ORIGINAL ARTICLE

To what extent does aortic pulse wave velocity estimate early atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic rabbits?

Shiho Hayashi^{1,2}, Shinju Obara², Masahiro Murakawa², Akihiro Hazama³, Masahiko Kusanagi⁴ and Shin-ichiro Katsuda³

Pulse wave velocity (PWV), an index of arterial stiffness, is dependent not only on structural alterations in arterial wall but also on distending pressure, which could be related to wall distensibility. We investigated the efficacy of aortic PWV (AoPWV) for detecting early to moderate atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. Pressure waves were recorded at the ascending, and proximal and distal abdominal aortas in 13 male normal and KHC rabbits aged 12 months under pentobarbital anesthesia when angiotensin II or sodium nitroprusside was infused intravenously. AoPWV was compared between the two rabbit groups at different mean arterial pressure (MAP) levels. Vascular diameter (D_m) was measured using an intravascular ultrasound imaging system in the proximal thoracic and proximal abdominal aortas to determine pressure-strain elastic modulus (E_p). AoPWV was correlated to MAP in the two rabbit groups ($P < 0.001$). AoPWV was slightly but significantly greater ($< 0.5 \text{ m s}^{-1}$) in the KHC rabbit group than in the normal rabbit group at MAP level $> 80 \text{ mm Hg}$ ($P < 0.01$). The value of h/D_m (h , wall thickness) was significantly high in the KHC rabbit group at any MAP level ($P < 0.001$) in these aortic regions. There was no significant difference in E_p and D_m between the two rabbit groups at any MAP level. AoPWV showed only a slight but significant increase in the young KHC rabbits at different MAP levels, which was mainly due to the increased h/D_m . AoPWV could underestimate aortic stiffness due to early to moderate atherosclerosis if AoPWV was adjusted for arterial pressure level.

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INTRODUCTION

It is well known that pulse wave velocity (PWV) can be used as a predictor of cardiovascular mortality independently of age, blood pressure and cardiac mass in patients with hypertension,^{1–3} diabetes^{3,4} and end-stage renal failure.^{5,6} PWV has been frequently employed in recent years in epidemiological studies, mass health screening, and diagnosis and assessment of prognosis of cardiovascular disorders, as it can be easily quantified with high reproducibility to estimate arterial stiffness.

PWV is dependent not only on pathohistological alterations in the arterial wall but also changes in arterial pressure (AP).⁷ Many investigators have confirmed that PWV was correlated with age,^{8–10} systolic blood pressure (SBP),^{11,12} and diastolic blood pressure (DBP).^{11,13} Katsuda *et al.*¹⁴ demonstrated previously that PWV in the entire aorta (AoPWV) in young Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits, an animal model of familial hypercholesterolemia¹⁵ showed a slight (about 10%) but significant increase compared with that in age-matched control rabbits, and that SBP and

DBP were also significantly higher in the KHC rabbit group than in the control group. Some investigators reported that brachial-ankle PWV (baPWV), closely associated with AoPWV measured invasively,^{16,17} was also useful to predict the early stage of atherosclerosis in obese young-adults¹⁸ and Type 2 diabetic patients.¹⁹ PWV can be lowered in hypertensive patients after antihypertensive therapy is performed.^{20,21} The effects of atherosclerosis on PWV may be small at relatively early to middle stages if PWV is corrected for AP. It is necessary to clarify the efficacy of AoPWV for estimating early to moderate atherosclerosis (atheromatosis) in experimental studies before the usual clinical examinations or epidemiological investigations.

In the present study, we verified experimentally to what extent AoPWV was useful to predict atheromatosis in young KHC rabbits by comparing AoPWV between KHC and age-matched control rabbit groups before and after adjustment for AP, respectively. AoPWV was analyzed with respect to distensibility of atherosclerotic aorta at different mean arterial pressure (MAP) level altered by vasoactive drugs.

¹Department of Anesthesiology, Iwaki Kyoritsu Hospital, Iwaki, Japan; ²Department of Anesthesiology, Fukushima Medical University School of Medicine, Fukushima, Japan;

³Department of Cellular and Integrative Physiology, Fukushima Medical University School of Medicine, Fukushima, Japan and ⁴Japan Laboratory Animals, Inc., Tokyo, Japan

Correspondence: Dr S Hayashi, Department of Anesthesiology, Iwaki Kyoritsu Hospital, 16 Kusehara Uchigo-Mimaya-cho, Iwaki, Fukushima 973-8402, Japan.

E-mail: s-hayashi@iwaki-kyoritsu.iwaki.fukushima.jp

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METHODS

Animals

Thirteen male KHC (3.5 ± 0.2 kg body weight, mean $\pm$ s.d.) and 13 male Japanese White rabbits (3.8 ± 0.4 kg) (Japan Laboratory Animals, Tokyo, Japan) aged 10–12 months were used in the present study. They were given a cholesterol-free commercial rabbit chow (RC-4, Oriental Yeast, Tokyo, Japan) at 100 g a day and allowed freely access of tap water in an air-conditioned room (at $24 \pm 3^\circ\text{C}$ room temperature, 50–60% relative humidity and 12L/12D light and dark cycle). The experiment was approved by the Experimental Animal Committee of Fukushima Medical University and was administered in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Surgical procedure

The surgical procedure and experimental set-up was same as those reported previously.²² The rabbits were fixed in a supine position and intubated through tracheotomy under pentobarbital anesthesia at a dose of 30 mg kg^{-1} (Nembutal, Abbott Laboratories, North Chicago, IL, USA). Additional pentobarbital sodium was administered at 5 mg kg^{-1} every 15–20 min, if necessary. Procaine chloride was applied to the incision sites to reduce pain. A catheter-tip micromanometer (3 Fr, Millar Instruments, Dallas, TX, USA) was placed in the ascending aorta. Another catheter with two micromanometers at the tip (80-mm intervals) was advanced to the abdominal aorta from the left femoral artery. One micromanometer was located at the bifurcation of the left renal artery and the other in the proximal abdominal aorta (around bifurcation of the left renal artery). Two polyethylene catheters for drug infusion were inserted from the right maxillary vein to the superior vena cava.

Pressure wave recording

Angiotensin II or sodium nitroprusside was infused in 60 s at doses of $50\text{--}100\text{ ng kg}^{-1}\text{ min}^{-1}$ and $50\text{--}100\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$, until the MAP level reached about 150 or 50 mm Hg, respectively. Pressure waves were simultaneously measured at the ascending aorta and proximal and distal abdominal aortas and applied into a computer through an analogue-to-digital converter (PowerLab System/16s, AD Instruments, Sydney, Australia) at intervals of 0.1 ms when vasoactive drugs were infused intravenously.

Measurement of vascular diameter and elastic modulus of the wall

Measurement of vascular diameter and tensile characteristics in the aorta were similar to those reported previously.²² After the recording of pulse wave, intravascular ultrasound images in response to the same vasoactive drugs were recorded using an intravascular ultrasound system (In-Vision Tsunami, Endo-Sonic, Rancho, Cordova, CA, USA) in the origin of the descending aorta (about 20 mm distal to the bifurcation of the left subclavian artery) and proximal abdominal aorta (between the bifurcation of the superior mesenteric and left renal arteries) almost at the same MAP and heart rate levels as those used for determination of PWV. Systolic (D_s) and diastolic (D_d) internal diameters for five successive pulse were measured using a computer (PC-LL9307D, NEC Personal Products, Tokyo, Japan) at MAP levels of 60, 80, 100, 120 and 140 mm Hg in the ascending aorta after the video images had been digitized through an analogue-to-digital converter (GV-MVP/RZ, I/O Data System, Kanazawa, Japan). The pressure-strain elastic modulus (E_p) was calculated as $E_p = PP / ((D_s - D_d) / D_m)$, where PP and D_m denote pulse pressure and mean diameter ($(D_s + D_d) / 2$), respectively.²³ PP in the ascending aorta and proximal abdominal aorta were used for calculation of E_p in the origin of the descending aorta and proximal abdominal aorta, respectively.

Determination of PWV

The distance between the micromanometer at the ascending aorta and the entry site of the catheter-transducer at the left femoral artery ($\Delta D_{\text{AA-fem}}$) was precisely measured *in situ* using a string after euthanizing by overdose injection of pentobarbital sodium. The distance between the two micromanometers placed at the ascending aorta and distal abdominal aortas (ΔD) was calculated as $\Delta D_{\text{AA-fem}} - 80\text{ mm}$. The rising time of the pressure waves was determined as the second peak of the second derivative of the original waves.¹⁴ AoPWV was

calculated as $\Delta D / \Delta T$, where ΔT was the difference in the rise time of pressure waves recorded at the ascending and distal abdominal aortas.

Measurement of percent lesioned area and wall thickness

The aorta was removed from its origin to the bifurcation of the common iliac arteries and xerox copied. The outline of the aorta and atheromatous plaques was carefully traced and fed into a personal computer through an image scanner. The lesioned and intact areas were displayed in black and white, respectively. The area of the plaques (A_{lesion}) and the entire inner surface of the aorta (A_{total}) were calculated using freely available software (NIH Scion Image, Frederick, MD, USA). The percent lesioned area was determined as $A_{\text{lesion}} / A_{\text{total}} \times 100$. The aorta was stored at -85°C and thawed rapidly at 37°C before measurement of wall thickness.

Circumferential strips 3 mm in width were excised at in the proximal thoracic and proximal abdominal aortas where intravascular ultrasound images had been recorded, and weighed precisely with a precision balance. The wall thickness (h) was calculated as $h = W / (1.06\pi \times D_m \times W_d)$, where 1.06 ,²⁴ W and W_d denoted the density of the wall (g cm^{-3}), sample weight (kg) and sample width (mm), respectively.

Histological sections

After weighing the samples, wall strips were fixed in 10% neutral buffered formalin solution. The strips were embedded in paraffin and sectioned at $5\text{ }\mu\text{m}$. Sections were stained with Elastica-van Gieson stain.

Statistical analysis

PWV, E_p , D_m , h and h/D_m at different MAP levels were tested by repeated measures two-way analysis of variance. If significant differences were observed, PWV, E_p , D_m , h and h/D_m at MAP levels of 60, 80, 100, 120 and 140 mm Hg were compared between normal and KHC rabbit groups using Scheffe's multi-comparison test. Correlation analysis was performed between MAP and PWV, and between MAP and E_p , D_m , h and h/D_m , respectively. Pearson's simple correlation coefficient was calculated and tested using Student's *t*-test. Significance was accepted at $P < 0.05$.

RESULTS

Pulse wave velocity before infusion of vasoactive drugs

Table 1 illustrates AoPWV, SBP, DBP and MAP before the infusion of vasoactive drugs in the KHC and control rabbit groups. AoPWV ($P < 0.001$), SBP ($P < 0.001$), DBP ($P < 0.001$) and MAP ($P < 0.001$) were significantly greater in the KHC rabbit group than in the control rabbit group.

Relationships between MAP and PWV

Figure 1 shows the correlation of AoPWV with MAP (a), SBP (b), DBP (c) and PP (d) in the normal and KHC rabbit groups. AoPWV was significantly correlated with MAP level in the normal ($r = 0.95$; $P < 0.001$) and KHC ($r = 0.95$; $P < 0.001$) rabbit groups. There were also significant positive correlations between SBP and AoPWV ($r = 0.97$; $P < 0.001$ and $r = 0.95$; $P < 0.001$, respectively for the normal and KHC rabbit groups), between DBP and AoPWV ($r = 0.96$;

Table 1 AoPWV, SBP, DBP, MAP and PP in normal and KHC rabbit groups before the infusion of vasoactive drugs

	AoPWV	SBP	DBP	MAP	PP
Normal	5.30 ± 0.24	126.7 ± 5.6	103.4 ± 5.2	114.6 ± 5.7	23.3 ± 3.9
KHC	$6.29 \pm 0.41^{***}$	$141.2 \pm 6.1^{***}$	$113.8 \pm 4.1^{***}$	$128.1 \pm 4.7^{***}$	$27.4 \pm 4.0^{**}$

Abbreviations: AoPWV, aortic pulse wave velocity; DBP, diastolic blood pressure; KHC, Kurosawa and Kusanagi-hypercholesterolemic; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

The data are mean $\pm$ s.d.

*** $P < 0.001$.

** $P < 0.01$.

Figure 1 Correlation diagrams between MAP and AoPWV (a), between SBP and AoPWV (b), between DBP and AoPWV (c) and between PP and AoPWV (d) in normal and KHC rabbit groups. The data are mean $\pm$ s.d. SBP and DBP are measured at MAP levels at 60, 80, 100, 120 and 140 mm Hg and averaged within each MAP level, respectively. PP is determined at these MAP levels. Correlation coefficient, significant difference and regression formula are as follows: (a) $r=0.95$, $P<0.001$, $y=0.027x+2.21$, for normal rabbit group. $r=0.95$, $P<0.001$, $y=0.033x+1.86$, for KHC rabbit group. (b) $r=0.97$, $P<0.001$, $y=0.028x+1.78$, for normal rabbit group. $r=0.95$, $P<0.001$, $y=0.034x+1.56$, for KHC rabbit group. (c) $r=0.96$, $P<0.001$, $y=0.028x+2.36$, for normal rabbit group. $r=0.94$, $P<0.001$, $y=0.038x+1.93$, for KHC rabbit group. (d) $r=0.11$; $P>0.05$, for normal rabbit group. $r=0.79$; $P<0.001$, $y=0.183x+1.29$, for the KHC rabbit group. MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; AoPWV, aortic pulse wave velocity.

$P<0.001$ and $r=0.94$; $P<0.001$, respectively for the normal and KHC rabbit groups), and between PP and AoPWV ($r=0.79$; $P<0.001$ for the KHC rabbit group), whereas AoPWV showed no correlation with PP in the normal rabbit group ($r=0.11$; $P>0.05$). AoPWV in the KHC rabbit group showed only a slight but significant increase at MAP levels of 80 mm Hg ($P<0.01$), 100 mm Hg ($P<0.01$), 120 mm Hg ($P<0.01$) and 140 mm Hg ($P<0.01$) when compared with that in the normal rabbit group. The difference in AoPWV between the two rabbit groups tended to increase gradually as the MAP level increased.

Relationships between MAP and E_p , h , D_m , h/D_m

Figure 2 shows the correlation between MAP and E_p (a), between MAP and h (b), between MAP and D_m (c) and between MAP and h/D_m (d) in the proximal thoracic aorta. E_p was significantly correlated with MAP ($r=0.58$; $P<0.001$ and $r=0.81$; $P<0.01$, respectively for normal and KHC rabbit groups). D_m was also significantly correlated with MAP ($r=0.73$; $P<0.001$ and $r=0.59$; $P<0.001$, respectively for normal and KHC rabbit groups). However, there were no significant differences in E_p and D_m between the two rabbit groups at any MAP level in this aortic region. There were significant negative correlation between MAP and h ($r=-0.74$; $P<0.001$ and $r=-0.30$; $P<0.001$, respectively for normal and KHC rabbit groups), between MAP and h/D_m ($r=-0.81$; $P<0.001$ and $r=-0.52$; $P<0.001$, respectively for normal and KHC rabbit groups). The value of h and h/D_m were significantly greater in the KHC rabbits group at any MAP level ($P<0.001$).

Figure 3 depicts the correlation between MAP with E_p (a), between MAP and h (b), between MAP and D_m (c) and between MAP and h/D_m (d) in the proximal abdominal aorta. The value of E_p ($r=0.74$; $P<0.001$ and $r=0.82$; $P<0.001$, respectively for normal and KHC

rabbit groups) and D_m ($r=0.69$; $P<0.001$ and $r=0.61$; $P<0.001$, respectively for normal and KHC rabbit groups) showed significant positive correlation with MAP. No significant difference in E_p and D_m was observed between the two rabbit groups at any MAP level. There was a significant negative correlation between h and MAP ($r=-0.55$; $P<0.001$ and $r=-0.27$; $P<0.05$, respectively for normal and KHC rabbit groups). The value of h/D_m also showed significant negative correlation with MAP ($r=-0.72$; $P<0.001$ and $r=-0.49$; $P<0.001$, respectively for normal and KHC rabbit groups). The values of h and h/D_m were significantly greater in the KHC rabbits group than in the normal rabbit group at any MAP levels ($P<0.01$ and $P<0.001$, respectively for h and h/D_m).

Macroscopic and microscopic findings of the aorta

Figure 4 shows examples of photocopies of the aorta in KHC rabbits aged 12 months. In the KHC rabbits atheromatous plaques were located in the ascending aorta and around bifurcations of the main branch arteries, such as the intrecostal, celiac, mesenteric and renal arteries. The extent of sclerotic lesions was considerably varied. Percent lesioned area was $54.3 \pm 20.5\%$ (mean $\pm$ s.d.). Photomicrographs of transverse histological sections in the proximal thoracic (a) and proximal abdominal (b) aortas are shown in Figure 5. Marked intimal thickening with abundant foam cells were dominant in the tested regions. Fibrous proliferation was partly observed among foam cells. The inner elastic lamina was partly injured and duplicated.

DISCUSSION

PWV has been frequently employed as a direct measure of arterial stiffness in recent years for mass physical screening, diagnosis and prognosis of cardiovascular disease. Arterial stiffness is an independent

Figure 2 Correlation diagrams between MAP and E_p (a), between MAP and h (b), between MAP and D_m (c) and between MAP and h/D_m (d) in the proximal thoracic aorta in the normal and KHC rabbit groups. The data are mean $\pm$ s.d. Correlation coefficient, significant difference and regression formula are as follows: (a) $r=0.58$, $P<0.001$, $y=7.22x-91.72$, for normal rabbit group. $r=0.81$, $P<0.01$, $y=10.21x-337.94$, for KHC rabbit group. (b) $r=-0.74$, $P<0.001$, $y=-0.001x+0.38$, for normal rabbit group. $r=-0.30$, $P<0.001$, $y=-0.003x+0.86$, for KHC rabbit group. (c) $r=0.73$, $P<0.001$, $y=0.025x+3.32$, for normal rabbit group. $r=0.59$, $P<0.001$, $y=0.029x+3.27$, in KHC rabbit group. (d) $r=-0.81$, $P<0.001$, $y=-0.0003x+0.072$, in normal rabbit group. $r=-0.52$, $P<0.001$, $y=-0.0009x+0.19$, in KHC rabbit group. E_p , pressure-strain elastic modulus; D_m , mean diameter; h , the thickness of the wall; MAP, mean arterial pressure.

Figure 3 Correlation diagrams between MAP and E_p (a), between MAP and h (b), between MAP and D_m (c) and between MAP and h/D_m (d) in the proximal abdominal aorta in the normal and KHC rabbit groups. The data are mean $\pm$ s.d. Correlation coefficient, significant difference and regression formula are as follows: (a) $r=0.74$, $P<0.001$, $y=13.31x-519.82$, for normal rabbit group. $r=0.82$, $P<0.001$, $y=13.14x-572.41$, for KHC rabbit group. (b) $r=-0.55$, $P<0.001$, $y=-0.0007x+0.29$, for normal rabbit group. $r=-0.27$, $P<0.05$, $y=-0.001x+0.40$, for KHC rabbit group. (c) $r=0.69$, $P<0.001$, $y=0.019x+2.75$, for normal rabbit group. $r=0.61$, $P<0.001$, $y=0.012x+3.01$, in KHC rabbit group. (d) $r=-0.72$, $P<0.001$, $y=-0.0003x+0.065$, in normal rabbit group. $r=-0.49$, $P<0.001$, $y=-0.0004x+0.11$, in KHC rabbit group. E_p , pressure-strain elastic modulus; D_m , mean diameter; h , the thickness of the wall; MAP, mean arterial pressure.

predictor of cardiovascular²⁵ and all-cause²⁶ mortality, which is associated with age,⁸⁻¹⁰ atherosclerosis,²⁷ hypertension,^{1,2} Type 2 diabetes⁴ and end-stage renal failure.^{5,6}

PWV can be theoretically expressed by the Moens-Korteweg equation,^{28,29} $PWV = k\sqrt{Eh/\rho D}$, where k , E , h , ρ and D are a constant, elastic modulus, wall thickness, the density of the blood and arterial

internal diameter, respectively. PWV is dependent not only on structural alterations of the arterial wall such as atherosclerosis but also on distending pressure, which could be directly related to the internal diameter of the artery or the elastic modulus of the artery wall.

In the present study, AoPWV in the KHC rabbit group showed only a slight but significant increase at MAP levels >80 mm Hg (at most <0.5 m s $^{-1}$ at each MAP level) when compared with that in the age-matched control rabbit group at the same MAP level, even though 54% of the aortic intimal surface was covered with atheromatous plaques on average. In contrast, AoPWV was significantly greater by about 1.0 m s $^{-1}$ in the KHC rabbit group than in the control rabbit group before the infusion of vasoactive drugs, indicating that the significantly increased AP level in the KHC rabbit group contributed to elevate AoPWV. Similar findings have been reported previously in KHC rabbits.¹⁴ Sclerotic lesions in young-adult KHC rabbits consisted mainly of abundant foam cells, which were thought to endow the wall with viscoelastic properties. Katsuda *et al.*³⁰ demonstrated previously that the aortic walls of KHC rabbits aged 10–12 months were less rigid in an *ex vivo* tensile study. Newman *et al.*³¹ also observed similar findings in cholesterol-fed cockerels. Though the value of ρ was not measured in the present study, Hasegawa *et al.*³² reported no significant difference in ρ between normal and heritable hyperlipidemic rabbit groups. We consider that the significantly increased h at any MAP level likely contributes to the slight but significant increase in AoPWV in the KHC rabbit group, because there were no significant

differences in E_p and D_m at any MAP level between the two rabbit groups. Appearance of significant statistical differences in AoPWV between the two rabbit groups would be partly due to the considerably smaller variation in AoPWV at any MAP level, though the difference in AoPWV between the two rabbit groups was very slight. It is also considered that effect of AP on the aortic wall is buffered by cholesterol-rich atheromatous plaques, which would be partly responsible for minimizing difference in AoPWV between the two rabbit groups at the same MAP level.

Early detection of cardiovascular risk factors, such as hyperlipidemia, obesity and high blood pressure were identified in childhood^{33–35} or adolescence^{18,36} and establishment of appropriate primary prevention programs could influence health in later life. There are pathological studies indicating that atherosclerotic lesions begin even in childhood.^{37,38} Several investigators have demonstrated that baPWV showed a significant correlation with MAP in young adults^{18,36} and with SBP and DBP in children.^{35,39} In the Bogalusa Heart Study, Li *et al.*³⁴ reported that childhood blood pressure was a predictor of arterial stiffness in adulthood. AP level is thought to contribute considerably to arterial stiffness. To what extent PWV is useful to assess relatively early atherosclerotic lesion *per se*?

Carotid intima-media thickness has frequently been measured using ultrasonography to estimate early atherosclerosis. Strong positive correlation of intima-media thickness with carotid-femoral PWV has been observed in large population cohort studies²⁷ and in patients with diabetes.^{19,40} baPWV^{16,17} was also useful to predict the early stages of atherosclerosis.^{18,19} In contrast, Nishi *et al.*⁴¹ also observed a significant but poor correlation between baPWV and intima-media thickness in subjects with diabetes and no significant relationship with baPWV in the presence of plaques. Further investigation is needed to assess usefulness of baPWV for detection of early atherosclerosis.

Hypertension has been well shown to be one of the major risk factors for cardiovascular events. Therefore, we also raise the question of whether PWV should be corrected for AP or not? Hasegawa⁴² previously established a precise nomogram of carotid-femoral PWV adjusted for DBP, age and sex in large number of healthy subjects. They used a PWV corrected for DBP at 80 mmHg using their nomogram for diagnosis and clinical follow-up of vascular disease,

Figure 4 Example of photocopies of the aorta in KHC rabbits aged 12 months.

Figure 5 Photomicrographs of transverse histological sections in the proximal thoracic (a) and proximal abdominal (b) aortas in KHC rabbits aged 12 months.

which made it possible to estimate arterial stiffness without the influence of distending pressure and to compare cardiovascular findings among subjects. The corrected PWV, however, would not be effective for assessing the rigidity of vessel walls due to atherosclerosis under conditions of hypertension. Zureik *et al.*⁴³ reported that the significant relationship between carotid intima-media thickness and carotid-femoral PWV disappeared after further adjustment for age and blood pressure, while the relationship remained significant after adjusting for gender. In recent years, PWV has been little adjusted in most countries, although a nomogram of baPWV⁴⁴ corrected for AP, age and gender has been established. Antihypertensive therapy could lower PWV.^{20,21} It can be considered that adjustment of PWV for AP is not necessarily required to assess early atherosclerosis in the sight of predicting cardiovascular risk.

In conclusion, AoPWV could underestimate alteration in aortic stiffness due to atherosclerotic plaques at the early to middle stages if AoPWV was corrected for AP. It is desirable to employ AoPWVs with and without adjusting for AP to estimate arterial stiffness precisely. We believe that the present study will offer beneficial information for assessment of early to moderate atherosclerosis using PWV for diagnosis and clinical follow-up in patients treated for hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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