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ORIGINAL ARTICLE

Autonomic nervous activation triggered during induction of reactive hyperemia exerts a greater influence on the measured reactive hyperemia index by peripheral arterial tonometry than on flow-mediated vasodilatation of the brachial artery in patients with hypertension

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Flow-mediated vasodilatation of the brachial artery (FMD) and reactive hyperemia index (RHI) measured by peripheral arterial tonometry are known to be weakly associated with one another, but the mechanisms underlying this weak association remain to be clarified. We examined whether the autonomic nervous activation induced by the 5 min forearm clamping used to induce reactive hyperemia might exert any influence on the FMD and RHI in subjects with hypertension. In 115 subjects with hypertension (age 61 ± 1 years), the FMD and RHI were measured simultaneously, and the heart rate variability (HRV) parameters (low-frequency component (LF), high-frequency component (HF), and the ratio (LF/HF) between the two) were calculated from the electrocardiographic recordings obtained before and after the start of forearm clamping. A multivariate linear regression analysis with adjustments for confounding variables demonstrated that the RHI, but not the FMD, was significantly associated with the percent change of the LF/HF associated with forearm clamping (beta = −0.204, $P = 0.043$). In conclusion, autonomic nervous system activation, especially sympathetic activation, induced by 5-min forearm clamping utilized to provoke reactive hyperemia may significantly affect the RHI rather than FMD in subjects with hypertension.
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INTRODUCTION

Endothelial dysfunction is known to have a pivotal role in the initiation/progression of atherosclerosis.[1] In patients with hypertension, flow-mediated vasodilatation of the brachial artery (FMD), a marker of conduit arterial endothelial function, is known to be a predictor of future cardiovascular events.[2–4] The reactive hyperemia index (RHI), a marker of resistance arterial endothelial function measured by peripheral arterial tonometry, is available in clinical settings. Although a significant correlation has been observed between the FMD and RHI in subjects with coronary artery disease,[5,6] a weak to almost no association between the two indicators has been found in the general population.[7,8] Thus, it is still unknown whether the FMD and RHI might reflect similar pathophysiological abnormalities associated with endothelial function, and whether the significance of the relationship between these two indicators may differ among different pathophysiological conditions such as cardiovascular disease, dyslipidemia, obesity, diabetes mellitus and hypertension.

Nitric oxide (NO), derived from the vascular endothelium, is one of the major factors contributing to vasodilatation caused by reactive hyperemia, and its effect is more prominent in the conduit arteries than in the peripheral resistance arterioles.[9] However, Hijmering *et al.*[10] demonstrated that autonomic nervous system activation was also associated with FMD. Several other studies have

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shown an association between autonomic nervous system activity not only with FMD, but also with RHI.[10–13] The 5-min forearm clamping procedure to induce reactive hyperemia is indispensable for both assessments, and it is possible that this procedure affects autonomic nervous system activity.[14] Such activation may influence the tone of the peripheral resistance arterioles rather than that of the conduit arteries. Several studies have also demonstrated that mental stress may affect the peripheral hemodynamics in subjects with hypertension.[15,16]

We hypothesized that FMD may not be closely associated with the RHI, and furthermore, that autonomic nervous system activation induced by 5-min forearm clamping may exert a greater influence on the measured value of the RHI by applanation tonometry than on the FMD in subjects with hypertension. The present study was conducted to clarify whether autonomic nervous activity induced by 5-min forearm clamping may have a greater influence on the measured RHI than on the FMD in subjects with hypertension.

METHODS

Study subjects

The study subjects were hypertensive patients, well-controlled with treatment (blood pressure < 150/95 mm Hg), who were in follow-up care at the outpatient department of the Department of Cardiology of Tokyo Medical University Hospital for at least 6 months. The exclusion criteria were: patients taking beta-blocker medication; patients with coronary artery disease including a history of percutaneous coronary intervention or coronary bypass surgery, severe valvular heart disease, arrhythmia requiring treatment (that is, atrial fibrillation, atrial flutter, permanent pacemaker implantation or frequent ventricular premature beats) or impaired left ventricular systolic function (left ventricular ejection fraction <40%); patients with malignancy; patients receiving treatment with steroids, nonsteroidal anti-inflammatory drugs or immunosuppressive drugs; patients with a serum creatinine level greater than 2.5 mg dl^{-1}; patients with stroke, aortic disease (except peripheral arterial disease) or serious liver disease. Written informed consent was obtained from all the subjects before their participation in the study. The protocol of the study was approved by the Ethics Committee of Tokyo Medical University.

From April 2012 through February 2013, a total of 132 patients with hypertension were enrolled in the study, and their FMD, RHI and heart rate variability (HRV) were measured simultaneously. Of the 132 patients, 17 were excluded because they were being treated with beta-blockers that are well-known to affect autonomic nervous system activity.

Measurements

Flow-mediated vasodilatation of the brachial artery. The method of measurement of FMD is described elsewhere in detail.[17] The subjects were instructed to fast overnight and to abstain from alcohol, smoking, caffeine and antioxidant vitamins for at least 12 h before the measurements. They were asked to rest in the sitting position in a quiet, dark, air-conditioned room (22 °C to 25 °C) for 5 min. Then, they were asked to rest again for at least 15 min in the supine position in the same room before the measurement of FMD. Blood pressure was then measured by the oscillometric method (UA 767; A&D, Saitama, Japan). We performed ultrasound measurements according to the guidelines for ultrasound assessment of FMD.[18]

Using high-resolution ultrasound with a 10-MHz linear array transducer, longitudinal images of the right brachial artery were recorded at baseline and then continuously from 30 s before to 2 min or more after cuff deflation following suprasystolic compression (50 mm Hg over the systolic blood pressure) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with a software for monitoring the brachial artery diameter (Unex, Nagoya, Japan). In brief, continuous recordings of two-dimensional gray-scale images and A-mode waves of the brachial artery in the longitudinal plane were obtained with a novel stereotactic probe-holding device. A segment with clear anterior (intima–lumen) and posterior (lumen–intima) interfaces was manually determined. These border interfaces were identified automatically on the A-mode waves as a signal from the intima–media complex, and the diastolic diameter of the brachial artery beats was synchronized with the electrocardiographic R-waves and tracked automatically. The changes in the diastolic diameter were continuously recorded. Then, FMD was estimated as the percent change of the diameter of the brachial artery at maximal dilatation during reactive hyperemia compared with the baseline value. The reproducibility of the measurement of FMD at our institute is described elsewhere.[18]

Peripheral arterial tonometry. The methodology for measurement of the RHI is described elsewhere.[3,19] During the measurement of FMD, before the forearm cuff occlusion, a peripheral arterial tonometry device (Endo-PAT2000; Itamar Medical, Caesarea, Israel) was placed on the index finger of each hand. Then, the pulse amplitude detected by this device was electronically recorded throughout the FMD measurement. The extent of reactive hyperemia was calculated as the ratio of the average pulse amplitude of the device signal over a 1-min time interval starting 1.5 min after cuff deflation to the average pulse amplitude of the device signal over a 2.5-min time period before cuff inflation. The RHI value was calculated as the ratio of the reactive hyperemia between the two hands.

Heart rate variability. After a 10-minute rest in the supine position in preparation for the measurement of FMD, recording of the electrocardiographic R-R intervals was begun. The recording was not controlled for the respiratory phase. The parameters of HRV (HRVs) were determined from these sample recordings, and the power spectra of the R-R interval were calculated using the maximum entropy method using MemCalc software (Cross-well, Yokohama, Japan).[20,21] Then, the R-R interval, high-frequency power (HF) component, low-frequency power (LF) component and the ratio of the LF to HF (LF/HF ratio) were calculated. These parameters were calculated in three phases (that is, the HRVs calculated from the total of 15-min electrocardiographic recording during the measurement of FMD (HRVs total), from the 5-min electrocardiographic recording from the start of the measurement of FMD to just before forearm cuff occlusion (HRVs measured before clamping), and from the 10-min electrocardiographic recording from the start of cuff occlusion to the end of measurement of the FMD (HRVs after the clamping)). The changes of the HRVs induced by 5-min forearm clamping were calculated as follows: ((HRVs after clamping − HRVs before clamping)/HRVs before clamping) × 100 (%).

Laboratory measurements. Serum levels of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and creatinine, and blood glucose were measured enzymatically in fasting blood samples collected from the subjects.

Statistics. All the data are expressed as the mean ± s.d. The relationships among the variables were assessed by univariate linear regression and multivariate linear regression analyses. The differences in the values of the variables measured before and after the 5-min forearm clamping procedure were assessed by the paired *t*-test. The differences between the groups were assessed by the Mann-Whitney *U*-test or χ^2 test. In all the analyses, *P*-values less than 0.05 were considered to indicate a statistically significant difference. All the analyses were conducted using IBM SPSS (Statistical Package for the Social Sciences) software (19.0J for Windows, IBM/SPSS, Chicago, IL, USA).

RESULTS

Table 1 shows the clinical characteristics of the study subjects. Univariate linear regression analysis did not reveal any significant relationship between FMD and the RHI (nonstandardized coefficient = 0.027, 95% confidence interval = −0.015 to 0.069, standardized coefficient (β) = −0.121, $P = 0.200$).

In the overall study population, the 5-min forearm clamping procedure did not affect either the HRVs or the heart rate (from before the clamping procedure to after the clamping procedure: LF = from 334 ± 625 to 403 ± 1221 ms^2, $P = 0.455$; HF = from

Table 1 Clinical characteristics of the study population

Number of subjects	115
Gender (men/women)	75/40
Age (years)	61 ± 9
SBP (mm Hg)	130 ± 12
DBP (mm Hg)	84 ± 8
Smoking (no. of subjects) (%)	18 (16)
BMI	24.1 ± 3.8
LDL (mmol l^{-1})	2.9 ± 0.8
HDL (mmol l^{-1})	1.4 ± 0.5
TG (mmol l^{-1})	1.5 ± 1.0
FBG (mmol l^{-1})	5.6 ± 1.1
Crnn (μmol l^{-1})	69 ± 19
FMD (%)	4.0 ± 2.5
Baseline diameter (mm)	4.2 ± 0.6
Maximum diameter (mm)	4.4 ± 0.6
RHI	2.2 ± 0.6
LF-total (ms^2)	376 ± 912
HF-total (ms^2)	217 ± 382
LF/HF-total	2.3 ± 1.6
Heart rate-total (beats per min)	61 ± 8
Medications (n(%))	
For diabetes mellitus	11 (10)
For dyslipidemia (statins)	25 (22)
Antihypertensive medication (n(%))	
CCB	74 (64)
ARB/ACEi	88 (77)
Diuretics	33 (29)

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, number of subjects prescribed angiotensin receptor blockers; Baseline diameter, baseline diameter of the brachial artery; BMI, body mass index; CCB, number of subjects prescribed calcium channel blockers; Crnn, serum levels of creatinine; DBP, diastolic blood pressure; diuretics, number of subjects prescribed diuretics; FBG, fasting blood glucose levels; FMD, flow-mediated dilatation of the brachial artery; for dyslipidemia (statins), number of subjects prescribed statins (no subjects prescribed any other drugs than statins); HDL, serum levels of high-density lipoprotein cholesterol; HF, high-frequency power component; LDL, serum levels of low-density lipoprotein cholesterol; LF, low-frequency power component; LF/HF, the LF to HF ratio; maximum diameter, diameter of the brachial artery at maximum dilatation; RHI, reactive hyperemia index measured by peripheral arterial tonometry; SBP, systolic blood pressure; TG, serum levels of triglycerides; -total, calculated from a total of 15-min electrocardiographic recording during the measurement of flow-mediated vasodilatation.

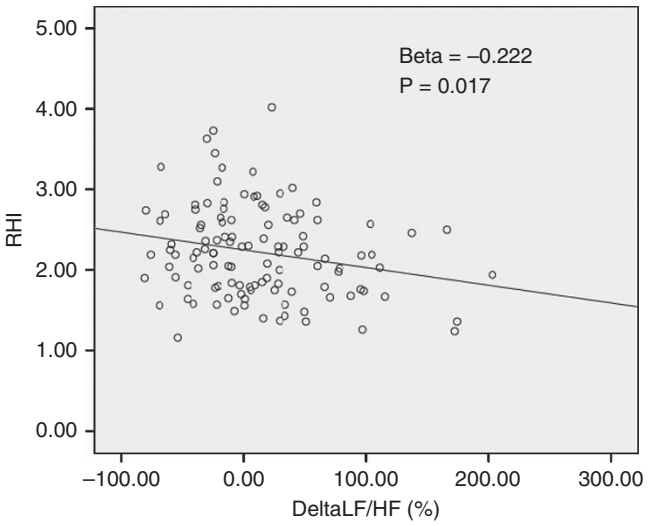

Figure 1 Scatter-plots showing the changes in the ratio between the low-frequency power component and high-frequency power component induced by 5-min forearm clamping and the reactive hyperemia index measured by peripheral arterial tonometry. deltaLF/HF, change of the ratio between the low-frequency power component and high-frequency power component induced by 5-min forearm clamping; RHI, reactive hyperemia index measured by peripheral arterial tonometry.

Table 2 Results of multivariate linear regression analysis to assess the significance of the relationship of the change of LF/HF with the reactive hyperemia index

	RHI ($R^2 = 0.149$)		
Variables	B *(95% CI)*	*Beta*	P-*value*
DeltaLF/HF	−0.002 (−0.004 to 0.000)	−0.204	0.043

Abbreviations: *B*, nonstandardized coefficient; beta, standardized coefficient; CI, confidence interval; Crnn, serum levels of creatinine; delta, change of variables by 5-min forearm clamping; HDL, serum levels of high-density lipoprotein cholesterol; LDL, serum levels of low-density lipoprotein cholesterol; LF/HF, the LF to HF ratio; TG, serum levels of triglycerides. The adjusted variables were age, gender, body mass index, smoking status, systolic blood pressure, heart rate, medication (for diabetes mellitus or dyslipidemia (statins), calcium channel blockers, angiotensin II receptor blocker/angiotensin-converting enzyme inhibitors or diuretics), serum levels of LDL, HDL, TG and Crnn, and blood glucose.

196 ± 266 to 229 ± 473 ms^2, $P = 0.223$; LF/HF = from 2.4 ± 1.7 to 2.3 ± 1.7, $P = 0.626$; heart rate = from 61 ± 8 to 61 ± 8 beats per minute, $P = 0.423$). As shown in the horizontal axis of Figure 1, this procedure was associated with an increase of the LF/HF ratio in 61 of the 115 subjects (53%).

As shown in Figure 1, univariate linear regression analysis revealed a significant relationship between the change of the LF/HF ratio associated with 5-min forearm clamping and the RHI (non-standardized coefficient = −0.002, 95% confidence interval = −0.004 to 0.000, β = −0.222, $P = 0.017$), but not FMD (nonstandardized coefficient = 0.002, 95% confidence interval = −0.006 to 0.010, β = 0.053, $P = 0.574$). Multivariate linear regression analysis with the adjustments revealed a significant relationship between the change of the LF/HF ratio associated with 5-min forearm clamping and the RHI (Table 2). However, no significant associations were observed between any of the HRV parameters (other than the change of LF/HF) and either the FMD or RHI.

The patients were classified into tertile ranges of the change of the LF/HF ratio associated with 5-min forearm clamping: (T1, 203–29%; T2, 28 to −18%; T3, −18 to −81%). Then, they were divided into two groups (that is, T1 *vs.* T2–3). Although clinical characteristics and the FMD were similar between both groups, the RHI was significantly lower in the T1 group than in the T2–3 group (Table 3).

DISCUSSION

To the best of our knowledge, the present study is the first to examine whether the change in autonomic nervous activity associated with the 5-min forearm clamping procedure utilized to induce reactive hyperemia might influence FMD or the RHI.

The findings of the present study were consistent with the results of the Framingham study in which only a weak association was found between the PAT and FMD.[5] This outcome suggests that the pathophysiological abnormalities related to FMD may differ from those associated with the RHI. The reduced NO release in response to stimuli has a central role in the pathophysiology of endothelial dysfunction in the conduit arteries reflected by FMD,[22] whereas metabolic and other factors such as endothelium-derived hyperpolarizing factors are thought be important in the pathophysiology of endothelial dysfunction in the peripheral resistance arteries that are reflected by the RHI.[4,23]

Table 3 Clinical characteristics and endothelial functions between the groups divided by tertile ranges of the change of the ratio between the low-frequency power component and high-frequency power component induced by 5-min forearm clamping

	T1 (n = 38)	*T2–3 (n = 77)*	P-*value*
Gender (men/women)	27/11	48/29	0.239
Age (years)	60 ± 9	62 ± 9	0.166
SBP (mm Hg)	127 ± 12	131 ± 12	0.070
DBP (mm Hg)	84 ± 8	84 ± 7	0.624
Smoking (no. of subjects) (%)	5 (13)	13 (17)	0.412
BMI	24.4 ± 3.3	23.9 ± 4.1	0.515
LDL (mmol l^{-1})	2.9 ± 0.9	2.9 ± 0.8	0.990
HDL (mmol l^{-1})	1.4 ± 0.4	1.4 ± 0.5	0.771
TG (mmol l^{-1})	1.4 ± 0.9	1.5 ± 1.0	0.389
FBG (mmol l^{-1})	5.7 ± 1.1	5.5 ± 1.2	0.322
Crnn (μmol l^{-1})	70 ± 16	69 ± 20	0.747
FMD (%)	4.4 ± 2.5	3.8 ± 2.5	0.147
Baseline diameter (mm)	4.3 ± 0.7	4.2 ± 0.6	0.402
Maximum diameter (mm)	4.4 ± 0.6	4.3 ± 0.6	0.365
RHI	2.0 ± 0.6	2.3 ± 0.6	0.019
Medications (n(%))			
For diabetes mellitus	5 (13)	6 (8)	0.274
For dyslipidemia (statins)	5 (13)	20 (17)	0.090
Antihypertensive medication (n(%))			
CCB	24 (65)	50 (63)	0.505
ARB/ACEi	30 (75)	58 (80)	0.569
Diuretics	11 (29)	22 (29)	0.253

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, number of subjects prescribed angiotensin receptor blockers; Baseline diameter, baseline diameter of the brachial artery; BMI, body mass index; CCB, number of subjects prescribed calcium channel blockers; Crnn, serum levels of creatinine; DBP, diastolic blood pressure; diuretics, number of subjects prescribed diuretics; FBG, fasting blood glucose levels; FMD, flow-mediated dilatation of the brachial artery; for dyslipidemia (statins), number of subjects prescribed statins (no subjects prescribed any other drugs than statins); HDL, serum levels of high-density lipoprotein cholesterol; LDL, serum levels of low-density lipoprotein cholesterol; maximum diameter, diameter of the brachial artery at maximum dilatation; RHI, reactive hyperemia index measured by peripheral arterial tonometry; SBP, systolic blood pressure; T1, subjects with the highest tertile range of the change of the ratio between the low-frequency power component and high-frequency power component induced by 5-min forearm clamping; T2–3, subjects with the middle and lowest tertile ranges of the change of the ratio between the low-frequency power component and high-frequency power component induced by 5-min forearm clamping; TG, serum levels of triglycerides.

The Framingham study suggested that conventional risk factors for cardiovascular disease had different associations with the FMD and RHI.[5] However, thus far, no study has examined the difference in the association of autonomic nervous system activity with the FMD and PAT.

Mental stress has been reported to both increase[24] and decrease the HRV.[25] Thus, the effect of mental stress on autonomic nervous system activity might differ depending on the method used to induce mental stress. The 5-min forearm clamping procedure induces slight mental stress in participants; however, no study thus far has examined the effect of the 5-min forearm clamping procedure on autonomic nervous system activity. In the present study, although the 5-min forearm clamping procedure did not induce a change in the HRV in the entire study population, half of the patients showed a change in the HRV with the clamping procedure. Thus, although mental stress is thought to exert a more pronounced effect on autonomic nervous activity in subjects with hypertension than in normotensive subjects,[26] in the present study, the change in autonomic nervous activity induced by the 5-min clamping procedure differed even among individuals with hypertension.

Sympathetic nervous system activity has a pivotal role in the pathophysiological abnormalities underlying the development of hypertension,[27] and sympathetic activation attenuates the skin blood flow by causing microvascular vasoconstriction.[13] Vasodilatation and vasoconstriction coordinate the control of blood flow in the peripheral resistance arteriole network,[28] and reactive hyperemia causes vasodilatation in the resistant arterioles independent of sympathetic nervous activation.[29] Several studies have demonstrated an effect of autonomic nervous activity on the endothelial function.[10–13] Ghiadoni *et al.*[30] reported that mental stress attenuates FMD. Basically, RHI is programmed such that the values are normalized by simultaneous measurement in the contralateral arm.[14] Nevertheless, Chen *et al.*[12] reported that mental stress also attenuates the RHI. In the present study, the change in the LF/HF ratio, a marker of the sympathetic nervous tone, induced by 5-min forearm clamping was negatively associated with the RHI, but not with the FMD. Therefore, in the present study, sympathetic nervous system activation induced by 5-min forearm clamping appeared to affect the RHI, but not the FMD, presumably via a more pronounced effect on the peripheral resistance arterioles than on the conduit arteries.

Watanabe *et al.*[31] recorded the HRV during the FMD measurements and reported that the LF/HF ratio calculated from the total recording of the R-R intervals during the FMD measurement was negatively correlated with the FMD in patients with coronary artery disease, although the authors did not evaluate the change of the HRV associated with the 5-min forearm clamping procedure. In the present study, unlike the change of the HRV induced by the 5-min forearm clamping procedure, the HRV recorded completely during simultaneous measurement of the FMD and RHI were related to neither FMD nor the RHI. Progressive impairment of the HRV and FMD has been reported to occur together with the progression of atherosclerosis.[1,4,32] The subjects in the present study were patients with hypertension, whereas Watanabe's study subjects had coronary artery disease. Therefore, some of the study subjects of Watanabe might have had advanced atherosclerosis. The HRV calculated from the total recording of the R-R intervals during the measurement of FMD might therefore have been related to FMD in the Watanabe study.

The present study has the following limitations: (1) Some prospective studies have demonstrated that the RHI may be a predictor of future cardiovascular events. A recent meta-analysis reported that acute mental stress adversely affected future cardiovascular risk.[33] We proposed to conduct a study to clarify whether the change in autonomic nervous activity by 5-min forearm clamping might be one of the mechanisms that could explain the predictive values of RHI; (2) The findings of the present study, in which autonomic nervous activation induced by 5-min forearm clamping affected the RHI, need to be confirmed in a variety of conditions such as diabetes mellitus and coronary heart disease, as well as in healthy subjects.

CONCLUSIONS

Autonomic nervous system activation, especially sympathetic activation, induced by 5-min forearm clamping utilized to induce reactive hyperemia may significantly affect the RHI rather than FMD in subjects with hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; **115**: 1285–1295.
2 Ghiadoni L, Versari D, Giannarelli C, Faita F, Taddei S. Non-invasive diagnostic tools for investigating endothelial dysfunction. *Curr Pharm Des* 2008; **14**: 3715–3722.
3 Bonetti PO, Pumper GM, Higano ST, Holmes Jr DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol* 2004; **44**: 2137–2141.
4 Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Lüscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation* 2012; **126**: 753–767.
5 Woo JS, Jang WS, Kim HS, Lee JH, Choi EY, Kim JB, Kim WS, Kim KS, Kim W. Comparison of peripheral arterial tonometry and flow-mediated vasodilation for assessment of the severity and complexity of coronary artery disease. *Coron Artery Dis* (e-pub ahead of print 27 February 2014).
6 Onkelinx S, Cornelissen V, Goetschalckx K, Thomaes T, Verhamme P, Vanhees L. Reproducibility of different methods to measure the endothelial function. *Vasc Med* 2012; **17**: 79–84.
7 Lind L. Relationships between three different tests to evaluate endothelium-dependent vasodilation and cardiovascular risk in a middle-aged sample. *J Hypertens* 2013; **31**: 1570–1574.
8 Hamburg NM, Palmisano J, Larson MG, Sullivan LM, Lehman BT, Vasan RS, Levy D, Mitchell GF, Vita JA, Benjamin EJ. Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011; **57**: 390–396.
9 Tagawa T, Imaizumi T, Endo T, Shiramoto M, Harasawa Y, Takeshita A. Role of nitric oxide in reactive hyperemia in human forearm vessels. *Circulation* 1994; **90**: 2285–2290.
10 Hijmering ML, Stroes ES, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ. Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *J Am Coll Cardiol* 2002; **39**: 683–688.
11 Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA, Joyner MJ. Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *J Physiol* 1997; **504**(Pt 1), 211–220.
12 Chen Y, Dangardt F, Osika W, Berggren K, Gronowitz E, Friberg P. Age- and sex-related differences in vascular function and vascular response to mental stress. Longitudinal and cross-sectional studies in a cohort of healthy children and adolescents. *Atherosclerosis* 2012; **220**: 269–274.
13 Söderström T, Stefanovska A, Veber M, Svensson H. Involvement of sympathetic nerve activity in skin blood flow oscillations in humans. *Am J Physiol Heart Circ Physiol* 2003; **284**: H1638–H1646.
14 Axtell AL, Gomari FA, Cooke JP. Assessing endothelial vasodilator function with the Endo-PAT 2000. *J Vis Exp* 2010: pii: 2167.
15 de Visser DC, van Hooft IM, van Doornen LJ, Hofman A, Orlebeke JF, Grobbee DE. Cardiovascular response to mental stress in offspring of hypertensive parents: the Dutch Hypertension and Offspring Study. *J Hypertens* 1995; **13**: 901–908.
16 Schwartz CE, Durocher JJ, Carter JR. Neurovascular responses to mental stress in prehypertensive humans. *J Appl Physiol* 2011; **110**: 76–82.
17 Tomiyama H, Matsumoto C, Yamada J, Teramoto T, Abe K, Ohta H, Kiso Y, Kawauchi T, Yamashina A. The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. *Hypertens Res* 2008; **31**: 2019–2025.
18 Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; **39**: 257–265.
19 Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, Pumper GM, Lerman LO, Lerman A. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *Eur Heart J* 2010; **31**: 1142–1148.
20 Cerutti C, Barres C, Paultre C. Baroreflex modulation of blood pressure and heart rate variabilities in rats: assessment by spectral analysis. *Am J Physiol* 1994; **266**: H1993–H2000.
21 Ito K, Hirooka Y, Kimura Y, Sagara Y, Sunagawa K. Ovariectomy augments hypertension through rho-kinase activation in the brain stem in female spontaneously hypertensive rats. *Hypertension* 2006; **48**: 651–657.
22 Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Lüscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 1995; **91**: 1314–1319.
23 Trochu JN, Bouhour JB, Kaley G, Hintze TH. Role of endothelium-derived nitric oxide in the regulation of cardiac oxygen metabolism: implications in health and disease. *Circ Res* 2000; **87**: 1108–1117.
24 Sato N, Kamada T, Miyake S, Akatsu J, Kumashiro M, Kume Y. Power spectral analysis of heart rate variability in type A females during a psychomotor task. *J Psychosom Res* 1998; **45**: 159–169.
25 Sloan RP, Shapiro PA, Bagiella E, Bigger Jr JT, Lo ES, Gorman JM. Relationships between circulating catecholamines and low frequency heart period variability as indices of cardiac sympathetic activity during mental stress. *Psychosom Med* 1996; **58**: 25–31.
26 Wallin BG, Delius W, Hagbarth KE. Comparison of sympathetic nerve activity in normotensive and hypertensive subjects. *Circ Res* 1973; **33**: 9–21.
27 Grassi G. Sympathetic neural activity in hypertension and related diseases. *Am J Hypertens* 2010; **23**: 1052–1060.
28 Segal SS. Integration of blood flow control to skeletal muscle: key role of feed arteries. *Acta Physiol Scand* 2000; **168**: 511–518.
29 Pohl U, Herlan K, Huang A, Bassenge E. EDRF-mediated shear-induced dilation opposes myogenic vasoconstriction in small rabbit arteries. *Am J Physiol* 1991; **261** (6 Pt 2), H2016–H2023.
30 Ghiadoni L, Donald AE, Cropley M, Mullen MJ, Oakley G, Taylor M, O'Connor G, Betteridge J, Klein N, Steptoe A, Deanfield JE. Mental stress induces transient endothelial dysfunction in humans. *Circulation* 2000; **102**: 2473–2478.
31 Watanabe S, Amiya E, Watanabe M, Takata M, Ozeki A, Watanabe A, Kawarasaki S, Nakao T, Hosoya Y, Omori K, Maemura K, Komuro I, Nagai R. Simultaneous heart rate variability monitoring enhances the predictive value of flow-mediated dilation in ischemic heart disease. *Circ J* 2013; **77**: 1018–1025.
32 Gautier C, Stine L, Jennings JR, Sutton-Tyrrell K, Muldoon MB, Kamarck TW, Kaplan GA, Salonen J, Manuck SB. Reduced low-frequency heart rate variability relates to greater intimal-medial thickness of the carotid wall in two samples. *Coron Artery Dis* 2007; **18**: 97–104.
33 Chida Y, Steptoe A. Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. *Hypertension* 2010; **55**: 1026–1032.