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VISIT-TO-VISIT AND 24-H BLOOD PRESSURE VARIABILITY: ASSOCIATION WITH ENDOTHELIAL AND SMOOTH MUSCLE FUNCTION IN AFRICAN AMERICANS

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Abstract

The purpose of this study was to investigate the association of visit-to-visit and 24-h BP variability with markers of endothelial injury and vascular function. We recruited 72 African Americans who were non-diabetic, non-smoking, and free of cardiovascular and renal disease. Office BP was measured at three visits and 24-h ambulatory BP monitoring was conducted to measure visit-to-visit and 24-h BP variability, respectively. The 5-min time-course of brachial artery flow-mediated dilation and nitroglycerin-mediated dilation were assessed as measures of endothelial and smooth

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muscle function. Fasted blood samples were analyzed for circulating endothelial microparticles. Significantly lower CD31+CD42− endothelial microparticles were found in participants with high visit-to-visit SBP variability or high 24-h DBP variability. Participants with high visit-to-visit DBP variability had significantly lower flow-mediated dilation and higher nitroglycerin-mediated dilation at multiple time-points. When analyzed as continuous variables, 24-h mean arterial pressure variability was inversely associated with CD62+ endothelial microparticles; visit-to-visit DBP variability was inversely associated with flow-mediated dilation normalized by smooth muscle function and was positively associated with nitroglycerin-mediated dilation; and 24-h DBP variability was positively associated with nitroglycerin-mediated dilation. All associations were independent of age, gender, BMI, and mean BP. In conclusion, in this cohort of African Americans visit-to-visit and 24-h BP variability were associated with measures of endothelial injury, endothelial function, and smooth muscle function. These results suggest that BP variability may influence the pathogenesis of cardiovascular disease, in part, through influences on vascular health.

Keywords

Blood pressure variability; endothelial function; endothelial microparticles; smooth muscle function; blood pressure; ambulatory blood pressure monitoring

INTRODUCTION

Evidence has accumulated to show that blood pressure variability (BPV) has a striking relationship with cardiovascular (CV) risk. Most notably, several general-population based studies have reported that measures of BPV within a 24-h period were independent predictors of CV events and all-cause mortality[1–3]. In addition to fluctuations in BP from reading-to-reading over a 24-h period, recent retrospective analyses of several randomized controlled trials and general-population based studies have shown that visit-to-visit variability in clinic BP is also a powerful predictor of CV events and all-cause mortality, independent of mean BP[4–6].

Despite the mounting evidence, scant attention has been paid to the mechanisms by which high BPV may confer greater CV risk. Moreover, it is still not clear whether 24-h and visit-to-visit BPV are two risk markers with different underlying causes and sequelae. Data from sinoaortic denervation (SAD) rats, the animal model of high BPV, suggests that the augmented mechanical stress placed on the vasculature as a result of increased BPV leads to the impairment and injury of the endothelium[7–9]. In accordance with these findings, we reported in a preliminary study that both 24-h and visit-to-visit BPV were associated with measures of endothelial- and smooth muscle-dependent vasodilation[10]. To the best of our knowledge, outside of this preliminary study, no previous clinical study has assessed the relationship between BPV and measures of endothelial health to elucidate the potential pathogenic effects of high BPV on the vasculature.

Endothelial dysfunction has conventionally been considered to be solely an impairment in endothelial-dependent vasodilation. However, this narrow focus has provided insight concerning only one aspect of endothelial cell function (i.e. their ability to induce arterial

vasodilation). In recent years, laboratory-based investigations have elucidated the processes underlying endothelial dysfunction and have identified endothelial microparticles (EMPs) as a novel direct biomarker of endothelial cell injury[11]. These endothelial-derived microparticles are submicroscopic fragments that are released from the plasma membrane of endothelial cells into circulation when endothelial cells are subjected to a number of stress conditions, including cell activation or cell apoptosis. Although long thought to be cellular debris, EMPs present in the blood are now widely considered to be a biological marker reflective of endothelial cell activation, degeneration, and apoptosis which have been shown to be elevated in a number of disease states[11].

The purpose of this study was to extend upon our preliminary findings by comprehensively investigating the association of 24-h and visit-to-visit BPV with measures of endothelial and smooth muscle function through the assessment of the 5-min time-course vasodilatory response to reactive hyperemia and nitroglycerin, respectively, in a cohort of African Americans with a range of BP levels. In order to evaluate the relationship between BPV and endothelial health beyond just one aspect of endothelial cell function, as a secondary purpose we also investigated the association of 24-h and visit-to-visit BPV with circulating levels of endothelial microparticles (EMPs), a measure of endothelial cell injury.

METHODS

Participants

African Americans within the city of Philadelphia were recruited via newspaper advertisements. Participants were required to be 40–75 years old, sedentary, non-diabetic, non-smoking, have a clinic BP < 160/100 mmHg, and have no evidence or history of CV disease, hypercholesterolemia, or renal disease. Participants on medications that effect CV hemodynamics or who were on more than one antihypertensive medication were excluded from the study. Any participant receiving antihypertensive monotherapy (n = 17) was tapered off their medication. Medications were withdrawn for a 2-week washout period, at minimum, before testing. These inclusion criteria were used to create a more homogeneous group of middle- to older-age African Americans who were at low-to-moderate risk for CV disease, but who were free of substantial medical co-morbidities that could confound data interpretation. Each participant gave written informed consent. The protocol was approved by the Temple University Institutional Review Board.

Office BP Measurements

Office BP measurements were obtained in accordance with JNC-7 guidelines by laboratory personnel on three separate visits in a quiet, temperature controlled room[12]. Measurements were obtained in the morning, except under rare circumstances when participant scheduling issues required afternoon measurement. Seated BP measurements were measured using an aneroid sphygmomanometer (Omron, Model 11-675D, Vernon Hills, IL) after 5-min of quiet rest. An appropriate size cuff was determined by upper arm circumference. For each visit, BP measurements were performed in triplicate, 5-min apart, and the average of the three values was used as the representative BP for the visit. For primary analyses, the standard deviation was calculated to define visit-to-visit BPV. As a sensitivity analysis,

visit-to-visit BPV was also expressed as the coefficient of variation. The mean duration between visits 1 and 2 and visits 2 and 3 were 12 ± 2 days and 14 ± 3 days, respectively.

24-h ABPM

Participants underwent 24-h ABPM using a non-invasive portable BP monitor (SpaceLabs, Model 90219, Redmond, WA) as previously described[13]. Briefly, monitoring began in the morning of each participant's typical day. A BP cuff was fitted to the participant's non-dominant arm with cuff size determined by upper arm circumference. BP measures were obtained at 30-min intervals during the day (6:00am–10:00pm) and 60-min intervals at night (10:00pm–6:00am). Only recordings of good technical quality (≥ 80% of valid measurements) were included in final analyses.

Mean values were calculated for 24-h SBP, DBP, mean arterial pressure (MAP), and heart rate (HR). Systolic BPV (SBPV), diastolic BPV (DBPV), MAP variability (MAPV), and HR variability (HRV) during the 24-h period were calculated using the average real variability (ARV) index[13].The rationale for selecting the ARV index to calculate 24-h BPV is based on previous studies that have reported the ARV index to be a more reliable representation of time series variability than standard deviation or coefficient of variation[14,15].

Forearm Hemodynamics Studies

Brachial artery diameter was measured in response to increased flow (flow-mediated vasodilation: FMD) and nitroglycerin (nitroglycerin-mediated vasodilation: NMD) as previously described[16]. Briefly, measurements were performed in the morning following an overnight fast in a quiet, temperature controlled room. All measurements of brachial artery diameter were taken after 10-min of lying in the supine position. Baseline images of the right brachial artery were first obtained. Reactive hyperemia was then induced by distal occlusion using a cuff inflated to suprasystolic pressure for 5-min on the right forearm. Brachial artery diameter was imaged immediately after and 1-min, 2-min, 3-min, 4-min, and 5-min post-cuff release. After at least 15-min of rest, new baseline images were obtained and a 0.4mg nitroglycerin tablet was administered sublingually to assess smooth muscle-dependent vasodilation. Images were recorded immediately after nitroglycerin administration and 1-min, 2-min, 3-min, 4-min, and 5-min post-nitroglycerin administration.

Data Analysis of Forearm Hemodynamics Studies

Arterial diameter was measured from the anterior to the posterior 'm' line at end-diastole, incident with the R-wave on the electrocardiogram. FMD and NMD were calculated as the relative (%) increase in diameter from baseline at each time-point. Peak % increases in diameter ($\%FMD_{peak}$ and $\%NMD_{peak}$) and the 5-min time-course area under the curve (AUC; FMD_{AUC} and NMD_{AUC}) are reported as measures of endothelial and smooth muscle function. As an additional index of endothelial function, the FMD/NMD ratio was calculated using the peak % increase in diameter from FMD and NMD in order to correct for vascular smooth muscle function in each participant.

Circulating EMP Measurements

Circulating EMPs were quantified using a method we have previously described[17,18]. Briefly, fasted blood samples were collected into EDTA tubes and were centrifuged at 2,000 *g* for 20-min at 4°C immediately after collection. Plasma samples were then stored at −80°C until measurement. On the day of analysis, samples were thawed and centrifuged at 1500 *g* for 20-min at room temperature to obtain platelet poor plasma (PPP). The top two-thirds volume of PPP were then further centrifuged at 1500 *g* for 20-min at room temperature. A volume of 100 μl supernatant was then incubated with two different flurochrome-labeled antibody combinations to distinguish between EMP subpopulations: CD31-PE with CD42b-FITC; and CD62E-PE alone. Samples were analyzed using BD LSRII flow cytometer (BD Biosciences, San Jose, CA) and BD FACSDIVA software (v 6.1.3; BD Biosciences). Forward scatter (FSC), side scatter (SSC) and each fluorescent channel were set in logarithmic scale. The upper limit gate on FSC/SSC plot was determined by 0.9 μm standard beads. Events within this gate were further analyzed on FITC/PE dot plot. CD31+ CD42− or CD62E+ events were defined as EMPs and were expressed as events per μl plasma.

Statistical Analyses

Of the 72 participants enrolled in the study, 8 participants did not have office BP measures on 3 separate visits and were excluded from all analyses of visit-to-visit BPV. Data are presented as means ± s.e. The univariate relationship of each clinical, demographic, and BPV variable with FMD, NMD, the FMD/NMD ratio, CD31+CD42− EMPs, and CD62+ EMPs were tested with Spearman's rank correlation coefficient (r_s). All variables significantly associated with a measure of vascular function in univariate analyses was then inserted separately into a multivariate regression model containing variables known to impact vascular function: age, gender, BMI, and mean BP (corresponding clinic or 24-h BP). In a sensitivity analysis, all univariate and multivariate models were repeated using visit-to-visit BPV expressed as the coefficient of variation. Each model was evaluated for multicollinearity among variables using the variance inflation factor. The variance inflation factor was < 5 for all models (range: 1.099–3.499).

For secondary analyses, participants were classified into groups according to their BPV. Any participant at or above the median for the BPV measure were classified as having high BPV, while any participant below the median were classified as having low BPV. Comparisons between groups were tested using the Mann-Whitney U test for continuous variables and Pearson's χ^2-test for dichotomous variables. Two-way repeated-measures ANOVA examining the main effects of time and BPV group on time-course FMD and NMD were run using Greenhouse-Geisser correction as appropriate. All FMD and NMD measures were log transformed for ANOVAs to account for non-normal distribution. Repeated-measures ANCOVA was conducted to evaluate group differences after adjustment for age, gender, BMI, and mean BP (corresponding clinic or 24-h BP). Post-hoc comparisons were conducted using the Mann-Whitney U test. Statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL).

Power analyses were conducted using the PS software (Power and Sample Size Calculations, version 3.0.12, Vanderbilt University, Nashville, TN). Power calculations

were based on the relationship of SBPV from ABPM with %FMD and, separately, the FMD/NMD ratio. The sample provided 80% power to detect an association between SBPV and %FMD with a slope of 0.37 or greater (α level 0.05, two-sided) and between SBPV and the FMD/NMD ratio of 0.03 or greater.

RESULTS

Study population characteristics

The study population consisted of 72 African Americans, including 12 males (16.7%) and 60 females (83.3%). Mean age was 51.7 ± 0.7 years and mean BMI was 32.7 ± 0.6 kg/m^2. The mean office SBP and DBP were 126.7 ± 1.5 mmHg and 81.1 ± 1.0 mmHg, respectively. Of the 72 participants, 24 (33.3%) were normotensive, 32 (44.4%) were pre-hypertensive, and 15 (20.8%) were hypertensive. For ABPM, mean 24-h SBP and DBP were 128.0 ± 0.5 mmHg and 78.9 ± 1.1 mmHg, respectively. According to AHA criteria[19], 27 participants (37.5%) had abnormal 24-h BP levels (24-h BP ≥ 135/85 mmHg).

Correlates of Endothelial-Dependent and Smooth Muscle-Dependent Vasodilation

The association of all clinical variables and BP parameters with any of the vascular measures in univariate and multivariate analyses are presented in Table 1 and Table 2, respectively. Age and HDL-C were negatively associated with %FMD$_{peak}$, however neither remained a significant predictor in multivariate analyses. For FMD$_{AUC}$, LDL-C and triglycerides were positively associated, while HDL-C and visit-to-visit DBPV were negatively associated with FMD$_{AUC}$. In multivariate analysis, only LDL-C and triglycerides remained significant predictors of FMD$_{AUC}$. When endothelial function was assessed by normalizing FMD by smooth muscle function (FMD/NMD ratio), the only variables associated with the FMD/NMD ratio in univariate analyses were body weight and visit-to-visit DBPV. In multivariate analyses, body weight and visit-to-visit DBPV remained significant predictors of the FMD/NMD ratio.

For measures of smooth muscle function, HDL-C, visit-to-visit SBPV, visit-to-visit DBPV, and 24-h DBPV all were significantly associated with %NMD$_{peak}$, while visit-to-visit SBPV, visit-to-visit DBPV, and 24-h DBPV were significantly associated with NMD$_{AUC}$. After adjusting for covariates in multivariate regression analyses, visit-to-visit DBPV remained the only significant predictor of %NMD$_{peak}$, while visit-to-visit DBPV and 24-h DBPV remained the only significant predictors of NMD$_{AUC}$.

Mean BP levels from office or 24-h ABPM were not significantly associated with any measure of endothelial or smooth muscle function. In sensitivity analyses, all results were similar when visit-to-visit BPV was expressed as the coefficient of variation instead of the standard deviation (data not shown).

When the relationships between brachial artery diameter and FMD and NMD derived variables were tested in univariate analyses, a significant positive association was found between FMD$_{peak}$ and NMD$_{peak}$ (r_s = 0.41; p = 0.01), but not between FMD$_{AUC}$ and NMD$_{AUC}$ (r_s = 0.11; p = 0.53). Brachial artery diameter was associated with NMD$_{peak}$ (r_s = −0.37; p = 0.006), but not with FMD$_{peak}$ (r_s = 0.11; p = 0.48). The FMD/NMD ratio was

significantly associated with FMD_{peak} (r_s = 0.44; p = 0.005), NMD_{peak} (r_s = −0.56; p = < 0.001), FMD_{AUC} (r_s = 0.35; p = 0.03), and NMD_{AUC} (r_s = −0.61; p < 0.001).

Correlates of EMPs

In univariate analyses, body weight (r_s = −0.32; p = 0.02) and 24-hour DBPV (r_s = −0.33; p = 0.02) were negatively associated with CD31+CD42− EMPs. In multivariate analysis adjusting for age, gender, BMI, and mean BP, only body weight (B = −0.04 [95%CI: −0.08–−0.01], partial correlation = −0.24; p = 0.01) remained a significant predictor of CD31+CD42− EMPs. For CD62+ EMPs, triglyceride levels (r_s = 0.33; p = 0.01) was positively associated, while 24-h MAPV (r_s = −0.29; p = 0.03) was negatively associated with CD62+EMPs. After adjustment for age, gender, BMI, and mean BP, triglyceride levels (B = 0.21 [95%CI: 0.04–0.37], partial correlation = 0.34; p = 0.02) and 24-h MAPV (B = −4.79 [95%CI: −8.78–−0.81], partial correlation = −0.33; p = 0.02) both remained significant predictors of CD62+ EMPs. Mean BP levels from office or 24-h ABPM were not significantly associated with either EMP marker.

Visit-to-Visit BPV Group Comparisons

Participants with higher visit-to-visit SBPV had significantly lower CD31+CD42− EMPs and a trend for lower CD62+ EMPs when compared to participants with lower visit-to-visit SBPV (Supplemental Table S1). There were no significant differences between the two groups for any measures of endothelial function. For smooth muscle function measures, participants with higher visit-to-visit SBPV had significantly higher $\%NMD_{peak}$ and NMD_{AUC}. Repeated-measures ANOVA for the time-course NMD response showed a significant main effect of visit-to-visit SBPV group (F = 4.90; p = 0.03). The main effect of visit-to-visit SBPV group, however, was no longer significant (F = 2.89; p = 0.10) after adjusting for age, gender, BMI, and mean BP.

Participants with higher visit-to-visit DBPV showed a trend for lower CD62+ EMPs when compared to participants with lower visit-to-visit DBPV, however this difference did not reach statistical significance (Supplemental Table S2). For measures of endothelial function, the high visit-to-visit DBPV group had significantly lower FMD_{AUC} and FMD/NMD ratio. Significant differences were also observed for measures of smooth muscle function as the high visit-to-visit DBPV group had significantly higher $\%NMD_{peak}$ and NMD_{AUC} when compared to the low visit-to-visit DBPV group.

Figure 1 shows the time-course %change in brachial artery diameter across the 5-min after FMD for the visit-to-visit DBPV groups. Repeated-measures ANOVA showed a significant main effect of visit-to-visit DBPV group (F = 5.28; p = 0.03). Post-hoc comparisons showed that %FMD was significantly lower in the high visit-to-visit DBPV group immediately post-cuff release (p = 0.03), and 2-min (p = 0.005), 3-min (p = 0.001), and 5-min (p = 0.02) post-cuff release. The main effect of visit-to-visit DBPV group, however, was no longer significant (F = 3.06, p = 0.09) after adjusting for age, gender, BMI, and mean BP. Figure 2 shows the time-course %change in brachial artery diameter across the 5-min after nitroglycerin administration for the visit-to-visit DBPV groups. Repeated-measures ANOVA showed a significant effect of visit-to-visit DBPV group (F = 13.55; p = 0.001).

The main effect of visit-to-visit DBPV group (F = 12.39; p = 0.001) remained significant after adjusting for age, gender, BMI, and mean BP. Post-hoc comparisons showed that %NMD was significantly higher in the high visit-to-visit DBPV group at 1-min (p = 0.002), 2-min (p = 0.001), 3-min (p = 0.001), 4-min (p = 0.001), and 5-min (p = 0.001) post-nitroglycerin administration.

24-h BPV Group Comparisons

There were no significant differences between participants with higher vs. lower 24-h SBPV for EMP levels, endothelial function measures, or smooth muscle function measures (Supplemental Table S3).

Participants with higher 24-h DBPV had lower CD31+CD42− and CD62+ EMPs when compared to participants with lower 24-h DBPV, however, these differences reached statistical significance only for CD31+CD42− EMPs (Supplemental Table S4). For measures of endothelial function, there were no differences between the two groups for any measure of endothelial function. For measures of smooth muscle function, trends were observed as participants with high 24-h DBPV had trends for higher $\%NMD_{peak}$ (p = 0.05) and NMD_{AUC} (p = 0.06).

DISCUSSION

In this preliminary study, we investigated the relationship of two different measures of BPV with markers of endothelial injury, endothelial function, and smooth muscle function in a cohort of African Americans with a wide range of BP levels. Several important findings were observed. First, our results showed that African Americans with increased visit-to-visit DBPV had decreased endothelial function when assessed using the FMD_{AUC} or the FMD/NMD ratio. Second, we found that higher visit-to-visit DBPV and 24-h DBPV were both associated with a greater vasodilatory response to NMD. Finally, our results unexpectedly showed that African Americans with increased BPV (visit-to-visit or 24-h) had lower circulating EMP levels.

In our previous preliminary study, we showed that increased visit-to-visit BPV was associated with diminished endothelial function assessed using the conventional approach to FMD calculation that expresses the brachial artery diameter using the arbitrary time point of 60-sec post-cuff deflation[10]. However, it has been demonstrated that FMD calculated by this method (i.e. 60-sec diameter calculations) significantly underestimates true peak FMD[20], thus our previous findings could have been confounded. In the present study, our finding that African Americans with high visit-to-visit DBPV have decreased endothelial function expressed as an AUC over a 5-min time course or as the normalized true peak in FMD, confirms and extends upon our preliminary study findings and provides some of the first clinical data that high BPV may be associated with impaired endothelial function. Nonetheless, as a result of the cross-sectional design of this study, it is difficult to ascertain whether high BPV precedes impaired endothelial function or vice versa.

It has been proposed by some that the FMD/NMD ratio is the best available marker of endothelial function because differences in the vascular smooth muscle response to nitric

oxide can be accounted for[21,22]. The most supportive data for the FMD/NMD ratio serving as the best available marker of endothelial function, comes from a study by Chan et al. who showed that the FMD/NMD ratio was a more powerful predictor of vascular events than FMD in coronary artery disease patients[23]. In the present study, the FMD/NMD ratio was negatively associated with measures of visit-to-visit DBPV; suggestive that endothelial-specific vasodilatory mechanisms are impaired in African Americans with increased visit-to-visit DBPV. Unfortunately, although the FMD/NMD ratio is used by some investigators, it is frequently not reported, thus its true prognostic value has not been clearly established. Therefore, although provocative, our findings may be tempered to some extent by a lack of studies investigating the prognostic importance of the FMD/NMD ratio. It should however be noted that there was a positive association found between $\%FMD_{peak}$ and $\%NMD_{peak}$ in the present study, which could suggest that smooth muscle function may be underlying and/or confounding the FMD response. Thus, normalizing FMD by NMD may have been warranted in our study.

In our previous preliminary study, we also reported that BPV was positively associated with %NMD at 4-min post-nitrogyclerin administration[10], the conventional time point used to asses smooth muscle function. To further investigate this relationship, we analyzed NMD at multiple time-points and found that individuals with high visit-to-visit BPV had greater %NMD as early as 1-min post-nitroglycerin administration, which persisted up to 5-min post administration. Although purely speculative, we suspect that differences in vascular reactivity to vasoactive agents could be an underlying mechanism contributing to the greater CV risk in individuals with increased BPV, and itself could be a causative factor for higher BPV. Previous animal studies have reported differences in vascular reactivity to contractile and vasodilatory agonists between SAD rats and sham-operated rats which investigators attributed to differences in gap junction-mediated vascular smooth cell communication[24]. Based on these experimental findings, we hypothesize that a potential mechanism for the association between high BPV and a greater vasodilatory response to nitroglycerin observed in the present study could be the result of increased gap junction communication in the arteries of individual's with high BPV. Additional clinical studies may be needed to further elucidate the relationship between BPV and vascular reactivity to other vasoactive agents (e.g. endothelin-1; angiotensin II); as such findings could provide insights as to the underlying mechanisms linking BPV to CV disease risk.

In recent years, EMPs have emerged as a novel biomarker that provides valuable information as to the biological status of the endothelium. Based on both experimental evidence that has demonstrated an active role for EMPs in the promotion and propagation of inflammation, coagulation, and thrombosis, as well previous clinical studies which have shown EMP levels to be elevated in a number of disease states[11], we had hypothesized that individuals with higher BPV would have higher levels of EMPs. Contrary to our hypothesis, we observed that individuals with higher 24-h DBPV or visit-to-visit BPV had lower EMP levels. A potential explanation for these findings was recently introduced by Tushuizen et al. who contended that microparticles serve a critical role in cellular homeostasis and have protective properties in healthy and/or early disease states, and argued that impairment of the ability of the endothelium to release EMPs would result in the deterioration of endothelial cell integrity and/or viability[25]. According to this hypothesis, our finding that

higher BPV is associated with lower EMP levels may still be in agreement with our hypothesis that increased BPV elicits endothelial damage. Intuitively, lower levels of circulating EMPs could indicate that the ability of the endothelium to release EMPs has become impaired. Consequently, the integrity and viability of endothelial cells could be diminished in those participants with higher BPV. Coinciding with our findings and the hypothesis proposed by Tushuizen et al., it has been demonstrated that patients with subclinical atherosclerosis have higher circulating EMP levels when compared to patients with symptomatic atherosclerosis[26,27]. However, outside of these studies, little clinical evidence exists to substantiate the hypothesis that increased circulating EMP levels are indicative of a healthy endothelium.

It is also noteworthy to mention that although visit-to-visit and 24-h BPV have both been associated with clinical outcomes, seldom have they been investigated together in the same population. Here we report separate but similar relationships for measures of 24-h and visit-to-visit BPV with measures of smooth muscle function, particularly for DBPV. Although they are certainly not interchangeable entities, our findings may provide early evidence that 24-h BPV and visit-to-visit BPV could have some similar underlying causes and/or sequelae. Interestingly, in the only other study to date to compare visit-to-visit and 24-h BPV with clinical outcomes in the same population, it was observed that visit-to-visit BPV was a stronger predictor of vascular events in the ASCOT-BPLA trial than BPV derived from ABPM[4]; suggestive that visit-to-visit BPV may be more closely aligned with CV risk. Our study findings may be in agreement with this sentiment, as we showed that visit-to-visit BPV was more strongly associated with endothelial and smooth muscle function measures than 24-h BPV. However, the mechanisms for the stronger associations of visit-to-visit BPV with CV risk still remains to be elucidated.

Several limitations must be noted when interpreting our study findings. First, the reproducibility of visit-to-visit BPV when quantified using three visits may be low[28]. Second, it has been recommended that FMD should be normalized by dividing the percentage change of FMD by shear rate AUC in order to account for the heterogeneity of blood flow responses across subjects[29]. In our study, however, we did not normalize FMD by shear rate. Third, there are presently no standardized methods for the measurement of microparticles. Processing and analyzing techniques differ from investigator to investigator, thus comparisons across studies for EMPs should be done so cautiously. Fourth, only African Americans were included in the study. Therefore, our study findings are not generalizable to other race-ethnicities. Moreover, the sample population was predominately female, thus our findings also may have limited generalizability to male populations. Fifth, because of our recruitment procedures (e.g. advertisements), it is possible that our study could be confounded by a volunteer bias[30]. Finally, analyses were not adjusted for multiple testing, thus we cannot rule out the possibility of type I errors.

In conclusion, our findings showed that visit-to-visit and 24-h BPV were associated with measures of endothelial injury, endothelial function, and smooth muscle function. These early preliminary results may suggest that high BPV could influence the pathogenesis of CV disease, in part, through influences on vascular health. Additional research, however, will be needed to confirm these results, determine the clinical relevance of the FMD/NMD ratio,

and resolve the conflicting hypotheses regarding the interpretation of circulating EMP levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary Table

What is known about the topic

- Data from randomized controlled trials and general-population, propspetive studies have shown that both 24-hour blood pressure variablity and visit-to-visit variability in clinic blood pressure are associated with cardiovascular events and mortaility, independent of mean BP and other explanatory factors.
- Experimental studies in sinoaortic denervation rats, the animal model of blood pressure variability, have provided some evidence that high blood pressure variability could confer greater cardiovascular risk by elicting damage and injury to the endothelium.

What this study adds

- This cross-sectional study shows that visit-to-visit blood pressure variability, but not 24-hour blood pressure variability, is associated with endothelial-dependent vasodilation normalized for smooth muscle function in African Americans.
- Both 24-hour blood pressure varialbility and visit-to-visit blood pressure variability are associated with vascular smooth muscle-dependent vasodilation in African Americans.
- Visit-to-visit blood pressure variability is more closely with measure of vascular health/function than 24-hour blood pressure variability in African Americans.

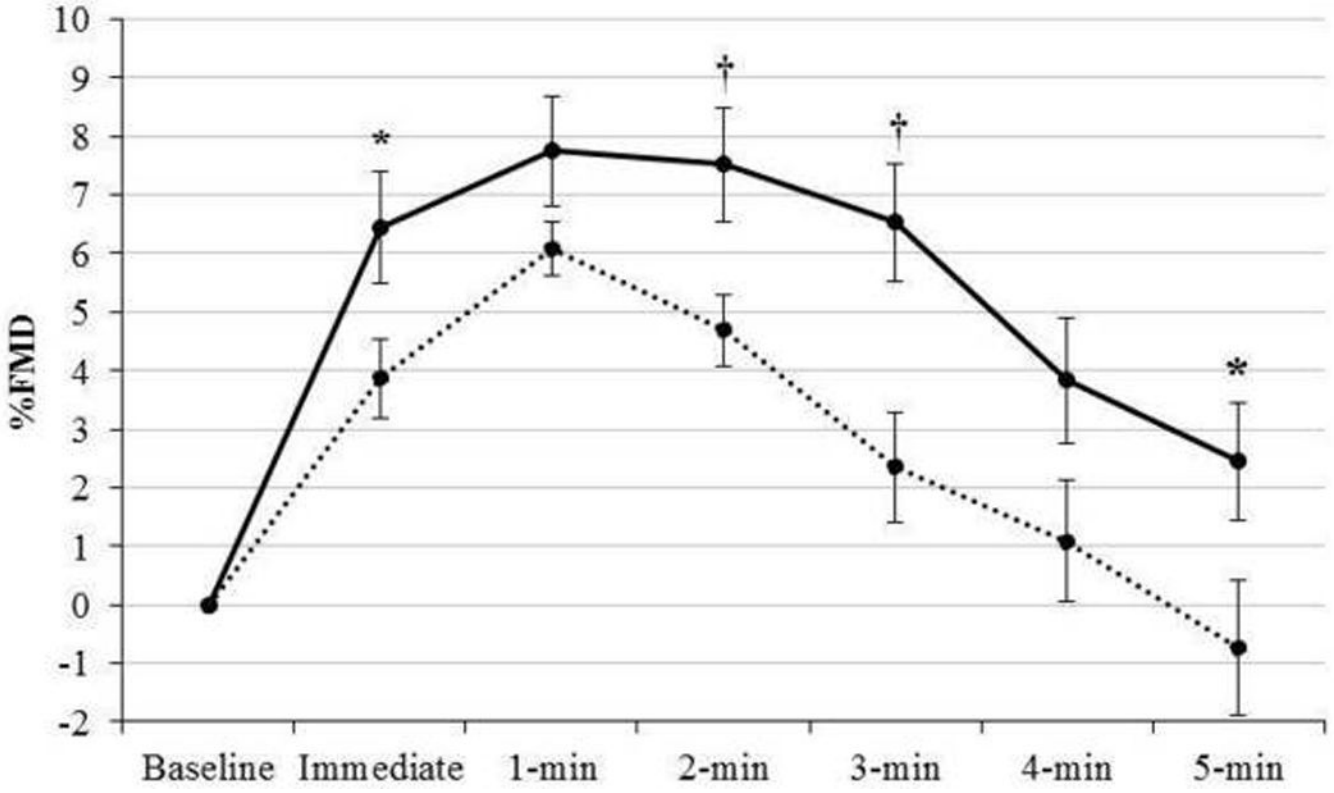

Figure 1.

Time-course % change in brachial artery FMD in participants categorized as having low visit-to-visit diastolic blood pressure variability (solid line) or high visit-to-visit diastolic blood pressure variability (dotted line). * $P < 0.05$, † $P < 0.01$ between groups.

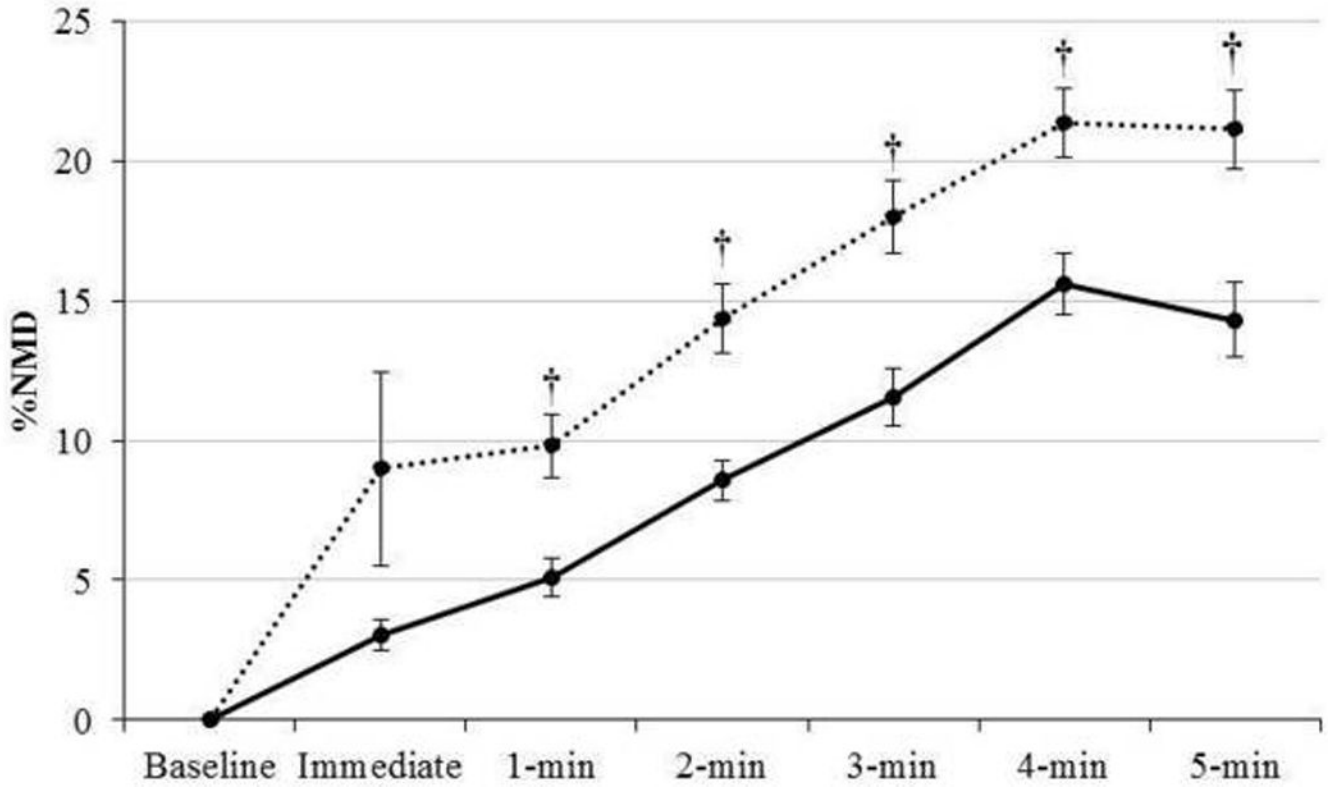

Figure 2.

Time-course % change in brachial artery nitroglycerin-mediated dilation in participants categorized as having low visit-to-visit diastolic blood pressure variability (solid line) or high visit-to-visit diastolic blood pressure variability (dotted line). † $P < 0.01$ between groups.

Table 1

Univariate correlation analyses of the association between clinical variables and vascular function measures.

Variable	%FMD$_{peak}$		FMD$_{AUC}$		%NMD$_{peak}$		NMD$_{AUC}$		FMD/NMD Ratio	
	r_s	*P-Value*	r_s	*P-Value*	r_s	*P-Value*	r_s	*P-Value*	r_s	*P-Value*
Age	**−0.31**	**0.04**	−0.28	0.06	−0.22	0.12	−0.08	0.56	−0.13	0.42
Total Cholesterol	0.07	0.63	0.27	0.07	−0.25	0.07	−0.22	0.11	0.23	0.17
Triglycerides	0.09	0.57	**0.30**	**0.04**	0.00	0.98	−0.02	0.87	0.07	0.66
HDL-C	**−0.35**	**0.02**	**−0.41**	**0.01**	**−0.29**	**0.03**	−0.21	0.14	−0.03	0.88
LDL-C	0.12	0.44	**0.31**	**0.04**	−0.11	0.42	−0.16	0.26	0.26	0.11
Fasting Glucose	−0.12	0.41	−0.05	0.74	0.09	0.55	0.06	0.67	−0.01	0.93
CRP	0.14	0.37	0.06	0.68	0.03	0.82	−0.07	0.61	0.23	0.18
Weight	0.16	0.29	0.19	0.21	0.06	0.70	−0.01	0.93	**0.33**	**0.04**
BMI	0.14	0.37	0.18	0.24	0.08	0.56	0.02	0.89	0.23	0.17
Office SBP	0.19	0.22	0.08	0.63	0.03	0.81	0.06	0.70	0.16	0.35
Office DBP	0.16	0.30	0.07	0.67	−0.02	0.90	0.03	0.85	0.18	0.30
Visit-to-Visit SBPV	0.06	0.74	0.02	0.90	**0.42**	**0.01**	**0.38**	**0.01**	−0.16	0.39
Visit-to-Visit DBPV	−0.12	0.51	**−0.32**	**0.05**	**0.56**	**< 0.001**	**0.62**	**< 0.001**	**−0.72**	**< 0.001**
24-h SBP	0.11	0.52	0.01	0.97	−0.19	0.22	−0.18	0.26	0.18	0.34
24-h DBP	−0.05	0.78	−0.14	0.42	−0.16	0.31	−0.10	0.52	0.08	0.70
24-h MAP	0.03	0.86	−0.08	0.64	−0.14	0.38	−0.09	0.55	0.10	0.59
24-h HR	0.11	0.53	0.00	0.98	0.21	0.18	0.25	0.10	0.09	0.65
24-h SBPV	−0.18	0.29	−0.22	0.21	0.09	0.56	0.21	0.17	−0.19	0.31
24-h DBPV	0.11	0.52	0.01	0.95	**0.34**	**0.02**	**0.30**	**0.04**	0.05	0.82
24-h MAPV	−0.03	0.86	−0.1	0.46	**0.18**	**0.25**	**0.18**	**0.26**	0.10	0.60
24-h HRV	0.13	0.43	0.05	0.79	0.17	0.28	0.21	0.18	−0.18	0.35

AUC, area under curve; CRP, C-reactive protein; DBP, diastolic blood pressure; DBPV, diastolic blood pressure variability; FMD, flow-mediated dilation; HDL, high density lipoprotein; HR, heart rate; HRV, heart rate variability; LDL, low density lipoprotein; MAP, mean arterial pressure; MAPV, mean arterial pressure variability; NMD, nitroglycerin-mediated dilation; r_S, Spearman's rank correlation coefficient; SBP, systolic blood pressure; SBPV, systolic blood pressure variability.

Table 2

Multivariable analyses predicting endothelial and smooth muscle function measures.

Variable	$\%FMD_{peak}$			FMD_{AUC}			$\%NMD_{peak}$			NMD_{AUC}			FMD/NMD Ratio		
	B (95% CI)	R^2	*P-Value*	*B (95% CI)*	R^2	*P-Value*	*B (95% CI)*	R^2	*P-Value*	*B (95% CI)*	R^2	*P-Value*	*B (95% CI)*	R^2	*P-Value*
Age†	−0.16 (−0.34, 0.02)	26.2	0.07	-	-	-	-	-	-	-	-	-	-	-	-
HDL-C	−0.01 (−0.01, 0.08)	26.4	0.81	−0.14 (−0.53, 0.26)	22.3	0.47	−0.08 (−0.29, 0.13)	21.0	0.45	-	-	-	-	-	-
LDL-C	-	-	-	0.28 (0.03, 0.55)	32.9	0.03	-	-	-	-	-	-	-	-	-
Triglycerides	-	-	-	0.14 (0.02, 0.26)	33.7	0.03	-	-	-	-	-	-	-	-	-
Visit-to-Visit DBPV	-	-	-	−1.11 (−3.65, 1.44)	14.6	0.38	2.49 (0.91, 4.07)	33.5	0.003	6.97 (3.54, 10.40)	35.2	< 0.001	−0.04 (−0.06, −0.01)	38.3	0.006
Visit-to-Visit SBPV	-	-	-	-	-	-	0.24 (−0.98, 1.46)	13.2	0.69	1.11 (−1.71, 3.95)	3.6	0.43	-	-	-
24-h DBPV	-	-	-	-	-	-	0.86 (−1.32, 3.05)	21.1	0.43	4.89 (0.03, 9.74)	19.6	0.04	-	-	-
Weight††	-	-	-	-	-	-	-	-	-	-	-	-	0.004 (0.000, 0.008)	20.7	0.10

AUC, area under curve; DBPV, diastolic blood pressure variability; FMD, flow-mediated dilation; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NMD, nitroglycerin-mediated dilation; SBPV, systolic blood pressure variability. Variables listed were entered seperately into a multivariable regression model containing age, gender, BMI, mean SBP, and mean DBP (model 1).

† Age entered into model containing gender, BMI, mean 24-h SBP, and mean 24-h DBP.

†† Weighted entered into model containing age, gender, mean 24-h SBP, and mean 24-h DBP.