1 ORIGINAL RESEARCH

2 Manami Watahiki et al

Comparing the heart–thigh and thigh–ankle arteries with the heart–ankle arterial segment for arterial stiffness measurements

6 Running title: Impact of segmental arterial stiffness

7 Manami Watahiki[1]

8 Shigeo Horinaka[1]

9 Toshihiko Ishimitsu[2]

10 Shigeru Toyoda[1]

11 Teruo Inoue[1]

12

13 [1]Department of Cardiovascular Medicine, Dokkyo Medical University, Mibu, Japan; [2]Department of

14 Kidney and Hypertension, Dokkyo Medical University, Mibu, Japan

15

16 Correspondence: Shigeo Horinaka

17 Department of Cardiovascular Medicine, Dokkyo Medical University, 880 Kitakobayashi, Mibu,

18 Shimotsuga, Tochigi, Japan, 321-0293

19 Email horinaka@dokkyomed.ac.jp

20

21 **Abstract:**

22 **Purpose:** The cardio-ankle vascular index, applying the stiffness parameter β theory, was

23 calculated using the pulse wave velocity and blood pressure from the aortic orifice to ankle.

24 Accordingly, the impact of the stiffness of the aorta [heart–thigh β (htBETA)] and medium-sized

25   muscular artery [thigh–ankle β (taBETA)] on the stiffness of the heart–ankle β (haBETA) was

26   investigated; further, whether the htBETA (haBETA − taBETA) improved the power of diagnosis of

27   coronary artery disease (CAD) was examined.

28   **Patients and methods:** Segmental βs were calculated using VaSela (Fukuda Denshi, Tokyo) with

29   an additional thigh cuff and compared using the receiver operating characteristic (ROC) curve

30   analysis to evaluate CAD.

31   **Results:** Overall, 90 healthy subjects and 41 patients with CAD were included. In both groups,

32   haBETA and htBETA, but not taBETA, correlated with age, and taBETA was three times higher

33   than htBETA ($p < 0.01$). Multiple regression analysis revealed that haBETA can be estimated using

34   htBETA and taBETA in healthy subjects and patients with CAD ($r = 0.86$, $r = 0.67$, respectively, $p <$

35   0.01), and two-thirds of the haBETA components can be estimated by htBETA using the component

36   analysis. The area under the ROC curve (AUC) for CAD in taBETA (0.493, p = n.s.) was smaller

37   than that in haBETA (0.731, $p < 0.01$) or htBETA (0.757, $p < 0.01$); no difference was observed in

38   AUC between haBETA and htBETA.

39   **Conclusion:** The stiffness of medium-sized muscular arteries of the age-independent thigh–ankle

40   segment (taBETA) was constant, which was three times greater than that of the elastic artery of the

41   heart–thigh artery (htBETA). Two-thirds of the haBETA components could be estimated using

42   htBETA. The ROC curve analysis revealed that the AUC of haBETA could be replaced by that of

43   htBETA, prolonging the measurement segment without affecting the diagnostic power for CAD.

44   **Keywords:** Arterial stiffness, Stiffness parameter, Cardio-ankle vascular index, Coronary artery

45   disease

Introduction

47   Carotid–femoral pulse wave velocity (cfPWV) is typically considered the most simple,

48   noninvasive, and popular method to determine arterial stiffness worldwide, as evidenced by the

49   epidemiological studies that have demonstrated its predictive value for cardiovascular events.[1,2] By

50   contrast, PWV measured outside the aortic trunk at the limb (from femoral to posterior tibial arteries)

51 has no predictive value for end-stage renal disease.[3] Therefore, cfPWV is considered the gold

52 standard for arterial stiffness measurement because most elastic arteries are located here.[4]

53 However, PWV is intrinsically pressure dependent considering that arterial compliance (dV/dP)

54 decreases with increasing pressure owing to the curvilinear relationship between arterial pressure

55 and volume and volume (V) increases with increasing pressure, thereby directly increasing PWV, as

56 shown in the Bramwell–Hill derived equation.[5]

57   For incompressible blood in a compressible elastic artery,

58 $$PWV = \sqrt{V \cdot dP/\rho \cdot dV}$$

59 where PWV is in cm/s, V (cm^3) is the volume per unit length, P (dyne/cm^2) is the pressure, and ρ

60 (g/cm^3) is the blood density.

61 Spronk et al[6] demonstrated that short-term changes in local carotid PWV—calculated based on the

62 relationship between echo-acquired cross-sectional area and tonometric blood pressure

63 measurement—that are concurrent with a decrease in blood pressure can be deemed blood

64 pressure dependent at a rate of approximately 1 m/s per 10 mmHg diastolic blood pressure. To

65 overcome this limitation, the cardio-ankle vascular index (CAVI) is used considering that it is less

66 pressure dependent and derived from stiffness parameter β,[7] which is calculated based on the

67 blood pressure and PWV[8] as follows:

68 $$\text{CAVI} = a((\ln (Ps/Pd) \cdot 2\rho/\Delta P) \cdot PWV^2) + b$$

69 where a and b are coefficients,[9] Ps (dyne/cm^2) is the systolic blood pressure, Pd is the diastolic

70 blood pressure, ΔP is Ps – Pd, ρ (g/cm^3) is the density of the blood, and PWV (cm/s) is measured

71 by the transient time from the aortic orifice to the ankle. However, the main issue associated with

72 CAVI is that it is calculated from PWV, including the measurement segment of the lower limb (from

73 femoral to posterior tibial arteries), which has no predictive value.[3] In clinical practice, CAVI has

74 widely been used as a surrogate arteriosclerosis maker,[10] and this equation includes the

75 coefficients "a" and "b" to adjust it to the value of Hasegawa's PWV, which is compensated for 80

76 mmHg of diastolic pressure.[11] Recently, Takahashi et al[9] demonstrated that CAVI can interpret

77 heart–ankle β (haBETA) in epidemiological and clinical studies; it can be considered that application

78 of CAVI without the coefficients "a" and "b" is more reasonable in various arterial segments.

79 Therefore, the segmental stiffness parameter β was applied to compare the clinical and

80 physiological implications in the present study using an additional thigh cuff. Although cfPWV can

81 be measured using a thigh cuff,[12,13] to the best of our knowledge, only one study has analyzed the

82 segmental β as heart–thigh β (htBETA) and thigh–ankle β (taBETA) separately, which was mainly

83 evaluated as an acute effect of nitroglycerin.[14]

84 The present study aimed to compare the clinical and physiological implications between the

85 stiffness of elastic aortic arteries (htBETA) and medium-sized limb muscular arteries (taBETA) and

86 to investigate the effects of htBETA and taBETA on haBETA as well as evaluate whether diagnostic

87 power for coronary artery disease (CAD) differed between haBETA and htBETA.

88 # Material and methods

89 ### *Healthy subject and patient selection*

90 Community residents and employees of companies and governments who underwent a periodic

91 health examination from April 2015 to March 2016 in Sano City, Tochigi Prefecture, Japan, were

92 included in the study. Informed consent was obtained from all participants. The study design was

93 approved by the ethics community of the Sano Medical Association Hospital, and data were

94 collected from the database of this institution. The details of data collection and definition have

95 previously been reported,[15] and data regarding current medications, including antihypertensive,

96 hypoglycemic, and hypolipidemic drugs, were collected via a questionnaire. The study protocol was

97 approved by the ethics committee of Dokkyo Medical University according to the Declaration of

98 Helsinki.

99 Patients were referred to Dokkyo Medical University Hospital from April 2015 to March 2016 to

100 undergo their first coronary angiography. All patients who underwent coronary angiography

101 presented with chest pain and exhibited at least 75% stenosis of the proximal left anterior

102   descending or right coronary artery on coronary angiography. The therapeutic goals for patients

103   with suspicious CAD for primary prevention were as follows: systolic and diastolic blood pressure of

104   <130/85 mmHg, fasting blood glucose level of <129 mg/dL, glycated hemoglobin (HbA_{1c}) level of

105   <6.9%, serum low-density lipoprotein (LDL) cholesterol level of <100 mg/dL, high-density lipoprotein

106   (HDL) cholesterol level of >40 mg/dL, and triglyceride level of <150 mg/dL. Written informed

107   consent was obtained from all patients after the study protocol approval from the Institutional

108   Review Board of Dokkyo Medical University. Participants with acute coronary syndrome, distinct

109   aortic aneurysm, arteriosclerosis obliterans (ankle–brachial index, ≤0.90[16]), and atrial fibrillation

110   were excluded.

111   According to our previous data,[17] the area under the curve (AUC) of CAVI, instead of segmental β,

112   which has not been reported in single coronary vessel disease, was 0.648; the type I error rate was

113   0.05 in the one-side test, and the type II error rate was ≤0.15 (power ≥85% power). The number of

114   healthy subjects was two times as that of patients with CAD, and with 5% attrition, it was

115   determined that 81 healthy subjects and 41 patients with CAD were required to detect for

116   equivalence or difference in diagnostic accuracy using the receiver operating characteristic (ROC)

117   curve analysis.[18] For exclusion owing to CAD complications, such as ischemic electrocardiographic

118   change in healthy subjects, a larger sample size was used to ensure the necessary number of

119   healthy subjects. Ultimately, 90 healthy subjects and 41 patients with CAD who agreed to

120   participate were included in the study.

121   *BETA measurement*

122   To measure the volume change of the femoral artery in the inguinal area, a prototype of the thigh

123   cuff that can be used for volume plethysmography was produced in collaboration with Fukuda

124   Denshi, as shown in Supplementary File 1. The local volume change was accurately reflected when

125   the cuff was wrapped around both thighs at a shorter distance of approximately 20 mm than the

126   thigh circumference in the supine position.

127 The vascular length from the aortic valve to the thigh was calculated as the total distance from the

128 second intercostal space of the parasternal position to the femoral artery in the inguinal area (Lpf) ×

129 1.3, defined as AF[19], and the measured length from the femoral artery in the inguinal area to the

130 middle of the thigh cuff (L1), ie, AF + L1. Moreover, the vascular length from the thigh to the ankle

131 was measured from the middle of the thigh cuff to the middle of the ankle cuff (L2). Thereafter, the

132 vascular length from the aortic valve to the ankle was calculated as follows: AF + L1 + L2.

133 Considering the difficulty in determining the transient time from the aortic valve to the brachial from

134 the valve opening sound, the time is determined based on the time between the aortic valve closing

135 sound (II_A) of the phonocardiogram and notch of the brachial pulse wave (dicrotic notch). Therefore,

136 the traveling time of heart–thigh (Tht), thigh–ankle (Tta), and heart–ankle (Tha) was automatically

137 calculated using the VS-1500 vascular screening system (Fukuda Denshi Co., Tokyo, Japan).

138 PWV was calculated by dividing the vascular length by the traveling time and was recorded in the

139 comma-separated value format as follows:

140 htPWV = (AF + L1)/Tht

141 taPWV = L2/Tta

142 haPWV = (AF + L1 + L2)/ Tha

143 Using the original CAVI formula[8] and removing the coefficient values of a and b,[9] segmental β was

144 calculated as follows:[14]

145 Segmental β = ln (Ps/Pd) × 2ρ/ΔP × PWV^2

146 where Ps and Pd are the systolic and diastolic blood pressures, respectively, ΔP = Ps − PD, ρ is the

147 blood density, and PWV is the value calculated for each segment.

148 Accordingly, the following were determined:

149 htBETA = ln (Ps/Pd) × 2ρ/ΔP × $htPWV^2$

150 taBETA = ln (Ps/Pd) × 2ρ/ΔP × $taPWV^2$

151 haBETA = ln (Ps/Pd) × 2ρ/ΔP × $haPWV^2$

152 First, to clarify the characteristics of htBETA, taBETA, and haBETA, their correlation was evaluated,

153 and their relationship with the clinical characteristics was examined in healthy subjects and patients

154 with CAD who underwent arterial stiffness measurements using volume plethysmography ten

155 minutes before coronary angiography in the catheter laboratory. Thereafter, we compared htBETA,

156 taBETA, and haBETA between healthy subjects and patients with CAD to determine the superior

157 index for discerning the presence of CAD.

Statistical analysis

159 Results were presented as mean ± standard deviation for continuous data and as numbers and

160 percentages for categorical data. Data were compared using Student's *t*-test or analysis of variance

161 for continuous variables and using chi-square test for categorical variables. Correlation coefficients

162 were calculated for paired data. Furthermore, multiple regression analysis was performed, and

163 principal component analysis was conducted.

164 ROC curves were used to visualize the sensitivity and specificity depending on the threshold. AUC

165 and its standard error (SE) were obtained. The statistical comparison of the areas under two ROC

166 curves was derived by the method described by Hanley and McNeil,[20] who demonstrated that the

167 difference in AUC of two ROC curves derived from the same set of patients can be determined to

168 be random or real from the critical ratio Z, which is defined as follows:

169
$$Z = \frac{A1 - A2}{\sqrt{SE1^2 + SE2^2 + 2rSE1 \times SE2}}$$

170 where A1 and SE1 are the observed area and estimated SE of AUC associated with test 1,

171 respectively; A2 and SE2 refer to the corresponding quantities for test 2. In addition, r was derived

172 from (A1 + A2)/2 and (r_n + r_a)/2, wherein r_n and r_a are correlation coefficients between measurement

173 values of tests 1 and 2 in the control groups and those of tests 1 and 2 in the diseased groups,

174 respectively. Then, the obtained Z value was above the cutoff value, it was referred to the table of

175 the normal distribution, which was considered as evidence that the AUC was truly different.

176 A P-value of <0.05 was considered statistically significant. All calculations were performed using

177 JMP version 10.0 (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics and treatments

Overall, 90 healthy subjects and 41 patients with CAD were included in this study. The healthy subjects were 10 years younger than the patients with CAD [mean age, 54.0 (range, 23–84) and 64.1 (range 46–86) years, respectively]. Table 1 shows that patients with CAD were more frequently men and obese. Although antihypertensive and hypoglycemic drugs were more frequently used in patients with CAD than in healthy subjects, systolic and diastolic blood pressures and serum glucose and HbA_{1c} levels remained high (Table 1). By contrast, serum total cholesterol and LDL cholesterol levels were lower and HDL cholesterol level was higher in patients with CAD who were more frequently treated with the hypolipidemic drugs compared with the healthy subjects (Table 1).

Relationship among htBETA, taBETA, and haBETA in healthy subjects and patients with CAD

HaBETA was correlated with htBETA (r = 0.78, $p < 0.01$) and taBETA (r = 0.46, $p < 0.01$) in healthy subjects (Figure 1). In patients with CAD, haBETA was correlated only with htBETA (r = 0.56, p < 0.01, Figure 2). Moreover, htBETA and taBETA were unrelated in healthy subjects and patients with CAD (Figures 1 and 2).

Multiple regression analysis revealed that haBETA can be estimated using htBETA and ta htBETA in healthy subjects (haBETA = 0.828 htBETA + 0.094 taBETA + 1.406, r = 0.86, $p < 0.01$, Figure 3) and patients with CAD (haBETA = 0.546 htBETA + 0.073 taBETA + 4.259, r = 0.67, $p < 0.01$, Figure 4). Moreover, principal component analysis indicated that the plots were horizontally distributed for healthy subjects, whereas the plots were equally distributed in horizontal and vertical directions for patients with CAD (Figure 3). The proportions of variance of htBETA and taBETA to haBETA were 65.4% (component 1, relationship between htBETA and haBETA) and 29.7% (component 2,

201   relationship between taBETA and haBETA) in healthy subjects and 52.7% (component 1) and

202   37.2% (component 2) in patients with CAD (Figure 3).

203   These data suggested that compared with patients with CAD, healthy subjects had a greater effect

204   on the variance of htBETA to haBETA.

Relationship between htBETA, taBETA, haBETA, and clinical characteristics in healthy subjects and patients with CAD

207   Table 2 shows that the thigh circumference, height, and body weight were not related to all

208   segmental βs. In healthy subjects and patients with CAD, age was correlated with haBETA (r =

209   0.626 and r = 0.387, respectively) and htBETA (r = 0.560 and r = 0.406, respectively) but not

210   taBETA ($p < 0.01$). Systolic and diastolic blood pressures were significantly correlated with taBETA

211   in healthy subjects (r = 0.338, p < 0.01, and r = 0.273, $p < 0.05$, respectively) and patients with CAD

212   (r = 0.361, $p < 0.05$, and r = 0.395, $p < 0.05$, respectively). Moreover, in healthy subjects, systolic (r

213   = 0.331, $p < 0.01$) and diastolic (r = 0.224, $p < 0.05$) blood pressures were correlated with haBETA

214   and only the systolic blood pressure was correlated with htBETA (r = 0.297, $p < 0.01$). In patients

215   with CAD, the body mass index was correlated with haBETA (r = 0.372, $p < 0.05$) and heart rate

216   was correlated with htBETA (r = 0.366, $p < 0.05$).

217   In summary, haBETA and htBETA were significantly related to age, whereas taBETA was

218   associated with systolic and diastolic blood pressures.

Comparison of htBETA, taBETA, and haBETA between healthy subjects and patients with CAD

221   As shown in Figure 4, taBETA was three times higher than htBETA in healthy subjects and patients

222   with CAD ($p < 0.01$). Moreover, haBETA and htBETA were significantly lower in healthy subjects

223   compared with those in patients with CAD ($p < 0.01$). However, taBETA did not differ between the

224   two groups.

ROC curve of haBETA, htBETA, and taBETA in diagnosis of CAD

The ROC curves of haBETA, htBETA, and taBETA in the diagnosis of CAD were computed (Figure

5), and the ROC curves of haBETA and htBETA were more upward and shifted to the left side

compared with that of taBETA. The AUC ± SE value of haBETA (0.731 ± 0.046) and htBETA (0.757

± 0.043) was significantly higher than that of taBETA (0.493 ± 0.054) ($p < 0.01$, respectively),

although that of haBETA did not differ with that of htBETA ($p = 0.49$). Therefore, haBETA and

htBETA were superior to taBETA in discerning the presence of CAD, because taBETA exhibited no

discerning ability.

Diagnostic power for CAD with a threshold of haBETA, htBETA, and taBETA

To determine the optimal threshold for discerning the presence of CAD, the optimal intersection

point between sensitivity and 1-specificity curves of haBETA, htBETA, and taBETA in CAD was

computed. The optimal cutoff points were 9.20, 7.72, and 21.0 for haBETA, htBETA, and taBETA,

respectively (Figure 5). Using the threshold of 9.20 in haBETA, 7.72 in htBETA, and 21.0 in taBETA,

sensitivity of 80.5%, 75.6%, and 65.9% and specificity of 63.3%, 68.9%, and 45.6%, respectively,

were obtained (arrows indicate each threshold in Figure 5). Therefore, sensitivity and specificity for

discerning the presence of CAD were higher in haBETA and htBETA than in taBETA. However,

haBETA and htBETA were not significant ($p = 0.49$).

Discussion

The present study demonstrated that segmental βs, such as htBETA and taBETA, were different. In

the healthy subjects and patients with CAD, htBETA was age dependent but taBETA was not

(Table 2). Moreover, taBETA was three times higher than htBETA in healthy subjects as well as

patients with CAD, suggesting that the stiffness of the medium-sized extremity muscle artery from

the thigh–ankle artery was significantly higher than that of the elastic artery in the aorta and that the

249 muscle artery was independent of age. Nichols et al[21] suggested that stiffness of elastic arteries

250 increased with age, which has primarily been attributed to the degeneration of the medial layer of

251 the arterial walls. By contrast, compared with the elastic arteries, the medium-sized muscular

252 arteries are barely affected by age and less distensible.[22,23] Moreover, the stiffness of medium-sized

253 muscular arteries is modulated by the vasomotor tone depending on either the endothelial function,

254 sympathetic nervous system,[24,25] or renin–angiotensin system.[26] Moreover, Wohlfahrt et al[27]

255 reported that the stiffness of the lower-extremity artery, which was determined using PWV from the

256 femoral artery to dorsal pedal/posterior tibial arteries, was affected to a lesser extent by age and

257 cardiovascular risk factors than aortic stiffness (cfPWV); further, increased ankle systolic blood

258 pressure was associated with the stiffness of the lower-extremity artery in a random sample from

259 the Czech population. Because PWV is dependent on blood pressure, these associations may be

260 observed; our taBETA was less dependent on blood pressure. However, the measurement

261 segment of taBETA is a functional medium-sized muscular artery, which is modulated by the

262 vasomotor tone, particularly in young age. The association between blood pressure and stiffness of

263 the lower-extremity artery, which was calculated by the stiffness parameter β theory, was observed

264 in the present study, suggesting that blood pressure is one of the important factors associated with

265 the stiffness of medium-sized muscular arteries.

266 Data regarding aging of the lower-extremity arteries are discrepant: in some studies,[23,28] no

267 increase in stiffness with age was observed, whereas in other studies,[29,30] stiffness was found to

268 increase with age. This discrepancy may be explained by the minor effect of age on the stiffness of

269 the lower-extremity arteries and by the different methods of arterial stiffness measurement.

270 The stiffness of the muscle artery (taBETA) did not differ between the healthy subjects and patients

271 with CAD in the present study. By contrast, Yamamoto et al[14] reported lower taBETA in their healthy

272 group (14.10 ± 4.14) than that in our healthy subjects (21.27 ± 6.68), and it was higher in their

273 patients with atherosclerosis (25.45 ± 22.31) than in our patients with CAD (21.14 ± 8.36). These

274 discrepancies may be explained by the age and sex differences between both studies, considering

275 that the age of the healthy subjects and patients were 30.9 vs. 54.0 and 72.0 vs. 64.1 years and the

276 proportion of men were 100% vs. 39% and 84% vs. 76% (Yamamoto et al's data vs. our data),

277 respectively. Therefore, although taBETA may be lower in younger healthy subjects aged

278 approximately 30 years, the changes in the stiffness of muscular artery (taBETA) appear to be

279 extremely small in elderly individuals aged >50 years who are predominantly at risk of

280 atherosclerosis.

281 Furthermore, haBETA was more strongly correlated with htBETA (r = 0.78) than with taBETA (r

282 =0.46) in healthy subjects (Figure 1), and the multiple regression analysis revealed that haBETA

283 can be almost precisely estimated using htBETA and taBETA (Figure 4). However, these

284 relationships were weakened in patients with CAD (Figure 4). Furthermore, the proportion of the

285 variance of htBETA to haBETA was greater and that of taBETA to haBETA was smaller in healthy

286 subjects compared with the proportions in patients with CAD (Figure 3). Therefore, the

287 nonuniformity of the arterial system between elastic and medium-sized muscular arteries would

288 disappear in patients with CAD, which is typically observed in healthy young subjects.

289 Previously, we have demonstrated that CAVI, which includes the coefficients "a" and "b" on the

290 haBETA formula, was significantly correlated with the regional stiffness parameter β of the

291 ascending and descending aorta calculated from electrocardiogram-gated multidetector row

292 computed tomography and that the ratio of the thoracic aorta pulse wave propagation time (heart–

293 thigh) to the entire pulse wave propagation time from the heart to the ankle was large, which may

294 substantially impact the entire PWV (heart–ankle).[31] In addition, Wohlfahrt et al.[32] reported that

295 cfPWV was positively correlated with carotid–ankle PWV, and the addition of thigh–ankle PWV to

296 cfPWV decreased the association with age, which can only be explained by the minor effect of this

297 factor on the arterial stiffness of medium-sized extremity muscular artery. Accordingly, these studies

298 suggested that CAVI (haBETA) shows the highest dependence on the stiffness of the central artery,

299 such as thoracic aortas, and age is a major confounder of this stiffness.

300 Recently, Fico et al[33] determined cfPWV using an automatic vascular screening device (VP-1000

301 Plus, Omron Healthcare, Kyoto, Japan), and the heart–thigh PWV (htPWV) was determined with

302 the same device in our study of 50 healthy subjects (18–79 years old). The mean values of cfPWV

303   (713 ± 145 cm/s) and htPWV (699 ± 150) did not differ (p = 0.43), and these correlations were high

304   (r = 0.64, p < 0.001). The regression line was derived from the line of identity in the Bland–Altman

305   plot. Further, these results suggested that htBETA has good potential for assessing arterial stiffness

306   in the clinical setting in comparison to cfPWV.

307   The comparison of the segmental βs shows that htBETA, but not taBETA, was significantly higher

308   in patients with CAD than in healthy subjects (Table 3). However, the ROC curve analysis showed

309   that htBETA did not improve the diagnostic power for CAD compared with haBETA, which

310   comprises htBETA and taBETA. These data may be extremely important because the lengthening

311   of the measurement site from the heart–thigh distance (elastic artery) to the heart–ankle distance

312   (elastic artery plus medium-sized muscular artery) does not decrease the diagnostic power for

313   middle-aged patients with CAD.

314   ### *Study limitations*

315   Several healthy subjects were using antihypertensive (10%), hypoglycemic (2.2%), and

316   hypolipidemic (6.7%) drugs, and patients with CAD were using some medications, which may have

317   influenced our results.

318   Moreover, although coronary arteriography or computed tomography of the coronary artery was not

319   performed, the ischemic change in the electrocardiogram at rest for all healthy subjects was not

320   observed. For comparing each β between healthy subjects and patients with CAD, both study

321   populations were heterogeneous. However, the characteristics of healthy subjects appear to

322   represent the status of the subjects undergoing general medical examinations in Japan.

323   ## Conclusion

324   The stiffness of the medium-sized thigh–ankle artery was three times greater than the elastic heart–

325   ankle artery. Its stiffness was constant and that of the elastic aorta correlated with age. It was

326   possible to estimate two-thirds of the components of the stiffness of the heart–ankle artery using the

327   stiffness of the heart–thigh artery. The ROC curve analysis revealed that the stiffness of the heart–

328   ankle artery could be replaced by that of the heart–thigh artery, prolonging the measurement

329   segment without affecting the diagnostic power for CAD.

330   Disclosure

331   The authors have no conflicts of interest in this manuscript.

332   References

333   1.   Shokawa T, Imazu M, Yamamoto H, et al. Pulse wave velocity predicts cardiovascular

334   mortality: findings from the Hawaii-Los Angeles-Hiroshima study. *Circ J.* 2005;69(3):259–264.

335   2.   Willum-Hansen T, Staessen JA, Torp-Pedersen C, et al. Prognostic value of aortic pulse wave

336   velocity as index of arterial stiffness in the general population. *Circulation.* 2006;113(5):664–

337   670.

338   3.   Pannier B, Guérin AP, Marchais SJ, Safar ME, London GM. Stiffness of capacitive and conduit

339   arteries: prognostic significance for end-stage renal disease patients. *Hypertension.*

340   2005;45(4):592–596.

341   4.   Laurent S, Cockcroft J, Van Bortel L, et al; European Network for Non-invasive Investigation of

342   Large Arteries. Expert consensus document on arterial stiffness: methodological issues and

343   clinical applications. *Eur Heart J.* 2006;27(21):2588–2605.

344   5.   Bramwell JC, Hill AV. Velocity of transmission of the pulse and elasticity of arteries. *Lancet.*

345   1922;1:891.

346   6.   Spronck B, Heusinkveld MH, Vanmolkot FH, et al. Pressure-dependence of arterial stiffness:

347   potential clinical implications. *J Hypertens.* 2015;33(2):330–338.

348   7.   Hayashi K, Handa H, Nagasawa S, Okumura A, Moritake K. Stiffness and elastic behavior of

349   human intracranial and extracranial arteries. *J Biomech.* 1980;13(2):175–184.

350   8.   Shirai K, Utino J, Otsuka K, Takata M. A novel blood pressure-independent arterial wall

351   stiffness parameter; cardio-ankle vascular index (CAVI). *J Atheroscler Thromb.*

352   2006;13(2):101–107.

353   9.   Takahashi K, Yamamoto T, Tsuda S, et al. Coefficients in the CAVI equation and the

354   comparison between CAVI with and without the coefficients using clinical data. *J Atheroscler*

355   *Thromb.* 2019;26(5):465–475.

356   10. Hayashi K, Yamamoto T, Takahara A, Shirai K. Clinical assessment of arterial stiffness with

357   cardio-ankle vascular index: theory and applications. *J Hypertens.* 2015;33(9):1742–1757.

358   11. Hasegawa M, Arai C. Clinical estimation of vascular elastic function and practical application.

359   *Connective Tissue.* 1995;27(2):149–157.

360   12. Hickson SS, Butlin M, Broad J, Avolio AP, Wilkinson IB, McEniery CM. Validity and

361   repeatability of the Vicorder apparatus: a comparison with the SphygmoCor device. *Hypertens*

362   *Res.* 2009;32(12):1079–1085.

363   13. Butlin M, Qasem A, Battista F, Bozec E, et al. Carotid-femoral pulse wave velocity assessment

364   using novel cuff-based techniques: comparison with tonometric measurement. *J Hypertens.*

365   2013;31(11):2237–2243.

366   14. Yamamoto T, Shimizu K, Takahashi M, Tatsuno I, Shirai K. The effect of nitroglycerin on

367   arterial stiffness of the aorta and the femoral-tibial arteries. *J Atheroscler Thromb.*

368   2017;24(10):1048–1057.

369   15. Yonezawa Y, Horinaka S, Shirakawa C, Kogure Y. Estimated glomerular filtration ratio is a

370   better index than creatinine clearance (Cockcroft-Gault) for predicting the prevalence of atrial

371   fibrillation in the general Japanese population. *Hypertens Res.* 2018;41(6):451–459.

372   16. Gerhard-Herman MD, Gornik HL, Barrett C, et al. 2016 AHA/ACC Guideline on the

373   Management of Patients With Lower Extremity Peripheral Artery Disease: A Report of the

374   American College of Cardiology/American Heart Association Task Force on Clinical Practice

375   Guidelines. *Circulation.* 2017;135(12):e726–e779.

376   17. Horinaka S, Yabe A, Yagi H, et al. Comparison of atherosclerotic indicators between cardio

377   ankle vascular index and brachial ankle pulse wave velocity. *Angiology.* 2009;60(4):468–476.

378   18. Obuchowski NA, Lieber ML, Wians FH Jr. ROC curves in clinical chemistry: uses, misuses,

379   and possible solutions. *Clin Chem.* 2004;50(7):1118–1125.

380   19. Nye ER. The effect of blood pressure alteration on the pulse wave velocity. *Br Heart J.*

381   1964;26(2):261–265.

382   20. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating

383   characteristic (ROC) curve. *Radiology.* 1982;143(1):29–36.

384   21. Valchopoulos C, Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries: Theoretical,*

385   *Experimental and Clinical Principles.* 6th ed. London: CRC Press; 2011.

386   22. van der Heijden-Spek JJ, Staessen JA, Fagard RH, Hoeks AP, Boudier HA, van Bortel LM.

387   Effect of age on brachial artery wall properties differs from the aorta and is gender dependent:

388   a population study. *Hypertension.* 2000;35(2):637–642.

389   23. Boutouyrie P, Laurent S, Benetos A, Girerd XJ, Hoeks AP, Safar ME. Opposing effects of

390   ageing on distal and proximal large arteries in hypertensives. *J Hypertens.* 1992;10(6):S87–

391   S91.

392   24. Boutouyrie P, Lacolley P, Girerd X, Beck L, Safar M, Laurent S. Sympathetic activation

393   decreases medium-sized arterial compliance in humans. *Am J Physiol.* 1994;267(4 Pt

394   2):H1368–H1376.

395   25. Giannattasio C, Failla M, Lucchina S, et al. Arterial stiffening influence of sympathetic nerve

396   activity: evidence from hand transplantation in humans. *Hypertension.* 2005;45(4):608–611.

397   26. Giannattasio C, Failla M, Stella ML, et al. Angiotensin-converting enzyme inhibition and radial

398   artery compliance in patients with congestive heart failure. *Hypertension.* 1995;26(3):491–496.

399   27. Wohlfahrt P, Krajčoviechová A, Seidlerová J, et al. Lower-extremity arterial stiffness vs. aortic

400   stiffness in the general population. *Hypertens Res.* 2013;36(8):718–724.

401   28. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME. Arterial alterations with aging and

402   high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb.*

403   1993;13(1):90–97.

404   29. Lo CS, Relf IR, Myers KA, Wahlqvist ML. Doppler ultrasound recognition of preclinical changes

405   in arterial wall in diabetic subjects: compliance and pulse-wave damping. *Diabetes Care.*

406   1986;9(1):27–31.

407   30. Blacher J, Demuth K, Guerin AP, Safar ME, Moatti N, London GM. Influence of biochemical

408   alterations on arterial stiffness in patients with end-stage renal disease. *Arterioscler Thromb*

409   *Vasc Biol.* 1998;18(4):535–541.

410   31. Horinaka S, Yagi H, Ishimura K, et al. Cardio-ankle vascular index (CAVI) correlates with aortic

411   stiffness in the thoracic aorta using ECG-gated multi-detector row computed tomography.

412   *Atherosclerosis.* 2014;235(1):239–245.

413   32. Wohlfahrt P, Krajčoviechová A, Seidlerová J, et al. Arterial stiffness parameters: how do they

414   differ? *Atherosclerosis*. 2013;231(2):359–364.

415   33. Fico BG, Gourley DD, Wooten SV, Tanaka H. Heart-thigh cuff pulse wave velocity: A novel

416   nontechnical measure of arterial stiffness. *Am J Hypertens.* 2019;32(11):1051–1053.

417

418   **Figure 1** Correlation between each segmental β in healthy subjects.

419   haBETA, heart–ankle β; htBETA, heart–thigh β; taBETA, thigh–ankle β; r, correlation coefficient

420   **Figure 2** Correlation between each segmental β in patients with coronary artery disease.

421   **Figure 3** Principal component analysis in healthy subjects and patients with coronary artery disease.

422   Component 1: horizontal (relationship between htBETA and haBETA)

423   Component 2: vertical (relationship between taBETA and haBETA)

424   haBETA, heart–ankle β; htBETA, heart–thigh β; taBETA, thigh–ankle β

425   **Figure 4** Multiple linear regression model for haBETA with htBETA and taBETA in healthy subjects

426   and patients with coronary artery disease.

427   haBETA, heart–ankle β; htBETA, heart–thigh β; taBETA, thigh–ankle β

428   **Figure 5** Reciever operating characteristic (ROC) curves of haBETA, htBETA, and taBETA in

429   coronary artery disease (CAD).

430   ——— haBETA: cutoff value 9.20, sensitivity 80.5, specificity 63.3%

431   ——— htBETA: cutoff value 7.72, sensitivity 75.6, specificity 68.9%

432   ——— taBETA: cutoff value 21.0, sensitivity 65.6, specificity 45.6%

433   Each arrow indicates the optimal threshold (cutoff value) of haBETA, htBETA, and taBETA for the

434   discernment of the presence of CAD, respectively.

435   The area under the ROC curve (AUC ± SE) of haBETA, htBETA, and taBETA were 0.731 ± 0.046

436   ($p < 0.01$), 0.757 ± 0.043 ($p < 0.01$), and 0.493 ± 0.054 ($p = 0.49$), respectively.

437   SE, standard error; haBETA, heart–ankle β; htBETA, heart–thigh β; taBETA, thigh–ankle β.