Original Article

Cardiorespiratory Fitness is a Strong Predictor of the Cardio-ankle Vascular Index in Hypertensive Middle-aged and Elderly Japanese Men

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Aim: This study aimed to examine whether cardiorespiratory fitness (CRF) is associated with arterial stiffening, evaluated using the cardio-ankle vascular index (CAVI), independent of visceral fat (VF) in middle-aged and elderly Japanese men. We also examined whether the relationship between CRF and the CAVI is modified by age and/or hypertension.

Methods: The CAVI was determined in 157 Japanese men (age range, 30-79 years), including 96 hypertensive subjects (61.1%). CRF was assessed by measuring the peak oxygen uptake ($\dot{V}O_{2peak}$). The subjects were divided into low- and high-CRF groups, and the VF area was assessed using magnetic resonance imaging.

Results: The $\dot{V}O_{2peak}$ correlated with the CAVI following adjustment for age and body mass index in the middle-aged and elderly groups (all the subjects: $r = -0.285$, $p < 0.001$; middle-aged: $r = -0.240$, $p = 0.040$; elderly: $r = -0.225$, $p = 0.049$). VF also correlated with the CAVI ($r = 0.230$, $p = 0.004$). A multiple linear regression analysis revealed that age ($\beta = 0.406$, $p < 0.001$) and the $\dot{V}O_{2peak}$ ($\beta = -0.186$, $p = 0.015$) were associated with the CAVI independently of VF and the mean blood pressure. Two-way ANCOVA adjusted for age demonstrated that the hypertensive individuals had higher CAVI values than the normotensive individuals in the low-CRF group, whereas no significant differences in the CAVI were observed in the high-CRF group (p for interaction < 0.05).

Conclusions: In the present study, CRF was found to be associated with the CAVI, independent of age and VF, in hypertensive middle-aged and elderly Japanese men.

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Key words: Cardiorespiratory fitness, Visceral fat, Arterial stiffness, Hypertension, Cardio-ankle vascular index

Introduction

Aging is associated with structural and functional changes in the vessel walls that subsequently result in decreased vascular elasticity and elevated arterial stiffness^{1, 2}. Increased arterial stiffness is an independent

predictive factor of mortality from cardiovascular disease (CVD)³. Although age-related declines in the arterial function are inevitable, numerous epidemiological studies have indicated that a high level of cardiorespiratory fitness (CRF) is associated with a reduced risk of CVD^{4, 5}, which can, in part, be explained by the beneficial effects of a high CRF on arterial stiffness⁶. Moreover, a high CRF is associated with a low level of visceral fat (VF), independent of body mass index (BMI)⁷. Several studies have reported that arterial stiffness is closely associated with visceral obesity^{8, 9}, indicating that the beneficial effects of a high CRF on

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the arterial function may be mediated via a decrease in VF. Therefore, it is debatable as to whether a high CRF level *per se* contributes to improving arterial stiffening independent of a high CRF-related decrease in VF.

Several previous studies have examined whether CRF is associated with arterial stiffening independent of indicators of body fatness, with inconsistent results. For example, Christou *et al.*¹⁰⁾ demonstrated that the association between CRF and arterial stiffening is lost after controlling for body fatness in terms of BMI, body fat percentage and waist circumference in healthy men. In contrast, Boreham *et al.*¹¹⁾ indicated that CRF is independently associated with arterial stiffening after adjusting for age and skinfold thickness, a representative measure of subcutaneous fat (SF). However, these studies only measured simple anthropometric indices, and the VF-adjusted association between CRF and arterial stiffening has not been previously examined. Therefore, whether the beneficial effects of a high CRF value on arterial stiffening are independent of VF reduction remains unclear. Furthermore, most previous studies evaluated healthy young or middle-aged subjects, and trials focusing on older patients are relatively limited. Furthermore, previous analyses have reported that the age-related increase in arterial stiffness is particularly higher in older individuals with elevated blood pressure^{12, 13)}. Therefore, the relationships between the CRF and VF levels and arterial stiffening may differ according to age and the presence of hypertension.

Among a number of noninvasive measurements of arterial stiffness, the pulse wave velocity (PWV) has become widely used¹⁴⁾. However, the PWV is essentially influenced by blood pressure^{15, 16)}, and a previous study demonstrated that systolic blood pressure (SBP) is a major determinant of the brachial-ankle PWV¹⁷⁾. Therefore, it is difficult to assess arterial stiffness using the PWV in older individuals with high blood pressure. Recently, a new arterial stiffness index, the cardio-ankle vascular index (CAVI), was developed^{18, 19)}. The CAVI represents the degree of stiffness in the aorta and femoral and tibial arteries as a whole and is theoretically independent of blood pressure changes, as it is calculated based on the stiffness parameter β . Therefore, we speculate that the impact of CRF on arterial stiffness can be correctly evaluated by measuring the CAVI, even in older individuals with high blood pressure.

Aim

The aim of this study was to examine whether

CRF is associated with arterial stiffening, as evaluated according to the CAVI, independent of VF in middle-aged and elderly Japanese men. We also aimed to examine whether the relationship between CRF and CAVI is modified by aging and the presence of hypertension.

Methods

Subjects

One hundred and fifty-seven middle-aged (age range: 30-64 years, $n=77$) and elderly Japanese men (65-79 years, $n=80$) participated in this study. The subjects had no history of diabetes or coronary heart disease and were free of other chronic diseases, such as cancer, chronic kidney failure, non-alcoholic steatohepatitis and autoimmune disorders. Thirty-three subjects (21.0%) were treated with antihypertensive drugs, and 11 subjects (7.0%) received lipid-lowering drugs. All participants were instructed not to take any medications on the morning of the day of the experiment. Ninety-six subjects (61.1%) had hypertension, defined as a SBP of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg or the use of antihypertensive drugs. The current or former smoking status was recorded using a questionnaire, and daily alcohol intake was assessed using a brief self-administered diet history questionnaire²⁰⁾. All subjects provided their written informed consent prior to enrollment in the study, which was approved by the ethics committee of Waseda University. The study protocol was conducted in accordance with the Declaration of Helsinki.

Anthropometric Characteristics

The body weight and body fat percentage were measured using an electronic scale (Inner Scan BC-600, Tanita Inc., Tokyo, Japan), while height was measured using a stadiometer (YL-65, YAGAMI Inc., Nagoya, Japan). The BMI was calculated from the body weight and height measurements, and the VF and SF areas were measured on magnetic resonance imaging (MRI; Signa 1.5 T, General Electric Inc., Milwaukee, WI, United States). The imaging conditions included a T1-weighted spin-echo and axial-plane sequence with a slice thickness of 10 mm, repetition time of 140 ms and echo time of 12.3 ms²¹⁾. Cross-sectional images of the umbilical region were obtained. During the scan, the subjects were asked to hold their breath for approximately 30 seconds after inhalation to reduce respiratory motion artifacts. The MRI scans were transferred to a personal computer in the Digital Imaging and Communications in Medi-

cine file format, and the cross-sectional area of the VF at the umbilical region was determined using an image analysis software program (sliceOmatic 4.3 for Windows, TomoVision, Montreal, Quebec, Canada). In order to minimize interobserver variation, the same investigator performed all analyses, and the coefficient of variation was 0.4% for the cross-sectional areas of the umbilical region. The VF/SF ratio was calculated from the obtained measurements.

Cardiorespiratory Fitness

CRF was assessed according to a maximal graded exercise test using a cycle ergometer (Ergomic 828E, Monark, Varberg, Sweden) and quantified as the $\dot{V}O_{2peak}$. The graded cycle exercise began at a workload of 45–90 W, which was then increased by 15 W/min until the subject could no longer maintain the required pedaling frequency of 60 rpm. The subject's heart rate and rating of perceived exertion were monitored every minute during exercise, and expired gas was collected during the incremental portion of the test. The O_2 and CO_2 concentrations were subsequently measured and averaged over 30-second intervals using an automated gas analyzing system (Aeromonitor AE-300, Minato Medical Science, Tokyo, Japan). The highest $\dot{V}O_2$ value recorded during the exercise test was considered to be the $\dot{V}O_{2peak}$ (mL/kg/min). The subjects were then subsequently divided into low- and high-CRF groups according to the median $\dot{V}O_{2peak}$ value for each age group, as follows (in mL/kg/min): 41.8 for 30–39 years, 36.4 for 40–49 years, 38.9 for 50–59 years, 32.6 for 60–64 years, 30.5 for 65–69 years and 27.5 for 70–79 years.

Cardio-ankle Vascular Index

The CAVI was measured with the VaSera VS-1500 device (Fukuda Denshi, Tokyo, Japan) using blood pressure and PWV measurements while monitoring electrocardiographic changes and heart sounds^{18, 19, 22}. The PWV was calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the time between the aortic valve closing sound and the notch of the brachial pulse wave and the time between the increase in the brachial pulse wave and that of the ankle pulse wave. The CAVI was calculated using the following equation:

$$CAVI = a \times [(2\rho/\Delta P) \times \ln(P_1/P_0) \times PWV^2] + b,$$

where P_1 and P_0 are the SBP and DBP values, respectively, ΔP is $P_1 - P_0$, PWV is the pulse wave velocity between the heart and ankle, ρ is the blood density and a and b are constants. The day-to-day coefficient of variation for the CAVI was $2 \pm 1\%$ ²².

Brachial Blood Pressure

The brachial SBP and DBP values and mean blood pressure (MBP) at rest were measured using the oscillometric method (VaSera VS-1500)¹⁸. All recordings were made in duplicate with the participant in the supine position.

Collection and Analysis of Blood Samples

Blood samples were collected between 0830 and 1100 after a 12-hour overnight fast and then centrifuged at $3,000 \times g$ for 15 minutes at $4^\circ C$. The serum and plasma were stored at $-80^\circ C$ until the time of the analysis. The concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and fasting glucose were determined using standard enzymatic techniques (BML, Inc., Tokyo, Japan). In addition, the glycated hemoglobin (HbA1c) levels were determined using the latex coagulation method (BML, Inc.), and the ratio of the LDL cholesterol level to the HDL cholesterol level (LDL cholesterol/HDL cholesterol) was calculated from the above measurements.

Statistical Analysis

All statistical analyses were performed using the SPSS version 21.0 software program (SPSS Inc., Chicago, IL, United States). Several variables were log transformed to obtain a normal distribution before the analysis. Student's t -test (for normally distributed variables) and the Mann-Whitney U test (for non-normally distributed variables) or chi-squared test (for categorical variables) were used to evaluate the differences between the middle-aged and elderly groups. The associations among the variables were determined using the Pearson correlation coefficient, and a partial correlation analysis adjusted for age was performed. Moreover, we examined the correlation between the CAVI and $\dot{V}O_{2peak}$ values in each age group, and general linear models were used to assess differences in the slope and intercept of the regression line between the age groups. Furthermore, a multiple linear regression analysis was performed to determine factors that influence the CAVI. We first selected age, VF (or VF/SF), $\dot{V}O_{2peak}$ and MBP as independent variables and the CAVI as the dependent variable. We next added the interaction terms " $\dot{V}O_{2peak} \times VF$ (or $\dot{V}O_{2peak} \times VF/SF$)" and " $\dot{V}O_{2peak} \times MBP$ " to the first model. All the models were adjusted for alcohol consumption, current or former smoking, antihypertensive drug use, lipid-lowering drug use and variables that exhibited significant partial correlations with the CAVI values after adjustment for age and BMI. Multicollinearity among the variables was examined by calculating the variance inflation factor. The associations between the

Table 1. Characteristics of the subjects ($n = 157$)

	Middle-aged	Elderly	p^*
n	77	80	
<i>Anthropometric characteristics</i>			
Age (years)	52.9 $\pm$ 11.5	70.2 $\pm$ 3.9	<0.001
BMI (kg/m ²)	23.6 $\pm$ 2.3	23.5 $\pm$ 2.4	0.710
Body fat (%)	19.6 $\pm$ 4.5	21.0 $\pm$ 4.7	0.069
VF (cm ²)	99.1 $\pm$ 42.8	117.0 $\pm$ 49.1	0.016
SF (cm ²)	102.3 $\pm$ 47.6	97.9 $\pm$ 40.9	0.539
VF/SF	1.08 $\pm$ 0.61	1.33 $\pm$ 0.71	0.022
$\dot{V}O_2$ peak (mL/kg/min)	37.3 $\pm$ 6.7	29.1 $\pm$ 5.4	<0.001
<i>Vascular characteristics</i>			
SBP (mmHg)	134.7 $\pm$ 15.8	142.2 $\pm$ 14.1	0.002
DBP (mmHg)	89.1 $\pm$ 10.4	90.8 $\pm$ 9.0	0.273
MBP (mmHg)	107.4 $\pm$ 13.7	113.9 $\pm$ 12.8	0.002
CAVI	7.50 $\pm$ 0.91	8.78 $\pm$ 1.01	<0.001
<i>Biochemical characteristics</i>			
Total cholesterol (mg/dL)	209.2 $\pm$ 35.5	215.3 $\pm$ 30.5	0.248
HDL cholesterol (mg/dL)	57.0 (50.0-68.8)	62.0 (52.0-68.0)	0.307
LDL cholesterol (mg/dL)	121.4 $\pm$ 30.6	124.3 $\pm$ 27.6	0.542
LDL cholesterol/HDL cholesterol	2.11 $\pm$ 0.67	2.11 $\pm$ 0.70	0.932
Triglycerides (mg/dL)	94.5 (67.3-125.0)	83.0 (65.0-118.0)	0.361
Fasting glucose (mg/dL)	93.9 $\pm$ 9.3	98.0 $\pm$ 8.3	0.004
HbA1c (%)	4.93 $\pm$ 0.25	5.02 $\pm$ 0.28	0.048
<i>Confounders</i>			
Alcohol consumption (g/day)	17.4 (5.2-38.4)	21.7 (6.4-43.4)	0.459
Current or former smoking status (%)	42.9	52.5	0.264
Hypertension (%)	50.6	71.3	0.009
Antihypertensive drug use (%)	13.0	28.8	0.019
Lipid-lowering drug use (%)	7.8	6.3	0.763

BMI: body mass index; VF: visceral fat; SF: subcutaneous fat; VF/SF: ratio of visceral fat to subcutaneous fat; $\dot{V}O_2$ peak: peak oxygen uptake; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; CAVI: cardio-ankle vascular index; LDL cholesterol/HDL cholesterol: ratio of LDL cholesterol to HDL cholesterol; HbA1c: glycated hemoglobin. The data are presented as the mean $\pm$ SD or median (interquartile range) and were analyzed using Student's t -test (for normally distributed variables), Mann-Whitney's U test (for non-normally distributed variables) or the chi-squared test (for categorical variables). Boldface text indicates significance ($p < 0.05$). *Middle-aged vs. elderly.

fitness group, presence of hypertension and CAVI values were assessed using a two-way ANCOVA adjusted for age. A *post hoc* test with Bonferroni correction was employed to identify significant differences between mean values if a significant interaction was identified. All measurements and calculated values are presented as the mean $\pm$ SD (for normally distributed variables), median (interquartile range) (for non-normally distributed variables) or adjusted mean $\pm$ SE (for the ANCOVA results), with statistical significance set at a p value of < 0.05 .

Results

The characteristics of the subjects are presented

in **Table 1**. The elderly group had lower $\dot{V}O_2$ peak values and higher VF, VF/SF, CAVI, fasting glucose and HbA1c values than the middle-aged group ($p < 0.05$). The proportions of subjects with hypertension and a history of antihypertensive drug use were significantly higher in the elderly group than in the middle-aged group ($p < 0.05$).

Table 2 shows the correlations between the CAVI values and the other variables. The cross-sectional analysis indicated that the CAVI was positively correlated with age, VF, VF/SF, SBP, DBP, MBP, fasting glucose and HbA1c and negatively correlated with the $\dot{V}O_2$ peak ($p < 0.05$). The CAVI was also found to be significantly correlated with VF, VF/SF, $\dot{V}O_2$ peak, SBP, MBP and HbA1c following adjustment for age and

Table 2. Correlations between the CAVI and other variables

Variables	CAVI		CAVI (age- and BMI-adjusted)	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Age (years)	0.663	<0.001		
BMI (kg/m ²)	−0.100	0.214		
Body fat (%)	0.088	0.272	0.070	0.386
VF (cm ²)	0.221	0.006	0.230	0.004
SF (cm ²)	−0.098	0.224	−0.022	0.785
VF/SF	0.305	<0.001	0.210	0.009
$\dot{V}O_{2peak}$ (mL/kg/min)	−0.553	<0.001	−0.285	<0.001
SBP (mmHg)	0.369	<0.001	0.177	0.028
DBP (mmHg)	0.282	<0.001	0.132	0.102
MBP (mmHg)	0.411	<0.001	0.214	0.007
Total cholesterol (mg/dL)	0.133	0.100	−0.063	0.438
HDL cholesterol (mg/dL)	0.093	0.251	−0.124	0.126
LDL cholesterol (mg/dL)	0.077	0.339	−0.001	0.992
LDL cholesterol/HDL cholesterol	−0.008	0.920	0.059	0.465
Triglycerides (mg/dL)	0.019	0.811	0.094	0.246
Fasting glucose (mg/dL)	0.352	<0.001	0.127	0.120
HbA1c (%)	0.383	<0.001	0.224	0.006

BMI: body mass index; VF: visceral fat; SF: subcutaneous fat; VF/SF: ratio of visceral fat to subcutaneous fat; $\dot{V}O_{2peak}$: peak oxygen uptake; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; CAVI: cardio-ankle vascular index; LDL cholesterol/HDL cholesterol: ratio of LDL cholesterol to HDL cholesterol; HbA1c: glycated hemoglobin. The data are presented as Pearson's correlation coefficients. The HDL cholesterol and triglyceride levels were log transformed for the analysis. Boldface text indicates significance ($p < 0.05$).

BMI ($p < 0.05$).

In order to evaluate the influence of age on the relationship between the CAVI and CRF, we analyzed the correlation between the CAVI and $\dot{V}O_{2peak}$ in each age group (**Fig. 1**). In the middle-aged and elderly groups, the CAVI values were negatively correlated with the $\dot{V}O_{2peak}$ values ($r = -0.326$, $p = 0.003$ and $r = -0.361$, $p = 0.001$, respectively). These correlations remained significant after adjusting for age and BMI ($r = -0.240$, $p = 0.040$ and $r = -0.225$, $p = 0.049$, respectively). The slope of the regression line did not differ significantly between the age groups ($p = 0.603$), whereas the intercept did ($p < 0.001$).

In order to determine independent predictors of the CAVI, we performed a multiple linear regression analysis with the CAVI as the dependent variable (**Table 3**). In model 1, age was found to be most strongly associated with the CAVI after adjusting for the HbA1c level, alcohol consumption, current or former smoking, antihypertensive drug use and lipid-lowering drug use ($p < 0.001$). Furthermore, the $\dot{V}O_{2peak}$ was also identified to be a significant predictor of the CAVI ($p = 0.015$), whereas VF and MBP were not shown to be associated with the CAVI, inde-

pendent of age and $\dot{V}O_{2peak}$ ($p = 0.104$ and $p = 0.182$, respectively). In order to examine the synergic effects between the $\dot{V}O_{2peak}$, VF and MBP values on the CAVI, we further added the interaction terms " $\dot{V}O_{2peak} \times VF$ " and " $\dot{V}O_{2peak} \times MBP$ " to the model. Model 2 showed a significant interaction between $\dot{V}O_{2peak}$ and MBP ($p = 0.020$), whereas no such interactions were observed between $\dot{V}O_{2peak}$ and VF ($p = 0.475$). When VF/SF and the interaction term " $\dot{V}O_{2peak} \times VF/SF$ " were entered into the model instead of VF and " $\dot{V}O_{2peak} \times VF$ ", VF/SF and the interaction term " $\dot{V}O_{2peak} \times VF/SF$ " were not found to be independently associated with the CAVI, whereas the associations between the other independent variables and the CAVI did not change (age: $\beta = 0.464$, $p < 0.001$; $\dot{V}O_{2peak}$: $\beta = -0.178$, $p = 0.018$; VF/SF: $\beta = 0.068$, $p = 0.265$; MBP: $\beta = 0.089$, $p = 0.148$; $\dot{V}O_{2peak} \times VF/SF$: $\beta = -0.094$, $p = 0.125$; $\dot{V}O_{2peak} \times MBP$: $\beta = -0.133$, $p = 0.020$; model $r^2 = 0.620$).

A multiple linear regression analysis indicated a significant interaction between $\dot{V}O_{2peak}$ and MBP, suggesting that blood pressure modifies the relationship between CRF and the CAVI. We thus compared the CAVI values between the low- and high-CRF

Fig. 1. Association between the CAVI and $\dot{V}O_{2\text{peak}}$ values in the middle-aged and elderly Japanese men. Boldface text indicates significance ($p < 0.05$).

Table 3. Multiple linear regression analysis with the CAVI as the dependent variable

Independent variables	β	t	p
Model 1			
Age (years)	0.406	5.425	<0.001
$\dot{V}O_{2\text{peak}}$ (mL/kg/min)	-0.186	-2.458	0.015
VF (cm ²)	0.135	1.635	0.104
MBP (mmHg)	0.085	1.342	0.182
Model 2			
Age (years)	0.468	6.085	<0.001
$\dot{V}O_{2\text{peak}}$ (mL/kg/min)	-0.158	-2.089	0.039
VF (cm ²)	0.127	1.568	0.119
MBP (mmHg)	0.069	1.099	0.274
$\dot{V}O_{2\text{peak}} \times \text{VF}$	-0.042	-0.716	0.475
$\dot{V}O_{2\text{peak}} \times \text{MBP}$	-0.136	-2.358	0.020

CAVI: cardio-ankle vascular index; $\dot{V}O_{2\text{peak}}$: peak oxygen uptake; VF: visceral fat; MBP: mean blood pressure; β : Standardized coefficient. The model was adjusted for BMI, MBP, HbA1c, alcohol consumption, current or former smoking, antihypertensive drug use and lipid-lowering drug use. Model 1, $r^2=0.593$ ($p < 0.001$); Model 2, $r^2=0.614$ ($p < 0.001$). Boldface text indicates significance ($p < 0.05$).

groups according to the presence of hypertension. The two-way ANCOVA adjusted for age detected a significant interaction between the CRF group and the presence of hypertension on the CAVI (**Fig. 2**). In the low-CRF group, the hypertensive individuals had higher CAVI values than the normotensive individuals ($p < 0.05$), whereas no differences in the CAVI values

were observed between the hypertensive and normotensive individuals in the high-CRF group. Furthermore, although the hypertensive individuals in the high-CRF group had lower CAVI values than those in the low-CRF group, no differences in blood pressure (SBP, DBP and MBP) were noted between the CRF groups (**Table 4**). Because the CAVI values differed

Fig. 2. Associations between the CRF group, presence of hypertension and CAVI values. The data are presented as adjusted means (SE) and were analyzed using a two-way analysis of covariance adjusted for age. Boldface text indicates significance ($p < 0.05$). * $p < 0.05$ vs. low CRF among the hypertensive subjects. † $p < 0.05$ vs. normotension within the same CRF group.

between the CRF groups only among the hypertensive individuals, we additionally examined the correlation between the $\dot{V}O_{2\text{peak}}$ and CAVI values in the normotensive group. Consequently, the $\dot{V}O_{2\text{peak}}$ was found to be correlated with the CAVI in the normotensive individuals ($r = -0.430$, $p = 0.001$); however, the association was lost after adjusting for age ($r = -0.041$, $p = 0.757$).

Discussion

In the present study, we demonstrated that CRF is a significant predictive factor of the CAVI, independent of age and VF, in middle-aged and elderly men (**Table 3**). Furthermore, the subsequent analysis demonstrated that the CRF values were robustly associated with the CAVI values only in the hypertensive individuals, without affecting blood pressure (**Fig. 2**). This is the first study to show that a high CRF is associated with reduced arterial stiffness, regardless of the VF area and blood pressure, in hypertensive middle-aged and elderly men.

To our knowledge, Lindholm *et al.*²³⁾ conducted one of the few studies to investigate the relationship between CRF and the CAVI. They reported that the annual decline in maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) during a 13-year follow-up was significantly correlated with the CAVI after the follow-up period. However, the authors failed to detect a significant association

between the $\dot{V}O_{2\text{max}}$ and CAVI independent of age in their cross-sectional observations. Because the CRF level inevitably declines with age^{24, 25)}, the relationship between CRF and the CAVI may be strongly mediated by aging. Nevertheless, in the present study, the correlation between the $\dot{V}O_{2\text{peak}}$ and CAVI remained significant, even after adjustment for age (**Table 2**), suggesting that a high CRF has positive effects on the CAVI via several mechanisms, excluding aging.

Moreover, a negative relationship between CAVI and $\dot{V}O_{2\text{peak}}$ was observed among the middle-aged and elderly subjects in the present study, suggesting that a high CRF has a beneficial effect on arterial stiffening regardless of age (**Fig. 1**). However, the significant difference in the intercept of the regression line between the middle-aged and elderly groups, as shown in **Fig. 1**, indicates that the effect of aging on the CAVI certainly remains, even in subjects with high CRF levels. We should note that the age-related increase in the CAVI is not completely abolished by maintaining a high CRF level.

Previous studies have reported that the presence of hypertension accelerates the age-related increase in arterial stiffness in older individuals^{12, 13)}. We found it interesting that the CRF values were strongly associated with the CAVI values in the hypertensive individuals (**Fig. 2**). Inconsistent with our finding, Kraft *et al.*²⁶⁾ reported that arterial stiffness, as assessed using the PWV, did not differ between the high- and low-

Table 4. Associations between the CRF group, presence of hypertension and characteristics of the subjects

	Normotension		Hypertension		CRF	Hypertension	Interaction
	High CRF	Low CRF	High CRF	Low CRF	<i>p</i>	<i>p</i>	<i>p</i>
<i>n</i>	36	25	53	43			
Age (years)	56.9 ± 1.8	53.2 ± 2.2	65.6 ± 1.7	65.8 ± 1.5	0.348	<0.001	0.278
BMI (kg/m ²)	23.0 ± 0.4	23.5 ± 0.5	23.5 ± 0.4	24.1 ± 0.3	0.202	0.176	0.952
VF (cm ²)	86.8 ± 7.4	100.7 ± 9.1	109.0 ± 6.9	125.9 ± 6.1	0.036	0.004	0.838
SF (cm ²)	84.9 ± 7.5	112.8 ± 9.2*	103.1 ± 6.9	102.0 ± 6.2	0.069	0.649	0.049
VF/SF	1.09 ± 0.11	1.00 ± 0.13	1.15 ± 0.10	1.43 ± 0.09	0.360	0.040	0.077
VO ₂ peak (mL/kg/min)	36.8 ± 0.7	28.5 ± 0.8	37.8 ± 0.6	28.9 ± 0.6	<0.001	0.312	0.624
SBP (mmHg)	126.3 ± 2.0	127.3 ± 2.4	146.6 ± 1.8	145.7 ± 1.6	0.979	<0.001	0.623
DBP (mmHg)	80.3 ± 1.1	81.6 ± 1.4	94.9 ± 1.0	96.4 ± 0.9	0.190	<0.001	0.891
MBP (mmHg)	98.0 ± 1.5	100.0 ± 1.9	117.0 ± 1.4	119.3 ± 1.3	0.150	<0.001	0.921
CAVI	8.04 ± 0.14	8.02 ± 0.18	7.91 ± 0.13	8.50 ± 0.12*†	0.043	0.251	0.032

BMI: body mass index; VF: visceral fat; SF: subcutaneous fat; VF/SF: ratio of visceral fat to subcutaneous fat; VO₂peak: peak oxygen uptake; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; CAVI: cardio-ankle vascular index. The data are presented as the adjusted mean ± SE and were analyzed using a two-way analysis of covariance adjusted for age. Boldface text indicates significance ($p < 0.05$). * $p < 0.05$ vs. low CRF within the normotensive or hypertensive groups. † $p < 0.05$ vs. normotension within the same CRF group.

CRF groups of hypertensive subjects in their study ($n=61$, mean age: 57.9 ± 14.3 years). Because the blood pressure values did not differ according to the CRF levels in that study and the PWV is strongly influenced by blood pressure^{15, 16}, the true relationship between CRF and arterial stiffness in hypertensive subjects may have been overlooked. In contrast, we assessed arterial stiffness by measuring the CAVI, which is theoretically independent of blood pressure^{18, 19}. Notably, our data showed that, although the hypertensive individuals with a high CRF level had a low CAVI, the blood pressure values of these subjects did not differ from those of the hypertensive individuals with a low CRF (Table 4). This finding suggests that a low CAVI in hypertensive individuals with a high CRF is not accompanied by a decrease in blood pressure. Several epidemiological studies have demonstrated that hypertensive individuals with a high CRF exhibit a lower risk of CVD-related mortality, despite having comparable blood pressure values to those observed in low-CRF individuals^{27, 28}. Therefore, it is reasonable that a high CRF level is associated with reduced arterial stiffness independent of blood pressure. Hence, the methodological superiority of the CAVI may contribute to the identification of the true association between CRF and arterial stiffness, independent of blood pressure, in hypertensive individuals.

Although the mechanisms underlying our findings remain unknown, hypertension has been reported to induce arterial remodeling²⁹, which involves structural alternations in the arterial wall. In contrast, a high CRF has been shown to attenuate age-related

arterial remodeling³⁰. Because age- and hypertension-related structural changes in the arterial wall are closely related to increased arterial stiffness³¹, we speculate that a high CRF attenuates hypertension-related arterial stiffening in part by reducing arterial remodeling. Because the proportion of hypertensive subjects in the present study (mean age: 61.7 ± 12.1 years; hypertension: 61.1%) is comparable to that of the age-matched general Japanese male population (60–69 years: 68.0%; 70–79 years: 78.1%; National Health and Nutrition Survey Japan, 2011, <http://www.mhlw.go.jp/english/>), the maintenance of a high CRF may attenuate arterial stiffening in most middle-aged and elderly Japanese men.

In contrast to that observed in the hypertensive individuals, the association between CRF and the CAVI was lost after adjusting for age in the normotensive individuals. A previous study failed to detect an independent association between CRF and arterial stiffening in healthy individuals, as noted in the present study¹⁰. However, an age-independent significant association has also been reported^{6, 11}. Fujie *et al.*³² reported that the carotid β -stiffness values did not significantly differ according to the CRF levels in a healthy Japanese population ($n=837$, mean age: 44 ± 1 years), whereas the β -stiffness values were elevated only in the low-CRF individuals with a specific genetic component. Therefore, it is possible that the normotensive subjects with a low CRF in the present study had genetic factors conferring resistance to arterial stiffness. Due to our limited sample size, further investigations using larger cohorts are required to clar-

ify whether CRF is associated with the CAVI, even in normotensive individuals, regardless of their genetic predisposition.

Several studies have reported that, other than age, VF is the strongest predictor of the CAVI^{33, 34}; however, the VF values were not associated with the CAVI independent of age and CRF in the present study (**Table 3**). Nagayama *et al.*³³ assessed the changes in the CAVI values in obese subjects who enrolled in a weight reduction program and reported that the improvement in the CAVI was mainly explained by a decrease in VF. Ohashi *et al.*³⁴ also demonstrated that the VF area is associated with the CAVI in middle-aged healthy Japanese adults following adjustment for age. However, they did not evaluate the individual CRF levels, even though the CRF is inversely associated with the VF area and is an important determinant of arterial stiffening^{6, 7}. Given the results of the current study, the observed association between the CAVI and VF in previous studies may have been mediated by CRF. Nevertheless, as many studies reported that visceral obesity is strongly associated with arterial stiffness and the incidence of CVD^{8, 9, 35}, further investigations with a large sample size are needed to draw conclusions on this issue.

We measured the blood lipid levels to determine whether the beneficial effect of a high CRF on the CAVI is mediated by an improvement in the blood lipid profile. In our study population, however, no blood lipid parameters were associated with the age-adjusted CAVI (**Table 2**). In concordance with these results, several studies have failed to detect a significant association between the CAVI and blood lipid levels³⁶⁻³⁸. Higashiyama *et al.*³⁶ reported that the CAVI is more strongly correlated with the high-sensitivity C-reactive protein (hs-CRP) level than with the LDL cholesterol level. Furthermore, Masugata *et al.*³⁷ demonstrated that the CAVI is independently associated with the urinary 8-hydroxydeoxyguanosine (8-OHdG) level, but not any blood lipid parameters, in hypertensive patients. The hs-CRP and 8-OHdG levels are indicators of chronic inflammation and oxidative stress, respectively, and a high CRF is associated with low levels of these indicators^{39, 40}. Therefore, the association between the CAVI and CRF is possibly mediated by a decrease in chronic inflammation and oxidative stress rather than improvements in blood lipid profiles.

The present study is associated with several limitations. First, our sample size was relatively small, which may have led to a type 2 error. Second, because this was a cross-sectional study, further prospective and interventional studies are needed to elucidate

whether a high CRF significantly attenuates the age-related increase in the CAVI. Third, our study population included only men. Women are well known to generally have less VF than men⁴¹; therefore, the relationships between CRF, VF and the CAVI may differ according to sex. Further research is thus needed to confirm our findings in women.

Conclusion

In conclusion, the present study identified CRF to be a significant predictive factor of the CAVI, independent of age and VF, in hypertensive middle-aged and elderly Japanese men.

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Conflicts of Interest

The authors do not have any conflicts of interest to disclose.

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