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ASSOCIATIONS OF CENTRAL AORTIC PRESSURE AND BRACHIAL BLOOD PRESSURE WITH FLOW MEDIATED DILATATION IN APPARENTLY HEALTHY JAPANESE MEN: THE CIRCULATORY RISK IN COMMUNITIES STUDY (CIRCS)

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Highlights

・We examined the associations of central systolic aortic pressure and brachial systolic pressure with flow mediated dilatation in apparently healthy Japanese men.

・Higher central aortic pressure rather than higher brachial blood pressure was associated with lower flow mediated dilatation; the association was evident for men without antihypertensive medication.

・Our finding suggests that central systolic aortic pressure, rather than brachial systolic blood pressure, is a useful marker for endothelial dysfunction in men.
Background and aims. Endothelial dysfunction is considered the first stage in the development of atherosclerosis and cardiovascular disease, and brachial flow-mediated dilation (FMD) is a measure of endothelial function. It is uncertain which of central systolic aortic pressure (CAP) or brachial systolic blood pressure (SBP) is more strongly associated with FMD. Therefore, we examined the correlations of CAP and SBP with FMD in Japanese men.

Methods. The study subjects comprised 507 male volunteers aged 30–79 years that were residents in two communities under the Circulatory Risk in Communities Study (CIRCS) between 2013 and 2015. The low percent change of FMD (%FMD) ≤5.0% after 5 minutes of reactive hyperemia evaluated by the brachial artery was used to assess endothelial dysfunction. Values of CAP and SBP were divided into tertiles, with the lowest tertile used as a reference.

Results. After adjustment for cardiovascular risk factors, the multivariable odds ratio (95% CI) of low FMD for the highest versus the lowest tertile of CAP was 1.5(0.9–2.6) for total subjects and 1.3(0.7–2.5) for those with and 2.4(1.2–4.8) for those without antihypertensive medication use. The corresponding odd ratios for the highest versus lowest tertile of SBP were 0.9(0.5–1.5), 0.8(0.3–2.2), and 1.3(0.7–2.5).

Conclusions. Higher CAP levels were associated with low FMD for men without antihypertensive medication, but such an association was not found for SBP levels. (word count: 227)

Key words: Central aortic pressure ■ Endothelial function ■ Japanese men ■
Cross sectional study
1. Introduction

Cardiovascular diseases remain the major cause of morbidity and mortality in developed countries, with atherosclerosis being the leading underlying cause (1). Endothelial dysfunction is considered the first stage in the development of atherosclerosis and cardiovascular disease (2, 3). Endothelial cells form the inner lining of all blood vessels and play a central role in vascular homeostasis; they respond to stimuli, such as hemodynamic changes or blood-borne signals by releasing vasoactive substances (4). Brachial flow-mediated dilation (FMD) is a measure of the release of nitric oxide by the endothelium due to a transient flow stimulus (5) and low brachial FMD was regarded as a cardiovascular disease risk factor (4, 5).

Hypertension is a recognized risk factor for the development of atherosclerosis and cardiovascular disease (6-8). Central systolic aortic pressure (CAP) has been reliably determined by mathematically transforming the radial artery pulse waveform to the aortic pulse waveform (9-10). Several studies have also reported that CAP levels were strongly associated with risk of mortality from cardiovascular disease (11, 12). The Circulatory Risk in Communities Study (CIRCS) of 3,002 Japanese men and women reported that CAP levels were correlated more strongly with cardiovascular risk factors than brachial systolic blood pressure (SBP) levels (13). However, evidence for the correlation of CAP with FMD is limited. Additionally, to date, it is unclear which of CAP or SBP is more strongly associated with flow-mediated dilatation. In this study, we
investigated the relationship of CAP and SBP with FMD in the general population.
2. Materials and Methods

2.1. Subjects

FMD and CAP measurements were conducted in two communities of the CIRCS, a dynamic cohort study of the Japanese population: Yao City, Osaka Prefecture and Ikawa town, Akita Prefecture under a nationwide study. We recruited 507 men aged 30–79 years one by one from January 2013 to May 2015 from participants who underwent annual cardiovascular risk surveys. Informed consent was obtained from community representatives based on guidelines of the Council for International Organizations of Medical Science to perform an epidemiological study (14). The study protocol was approved by the Ethics Committee of the Osaka University.

2.2. Measurement of FMD and CAP

All participants had five minutes of rest in the seated posture, using a standard protocol (15). FMD was measured with high-resolution ultrasonography and forearm occlusive cuff by technicians. High-resolution ultrasound with a 10-MHz linear array transducer was used to record longitudinal images of the right brachial artery. This transducer system can accurately capture and track the edge of target artery automatically once the probe is placed at the proper position. To standardize the position of the probe, we used a specially designed arm-rest and probe holder. The brachial artery diameter at baseline was measured by this system and then the brachial cuff was inflated to 50mm Hg above SBP for 5
minutes and deflated. Computer-assisted analysis software (UNEX Co. Ltd., Nagoya, Japan) was used to determine brachial artery diameter semi-automatically, as previously described (16).

The baseline longitudinal image of the artery was acquired for 30 seconds, after which the blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 minutes. FMD change (%FMD) was defined by the following formula: %FMD = ((maximal artery lumen diameter after cuff release - artery lumen diameter at baseline) / artery-lumen diameter at baseline) × 100, according to published guidelines for determining endothelial function (17). The coefficient of inter-observer variability for FMD measurements in our laboratory was 5.7 %, while that of intra-observer variability were 11.1% apart from 2 months and 10.8% apart from 4 months. In previous studies, the coefficient of inter-observer variability for FMD measurement was 1.3% to 3.5% (18, 19), and that for intra-observer variability was 15.6% apart from 48 hours and 18.3% apart from 3 months (20).

CAP was measured by technicians with an automated tonometer, HEM-9000AI (Omron, Healthcare Co., Kyoto, Japan). A previous clinical study used both HEM-9000AI and standard cardiac catheterization to examine the validity and reproducibility of CAP levels among 18 hypertension patients aged 47–78 years. The correlation coefficient was 0.95 (p < 0.001) between CAP levels by the two measurement systems, and 0.93 (p < 0.001) between the repeated CAP measurement by HEM-9000AI (6).
2.3. Measurement of cardiovascular risk factors

We previously reported the protocols for measuring cardiovascular risk factors, such as blood pressure, serum lipids, body mass index (BMI), assessment of smoking and drinking habits, hypertension, and diabetes mellitus (10, 21, 22).

Height in stocking feet and weight in light clothing were measured. Body mass index (BMI) (kg/m²) was calculated as weight in kilograms divided by height in square meters. Trained observers measured SBP and diastolic blood pressure (DBP) using a standard mercury sphygmomanometer on the right arm after participants had rested for 5 minutes (23). An interview was conducted to confirm information on habits, including drinking status, tobacco status, hypertension, and diabetes mellitus medication use. For drinking status, persons who reported consuming 0.3 gō (equivalent to 7 grams of ethanol) or more per week were regarded as current drinkers. Former drinkers were defined as abstainers for the previous 3 months or more. Trained interviewers also determined information on smoking status, use of antihypertensive agents, and medical history. Persons who smoked ≥1 cigarette per day were defined as current smokers.

Blood samples were obtained on the same day as annual cardiovascular risk surveys from participants and the serum was separated immediately. Measurements of serum triglycerides were performed using a fluorometric method by an Autoanalyzer II (Technicon, Tarrytown, NY, U.S.A.), while total cholesterol and high density lipoprotein (HDL)-cholesterol measurements were
performed at the Osaka Medical Center for Health Science and Promotion lipid reference laboratory, a certified member of the US National Cholesterol Reference Method Laboratory Network (CRMLN), using enzymatic methods by an auto analyzer Olympus AU 2700 (24). Serum glucose measurements were performed by the hexokinase method, using the same instrument. Diabetes mellitus was defined as a fasting glucose level of ≥7.8 mmol/L, a non-fasting glucose level of ≥11.1 mmol/L, or use of medication for diabetes mellitus (25). Hypertension was defined as SBP ≥140 mmHg, DBP ≥90 mmHg, or use of antihypertensive medication (26).

2.4. Statistical analysis

We defined the low FMD as %FMD≤5.0 (lowest 30 percentile) based on previous reports that used the receiver-operating characteristic analysis (27, 28). Values of cardiovascular risk factors in subjects with %FMD≤5.0 and >5.0 are presented as mean ± standard deviation (SD) or proportions (%). The odd ratios (OR) with the respective 95% confidence intervals (CIs) of the low FMD were calculated according to tertiles of and 1-SD increment of CAP and SBP levels, by using logistic regression analysis, after adjusting for age in one model, and further adjustment for potential confounding factors including area of residence, heart rate, brachial artery baseline diameter, total serum cholesterol, serum triglycerides, history of diabetes mellitus, drinking status and smoking status. The analyses were repeated by stratifying antihypertensive medication use. All statistical analyses were performed with SAS version 9.4 software (SAS
Institute Inc., Cary, NC, USA). All probability values for statistical tests were
two-tailed and values of p < 0.05 were regarded as statistically significant.
3. Results

The characteristics of 507 Japanese men are summarized in Table 1. The mean values of %FMD, age and BMI were 6.7, 54.1 years and 24.2kg/m², respectively. Compared with participants in the group of %FMD≤5, those in the group of %FMD>5 had lower CAP, lower SBP levels and smaller brachial artery baseline diameter, and were less likely to be drinkers, diabetics and hypertensive. The ORs (95% CI) of the low FMD according to tertiles and 1-SD increment for CAP and SBP levels are given in Table 2. Among total 507 subjects, the multivariable ORs (95% CI) of the low FMD was 1.5(0.9–2.6) for the highest versus lowest tertiles of CAP, and 1.2(1.0-1.5) for 1-SD increment (16.3 mmHg) of CAP levels; while were 0.9(0.5–1.5) for the highest versus lowest tertiles of SBP, and 1.0(0.8-1.3) for 1-SD increment (13.9 mmHg) of SBP levels. When these associations were stratified by antihypertensive medication use, significant positive associations between CAP and the low FMD were observed primarily in subjects without antihypertensive medication use; the multivariable ORs (95% CI) of the low FMD was 2.4(1.2–4.8) for the lowest versus highest tertiles and 1.3(1.0-1.7) for 1-SD increment of CAP levels. There were no difference in the associations between SBP and low FMD in participants with and without the use of antihypertensive medication.
4. Discussion

In the present community-based study of 507 Japanese men aged 30–79 years, CAP, but not brachial SBP, levels were correlated with the low FMD. The association between CAP and low FMD levels was evident for men who did not use antihypertensive medications.

Low FMD is a surrogate marker of early atherosclerosis in Japanese (29), American (30), and European subjects (31). In a clinical study of 384 patients with suspected cardio metabolic disorders, %FMD was significantly reduced in patients with nonalcoholic fatty liver disease, diabetes, history of coronary heart disease, metabolic syndrome, and in those taking antihypertensive drugs (31).

The Multi-Ethnic Study of Atherosclerosis for 2,936 men and women (mean age 61 years) showed that a 1-SD (2.8%) increase in %FMD values was associated with lower risk of incident auricular fibrillation [Hazard ratio (HR) =0.84, 95%CI=0.70, 0.99], suggesting that markers of endothelial dysfunction contributes to the pathogenesis of auricular fibrillation (30).

To our knowledge our study is the first to show that CAP levels were associated more with reduced %FMD than SBP levels in men without use of antihypertensive medication. Lind L has reported that CAP measurement was not superior over traditional blood measurements regarding its relation to endothelium-dependent vasodilatation or FMD (32). However, that study was conducted only among elder participates over 70 years old. It was previously shown that the absolute difference between aortic and brachial systolic pressures
declined with age (<20y up to 69 years) and then the difference plateaued after ages≥70 years (33). Our study supports the previous finding from a clinical study of 201 type 2 diabetes patients that an ankle-brachial index, a surrogate marker of atherosclerosis, was more strongly correlated with CAP than SBP levels (CAP: r=0.162, p=0.04, SBP: r=0.083, p=0.30) (34). Compared with a 10 mmHg increment of SBP, the same increment of CAP was more strongly associated with mortality risk from cardiovascular disease in a cohort study of normotensive and untreated hypertensive Taiwanese; the multivariable HR (95% CI) of cardiovascular mortality was 1.34(1.10–1.49) for CAP and 0.96(0.79–1.16) for SBP (12). Our previous study found that CAP levels were associated with subclinical damage expressed by minor ST-T ECG abnormalities (8). Furthermore, a clinical study of 146 hypertensive patients reported that left ventricular mass change was more strongly correlated with CAP than SBP (35). These findings support CAP as a more sensitive marker for the loading conditions on the heart and coronary arteries than SBP.

In the current study, the lack of association between CAP levels with %FMD among subjects with antihypertensive medication use may be due to the dilution of association after lowering CAP levels by various amounts. On the other hand, the lack of association between SBP levels and %FMD in total subjects regardless of antihypertensive medication use might probably due to the small number of severe high blood pressure patients (SBP≥160 mmHg, n=21).

The strengths of the present study include the use of a noninvasive technique
for measuring CAP and FMD and the standardized measurements of other cardiovascular risk factors in community population-based samples (22).

However, this study has several limitations as follows: first, details of antihypertensive drug treatment were not available. Antihypertensive drugs such as calcium channel antagonist, ACE-inhibitors and AT1-receptor antagonists can improve endothelial function (36). However, we could not investigate whether the lack of association between CAP levels and reduced %FMD in men with antihypertensive medication use might be attributable to the effects of those drugs or not. Second, reduced %FMD was the only indicator for endothelial dysfunction in the current study; no data were available for nitrate-induced vasodilatation. Last, our subjects were not recruited randomly, but they were selected consecutively, and thus generalizability of our findings is limited. We also were unable to include women in the current analysis because of very small sample size, and further investigation will be necessary.

In conclusion, higher CAP levels were associated with low FMD for men without antihypertensive medication, but such an association was not found for SBP levels.

Conflict of interest

None declared.

Financial support

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**Author contributions**

Keyang Liu, Renzhe Cui, Ehab S. Eshak, Jia-Yi Dong, Meishan Cui and Masahiko Kiyama participated in the study design and data collection; Keyang Liu, Renzhe Cui and Ehab S. Eshak analyzed the data; Keyang Liu, Renzhe Cui, Akihiko Kitamura and Hiroyasu iso participated in interpretation of data and drafting of the manuscript; Keyang Liu, Renzhe Cui and Ehab S. Eshak provided statistical expertise. Takeo Okada, Akihiko Kitamura, Mitsumasa Umesawa, Kazumasa Yamagishi, Hironori Imano, Tetsuya Ohira and Hiroyasu Iso participated in the study concept and design, acquisition of data and interpretation of data, and critical revision of the manuscript.

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**Appendix**

The CIRCS study is a collaborative study managed by the Osaka Center for Cancer and Cardiovascular Disease Prevention, University of Tsukuba, Osaka University and Ehime University. The authors thank the CIRCS investigators who contributed to this study: Professor Emeritus Yoshio Komachi (University of Tsukuba), Professor Emeritus Hideki Ozawa (Medical College of Oita), Former
professor Minoru Iida (Kansai University of Welfare Sciences), Professor Emeritus Takashi Shimamoto (University of Tsukuba), Dr. Yoshinori Ishikawa (Consultant of Osaka Center for Cancer and Cardiovascular Disease Prevention), Professor Yoshihiko Naito (Mukogawa Women’s University), and Professor Tomonori Okamura (Keio University).
References


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Table 1. Mean values ± standard deviations and proportions of cardiovascular risk factors among 507 Japanese men.

<table>
<thead>
<tr>
<th></th>
<th>Total number</th>
<th>%FMD</th>
<th>P for difference a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>507</td>
<td>153</td>
<td>354</td>
</tr>
<tr>
<td>Mean % flow-mediated dilation</td>
<td>6.7±0.1</td>
<td>3.7±0.1</td>
<td>6.3±0.1</td>
</tr>
<tr>
<td>%FMD≤5, n</td>
<td>153</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachial artery baseline diameter, mm</td>
<td>4.5±0.6</td>
<td>4.7±0.6</td>
<td>4.4±0.5                &lt;0.01</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.1±0.5</td>
<td>56.9±0.7</td>
<td>53.0±0.6                &lt;0.0001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.2±0.2</td>
<td>24.3±0.3</td>
<td>24.2±0.2                0.6</td>
</tr>
<tr>
<td>Central aortic pressure, mmHg</td>
<td>124.5±0.8</td>
<td>127.9±1.4</td>
<td>123.1±0.9                &lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>129.2±0.7</td>
<td>132.1±1.3</td>
<td>127.9±0.9                0.05</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>82.3±0.5</td>
<td>83.2±0.8</td>
<td>82.6±0.6                0.56</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>202.2±1.5</td>
<td>200.2±2.7</td>
<td>203.0±1.7                0.47</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>135.9±4.7</td>
<td>141.0±8.4</td>
<td>133.7±5.7                0.58</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>56.6±0.7</td>
<td>57.1±1.1</td>
<td>56.4±0.8                0.88</td>
</tr>
<tr>
<td>Current drinkers, %</td>
<td>74</td>
<td>76</td>
<td>73</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>33</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>8</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>35</td>
<td>45</td>
<td>31</td>
</tr>
<tr>
<td>Antihypertensive medication use, %</td>
<td>26</td>
<td>35</td>
<td>21</td>
</tr>
</tbody>
</table>

a Chi-square test was used for categorical variables; ANOVA was used for continuous variables.
Table 2. Age- and multivariable-adjusted odds ratio (95% CI) of low FMD according to tertiles of central aortic pressure and systolic blood pressure in Japanese men.

<table>
<thead>
<tr>
<th>Tertiles of central systolic aortic pressure (mmHg)</th>
<th>OR per 1-SD increment b</th>
<th>Tertiles of brachial systolic blood pressure (mmHg)</th>
<th>OR per 1-SD increment b</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Low)</td>
<td>T2</td>
<td>T3 (High)</td>
<td>T1 (Low)</td>
</tr>
<tr>
<td>Total subjects, No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>173</td>
<td>165</td>
<td>165</td>
</tr>
<tr>
<td>Range of pressure</td>
<td>≤115</td>
<td>116-130</td>
<td>≥131</td>
</tr>
<tr>
<td>Mean %FMD ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted %FMD</td>
<td>6.9±0.2</td>
<td>6.7±0.2</td>
<td>6.4±0.2</td>
</tr>
<tr>
<td>Multivariable-adjusted %FMD a</td>
<td>6.8±0.2</td>
<td>6.8±0.2</td>
<td>6.4±0.2</td>
</tr>
<tr>
<td>Low FMD, No.</td>
<td>41</td>
<td>51</td>
<td>61</td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>1.1(0.7-1.8)</td>
<td>1.5(0.9-2.4)</td>
</tr>
<tr>
<td>Multivariable-adjusted OR a</td>
<td>1</td>
<td>1.1(0.6-1.9)</td>
<td>1.5(0.9-2.6)</td>
</tr>
<tr>
<td>Subjects without antihypertensive medication use</td>
<td>142</td>
<td>124</td>
<td>111</td>
</tr>
<tr>
<td>Range of pressure</td>
<td>≤113</td>
<td>114-128</td>
<td>≥129</td>
</tr>
<tr>
<td>Mean %FMD ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted %FMD ± SD</td>
<td>7.3±0.3</td>
<td>6.9±0.3</td>
<td>6.6±0.3</td>
</tr>
<tr>
<td>Multivariable-adjusted %FMD ± SD a</td>
<td>7.1±0.3</td>
<td>7.0±0.3</td>
<td>6.6±0.3</td>
</tr>
<tr>
<td>Low FMD, No.</td>
<td>29</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>1.5(0.8-2.7)</td>
<td>2.0(1.1-3.7)</td>
</tr>
<tr>
<td>Multivariable-adjusted OR a</td>
<td>1</td>
<td>1.9(0.9-3.9)</td>
<td>2.4(1.2-4.8)</td>
</tr>
<tr>
<td>Subjects using antihypertensive medication</td>
<td>27</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td>Range of pressure</td>
<td>≤123</td>
<td>124-137</td>
<td>≥138</td>
</tr>
<tr>
<td>Mean %FMD ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted %FMD ± SD</td>
<td>5.6±0.5</td>
<td>6.1±0.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>Low FMD, No.</td>
<td>12</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>-------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Age-adjusted OR</td>
<td>1</td>
<td>1.2(0.5-2.8)</td>
<td>1.3(0.6-3.1)</td>
</tr>
<tr>
<td>Multivariable-adjusted OR</td>
<td>1</td>
<td>1.1(0.4-3.0)</td>
<td>1.4(0.5-3.8)</td>
</tr>
</tbody>
</table>

1-SD for CAP= 16.3 mmHg, and 1-SD for SBP= 13.9 mmHg.

a Adjusted for age, area of residence, heart rate, brachial artery baseline diameter, total serum cholesterol, serum triglycerides, history of diabetes mellitus, drinking status, and smoking status.

b Calculated by linear regression analysis.