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ORIGINAL ARTICLE

Predictors of vascular remodelling in hypertensive subjects with well-controlled blood pressure levels

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We evaluated the structural/functional characteristics of the arterial wall in a cohort of hypertensives with well-controlled blood pressure (BP) levels. We studied 40 hypertensives with well-controlled BP. We assessed by B-mode ultrasound the mean intima-media thickness (mean-IMT) and maximum-IMT (M-MAX) of carotid artery (common, bulb, internal) bilaterally. Endothelial function was evaluated by post-occlusion flow-mediated dilation (FMD) of the brachial artery. Along with traditional risk factors, we studied the impact of serum high-sensitivity C-reactive protein (hs-CRP) and osteoprotegerin (OPG). Forty normotensive subjects served as controls. In the hypertensives, the BP levels were well controlled (office BP: 129/79 mm Hg, ambulatory BP monitoring: 121/75 mm Hg). Compared with controls, higher BP levels and body mass index were present in hypertensives, whereas age and metabolic parameters were similar. In hypertensives, the IMT (mean-IMT 0.68 mm, M-MAX 0.81 mm) was significantly higher than in controls (mean-IMT 0.60 mm, M-MAX 0.71 mm). FMD was impaired in hypertensives (5.9%) compared with controls (9.2%). In multivariate analyses, it turned out that in hypertensives IMT parameters were related to age, hs-CRP and OPG. Low-density lipoprotein (LDL) cholesterol was the only factor related to FMD. IMT and FMD had no relationship with BP levels. In conclusion, in hypertensives with well-controlled BP, the pro-atherogenic remodelling (IMT) is mainly dependent on age and the inflammatory cytokines, OPG in particular. The functional impairment of the arterial wall (FMD) was related to the levels of LDL cholesterol. Under these conditions, when the impact of BP is minimized, the role of inflammatory cytokines and lipids on structural/functional remodelling becomes predominant.

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INTRODUCTION

Compared with normotensive controls, in hypertensive subjects an excess prevalence of intimal thickening and atherosclerotic lesions was reported.[1,2] Several studies have been performed in hypertensive patients using ultrasound assessment of carotid intima-media thickness (IMT), a parameter that is generally considered as a reliable surrogate of intimal thickening. These studies showed that carotid IMT is greater in hypertensives than in normotensive subjects and that it correlates with blood pressure (BP) values.[3–5] Besides ultrasound IMT measurement, the evaluation of the endothelial function by flow-mediated vasodilation (FMD) represents another non-invasive method that analyzes the functional properties of the vessel wall.[6,7] Endothelial dysfunction is considered as the first step to atherosclerosis and has an important role in the pathogenesis of cardiovascular disease. Hence, FMD has been recently investigated as a putative tool for detecting atherosclerosis and as a possible therapeutic target.[8–10] Finally, impaired endothelial function is common in hypertensives and some pharmacological antihypertensive interventions have been shown to be effective in restoring endothelium-dependent vasodilation.[11]

Atherosclerosis can be considered as a chronic inflammatory condition of the vessel wall. Several immune/autoimmune factors have their own role in atherogenesis and a complex interplay of molecules and cells is responsible for the increase in the intima layer eventually leading to plaque development and progression.[12] The receptor activator of nuclear factor kappa B (RANK), osteoprotegerin (OPG) and their ligand RANK ligand (RANKL) are a set of proteins within the TNF/TNF receptor families that are required for the control of bone remodelling. Many studies revealed the involvement of the OPG/RANK/RANKL molecular system in the remodelling of vascular structures, through its interplay with immune and procalcific cells.[13,14]

In previous studies, we evaluated a cohort of young subjects with grade I hypertension enroled at the University of Padova within the frame of the Hypertension and Ambulatory Recording VEnetia STudy (HARVEST). It turned out that in never-treated hypertensives, carotid IMT is greater and grows faster than in normotensive subjects.[4,15] BP levels and plasma lipids were among the main determinants of carotid IMT. However, it is not known whether in uncomplicated hypertension the achievement of a good BP control can slow down the carotid IMT progression and bring back endothelial function to normal.

We therefore sought to establish the relationship between carotid artery remodelling along with the endothelial function in a cohort of hypertensive subjects with long-time well-controlled BP levels. Parameters of pro-atherogenic remodelling were compared with those in a group of normotensive subjects and were correlated with traditional and non-traditional risk factors, markers of inflammation and serum OPG levels in particular.

SUBJECTS AND METHODS

Study groups and inclusion criteria

We studied 40 stage I essential hypertensive subjects (HT) with well-controlled BP levels (office BP permanently $< 140/90$ mm Hg[16]) attending

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hospital outpatient clinics enroled in the HARVEST centre of Padova. The HARVEST is a prospective study aimed at evaluating the prevalence and the progression of markers of target organ damage in young subjects at low cardiovascular risk screened for stage I hypertension.[4] Subjects having a systolic BP in the 140–159 mm Hg range or a diastolic BP in the 90–99 mm Hg range at screening were considered for inclusion in the HARVEST.[17] Patients with diabetes mellitus, nephropathy and cardiovascular disease were excluded.[17] Only two patients among hypertensives and three in the normotensive group were taking statins. For the present study, we enroled 40 patients who had been kept either on pharmacological treatment (31 of 40) or on lifestyle modification (9 of 40 patients) for at least 6 months. The goal was to maintain office BP levels below the target of 140/90 mm Hg. Office BP was measured at baseline and at each follow-up visit three times in the sitting position, after 5 min of rest and the average of the three consecutive readings was used.[16] All of the HT underwent 24-h ambulatory BP monitoring during a routine working day. During the recordings, subjects were invited to follow their ordinary routine. Subjects were asked to go to bed no later than 2300 h. BP and heart rate were measured every 10 min during waking hours (0600 to 2300 h) and every 30 min during the night time. For the statistical analyses, the office BP measured at the ultrasound study and the nearest ambulatory BP monitoring to the time of the ultrasound study was used. As controls, we studied 40 normotensive healthy subjects (NT) comparable by sex and age. All subjects gave informed consent to the study protocol, which was approved by the local ethics committee.

Biochemical and molecular analyses

Fasting serum samples were collected and stored at − 80 °C until use. We assessed serum lipid profile (total cholesterol, low-density lipoproteins (LDL-C), high-density lipoproteins, triglycerides), fasting blood glucose, high-sensitivity C-reactive protein (hs-CRP) and TNF-α using standard assays from the commerce. Serum OPG concentrations were determined by the DuoSet ELISA Development System for human OPG (R&D Systems, Minneapolis, MN, USA). Monoclonal mouse anti-human OPG antibody was used to capture OPG from serum and a biotinylated polyclonal goat anti-human OPG antibody was used for detection. In our laboratory, the coefficient of variation, as judged from duplicate measurements, was 7.9%.

Ultrasound studies

Ultrasound examination of carotid arteries. Carotid ultrasound examinations were performed using the Aspen Advanced Ultrasound System (Acuson, Mountain View, CA, USA) equipped with a linear probe (7–10 MHz). The procedure was carried out according to the Mannheim Intima-Media Thickness Consensus.[6] All subjects were examined in the same room in dim light, lying comfortably in a supine position. The right and left carotid arteries of each subject were examined by the same sonographer. Once an optimal longitudinal image was obtained, it was stored on 1/2-inch super VHS videotape. Images were analysed using a high-resolution video recorder, coupled with a mouse-driven image analysis system. IMT, defined as the distance between the lumen-intima and the media-adventitia interfaces, was measured at end diastole in the far wall of the right and left sides of the common carotid artery, the bulb and the internal carotid artery.[15] IMT measurements were expressed as cumulative mean of mean-IMT (mean-IMT) and as cumulative mean of maximum-IMT (M-MAX) recorded in each vascular segment. To rule out potential interference of arterial enlargement with IMT measurements, the intraluminal diameter of common carotid artery 1 cm proximal to the dilatation of the bulb was measured at end diastole in lateral projection. In our lab, reproducibility of IMT measurements on separate visits displayed a coefficient of variation of 4.4 and 9.6%, for mean-IMT and maximum-IMT, respectively. The Bland–Altman statistics confirmed the good reproducibility of measurements.

FMD of brachial arteries. Nitirc oxide-dependent and -independent vasodilatation was determined by a B-mode scan of the right brachial artery in longitudinal section above the elbow using a 7–10 MHz linear array transducer and a standard Aspen Advanced Ultrasound System (Acuson, USA). The transducer was held at the same point throughout the scan by a stereotactic clamp to ensure consistency of the image.[7] A cuff was placed around the forearm just below the elbow. Measurements were obtained using a dedicated automatic system for computing the brachial artery diameter in real-time by analyzing B-mode ultrasound images.[10] Endothelium-dependent response was assessed as dilation of the brachial artery to increased flow (FMD). After 1 min of acquisition for measuring basal diameter, the cuff was inflated for 5 min at 250 mm Hg and then deflated to induce reactive hyperaemia. Endothelium-independent dilation was obtained by administration of a low dose (25 μg) of sublingual glyceril trinitrate (GTN). Ten minutes were allowed to recover baseline diameter after cuff deflation or GTN administration. FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline. In our lab, the reproducibility of FMD on separate visits displayed a coefficient of variation of 8.4%. The response to GTN was similar in the two groups, confirming the effectiveness of the technique we used.

Statistical analysis

All data are presented as mean (± s.d.) or median (interquartiles range). Differences between groups were compared by analysis of (co)variance and the two-sided Tukey's *post hoc* test for normally distributed parameters. The Kruskal–Wallis and the Mann–Whitney *U*-tests were used to compare non-normally distributed data. Prevalence of categorical variables were evaluated with two-way contingency tables, expressed as a percentage rate, and compared by means of the Pearson χ^2-test. The univariate correlations between mean-IMT, M-MAX and FMD and other continuous variables were evaluated by Pearson correlation coefficient with Bonferroni's adjusted probabilities. The IMT parameters and FMD were also played into a multiple regression analysis, obtaining one multiple correlation coefficient (*r*). A two-tailed probability value < 0.05 was considered significant. The SPSS Statistics (SPSS Inc., Chicago, IL, USA) package was used.

RESULTS

Demographic data of HT and NT subjects are presented in Table 1. Compared with NT, higher BP levels, body mass index and waist circumference were present in HT, whereas age, serum

Table 1. General and ultrasound data of the two populations

	Normotensive subjects (n = 40)	*Hypertensive subjects (n = 40)*	P-*value*
Age (years)	52.2 ± 9.7	50.3 ± 10.2	NS (0.401)
BMI (kg m^{-2})	24.6 ± 3.2	26.5 ± 3.3	0.008
Waist (cm)	87.3 ± 10.7	96.9 ± 12.3	< 0.0005
Systolic BP (mm Hg)	118.1 ± 9.7	128.8 ± 10.9	< 0.0005
Diastolic BP (mm Hg)	75.4 ± 6.7	78.6 ± 6.9	0.043
MAP (mm Hg)	89.7 ± 7.1	95.3 ± 7.2	0.001
Heart rate (b.p.m.)	68.1 ± 9.9	69.5 ± 13.2	NS (0.614)
Total cholesterol (mmol l^{-1})	5.38 ± 1.04	5.17 ± 0.91	NS (0.347)
LDL cholesterol (mmol l^{-1})	3.38 ± 0.96	3.27 ± 0.92	NS (0.592)
HDL cholesterol (mmol l^{-1})	1.33 ± 0.38	1.39 ± 0.34	NS (0.467)
Non-HDL cholesterol (mmol l^{-1})	4.05 ± 0.99	3.78 ± 0.98	NS (0.231)
Triglycerides (mmol l^{-1})	1.41 ± 0.88	1.21 ± 0.47	NS (0.224)
Glucose (mmol l^{-1})	4.91 ± 0.56	4.98 ± 0.57	NS (0.591)
hs-CRP (mg l^{-1}), mean	1.20 ± 1.37	1.61 ± 1.99	NS (0.344)
median	0.71 (0.50–1.16)	0.96 (0.51–1.77)	NS (0.179)
OPG (pg ml^{-1}), mean	986 ± 254	974 ± 216	NS (0.868)
median	884 (840–1195)	991 (791–1126)	NS (0.817)
TNF-α (pg/ml), mean	6.63 ± 2.40	8.46 ± 6.25	NS (0.424)
median	6.10 (4.90–7.90)	7.45 (6.43–8.40)	NS (0.052)
Mean-IMT (mm)	0.604 ± 0.090	0.679 ± 0.135	0.004
M-MAX (mm)	0.707 ± 0.125	0.813 ± 0.145	0.001
FMD (%)	9.2 ± 2.9	5.8 ± 1.8	< 0.0005
GTN (%)	9.1 ± 3.0	8.0 ± 2.4	NS (0.079)

Abbreviations: BMI, body mass index; BP, blood pressure; b.p.m., beats per min; FMD, flow-mediated dilation; GTN, glyceril trinitrate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; LDL, low-density lipoprotein; MAP, mean arterial pressure; mean-IMT, cumulative mean of mean-IMT recorded in each carotid artery segments in both sides; M-MAX, cumulative mean of maximum-IMT recorded in each carotid artery segments in both sides; NS, not significant; OPG, serum osteoprotegerin; TNF-α, tumour necrosis factor alpha. Data are presented as mean value ± s.d. or median (interquartile range). *P*-value: *t*-test or Mann–Whitney *U*-test.

cholesterol, high-density lipoprotein cholesterol, triglycerides and glucose were similar. Comparable levels of inflammatory markers were present in HT and NT. In the hypertensive cohort, office BP was well controlled in all subjects, with a mean sitting office BP of 129 ± 11/79 ± 7 mm Hg. The good BP control was confirmed by the results of ambulatory BP monitoring, with a mean 24-h BP of 121 ± 8/75 ± 6 mm Hg.

In HT, the IMT measurements were within the normal range, usually considered as < 0.9 mm, but values were significantly higher than those of NT (Table 1). FMD was impaired in HT compared with NT (Table 1).

As far as the role played by the classic and new risk factors in determining the vascular remodelling is concerned, in the whole population BP levels and inflammatory markers, hs-CRP and OPG in particular, were factors related to mean-IMT and M-MAX at the univariate analyses (Supplementary Table A). Systolic BP was the only factor inversely related to FMD (Supplementary Table A).

Compared with NT, in HT the crude odd ratios for the presence of advanced vascular damage (M-MAX $\geqslant 0.9$ mm) was three times greater, which turned into five times after adjustment for age (Table 2). Although in the whole population the multivariate analyses showed that IMT measurements were related to BP levels (not shown), in HT there was no relationship of IMT and FMD with BP levels. In the univariate analyses, in the HT, hs-CRP and OPG were the only factors related to IMT measurements (Figure 1, Supplementary Table B), whereas total serum cholesterol and LDL cholesterol were related inversely to FMD (Supplementary Table B). Ultrasound parameters were played into a multiple regression analysis including as independents variables those related to classical cardiovascular risk factors, such as age, body mass index, LDL cholesterol level, high-density lipoprotein cholesterols, triglycerides, glucose and those related to new risk factors, hs-CRP and OPG in particular. The multivariate step analyses confirmed that both mean-IMT and M-MAX were related to age and OPG (Table 3). LDL cholesterol was the only factor related to FMD (Table 3).

DISCUSSION

The aim of our study was to evaluate the extent of pro-atherogenic remodelling in hypertensive patients with long-time well-controlled BP levels. Our approach was based on measurements of carotid IMT by ultrasound and of brachial artery vasodilation by FMD.[6,7] The present results showed that in HT with well-controlled BP, IMT was increased and endothelial function was impaired compared with a group of control subjects.

Table 2. ORs for the presence of vascular damage (M-MAX $\geqslant 0.9$ mm) in hypertensives and controls

Groups	*M-MAX < 0.9 mm*	*M-MAX $\geqslant 0.9$ mm*	*Crude OR (95% CI)*	P-*value*	*Adjusted OR* (95% CI)*	P-*value*
NT	36	4	Reference	—	Reference	—
HT	30	10	3.00 (0.85–10.54)	0.077	5.10 (1.18–22.13)	0.029

Abbreviations: CI, confidence interval; HT, hypertensive subjects; M-MAX, cumulative mean of maximum-IMT recorded in each carotid artery segments in both sides; NT, normotensive subjects; OR, odds ratio; * OR adjusted for age.

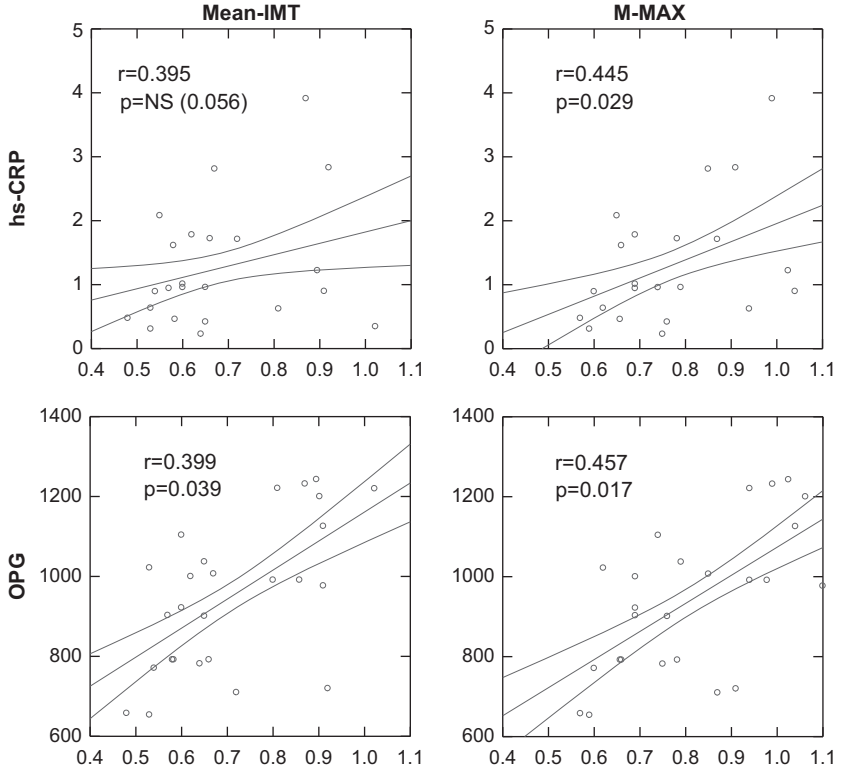

Figure 1. Relationship of mean-IMT and M-MAX to hs-CRP and OPG in the hypertensive cohort.

Table 3. Multivariate regression analysis for factors potentially linked to ultrasound parameters in hypertensive subjects ($n = 40$; best models)

Individual variables	*Coefficent*	*S.e.*	P-*value*
Mean-IMT, multiple r^2 0.507, $P < 0.0005$			
Intercept	−0.019	—	—
Age	0.679	0.003	< 0.0005
OPG	0.261	0.0005	NS (0.076)
M-MAX, multiple r^2 0.570, $P < 0.0005$			
Intercept	−0.062	—	—
Age	0.627	0.003	< 0.0005
OPG	0.339	0.0005	0.029
FMD, multiple r^2 0.100, $P = 0.026$			
Intercept	7.842	—	—
LDL cholesterol	−0.635	0.275	0.026

Abbreviations: FMD, flow-mediated dilation; IMT, intima-media thickness; LDL, low-density lipoprotein; mean-IMT, cumulative mean of mean-IMT recorded in each carotid artery segments in both sides; M-MAX, cumulative mean of maximum-IMT; OPG, serum osteoprotegerin.

Although medial hypertrophy may contribute to increased IMT in hypertensives and could represent a sort of target organ damage, there is evidence for an excess prevalence of intimal thickening and true atherosclerotic lesions in patients suffering from definite hypertension when compared with normotensives.[18,19] For these reasons, an increased carotid IMT has been considered as a marker of subclinical atherosclerosis.[20] Although IMT is a weak predictor of cardiovascular events,[21] carotid IMT has also been taken as a surrogate end point for clinical events in several intervention trials using antihypertensive medications.[3]

Hypertension accelerates the atherosclerotic process not only through haemodynamic factors, bur also via the vasoactive peptides angiotensin II and endothelin-1, by triggering inflammatory mechanisms of oxidative stress in the arterial wall, by inducing overexpression of adhesion molecules and inflammatory mediators, activation of NF-kB, increased breakdown of NO and uncoupling of NO synthase.[22] Ultimately, this would result in endothelial dysfunction and growth of myointimal cells. Moreover, several studies have reported an association between lipid levels and arterial function impairment as measured by FMD[23] and serum OPG was shown to act as an independent risk factor for the progression of carotid atherosclerosis and the onset of cardiovascular disease in population-based studies.[24]

In the present study, in HT we observed that, even when hypertension is well controlled, there is an increased risk of pro-atherogenic remodelling (Tables 2 and 3). As shown in Table 2, the presence of advanced vascular damage as represented by M-MAX was three to five times greater in HT compared with NT. In the literature, the continued impact of a more elevated BP burden seems to be responsible for the progression in mean-IMT, which is also likely to reflect some hypertrophy of the arterial media layer.[19,20] Taking into account the BP difference between the HT and NT in the present study, we cannot rule out that some 'pseudo-normalization' of the BP levels could be sufficient to promote the atherosclerotic process in our HT. However, in our HT, both mean-IMT and M-MAX were dependent on age and the inflammatory cytokines, OPG in particular, rather than on BP levels (Table 3). When the NH were examined separately, there was no impact of inflammatory markers on both FMD and IMT (not shown) meaning that the relationship between inflammatory markers and IMT parameters was mainly sustained by the hypertensive status.

As far as brachial artery FMD is concerned, this parameter was impaired in HT compared with NT, apparently supporting the view that endothelial dysfunction was affected by BP levels, which were still higher than in NT (Table 1). However, looking at the univariate and multivariate analyses, both total serum cholesterol and LDL cholesterol rather than BP levels were the factors related to FMD. An inverse relationship of serum cholesterol to FMD is not surprising.[25] Despite interventional studies having shown that some antihypertensive therapies are able to restore the endothelial function,[11] this was not observed in our series. Of course, our study was not designed to address the issue of the impact of pharmacological treatment and lifestyle modification on the arterial wall remodelling. Nevertheless, we can argue that when the impact of BP is minimized as in our study, the persistence of endothelial dysfunction seems to depend on cholesterol rather than on BP level (Table 3, Supplementary Table B).

As mentioned above, within the whole series and in HT in particular, OPG level was related to the degree of both mean-IMT and M-MAX but not to FMD (Figure 1, Table 3, Supplementary Tables A and B). A potential mechanism for a role of OPG in the vascular remodelling of HT is represented by the inflammatory process underlying the atherogenic process.[26] In particular, the abundance of activated monocytes could induce the overexpression of OPG by vascular cells.[27] Although the participation of OPG to atherogenesis is still controversial some studies showed a direct effect of this mediator in promoting smooth muscle cell migration/proliferation,[28] a well-known step in carotid IMT progression.[29] Specific studies are warranted to address this issue.

The relatively small size of the series represents a main limitation of the study. This was due to the difficulty of enroling stage I hypertensives with well-controlled BP, subjected to a close follow-up and strict exclusion criteria. Another limitation is the lack of information about the potential impact on vascular remodelling of the different antihypertensive medications used.

In conclusion, in HT with well-controlled BP, an increase of IMT takes place along with impaired endothelial function compared with normotensive controls. The pro-atherogenic remodelling (IMT) was mainly dependent on age and the inflammatory cytokines, OPG in particular, whereas the functional impairment of the arterial wall (FMD) was related to levels of LDL cholesterol. This suggests that when the impact of BP is minimized by treatment or lifestyle changes, the role of inflammatory cytokines and lipids on structural/functional remodelling of the arterial wall may become predominant.

What is known about topic

- Increased prevalence of intimal thickening and atherosclerotic lesions have been observed in hypertensive subjects.

What this study adds

- In hypertensives with well-controlled BP, pro-atherogenic remodelling is enhanced and vascular reactivity are reduced compared to normotensive controls.
- The structural pro-atherogenic remodelling (IMT) is mainly dependent on age and the inflammatory cytokines, osteoprotegerin in particular, whereas the functional impairment of the arterial wall (FMD) was related to the levels of LDL cholesterol.
- When the impact of BP is minimized, the role of inflammatory cytokines and lipids on structural/functional remodelling becomes predominant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Journal of Human Hypertension website (http://www.nature.com/jhh)