Are you as old as your arteries? Comparing arterial aging in Japanese and European patient groups using cardio-ankle vascular index

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TITLE PAGE

Are you as old as your arteries? Comparing arterial aging in Japanese and European patient groups using CAVI

Short title – Arterial aging in Japanese vs European groups

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We certify that this work is **A) novel**. This is the first study to directly compare raw data on arterial stiffness between a European and Japanese group, showing higher levels of CAVI in the Japanese group and differences across sexes and levels of cardiovascular risk.

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Key words – arterial stiffness, cardio-ankle vascular index, vascular aging, international comparison

Key Points – Arterial stiffness was higher in Japanese patient groups than Europeans while controlling for cardiovascular risk factors.

- Europeans had greater increases in arterial stiffness with age in healthy individuals, particularly for males.
- The addition of cardiovascular risk factors had a greater impact on arterial aging in the Japanese group.

Why does this paper matter? - This is the first study to directly compare raw data on arterial stiffness between a European and Japanese group, showing higher levels of CAVI in the Japanese group and differences across sexes and levels of cardiovascular risk. This offers insight into the different patterns of cardiovascular disease seen across these geographic regions and highlights the potential role of CAVI in demonstrating cardiovascular risk beyond traditional risk factors. Arterial stiffness differs across geographic regions, as does the relationship between vascular compliance with age and cardiovascular risk factors. Our study suggests that there may be inherent differences in vascular structure and function between groups from different geographic regions, controlling for conventional cardiovascular risk factors, which may partly explain differences in cardiovascular outcomes.

Author's roles

KIRKHAM - study concept and design, acquisition of subjects and/or data, analysis and interpretation of data, and preparation of manuscript.

MILLS – study concept and design, acquisition of subjects and/or data, analysis and interpretation of data, and preparation of manuscript

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Abstract 249 words

Background Most comparisons of arterial stiffness between ethnic groups focus on pulse wave velocity. This study used the cardio-ankle vascular index (CAVI) in European compared to Japanese individuals to investigate how cardiovascular risk factors affect arterial aging across geographic regions.

Methods 494 European and 1044 Japanese individuals underwent measurements of CAVI, blood pressure and information on cardiovascular risk factors. Both datasets included individuals with 0-5 cardiovascular risk factors.

Results Average CAVI was higher in the Japanese than the European group in every age category, with significant differences up to 75 years for males and 85 for females. The correlation of CAVI with age, controlled for cardiovascular risk factors, was slightly higher in Japanese females ($r=0.594$ vs Europeans $r=0.542$) but much higher in European males ($r=0.710$ vs Japanese $r=0.511$). There was a significant correlation between CAVI and total cardiovascular risk factors in the Japanese ($r=0.141$, $p<0.001$) but not the European group. On linear regression, average CAVI was significantly dependent on age, sex, diabetes, BMI, SBP and geographic region. When divided into 'healthy' vs 'high risk', the healthy group had a steeper correlation with age for Europeans ($r=0.644$ vs Japanese $r=0.472$, Fisher's Z $p<0.001$), whereas in the high-risk group, both geographic regions had similar correlations.

Conclusion Japanese patient groups had higher arterial stiffness than Europeans, as measured by CAVI, controlling for cardiovascular risk factors. Europeans had greater increases in arterial stiffness with age in healthy individuals, particularly for males. However, cardiovascular risk factors had a greater impact on the Japanese group.

Condensed abstract (95 words)

This study of 494 European and 1044 Japanese individuals used the cardio-ankle vascular index (CAVI) in European compared to Japanese individuals to investigate how cardiovascular risk factors affect arterial aging across regions.

Average CAVI was higher in the Japanese than the European group in every age category. The correlation of CAVI with age, controlled for cardiovascular risk factors, was slightly higher in Japanese females ($r=0.594$ vs Europeans $r=0.542$) but much higher in European males ($r=0.710$ vs Japanese $r=0.511$). On linear regression, average CAVI was significantly dependent on age, sex, diabetes, BMI, SBP and geographic region.

Key words – arterial stiffness, cardio-ankle vascular index, vascular aging, international comparison

Introduction

The impact of age on cardiovascular health has been the source of inquisition for centuries, since Sir Thomas Sydenham famously purported that "a man is as old as his arteries" [1]. In the past 30 years, burgeoning understanding of the biomechanical configuration and function of the heart and great vessels has focused on clinical consequences including the fragmentation of elastic tissue by cyclical pulsatile stress and, thus, implications for blood pressure (BP) [2], cardio- and cerebro-vascular disease [3]. Since the development of carotid to femoral pulse wave velocity, PWV has been pronounced as the gold standard in assessment of arterial stiffness due to its well-documented correlation with cardiovascular disease outcomes [4]. However, difficulties inherent to the calculation of PWV relate to the heterogeneity of vessel stiffness along the arterial tree, relative differences in method and its intrinsic dependence on blood pressure [5, 6]. More recently, recommendations have been made for standardisation of measurements and reference values to guide clinical use [7].

The cardio-ankle vascular index (CAVI) was established as an alternative to PWV, ostensibly less blood pressure dependent and incorporating the ascending aorta to give a whole body estimation of the vascular stiffness parameter β [8]. Traditionally, CAVI has been advantageous compared to PWV as it has been found to be relatively independent of blood pressure at the time of measurement [9, 10]. However, Spronck et al recently suggested that there may be an inherent dependence on BP and thus proposed a novel formula for calculating CAVI to reduce the impact of transient BP fluctuations [11]. This has since been countered by Shirai et al [12]. Proposed clinical applications of CAVI have concentrated on recognition and diagnosis of cardiovascular disease, with various significant studies exploring its association with cardiovascular risk factors [13], largely in Asian patient groups. In one study, a CAVI value over 9.0 was found to be associated with a level of coronary artery stenosis of at least 75% [14]. It is known that CAVI increases with age and a comprehensive study of over 32,000 participants demonstrated the relationship between CAVI and biological age in a Japanese population in order to propose potential cut-off points for use in clinical practice [15]. Whether different cut offs should be established for different geographical populations has not yet been established, as it is as yet unclear how CAVI changes with age in different groups and the potential genetic and environmental factors which may contribute to this.

Differences in cardiovascular risk between ethnic groups have been demonstrated across geographic regions [16]. Studies have examined differences in arterial compliance between ethnic groups living in the same country, finding higher levels of PWV and augmentation index in South Asians compared to Europeans [17]. However, while some age-specific reference values have been proposed for CAVI in a random sample of Europeans [18], compared to published data from Japan, the association with age and cardiovascular risk factors has not been fully evaluated in a Caucasian compared to a Japanese patient group.

This study measured CAVI in 494 European individuals, including the UK and Italy, and 1044 Japanese individuals. Both datasets included individuals with 0 to 5 cardiovascular risk factors. Our aim was to assess the changes in arterial stiffness with age in a European group compared to a Japanese group, and to investigate how cardiovascular risk factors affect these correlations in patients from different geographic regions.

Methods

Data on CAVI and cardiovascular risk factors were collected from multiple sites across Europe (London, South East England and Italy) and Japan (Toho University Medical Center, Sakura Hospital, Japan). Patients were recruited for data collection based on a range of cardiovascular risk factors including pre-diabetes and cerebrovascular events, as well as healthy individuals. The total patient number was 494 from Europe (222 female, 272 male) and 1044 from Japan (519 female, 525 male). This included 83 age- and sex-matched individuals with no cardiovascular risk factors from each geographic region. The remaining participants had a range of cardiovascular risk factors, defined as: Body mass index (BMI) greater than 30 for Caucasian patients, BMI 25 or greater for Asian patients, clinic or ambulatory average blood pressure of 140/90 or more, total serum cholesterol ≥ 6.2 millimoles per litre (mmol/l) (240 milligrams per decilitre, mg/dl) [19], fasting plasma glucose ≥ 7.0mmol/l (126mg/dl) [18] or 2–h plasma glucose ≥ 11.1mmol/l (200mg/dl) [20], previously diagnosed hypertension, hyperlipidaemia or Diabetes Mellitus, known current or former smoker. The average age of participants was 63.9 ±11.4 (mean ±SD), ranging from 16-93 years. The baseline characteristics of the population are presented in Table 1.

Inclusion and exclusion criteria varied according to the data source, as six different trials contributed data to the databank. See Appendix 1 for details. Written informed consent was obtained from all participants.

The following were measured: medical history and cardiovascular risk factors, BMI, CAVI using VaSera VS-1500N® (Fukuda Denshi, Japan); brachial blood pressure using OMRON705-IT.

Statistical analysis

Data are presented as mean +/- standard deviation with frequencies for each age group across geographic regions. We used a paired t test and ANOVA to compare data between groups and bivariate Pearson correlation analyses to look at the correlation between arterial stiffness and age, calculated separately for each sex and corrected for cardiovascular risk factors. Correlations were compared between groups using two-tailed Fisher's Z transformation. We stratified patients into two groups based on the number of cardiovascular risk factors – the 'healthy' group consisted of patients with 0-2 cardiovascular risk factors, while the 'high-risk' group consisted of patients with 3-5 cardiovascular risk factors. We performed a subgroup analysis to assess the relationship of CAVI with aging in diabetic individuals from both geographic regions. We used simple linear regression analyses to look at the impact of ethnic group and cardiovascular risk factors on CAVI. We used binary logistic regression to look at the impact of average CAVI and cardiovascular risk factors on predicting ethnic group. We used a general linear model to evaluate the impact of different age and geographic groups on CAVI. CAVI0 was calculated according to the method described by Spronck et al. [11], adjusting for diastolic pressure to produce a pressure-normalised equation for CAVI calculation. Statistical analyses were performed using a statistical software package (SPSS Version 24) and a p value of less than 0.05 was considered to be statistically significant.

Results

The average age of the European group was 65.95 (±11.9) vs 62.92 (±10.9) in the Japanese group. There were also significant differences in the distribution of cardiovascular risk factors, with the UK group having more hypertension, smokers and higher BMI while the Japanese group had more hyperlipidaemia, diabetes and marginally higher diastolic BP (Table 1). There was no significant difference in systolic BP or sex between groups. In the whole dataset, average CAVI was higher in the Japanese group than the European group in every age group for both males and females. The differences were significant on ANOVA and independent samples t test up to the age of 75 years for males and 85 years for females (Table 2). The correlation of CAVI with age, controlled for cardiovascular risk factors, was slightly higher in the Japanese group for females ($r=0.594$, $p<0.001$ vs

Europeans r=0.542, p<0.001) but much higher in the European group for males (r=0.710, p<0.001 vs Japanese r=0.511, p<0.001). On two-tailed Fisher's Z transformation, this was statistically significant for males with p<0.001 but not females.

When looking at the relationship of CAVI to cardiovascular risk factors, there was a significant correlation in the Japanese group after correction for sex (r=0.141, p<0.001) but no significant correlation in the European group (Figure 1). When divided into 'healthy' (0-2 cardiovascular risk factors) vs 'high risk (3-5 cardiovascular risk factors), the Japanese group had significantly higher CAVI in both groups up to the age of 75 years, however the European group overtook the Japanese group in the healthy group over this age (Table 3). In the healthy group, there was a much steeper correlation with age for Europeans (r=0.644 vs Japanese r=0.472, p<0.001 on two-tailed Fisher Z transformation) after correction for sex, whereas in the high-risk group, both geographic regions had similar correlations of CAVI and age (Europeans r=0.657 vs Japanese r=0.619, non-significant on Fisher Z transformation). In the healthy group, there was a larger difference in correlation with age for males, with a much steeper correlation in European males (p<0.001), whereas in the high-risk group, the difference was most pronounced in females, with Japanese females having a steeper correlation with age (p=0.06) (Figure 2).

On linear regression, average CAVI was significantly dependent on age, sex, diabetes, BMI, SBP and geographic region (Table 4). In the European group, CAVI was significantly dependent on age, sex, diabetes, BMI and systolic BP. in the Japanese group, CAVI was significantly dependent on age, sex, diabetes, and systolic BP (Supplementary Material – Appendix 2a and b). On binary logistic regression, average CAVI was a significant predictor of geographic region with OR 1.154 (95% confidence interval 1.013-1.315, p=0.031) (Supplementary Material – Appendix 2c). On subgroup analysis, this remained significant for males but became non-significant for females.

Discussion

Geographic differences in arterial stiffness:

Cardiovascular risk varies across populations from different geographic regions, with previous studies showing higher stroke and ischaemic heart disease mortality in South Asian groups compared to White British [16], and higher stroke mortality with lower coronary heart disease in Japan compared to the US and Europe [21]. Ethnic differences in PWV have been demonstrated in migrant populations to the UK, suggesting that arterial stiffness may be a key factor explaining differences in cardiovascular outcomes beyond conventional cardiovascular risk factors [22]. However, the Helius study found that ethnic differences in PWV within the Dutch population were predominately explained by conventional risk factors [23]. In the US, a large population-based cohort study, the Atherosclerosis Risk in Communities (ARIC) study, has shown significant differences between African-American and Caucasian-American groups including stiffer carotid arteries [24] and higher cfPWV [25]. The relatively recent development of CAVI as a widely used measure of arterial stiffness means that there are comparatively few in-depth studies using CAVI to assess these differences. As CAVI is operator and blood pressure independent, this has the potential to offer an easily-accessible tool for clinicians in assessing cardiovascular risk and thus understanding the changes in CAVI that occur with age and cardiovascular risk in different cohorts could aid this assessment.

Our study found higher levels of arterial stiffness in Japanese individuals at all age groups for both sexes, with regression analyses suggesting these differences could not be explained by differences in cardiovascular risk factors. This is in keeping with comparisons by Wohlfahrt et al, who compared

CAVI data from a random sample of the Czech population with published Japanese data, but only up to the age of 65 years [18]. Although there were fewer participants in the oldest age group in our study, the addition of participants up to the age of 85y+ adds significantly to the existing knowledge along with the analysis of health and high risk groups. In contrast, a study comparing healthy Japanese and US adults found that there were similar rates of aging between the two counties, except for females, where Japanese females exhibited slower arterial stiffening [26]. However, this study only went up to the age of 69 years using carotid-femoral and brachial-ankle PWV and did not address the impact of cardiovascular risk factors. Our study suggests that there may be inherent differences in vascular structure and function between groups from different geographic regions, controlling for conventional cardiovascular risk factors, which may explain differences in cardiovascular outcomes, although whether these are environmental or genetic is yet to be determined.

In one study, family history of stroke but not ischaemic heart disease was shown to be associated with higher arterial stiffness in Japanese patients, independent of other risk factors [27]. Genetic influences on cardiovascular risk have been noted to differ between the two regions, for example AB0 gene variants in lipid profiling were included in risk profiling for Japanese but not Caucasian groups [28]. Similarly, some polymorphisms have been linked to arterial stiffness in European American groups but not Japanese [29]. More studies are needed to pinpoint specific genetic differences between the populations if a genetic cause is suspected. As the changing cardiovascular profile of Japanese individuals continues, it is likely we will be able to assess whether increasing environmental similarity to European counterparts may be borne out in a more similar level of cardiovascular risk or whether remaining differences can be attributed to some of these purported genetic elements.

Changes in CAVI with age and cardiovascular risk factors:

CAVI increases with biological age, as has been shown in numerous populations, but the factors that influence the gradient of this relationship are not yet fully understood. For CAVI, comparisons have been presented between Japanese and Chinese populations [30], and published data is available from multiple trials in Japan [17] and Korea [31], showing the increase of CAVI with age and association with cardiovascular risk factors. In a Korean population, the correlation with age was stronger for females compared to males, which was echoed in our findings for the Japanese group, but the opposite to our European group which had a stronger correlation for males.

A study of Japanese participants demonstrated the influence of cardiovascular risk factors on CAVI [32], but this has not been previously established in a UK group. In studies of ethnic differences within the UK population, the impact of cardiovascular risk factors on arterial stiffness, measured through central pulse pressure, has been shown to be greater in individuals of South Asian origin compared to European and African Caribbean [33]. Our study showed that the slope of CAVI vs age was steeper for Europeans in the healthy group, suggesting that baseline changes in arterial stiffness with age are more pronounced in Europeans but that cardiovascular risk factors have a bigger effect in the Japanese group. While currently the mortality from cardiovascular disease is lower in Japan than Europe, with increasing levels of cardiovascular risk factors in Japan, these findings suggest such trends may change in the near future, as the influence of this increased risk on outcomes becomes evident. A CAVI of 8 or more has been proposed as a cut off for using CAVI in secondary prevention of cardiovascular events in an Asian population [34] and a similar value for a UK group should be determined to enable its use in clinical practice.

As there was a high prevalence of diabetes in the Japanese group, analysis was performed on just the diabetic participants to see if the difference in prevalence of diabetes was responsible for the differences between geographic groups. On binary logistic regression, average CAVI was a significant predictor of geographic region for both diabetic individuals and non-diabetic individuals with OR for Japanese vs European of 1.399 for diabetics (95% CI 1.142-1.715, p=0.001) and 1.362 for non-diabetics (95%CI 1.180-1.572, p<0.001).On linear regression, average CAVI amongst diabetics was significantly dependent on geographic region (p<0.001), age, sex and systolic BP. These results suggest that the differences in levels of diabetes between groups were not responsible for the overall results.

CAVI vs CAVI0 for comparing ethnic groups:

CAVI's independence from blood pressure at the time of measurement has long been purported to be one of its main advantages when compared to pulse wave velocity. However, this has been the source of much debate in recent years, with work by Spronck et al [11] suggesting a new formula is required to calculate a CAVI value that is truly blood pressure independent, substituting diastolic blood pressure for a reference pressure. However, this was refuted by Shirai et al [12], commenting that the aforementioned Japanese studies had already demonstrated CAVI's independence from BP, suggesting that both the original and new formula are valid methods for calculating CAVI. Our study showed similar differences between geographic groups and correlations for both CAVI and CAVI0, thus the calculation of CAVI0 did not change our results (Supplementary Material – Appendix 3). The ARIC study suggested that CAVI was less useful as a short term prognostic indicator compared to cfPWV, and found no difference between CAVI and CAVI0 in predicting cardiovascular outcomes [35], but did not look directly at the use of different measures in comparing arterial aging between ethnic groups. The optimal tool for assessing arterial stiffness across geographic regions has yet to be determined, but the operator-independence of CAVI make it a useful tool for making direct comparisons, and our results suggest no advantage to CAVI0 in this context.

CAVI and CAVI0 are based on stiffness index b and a wave equation derived from Newton's second law. The difference between CAVI and CAVI0 is that CAVI employs b over a range of diastolic to systolic pressures and CAVI0 employs b at diastolic pressure. If the length of the arterial pathway being measured is short enough, diastolic pressure would not significantly change. However, it is known that diastolic pressure decreases, and systolic pressure increases from the origin of the aorta to the peripheral arteries. Therefore, in case of the long arterial pathway, adoption of one point of diastolic pressure only becomes less accurate as a reference value representing the entire length of the pathway. In facts, The CAVI values of the Japanese hypertensive group were higher than those of healthy group in both men and women, but the CAVI0 values in young women of the hypertensive group was significantly lower than that of the corresponding healthy group [12].

The main component of CAVI0 is composed of PWV^2/diastolic pressure, so it is supposed that CAVI0 is overly negatively influenced by diastolic pressure. This might be the reason for lower CAVI0 with higher diastolic pressure and higher CAVI0 with lower diastolic pressure.

Limitations

This was a cross-sectional study so we cannot draw conclusions about causality. Although we included a large sample size, there were relatively few participants with no cardiovascular risk factors, so our conclusions cannot be definitive regarding the impact of aging on arterial stiffness in purely healthy individuals. However, as the majority of participants were in older age groups, we do not feel this limits the applicability of our findings, as this is an accurate representation of the variety of risk factors present in the aging population and thus pertinent in a real-world context. Due to the nature of international collaboration, the recruitment methods for each databank varied. However, strict definitions of cardiovascular risk factors were defined before the study and agreed by all participating institutions, and these were considered and adjusted for in all analyses. Equally, as CAVI is operator-independent and the VaSera machines were used by all participating institutions, there should be no confounding due to instrumentation bias. We chose to focus purely on CAVI in this study, as extensive evidence exists on comparing arterial stiffness across regions using PWV and CAVI's operator-independence makes it ideal for international collaboration in this manner. The high number of diabetic participants in the Japanese group may represent a confounding factor, however on subgroup analyses of diabetic groups, this did not significantly affect our results as discussed. Similarly, the differing definition and prevalence of BMI between the regions was determined based on international guidance for the regions in question to be representative of the clinical utility of these measures in the locality in question.

Conclusion

We found that arterial stiffness is higher in Japanese patient groups than Europeans, as measured by CAVI while controlling for cardiovascular risk factors. Europeans had greater increases in arterial stiffness with age in healthy individuals, particularly for males. However, the addition of cardiovascular risk factors had a greater impact on the Japanese group. Further studies looking deeper into the possible differences between these geographic areas may help to explain the results and the differential patterns of cardiovascular disease seen across regions. Future trials should address the association with clinical outcomes in long term prospective studies to assess prognostic value of measures of arterial stiffness in different geographically-determined patient groups.

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For the Italian cohort – no funding was provided for this study

Conflict of interest/disclosure none

Novelty and Significance

What is new

This is the first study to directly compare raw data on arterial stiffness between a European and Japanese group, showing higher levels of CAVI in the Japanese group and differences across sexes and levels of cardiovascular risk.

What is relevant

This offers insight into the different patterns of cardiovascular disease seen across these geographic regions and highlights the potential role of CAVI in demonstrating cardiovascular risk beyond traditional risk factors.

Summary

Arterial stiffness differs across geographic regions, as does the relationship between vascular compliance with age and cardiovascular risk factors.

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Figure Legends

Figure 1 – Box plot of changes in average cardio-ankle vascular index (CAVI) with age stratified by number of cardiovascular risk factors

Figure 1a – Japanese group

Figure 1b – European group

Figure 2 – Scatter plots showing change in average cardio-ankle vascular index (CAVI) with age for Europeans vs Japanese in healthy and high-risk groups

2a) Healthy group (0-2 cardiovascular risk factors) – male

2b) Healthy group (0-2 cardiovascular risk factors) – female

2c) High-risk group (3-5 cardiovascular risk factors) – male

2d) High-risk group (3-5 cardiovascular risk factors) – female

Table 1 – baseline demographics

Parameter	All (n=1538) Mean (±SD)	European group (n=494) Mean (±SD)	Japanese group (n=1044) Mean (±SD)	P value
Age (years)	63.89 (±11.36)	65.95 (±11.93)	62.92 (±10.95)	<0.001
Sex (%M:%F)	52:48	55:45	50:50	0.08
Hypertension (%)	66	72	64	0.001
Diabetes (%)	43	18	55	<0.001
Hyperlipidaemia (%)	56	44	61	<0.001
Current or former smoker (%)	28	43	21	<0.001
Body Mass Index	25.4 (±4.9)	28.7 (±5.4)	23.9 (±3.8)	<0.001
Brachial systolic blood pressure (mmHg)	137.2 (±20.9)	138.5 (±18.3)	136.7 (±22.0)	0.12
Brachial diastolic bloods pressure (mmHg)	80.2 (±11.5)	78.3 (±11.0)	81.1 (±11.6)	<0.001
Cardio-ankle vascular index (CAVI)	9.133 (±1.439)	8.936 (±1.468)	9.227 (±1.416)	<0.001
CAVI0	15.105 (±4.099)	14.709 (±4.048)	15.255 (±4.110)	0.024

Table 2 – Cardio-ankle vascular index (CAVI) comparison between geographic regions across age groups

Table 2a – female

Female	European		Japanese		
Age group	**N**	**Average CAVI ±SD**	**N**	**Average CAVI ±SD**	**P value**
Under 55	28	7.12 ±1.0	97	7.94 ±1.1	0.000
55-64	56	8.43 ±1.2	188	8.76 ±1.0	0.033
65-74	99	8.84 ±1.0	165	9.47 ±1.2	0.000
75-84	30	9.34 ±1.3	65	10.10 ±1.3	0.009
85+	9	10.13 ±1.2	4	11.22 ±1.2	NS

Table 2b – male

Male	European		Japanese		
Age group	**N**	**Average CAVI ±SD**	**N**	**Average CAVI ±SD**	**P value**
Under 55	47	7.48 ±0.9	96	8.41 ±1.3	0.000
55-64	62	8.70 ±1.3	167	9.09 ±1.1	0.022
65-74	96	9.40 ±1.1	196	9.85 ±1.4	0.006
75-84	52	10.50 ±1.3	58	10.51 ±1.8	NS
85+	15	10.54 ±2.0	8	10.55 ±0.7	NS

Table 3 – Cardio-ankle vascular index (CAVI) comparison between geographic regions according to level of cardiovascular risk

Table 3a – comparison of participants who had between 0-2 cardiovascular risk factors (healthy group, n=901)

	European		Japanese		
Age group	**N**	**Average CAVI ±SD**	**N**	**Average CAVI ±SD**	**P value**
Under 55	35	7.30 ±1.1	88	8.08 ±1.2	0.001
55-64	79	8.55 ±1.2	210	8.80 ±1.0	0.072
65-74	137	9.08 ±1.0	201	9.47 ±1.3	0.003
75-84	56	10.04 ±1.4	70	9.94 ±1.7	NS
85+	17	10.69 ±1.6	8	10.56 ±0.8	NS

Table 3b – comparison of participants who had between 3-5 cardiovascular risk factors (high-risk group, n= 637)

	European		Japanese		
Age group	**N**	**Average CAVI ±SD**	**N**	**Average CAVI ±SD**	**P value**
Under 55	40	7.38 ±0.8	105	8.25 ±1.2	0.000
55-64	39	8.63 ±1.3	145	9.10 ±1.1	0.024
65-74	58	9.19 ±1.3	160	9.94 ±1.2	0.000
75-84	26	10.15 ±1.5	53	10.76 ±1.3	0.060
85+	7	9.65 ±2.0	4	11.21 ±1.0	NS

Table 4 – linear regression with average cardio-ankle vascular index (CAVI) as the dependent factor

Variable	Unstandardised B	Standard Error	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Significance
Age*	.066	.003	0.060	0.071	.000*
Sex*	.398	.060	0.280	0.516	.000*
Hypertension	.092	.075	-.055	0.238	.220
Diabetes*	.479	.064	0.354	0.604	.000*
Hyperlipidaemia	.090	.060	-0.028	0.208	.136
Smoking	.087	.069	-0.047	0.221	.204
Body Mass Index*	-.036	.007	-0.051	-0.022	.000*
Average lying systolic blood pressure*	.011	.002	0.008	0.014	.000*
Geographic region *	.205	.081	0.047	0.364	.011*

***statistically significant at the p<0.05 level**

Figure 1 – Box plot of changes in average cardio-ankle vascular index (CAVI) with age stratified by number of cardiovascular risk factors

Figure 1a – Japanese group

Figure 1b – European group

Figure 2 – Scatter plots showing change in average cardio-ankle vascular index (CAVI) with age for Europeans vs Japanese in healthy and high-risk groups

2a) Healthy group (0-2 cardiovascular risk factors) – male

2b) Healthy group (0-2 cardiovascular risk factors) – female

2c) High-risk group (3-5 cardiovascular risk factors) – male

2d) High-risk group (3-5 cardiovascular risk factors) – female

Supplementary Material

Appendix 1 – inclusion and exclusion criteria

Brighton and Sussex CMV Vasc trial used an existing patient research cohort and acquired new participants via GPs.

- Inclusion criteria: 1) White British females and males, 2) Aged 60+.
- Exclusion criteria: 1) immunodeficiency 2) organ transplantation, 3) use of immunosuppressive or immunomodulating drugs within the last year, 4) cancer or treatment within the previous 5 years, 5) insulin dependent diabetes mellitus 6) renal failure (estimated GFR < 20 ml/min), 7) severe liver disease 8) endocrine disorders , 9) autoimmune disease 10) dementia/mental incompetence, 11) alcohol or drug abuse, 12) Inability to lie flat (required for PWV). Participants with acute infections or very recent infections had appointments rescheduled for at least 4 weeks after full recovery.

Brighton and Sussex Arterial Stiffness In lacunar Stroke and TIA (ASIST) trial recruited participants from TIA clinic or inpatients.

- Inclusion criteria: 1) Age 40 years and above (to exclude atypical or rare causes of TIA/stroke). 2) Confirmed diagnosis of TIA or lacunar stroke
- Exclusion criteria: 1) Malignancy, with current active treatment, 2) Patient unable to give informed consent

King's College diabetes trial recruited participants from Guy's and St Thomas' NHS Foundation Trust and surrounding between 2013-2015

- Inclusion criteria: 1) age 18-80 years, 2) previously clinically diagnosed T2DM (n=96) or at risk of T2DM (any of body mass index (BMI) ≥27 kg/m2, a positive family history or glucose intolerance 2 hours after 75 g challenge) (n=58)
- Exclusion criteria: 1) chronic illness of any type likely to interfere with participation, 2) previous adverse reaction to either drug, known allergy to beetroot, 3) impaired renal function (eGFR < 45 mL min-1), 4) HbA1c >11 % = 97 mM/M, 5) pregnant or breast feeding and 6) atrial fibrillation.

Toho University, Sakura Medical Center (Chiba, Japan) recruited outpatients with metabolic disorders underwent CAVI measurement.

- Inclusion criteria: 1) Japanese females and males, 2) Aged 15-92 years, 3) Previously clinically diagnosed T2DM, hypertension and/or hyperlipidaemia.
- Exclusion criteria: 1) Patients with low ankle brachial index (< 0.9), 2) past history of cerebro-cardiovascular events, 3) atrial fibrillation, 4) malignancy, with current active treatment, and 5) Patients unable to give informed consent.

Cardio-vascular disease and cancer screening program organized by the Japan Health Promotion Foundation.

- Inclusion criteria: 1) Japanese females and males, 2) Aged 16-91 years.

- Exclusion criteria: 1) Subjects with low ankle brachial index (< 0.9), 2) taking any medication and had no history of heart disease, hypertension, stroke, diabetes, nephritis or gout, 3) malignancy, with current active treatment, and 4) Patients unable to give informed consent.

Appendix 2 – additional regression tables

2a) Linear regression with average cardio-ankle vascular index (CAVI) as the dependent factor for European group

Variable	Unstandardised B	Standard Error	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Significance
Age*	.198	.014	.170	.226	.000*
Sex*	.863	.303	.267	1.459	.005*
Hypertension*	1.305	.397	.523	2.086	.001*
Diabetes*	1.387	.401	.599	2.175	.001*
Hyperlipidaemia	.052	.311	-.561	.664	.868
Smoking	-.217	.301	-.808	.374	.470
Body Mass Index*	-.191	.033	-.256	-.125	.000*
Average lying systolic blood pressure*	.043	.009	.024	.061	.000*

***statistically significant at the p<0.05 level**

2b) Linear regression with average CAVI as the dependent factor for Japanese group

Variable	Unstandardised B	Standard Error	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Significance
Age*	.193	.010	.174	.212	.000*
Sex*	.728	.212	.313	1.144	.001*
Hypertension	-.065	.252	-.559	.429	.796
Diabetes*	1.711	.204	1.311	2.110	.000*
Hyperlipidaemia	.105	.208	-.304	.514	.615
Smoking	.183	.258	-.323	.689	.477
Body Mass Index	-.037	.028	-.091	.018	.186
Average lying systolic blood pressure*	.045	.005	.034	.055	.000*

***statistically significant at the p<0.05 level**

2c) Binary logistic regression with geographic region as the dependent factor

Variable	Exp (B)	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)	Significance
Age*	.935	.919	.951	.000*
Sex	1.073	.785	1.467	.659
Hypertension*	4.162	2.989	5.794	.000*
Diabetes	1.043	.712	1.526	.829
Hyperlipidaemia	.137	.096	.195	.000*
Smoking	.363	.267	.494	.000*
Body Mass Index*	.726	.697	.757	.000*

Average CAVI*	1.154	1.013	1.315	.031*
Average lying systolic blood pressure	1.000	.991	1.008	.935

Appendix 3 – CAVI0 comparison between geographic regions across age groups

3a) Female

Female	European		Japanese		
Age group	**N**	**Average CAVI0 ±SD**	**N**	**Average CAVI0 ±SD**	**P value**
Under 55	14	10.0 (1.8)	97	11.8 (2.4)	0.009
55-64	44	13.1 (2.8)	188	13.9 (2.8)	0.078
65-74	79	14.5 (2.8)	165	16.0 (3.7)	0.001
75-84	26	16.4 (3.9)	65	18.6 (4.2)	0.025
85+	7	19.5 (4.4)	4	23.2 (4.3)	NS

3b) Male

Male	European		Japanese		
Age group	**N**	**Average CAVI ±SD**	**N**	**Average CAVI ±SD**	**P value**
Under 55	39	10.7 (1.8)	96	12.8 (3.1)	<0.001
55-64	56	13.4 (3.4)	167	14.4 (3.0)	0.054
65-74	84	15.3 (3.2)	196	16.9 (4.2)	0.003
75-84	37	19.4 (3.7)	58	19.3 (4.9)	NS
85+	12	21.2 (3.6)	8	19.6 (3.0)	NS

3c) Comparison of correlation with age between CAVI and CAVI0 in subgroups corrected for cardiovascular risk factors

Subgroup	r for CAVI	p for CAVI	r for CAVI0	p for CAVI0
European males	0.701	<0.001	0.710	<0.001
Japanese males	0.511	<0.001	0.523	<0.001
European females	0.594	<0.001	0.601	<0.001
Japanese females	0.542	<0.001	0.548	<0.001

3d) Comparison of correlation with cardiovascular risk factors between CAVI and CAVI0 in subgroups corrected for sex

Subgroup	r for CAVI	p for CAVI	r for CAVI0	p for CAVI0
European group	0.131	NS	-0.031	NS
Japanese group	0.111	<0.001	0.141	<0.001

3e) Comparison of correlation with age between CAVI and CAVI0 in subgroups based on cardiovascular risk factors – healthy group =0-2 risk factors, high-risk group = 3-5 risk factors

Subgroup	r for CAVI	p for CAVI	r for CAVI0	p for CAVI0
European healthy group	0.644	<0.001	0.635	<0.001
Japanese healthy group	0.472	<0.001	0.494	<0.001
European high-risk group	0.657	<0.001	0.654	<0.001
Japanese high-risk group	0.619	<0.001	0.611	<0.001