

Decreased fibrinolytic activity is associated with carotid artery stiffening in arterial hypertension

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Background: A prothrombotic state is associated with the presence and severity of organ damage in hypertensive patients. In these patients, evidence of subclinical carotid functional changes anticipates major cardiovascular events. The aim of this study was to investigate the association of hemostatic markers with carotid artery stiffness in hypertension. **Materials and Methods:** In 116 untreated essential hypertensive patients recruited at a referral center in the University of Udine, we assessed common carotid artery stiffness by B-mode ultrasonography and measured plasma fibrinogen, D-dimer, plasminogen activator inhibitor-1 (PAI-1), and homocysteine by the currently available methods. For statistical reasons, the patients were divided according to the median value of each index of carotid stiffness, and continuous variables were further analyzed by univariate correlation and stepwise multivariate regression analysis. **Results:** PAI-1 levels were significantly higher in patients with low coefficient of distensibility ($P = 0.018$) and high Young's elastic modulus ($P = 0.012$), whereas no association of fibrinogen, D-dimer, and homocysteine levels was observed with carotid coefficient of distensibility, Young's elastic modulus, and β -stiffness. On univariate analysis, Young's elastic modulus was significantly and positively correlated with PAI-1 levels ($r = 0.286$, $P = 0.002$), a correlation that on multivariate regression resulted to be independent of other confounders ($\beta = 0.289$, $P = 0.028$). **Conclusion:** An independent association of plasma PAI-1 levels with carotid artery stiffness suggests a possible contribution of decreased fibrinolytic activity to the early functional abnormalities of arterial vessels in hypertensive patients. This contribution might be relevant for subsequent development of hypertension-related cardiovascular complications.

Key words: Beta stiffness, carotid artery, fibrinolysis, plasminogen activator inhibitor-1, primary hypertension, Young's elastic modulus

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INTRODUCTION

In addition to traditional cardiovascular risk factors, other factors that are commonly referred to as "emerging" or "nontraditional" are possibly involved in the occurrence of atherosclerosis-related events.^[1] Among these emerging risk factors, the coagulation system has been called into play and robust epidemiological evidence indicates its involvement in the pathophysiology of atherosclerotic vascular disease.^[2] For instance, an activated coagulation system is associated with more severe cardiovascular complications^[3] and left ventricular changes^[4] in individuals with high blood pressure, suggesting a contribution of a prothrombotic state.

The early stages of atherosclerosis are characterized by subtle functional and/or structural changes of arterial vessels that could be assessed noninvasively by ultrasound examination of carotid arteries.^[5] Carotid stiffness is a reproducible marker of early vascular disease that predicts major cardiovascular events^[5,6] and development of hypertension,^[7] providing important information for stratification of cardiovascular risk.^[8] A significant association of an activated hemostatic system with early arterial changes has been reported in individuals without clinically overt atherosclerotic disease, suggesting a contribution of a prothrombotic state.^[9,10] In hypertensive patients, changes of carotid arteries have specific relevance because they are a reliable marker of the overall vascular damage. In these patients, the carotid intima-media thickness (IMT) has

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been found to be independently associated with fibrin D-dimer,^[11] a breakdown fragment of fibrin and a marker of the overall activity of the hemostatic system. However, data on the possible association of a prothrombotic state with carotid artery stiffness are not available, and this is why we sought to investigate the relationship of hemostatic variables with carotid distensibility in hypertensive patients.

MATERIALS AND METHODS

Patients

One-hundred and sixteen patients (63 males, 53 females; age 49 ± 13 years) with Grade 1–2 primary hypertension who were consecutively referred from July 2014 to December 2015 to the hypertension clinic at the University of Udine were included in the cross-sectional study. All patients were light-skinned people and were representative of the hypertensive population in this area. Blood pressure was measured with an automated device (Omron M6, OMRON Healthcare Co., Kyoto, Japan) after each individual had been supine for 15 min, and the average of three readings was recorded. Diagnosis of hypertension was established in all patients according to the current guidelines.^[8] Patients younger of 18 years and older than 80 years were excluded, together with patients with glomerular filtration rate of <30 ml/min/1.73 m,^[2] cardiovascular complications, secondary hypertension, use of drugs that could interfere with hemostatic variables, and acute illness. Cardiovascular complications were identified for exclusion by the analysis of medical records, physical examination, electrocardiography (ECG), echocardiography, and ultrasound examination of aorta, carotid, iliac, and femoral arteries. Additional evaluations included exercise testing, myocardial scintigraphy, and angiography and were done when needed.^[12] Secondary forms of hypertension were excluded after extensive clinical and laboratory investigations that included duplicate measurements of 24-h creatinine clearance, plasma active renin and aldosterone, urinary cortisol and catecholamines, and renal ultrasound examination with the measurement of renal resistance index. Renal angio magnetic resonance imaging/computed tomography scan and additional functional tests were performed when appropriate.^[8,13]

Forty-eight (41%) of 116 patients had never been treated with antihypertensive drugs. The remaining 68 patients (59%) were treated with drugs that were washed out for a minimum of 2 weeks before the study. All patients were closely monitored during the wash-out period, and in those with blood pressure persistently higher than 180/110 mmHg, alpha-blockers and/or calcium-channel blockers were given. Before evaluation, patients ate a standard diet for 7 days to keep a sodium intake of 100–150 mmol/day that was checked with the measurement of sodium excretion in

24-h urine collections. Patients were defined as smokers if they had smoked for at least 5 years and up to 1 year before the study, and smoking habit was quantified by the average number of cigarettes/day. Alcohol intake was estimated by a questionnaire^[14] as g/day. The study was performed in accordance with the principles of the Declaration of Helsinki and received approval from the local Institutional Review Board. Informed consent was obtained from all patients

Assessment of carotid artery distensibility

Carotid arteries were examined with a duplex scanner (Toshiba Aplio CV, Japan) using a 7 MHz linear array transducer.^[15] The same trained operator performed all examinations. B-mode ultrasound examination of carotid arteries permits assessment of carotid artery stiffness with a good degree of reproducibility and provides information comparable to other echo-tracking methods with specificity and sensitivity of more than 90%.^[16] After patients had been laying quietly in a room for 15 min, and immediately before performing carotid artery ultrasound examination, blood pressure was measured at the left arm. The average of the last 2 of 3 consecutive measurements obtained in 5 min was considered, and the pulse pressure (PP) was calculated as the difference between systolic and diastolic blood pressure. Longitudinal scan of right and left common carotid arteries was performed under ECG guide to measure carotid systolic diameter (Ds) and diastolic diameter (Dd), and the average of measurements obtained in three consecutive cardiac cycles was calculated for both arteries according to consensus.^[16] Measurements used to calculate carotid distensibility are shown in Table 1 together with the formulas that have been used for calculation and the respective units. Intra-observer variability of all ultrasound measurements was below 8%, and the correlation coefficient (*r*) of duplicate measurements for all parameters was >0.850 showing good reproducibility of these measurements.

Laboratory measurements

A sample of venous blood was collected in the morning after an overnight fast with the patients in sitting position. Blood was collected into silicone-treated tubes containing trisodium citrate, and plasma was immediately separated and frozen at -80°C until assaying, usually within 1 month from sampling. Plasma glucose was assayed

Table 1: Indices of carotid artery distensibility that were measured in the study patients and formulas that are used for their calculation

Distensibility	(Ds–Dd)/Dd (%)
Coefficient of distensibility	$2 \times (Ds - Dd) / Dd / PP$ ($10^{-3} / \text{kPa}$)
Young's elastic modulus	$Dd / (IMT \times DC)$ ($10^3 / \text{kPa}$)
β -stiffness	$\ln (SBP / DBP) / [(Ds - Dd) / Dd]$

Ds = Carotid systolic diameter; Dd = Carotid diastolic diameter; PP = Pulse pressure; IMT = Carotid intima-media thickness; DC = Coefficient of carotid distensibility; SBP = Systolic blood pressure; DBP = Diastolic blood pressure

using the glucose-oxidase method. Total cholesterol and triglycerides were assayed enzymatically by an automated method. High-density lipoprotein cholesterol was assayed enzymatically after magnesium chloride-dextran sulfate precipitation of apolipoprotein B-containing lipoproteins, and low-density lipoprotein cholesterol was calculated with the formula of Friedewald. Glomerular filtration rate was measured by duplicate measurement of 24-h creatinine clearance and normalized for body surface area. Hemostatic variables were measured as reported previously.^[4,17] Briefly, plasma fibrinogen was determined by a functional assay in an automated coagulometer autoanalyzer (Instrumentation Laboratory, Lexington, Massachusetts, USA; inter- and intra-assay coefficient of variation: 6.8% and 5.1%, respectively), D-dimer was measured immunoenzymatically (according to the method of Rylatt; inter- and intra-assay coefficient of variation: 7.1% and 5.3%, respectively), and plasminogen activator inhibitor-1 (PAI-1) was assayed by enzyme immunoassay (Innotest PAI-1; Byk-Sangtec, Dietzenbach, Germany; inter- and intra-assay coefficient of variation: 6.8% and 4.6%, respectively). Homocysteine was determined by a nephelometric method (Dimension Vista System, Siemens Healthcare Diagnostics, Milan, Italy; inter- and intra-assay coefficients of variation: 8.2% and 7.0%, respectively).^[18]

Statistical methods

Values are expressed as mean \pm standard deviation for normally distributed variables, with median and interquartile ranges used for variables with skewed distributions. Normality of distribution was assessed with Kolmogorov-Smirnov test, and variables with skewed distributions were analyzed after logarithmic transformation. The Student's *t*-test was used for comparison between two independent groups after adjustment for age, body mass index, and blood pressure values. Pearson's Chi-square test was used to compare frequency distributions. Relationships between continuous variables were examined through linear regression analysis, with correlation expressed by Pearson's correlation coefficient. Stepwise multivariate regression analysis was used to ascertain which variables were independently related to indices of carotid stiffness. The sample size was calculated to provide a power >90% with a probability of <5% in the detection of a 20% difference in hemostatic variables between individuals with low or high carotid stiffness. Probability values of <5% were considered to indicate statistical significance. Data analyses were done with the Stata 9.2 software system (StataCorp LP, College Station, TX, USA).

RESULTS

The clinical characteristics, biochemical variables, and indices of carotid artery stiffness of the patients included in the study are summarized in Table 2. Patients were divided

according to the median value of the distribution of each index of carotid stiffness, and plasma levels of fibrinogen, D-dimer, PAI-1, and homocysteine are reported in Table 3. After adjustment for the confounding effects of age, body mass index, and blood pressure value, PAI-1 levels were significantly higher in patients with low values of coefficient of distensibility and high values of the Young's elastic modulus. No significant differences were observed in fibrinogen, D-dimer, and homocysteine between patients with values below or above the median of carotid distensibility, coefficient of distensibility, Young's elastic modulus, and β -stiffness.

Because the coefficient of distensibility and Young's elastic modulus are, respectively, the markers that best define the

Table 2: Clinical characteristics, biochemical variables, and markers of carotid artery distensibility of the study patients

Variables	Study patients (n=116)
Clinical characteristics	
Age (years)	49 \pm 13
Body mass index (kg/m ²)	27.4 \pm 4.8
Heart rate (bpm)	72 \pm 12
SBP (mmHg)	150 \pm 19
DBP (mmHg)	93 \pm 12
PP (mmHg)	57 \pm 15
Duration of hypertension (years)	6.0 \pm 6.9
Alcohol intake (g/day)	9 \pm 20
Antihypertensive treatment, n (%)	60 (53)
Males, n (%)	61 (54)
Smokers, n (%)	26 (25)
Biochemical variables	
Serum creatinine (mg/dl)	0.96 \pm 0.21
Creatinine clearance (ml/min/1.73 m ²)	56 \pm 18
Glucose (mg/dl)	91 \pm 15
Total cholesterol (mg/dl)	203 \pm 44
HDL cholesterol (mg/dl)	56 \pm 17
LDL cholesterol (mg/dl)	123 \pm 37
Uric acid (mg/dl)	5.18 \pm 1.26
Triglycerides (mg/dl)	96 (69-143)
Fibrinogen (mg/dl)	383 (331-438)
D-dimer (ng/ml)	240 (150-342)
PAI-1 (ng/ml)	9.0 (5.6-17.9)
Homocysteine (μ mol/l)	11.9 (9.3-14.5)
Carotid artery variables	
Systolic diameter (mm)	6.38 \pm 0.78
Diastolic diameter (mm)	5.83 \pm 0.76
Distensibility (%)	9.85 \pm 3.73
Coefficient of distensibility (10 ⁻³ /kPa)	35.87 \pm 14.67
Young's elastic modulus (10 ³ /kPa)	0.49 \pm 0.46
β -stiffness	6.28 \pm 3.87

Values are expressed as mean \pm SD or median (IQR) for variables with skewed distribution. To convert to international units, multiply creatinine by 88.4 (μ mol/l), glucose by 0.05551 (nmol/l), insulin by 7.175 (pmol/l), cholesterol by 0.0259 (mmol/l), triglycerides by 0.0113 (mmol/l), uric acid by 59.485 (μ mol/l). SBP=Systolic blood pressure; DBP=Diastolic blood pressure; PP=Pulse pressure; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; PAI-1=Plasminogen activator inhibitor-1; IQR=Interquartile range; SD=Standard deviation

Table 3: Plasma concentrations of hemostatic markers according to values (below or above the median of the distribution) of six different indices of carotid artery distensibility

Variables	Distensibility (%)			Coefficient of distensibility ($10^{-3}/\text{kPa}$)		
	Median (IQR)		P	Median (IQR)		P
	Below	Above		Below	Above	
Fibrinogen (mg/dl)	386 (331-466)	382 (332-433)	0.138	386 (334-453)	383 (328-437)	0.172
D-dimer (ng/ml)	261 (147-418)	239 (160-317)	0.338	248 (160-319)	235 (146-356)	0.496
PAI-1 (ng/ml)	11.0 (4.8-18.9)	8.9 (6.3-14.7)	0.826	12.0 (8.1-19.6)	7.7 (4.4-14.0)	0.018
Homocysteine ($\mu\text{mol/l}$)	11.2 (9.1-14.1)	12.5 (9.6-15.2)	0.636	11.9 (9.7-15.0)	11.8 (9.2-14.3)	0.701

Variables	Young's elastic modulus ($10^3/\text{kPa}$)			β -stiffness		
	Median (IQR)		P	Median (IQR)		P
	Below	Above		Below	Above	
Fibrinogen (mg/dl)	367 (326-452)	396 (347-437)	0.920	380 (330-455)	391 (333-434)	0.094
D-dimer (ng/ml)	200 (143-268)	284 (204-363)	0.186	254 (150-420)	234 (153-307)	0.394
PAI-1 (ng/ml)	6.8 (3.4-12.7)	12.9 (7.8-19.8)	0.012	8.3 (5.4-18.0)	10.0 (6.3-17.6)	0.538
Homocysteine ($\mu\text{mol/l}$)	11.0 (8.9-13.5)	13.0 (9.8-15.8)	0.106	11.0 (8.9-13.4)	12.6 (9.8-15.6)	0.313

Values are expressed as median and IQR. Comparisons between patients with or without the metabolic syndrome were done by the Student's *t*-test after log transformation for variables with skewed distribution. Median values were: Distensibility 9.36%, coefficient of distensibility $34.88 \times 10^{-3}/\text{kPa}$, Young's elastic modulus $0.356 \times 10^3/\text{kPa}$, β -stiffness 4.95. PAI-1=Plasminogen activator inhibitor-1; IQR=Interquartile range

relationship of vascular dimension changes through cardiac cycle with the blood pressure change (PP) and geometry of the vascular wall (relative wall thickness),^[8] we combined these two variables to select among the study patients a group of individuals with the worst carotid stiffness profile (coefficient of distensibility below the median and Young's elastic modulus above the median) and compared these with the remaining patients [Table 4]. Patients with higher carotid artery stiffness were older and had greater body mass index, systolic pressure and PP, fasting glucose, and PAI-1 levels than patients with better carotid stiffness profile, whereas no differences were observed in fibrinogen, D-dimer, and homocysteine.

Univariate regression analysis indicated an inverse significant relationship of the coefficient of distensibility with age ($r = -0.226$, $P = 0.015$), body mass index ($r = -0.220$, $P = 0.018$), log-transformed plasma glucose ($r = -0.219$, $P = 0.018$), triglycerides ($r = -0.270$, $P = 0.003$), and PAI-1 levels ($r = -0.197$, $P = 0.034$). Young's elastic modulus was directly and significantly correlated with age ($r = 0.349$, $P < 0.001$) and PAI-1 levels ($r = 0.286$, $P = 0.002$). No further significant correlation was observed between hemostatic markers and indices of carotid stiffness [Table 5]. Stepwise multivariate regression analysis that included demographic, anthropometric, and plasma lipid variables indicated that the Young's elastic modulus was independently related with both age ($\beta = 0.360$, $P = 0.011$) and PAI-1 ($\beta = 0.289$, $P = 0.028$).

DISCUSSION

Many nontraditional cardiovascular risk factors are associated with the presence and severity of organ damage in hypertensive patients. Definition of the pathophysiology of subclinical vascular damage in these patients is of great relevance because its detection anticipates major

cardiovascular events. Moreover, previous studies indicate an involvement of the hemostatic system in the development of structural changes of the carotid arteries. The present study has examined the association of hemostatic markers with early functional changes of the carotid arteries. Findings indicate that plasma levels of PAI-1 are directly and independently related to carotid artery stiffness as assessed by the coefficient of distensibility and Young's elastic modulus, suggesting a contribution of decreased fibrinolytic activity to the early functional abnormalities of arterial vessels.

Increased arterial stiffness has long been considered a consequence of aging. In fact, progressive fragmentation and derangement of the elastic laminae of the arterial wall occur in major central arteries resulting in age-related vascular stiffening.^[19] In addition to aging, however, other cardiovascular risk factors contribute to reduction of distensibility of the vascular wall including smoking,^[20] dyslipidemia,^[21] diabetes,^[22] and hypertension.^[6] Moreover, cross-sectional studies have reported that increased carotid stiffness is associated with greater cardiovascular risk in patients with atherosclerotic risk factors or who have already developed vascular disease.^[7] Evidence of the role of increased arterial stiffness in prediction of cardiovascular events was obtained also in longitudinal studies that were conducted in patients with hypertension,^[23] impaired glucose tolerance,^[24] and end-stage renal disease.^[25]

Hypertension causes structural changes of the arterial wall that are associated with hypertrophy of the muscular layer, increase in extracellular matrix, and geometric remodeling,^[26] resulting in functional abnormalities that anticipate major hypertension-related cardiovascular complications.^[5,7] In addition to increased blood pressure, other conditions including a prothrombotic state might contribute to these

Table 4: Clinical characteristics, biochemical variables, and indices of carotid artery distensibility of study patients who were grouped according to severity of aortic stiffness as Group A (patients with coefficient of distensibility below or Young's elastic modulus above median of the distribution) and Group B (patients with both coefficient of distensibility below and Young's elastic modulus above the median of the distribution)

Variables	Group A (coefficient of distensibility >median and/or Young's elastic modulus <median) (n=77)		Group B (coefficient of distensibility <median and Young's elastic modulus >median) (n=39)		P
Clinical characteristics					
Age (years)	47±12		53±11		0.007
Body mass index (kg/m ²)	26.6±4.1		29.4±5.7		0.008
Heart rate (bpm)	72±12		74±13		0.455
SBP (mmHg)	146±17		158±20		0.002
DBP (mmHg)	93±13		94±11		0.424
PP (mmHg)	53±14		64±16		0.002
Duration of hypertension (years)	6±7		6±8		0.663
Alcohol intake (g/day)	8±20		11±21		0.550
Males, n (%)	38 (51)		23 (60)		0.320
Anti-hypertensive treatment, n (%)	36 (48)		24 (63)		0.127
Smokers, n (%)	19 (27)		7 (22)		0.571
Biochemical variables					
Serum creatinine (mg/dl)	0.94±0.19		0.96±0.16		0.536
Creatinine clearance (ml/min/1.73 m ²)	55±19		56±15		0.613
Glucose (mg/dl)	89±11		96±20		0.048
Total cholesterol (mg/dl)	203±46		201±31		0.747
HDL cholesterol (mg/dl)	56±16		56±21		0.895
LDL cholesterol (mg/dl)	125±39		120±24		0.394
Uric acid (mg/dl)	4.9±1.4		5.4±1.5		0.104
Triglycerides (mg/dl)	93 (66-126)		98 (85-169)		0.082
Fibrinogen (mg/dl)	367 (326-453)		397 (370-437)		0.611
D-dimer (ng/ml)	226 (150-346)		276 (202-344)		0.713
PAI-1 (ng/ml)	7.8 (3.6-14.6)		14.9 (10.2-22.2)		0.003
Homocysteine (μmol/l)	11.2 (9.3-13.8)		12.5 (9.3-15.5)		0.906
Carotid artery variables					
Systolic diameter (mm)	6.22±0.68		6.71±0.67		<0.001
Diastolic diameter (mm)	5.60±0.64		6.27±0.59		<0.001
Distensibility (%)	11.3±3.5		7.0±2.4		<0.001
Coefficient of distensibility (10 ⁻³ /kPa)	42.53±12.31		21.80±6.31		<0.001
Young's elastic modulus (10 ³ /kPa)	0.30±0.10		0.91±0.59		<0.001
β-stiffness	4.63±1.40		7.25±1.70		<0.001

Values are expressed as mean±SD or median (IQR) for variables with skewed distribution. Comparisons between patients with or without the metabolic syndrome were done by the Student's t-test after log transformation for variables with skewed distribution. Pearson's Chi-square test was used to compare frequency distributions. To convert to international units, multiply creatinine by 88.4 (μmol/l), glucose by 0.05551 (nmol/l), insulin by 7.175 (pmol/l), cholesterol by 0.0259 (mmol/l), triglycerides by 0.0113 (mmol/l), uric acid by 59.485 (μmol/l). SBP=Systolic blood pressure; DBP=Diastolic blood pressure; PP=Pulse pressure; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; PAI-1=Plasminogen activator inhibitor-1; IQR=Interquartile range; SD=Standard deviation

Table 5: Univariate correlation analysis of hemostatic markers and indices of carotid artery distensibility

Variables	Distensibility		Coefficient of distensibility		Young's elastic modulus		β-stiffness	
	r	P	r	P	r	P	r	P
Fibrinogen	0.068	0.468	0.009	0.924	-0.133	0.155	-0.157	0.092
D-dimer	0.107	0.252	0.024	0.798	-0.097	0.300	-0.077	0.411
PAI-1	-0.124	0.184	-0.194	0.037	0.286	0.002	0.167	0.073
Homocysteine	-0.001	0.991	-0.018	0.848	0.020	0.831	0.019	0.839

PAI-1=Plasminogen activator inhibitor-1

vascular changes and the related complications. In a study conducted in hypertensive patients, a relationship of plasma fibrinogen and D-dimer levels with the presence and severity of target-organ damage that was independent

of age, blood pressure, and duration of hypertension was reported.^[3] In another group of patients with hypertension, it was shown that elevated levels of fibrinogen, fibrin D-dimer, and homocysteine are associated with high

carotid IMT although this association was independent of possible confounders only for D-dimer.^[11] These findings together with those of other studies conducted in the general population^[27,28] and patients with type-2 diabetes^[29] indicate a contribution of a prothrombotic state to the early structural changes of carotid arteries. Moreover, these findings set the stage for the present study in which we have tested the hypothesis that an activated hemostatic system might contribute also to carotid artery stiffness in patients with hypertension. Our findings indicate that levels of PAI-1 are significantly and independently related to carotid artery stiffening, suggesting a role of decreased fibrinolytic activity. However, in agreement with previous studies,^[30] we have not observed associations of carotid stiffness with plasma fibrinogen, D-dimer, and homocysteine.

Limitations of this study should be highlighted. First, use of a clinic sample of patients with hypertension limits the possibility to extrapolate the present observations to the general population. Second, inclusion of a relevant percentage of hypertensive patients who were not treatment-naïve might have introduced, despite a relatively long wash-out period, a possible confounder affecting some of the variables measured in this study. It must be noticed, however, that no differences in indices of carotid distensibility or hemostatic markers were observed between untreated patients and patients who were treated with different classes of antihypertensive drugs.

CONCLUSION

This study demonstrates a significant and independent relationship of plasma PAI-1 levels with carotid artery stiffness, suggesting a possible contribution of decreased fibrinolytic activity to the early functional abnormalities of arterial vessels that are commonly detected in patients with high blood pressure. This relationship might be relevant for the subsequent development of hypertension-related cardiovascular complications. Further research in appropriately designed longitudinal studies would be warranted to better explore this hypothesis.

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Conflicts of interest

The authors have no conflicts of interest.

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