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ORIGINAL ARTICLE

Endothelial function in normotensive and high-normal hypertensive subjects

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To evaluate the impact of a mild increment in blood pressure level on endothelial function, we evaluated 61 healthy volunteers (24 women, 37 men, and aged 35–50 years). All subjects underwent a blood chemistry panel to exclude any metabolic abnormalities and were submitted to a Doppler ultrasound of the brachial artery to assess endothelial function. We assessed the endothelial response to reactive hyperaemia and exogenous nitric oxide administration considering an increase in systolic blood pressure (SBP) at each 10-mm Hg interval. Our study population was divided as follows: SBP <115 mm Hg (SG1, $n = 13$), SBP $\geqslant 115$ mm Hg and <125 mm Hg (SG2, $n = 20$), SBP $\geqslant 125$ mm Hg and <135 mm Hg (SG3, $n = 13$) and SBP $\geqslant 135$ mm Hg and <140 mm Hg (SG4, $n = 15$). We found a significant difference in flow-mediated dilation among SG2, SG3 and SG4, 16.2 ± 5.6, 13.4 ± 5.2 and $11.5 \pm 3.6\%$, $P < 0.05$, respectively). After nitrate administration, we observed a nonsignificant decrease in brachial artery dilation among groups, $P = 0.217$. Our data showed in a healthy normotensive population, without any risk factor for atherosclerotic disease that small increases in SBP but not in diastolic blood pressure may impair endothelial function even in subjects considered as high-normal, meaning that this population deserves more attention than usually ascribed to intervene and prevent complications, as endothelial dysfunction may represent an early change in those who develop hypertension later in life.
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Introduction

The impact of blood pressure levels on cardiovascular complications is more and more frequently reported in epidemiological as well as in interventional studies. Recently, the VII Joint National Committee (JNC VII) published in May 2003, pointed out that '...the risk of cardiovascular diseases (CVD) beginning at 115/75 mm Hg doubles with each increment of 20/10 mm Hg...' and those individuals previously categorized as high-normal (HN), that is, systolic blood pressure (SBP) between 130–139 mm Hg and/or diastolic blood pressure between 85 and 89 mm Hg should be considered as prehypertensives, requiring lifestyle changes to prevent CVD.[1] Although not accepted by all international guidelines, this new classification led to some considerations regarding the structural and functional changes presented by these individuals with HN blood pressure levels.

The relationship between blood pressure levels and cardiovascular events, such as stroke, heart failure and myocardial infarction are well known as a continuum; thus, the higher the blood pressure the greater the chance of cardiovascular complications. The Framingham study showed an increased risk of 1.6 in men and 2.8 in women in HN individuals compared with individuals with optimal blood pressure levels for cardiovascular events during a 12-years follow-up.[2]

It is also known that one of the earliest changes observed in hypertensive subjects, either in stage 1 of hypertension or in normotensive (NT) subjects with a family history of hypertension is an impaired endothelial response to reactive hyperaemia.[3–6]

Previous data from our group have detected a graded impairment in vasodilatatory response from NT healthy subjects compared to smokers, hypertensive and those with hypertension and diabetes mellitus, suggesting that not only blood pressure levels but the presence or coexistence of other risk factors may decrease this vasodilatatory response.[7]

Endothelial dysfunction was initially described as a lack in nitric oxide (NO) release and/or excess of free radicals, suggesting an imbalance between vasodilators and vasoconstrictor agents present at

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endothelial cells (functional change). Once hypertension is established, there is also impairment in non-endothelium-dependent vasodilatatory response probably owing to structural changes in blood vessels. Several studies have been developed to evaluate these changes by a non-invasive methodology described by Celermajer,[8] the brachial artery by B mode ultrasound, which is safe and reliable.

Thus, considering the data shown in JNC VII based on a meta-analysis of 61 observational studies, and considering data from scientific literature that provides a support in the evaluation of endothelial response to different stimuli, our study aimed to assess the endothelium-dependent (flow-mediated dilation (FMD)) and non-endothelium dependent (nitrate-mediated dilation (NMD)) vasodilatatory response in NT and HN subjects. Second, we divided blood pressure levels in a 10-mm Hg interval for both, systolic and diastolic blood pressure (SBP), to investigate the brachial artery dilation through a non-invasive method using B mode ultrasound.

Patients and methods

This study had a cross-sectional design and the study population consisted of 61 healthy volunteers of both sexes (24 women, 37 men, aged 35–50 years) recruited from the community. None of them were smokers, or had a previous history of hypertension, dislipidemia, diabetes mellitus or any other clinical condition that might be considered as a confounder in the interpretation of the results. There were two groups: NT subjects ($n = 33$) who had normal blood pressure levels ($< 120/80$ mm Hg) and HN subjects ($n = 28$) defined as a SBP between 130 and 139 mm Hg and/or diastolic blood pressure between 85 and 89 mm Hg. All subjects were informed on study purposes and after they signed an informed consent they came to the office for study procedures including blood pressure measurement and determination of weight and height to further calculate body mass index. At this visit they also had blood sample drawn after 10 h of overnight fasting from antecubital vein for blood chemistry analyses to confirm the absence of any metabolic abnormalities that might influence the interpretation of the tests. A 12-lead electrocardiogram was also performed. Following these tests, all subjects were scheduled to perform a Doppler ultrasound of the brachial artery to assess endothelial response. Endothelial function was assessed in the next day after confirmation of their eligibility after a 10 h of overnight fast. This study was submitted to the Ethics Committee of the Hospital São Paulo; Federal University of São Paulo under protocol # 360/00.

Methods
Blood pressure measurement. Blood pressure levels were determined after 10 min of rest in sitting position using a standard mercury sphygmomanometer. Blood pressure levels were measured three times within a 1-min interval. Following these measurements, subjects assumed the upright position and after 2 min their blood pressure was measured once. The same physician performed these measurements. The average of the three values obtained in sitting position was used in the subsequent analyses. Systolic and diastolic blood pressure corresponded to phases I and V of Korotkoff sound, respectively.

Biochemistry. The tests performed were as follows: fasting serum glucose, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol and triglycerides, serum creatinine. All blood tests were performed at the Central Laboratory of the Hospital do Rim e Hipertensão. Cholesterol levels were determined using Bio-Chromatic analyser series II-F from Abbott, and normal range was based on the Brazilian Guidelines of Dyslipidemia, 1994. Serum creatinine was determined by Jaffé technique and fasting glucose by orthotoluidine method.

Brachial artery B mode ultrasound. The endothelial function test was performed by high-resolution B-mode ultrasound images using a non-invasive methodology described by Celermajer et al.[3] with modifications.[9] The equipment used in this study was an Ultramark HDI 3000 (ATL ultrasound incorporation), with a linear transducer of L7–4 MHz. The test consisted of four phases as follow: rest, after reactive hyperaemia (endothelium-dependent phase – FMD), again with the subject at rest, and finally, and after administration of sublingual nitrate (endothelium-independent phase – NMD). All measurements were performed in the same place, in longitudinal section 5–10 cm above the antecubital fossa of the right upper arm.

At the end of the first phase of resting when a satisfactory position was found to carry on the endothelial study, the skin was marked and the arm remained in the same position throughout the test. At this moment, the diameter and blood flow velocity were determined in triplicate for the first phase. To obtain an increased flow, a cuff was placed on the right upper arm and it was inflated to 300 mm Hg, resulting in a complete interruption of blood flow during a 5-min period, and then the cuff was deflated. A second and third scan were obtained after 15 and 90 s after the cuff was released (known as reactive hyperaemia phenomenon that was followed by a brachial FMD. The maximum blood flow (mm/min) was determined in the first 15 s after the cuff release. Ninety seconds after ischaemia, three measurements of the diameter of the brachial artery were taken at the diastolic period (FMD). A 10-min of rest was then allowed for recovery of the vessel and at the end of this period a sublingual tablet of isossorbide dinitrate 5.0 mg,

was given to the subjects. Five minutes after isossorbide dinitrate administration three measurements were obtained for brachial artery diameter and blood flow velocity to determine nitrate-induced vasodilation of the arterial wall (known as endothelium-independent vasodilation). The mean of these values was used in subsequent analyses. FMD response was expressed as the change in end-diastolic diameter of the brachial artery during reactive hyperaemia compared with the baseline (rest) measurement and used as a measure of endothelium-dependent vasodilation. The mean of these values were used in subsequent analyses.

To determine the confidence of the results, vascular tests (vasodilation after reactive hyperaemia and nitrate-stimulated dilation) were performed by two independent observers in 10 healthy volunteers. Intra- and interobserver variability for repeated measurements of the same recording of the brachial artery diameter was 2.16 ± 1.7, 2.41 ± 1.9 and $6.7 \pm 4.0\%$, respectively.

Data analysis
Statistical plan. To assess the endothelial response after the initial analysis (NT vs HN group), we divided the study population according to a 20-mm Hg interval for SBP and 10-mm Hg for diastolic blood pressure. A final analysis was carried out in the study population considering at this time a 10-mm Hg interval for SBP.

Statistical analysis
Data were stored and analyzed with SigmaStat for Windows version 2.0, (Jandel Corporation, Chicago). Data are expressed as mean$\pm$s.d. or median and its confidence intervals and comparison were made by using Student's *t*-test and analysis of variance. Statistical significance was considered for differences when the two-tailed *P*-value was less than 0.05.

Results

Subjects (24 women and 37 men) had a mean age of 41.8 ± 4.7 years (range, 35–50). Table 1 shows demography, clinical and laboratorial values in both groups, NT and HN subjects. Except for a statistical difference in mean body weight in HN subjects, no other statistically significant differences were observed between the two groups.

Although we had more women in NT group ($n = 16$) than in HN group ($n = 8$), we did not find any statistically significant difference in age (42.3 ± 4.4 vs 44.7 ± 3.6 years, NS), post-menopausal or childbearing potential status as well as in any variable assessed in this study. Among all laboratorial parameters evaluated, we did not observe a statistically significant difference, except for serum creatinine levels, where men showed a higher level of serum creatinine than women ($P = 0.0015$).

Table 1 Clinical and demographic characteristics of NT and HN groups

Variables/groups	NT subjects (n = 33)	HN subjects (n = 28)
Sex	17 M/16 F	20 M/8 F
Age (years)	41.6 ± 4.6	42.0 ± 4.7
Weight (kg)	67.4 ± 8.2	$77.6 \pm 15.4*$
Height (m)	1.65 ± 0.07	1.70 ± 0.10
BMI (kg/m²)	24.7 ± 2.1	26.8 ± 3.5
SBP (mm Hg)	114 ± 7	$134 \pm 5*$
DBP (mm Hg)	75 ± 7	$83 \pm 5*$
FPG (mg/dl)	87.6 ± 10.6	88.6 ± 8.9
Creatinine (mg/dl)	0.92 ± 0.18	0.97 ± 0.14
Total cholesterol (mg/dl)	187.2 ± 23.4	181.4 ± 24.3
LDL-cholesterol(mg/dl)	120.6 ± 27.3	114.5 ± 27.4
HDL-cholesterol (mg/dl)	49.0 ± 11.3	46.7 ± 8.5
Triglycerides (mg/dl)	110.0 ± 48.4	133.2 ± 51.3

*$P < 0.05$ versus normotensives.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-cholesterol, high-density lipoprotein cholesterol; HN, high-normal; LDL-cholesterol, low-density lipoprotein cholesterol; NT, normotensive; SBP, systolic blood pressure.

Normotensive versus high-normal subjects
Flow-mediated vasodilation. The mean FMD in brachial artery following reactive hyperaemia in NT group ($n = 33$) was $13.9 \pm 8.2\%$, whereas in HN ($n = 28$) subjects the mean dilation was $9.8 \pm 7.0\%$, which was a statistically significant difference ($P < 0.05$), as shown in Figure 1. The percentual change in FMD in HN subjects compared to HN was 29.5% lower.

When we compared the results according to sex, women showed a greater vasodilation $14.5 \pm 8.0\%$ than men 10.4 ± 7.5 ($P < 0.05$).

Nitrate-mediated dilation
The mean NMD of brachial artery after exogenous nitrate administration in NT group ($n = 33$) was $13.8 \pm 8.1\%$ whereas in HT ($n = 28$) subjects, NMD was slightly lower, $11.1 \pm 5.6\%$ and represented a percent change of -19.5%, although this difference did not reach statistical significance.
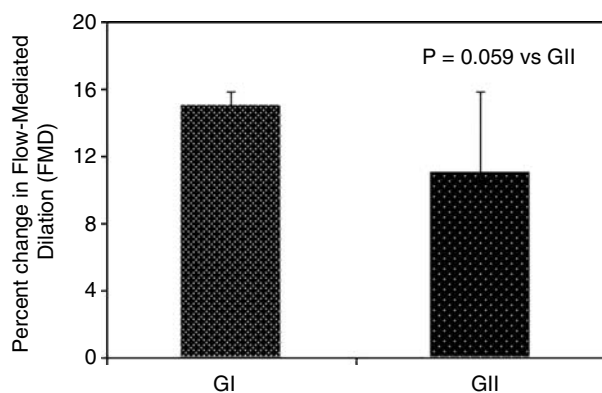
When the pooled data were compared to each sex, we did not find any difference: $12.5 \pm 7.2\%$ (pooled data), 12.5 ± 7.5 and 12.6 ± 7.1, women and men, respectively.

Blood pressure intervals
20-mm Hg interval in SBP. To analyze the impact of blood pressure interval in FMD, we divided the whole group irrespective of sex, considering an interval of 20 mm Hg in SBP starting from a cutoff value of 115 mm Hg, thus obtaining a group where SBP $\geqslant 115$ mm Hg and < 135 mm Hg (GI, $n = 33$), and a second group with a SBP $\geqslant 135$ mm Hg but < 140 mm Hg (GII, $n = 15$). The remaining 13 subjects had SBP values $\leqslant 115$ mm Hg and were not considered in this analysis. The endothelium-dependent vasodilation response in GI showed a

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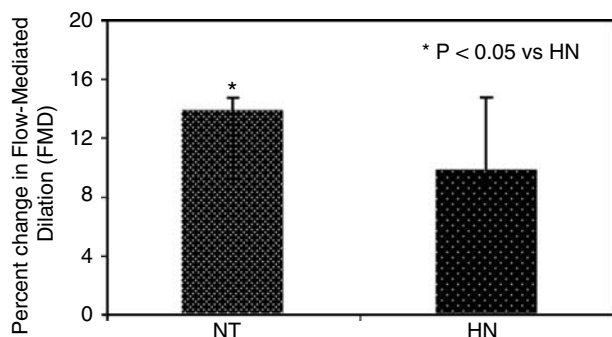
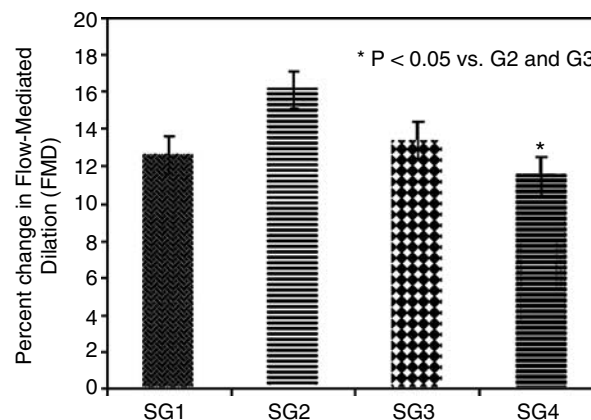**Figure 1** FMD in NT ($n = 33$) and HN subjects (HN, $n = 28$).

Figure 2 FMD following a 20 mm Hg increment in SBP.

Figure 3 FMD following a 10-mm Hg interval in SBP.

Table 2 Changes in brachial artery diameter after endothelium-dependent or independent stimuli with a 10 mm Hg increment in diastolic blood pressure

Parameter (%)	< 75 mm Hg (n = 17)	75–85 mm Hg (n = 35)	85–90 (n = 9)
FMD	13.9 ± 9.3	12.7 ± 6.1	10.0 ± 8.5
NMD	13.2 ± 6.9	13.1 ± 7.6	10.6 ± 5.2

Data expressed as mean ± s.d. or median and its confidence interval. Abbreviations: FMD, flow-mediated dilation; NMD, nitrate-mediated dilation.

trend towards a higher response 15% (9.75–20.00) when compared to GII 11% (8.0–14.25), $P = 0.059$. Figure 2 show the vasodilation response in both groups. The impact in NMD at 20-mm Hg interval did not show any statistical difference between groups.

10-mm Hg interval for SBP. We also performed an analysis considering an interval of 10 mm Hg in SBP to test the vascular response to endothelial stimuli. Subjects were divided in the following subgroups: SBP <115 mm Hg (SG1, $n = 13$), SBP ⩾115 mm Hg and <125 mm Hg (SG2, $n = 20$), SBP ⩾125 mm Hg and <135 mm Hg (SG3, $n = 13$) and SBP ⩾135 mm Hg and <140 mm Hg (SG4, $n = 15$). There was a statistically significant difference in FMD among the three subgroups, 16.2 ± 5.6, 13.4 ± 5.2 and $11.5 \pm 3.6\%$, SG2, SG3 and SG4, respectively) as shown in Figure 3. SG1 subjects showed a FMD of $12.6 \pm 8.8\%$.

When we compared the four subgroups regarding NMD, we did not observe any significant change in brachial artery dilation, as follows: 15.9 ± 8.0, 14.8 ± 6.5, 12.5 ± 4.8 and $11.5 \pm 5.0\%$, respectively, $P = 0.217$).

10-mm Hg interval in diastolic blood pressure. Taking into account a 10-mm Hg interval for diastolic blood pressure, we did not detect differ-ences in vessel diameter related to endothelium-dependent or-independent stimuli. Data are shown in Table 2.

Discussion

Our data showed in a healthy population with no risk factors for atherosclerotic disease that SBP assessed at 10- or 20-mm Hg intervals, but not diastolic blood pressure predisposed subjects to endothelial dysfunction, compromising vascular dilation.

It is well known that FMD reflects the relaxation of a vessel exposed to increased shear stress. On the other hand, the lack of response observed in endothelium-independent vasodilation, that is, a NMD may be explained by the fact that our subjects were NTs or had HN blood pressure levels, and probably these changes were not yet sufficient to promote a vascular smooth muscle dysfunction.

This impact was noted in the two different analyses, where within a 20-mm Hg interval for SBP there was a 26.7% reduction in vasodilatatory response, and within 10-mm Hg interval for SBP, we found a progressive and significant reduction of 17.3% (from SG2 to SG3) and an additional 14.2% (from SG3 to SG4) in vasodilatatory response as blood pressure raised, suggesting that a person with

no other risk factor for cardiovascular disease, presenting a SBP within the range of 125–135 mm Hg, which is considered adequate in clinical practice is, indeed, exposed to a greater risk of vascular damage later in life specially if he/she becomes hypertensive and is also predisposed to an earlier atherosclerotic process.

However, the most relevant finding was related to the 10-mm Hg interval, which allowed us to detect impairment in vasodilation. Comparing SG2 to SG4, there was a marked reduction in vasodilatatory capacity of 29%.

Our data are consistent with previous results from literature, which suggest the presence of endothelial dysfunction as a paraphysiological status of 'pre-hypertension'.[10] This assumption is corroborated by the findings in a prospective study involving more than 5000 subjects where those with prehypertension had an increased risk of coronary heart disease and myocardial infarction which was related to SBP level but not with diastolic.[11]

Another study using ABPM showed that NT subjects with endothelial dysfunction presented a higher 24-h blood pressure values, which is the reflex of consistent changes in the organisation of blood pressure circadian pattern probably related to vasopressor effects even in NT subjects.[12] There are some aspects that should be mentioned such as the impact of haemodynamic forces on arterial wall leading to damage and earlier atherosclerotic process that may be detected even in NT subjects, genetic predisposition and metabolic profile, which may individually or in association, play a role in the development of atherosclerosis. Also, in a population-based cohort study (NOMAS), the authors found a significant association between endothelial dysfunction and the presence of carotid plaque and this FMD impairment was more relevant among subjects with carotid plaques thicker than 2 mm, which in turn, was associated with a higher cardiovascular risk.[13]

In a meta-regression analysis, Witte et al.[14] reported a clearer relation between FMD and the principal cardiovascular risk factors only in the category with the lowest cardiovascular baseline risk, and found no relation in middle and high-risk populations. The authors considered that such difference among low-, middle- and high-risk populations could be partially explained by the fact that the endothelium ability to produce NO was affected at the initial stages of the atherosclerotic process, and beyond this point there could be no additional impairment in FMD.

The impact of body weight on endothelial function has been shown in literature[15,16] mainly when subjects are submitted to weight loss interventions such as bariatric surgery, dietary or exercise modifications, although some authors suggested that this impact is better observed among women than in men.[17] We did not find any impact on FMD related to body weight both in NT and HN subjects as

assessed by Pearson's correlation ($r = -0.20$ and $r = 0.10$, for FMD and NMD, $P > 0.05$, respectively) in NT subjects and ($r = -0.10$ and $r = 0.29$ for FMD and NMD, $P > 0.05$, respectively) in HN subjects. In both groups, most women were not overweight or obese and this may at least in part explain our finding.

Therefore, the relevance of our findings is the fact that for the first time it was shown that in a healthy population, within a graded level of blood pressure interval, it could be possible to detect an early impairment in vasodilation, or in other words, in the ability of vessel relaxation, and it should be taken into account as a possible cardiovascular risk marker assessed by a non-invasive method.

In summary, our data point out the need that in this population more attention than usually should be ascribed, to intervene and prevent complications later in life.

What is known about topic
- The risk of cardiovascular diseases doubles as blood pressure raises from 115/75 mm Hg doubles with each increment of 20/10 mm Hg, therefore prehypertensive subjects require earlier lifestyle changes to prevent CVD.[1]
- One of the earliest changes in hypertensive and/or normotensive subjects with family history is an impairment of endothelial function expressed as vasodilation in response to reactive hyperaemia.[3–6]

What this study adds
- Within 10-mm Hg interval for SBP, there was a progressive and significant reduction in vasodilatory response as blood pressure raised, of 17.3 and 14.2%, respectively.
- Starting from a SBP ⩾ 125 mm Hg up to a SBP < 140 mm Hg there was a marked reduction in vasodilation of 29%.
- Small increases in SBP but not in diastolic blood pressure impair endothelial function even in subjects considered as high-normal.

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