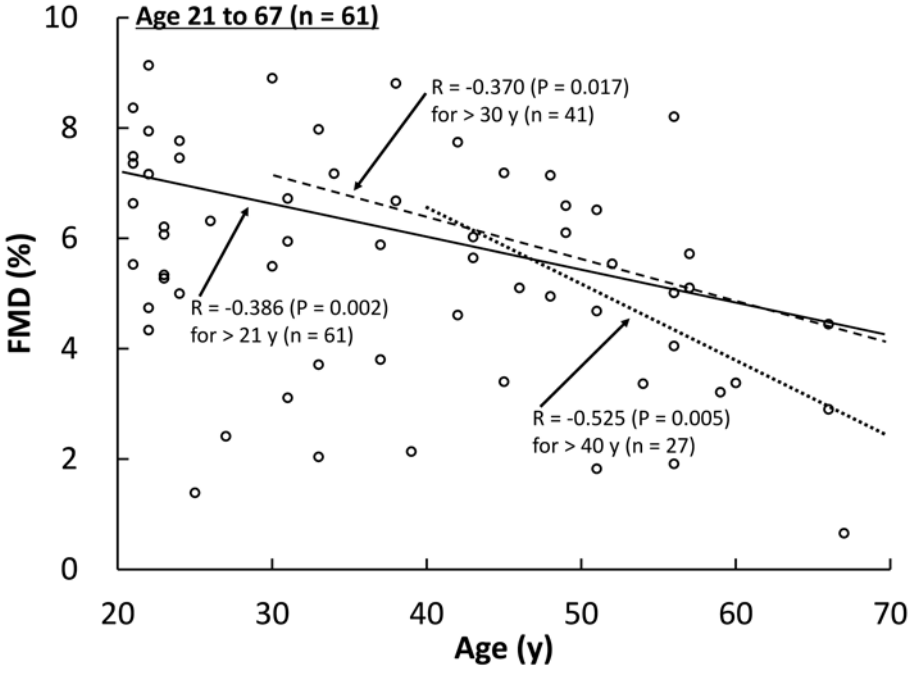


Fig. 2 The multifaceted evaluation system for artery function developed in the present study.

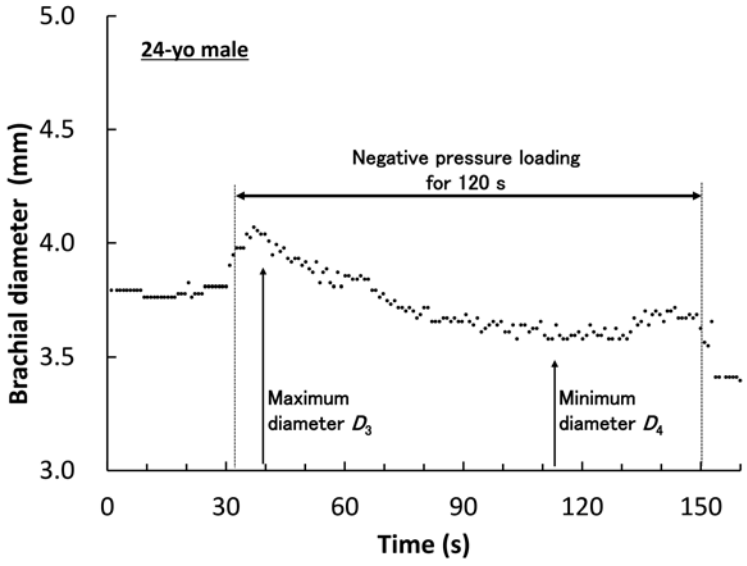


(A)

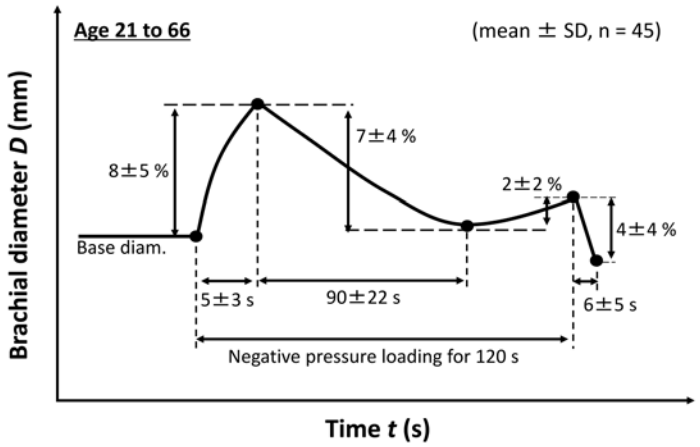


(B)

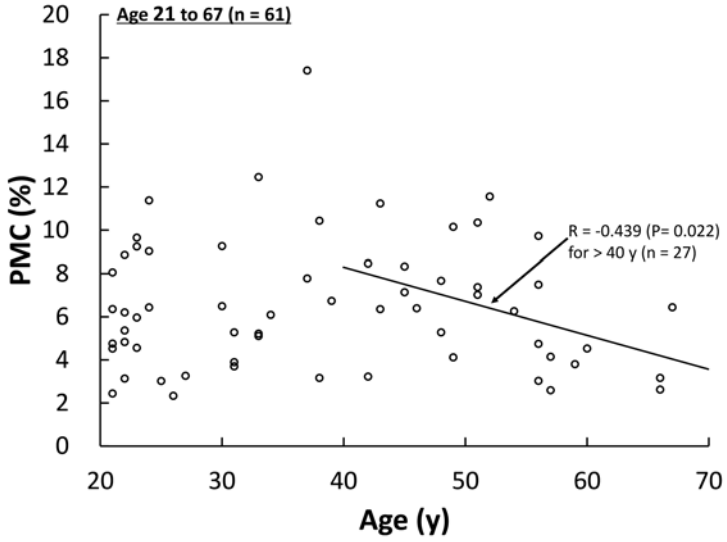
Fig. 3 A typical response of flow-mediated dilation (FMD) obtained in the present system (A) and correlation of FMD with age (B). Negative correlations were observed in all age ranges examined in the present study.



(A)

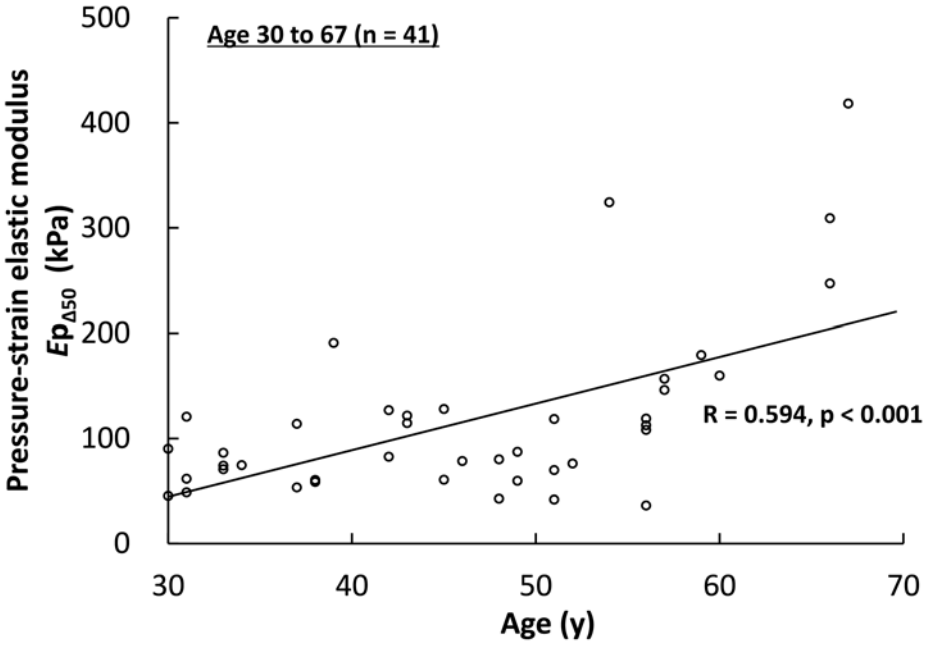


(B)

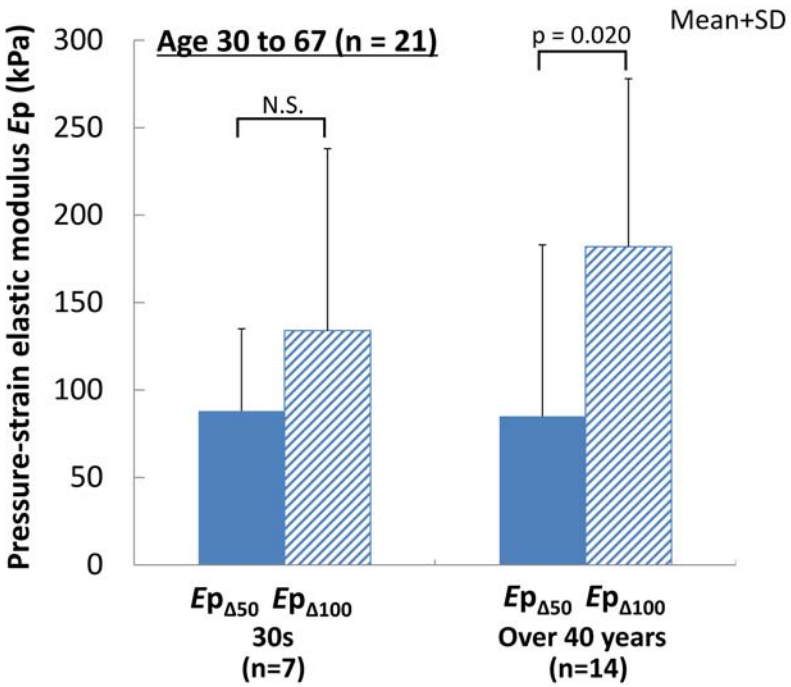


(C)

Fig. 4 A typical response of pressure-mediated contraction (PMC) obtained in the present system (A), average PMC response (B), and correlation between PMC and age (C). Negative correlation was observed with age in subjects aged >40 years.



(A)



(B)

Fig. 5 Correlation between the pressure-strain elastic modulus (E_p) obtained in a stepwise pressure increase of 50 mmHg ($E_{p\Delta 50}$) and age (A) and difference in $E_{p\Delta 50}$ and $E_{p\Delta 100}$ in subjects in their 30s and those aged >40 years (B). There was no significant difference in artery stiffness in a supra-physiological pressure range ($E_{p\Delta 100}$) and that in a supra-physiological range ($E_{p\Delta 100}$) in subjects in their 30s, while $E_{p\Delta 100}$ was significantly higher than $E_{p\Delta 100}$ in subjects aged >40 years.

******** Supplement ********

Preliminary examination for measurement protocols

We studied 11 healthy young men and women volunteers aged 21–49 years after obtaining their written informed consent. This study was approved by the Nagoya Institute of Technology Human Research Committee. The experimental conditions were the same as for the experiment described in the section on FMD and PMC measurements (Materials and Methods). An example of time course changes of brachial artery diameter in response to the application of stepwise negative and positive pressure is shown in Fig. A3. The diameter increased in a stepwise manner in response to negative pressure loading (−50 mmHg) and then decreased gradually to a diameter slightly greater than that before this loading. In response to positive pressure loading, the diameter decreased passively and then increased actively. We clearly confirmed that a myogenic response could be induced by transmural pressure manipulation.

We then determined the duration and amount of negative pressure loading, in which a stable myogenic response can be obtained with minimal load to patients. Our preliminary measurement of the brachial artery diameter in response to the stepwise application of negative pressure (−50 mmHg) indicated that the diameter became stable within 120 s in 82% of the 11 volunteers, and 120 s was taken as the loading time for PMC measurement. We then determined the value for negative pressure in six volunteers aged 21–46 years. A negative pressure of −10, −30, −50, or −70 mmHg was applied in the airtight chamber for 120 s to measure the PMC response (Fig. A4). The PMC value at −50 mmHg was significantly greater than that at −10 and −30 mmHg but was not significantly different from that at −70 mmHg. Thus, a negative pressure of −50 mmHg was taken as the loading pressure.

We finally confirmed the reproducibility of the PMC measurements and the effects of the FMD and PMC measurements on one another. For the sake of reproducibility, two sets of PMC measurements were performed at 10-min intervals for 10 volunteers aged between 21 and 45 years. There was no significant difference in the PMC values between the first (10.0 ± 2.8%, mean ± SD) and second measurements (10.7 ± 3.2%); this indicates that a 10-min interval is sufficient for obtaining reproducible results for PMC measurements. For multi-process variability, the FMD measurement was performed before the PMC measurement, and this set was repeated twice for four volunteers aged between 22 and 35 years, with less than a 5-min interval between the measurements (Fig. A5). There was no significant difference between the first and second measurements of FMD or PMC. These results indicate that the effect of the previous measurement is minimal if an interval of more than 5 min is taken between the measurements; furthermore, the effect of the order in which the measurements are taken has a negligible impact on the results.

Trial to measure pressure-diameter relationship

To determine the pressure-diameter relationship of the brachial artery, the pressure inside the airtight chamber was changed linearly with time. The chamber was first pressurized from 0 mmHg to diastolic pressure and then unloaded to 0 mmHg, after which it was depressurized from 0 to −100 mmHg and unloaded similarly. Typical pressure-diameter curves taken from four individuals on three different days are shown in Fig. A6. Pressure was changed at a rate of 3 mmHg/s. The shape of the curves was almost the same in each subject, suggesting that pressure-diameter curves can be obtained reproducibly. However, the diameter decreased despite a continuous increase in transmural pressure at some points, particularly in a high-pressure region. This may have been caused by the myogenic response of smooth muscle cells and/or an increase in vascular smooth muscle tone mediated by the nervous system. Such vascular smooth muscle contractions occur as a protective action to avoid wall vessel disruption at high pressure[A1-3]. The pressure-strain elastic modulus (E_p) is often used to evaluate the mechanical properties of arteries. This modulus is usually obtained from the slope of the pressure-diameter curve. In this study, however, smooth muscle contraction may have caused serious errors, particularly in the high-pressure region. We thus did not obtain E_p from the pressure-diameter curve but rather obtained it from the increase in diameter following the stepwise application of negative pressure.

Supplemental References

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Supplemental Figures

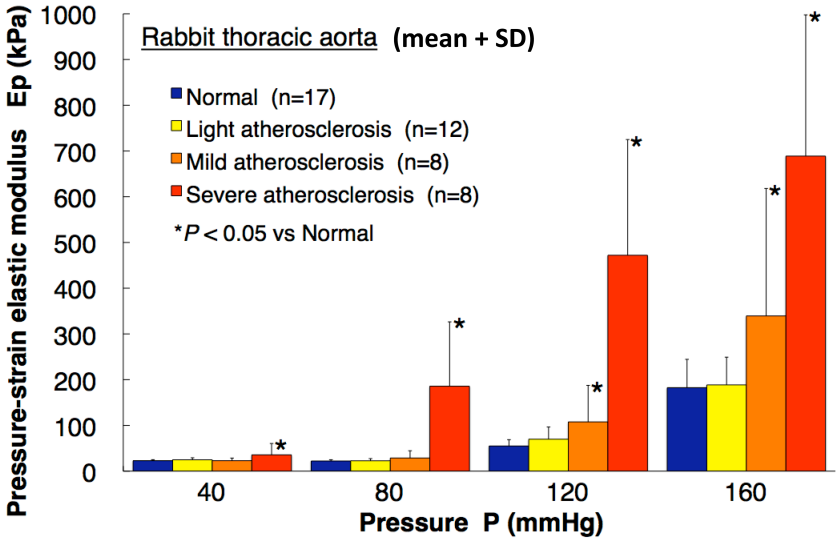

Fig. A1 Change in pressure-strain elastic modulus (E_p) during the progression of atherosclerosis. E_p in the lower pressure range (P = 40 and 80 mmHg) did not increase significantly until severe atherosclerosis, whereas it was significantly higher in the higher pressure range even in mild atherosclerosis (P = 120 and 160 mmHg). Data in the supplementary reference A4 were reanalyzed and plotted. Normal, Group A in the reference A1; light atherosclerosis, Group B0 + D0; mild atherosclerosis, Group B1 + D1; severe atherosclerosis, Group B2 + D2.

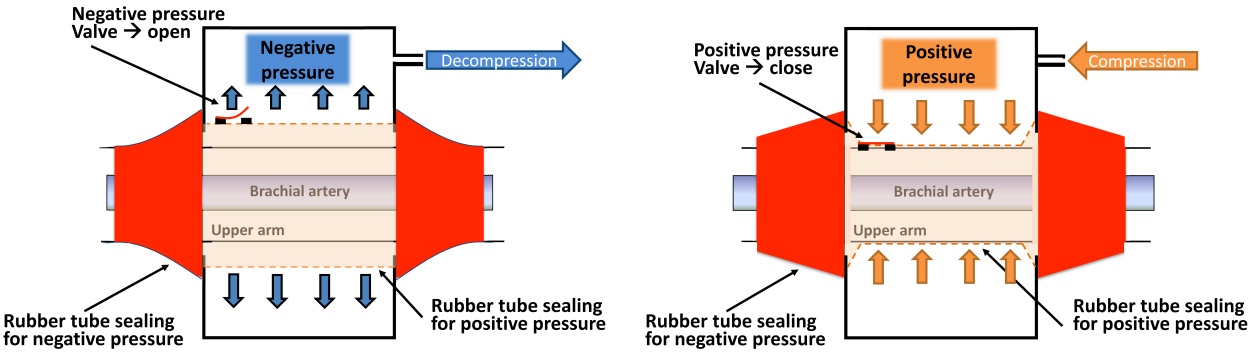

Fig. A2 Sealing mechanism for negative (left) and positive (right) pressures.

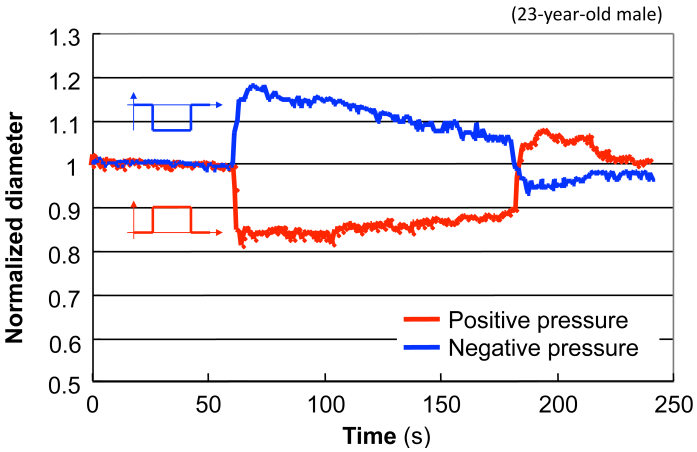

Fig. A3 An example of time course changes of brachial artery diameter in response to the application of stepwise negative or positive pressure for 120 s.

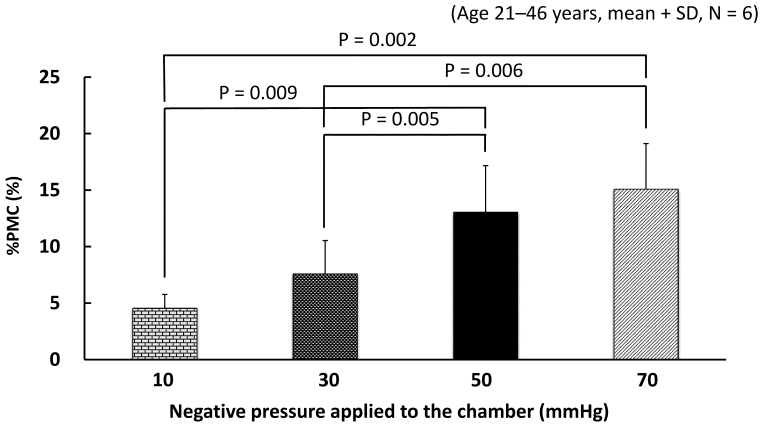

Fig. A4 Effects of the level of negative pressure on the pressure-mediated contraction of the brachial artery. The amount of contraction during the application of negative pressure for 120 s was normalized by the maximum vessel diameter just after the application of the negative pressure.

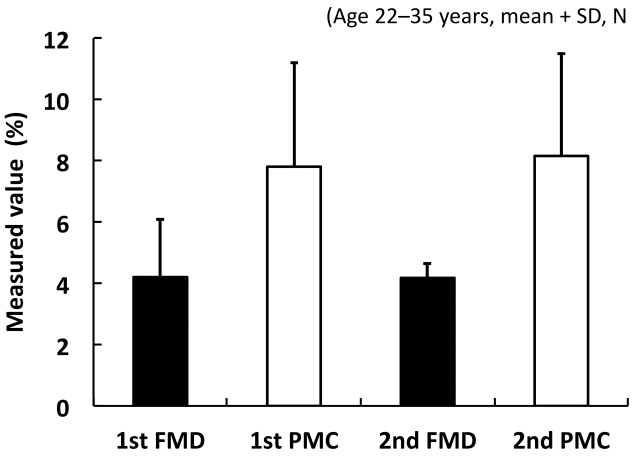

Fig. A5 Multi-process variability. Four measurements were performed at <5-min intervals. There was no significant difference between the first and second measurements of FMD or PMC. This indicates that the first PMC measurement inserted between the first and second FMD measurements has no effect on the results of the FMD measurements and vice versa (22–35-year-old males).

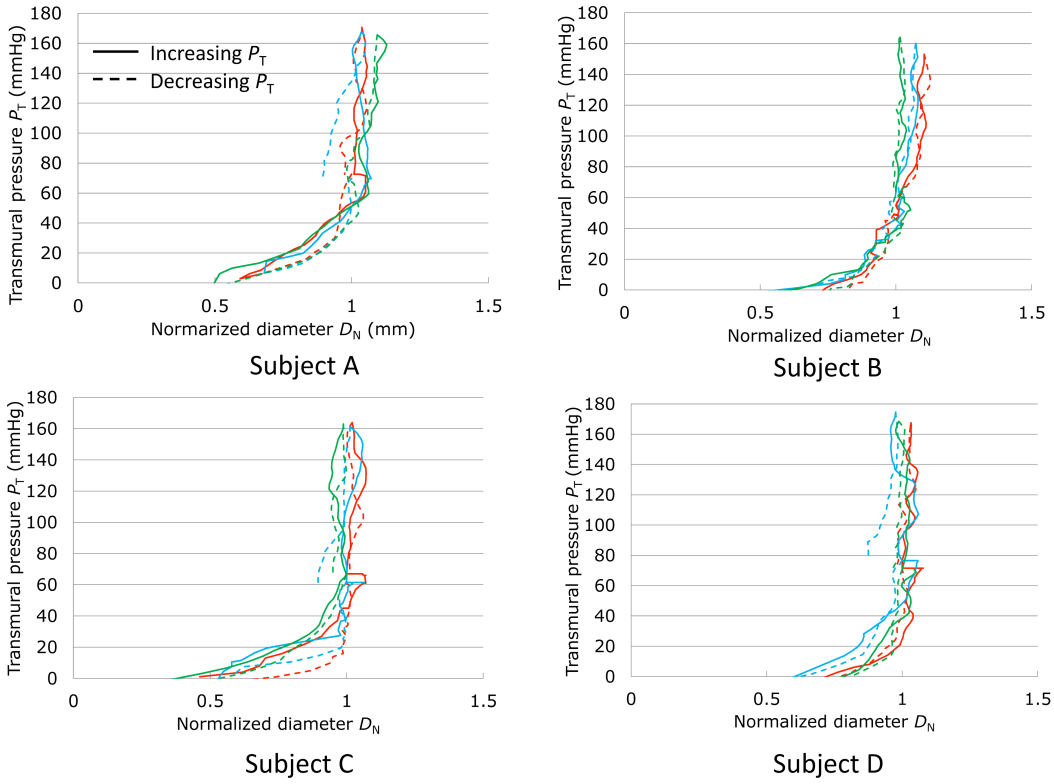

Fig. A6 Examples of pressure-diameter relationships of four individuals obtained on three different days (22–24-year-old males).

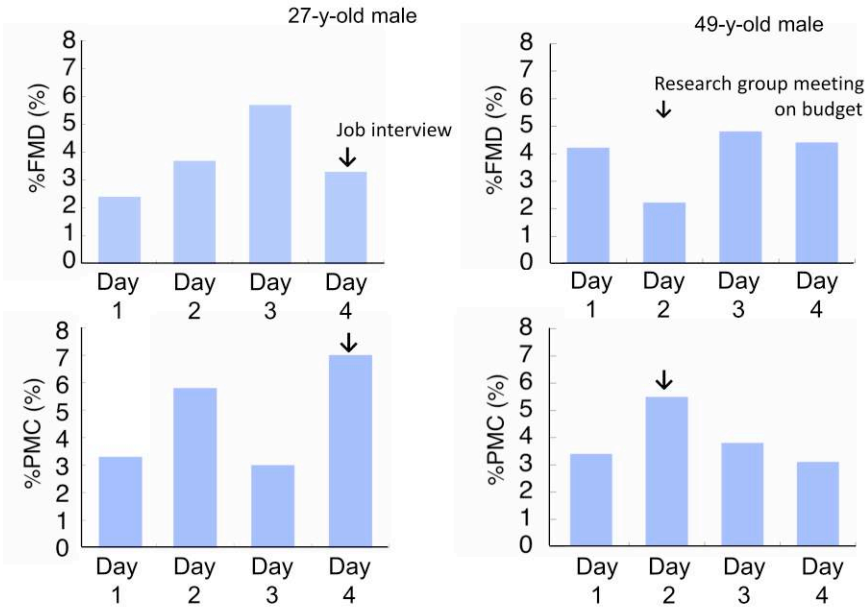

Fig. A7 Examples of the time course changes of %FMD and %PMC in healthy people. For both subjects, a decrease in FMD and an increase in PMC were observed on days with stressful events (i.e., job interview and research group meeting discussing budget). Interviews conducted with both subjects confirmed that the described events were indeed experienced as stressful.