Journal of the American College of Cardiology
© 2012 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 60, No. 16, 2012
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.05.049

Human Hypertension Is Characterized by a Lack of Activation of the Antihypertensive Cardiac Hormones ANP and BNP

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Objectives This study sought to investigate plasma levels of circulating cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and B-type or brain natriuretic peptide (BNP), in the general community, focusing on their relative differences in worsening human hypertension.

Background Although ANP and BNP are well-characterized regulators of blood pressure in humans, little is known at the population level about their relationship with hypertension. The authors hypothesized that hypertension is associated with a lack of activation of these hormones or their molecular precursors.

Methods The study cohort (N = 2,082, age >45 years) was derived from a random sample from Rochester, Minnesota, and each subject had a medical history, clinical examination, and assessment of different plasma forms of ANP and BNP. Patients were stratified by blood pressure. Multivariable linear regression was used to assess differences in natriuretic peptide levels in worsening hypertension.

Results Compared to normotensive, $BNP_{1–32}$ and N-terminal $proBNP_{1–76}$ (NT-$proBNP_{1–76}$) were significantly decreased in pre-hypertension ($p < 0.05$), with $BNP_{1–32}$ significantly decreased in stage 1 as well ($p < 0.05$). Although $proBNP_{1–108}$ remained unchanged, the processed form was significantly increased only in stage 2 hypertension ($p < 0.05$). $ANP_{1–28}$ remained unchanged, while NT-$ANP_{1–98}$ was reduced in pre-hypertension ($p < 0.05$).

Conclusions The authors demonstrated the existence of an impaired production and/or release of $proBNP_{1–108}$ along with a concomitant reduction of $BNP_{1–32}$ and NT-$proBNP_{1–76}$ in the early stages of hypertension, with a significant elevation only in stage 2 hypertension. Importantly, they simultaneously demonstrated a lack of compensatory ANP elevation in advanced hypertension. (J Am Coll Cardiol 2012;60:1558–65) © 2012 by the American College of Cardiology Foundation

Atrial natriuretic peptide 1–28 (ANP) and B-type natriuretic peptide 1–32 ($BNP_{1–32}$) are 28- and 32-amino acid hormones, respectively, that are normally produced and secreted by the human heart in response to cardiac injury and mechanical stretch. Both cardiac hormones regulate blood pressure (BP) homeostasis via their pleiotropic actions, including natriuresis, vasodilation, and renin-angiotensin-aldosterone system suppression, but their mechanisms of secretion and processing into active forms differ greatly (1,2). ANP is secreted and circulates as an active hormone, whereas a growing body of evidence indicates that the major secreted and circulating immunoreactive form of BNP is actually its 108-amino acid precursor, pro–B-type natriuretic peptide1–108 ($proBNP_{1–108}$), and not $BNP_{1–32}$ (3–6). Current evidence supports that this circulating prohormone, which has significantly reduced biological activity, is

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Manuscript received March 6, 2012; revised manuscript received May 1, 2012, accepted May 15, 2012.

cleaved by corin and/or furin into the biologically active $BNP_{1–32}$ and the inactive N-terminal $proBNP_{1–76}$ (NT-$proBNP_{1–76}$) in plasma or at the target tissue level (3,7,8). Although the ANP and BNP systems have been studied extensively, their simultaneous critical role in BP regulation has not yet been described at the population level.

Prior studies have reported that $BNP_{1–32}$ and NT-$proBNP_{1–76}$ are elevated in hypertension, especially when left ventricular hypertrophy (LVH) is present (9). However, Belluardo et al. (10) challenged this common view by showing in clinically and biochemically well-characterized subjects with essential hypertension that $BNP_{1–32}$ and NT-$proBNP_{1–76}$ are actually reduced in the early stages of human hypertension. Furthermore, the importance of ANP and BNP in BP regulation and in risk for development of hypertension was recently confirmed by the elegant studies of Newton-Cheh et al. (11). In that population study, genetic variants of the ANP and BNP gene, resulting in higher plasma concentrations of ANP and/or BNP, were associated with lower BP and reduced risk for hypertension. These findings are consistent with earlier animal studies in which ANP or natriuretic peptide receptor type A (*NPR-A*) gene deletion in mice resulted in hypertension and marked LVH and hypertension (12). Nonetheless, it is unknown whether decreased $BNP_{1–32}$ is accompanied by reduced circulating $proBNP_{1–108}$ and ANP, which, if present, would indicate that inadequate natriuretic peptide synthesis or secretion is a significant mechanism contributing to hypertension development. A lack of large, complete biomarker studies in hypertensive patients, with and without end-organ damage, has limited our understanding of the role of these 2 cardiac peptides (ANP and BNP) in worsening hypertension.

Using a large study from Olmsted County, Minnesota, derived from a population-based sample known as the PAVD (Prevalence of Asymptomatic Ventricular Dysfunction) cohort (N = 2,082, age >45 years), our objective was to confirm and extend in the general adult population our previous assessment of circulating levels of several molecular forms of BNP and ANP through simultaneous measurement of $proBNP_{1–108}$, $BNP_{1–32}$, NT-$proBNP_{1–108}$, $ANP_{1–28}$, and NT-$ANP_{1–98}$. As a corollary to the first observation of Belluardo et al. (10), we hypothesized that human hypertension is characterized by a lack of activation of these cardiac hormones, indicating the existence of a relative deficiency of their endogenous cardiorenal protective actions in this morbid condition. This finding would underscore an opportunity to intervene in hypertension, with improved therapeutics including natriuretic peptides and their chimerics, which are currently in the pipeline for clinical trials.

Methods

Study population. This study was approved by the Mayo Clinic institutional review board, and informed consent was obtained from all subjects. Participating subjects came from the PAVD cohort (N = 2,082, age >45 years), a study population derived from a random sample of subjects from the Rochester Epidemiology Project (13–15). PAVD investigators have previously reported each subject's medical history, clinical examination, detailed 2-dimensional echocardiography, and procedures for storage and measurement of BNP forms, including $BNP_{1–32}$ (16), NT-$proBNP_{1–76}$ (17), and $proBNP_{1–108}$ (5). Of note, although prior human studies used assays that measured $BNP_{1–32}$ and NT-$proBNP_{1–76}$, which cross-reacted with $proBNP_{1–108}$ (3), our novel approach used a newly available radioimmunoassay that is highly specific for $proBNP_{1–108}$ (18). Furthermore, we simultaneously measured 2 different forms of mature circulating ANP ($ANP_{1–28}$ and NT-$proANP_{1–98}$).

Abbreviations and Acronyms

- $ANP_{1–28}$ = atrial natriuretic $peptide_{1–28}$
- BMI = body mass index
- $BNP_{1–32}$ = B-type natriuretic $peptide_{1–32}$
- BP = blood pressure
- NT-$ANP_{1–98}$ = N-terminal atrial natriuretic $peptide_{1–98}$
- NT-$proBNP_{1–76}$ = N-terminal pro–B-type natriuretic $peptide_{1–76}$
- LV = left ventricular
- LVH = left ventricular hypertrophy
- $proBNP_{1–108}$ = pro–B-type natriuretic $peptide_{1–108}$

Subjects were classified according to office systolic BP and/or diastolic BP as normotensive or belonging to 1 of 3 grades of hypertension: pre-hypertensive, stage 1 hypertension, and stage 2 hypertension, in accordance with the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) guidelines (19). BP was measured 3 times at 5-min intervals at an outpatient clinic. A complete cardiovascular evaluation, including 2-dimensional Doppler echocardiograms, was performed the day of blood collection. Heart failure was defined as previously described in the publication from Redfield et al. (14).

LVH was defined as a LV mass index >134 for males and >110 for females, where the LV mass index was defined as the quotient of left ventricular mass (by echocardiogram) divided by the body surface area (20).

Radioimmunoassay. Natriuretic peptide levels were determined using the Shionogi (Florham Park, New Jersey) and Biosite (San Diego, California) assays for $BNP_{1–32}$, Roche (Indianapolis, Indiana) for NT-$proBNP_{1–76}$, Bio-Rad (Hercules, California) for $proBNP_{1–108}$, and Phoenix Pharmaceuticals (Mountain View, California) for $ANP_{1–28}$ and for NT-$proANP_{1–98}$ (21,22). The specific characteristics of these assays have previously been reported. The $BNP_{1–32}$ and NT-$proBNP_{1–76}$ assays have been reported to display cross-reactivities for precursor BNP forms, including $proBNP_{1–108}$ (3). However, they have been found to be highly specific with regard to processed $BNP_{1–32}$ and NT-$proBNP_{1–76}$, respectively.

Statistical methods. Summary statistics were separated by JNC 7 category as defined by BP at the time of clinical exam and displayed as frequencies (percentages) for categorical variables, and median, Q1 to Q3 range, and frequency (percentage) of missing entries for continuous variables.

Categorical variables were compared by chi-square test, and continuous variables (including levels of natriuretic peptide forms) were compared by the Kruskal-Wallis rank sum test. Multivariable linear regression was used to determine which variables were independently associated with plasma BNP-form levels using a step-wise model-building process. The variables for each multivariable linear regression model were selected from among the variables with $p < 0.10$ in univariate analysis (not shown). With the exception of age, continuous variables were classified into categories. Body mass index (BMI) was included as a 3 level variable: <25 kg/m^2 (reference), 25 to 30 kg/m^2, and >30 kg/m^2. Creatinine was defined as a dichotomous variable: <1.2 (reference) and ≥1.2. To account for potential non-linearity of NP effects with age, a quadratic term for age was also included. Progressive models added variables that were associated with the plasma concentration of the hormones and a final model included age, age^2, sex, BMI, LVH, creatinine level, and the number of antihypertensive medications (except for the analysis that was performed in the subjects who were not on antihypertensive medications). In order to satisfy modeling and regression assumptions, BNP and ANP values were transformed by taking the natural logarithm of the raw values. In order to visually display effects, the least squares adjusted means were calculated from the final model and estimates and 95% CI were back-transformed to original scale. Homogeneity, across hypertension groups, of age and gender effects were tested by inclusion of interaction terms with stage of hypertension. Age effects were found to be homogeneous. Due to some evidence that gender effects were non-homogeneous a sensitivity analysis was conducted to compare normotensive and pre-hypertensive subjects. All tests were 2-sided, and for all analyses, $p < 0.05$ was considered statistically significant.

Results

General characteristics. Table 1 displays the characteristics of our study population stratified by JNC 7 BP categories at initial visit. The covariates that were significantly different between the stages of hypertension were age, sex, BMI, chronic renal insufficiency defined as Cockroft-Gault calculated glomerular filtration rate <60 ml/min, diabetes, coronary artery disease, valvular heart disease, cerebrovascular accidents, LV mass index, LV geometry, proBNP$_{1–108}$, NT-proBNP$_{1–76}$, BNP$_{1–32}$, ANP$_{1–28}$, and NT-proANP$_{1–98}$. Of the 2,082 subjects, 1,465 (72%) had elevated BP (systolic BP >120 mm Hg and/or diastolic BP >80 mm Hg), further classified into 755 pre-hypertensive subjects (51% of all hypertensive subjects), 492 stage 1 hypertension (34%) subjects, and 218 stage 2 hypertension (15%) subjects. The overall prevalence of LVH in our study population was 12.4%. As expected, the percentage of subjects with LVH increased over each stage of hypertension, from 7.3% in normotensive subjects to 32.9% in subjects with stage 2 hypertension.

Natriuretic peptides in normotensive and in hypertensive subjects. Table 2 displays the effects of hypertension stage on natriuretic peptides levels, with no adjustment and, consistent with previous reports, shows progressive elevation of all NPs with the severity of hypertension. Progressive multivariable adjustments were performed on the entire study population, including those on antihypertensive medications. This regression accounted for important covariates that were previously identified in univariate analysis, including age, sex, BMI, LVH, and creatinine (Fig. 1). Although, proBNP$_{1–108}$ remained unchanged in the different stages of hypertension, processed BNP forms, including BNP$_{1–32}$ and NT-proBNP$_{1–76}$, were significantly decreased in pre-hypertension ($p < 0.05$), with BNP$_{1–32}$ significantly decreased in stage 1 as well ($p < 0.05$). The NT-proBNP$_{1–75}$ tended to increase, while BNP$_{1–32}$ was elevated in stage 2 hypertension ($p < 0.05$). ANP$_{1–28}$ levels, after adjustment, showed no association with stage of hypertension, whereas NT-proANP$_{1–98}$ was significantly decreased in pre-hypertension ($p < 0.05$), and showed no change with stages 1 and 2 hypertension. These results were similar when analyses were restricted to only normotensive and pre-hypertensive subjects (Table 3).

Because the effect of antihypertensive medications on natriuretic peptides levels cannot be completely corrected by multivariate adjustment, we reanalyzed the association between natriuretic peptide levels and hypertension, limiting our analysis to subjects who were not on antihypertensive medications (Table 3). After adjustment, we found that none of the BNP forms were elevated and that BNP$_{1–32}$ (Roche) and NT-proBNP$_{1–76}$ showed a slight trend downward in early hypertension. ANP$_{1–28}$ levels were not associated with stage of hypertension, whereas NT-proANP$_{1–98}$ levels were significantly decreased in pre-hypertensive subjects ($p < 0.05$) in models 1 to 3.

Although, it was not the aim of the study, potential interactions of hypertensive stage with gender and age were also assessed in this population. There was no evidence of interactions with age, but there was some evidence that there were differential effects by gender (Online Table 1).

Discussion

The purpose of the current investigation was to evaluate the hypothesis that there is a lack of elevation of circulating ANP and BNP in hypertension in the general population. By characterizing hypertension as a relative deficiency state of these cardiac peptides, we imply their key role in the development of human hypertension and their ability, if augmented, potentially to reduce disease burden. To test this hypothesis, we used the PAVD population, a well-characterized adult population from Olmsted County, Minnesota. We classified subjects according to their BP levels into normotensive, pre-hypertensive, and stage 1 and stage

Table 1 Demographics of the Study Population by BP

Variable	Normotensive	Pre-Hypertensive	Stage 1 Hypertensive	Stage 2 Hypertensive	Total Population	p Value
Age, yrs*	56.5 (50.3–64.8)	61.0 (53.9–69.2)	65.0 (56.9–72.7)	70.1 (62.6–77.1)	61.6 (53.5–70.5)	<0.001
Females	320 (56%)	360 (48%)	251 (51%)	126 (58%)	1057 (52%)	0.006
BMI, kg/m^2	26.2 (23.5–29.1)	27.9 (25.4–31.2)	28.9 (25.7–33.0)	28.3 (25.3–33.2)	27.7 (25.0–31.2)	<0.001
CRF	109 (20%)	168 (23%)	155 (33%)	86 (41%)	518 (26%)	<0.001
Diabetes	28 (5%)	53 (7%)	50 (10%)	19 (9%)	150 (7%)	0.010
CAD	62 (11%)	78 (10%)	68 (14%)	37 (17%)	245 (12%)	0.025
HF	13 (2%)	16 (2%)	15 (3%)	7 (3%)	51 (3%)	0.65
Atrial fibrillation	17 (3%)	39 (5%)	31 (6%)	12 (6%)	99 (5%)	0.07
Creatinine ≥1.2	128 (23%)	194 (26%)	148 (31%)	55 (26%)	525 (27%)	0.03
Valvular disease	62 (11%)	100 (13%)	83 (17%)	47 (22%)	292 (14%)	<0.001
CVA	6 (1%)	12 (2%)	3 (1%)	13 (6%)	34 (2%)	<0.001
LV mass index*	90.4 (78.5–101.4)	93.8 (81.5–108.1)	98.4 (86.1–113.7)	103.0 (87.6–125.3)	93.8 (81.9–108.7)	<0.001
LV geometry						<0.001
Normal	394 (78%)	387 (67%)	211 (59%)	59 (40%)	1,051 (66%)	
Concentric remodeling	73 (14%)	133 (23%)	91 (25%)	41 (28%)	338 (21%)	
Concentric hypertrophy	20 (4%)	29 (5%)	25 (7%)	22 (15%)	96 (6%)	
Eccentric hypertrophy	17 (3%)	26 (5%)	31 (9%)	27 (18%)	101 (6%)	
Antihypertensive medications	130 (25%)	260 (37%)	226 (49%)	112 (55%)	728 (39%)	<0.001
Antihypertensive medications, n						<0.001
0	441 (77%)	495 (66%)	266 (54%)	106 (49%)	1,308 (64%)	
1	69 (12%)	163 (22%)	126 (26%)	57 (26%)	415 (20%)	
2	53 (9%)	74 (10%)	68 (14%)	31 (14%)	226 (11%)	
3	6 (1%)	18 (2%)	26 (5%)	22 (10%)	72 (4%)	
4	2 (0%)	5 (1%)	6 (1%)	2 (1%)	15 (1%)	
Beta-blocker	68 (13%)	96 (14%)	87 (19%)	44 (21%)	295 (16%)	0.005
Calcium channel blocker	16 (3%)	51 (7%)	48 (10%)	33 (16%)	148 (8%)	<0.001
ACE inhibitor	35 (7%)	61 (9%)	55 (12%)	30 (15%)	181 (10%)	0.003
Diuretic	55 (11%)	118 (17%)	120 (26%)	53 (26%)	346 (18%)	<0.001
Nitrate	15 (3%)	27 (4%)	17 (4%)	15 (7%)	74 (4%)	0.05
Vasodilator	10 (2%)	19 (3%)	22 (5%)	12 (6%)	63 (3%)	0.01
ARB	2 (0.4%)	13 (2%)	15 (3%)	6 (3%)	36 (2%)	0.008
ProBNP$_{1–108}$ (Bio-Rad)*	17.5 (8.0–36.5)	18.0 (8.0–41.0)	21.0 (9.0–43.0)	31.0 (14.0–64.0)	20.0 (9.0–42.0)	<0.001
BNP$_{3–32}$ (Shionogi)*	12.9 (5.1–28.6)	13.7 (5.4–28.2)	15.8 (5.8–32.7)	23.6 (10.0–52.3)	14.6 (5.6–32.0)	<0.001
BNP$_{1–32}$ (Biosite Triage)*	20.7 (8.5–45.3)	21.2 (8.4–44.9)	24.6 (9.7–57.2)	51.9 (22.6–106.8)	23.6 (9.5–55.3)	<0.001
NT-proBNP$_{1–76}$ (Roche)*	56.3 (24.3–120.0)	59.2 (24.5–118.0)	78.1 (31.9–166.9)	142.3 (62.8–332.7)	68.3 (27.8–145.4)	<0.001
ANP$_{128}$ (Phoenix)*	11.0 (7.2–15.2)	11.1 (7.2–15.5)	12.3 (7.6–16.8)	14.2 (8.7–18.7)	11.6 (7.5–16.2)	<0.001
NT-ANP$_{198}$ (Phoenix)*	2,079.0 (1,420.0–3,023.0)	2,066.5 (1,297.0–3,112.0)	2,384.0 (1,543.0–3,512.0)	2,753.5 (1,725.5–4,242.0)	2,212.5 (1,415.0–3,281.0)	<0.001

Values are n (%) or median (interquartile range). *Kruskal-Wallis test used for continuous variables; all other variables were categorical and were compared by chi-square.

ACE = angiotensin-inhibiting enzyme; ANP = A-type natriuretic peptide; ARB = angiotensin receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; BP = blood pressure; CAD = coronary artery disease; CRF = chronic renal failure; CVA = cerebrovascular accident; HF = past heart failure diagnosis; LV = left ventricular; NT = N-terminal; ProBNP = pro–B-type natriuretic peptide.

Table 2 Univariate Analysis of Biomarkers and Stage of Hypertension

Hypertensive Stage	$proBNP_{1–108}$ Bio-Rad ($\beta \pm$ SE)	$BNP_{1–32}$ Shionogi ($\beta \pm$ SE)	$BNP_{1–32}$ Biosite ($\beta \pm$ SE)	NT-$proBNP_{1–76}$ Roche ($\beta \pm$ SE)	$ANP_{1–28}$ Phoenix Pharmaceuticals ($\beta \pm$ SE)	NT-$ANP_{1–98}$ Phoenix Pharmaceuticals ($\beta \pm$ SE)
All subjects						
Normotensive	Reference	Reference	Reference	Reference	Reference	Reference
Pre-hypertensive	0.06 ± 0.07	−0.00 ± 0.06	−0.02 ± 0.07	−0.01 ± 0.07	0.02 ± 0.04	−0.03 ± 0.04
Stage I hypertensive	0.16 ± 0.07*	0.12 ± 0.07	0.17 ± 0.08*	0.33 ± 0.08†	0.10 ± 0.04*	0.12 ± 0.04*
Stage II hypertensive	0.61 ± 0.09†	0.52 ± 0.08†	0.86 ± 0.10†	0.96 ± 0.11†	0.20 ± 0.05†	0.24 ± 0.06†
Subjects not on antihypertensive medications						
Normotensive	Reference	Reference	Reference	Reference	Reference	Reference
Pre-hypertensive	0.03 ± 0.07	0.04 ± 0.07	−0.06 ± 0.08	−0.06 ± 0.08	0.02 ± 0.05	−0.08 ± 0.05
Stage I hypertensive	0.13 ± 0.09	0.07 ± 0.08	0.11 ± 0.10	0.23 ± 0.10*	0.04 ± 0.05	0.04 ± 0.05
Stage II hypertensive	0.50 ± 0.12†	0.49 ± 0.11†	0.62 ± 0.14†	0.68 ± 0.14†	0.11 ± 0.08	0.20 ± 0.08*

Values are $\beta \pm$ SE in contrast to normotensive subjects. Logarithm of BNP/ANP values used to satisfy regression assumptions. *$p < 0.05$, †$p < 0.001$ compared with normotensive subjects. Abbreviations as in Table 1.

2 hypertensive. Indeed, our results, for the first time, support the existence of such a relative deficiency state, as indicated by the simultaneous lack of increase of $proBNP_{1–108}$ and $ANP_{1–28}$ in any of the hypertensive stages, concomitant with reduced levels of $BNP_{1–32}$ and NT-$proBNP_{1–76}$ in early stages of hypertension and with reduced NT-ANP in stage 1 hypertension (Fig. 1). Importantly, we are the first to our knowledge to show that the absence of an increase in all BNP forms was also not accompanied by a compensatory increase in ANP forms. A significant elevation of a mature BNP (Biosite) form was observed only in stage 2 hypertension.

These findings extend our previous study of a selected group of hypertensive patients (10). That study also indi-

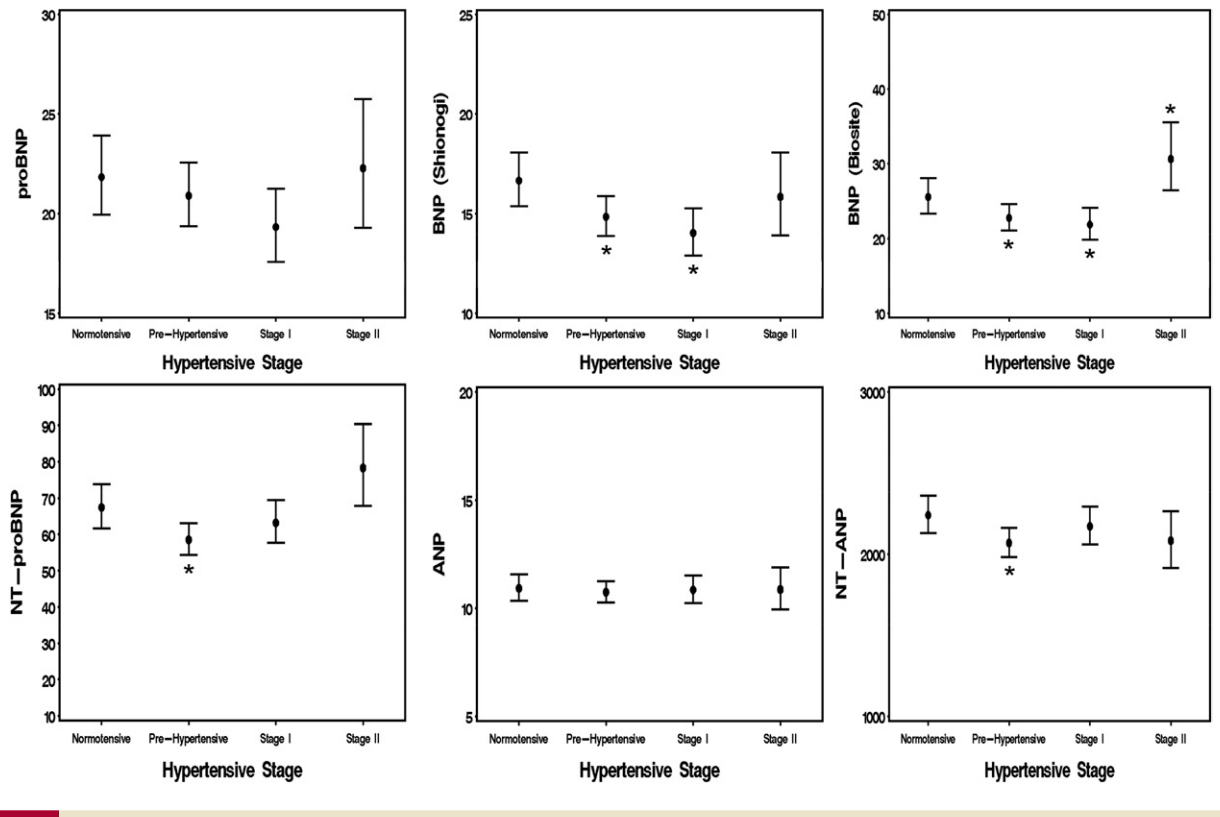


Figure 1 Results of Multivariable Analysis of All Subjects Adjusted for Age, Gender, BMI, LVH, Creatinine, and Number of Antihypertensive Medications

Figure displays least squares adjusted means for each stage of hypertension and each biomarker. ANP = atrial natriuretic peptide; BNP = B-type natriuretic peptide; NT-ANP = N-terminal atrial natriuretic peptide; NT-proBNP = N-terminal pro–B-type natriuretic peptide; ProBNP = pro–B-type natriuretic peptide.

Table 3 Univariate and Multivariate Analysis in Normotensive and Pre-Hypertensive Subjects

Biomarker	Univariate Model ($\beta \pm$ SE)	Model 1 ($\beta \pm$ SE)	Model 2 ($\beta \pm$ SE)	Model 3 ($\beta \pm$ SE)	Model 4 ($\beta \pm$ SE)	Model 5 ($\beta \pm$ SE)
Log $proBNP_{1–108}$	0.06 ± 0.07	−0.07 ± 0.06	−0.04 ± 0.06	−0.04 ± 0.06	−0.04 ± 0.06	−0.04 ± 0.06
Log $BNP_{1–32}$ (Shionogi)	−0.00 ± 0.06	−0.12 ± 0.05*	−0.11 ± 0.05*	−0.11 ± 0.05*	−0.11 ± 0.05*	−0.11 ± 0.05*
Log $BNP_{1–32}$ (Biosite)	−0.02 ± 0.07	−0.13 ± 0.06*	−0.12 ± 0.06	−0.12 ± 0.06	−0.12 ± 0.06	−0.12 ± 0.06*
Log $NT\text{-}proBNP_{1–76}$ (Roche)	−0.01 ± 0.07	−0.15 ± 0.06*	−0.13 ± 0.06*	−0.14 ± 0.06*	−0.13 ± 0.06*	−0.14 ± 0.06*
Log $ANP_{1–28}$ (Phoenix Pharmaceuticals)	0.02 ± 0.04	−0.01 ± 0.04	−0.00 ± 0.04	−0.01 ± 0.04	−0.01 ± 0.04	−0.01 ± 0.04
Log $NT\text{-}ANP_{1–98}$ (Phoenix Pharmaceuticals)	−0.03 ± 0.04	−0.11 ± 0.03*	−0.09 ± 0.03*	−0.08 ± 0.04*	−0.08 ± 0.03*	−0.08 ± 0.03*

Values are $\beta \pm$ SE in contrast to normotensive subjects. Model 1 = age+age^2+sex; Model 2 = age+age^2+sex+BMI; Model 3 = age+age^2+sex+BMI+LVH; Model 4 = age+age^2+sex+BMI+LVH+creatinine; Model 5 = hypertension+age+age^2+sex+BMI+LVH+creatinine+number of antihypertensive medications. ^Logarithm of BNP and ANP values used to satisfy regression assumptions. *$p < 0.05$, **$p < 0.001$ compared with normotensive subjects.

Abbreviations as in Table 1.

cated the presence of a BNP deficiency state in early stages of hypertension. However, the original investigation of Belluardo et al. (10) did not have access to $proBNP_{1–108}$ levels, so it was not possible to investigate whether reduced levels of processed forms of BNP, namely $NT\text{-}proBNP_{1–76}$ and $BNP_{1–32}$, in early hypertensive stages were also associated with an inadequate production/release of their precursor. Importantly, in the current study, when we looked at the entire population and adjusted for antihypertensive medications, we demonstrated that the reduced levels of mature $BNP_{1–32}$ in early stages of hypertension were accompanied by a lack of activation of its precursor, $proBNP_{1–108}$, and by a similar reduction of $NT\text{-}ANP_{1–98}$. All together this findings support the existence of a relative deficiency of such protective hormonal system possibly due to an impaired release and/or to an elevated metabolism of the mature and biologically active forms of these cardiac hormones.

Cardiac endocrine function in human hypertension. For the first time to our knowledge, both arms of the cardiac endocrine system were assessed in worsening hypertension in a large sample of the general community, adjusting for end-organ cardiac effects of hypertension, such as ventricular hypertrophy. Adjustment for important variables associated with natriuretic peptide levels reveals that most NP forms tend to decrease or are significantly decreased in early hypertension and then tend to increase only in late hypertension, when we consider early and late hypertension independently. Furthermore, using the JNC 7 classifications, $proBNP_{1–108}$, the precursor BNP form, was not significantly increased in any stage of hypertension. These observations point to a problem of production of substrate ($proBNP_{1–108}$), rather than a problem of downstream processing in hypertension.

Our conclusion regarding the presence of a relative deficiency of the entire BNP system in hypertension is also supported by recent evidence that some individuals in the community who have an elevated level of processed BNP forms have reduced, rather than elevated, BP. Specifically, polymorphisms in minor alleles of the *NPPA* gene were associated with lower BP and higher natriuretic peptide levels in subjects with coronary artery disease, although no correlation was seen with clinical outcome (23). Furthermore, Newton-Cheh et al. (11) also found polymorphisms of *NPPA* associated with higher natriuretic peptide levels and lower rates of hypertension in the general community. We have built on these important studies by characterizing levels of the precursor $proBNP_{1–108}$. At this time, it is unknown whether *NPPA* polymorphisms are associated with higher levels of $proBNP_{1–108}$ production. Thus, this study confirms that in human hypertension, there is a lack of activation of both BNP and ANP.

The question remains as to why there are inadequate or failed metabolic compensatory mechanisms, specifically $proBNP_{1–108}$ production and processing, in hypertensive patients. Therefore, there is a need for studies at the molecular and tissue levels of human myocardium to elucidate where $proBNP_{1–108}$ processing occurs in humans and whether chronically elevated BP affects the heart's production of $proBNP_{1–108}$ (such as through LVH) more than the peripheral processing to BNP (in plasma or at the level of a target organ, such as the kidney, heart, or adipose tissue). Finally, it may be possible to prevent late changes in the processing pathway by therapeutic supplementation of natriuretic peptides, which could reduce BP and improve cardiorenal structure and function. Indeed, a recent study by Cataliotti et al. (24) demonstrated that early vector delivery of a proBNP gene in spontaneously hypertensive rats, which resulted in constitutive overexpression of BNP, was associated with decreased BP and improved cardiac structure and function. The beneficial effect on controlling BP was recently confirmed in a patient with uncontrolled hypertension despite treatment with conventional anti-hypertensive medication, where a small dose of subcutaneously delivered BNP induced sustained normalization of BP without the need for further therapy (25).

Study limitations. An important limitation of our study is that it is epidemiological in nature and does not describe the molecular abnormalities that result in aberrant natriuretic peptide levels in hypertension. Another limitation of the current study is that residual confounding factors cannot be ruled out. We used multiple linear regression to attempt to control this cross-sectional comparison for marked age and gender differences between hypertension categories, and indeed some differences which were not apparent in unad-

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Table 4 Univariate and Multivariate Analysis in Normotensive and Pre-Hypertensive Subjects Not on Antihypertension Medications

Biomarker	Univariate Model ($\beta \pm$ SE)	Model 1 ($\beta \pm$ SE)	Model 2 ($\beta \pm$ SE)	Model 3 ($\beta \pm$ SE)	Model 4 ($\beta \pm$ SE)
Log $proBNP_{1–108}$	0.03 ± 0.07	−0.00 ± 0.07	0.02 ± 0.07	0.02 ± 0.07	0.02 ± 0.07
Log $BNP_{1–32}$ (Shionogi)	0.04 ± 0.07	−0.01 ± 0.06	−0.00 ± 0.06	0.00 ± 0.06	0.00 ± 0.06
Log $BNP_{1–32}$ (Biosite)	−0.06 ± 0.08	−0.07 ± 0.07	−0.05 ± 0.07	−0.05 ± 0.07	−0.05 ± 0.07
Log NT-$proBNP_{1–76}$ (Roche)	−0.06 ± 0.08	−0.08 ± 0.07	−0.06 ± 0.07	−0.06 ± 0.07	−0.06 ± 0.07
Log $ANP_{1–28}$ (Phoenix Pharmaceuticals)	0.02 ± 0.05	0.01 ± 0.05	0.03 ± 0.05	0.03 ± 0.05	0.03 ± 0.05
Log NT-$ANP_{1–98}$ (Phoenix Pharmaceuticals)	−0.08 ± 0.05	−0.12 ± 0.04*	−0.10 ± 0.04*	−0.09 ± 0.04*	−0.08 ± 0.04

Values are $\beta \pm$ SE in contrast to normotensive subjects. Model 1 = age+age^2+sex; Model 2 = age+age^2+sex+BMI; Model 3 = age+age^2+sex+BMI+LVH; Model 4 = age+age^2+sex+BMI+LVH+creatinine. Logarithm of BNP and ANP values used to satisfy regression assumptions. *$p < 0.05$.

Abbreviations as in Table 1.

justed analyses became apparent only after adjustment. This statistical adjustment procedure makes assumptions of linearity relating age and sex to log transformed peptide levels. We tested for non-linearity of age effects as well as for non-homogenous effects of these variables across hypertension categories. Although gender/hypertension category interactions were seen, a meta analysis based on gender stratified analysis gave very similar results to those presented using the simpler assumption. When we examined all hypertensive groups for evidence of interaction with age and gender, there was some evidence of gender effects that were differential by hypertensive stage. There was no evidence of differences by age, however. Table 1 of the Supplementary Data compares results of a simple gender adjustment with the results of combining separate male and female analyses with meta-analysis. These data indicate that circulating levels of NPs may be differently affected by gender.

Our study raises an important question regarding which part of the BNP system, the production of $proBNP_{1–108}$ or the processing into mature $BNP_{1–32}$, is more deficient in hypertension, and therefore, which of the 2 could be a better novel therapeutic target for the treatment of hypertension. Another important limitation is that we did not differentiate between essential hypertension and secondary hypertension. We also did not differentiate between the types of antihypertensive medications used in our analysis, and it may be that some individuals respond differently to different antihypertensive agents or may seek treatment at different points in the course of disease, making them more or less likely to benefit from their own endogenous natriuretic system. There is no definitive way to correct for medication usage, and we have, therefore, performed a variety of analyses to attempt to adjust for this factor. Specifically, we performed the analysis without adjusting for medications (Table 3, models 1 to 4), and adjusting for the medications (Fig. 1, Table 3, model 5), and also in the group of subjects who were not taking any medication (Table 4).

Perspective. Recent reports have described an underactivation of BNP forms in early hypertension. A seminal study by Newton-Cheh et al. (11) has now established in a cohort of 48,939 subjects that basal BP and risk for hypertension is associated with common genetic variants of the ANP (*NPPA*) and BNP (*NPPB*) genes that affect ANP and BNP levels. In that study, reduced ANP and BNP levels were characterized by elevated BP and increased risk for hypertension, leading the authors to propose the use of novel natriuretic peptides to treat hypertension. Furthermore, the presence of the single nucleotide polymorphism rs5068, characterized by a significantly higher level of circulating ANP and BNP, was associated with a 15% reduction in odds of hypertension. Thus, our data from a general adult population and the genetic studies from Newton-Cheh et al. support the existence of genetic predisposition to hypertension that is characterized by lower circulating levels of ANP and BNP. Newton-Cheh et al. have further proposed the beneficial effects of exogenous natriuretic peptides administration in the regulation of BP and support the need for their supplementation in hypertension. Recent studies from our group have confirmed such hypothesis both in animal models of hypertension and in a pilot study in human with uncontrolled hypertension (24,25). Indeed, the lack of ANP and BNP functionality, whether due to impaired processing or production, in patients in whom their biological properties would be highly beneficial, may have clinical and therapeutic implications that favor the use of such peptides for the treatment of this morbid condition. On this regard, Cataliotti et al. recently reported the favorable BP-lowering effects of subcutaneously (SQ) administered BNP in a patient with difficult to control hypertension and resistance to multiple drug regiment. Here a small dose of SQ BNP (10 μg/kg, twice daily) completely normalize BP without the need of further supplementation with conventional antihypertensive drugs (25). This study is the first clinical evidence of the efficacious blood pressure control with a cardiac hormone in a patient with difficult to control hypertension and opens to the possible use of BNP for the treatment of resistant hypertension.

Conclusions

We reported that although there appears to be no increase in mature forms of ANP and BNP in early hypertension, multivariable modeling instead shows significant deficiency of mature forms with a trend toward reduced $proBNP_{1–108}$. We conclude that secretion of $proBNP_{1–108}$ is spared in early hypertension, and that it does not increase with the

severity of hypertension, thus decreased levels of mature forms of BNP are better explained by either an impaired peripheral processing or an accelerated metabolism in this early stage, although a relative deficiency of $proBNP_{1–108}$ production does emerge as hypertension progresses, with compensatory processing likely only in late-stage hypertension. Because LVH represents reversible end-organ damage due to essential hypertension, which is likely the single biggest modifiable risk factor for cardiac and renal disease (26), the current findings encourage further studies to evaluate whether the use of natriuretic peptides or their chimeric derivates can be useful in the treatment of human hypertension.

Acknowledgments

The authors thanks the Cardiorenal Research Laboratory. Additionally, they also thank Drs. Ivan Nenadic, Wael Salem, Rishi Wadhera, Eddie Greene, Tony Windebank, and Thomas Wang for their most helpful and thoughtful discussions of this work.

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Key Words: ANP ■ BNP ■ hypertension ■ natriuretic peptide ■ NT-proBNP ■ proBNP.

APPENDIX

For supplementary data and table, please see the online version of this article.