ORIGINAL RESEARCH

N-Terminal Pro Brain, N-Terminal Pro Atrial Natriuretic Peptides, and Dynamic Cerebral Autoregulation

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BACKGROUND: Elevated natriuretic peptides (NP) are associated with adverse cerebrovascular conditions including stroke, cerebral small vessel disease, and dementia. However, the mechanisms underlying these associations remain unclear. In this study, we examined the relationship of NT-proBNP (N-terminal pro brain NP) and NT-proANP (N-terminal pro atrial NP) with cerebrovascular function, measured by cerebral autoregulation.

METHODS AND RESULTS: We included 154 participants (mean age 56±4 years old) from the CARDIA (Coronary Artery Risk Development in Young Adults) cohort. NT-proBNP and NT-proANP were measured in blood samples from the year 25 examination using electrochemiluminescence Immunoassay and enzyme-linked immunoassay, respectively. Dynamic cerebral autoregulation (dCA) was assessed at the year 30 examination by transcranial Doppler ultrasound, using transfer function analysis (phase and gain) of spontaneous blood pressure and flow velocity oscillations, where lower phase and higher gain reflect less efficient cerebral autoregulation. We used multivariable linear regression models adjusted for demographics, vascular risk factors, and history of kidney and cardiac diseases. Higher NT-proBNP levels at year 25 were associated with lower phase (β [95% CI]=−5.30 lower degrees of phase [−10.05 to −0.54]) and higher gain (β [95% CI]=0.06 higher cm/s per mm Hg of gain [0.004–0.12]) at year 30. Similarly, higher NT-proANP levels were associated with lower phase (β [95% CI]=−9.08 lower degrees of phase [−16.46 to −1.70]).

CONCLUSIONS: Higher circulating levels of NT-proBNP and NT-proANP are associated with less efficient dCA 5 years later. These findings link circulating NP to cerebral autoregulation and may be one mechanism tying NP to adverse cerebrovascular outcomes.

Key Words: autoregulation ■ brain ■ cerebrovascular disease ■ natriuretic peptides

Natriuretic peptides (NP) are a family of endogenous peptides that are abundantly expressed in cardiac myocytes and vascular endothelium.1 In the periphery, NP not only regulate blood pressure and body fluid homeostasis, but they also inhibit cardiac tissue and blood vessel remodeling.2 Dysregulated elevation in NP has been linked with adverse systemic and cerebrovascular outcomes. Evidence from epidemiological and clinical studies suggest that higher levels of NP in plasma are linked with a higher risk of stroke,3 cerebral small vessel disease,4 subarachnoid hemorrhage,5,6 and cognitive impairment.7 In line with these observations, studies in mammalian and rodent models have identified the presence of NP receptors in the endothelium of cerebral microvessels.8,9 Recently, it has been shown that NP and their receptors are abundantly present on human cerebral vessels’ smooth muscle cells and endothelium.10 These are the same
cellular components central to cerebral autoregulation; a key physiological mechanism that ensures adequate brain perfusion despite fluctuations in systemic blood pressure. In this study, we examined the relationship between NT-proBNP (N-terminal pro brain NP) and NT-proANP (N-terminal pro atrial NP) and cerebral autoregulation (dCA) in a bi-racial community-dwelling sample of participants from the CARDIA (Coronary Artery Risk Development on Young Adults) cohort. We hypothesized that higher peripheral levels of NP would be associated with less efficient dCA.

### CLINICAL PERSPECTIVE

**What Is New?**
- This study shows that higher levels of plasma natriuretic peptides are associated with less efficient cerebral autoregulation.
- The association between natriuretic peptides and cerebral autoregulation is independent of demographic and conventional cardiovascular risk factors.

**What Are the Clinical Implications?**
- Alterations in cerebral autoregulation may underlie the link between natriuretic peptides and adverse cerebrovascular outcomes.
- Our findings pave the path for future mechanistic studies to determine the potential diagnostic or therapeutic utility of natriuretic peptides as disease modifying agents in development and progression of cerebrovascular pathologies.

### Nonstandard Abbreviations and Acronyms

| Abbreviation | Definition                  |
|--------------|----------------------------|
| dCA          | dynamic cerebral autoregulation |
| ECLIA        | electrochemiluminescence immunoassay |
| MCA          | middle cerebral artery         |
| MFV          | mean flow velocity            |
| NT-proANP    | N-terminal pro atrial natriuretic peptide |

### STUDY POPULATION

Participants in this study were included from a sub-sample of the CARDIA cohort. The CARDIA study began in 1985–1986 to investigate cardiovascular risk factors in a cohort of bi-racial (White/Black) community-dwelling individuals aged 18 to 30 years (n=5115). The participants were recruited from 4 sites in the United States (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). At the 30th year of follow-up, participants from the Chicago site were invited to participate in the Cerebral Small Vessels in Motor and Cognitive Decline sub-study. This ancillary study aimed at investigating vascular biomarkers of cerebral small vessel disease using transcranial Doppler ultrasound. Separate written consent was obtained, and the study was approved by the institutional review board of Northwestern University. Out of a total of 202 participants recruited for the ancillary study, 154 had available blood samples that were collected at the last visit before the transcranial Doppler ultrasound examination. Figure S1 shows the flow diagram of included participants in this study. Characteristics of participants included in this study (n=154) were not significantly different compared to the rest of the Chicago field centers’ participants (n=591), except that those included in this study were more frequently male (Table S1).

### NT-proBNP and NT-proANP Measurement

NT-proBNP and NT-proANP were measured using frozen and stored blood samples (serum and plasma) from the 25th year of follow-up. The blood draw was performed before 10 am and participants were instructed to be in fasting state. Blood drawing, handling, aliquoting, and transportation have been standardized according to CARDIA protocols. NT-proBNP was measured using proBNP II electrochemiluminescence immunoassay (ECLIA) on a Cobas e411 chemistry analyzer (Roche Diagnostics, USA). The measurement range is 5.0 to 35 000 pg/mL (0.60–4130 pmol/L) and the inter-assay coefficient of variation is <5%. NT-proANP was measured using ELISA (Alpco, Salem, NH, USA), with a standard range 0 to 10 nmol/L and an inter-assay coefficient of variation <9%. All measurements were performed in the Comprehensive Metabolic Core Lab at Northwestern University, Feinberg School of Medicine.

### METHODS

Anonymized data are available from the CARDIA Coordinating Center (cardia.dopm.uab.edu/contact-cardia). A description of the National Heart, Lung, and Blood Institute policies governing the data and describing access to the data can be found online (https://www.cardia.dopm.uab.edu/study-information/nhlbi-data-repository-data).

### Cerebral Autoregulation Assessment

At the 30th year of CARDIA follow-up, participants reported to the Cerebrovascular Laboratory at Northwestern University, Feinberg School of Medicine, for cerebrovascular measures. They were
Dynamic Cerebral Autoregulation Quantification

The beat-to-beat cycles were determined by detecting the peak of each arterial pressure and cerebral flow velocity waveform. Using a custom program written in MATLAB, mean arterial pressure (MAP) and mean flow velocity (MFV) values were determined from the integrals of each waveform to calculate the systolic and diastolic blood flow velocity within each beat cycle. All MAP and MFV waveforms were visually inspected for artifacts, and only steady-state data were used for analyses. The frequency-domain transfer function analyses were used to quantify dCA, as previously described. This method examines the relationship between spontaneous beat-to-beat oscillations in MAP and cerebral MFV at rest. Briefly, the power spectral densities of MAP and MFV were estimated using the Welch algorithm of averaging periodograms. The waveforms were linearly detrended, smoothed through a Hanning Window, and transformed through Fast Fourier analysis. The periodograms were averaged across all widows to produce the spectrum estimate. The coherence between MAP and MFV were calculated using the cross-spectra and autospectra of the data segments. Using a custom MATLAB program, transfer function gain and phase values were calculated using the MAP and MFV signal autospectra over the very low frequency range. The phase and gain values were weighted by their precision in each frequency range to obtain the most accurate data for analysis. Furthermore, since the right and left dCA measures were not significantly different, these measures were averaged to obtain the global values. The transfer function gain quantifies the damping effect of cerebral autoregulation on the magnitude of blood pressure oscillations. A low gain indicates more efficient dCA, whereas a high gain indicates less efficiency. The phase shift represents the temporal difference between MFV oscillations in respect to MAP, and is considered a measure of the time delay in autoregulatory response. If the blood pressure and cerebral blood flow oscillations are synchronous, then the phase shift approaches zero degrees, indicating less efficient dCA, whereas a phase shift of 90° indicates more efficient dCA (hence, a lower phase indicates worse dCA).

Covariates

Other covariates were collected using standardized protocols at each CARDIA visit. Body mass index was calculated as weight (kg) divided by height in meters squared. Total cholesterol was determined on a chemistry analyzer using comparable enzymatic procedures (Hitachi 912; Roche Diagnostics). Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dL or self-reported use of diabetes mellitus medication. History of hypertension, antihypertensive medication use, and smoking status were self-reported. History of kidney problems was defined as any self-reported history of kidney problems, including pyelonephritis, kidney stone, nephritis, glomerulonephritis, or kidney failure. History of cardiac problems was defined as any self-reported heart problems, including heart attack, heart failure, angina, rheumatic heart disease, mitral valve prolapse, atrial fibrillation, and irregular heartbeat. Missing covariate data (n=2) were replaced with values acquired from a previous exam.

Statistical Analysis

Descriptive measures are reported as mean±SD, proportion, and median with interquartile ranges where applicable. Characteristics of participants across tertiles of NT-proBNP and NT-proANP were compared using one-way ANOVA for continuous variables and chi-squared test for categorical variables. Multivariable linear regression models were used to assess the association between NP (independent variable) and dCA (dependent variable). Adherence to assumptions of linear regression was examined by visual inspection of the distribution of residuals through histograms and normal probability plots. Ordinary least square linear regression models with restricted cubic spline were used to test for non-linear associations. All analyses were performed in 2 steps: in the first step (model 1), analyses were adjusted for sociodemographic factors: age, sex, and race. In the next step (model 2), vascular risk factors (smoking status, body mass index, total cholesterol level, history of hypertension, and diabetes mellitus history), history of cardiac problems, and...
renal history were additionally included as confounders. These variables were used as confounders based on previous literature showing their association with NP and/or dCA. To further test the effect of these vascular risk factors on the results, we performed additional sensitivity analyses by stratifying participants to those with and without vascular risk factors and testing for interaction. The P values for interaction were calculated using linear regression models by adding an interaction term produced by multiplying levels of NT-proBNP to vascular risk factors. In an extra analysis, to account for the time difference between NP and dCA measures, we calculated the time interval between year-25 and year-30 examinations and added it to the linear regressions models, which did not materially change the associations. All statistical analyses were conducted with SPSS (version 25.0) and R (version 3.5.1) software, and a P<0.05 was considered as statistically significant.

RESULTS

Table 1 presents the characteristics of participants. The mean age of our participants was 56 years old; a total of 69 (45%) participants were female and 70 (46%) were Black. The socio-demographic and vascular risk factors were not different across NT-proBNP tertiles, except that females had higher NT-proBNP levels (Table S2). Participants in the highest tertile of NT-proANP were older (Table S3). The NT-proBNP and NT-proANP levels ranged from 5 to 273.5 pg/mL and from 0.18 to 3.01 nmol/L, respectively.

Table 2 shows the prospective association of NT-proBNP and NT-proANP with transfer function measures of dCA. In model 1, each unit (pg/mL) increase in NT-proBNP was associated with a 0.12° lower phase (indicating less efficient dCA; 95% CI, −0.21 to −0.03) and a 0.001 cm/s per mm Hg higher gain (indicating less efficient dCA; 95% CI, 0.0002 to 0.0002). After full adjustments for vascular risk factors, and history of cardiac or renal problems, higher NT-proBNP remained associated with lower phase and higher gain (Table 2, model 2). Each unit (nmol/L) increase in NT-proANP was associated with a 9.08° lower phase (indicating less efficient dCA; 95% CI, −16.46 to −1.70) after full adjustments (Table 2, model 2).

Figure 2 shows the prospective relation of NT-proBNP and NT-proANP with transfer function measures of dCA in different subgroups of participants. The association between higher NT-proBNP and lower phase was stronger in males, Black patients, and those with a history of diabetes mellitus (all P for interaction <0.05). The association of higher NT-proBNP and higher gain was stronger in Black participants (P for interaction <0.05). The association of NT-proANP and transfer function measures of dCA was not different among the subgroups. Finally, after exclusion of

**Table 1. Characteristics of Participants**

|                     | n=154 | Demographics |
|---------------------|-------|--------------|
| Age, y, mean (SD)   | 55.7  (3.9) |
| Female, N (%)       | 69 (44.8) |
| Black, N (%)        | 70 (45.5) |
| Risk factors        |       |
| SBP, mm Hg, mean (SD) | 120.5 (15.4) |
| DBP, mm Hg, mean (SD) | 74.1 (11.2) |
| Body mass index, kg/m², mean (SD) | 30.0 (6.0) |
| Total cholesterol, mg/dL, mean (SD) | 191.8 (39.0) |
| Current smoker, N (%) | 22 (14.3) |
| History of hypertension, N (%) | 51 (33.1) |
| History of diabetes mellitus, N (%) | 24 (15.6) |
| History of cardiac problems, N (%) | 18 (11.7) |
| Renal history, N (%) | 12 (7.8) |
| TCD measures        |       |
| MCA flow velocity, cm/s, mean (SD) | 51.2 (12.1) |
| Phase, degree, mean (SD) | 42.24 (23.97) |
| Gain, cm/s per mm Hg, mean (SD) | 0.70 (0.3) |
| Coherence, mean (SD) | 0.51 (0.12) |

DBP indicates diastolic blood pressure; TCD, transcranial doppler; MCA, middle cerebra artery; SBP, systolic blood pressure; and transcranial Doppler ultrasound.

**Table 2. Prospective Association Between Natriuretic Peptides and Cerebrovascular Autoregulation**

|                     | NT-proBNP (pg/mL) Per 1 Unit Increase | NT-proANP (nmol/L) Per 1 Unit Increase |
|---------------------|---------------------------------------|---------------------------------------|
|                     | β (95% CI) P Value | β (95% CI) P Value |
| Phase, degree       |                         |                         |
| Model 1             | −0.12 (−0.21 to −0.03) 0.013 | −6.90 (−14.17 to 0.38) 0.063 |
| Model 2             | −0.10 (−0.20 to −0.01) 0.030 | −9.08 (−16.46 to −1.70) 0.016 |
| Gain, cm/s per mm Hg |                         |                         |
| Model 1             | 0.001 (0.0002 to 0.002) 0.022 | 0.03 (−0.05 to 0.12) 0.444 |
| Model 2             | 0.001 (0.0004 to 0.003) 0.008 | 0.04 (−0.05 to 0.13) 0.386 |

Beta (β) coefficient represents difference in phase (degree) and gain (cm/s per mm Hg), corresponding to 1 unit increase on NT-proBNP (picograms/milliliter) or NT-proANP (nanomoles/liter). Model 1 is adjusted for age, sex, and race. Model 2 is adjusted for age, sex, race, smoking status, body mass index, total cholesterol level, history of hypertension, history of diabetes mellitus, history of cardiac problem, and renal history. NT-proANP indicates N-terminal pro atrial natriuretic peptide; and NT-proBNP, N-terminal pro brain natriuretic peptide.
participants with history of cardiac disease (n=18), NT-proBNP ($\beta=-0.16$; 95% CI, $-0.26$ to $-0.05$) and NT-proANP ($\beta=-11.35$; 95% CI, $-19.67$ to $-3.03$) remain significantly associated with phase.

**DISCUSSION**

In this population-based study of middle age adults, we show that elevated circulating levels of NT-proBNP and NT-proANP are associated with less efficient cerebral autoregulation. This association was independent of socio-demographics and cardiovascular risk factors.

Evidence from neuropathological studies indicate that ANP and BNP are not only present in cardiac myocytes, but also in the central nervous system and its vascular beds. In fact, observations from animal studies suggest that NPs’ receptors are localized in the brain vessels, including in isolated cerebral microvessels and cultured brain capillary endothelium. For example, Chabrier et al were among the first to demonstrate specific ANP binding sites on bovine cerebral microvessel preparations. Further in vitro and in vivo experiments have shown the binding of ANP on rats’ brain microvessel endothelium. In humans, we have recently shown the immunohistochemical staining of ANP, BNP, and their receptors (including NP receptor types A, B, and C [NPR-A, NPR-B, and NPR-C]) in the smooth muscle and endothelium of cerebral vessels. Given these observations, it is plausible that NP contributes to cerebrovascular pathologies through modulation of cerebral hemodynamics. In this context, the present study extends the current knowledge on the roles of NP in cerebrovascular hemodynamics by reporting a close link between elevated systemic NP and less efficient cerebral autoregulation in a cohort of

**Figure 1.** Linear association between plasma natriuretic peptides and cerebrovascular autoregulation. The figure shows fitted restricted cubic splines and 95% CIs, indicating no evidence of non-linear association between plasma natriuretic peptides (NTproBNP and NTproANP) and measures of cerebral autoregulation (phase and gain). All P-values are >0.05. dCA indicates dynamic cerebral autoregulation; NT-proANP, N-terminal pro atrial natriuretic peptide; and NT-proBNP, N-terminal pro brain natriuretic peptide.
middle-age adults. To our knowledge, this is the first study to examine this relationship in a midlife cohort without significant cardiovascular disease burden.

The observed link between NP and dCA can be explained in different ways. Elevated NP and impaired dCA may both reflect vascular damage that share a common cause. Previous work shows that higher circulating NP levels strongly associate with cardiovascular mortality, cardiovascular diseases, and vascular risk factors. Similarly, cerebral autoregulation has been shown to be impaired in patients with hypertension and diabetes mellitus. In line with these observations, we found that the association between NP and dCA was stronger in those with a history of diabetes mellitus. However, adjustment of our analyses for measured vascular risk factors did not essentially change the association between NP and dCA. Nevertheless, we cannot rule out the effect of residual confounding and unmeasured risk factors. Alternatively, our findings could be attributed to autonomic dysfunction. The sympathetic nervous system is known to activate secretion of NP, and autonomic failure has been associated with altered dCA. Therefore, elevated NP and impaired dCA may reflect an epi-phenomenon representing early signs of autonomic dysregulation, activating secretion of NP and altering dCA in parallel.

It is widely accepted that dCA is a multifactorial phenomenon involving myogenic, autonomic, and...
metabolic mechanisms. Specifically, the myogenic response arises from the vascular smooth muscle activities in response to intravascular pressure changes, and it is crucial for maintaining vascular resistance. Furthermore, the endothelium also plays critical roles in the regulation of vascular tone and cerebral autoregulation through production of vasoactive mediators, such as NO. Previous work in experimental models shows that all NP exert their vasoactive properties by modulating intracellular calcium (Ca\(^{2+}\)) concentration in the brain. In particular, NP activate production of intracellular cyclic guanosine monophosphate (cGMP), which in turn reduces Ca\(^{2+}\) concentrations along with the activity of Ca\(^{2+}\)-activated potassium (K\(^+\)) channels and adenosine triphosphate (ATP) sensitive K\(^+\) channels, thereby affecting smooth muscle responses and changing vessel diameter. NP also stimulate the production of nitric oxide and exert vasodilatation by affecting nitric oxide synthase (NOS) production. The other hand, ANP is able to modulate the autonomic nervous system by affecting cardiac baroreceptor nerve endings and inhibiting sympathetic ganglionic neurotransmission. Given these local effects of NP on cerebral vessels, it is also possible that a long-lasting unregulated elevation in NP may hamper the response of smooth muscle and endothelium of cerebral vessels to intravascular pressure changes, thereby affecting cerebral vascular tone and autoregulation.

Our data show that higher plasma NT-proBNP and NT-proANP were both associated with less efficient dCA. Although the systemic effects of ANP and BNP have been extensively studied, their local impact on the brain vasculature and the interaction between systemic and central NP remains largely unexplored. It is worth noting that the third member of the NP family, C-type NP (CNP), is found to have higher concentrations in the central nervous system than in the systemic circulation. Therefore, future studies on humans are needed to investigate the local effects of NP on cerebrovascular function.

The strength of this study includes a well-characterized cohort of bi-racial middle age adults with extensive phenotyping of vascular profile that enabled us to correct for several potential confounders. Furthermore, to the best of our knowledge, this is the first study to examine the association between NP and cerebral autoregulation. Our study also has limitations. One important limitation is the 5 year time lag between NP and dCA measurements. Without knowing the longitudinal association between NP and dCA, our findings should be interpreted with caution. While MCA is considered the largest vascular territory in the brain, and hence a reasonable measure of global dCA, it is important to also note that the inability to acquire dCA in all vascular territories simultaneously is a limitation in all such studies. It should also be noted that NP were measured using stored blood samples. While using stored samples is a well-established approach in cohort studies and previous studies have shown stability of NP measures, obtaining fresh blood samples might decrease the chance of random errors and results should be further explored using fresh blood samples. Moreover, we measured NT-proANP, and more recent work suggests that mid-regional pro ANP may be a more stable marker of ANP, so this marker should be explored in the future. Finally, we would like to emphasize that this is an observational study and causality cannot be inferred. A better understanding of the underlying mechanisms discussed relies on future experimental models, as well as on interventional studies and collaborative effort across basic, translational, and clinical investigators.

In summary, our findings link elevated circulating levels of NP with less efficient cerebral autoregulation measured 5 years later, and this suggests that cerebrovascular dysregulation may be a potential mechanism linking elevated NP with adverse brain and cerebrovascular outcomes. Our observations have the potential to facilitate collaborative initiatives bridging basic, translational, and clinical efforts to advance our mechanistic understanding of the link between NP and cerebrovascular dysregulation, with an eye towards developing novel therapeutic targets.

### ARTICLE INFORMATION

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**Disclosures**

None.

**Supplementary Material**

Tables S1–S3

Figure S1
SUPPLEMENTAL MATERIAL
Table S1. Comparison between the participants from Chicago field center and those included in this study.

| Included in this study | No (n=591) | Yes (n=154) | p-value  |
|------------------------|------------|------------|----------|
| **Demographics at Y30** |            |            |          |
| Age, y                 | 55 ± 4     | 54 ± 4     | 0.080    |
| Female, %              | 60         | 45         | <0.001   |
| Blacks, %              | 46         | 46         | 0.954    |
| **Risk Factors at Y30** |            |            |          |
| SBP, mmHG              | 120 ± 17   | 120 ± 15   | 0.107    |
| DBP, mmHG              | 73 ± 11    | 74 ± 11    | 0.887    |
| Body Mass Index, kg/m2 | 30 ± 7     | 30 ± 6     | 0.003    |
| Total cholesterol, mg/dL | 189 ± 37  | 192 ± 38   | 0.976    |
| History of diabetes, % | 16         | 16         | 0.871    |
| History of HTN, %      | 40         | 33         | 0.131    |
| Current Smoker, %      | 15         | 14         | 0.990    |
| History of cardiac problem, No. (%) | 13     | 12         | 0.705    |
| Renal history, No. (%) | 9          | 8          | 0.595    |

Data are expressed as mean±SD or %. Characteristics were compared using t-test for continuous variables and chi-squared test for categorical variables. Chicago cohort had a total of 1108 participants at baseline. A total of 745 participants attended the Y30 follow-up examination (n=64 death before Y30 examination).
Table S2. Characteristics of participants in thirds of NTproBNP.

|                        | Low (5.0-21.97) | Middle (22.02-45.35) | High (45.72-273.50) | P-value<sup>a</sup> |
|------------------------|-----------------|----------------------|---------------------|---------------------|
|                        | n=51            | n=52                 | n=51                |                     |
| **Demographics at Year 25** |                 |                      |                     |                     |
| Age, y, mean (SD)      | 49.5 (3.9)      | 48.8 (3.9)           | 49.9 (3.8)          | 0.316               |
| Females, No. (%)       | 10 (19.6)       | 19 (36.5)            | 40 (78.4)           | <0.001              |
| Blacks, No. (%)        | 24 (47.1)       | 24 (46.2)            | 22 (43.1)           | 0.917               |
| **Risk Factors at Year 25** |                 |                      |                     |                     |
| SBP, mmHG, mean (SD)   | 116.5 (9.6)     | 118.8 (11.0)         | 117.2 (20.0)        | 0.704               |
| DBP, mmHG, mean (SD)   | 73.4 (9.2)      | 74.0 (10.4)          | 73.8 (15.4)         | 0.975               |
| Body Mass Index, kg/m², mean (SD) | 30.2 (5.7)  | 27.9 (5.0)           | 29.5 (6.8)          | 0.143               |
| Total cholesterol, mg/dL, mean (SD) | 190.6 (34.2) | 193.2 (42.6)         | 193.4 (35.3)        | 0.917               |
| History of diabetes, No. (%) | 7 (13.7)       | 5 (9.6)              | 8 (15.7)            | 0.645               |
| History of hypertension, No. (%) | 15 (29.4)    | 15 (29.0)            | 13 (26.0)           | 0.892               |
| Current Smoker, No. (%) | 9 (17.6)        | 8 (16.0)             | 9 (17.6)            | 0.151               |
| History of cardiac problem, No. (%) | 8 (15.7)     | 5 (9.6)              | 6 (11.8)            | 0.637               |
| Renal history, No. (%)  | 5 (9.8)         | 5 (9.6)              | 0                   | 0.071               |

<sup>a</sup> P-values were calculated using one-way ANOVA or chi-squared test were appropriate. Abbreviations: SBP: systolic blood pressure and DBP: diastolic blood pressure.
Table S3. Characteristics of participants in thirds of proANP.

| NT-proANP (nmol/L) thirds | Low 0.18-1.09 n=52 | Middle 1.10-1.63 n=51 | High 1.64-3.07 n=51 | p-value a |
|----------------------------|------------------|---------------------|-------------------|----------|
| Demographics at Year 25    |                  |                     |                   |          |
| Age, y, mean (SD)          | 48.4 (4.1)       | 49.6 (3.5)          | 50.3 (3.8)        | 0.038    |
| Female, No. (%)            | 17 (32.7)        | 26 (51.0)           | 26 (51.0)         | 0.097    |
| Blacks, No. (%)            | 26 (50.0)        | 22 (43.1)           | 22 (43.1)         | 0.721    |
| Risk Factors at Year 25    |                  |                     |                   |          |
| SBP, mmHG, mean (SD)       | 118.4 (11.0)     | 116.6 (11.0)        | 117.6 (19.2)      | 0.810    |
| DBP, mmHG, mean (SD)       | 75.5 (10.6)      | 73.0 (9.8)          | 72.7 (14.7)       | 0.426    |
| Body Mass Index, kg/m², mean (SD) | 29.5 (6.2)       | 29.0 (5.3)          | 29.0 (6.3)        | 0.892    |
| Total cholesterol, mg/dL, mean (SD) | 191.1 (41.8)     | 197.1 (35.0)        | 189.1 (35.1)      | 0.532    |
| History of hypertension, No. (%) | 17 (32.7)        | 12 (23.5)           | 14 (27.5)         | 0.582    |
| History of diabetes, No. (%) | 11 (21.2)        | 4 (7.8)             | 5 (9.8)           | 0.094    |
| Current Smoker, No. (%)    | 13 (25.5)        | 6 (12.0)            | 7 (13.7)          | 0.206    |
| History of cardiac problem, No. (%) | 10 (19.2)        | 9 (17.6)            | 0                 | 0.005    |
| Renal history, No. (%)     | 3 (5.8)          | 3 (5.9)             | 4 (7.8)           | 0.892    |

*p-values were calculated using one-way ANOVA or chi-squared test were appropriate. Abbreviations: SBP: systolic blood pressure and DBP: diastolic blood pressure
The CARDIA cohort enrolled 5115 adults aged 18-30 years beginning 1986 from four U.S. cities of Birmingham, Chicago, Minneapolis and Oakland. These participants were followed for 30 years through 8 in-person follow-up visits after 2, 5, 7, 10, 15, 20, 25 and 30 years. The current study includes a subset of participants from the Chicago center at year 25 and year 30 examinations. NP: natriuretic peptides; TCD: transcranial Doppler; dCA: dynamic cerebral autoregulation.