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# Biomarkers Associated With Pulse Pressure in African-Americans and Non-Hispanic Whites

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## Abstract

**BACKGROUND—**Pulse pressure (an indirect measure of arterial stiffness) is a robust predictor of cardiovascular events, but its pathophysiology remains poorly understood. To gain insight into the pathophysiology of arterial stiffness we conducted an exploratory investigation of the associations of 47 circulating biomarkers in etiologic pathways of arteriosclerosis with brachial artery pulse pressure.

**METHODS—**Participants included 1,193 African-Americans and 1,145 non-Hispanic whites belonging to hypertensive sibships. Blood pressure (BP) was measured with a random-zero sphygmomanometer. Multivariable linear regression was employed to assess the associations of biomarkers with pulse pressure after adjustment for age, sex, conventional risk factors, mean arterial pressure, heart rate, and use of aspirin, statins, estrogens, and antihypertensives. Statistical significance was set at $P \leq 0.001$ (Bonferroni correction for multiple testing).

**RESULTS—**Log N-terminal probrain natriuretic peptide (NT-proBNP) (African-Americans: $\beta = 2.11 \pm 0.52$, non-Hispanic whites: $\beta = 2.65 \pm 0.55$), log midregional proatrial natriuretic peptide (African-Americans: $\beta = 4.83 \pm 0.70$, non-Hispanic whites: $\beta = 3.70 \pm 0.67$), and log osteoprotegerin (African-Americans: $\beta = 4.64 \pm 1.02$, non-Hispanic whites: $\beta = 4.19 \pm 0.99$) were independently associated with pulse pressure ($P < 0.001$ for all) in both ethnicities. Log C-reactive protein (CRP) ($\beta = 1.56 \pm 0.35$), log midregional proadrenomedullin (MR-proADM) ($\beta = 5.53 \pm 1.19$) and log matrix metalloproteinase-2 ($\beta = 3.89 \pm 1.06$) were associated with greater pulse pressure in African-Americans only ($P \leq 0.001$ for all), whereas higher fibrinogen was associated with pulse pressure in non-Hispanic whites only ($\beta = 0.02 \pm 0.004$. $P < 0.001$).

**CONCLUSIONS—**Our results suggest that hemodynamic stress, vascular inflammation and calcification, and matrix remodeling may have a role in the pathogenesis and/or adverse consequences of increased pulse pressure.



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### Keywords

adrenomedullin; arterial stiffness; atrial natriuretic peptide; biomarkers; blood pressure; brain natriuretic peptide; fibrinogen; hypertension; matrix metalloproteinase-2; osteoprotegerin; pulse pressure

The blood pressure (BP) waveform consists of the summation of a steady component, mean arterial pressure, and a pulsatile component, the pulse pressure.[1] Pulse pressure results from intermittent ejection from the heart, coupled with cushioning properties of the arteries and the timing and intensity of wave reflections.[1] The rise in pulse pressure with aging appears to represent different properties of the arterial stiffening process, including central arterial stiffness and increased peripheral wave reflection. Pulse pressure is a readily available albeit indirect marker of arterial stiffness, making it convenient for analyses in large cohort studies.

In younger hypertensives (<60 years), systolic BP, diastolic BP, and mean arterial BP are each associated with adverse cardiovascular events.[2] However, in older hypertensive individuals, isolated systolic hypertension and elevated pulse pressure are more strongly associated with cardiovascular risk than elevated diastolic BP.[2] Pulse pressure is associated with increased mortality[3] and the risk of myocardial infarction,[4] new-onset atrial fibrillation,[5] stroke,[6] heart failure,[7] and cognitive decline.[8] Furthermore, the prevalence and severity of hypertension,[9] as well as risk of target-organ damage[10,11] is greater among African Americans than non-Hispanic whites. Despite this, African-Americans remain an understudied group, and the basis for the ethnic differences in the prevalence and pathophysiology of arterial stiffness remains obscure.

There is a significant gap in our knowledge of the pathophysiologic mechanisms that lead to arterial stiffening and increase in pulse pressure, their hemodynamic and pathologic consequences, and their differences based on ethnicity. Such knowledge could help identify individuals at higher cardiovascular risk while contributing to the development of therapeutic strategies aimed at arterial “destiffening.” We hypothesized that the pathways of inflammation, lipoprotein metabolism, adipocyte metabolism, calcification, thrombosis, and h emodynamic stress could play a role in the pathophysiology of arterial stiffness. To this end, we undertook an exploratory analysis of the associations of multiple candidate circulating biomarkers in the aforementioned pathways with pulse pressure in a biethnic cohort that included a large proportion of hypertensives and African-Americans.

## METHODS

### Study population

Study participants belonged to the Genetic Epidemiology Network of Arteriopathy (GENOA) study, and were ascertained from sibships in which at least two family members were diagnosed with hypertension before the age of 60 years. The cohort included non-Hispanic whites from Rochester, MN as well as African-American participants from Jackson, MS. Between January 2003 and December 2008, 2,561 participants (1,324 African Americans and 1,237 non-Hispanic whites) completed the study protocol. We excluded 223 participants with history of myocardial infarction or stroke, leaving 2,338 subjects for the final analysis. The project was approved by the Mayo Clinic’s and University of Mississippi’s institutional review boards and participants gave informed consent.

### Assessment of baseline characteristics and pulse pressure

Information about the medical history of the participants, as well as their medications was obtained from a comprehensive questionnaire filled out on the day of the study. Ethnicity was ascertained based on self-report. Blood was drawn by venipuncture after an overnight fast. Total cholesterol, high-density lipoprotein cholesterol, and fasting glucose were measured by standard enzymatic methods. Serum creatinine was measured by a spectrophotometric method. Estimated glomerular filtration rate was calculated with the validated modification of diet in renal disease equation.[12]

Hypertension was defined as a systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg on the day of the study visit, or report of a prior diagnosis of hypertension and current treatment with antihypertensive agents. Diabetes was considered present if a subject was being treated with insulin or oral agents or had a fasting glucose level ≥ 126 mg/dl (≥ 7.0 mmol/l). “Ever” smoking was defined as having smoked >100 cigarettes in the past. Weight was measured in kilograms by an electronic scale, height was measured in centimeters by a stadiometer, and body mass index was calculated in units of $kg/m^2$.

BP was measured in the supine position in a quiet room with controlled temperature. Brachial systolic BP and diastolic BP were obtained by a trained technician in the right arm three consecutive times by auscultating at 2-min intervals using a random-zero sphygmomanometer (Hawksley and Sons, London, UK). The average of the last two BP values was used for analyses. Pulse pressure was calculated as systolic BP–diastolic BP, in mm Hg.

### Circulating biomarkers

Forty seven circulating protein biomarkers were chosen to represent pathways implicated in arterial disease: inflammation, hemodynamic stress, lipoprotein metabolism, adipocyte metabolism, calcification, and thrombosis. The biomarkers and their abbreviations are listed in Table 1. Assays for the individual biomarkers, including precision, accuracy, and stability, as well as methods for quality control have been previously described.[13]

### Statistical methods

Continuous variables were reported as mean ± standard deviation (s.d.), and nominal variables were reported as n (%). Differences between groups were compared with t-test for normally distributed continuous variables, Wilcoxon signed-rank test for skewed continuous variables, and the chi-square test for nominal variables. Circulating levels of most serum biomarkers were log-transformed to reduce skewness. Linear regression analyses stratified by ethnicity were employed to assess the associations of each of the biomarkers with pulse pressure after adjustment for age and sex, and after further adjustment for hypertension, mean arterial pressure, diabetes, body mass index, history of smoking, total and high-density lipoprotein cholesterol, estimated glomerular filtration rate, and use of β-blockers, calcium-channel blockers, diuretics and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, aspirin, statins, and estrogens. Stepwise elimination was performed with criteria of $P \leq 0.10$ to enter and $P \leq 0.05$ to stay in the model. We included one biomarker at a time in the multivariable models, and subsequently created a separate model, adding all the significant biomarkers together while adjusting for the covariates listed above. Linear regression with similar adjustment was also performed in the subset of hypertensives ($n$ = 1,753). To reduce the possibility of type I error due to multiple testing, we employed the Bonferroni correction, and a $P$ value ≤0.001 (0.05 ÷ 47, since we analyzed 47 different biomarkers) was considered to be statistically significant.

As our cohort consisted of sibships, we used population-averaged generalized estimating equations to account for the possible impact of familial correlations on the analyses. Statistical analyses were performed with SAS v 8.2 (SAS Institute, Cary NC).

## RESULTS

Participant characteristics are outlined in Table 2. The mean ± s.d. pulse pressure (mm Hg) among African-Americans and non-Hispanic whites was 59.4 ± 17.2 and 56.8 ± 15.3, respectively. The distributions of the circulating biomarkers are shown in Supplementary Table S1 online.

In African-American participants, higher levels of the following biomarkers were significantly associated with greater pulse pressure after adjustment for age and sex: log C-reactive protein (CRP) ($\beta = 1.85 \pm 0.44$, $P < 0.001$), log interleukin-6 ($\beta = 2.98 \pm 0.75$, $P < 0.001$), log tumor necrosis factor receptor-II ($\beta = 3.96 \pm 1.21$, $P = 0.001$), log matrix metalloproteinase-2 (MMP-2) ($\beta = 5.69 \pm 1.32$, $P < 0.001$), log N-terminal probrain natriuretic peptide (NT-proBNP) ($\beta = 3.67 \pm 0.66$, $P < 0.001$), log midregional proatrial natriuretic peptide (MR-proANP) ($\beta = 6.31 \pm 0.96$, $P < 0.001$), log midregional proadrenomedullin (MR-proADM) ($\beta = 7.11 \pm 1.45$, $P < 0.001$), log C-terminal proendothelin ($\beta = 3.24 \pm 1.00$, $P = 0.001$), and log osteoprotegerin (OPG) ($\beta = 7.71 \pm 1.31$, $P < 0.001$). After further adjustment for potential confounders; higher levels of log CRP, log MMP-2, log NT-proBNP, log MR-proANP, log MR-proADM, and log OPG remained significantly associated with greater pulse pressure in African-American participants (Table 3). When all significant biomarkers were included in the multivariable model, higher levels of log CRP ($\beta = 1.58 \pm 0.34$, $P < 0.001$), log MR-proANP ($\beta = 3.98 \pm 0.71$, $P < 0.001$), and log OPG ($\beta = 3.72 \pm 1.01$, $P < 0.001$) remained significantly associated with greater pulse pressure.

After adjustment for age and sex, higher levels of the following biomarkers were significantly associated with greater pulse pressure in non-Hispanic whites: log NT-proBNP ($\beta = 2.58 \pm 0.64$, $P = 0.001$), log MR-proANP ($\beta = 4.18 \pm 0.82$, $P < 0.001$), log OPG ($\beta = 6.29 \pm 1.23$, $P < 0.001$), and fibrinogen ($\beta = 0.02 \pm 0.005$, $P = 0.001$). In the multivariable model, higher levels of log NT-proBNP, log MR-proANP, log OPG, and fibrinogen remained independently associated with greater pulse pressure (Table 3). When we included all significant biomarkers in the multivariable model, higher levels of log MR-proANP ($\beta = 2.73 \pm 0.74$, $P < 0.001$) and log OPG ($\beta = 3.17 \pm 0.99$, $P = 0.001$) remained significantly associated with greater pulse pressure.

When hypertensives were analyzed separately, similar results were noted (Table 3). After including all significant biomarkers in the same multivariable model, only higher levels of log CRP ($\beta = 1.91 \pm 0.42$, $P < 0.001$) and log MR-proANP ($\beta = 4.22 \pm 0.83$, $P < 0.001$) remained independently associated with increased pulse pressure in hypertensive African Americans, whereas log MR-proANP remained associated with pulse pressure in hypertensive non-Hispanic whites ($\beta = 3.17 \pm 0.94$, $P < 0.001$).

## DISCUSSION

The results of our exploratory analysis demonstrated that higher circulating levels of NT-proBNP, MR-proANP, and OPG are independently associated with greater pulse pressure, a measure of arterial stiffness, in a cohort of predominantly hypertensive African-American and non-Hispanic white individuals without history of myocardial infarction or stroke. Additionally, higher levels of CRP, MR-proADM, and MMP-2 in African-Americans, and fibrinogen in non-Hispanic whites were also associated with greater pulse pressure. This is

the first study to assess the associations of a comprehensive panel of circulating biomarkers in several different etiologic pathways of arteriosclerosis with pulse pressure in both African-Americans and non-Hispanic whites. Our findings are relevant for understanding the pathophysiology of increased pulse pressure and its hemodynamic consequences. Broadly, our results suggest that circulating markers of hemodynamic stress, vascular inflammation and calcification, and matrix turnover are associated with greater pulse pressure.

### Hemodynamic stress and pulse pressure

We found higher levels of three markers of hemodynamic stress, NT-proBNP, MR-proANP (in both ethnicities), and MR-proADM (in African-Americans) to be independently associated with greater pulse pressure. The associations of natriuretic peptides with different measures of arterial stiffness have been previously reported.[14,15] In the Framingham Heart Study,[14] N-terminal proatrial natriuretic peptide and NT-proBNP were positively associated with aortic pulse wave velocity (aPWV) in men, but inversely associated with aPWV in women. Furthermore, the association of natriuretic peptides with central pulse pressure was observed only in men. In contrast to our study, the majority of the participants in the Framingham Heart Study were normotensive, and central rather than brachial pulse pressure was the dependent variable. In 725 normotensive Japanese men, serum BNP was independently associated with both pulse pressure and brachial-ankle pulse wave velocity.[15]

Arterial stiffness may lead to left ventricular hypertrophy and demand ischemia, thereby increasing production of natriuretic peptides.[16] Others have hypothesized that elevated BNP levels in patients with heart failure may in fact represent a state of “BNP deficiency,” since levels of BNP 1–32 (the mature, biologically active form of the peptide) are very low while assays paradoxically detect high levels of BNP that are biologically inactive.[17] Based on this theory, a state of natriuretic peptide “dysfunction” (high levels with low biological activity) could also explain the association of BNP and ANP with higher pulse pressure observed in our study. However, further studies are necessary to confirm this hypothesis, and to determine whether natriuretic peptide therapy would decrease arterial stiffness.

We observed an independent association between MR-proADM and pulse pressure in African-American participants only. Previous studies[18–20] that investigated the associations of adrenomedullin with different measures of arterial stiffness in non-African-American populations yielded conflicting results. We have previously shown that higher levels of MR-proADM are independently associated with higher pulse pressure, left ventricular mass, and urine albumin-creatinine ratio in hypertensive African-American adults.[18] In the Framingham Heart Study Offspring Cohort[19] adrenomedullin was not associated with measures of arterial stiffness; whereas Kita *et al.*[20] reported a positive correlation between adrenomedullin and brachial-ankle pulse wave velocity in 126 Japanese subjects, 57% of whom were hypertensive.

Adrenomedullin is a peptide abundantly expressed in cardiovascular tissues.[21] Its physiologic effect consists of reduction of peripheral arterial resistance, leading to lowering of BP,[22] but it also promotes vascular regeneration and neovascularization, especially under hypoxic conditions. Therefore, it is possible that the association of MR-proADM and pulse pressure in African-Americans observed in our study could reflect a reactive mechanism, in which hypertension, shear stress, and arteriosclerosis caused by arterial stiffness enhance the production of MR-proADM, thereby limiting the adverse physiologic consequences of arterial stiffness and hypertension.

### Inflammation, matrix remodeling, and pulse pressure

We observed higher levels of two inflammation markers, CRP and MMP-2, to be associated with higher pulse pressure in African-American participants, whereas fibrinogen (a coagulation factor which is also an acute-phase reactant) was associated with pulse pressure in non-Hispanic whites. The association of CRP with increased pulse pressure has been demonstrated in previous studies of hypertensives[23] and also in the general population.[24] In contrast, CRP was not associated with pulse pressure in the Framingham Heart Study[14] or in the Copenhagen City Heart Study.[25] CRP is an acute-phase reactant linked to increased risk of adverse cardiovascular events.[26] It remains unclear whether CRP has a causal role in development of arterial stiffness and increased pulse pressure, or whether arterial stiffness causes inflammation and CRP elevation (increased pulse pressure has been shown to stimulate the inflammatory cascade and production of reactive oxygen species).[27]

The relationship of MMP-2 with arterial stiffness appears to be complex and depends upon the population studied. Vlachopoulos *et al.*[28] found MMP-2 levels were inversely associated with arterial stiffness (measured by aPWV) in 213 healthy subjects. In contrast, in patients with isolated systolic hypertension (thus, increased pulse pressure), a positive association between MMP-2 and aPWV was observed.[29] MMP-2 is an endopeptidase produced in the arterial vasculature[30] and involved in vascular remodeling, as one of its functions is to degrade components of the extracellular matrix, which in turn influences arterial elasticity. In the setting of inflammation, shear stress, increased transmural pressure, and oxidative stress, the expression and activity of MMP-2 are increased, favoring extracellular matrix degradation and vascular remodeling.[30] Thus, it is possible that increased vascular inflammation may represent the link between MMP-2 and pulse pressure observed in African Americans participants in our study.

We found higher levels of fibrinogen to be associated with greater pulse pressure among non-Hispanic whites participants. An association between fibrinogen and pulse pressure was demonstrated in the Strong Heart Study[31] and in the Rotterdam Study,[32] but not in the Framingham Offspring Study[14] or in the Copenhagen City Heart Study.[25] Fibrinogen is a coagulation factor, and also an acute-phase reactant.[33] The association of fibrinogen with arterial stiffness may be partially explained by inflammation, since fibrinogen enhances the expression of proinflammatory cytokines.[34] However, in our study the association of fibrinogen with pulse pressure was significant despite adjustment for CRP (analyses not shown). The mechanisms linking fibrinogen to pulse pressure need further investigation.

### OPG and pulse pressure

The association of circulating OPG with arterial stiffness has been reported in two previous studies. Kim *et al.*[35] showed that OPG was associated with brachial-ankle pulse wave velocity (49 diabetic patients and 72 controls) independently of age and sex. Schnabel *et al.*[36] studied the association of 12 inflammatory biomarkers with different measures of arterial stiffness in 2,409 volunteers from the community and reported that OPG was significantly associated with aPWV, but not with pulse pressure. Differently than our cohort, in their study, only 32% of the participants had treated hypertension. OPG is considered a modulator of vascular calcification[37] and an inhibitor of the inflammatory cascade due to its antiapoptotic mechanisms in the endothelium.[38] Since OPG is part of the tumor necrosis factor family, the association of OPG with pulse pressure may simply reflect a heightened inflammatory state that leads to vascular damage and stiffness. However, OPG remained associated with pulse pressure even after adjustment for other biomarkers of inflammation (analyses not shown). This suggests that the association of OPG with arterial stiffness may not reflect inflammation alone. Although the mechanisms through which OPG affects the cardiovascular system are still poorly understood, higher levels identify individuals at higher

risk.[39] Based on the results from our study, it is possible that increased pulse pressure may mediate the association of OPG with incident cardiovascular events.

### Strengths and limitations

The main strength of our study is a large cohort of 2,338 participants that included 1,145 African-American participants. Even though African-Americans have a higher prevalence of hypertension and are at higher risk of target-organ damage from hypertension, they remain an understudied group. Furthermore, we evaluated multiple candidate biomarkers implicated in different etiologic pathways that could lead to arterial stiffness or mediate its effects. We were also able to conduct subset analyses in participants with hypertension given that the majority in both cohorts was hypertensive.

Additional studies are necessary to further evaluate potential biomarkers associated with arterial stiffness in younger individuals, and in other ethnic groups. Further, the cross-sectional nature of our study does not allow us to establish causality or temporality of the associations. Another weakness is the lack of central pulse pressure data. However, in individuals older than 50 years, peripheral pulse pressure has been shown to approximate central pulse pressure.[40] Lastly, our use of pulse pressure as the main outcome variable in our study has strengths (pulse pressure is a robust prognostic marker, the complexity of pulse pressure physiology is related to several biological mechanisms, and pulse pressure can be simply measured in the office at no additional cost to the patient) and weaknesses (pulse pressure is not the gold-standard measure of arterial stiffness).

Our results suggest additional lines of investigation to better understand the pathophysiology of arterial stiffness and its consequences. Markers of hemodynamic stress, matrix remodeling, and vascular inflammation and calcification in clinical practice may identify higher risk individuals, and such knowledge may guide therapy to better control BP and risk factors. Furthermore, the results of our study should motivate randomized clinical trials to assess whether drugs that modulate these pathways can reduce arterial stiffness.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Table 1**

Circulating biomarkers, organized by etiologic pathway, and their abbreviations

| Inflammation | Lipoprotein metabolism | Thrombosis | Hemodynamic stress | Calcification | Adipocyte metabolism |
|---|---|---|---|---|---|
| C-Reactive protein (CRP) | Apolipoprotein A-I (APOA1) | FactorII (F2) | N-terminal probrain natriuretic peptide (NT-proBNP) | Osteopontin (OPN) | Leptin (LEP) |
| Serum amyloid A (SAA) | Apolipoprotein B (APOB) | FactorV (F5) | Midregional proatrial natriuretic peptide (MR-proANP) | Osteoprotegerin (OPG) | Adiponectin (ADIPOQ) |
| Intercellular adhesion molecule (ICAM) | Apolipoprotein C-III (APOC3) | FactorVII (F7) | C-Terminal proarginine vasopressin (CT-proAVP) | Osteonectin (ON) | Resistin (RETN) |
| Vascular cell adhesion molecule (VCAM) | Apolipoprotein E (APOE) | FactorVIII (F8) | Midregional proadrenomedullin (MR-proADM) | Osteocalcin (OCN) | |
| Interleukin-6 (IL6) | Low-density lipoprotein particle size (LDL size) | Von Willebrand factor (VWF) | C-terminal proendothelin (CT-proET) | | |
| Interleukin-18 (IL18) | Lipoprotein (a) [LPA] | D-Dimer | | | |
| Tumor necrosis factor receptor-1 (TNFR1) | Oxidized low-density lipoprotein (Ox-LDL) | Antithrombin III (AT3) | | | |
| Tumor necrosis factor receptor-2 (TNFR2) | Lipoprotein-associated phospholipase A2 mass (Lp-PLA2 mass) | Fibrinogen (FI) | | | |
| Monocyte chemotactic protein-1 (MCP1) | Lipoprotein-associated phospholipase A2 activity (Lp-PLA2 activity) | | | | |
| E-selectin (ESEL) | | | | | |
| P-selectin (SELP) | | | | | |
| Heat shock protein 27 (Hsp27) | | | | | |
| Myeloperoxidase (MPO) | | | | | |
| Receptor for advanced glycation end-products (RAGE) | | | | | |
| Matrix metalloproteinase-2 (MMP2) | | | | | |
| Matrix metalloproteinase-9 (MMP9) | | | | | |
| Tissue inhibitor of metalloproteinases-1 (TIMP1) | | | | | |
| Tissue inhibitor of metalloproteinases-2 (TIMP2) | | | | | |

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## Table 2

Baseline characteristics of the participants

| | African-Americans (n = 1,193) | Non-Hispanic whites (n = 1,145) | P value |
|---|---|---|---|
| Age, years | 63.3 ± 9.3 | 58.4 ± 10.2 | <0.001 |
| Men, *n* (%) | 335 (28.1%) | 480 (41.9%) | <0.001 |
| Body mass index, kg/m$^2$ | 31.6 ± 6.8 | 30.7 ± 6.3 | <0.01 |
| Waist circumference, cm | 103.5 ± 14.8 | 100.3 ± 16.0 | <0.001 |
| Hypertension, *n* (%) | 936 (78.5%) | 817 (71.4%) | <0.001 |
| Systolic blood pressure, mm Hg | 139 ± 20 | 131 ± 17 | <0.001 |
| Diastolic blood pressure, mm Hg | 79 ± 11 | 74 ± 9 | <0.001 |
| Mean arterial pressure, mm Hg | 99 ± 12 | 93 ± 10 | <0.001 |
| Pulse pressure, mm Hg | 59.4 ± 17.2 | 56.8 ± 15.3 | <0.01 |
| Diabetes, *n* (%) | 341 (28.6%) | 161 (14.1%) | <0.001 |
| History of smoking (past or current) | 462 (38.7%) | 546 (47.7%) | <0.001 |
| Total cholesterol, mg/dl | 202.2 ± 40.9 | 198.6 ± 34.6 | 0.054 |
| HDL cholesterol, mg/dl | 57.7 ± 18.1 | 52.2 ± 15.3 | <0.001 |
| eGFR (ml/min/1.73 m$^2$) | 76.0 ± 19.7 | 64.9 ± 13.5 | <0.001 |
| Statin use, *n* (%) | 205 (17.2%) | 298 (26%) | <0.001 |
| ACEi/ARB use, *n* (%) | 446 (37.4%) | 386 (33.7%) | 0.06 |
| β-Blocker use, *n* (%) | 177 (14.8%) | 384 (30.4%) | <0.001 |
| Calcium-channel blocker use, *n* (%) | 331 (27.7%) | 159 (13.9%) | <0.001 |
| Diuretic use, *n* (%) | 536 (44.9%) | 430 (37.6%) | <0.001 |
| Aspirin use (%) | 365 (30.6%) | 442 (38.6%) | <0.001 |

Continuous variables are reported as mean ± s.d. Nominal variables are reported as *n* (%). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.

**Table 3**

Novel markers independently associated with pulse pressure in African-American and non-Hispanic white adults

| | **Multivariable linear regression in the entire cohort (*n* = 2,338)[a]** | | | | | |
|---|---|---|---|---|---|---|
| | **African Americans (*n* = 1,193)** | | | **Non-Hispanic whites (*n* = 1,145)** | | |
| | **β ± s.e.** | **Model $R^2$ [d]** | ***P* value** | **β ± s.e.** | **Model $R^2$ [d]** | ***P* value** |
| Log MR-proANP[b] | 4.83 ± 0.70 | 0.48 | <0.001 | 3.70 ± 0.67 | 0.49 | <0.001 |
| Log NT-proBNP[b] | 2.11 ± 0.52 | 0.46 | <0.001 | 2.65 ± 0.55 | 0.49 | <0.001 |
| Log OPG[b] | 4.64 ± 1.02 | 0.46 | <0.001 | 4.19 ± 0.99 | 0.48 | <0.001 |
| Log CRP[b] | 1.56 ± 0.35 | 0.46 | <0.001 | — | — | — |
| Log MMP-2[b] | 3.89 ± 1.06 | 0.46 | <0.001 | — | — | — |
| Log MR-proADM[b] | 5.53 ± 1.19 | 0.46 | 0.001 | — | — | — |
| Fibrinogen (mg/dl)[c] | — | — | — | 0.02 ± 0.004 | 0.49 | <0.001 |
| | **Multivariable linear regression in the in the subset of hypertensives (*n* = 1,753)[a]** | | | | | |
| | **African Americans (*n* = 936)** | | | **Non-Hispanic whites (*n* = 817)** | | |
| | **β ± s.e.** | **Model $R^2$ [d]** | ***P* value** | **β ± s.e.** | **Model $R^2$ [d]** | ***P* value** |
| Log MR-proANP[b] | 5.04 ± 0.83 | 0.41 | <0.001 | 3.93 ± 0.83 | 0.45 | < 0.001 |
| Log NT-proBNP[b] | 2.42 ± 0.61 | 0.40 | <0.001 | 2.75 ± 0.67 | 0.44 | <0.001 |
| Log OPG[b] | 4.64 ± 1.22 | 0.40 | <0.001 | 3.98 ± 1.26 | 0.44 | <0.01 |
| Log CRP[b] | 1.97 ± 0.43 | 0.40 | <0.001 | — | — | — |
| Log MMP-2[b] | 4.28 ± 1.29 | 0.39 | 0.001 | — | — | — |
| Log MR-proADM[b] | 6.48 ± 1.41 | 0.40 | <0.001 | — | — | — |
| Fibrinogen (mg/dl)[c] | — | — | — | 5.42 ± 1.66 | 0.44 | 0.001 |

BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; MMP-2, matrix metalloproteinase-2; MR-proADM, midregional proadrenomedullin; MR-proANP, midregional proatrial natriuretic peptide; NT-proBNP, N-terminal probrain natriuretic peptide; OPG, osteoprotegerin; s.e., standard error.

[a]Markers were added to the multivariable model one at a time. Covariates included in the models were: age, sex, hypertension, mean arterial pressure, diabetes, BMI, history of smoking, total and HDL cholesterol, estimated glomerular filtration rate, and use of β-blockers, calcium-channel blockers, diuretics and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, aspirin, statins, and estrogens. Statistical significance was set at $P \leq 0.001$ to correct for multiple testing.

[b] $\beta$-Coefficients for MR-proANP, NT-proBNP, OPG, CRP, MMP-2, and MR-proADM are for 1 logarithmic unit increase in biomarker level. Untransformed units of measurement for these biomarkers were: pmol/l for MR-proANP, pg/ml for NT-proBNP, pg/ml for OPG, mg/l for CRP, ng/ml for MMP-2 and nmol/l for MR-proADM.

[c] Fibrinogen levels were not log-transformed because its distribution was normal in the sample.

[d] The $R^2$ for the baseline model (without biomarkers) was 0.47 in non-Hispanic whites and 0.45 in African Americans. In the subset of hypertensives, the $R^2$ for the baseline model was 0.43 in hypertensive non-Hispanic whites and 0.38 in hypertensive African Americans.