Hodgson JM, Woodman RJ, Croft KD, Ward NC, Bondonno CP, Puddey IB, Lukoshkova EV, Head GA. Relationships of vascular function with measures of ambulatory blood pressure variation. Atherosclerosis 2014;233(1):48-54.

http://hdl.handle.net/11187/2099

Original Article

Relationships of vascular function with measures of ambulatory blood pressure variation

Jonathan M Hodgson[a], Richard J Woodman[b], Kevin D Croft[a], Natalie C Ward[a], Catherine P Bondonno[a] , Ian B Puddey[a], Elena V Lukoshkova [c], Geoffrey A Head [d]

[a]School of Medicine and Pharmacology, University of Western Australia, Western Australia, Australia;
[b]Discipline of General Practice, Flinders University, Adelaide, South Australia, Australia;
[c]Department of Cardiovascular Regulation, Russian Cardiology Research Center, Moscow, Russia;
[d]BakerIDI Heart and Diabetes Institute, Melbourne, Australia

Background: Characteristics of short-term blood pressure (BP) variation may influence cardiovascular disease risk via effects on vascular function.
Objective: In a cross-sectional study of a group of normotensive and hypertensive subjects we investigated the relationships of measures of short-term BP variation with brachial artery vasodilator function.
Methods: A total of 163 normotensive (n=72) and treated hypertensive (n=91) men and women were recruited from the general population. Measures of systolic and diastolic BP variation were calculated from 24 h ambulatory BP assessments and included: (i) rate of measurement-to-measurement BP variation (SBP-var and DBP-var); and (ii) day-to-night BP dip (SBP-dip and DBP dip). Endothelium-dependent vasodilation was assessed as flow-mediated dilation (FMD) and endothelium-independent vasodilation was assessed in response to glyceryl trinitrate (GTN). Relationships were explored using univariate and multivariate linear regression.
Results: The relationships of brachial artery vasodilator function with BP variation were not significantly different between normotensive and treated hypertensive subjects, therefore these groups were combined for analysis. In univariate analysis, higher SBP-var (P<0.001) and lower DBP-dip (P=0.004) were associated with lower FMD; and higher SBP-var (P=0.002) and lower SBP-dip (P=0.003) and DBP-dip (P=0.001) were associated with lower GTN-mediated dilatation. In multivariate analysis, lower SBP-dip (P=0.007) and DBP-dip (P=0.03) were independently associated with lower GTN response.
Conclusions: Our results indicate that a lower day-to-night BP dip is independently associated with impaired smooth muscle cell function. Although rate of BP variation was associated with measures of endothelial and smooth muscle cell function, relationships were attenuated after accounting for age and BP.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death globally [1]. High blood pressure (BP) is the leading risk factor for CVD and total mortality [2] and the measurement of BP provides the primary marker of individual risk. However, other characteristics of BP in addition to its absolute level may also contribute to risk. Two measures linked to increased CVD risk are a high BP variability [3-5] and a blunted day-to-night BP dip [6-8]. High short-term BP variation [4, 9-11] and blunted day-to-night BP dip [7, 12-14] have also been associated with outcomes related to atherosclerosis and hypertensive end-organ damage. However, data supporting a link between vascular dysfunction and characteristics of short-term BP variation are limited [15-17].

The deterioration of vascular function is associated with the development of CVD [18]. Vascular function is influenced by both endothelial and smooth muscle cell function; change in endothelial function is an early event in the development of atherosclerosis and CVD [19, 20]; and endothelial and smooth muscle cell functions are impaired with CVD [21]. In addition, reduced brachial flow-mediated dilatation (FMD) is associated with elevated risk for future CVD events [22]. Hypertension appears to be causally related to the development of endothelial and smooth muscle cell dysfunction, but the mechanisms are not fully understood [23]. It is possible that short-term fluctuations in BP, even within the normotensive range, and a blunted day-to-night BP dip resulting from elevated night time BP could also contribute to the development of vascular dysfunction, independent of the level of BP.

The cross-sectional relationships of brachial artery vasodilator function, assessed using ultrasound, with measures of BP variation over 24 h were investigated in normotensive and treated hypertensive individuals. We have also explored whether any observed relationships are independent of BP, age and other traditional CVD risk factors.

Atherosclerosis. 2014;233:48-54
Correspondence to Dr Jonathan Hodgson, School of Medicine and Pharmacology, GPO Box X2213 Perth, Western Australia 6847 Tel: 618 9224 0267; Fax: 618 9224 0246 Email: Jonathan.Hodgson@uwa.edu.au

Conflicts of interest and source of funding: No conflicts of interest to be declared. JMH and GAH were supported by National Health and Medical Research Council Fellowships. NCW was supported by a Medical Research Foundation/University of Western Australia Postdoctoral Fellowship

Received 4 September 2013
Received in revised form
26 November 2013
Accepted 10 December 2013
Available online 8 January 2014

METHODS

Participants

Volunteers were recruited from the general population of Perth, Australia using print media advertisements. Both normotensive and treated hypertensive men and women were

targeted during recruiting. Normotensive participants had a 24 h ambulatory systolic BP (SBP) of between 100 and 135 mm Hg. All participants had a 24 h ambulatory SBP less than 160 mm Hg. All hypertensive participants had a previous physician diagnosis of hypertension and were taking one or more antihypertensive drugs for at least 3 months prior to the study. The study was conducted from the University of Western Australia School of Medicine and Pharmacology located at Royal Perth Hospital in Western Australia. All participants were aged 35 to 75 y; had a body mass index of 19 to 36 kg/m^2; were non-diabetic; were not taking nitrate medication, the oral contraceptive or hormone replacement; had no major current or recent (< 6 months) illness; and had not taken nutritional supplements or antibiotics at least 3 weeks prior to the study. Usual medication was taken as prescribed. All individuals provided a written informed consent. Ethics approval was obtained from the University of Western Australia Ethics Committee and the Royal Perth Hospital Human Ethics Committee and the research was carried out in accordance with the Declaration of Helsinki of the World Medical Association.

Design

The cross-sectional relationship between measures of ambulatory BP variation and vascular function were investigated. Data on traditional CVD risk factors were also collected. Participants attended the School of Medicine and Pharmacology research unit located at Royal Perth Hospital, where all measurements were performed.

Ambulatory blood pressure and measures of variation

BP was assessed as 24 h ambulatory BP with BP measured every 20 min during the day and every 30 min at night [24, 25]. Participants were instructed to continue their usual daily activities and to avoid any vigorous exercise. Measurements showing an error code or those with a pulse pressure of less than 20 mm Hg were excluded from the analysis. BP traces were considered complete if more than 80% of the recordings were valid.

The rate of variation of SBP and diastolic BP (DBP) (SBP-var and DBP-var) were calculated from the 24 h ambulatory BP traces for the whole 24 h period as well as day time (08:00–20:00) and night time (22:00-06:00) periods separately according to a previously described method [25]. Measurement-to-measurement BP variation was calculated using the slope of the change in SBP and DBP between each reading over time [25]. The slope for each data point in the recording is calculated and allocated to a particular hour. The absolute values of the slopes of each hour as well as the preceding and subsequent hour were averaged to give one value. This is a modification of the method described in detail by Zakopoulos et al [9]. We also assessed BP variability using the SD of each individual's measurements over the 24-hr period. The SD of BP measurements during the day time (08:00–20:00) and night time (22:00-06:00) periods were also calculated. The weighted SD (SBP-wSD and DBP-wSD) were calculated according to the method of Bilo et al [26]. The day-to-night dip in BP (SBP-dip and DBP-dip) were calculated as the difference between the mean day time and night time BP.

Vascular function: brachial artery vasodilator function

Brachial artery vasodilator function was assessed after a 12 h fast using ultrasound according to a previously published protocol [27, 28]. A single trained ultrasonographer performed all measurements. Endothelium-dependent vasodilation was assessed as response to forearm ischaemic FMD and endothelium-independent vasodilation was assessed in response to glyceryl trinitrate (GTN). The FMD response provides a guide to nitric oxide-mediated endothelial cell function [29]. The GTN response provides a guide to changes in smooth muscle cell function that may affect the observed changes in flow-mediated dilation [30]. Analysis of scans was performed with semiautomated edge-detection software [27, 28]. This automatically calculated the brachial artery diameter, corresponding to the internal diameter. This was gated to the R wave of ECG, with measurements taken at end diastole. Responses were calculated as the maximum percentage change in brachial artery diameter from baseline. The analysis was performed by an experienced observer. Reproducibility studies have previously demonstrated an intrasubject coefficient of variation of 14.7 and 17.6% for flow-mediated dilatation and glyceryl trinitrate-mediated dilatation respectively [28].

Body weight, biochemistry and health history

Body weight was recorded with participants wearing light clothing and no footwear using Wedderburn digital scales (20–200 kg) (Wedderburn, Perth, Western Australia, Australia). Height was measured at baseline using a wall-mounted stadiometer. Waist circumference was measured at the top of the iliac crest. Fasting lipids were measured in serum samples, using routine laboratory methods in the PathWest Laboratory at Royal Perth Hospital, Western Australia. A questionnaire was used to gather information about age, use of medication, smoking history and physical activity. Because there were only 4 current smokers included, smoking status was defined as ever smoked (which included current and ex-smokers) or never smoked (non-smokers). Level of physical activity was classed as either inactive or active. Participants recorded their usual levels of physical activity as either: 'inactive'; 'low and irregular'; 'moderate and irregular'; 'moderate and regular'; or 'substantial and regular'. Participants describing their usual levels of physical activity as inactive or low and irregular were then classed as inactive and all other participants were classed as active.

Statistical analysis

Data were analyzed using SPSS (version 15; SPSS Inc, Chicago, IL) and Stata (version 12.1; StataCorp, Texas, USA). Descriptive characteristics are presented as the range, mean and SD for quantitative variables and n for qualitative variables. The Pearson correlation coefficient was used for univariate analysis to explore the degree and direction of association between measures of BP variation and CVD risk factors, as well as the association between different measures of BP variation. The independent samples t-test was used to assess differences in measures of BP variation between groups. Before performing linear regression analysis of vascular function on BP variation, we assessed whether there was evidence that the relationship between measures of brachial artery vasodilator function and measures of BP variation were different between normotensive and treated hypertensive subjects using linear regression models for vascular function with terms for BP variation, hypertensive status and a BP variation X hypertensive status interaction term. Since the interaction terms were not significant, the study population as a whole was used to asses the relationships between FMD, GTN and measures of BP variation and traditional CVD risk factors using simple linear regression. Multivariate linear regression analysis was used to investigate whether the relationships of measures of SBP variation with FMD and GTN response were independent of traditional CVD risk factors. Three different model adjustments were performed; age-adjusted; age + 24 h SBP-adjusted; and a fully adjusted model which included age, 24 h SBP, gender, waist circumference, physical activity and serum triglycerides. Backwards stepwise linear regression analysis with a P-value for

inclusion of <0.05 was used to further explore whether SBP variation was associated with FMD and GTN response independently of traditional cardiovascular risk factors. Traditional CVD risk factors associated with measures of vascular function were considered for entry into the models.

RESULTS

Participant characteristics and cross-sectional associations

The study population included 163 participants (72 normotensive and 91 treated hypertensive participants). The characteristics of the participants are provided in Table 1. Correlations of measures of BP variation with each other and with traditional CVD risk factors for all participants and for the normotensive and treated hypertensive participants are presented in supplementary Table S1.

Univariate analysis: CVD risk factors, BP variation and vascular function

The relationships of brachial artery vasodilator function with BP variation were not significantly different between normotensive and treated hypertensive subjects (Table S2), therefore these groups were combined for analysis (Table 2). Increasing age, male gender, higher waist circumference, higher SBP and DBP, treated hypertension and higher serum triglycerides were all associated with lower endothelium-dependent FMD and endothelium-independent GTN-mediated dilatation of the brachial artery. A higher 24 h SBP-var and a higher 24 h SBP-wSD were both associated with lower FMD and GTN-mediated dilatation of the brachial artery. These associations were primarily driven by daytime rather than night-time BP variation. Measures of DBP variation were not associated with measures of vascular function. A lower DBP-dip was associated with lower FMD response, and a lower SBP-dip and DBP-dip were associated with a lower GTN response.

Multivariate analysis: BP variation and vascular function

The relationships of vascular function with measures of BP variation were next explored in multivariate analyses (Table 3). Adjustment for age and SBP substantially attenuated the relationships for both 24 h SBP-var (Figure 1) and 24 h SBP-wSD, such that they were no longer significantly associated with vascular function. A similar attenuation of the associations for daytime SBP-var and daytime SBP-wSD with measures of vascular function were observed, such that these were not significant (results not presented). Further adjustment for gender and other CVD risk factors did not alter these non-significant relationships. The univariate association of DBP-dip with FMD was substantially attenuated after adjusting for age. However, in multivariate analysis lower SBP-dip (Figure 2) and DBP-dip were independently associated with lower GTN response. Separate sub-group analyses of the normotensive and hypertensive groups resulted in similar relationships, and did not alter interpretation of the findings (results not presented).

The relationships of measures of BP variation with measures of vascular function were next explored using stepwise linear regression analysis. Age (partial r=-0.37, P<0.001), male gender (partial r=-0.22, P=0.006) and 24 h SBP (partial r=-0.23, P=0.004) were independently associated with FMD; and male gender (partial r=-0.20, P=0.02), 24 h SBP (partial r=-0.36, P<0.001) and SBP-dip were independently associated with GTN-mediated dilatation (Table S3). The relationship for gender is known to be primarily due to the differences between men and women in artery diameter, which strongly influence the measures of artery relaxation.

DISCUSSION

Our hypothesis was that features of the short-term fluctuations in BP may contribute to the development of vascular dysfunction, independently of BP. To explore this we investigated the relationships of measures of endothelium-dependent and independent brachial artery vasodilator function with measures of BP variation over 24 h in normotensive and treated hypertensive subjects. Measures of vascular function were negatively associated with the rate of SBP variation and positively associated with the day-to-night systolic and diastolic BP dip in univariate analysis. In multivariate analysis, relationships of vascular function with rate of SBP variation were attenuated and largely explained by a combination of age and SBP. A lower SBP-dip and DBP-dip were independent predictors of lower endothelium-independent vasodilation.

There is accumulating data in support of the idea that higher short-term BP variation [5, 31] and a blunted BP dip at night [7, 12-14] contribute to CVD risk. If a higher BP variation and a blunted night time BP dip are causally linked with tissue and organ damage, then vascular endothelial and smooth muscle cells are clear targets for this damage. Changes in shear stress, which correspond with changes in BP, regulate endothelial cell function via release of nitric oxide and other vasodilators [29, 32]. Continuous and ongoing rapid changes in shear stress and BP could contribute to endothelial dysfunction [32]. Our results indicate that any additional shear stress due to higher BP variation, beyond the shear stress caused by BP alone, may not significantly contribute to endothelial or smooth muscle cell dysfunction. Similarly, a blunted night time BP dip was not independently related to poorer endothelial cell function assessed using the FMD response. However, the finding that a lower BP dip was independently associated with reduced endothelium-independent GTN-mediated dilatation does suggest a link with smooth muscle cell function.

Few studies have explored the hypothesis that short-term fluctuations in BP could contribute to the development of vascular dysfunction, independently of BP. In a small cross-sectional study (n=36) Diaz et al [15] explored the relationships of 24 h ambulatory BP variation, assessed using two measures including the rate of BP variation, with endothelium-dependent and independent brachial artery vasodilation. Consistent with the present study, their analysis provided evidence that higher BP variation was univariately associated with a lower endothelium-dependent FMD, but that BP variation was not independently associated with FMD. However, Diaz et al [15] also found a significant positive association between BP variation and endothelium-independent dilatation of the brachial artery, which for DBP variation was independent of age and DBP. This result is in contrast to our results. which did not demonstrate any independent inverse relationship between DBP variation and endothelium-independent dilatation. Limited data also support the link between night time BP, and blunted night time BP dip specifically with endothelial function [16, 17]. The results of Higashi et al [17] indicated that endothelium-dependent vasodilation and night-time BP dip might be blunted as a result of a decrease in nitric oxide release. In contrast to our results, endothelium-independent vasodilatation was not related to dipping status [17]. The reasons for the discrepancies among the studies are not clear, but may relate to differences in the study design and study populations.

Using stepwise linear regression, we also further explored the relationships of all the factors measured with endothelium-dependent and independent dilatation of the brachial artery. We found that for FMD mediated dilatation age, gender and

SBP were the only independent predictors. This result is similar to the findings from the Framingham study, which included almost 3000 participants [33]. The gender relationship with vascular function is primarily due to differences between men and women in artery diameter, which in turn is strongly related to response.

We acknowledge that our study has a number of limitations. The cross-sectional nature of the study allows only exploration of the hypothesis but not testing of any causal link. In addition, the study population may not be representative of the general population, and although our sample size is larger than previous studies in this area, it is still modest. The study population did not include untreated hypertensive individuals. It is possible that relationships in this group may be different to both normotensive and treated hypertensive individuals, where drug treatment to a normotensive range may influence the relationship. The hypertensive population were on a variety of medications, which can alter BP variability [34]. This could obscure relationships in sub-populations.

In conclusion our results indicate that a lower day-to-night BP dip is independently associated with impaired smooth muscle cell function. Although we have shown that rate of systolic BP variation was associated with both endothelium-dependent and independent brachial artery vasodilator function in univariate analysis, relationships were attenuated after accounting for age and BP. Therefore, our study does not provide evidence that characteristics of BP variation over 24 h are independently associated with endothelial dysfunction. Additional research is needed to confirm our results and also determine whether a blunted night time BP dip is causally related to impaired smooth muscle cell function.

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Table 1 Characteristics of all participants, the normotensive participants and the treated hypertensive participants

	n	Minimum	Maximum	Mean	SD
All participants (n=163)	163	35	75	58.6	9.6
Age (years)	163	35	75	58.6	9.6
Male : Female (n)	163			80:83	
Body mass index (kg/m^2)	163	19.0	35.9	26.5	3.9
Waist circumference (cm)	162	63.5	120.5	88.4	13.5
Mean 24 h systolic BP (mm Hg)	161	104.3	159.8	127.3	11.9
Mean 24 h diastolic BP (mm Hg)	161	55.3	101.0	75.6	8.7
Ever smoked : Never smoke (n)	163			61:102	
Physically inactive : Physically active (n)	163			75:88	
Serum total cholesterol (mmol/L)	162	2.8	7.7	5.1	1.0
Serum triglycerides (mmol/L)	162	0.4	5.3	1.4	0.9
24 h weighted SD for systolic BP (mm Hg)	160	6.2	22.7	11.0	2.8
24 h weighted SD for diastolic BP (mm Hg)	160	4.7	16.0	8.5	1.8
24 h rate of systolic BP variation (mm Hg/h)	160	14.6	36.3	23.3	4.0
24 h rate of diastolic BP variation (mm Hg/h)	160	12.0	26.7	18.2	3.0
Day-to-night systolic BP dip (mm Hg)	160	-17.2	51.3	13.3	9.2
Day-to-night diastolic BP dip (mm Hg)	160	-11.5	30.0	11.4	6.2
Normotensive (n=72)*					
Age (years)	72	35	74	55.0	10.6
Male : Female (n)	72			24:48	
Body mass index (kg/m^2)	72	19.0	33.9	24.5	3.3
Waist circumference (cm)	72	63.5	120.0	79.9	10.5
Mean 24 h systolic BP (mm Hg)	71	104.6	134.6	120.0	9.1
Mean 24 h diastolic BP (mm Hg)	71	56.0	85.8	71.6	6.3
Ever smoked : Never smoke (n)	72			50:22	
Physically inactive : Physically active (n)	72			35:37	
Serum total cholesterol (mmol/L)	72	3.5	7.7	5.2	0.9
Serum triglycerides (mmol/L)	72	0.4	2.6	1.0	0.4
24 h weighted SD for systolic BP (mm Hg)	70	6.17	15.24	9.7	1.9
24 h weighted SD for diastolic BP (mm Hg)	70	4.87	12.03	7.9	1.4
24 h rate of systolic BP variation (mm Hg/h)	70	14.6	32.3	21.6	3.5
24 h rate of diastolic BP variation (mm Hg/h)	70	13.2	24.1	17.7	2.7
Day-to-night systolic BP dip (mm Hg)	70	-4.3	34.8	13.9	7.7
Day-to-night diastolic BP dip (mm Hg)	70	-1.9	24.6	12.1	5.3
Treated hypertensive (n=91)					
Age (years)	91	43	75	61.5	7.5
Male : Female (n)	91			56:35	
Body mass index (kg/m^2)	91	19.9	35.9	28.1	3.6
Waist circumference (cm)	90	65.0	120.5	95.1	11.8
Mean 24 h systolic BP (mm Hg)	90	104.3	159.8	133.1	10.75
Mean 24 h diastolic BP (mm Hg)	90	55.3	101.0	78.7	9.1
Ever smoked : Never smoke (n)	91			52:39	
Physically inactive : Physically active (n)	91			40:51	
Serum total cholesterol (mmol/L)	89	2.8	7.2	5.1	1.0
Serum triglycerides (mmol/L)	89	0.4	5.3	1.7	1.0
24 h weighted SD for systolic BP (mm Hg)	90	6.2	22.7	11.9	3.0
24 h weighted SD for diastolic BP (mm Hg)	90	4.7	16.0	9.0	2.0
24 h rate of systolic BP variation (mm Hg/h)	90	17.1	36.3	24.6	4.0
24 h rate of diastolic BP variation (mm Hg/h)	90	12.0	26.7	18.6	3.1
Day-to-night systolic BP dip (mm Hg)	90	-17.2	51.3	12.7	10.3
Day-to-night diastolic BP dip (mm Hg)	90	-11.5	30.0	10.8	6.8

* mean 24 h systolic BP < 135 mm Hg

Table 2 Univariate regression analysis for flow-mediated dilatation (FMD) and glyceryltrinitrate-mediated dilatation (GTN) of the brachial artery versus traditional cardiovascular disease risk factors and measures of blood pressure (BP) variation (n=163)

	FMD (%)				GTN (%)			
	B (SE)	95%CI	Adjusted r^2	P	B (SE)	95%CI	Adjusted r^2	P
Age (y)	-0.15 (0.03)	-0.20, -0.10	0.18	<0.001	-0.17 (0.06)	-0.28, -0.06	0.05	0.002
Gender (M)	-1.78 (0.53)	-2.82, -0.75	--	0.001	-4.08 (1.02)	-6.10, -2.07	--	<0.001
Body mass index (kg/m^2)	-0.07 (0.07)	-0.21, 0.07	0.01	0.32	-0.40 (0.14)	-0.67, -0.14	0.05	0.003
Waist circumference (cm)	-0.07 (0.02)	-0.11, -0.03	0.08	<0.001	-0.15 (0.04)	-0.23, -0.08	0.09	<0.001
24 h systolic BP (mm Hg)	-0.11 (0.02)	-0.15, -0.06	0.13	<0.001	-0.24 (0.04)	-0.32, -0.16	0.17	<0.001
Day systolic BP (mm Hg)	0.09 (0.02)	-0.14, 0.05	0.10	<0.001	-0.19 (0.04)	-0.28, -0.11	0.12	<0.001
Night systolic BP (mm Hg)	-0.10 (0.02)	-0.13, -0.06	0.13	<0.001	-0.23 (0.04)	-0.30, -0.16	0.21	<0.001
24 h diastolic BP (mm Hg)	-0.07 (0.03(	-0.13, -0.01	0.02	0.03	-0.20 (0.06)	-0.32, -0.09	0.07	0.001
Treated hypertension (yes)	-2.1 (0.5)	-3.1, -1.1	--	<0.001	-4.5 (1.0)	-6.5, -2.5	--	<0.001
Cigarette smoking status (ever smoked)	-0.6 (0.6)	-1.8, 0.5	--	0.24	-1.5 (1.1)	-3.7, 0.6	--	0.16
Physical activity (inactive)	-1.1 (0.5)	-2.2,0.7	--	0.04	-1.2 (1.1)	-3.3, 0.9	--	0.25
Serum total cholesterol (mmol/L)	0.3 (0.3)	-0.2, 0.9	0.01	0.24	0.8 (0.6)	-0.3, 1.9	0.01	0.14
Serum triglycerides (mmol/L)	-0.7 (0.3)	-1.3,-0.1	0.03	0.02	-2.0 (0.6)	-3.1, -0.8	0.06	0.001
24 h rate of systolic BP variation (mm Hg/h)	-0.27 (0.07)	-0.40, -0.14	0.10	<0.001	-0.41 (0.13)	-0.67, -0.16	0.06	0.002
Day rate of systolic BP variation (mm Hg/h)	-0.22 (0.05)	-0.32, -0.11	0.09	<0.001	-0.26 (0.11)	-0.48, -0.05	0.03	0.02
Night rate of systolic BP variation (mm Hg/h)	-0.08 (0.06)	-0.19, 0.03	0.01	0.15	-0.27 (0.11)	-0.49, -0.06	0.04	0.01
24 h rate of diastolic BP variation (mm Hg/h)	0.04 (0.09)	-0.14, 0.22	0.00	0.66	0.07 (0.18)	-0.29, 0.44	0.00	0.70
24 h weighted SD for systolic BP (mm Hg)	-0.28 (0.10)	-0.47, -0.09	0.05	0.004	-0.22 (0.19)	-0.59, 0.16	0.01	0.25
Day SD for systolic BP (mm Hg)	-0.33 (0.07)	-0.47, 0.19	0.11	<0.001	-0.37 (0.15)	-0.66, 0.09	0.04	0.01
Night SD for systolic BP (mm Hg)	0.06 (0.07)	-0.08, 0.20	0.01	0.39	0.19 (0.14)	-0.09, 0.46	0.01	0.17
24 h weighted SD for diastolic BP (mm Hg)	-0.09 (0.15)	-0.39, 0.21	0.00	0.56	-0.19 (0.29)	-0.78, 0.39	0.00	0.52
Day-to-night systolic BP dip (mm Hg)	0.05 (0.03)	-0.01, 0.11	0.01	0.08	0.17 (0.06)	0.06, 0.29	0.05	0.003
Day-to-night diastolic BP dip (mm Hg)	0.13 (0.04)	0.04, 0.21	0.05	0.004	0.27 (0.08)	0.11, 0.44	0.06	0.001

Table 3 Crude and adjusted multivariate linear regression analysis for endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent glyceryltrinitrate-mediated dilatation (GTN) with measures of blood pressure (BP) variation (n=163).

	FMD (%)				GTN (%)			
	B (SE)	95%CI	Adjusted r^2	P	B (SE)	95%CI	Adjusted r^2	P
24 h rate of systolic BP variation (mm Hg/h)								
Unadjusted	**-0.27 (0.07)**	**-0.40, -0.14**	**0.10**	**<0.001**	**-0.41 (0.13)**	**-0.67, -1.16**	**0.06**	**0.002**
Age-adjusted	**-0.16 (0.07)**	**-0.29, -0.03**	**0.20**	**0.02**	**-0.30 (0.14)**	**-0.57, -0.02**	**0.08**	**0.03**
Age + 24 h systolic BP-adjusted	-0.09 (0.07)	-0.22, 0.05	0.26	0.19	-0.07 (0.14)	-0.34, 0.20	0.18	0.61
Fully adjusted*	-0.09 (0.07)	-0.23, 0.05	0.26	0.20	-0.13 (0.14)	-0.40, 0.14	0.25	0.35
24 h weighted SD for systolic BP (mm Hg)								
Unadjusted	**-0.28 (0.10)**	**-0.47, -0.09**	**0.05**	**0.004**	-0.22 (0.19)	-0.59, 0.16	0.01	0.25
Age-adjusted	-0.13 (0.09)	-0.32, 0.05	0.19	0.16	-0.04 (0.20)	-0.43, 0.34	0.05	0.83
Age + 24 h systolic BP-adjusted	-0.03 (0.10)	-0.22, 0.16	0.23	0.75	0.25 (0.19)	-0.12, 0.62	0.19	0.18
Fully adjusted*	-0.08 (1.0)	-0.28, 0.13	0.26	0.46	0.12 (0.20)	-0.28, 0.51	0.25	0.56
Day-to-night systolic BP dip (mm Hg)								
Unadjusted	0.05 (0.03)	-0.01, 0.11	0.01	0.08	**0.17 (0.06)**	**0.06, 0.29**	**0.05**	**0.003**
Age-adjusted	0.02 (0.03)	-0.04, 0.07	0.17	0.54	**0.13 (0.06)**	**0.02, 0.29**	**0.09**	**0.02**
Age + 24 h systolic BP-adjusted	0.01 (0.03)	-0.04, 0.07	0.23	0.59	**0.13 (0.05)**	**0.03, 0.24**	**0.23**	**0.02**
Fully adjusted**	0.01 (0.03)	-0.05, 0.06	0.27	0.77	**0.15 (0.05)**	**0.04, 0.26**	**0.26**	**0.007**
Day-to-night diastolic BP dip (mm Hg)								
Unadjusted	**0.13 (0.04)**	**0.04, 0.21**	**0.05**	**0.004**	**0.27 (0.08)**	**0.11, 0.44**	**0.06**	**0.001**
Age-adjusted	0.06 (0.04)	-0.03, 0.14	0.18	0.18	**0.20 (0.08)**	**0.03, 0.38**	**0.09**	**0.03**
Age + 24 h systolic BP-adjusted	0.06 (0.04)	-0.03, 0.14	0.18	0.18	**0.20 (0.08)**	**0.04, 0.36**	**0.22**	**0.01**
Fully adjusted**	0.04 (0.04)	-0.04, 0.13	0.26	0.34	**0.18 (0.08)**	**0.02, 0.35**	**0.25**	**0.03**

* The fully adjusted model includes age, 24 h systolic BP, gender, waist circumference, physical activity, serum triglycerides, and day-to-night diastolic BP dip

** The fully adjusted model includes age, 24 h systolic BP, gender, waist circumference, physical activity, serum triglycerides and 24 h rate of systolic BP variation

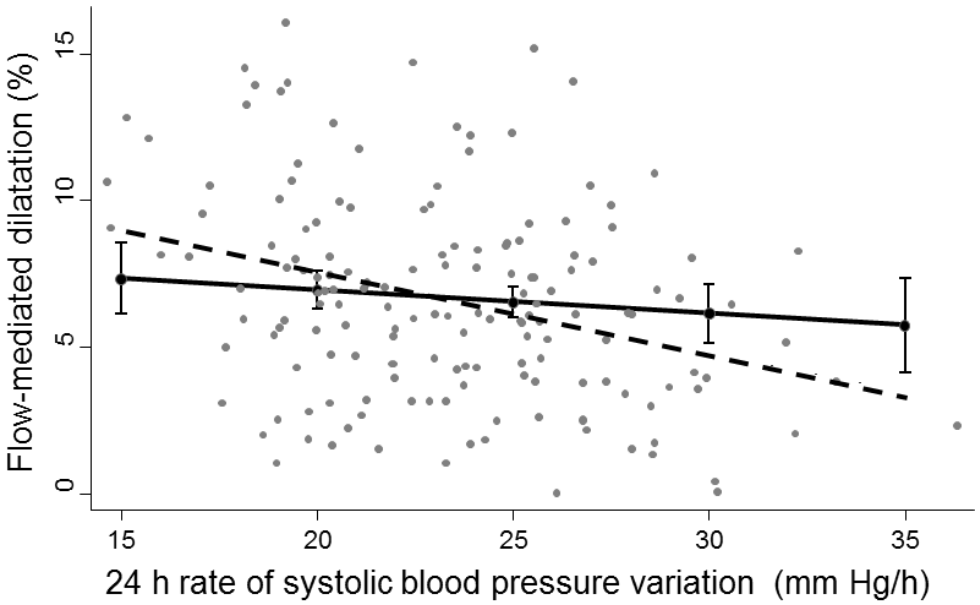

Figure 1 Relationship between flow-mediated dilatation of the brachial artery (%) and the 24 h rate of systolic blood pressure variation: unadjusted least squares linear regression (- - - -) and adjusted least squares linear regression (——). The adjusted model included age, gender, 24 h systolic BP, waist circumference, physical activity, serum triglycerides and day-to-night diastolic blood pressure dip.

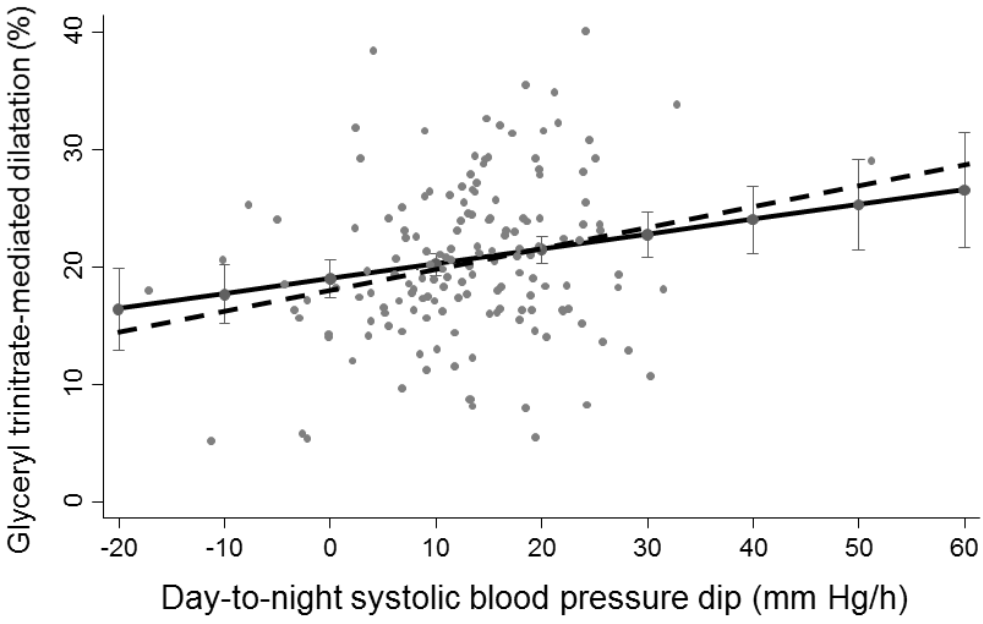

Figure 2 Relationship between glyceryl trinitrate-mediated dilatation of the brachial artery (%) and the day-to-night systolic blood pressure dip: : unadjusted least squares linear regression (- - - -) and adjusted least squares linear regression (——). The adjusted model included age, gender, 24 h systolic BP, waist circumference, physical activity, serum triglycerides and 24 h rate of systolic blood pressure variation.

SUPPLEMENTARY DATA

RELATIONSHIPS OF VASCULAR FUNCTION WITH MEASURES OF AMBULATORY BLOOD PRESSURE VARIATION

Table S1 Pearson correlation coefficients (r) for systolic and diastolic BP variation versus traditional risk factors for cardiovascular disease

	24 h weighted SD for systolic BP (mm Hg)	24 h weighted SD for diastolic BP (mm Hg)	24 h rate of systolic BP variation (mm Hg/h)	24 h rate of diastolic BP variation (mm Hg/h)	Day-to-night systolic BP dip (mm Hg)	Day-to-night diastolic BP dip (mm Hg)
All participants (n=163)						
Age (years)	0.309***	0.008	0.377***	-0.041	-0.224**	-0.322***
Body mass index (kg/m^2)	0.174*	0.288***	0.103	0.024	-0.054	-0.043
Waist circumference (cm)	0.265***	0.308***	0.211**	0.103	-0.058	-0.099
Serum total cholesterol (mmol/L)	0.097	0.179*	0.040	0.068	0.226**	0.187*
Serum triglycerides (mmol/L)	0.221**	0.348***	0.168*	0.137	-0.016	0.001
24 h systolic BP (mm Hg)	0.366***	0.248**	0.372***	0.126	-0.090	-0.105
24 h diastolic BP (mm Hg)	0.169*	0.262***	0.173*	0.131	-0.108	-0.026
24 h weighted SD for systolic BP (mm Hg)	1	0.656***	0.572***	0.171*	0.363***	0.234**
24 h weighted SD for diastolic BP (mm Hg)	0.656***	1	0.272***	0.490***	0.350**	0.401**
24 h rate of systolic BP variation (mm Hg/h)	0.572**	0.272***	1	.302***	0.113	0.055
24 h weighted SD for diastolic BP (mm Hg)	0.171*	0.490**	.302***	1	0.211**	0.230**
Day-to-night systolic BP dip (mm Hg)	0.363***	0.350***	0.113	0.211**	1	0.877***
Day-to-night diastolic BP dip (mm Hg)	0.234**	0.401***	0.055	0.230**	0.877***	1
Normotensive (n=72)						
Age (years)	0.461***	0.037	0.317***	-0.055	-0.078	-0.216
Body mass index (kg/m^2)	-0.025	0.159	-0.131	-0.163	-0.048	-0.097
Waist circumference (cm)	0.032	0.060	0.011	-0.024	0.018	-0.142
Serum total cholesterol (mmol/L)	0.250*	0.097	0.142	-0.115	-0.029	-0.037
Serum triglycerides (mmol/L)	-0.014	-0.144	-0.068	-0.170	-0.281*	-0.344**
24 h systolic BP (mm Hg)	0.419***	0.187	0.363***	0.051	-0.112	-0.186
24 h diastolic BP (mm Hg)	0.127	0.190	0.142	0.154	-0.091	0.056
24 h weighted SD for systolic BP (mm Hg)	1	0.589*8*	0.537***	0.135	0.294*	0.121
24 h weighted SD for diastolic BP (mm Hg)	0.589***	1	0.230	.425***	0.362**	0.424***
24 h rate of systolic BP variation (mm Hg/h)	0.537**	0.230	1	0.333**	0.095	-0.014
24 h weighted SD for diastolic BP (mm Hg)	0.135	0.425**	0.333**	1	0.334**	0.263*
Day-to-night systolic BP dip (mm Hg)	0.294*	0.362**	0.095	0.334***	1	0.831***
Day-to-night diastolic BP dip (mm Hg)	0.121	0.424***	0.014	0.263*	0.831***	1
Treated hypertensive (n=91)						

Age (years)	0.058	-0.217*	0.283**	-0.143	-0.350**	-0.413***
Body mass index (kg/m^2)	0.009	0.197	-0.047	0.020	-0.017	0.061
Waist circumference (cm)	0.074	0.239*	0.002	0.054	-0.051	-0.006
Serum total cholesterol (mmol/L)	0.089	0.264*	0.034	0.192	0.343**	0.289**
Serum triglycerides (mmol/L)	0.099	0.368***	0.041	0.157	0.077	0.147
24 h systolic BP (mm Hg)	0.115	0.080	0.147	0.063	-0.043	0.004
24 h diastolic BP (mm Hg)	-0.026	0.155	-0.024	0.047	-0.090	0.001
24 h weighted SD for systolic BP (mm Hg)	1	.627**	0.495***	0.123	0.472***	0.366***
24 h weighted SD for diastolic BP (mm Hg)	0.627**	1	0.165	0.497***	0.399***	0.463***
24 h rate of systolic BP variation (mm Hg/h)	0.495**	0.165	1	.230*	0.177	0.158
24 h weighted SD for diastolic BP (mm Hg)	0.123	0.497**	.230*	1	0.172	0.240*
Day-to-night systolic BP dip (mm Hg)	0.172	0.399***	0.177	0.472***	1	0.897***
Day-to-night diastolic BP dip (mm Hg)	0.240*	0.463***	0.158	0.366***	0.897***	1

* P<0.05; ** P<0.01; *** P<0.001

Table S2 Univariate regression analysis for flow-mediated dilatation (FMD) and glyceryltrinitrate-mediated dilatation (GTN) of the brachial artery versus measures of blood pressure (BP) variation in normotensive and hypetenstive participants

	FMD (%)				GTN (%)			
	B (SE)	95%CI	Adjusted r^2	P	B (SE)	95%CI	Adjusted r^2	P
Normotensive (n=72)*								
24 h rate of systolic BP variation (mm Hg/h)	-0.26 (0.11)	-0.49, -0.03	0.06	0.03	-0.24 (0.21)	-0.67, 0.19	0.02	0.26
24 h rate of diastolic BP variation (mm Hg/h)	0.14 (0.15)	-0.16, 0.44	0.00	0.35	0.31 (0.29)	-0.27, 0.89	0.02	0.29
Day-to-night systolic BP dip (mm Hg)	-0.00 (0.05)	-0.11, 0.10	0.00	0.91	0.18 (0.10)	-0.02, 0.38	0.05	0.07
Day-to-night diastolic BP dip (mm Hg)	0.04 (0.08)	-0.11, 0.20	0.01	0.57	0.31 (0.14)	0.04, 0.59	0.07	0.03
Hypertensive (n=91)								
24 h rate of systolic BP variation (mm Hg/h)	-0.17 (0.07)	-0.34, -0.00	0.03	0.05	-0.24 (0.17)	-0.58, 0.10	0.02	0.17
24 h rate of diastolic BP variation (mm Hg/h)	0.07 (0.11)	-0.15, 0.29	0.00	0.53	0.15 (0.22)	-0.29, 0.59	0.01	0.50
Day-to-night systolic BP dip (mm Hg)	0.07 (0.03)	0.00, 0.13	0.05	0.04	0.16 (0.07)	0.03, 0.29	0.06	0.02
Day-to-night diastolic BP dip (mm Hg)	0.14 (0.05)	0.05, 0.24	0.09	0.004	0.21 (0.10)	0.01, 0.41	0.05	0.04

* Mean 24 h systolic BP < 135 mm Hg

Table S3 Stepwise regression analysis of flow-mediated dilatation (FMD) and glyceryl trinitrate-mediated (GTN) dilatation of the brachial artery with measures of systolic blood pressure (BP) variation (n=163).

	FMD (%)				GTN (%)			
	B (SE)	95%CI	Partial correlation	P	B (SE)	95%CI	Partial correlation	P
Age (years)	-0.13 (0.03)	-0.17, -0.08	-0.37	<0.001	-0.08 (0.05)	-0.19, 0.02	-0.13	0.11
Gender (M)	-1.4 (0.5)	-2.3, -0.4	-0.22	0.006	-2.4 (1.0)	-4.3, -0.5	-0.20	0.02
24 h systolic BP (mm Hg)	-0.06 (0.02)	-0.11, -0.02	-0.23	0.004	-0.20 (0.04)	-0.28, -0.11	-0.36	<0.001
Day-to-night systolic BP dip (mm Hg)					0.12 (0.05)	0.02, 0.23	0.19	0.02

* Age was force entered into the model then other factors considered for inclusion in the model included gender, 24 h systolic BP, waist circumference, physical activity serum triglycerides, 24 h rate of systolic BP variation, 24 h weighted SD for systolic BP, age, Day-to-night systolic BP dip and Day-to-night diastolic BP dip.